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DIFFERENT DIETARY FIBRES INCLUDED IN THE GESTATION AND LACTATION DIET SHAPE SOW'S MICROBIOTA, MODIFY COLOSTRUM AND MILK COMPOSITION AND AFFECT PIGLET GROWTH AND HEALTH

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Abstract

This thesis reports three experimental studies that may contribute to understand how the sources or types of dietary fibres (DFs) included in sow diet with similar level of total DFs influence the composition of colostrum and milk and their related effects on offspring performance and gut microbiota. The first study showed that decreasing the level of hemicelluloses (HCs) in sow's lactation diet increased the proportion of butyrate and the concentration of volatile fatty acids (VFAs), copper and threonine in milk. Simultaneously, the post-weaning growth of low birthweight piglets was improved, and the diarrhoea occurrence was reduced during the second week post-weaning. The second study showed that the level of HCs in the diet of lactating sows affected their faecal microbiota, modified the VFA profile in sow's faeces during lactation and barely impacted the faecal microbiota of slow and fast growing piglets. The third study showed that replacing a source soluble DFs by one of insoluble DFs in sow's diet during late gestation and lactation reduced farrowing duration, increased total VFAs and lactoferrin concentrations in colostrum, improved growth performance from birth to 1 day of lactation, during the post-weaning period and throughout the study, and reduced diarrhoea occurrence during the first week post-weaning. Finally, a fourth study proposed a workflow to analyse low biomass samples from the umbilical cord blood aiming at investigating the existence of a pre-birth microbiota with no substantial findings to confirm this hypothesis. Overall, the results of these studies confirmed that, besides the level of DFs, the sources, and the types of DFs included in the sow's diet shape the sow's microbiota, influence the composition of colostrum and milk, and improve offspring performance, but with limited impacts on the microbiota of piglets.

List of original manuscripts included in the thesis

- **Manuscript 1:**

Decreasing the level of hemicelluloses in sow's lactation diet affects the milk composition and post-weaning performance of low birthweight piglets.

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- **Manuscript 2:**

The level of hemicelluloses in lactation diet affects faecal microbiota of sows and their piglets differing for slow and fast growth during suckling period.

- **Manuscript 3:**

Increasing the proportion of insoluble dietary fibres in sow's late gestation and lactation diet reduce the farrowing duration, affects the colostrum composition, and enhance the performance of the piglets.

- **Manuscript 4:**

A workflow to study the microbiota profile of piglet's umbilical cord blood: from sampling to data analysis.

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Background

Early life colonisation of the piglet intestine is one of the most important stages in modulating both gut health and development; changes in intestinal microbiota during this period might have long-term effects on the host (Nowland et al., 2021). Luise et al. (2021) reported that the supplementation of probiotics at birth improved piglet growth from birth until two weeks post-weaning, promoted the growth of beneficial bacteria such as those belonging to the Lactobacillus genus in the piglet faecal microbiota during the suckling period and reduced post-weaning diarrhoea. The first colonisation of the piglet intestinal microbiota begins at birth, during the passage across the vaginal tract of the mother, and with contact with the skin and faeces of the sow (Trevisi et al., 2021). Moreover, colostrum and milk not only provide the nutrients and bioactive compounds (e.g., immunoglobulins) essential for the growth and immunity of the offspring; however, they can also be important drivers in shaping the piglet intestinal microbiota (Csapó et al, 1996; Klobasa et al, 1987; Salcedo et al., 2016; Chen et al., 2018a). In fact, colostrum and milk contain several compounds, such as oligosaccharides and microorganisms, which could exert either a prebiotic or a probiotic effect, respectively, on piglet intestinal health (Chen et al., 2018a; Zhang et al 2018). In this context, both gestation and lactation diets can shape sow microbiota, modifying colostrum and milk quality and exerting a beneficial effect on the development and the health of the offspring (Tian et al., 2020).

Dietary fibres (DFs) are plant carbohydrates which are mainly fermented in the large intestine by cellulolytic bacteria, producing volatile fatty acids (VFAs) (Jha and Berrocoso, 2016). Subsequently, VFAs are absorbed from the large intestine and, by means of the bloodstream, they could arrive at the mammary glands where they could be used for fat synthesis in colostrum and milk (Tian et al., 2020). A previous study by Loisel et al. (2013)

reported that adding up to 20% of DFs to the sow gestation diet increased colostrum fat content. In fact, DFs could increase the concentration of VFAs in plasma, and subsequently the mammary glands could use these VFAs for fat synthesis (Theil et al., 2012). In addition to the inclusion level of DFs, recent studies have also observed that the physiochemical properties of DFs, such as solubility in water in the maternal diet could affect the piglet intestinal microbiota. For instance, Li et al. (2019) have reported that the ratio between insoluble and soluble DFs in the sow gestation diet affected the composition of the faecal microbiota of the dam as well as that of their offspring microbiota. At the same time, Paßlack et al. (2015) showed that including 3% inulin as a source of soluble DFs in the late gestation and lactation diet of the sow reduced piglet faecal Enterobacteriaceae populations during the suckling period. The following paragraphs will highlight the main nutrients and microorganisms contained in colostrum and milk. Subsequently, the focus will be on the main nutritional strategies in the maternal diet which could affect colostrum and milk quality, offspring growth and intestinal health. Finally, greater emphasis will be placed on the effects of the levels, types, and sources of DFs in the sow diet during gestation and lactation on reproductive performance, mammary secretion composition, and intestinal microbiota, and their related effect on piglet development and intestinal health.

1 Chemical composition and microbiota of sow colostrum and milk

1.1 The importance of colostrum and milk

Porcine colostrum and milk represent the first source of nutrients for newborn piglets (Quesnel et al, 2012). Their composition is mainly characterised by several sources of nutrients and immunological compounds, such as carbohydrates, lipids, and proteins (immunoglobulins) (Csapó et al, 1996; Klobasa et al, 1987). Furthermore, recent studies have reported that additional novel compounds, such as oligosaccharides, bacteria, exosomes, and leukocytes can exert a beneficial effect on piglet development and health (Zhang et al, 2018). More specifically, oligosaccharides and bacteria can have either a prebiotic or a probiotic effect on the piglet intestinal environment by increasing the population of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium* spp., at the expense of the pathogenic ones, such as *Salmonella* spp (Nowland et al., 2021). At the same time, previous studies have observed that exosomes in porcine milk can regulate the proliferation of the enterocytes and digestive tract development while leukocytes can be used as a source of immunity together with immunoglobulins (Chen et al., 2016; Pomorska-Mól et al., 2010). However, colostrum and milk chemical composition is strongly dependent on the stage of lactation. In fact, colostrum represents the first mammary secretion which is partially synthetised at the end of gestation (Quesnel et al, 2015). Due to its biochemical value, colostrum is considered to be the “elixir of life” for the newborn piglet (Theil et al, 2014). It contains a high concentration of immunoglobulins as compared to milk. Nevertheless, its production persists for up to 24 hours (Csapó et al, 1996; Klobasa et al, 1987). Therefore, during the first hours of life, it is of vital importance for the piglet to reach an adequate intake of colostrum (200-300 g) inasmuch, as the protein level and, therefore, the immunoglobulin concentration constantly decreases after birth

(Devillers et al, 2007). Transient milk is defined as the secretion by the mammary glands after colostrum and continues up to the fourth day of lactation. It contains more lipids as compared to the colostrum while its level of protein significantly decreases, primarly due to the decreasing immunoglobulin concentration. Finally, during mid-lactation (after the fourth day), the chemical composition of milk reaches a sort of stability. Hence, from 10 days of lactation onwards milk is defined as “mature milk”. It is mainly characterised by a higher level of lactose as compared to transient milk and colostrum (Csapó et al, 1996; Klobasa et al, 1987).

1.2 Nutrient precursors for colostrum and milk synthesis

The last month of gestation represents the time in which the major part of mammary growth and development occurs in sows (Farmer and Hurley, 2015). During this period, colostrum secreting capacity develops as the mammary gland converts from a merely ductal tree to a highly functioning exocrine organ with lobolo-alveolar expansive structures. (McManaman and Neville, 2003). The main components of colostrum and milk, such as lactose, protein and lipids are synthesised from the nutrients absorbed by the mammary glands through the bloodstream and delivered to the secretory epithelial cells (Farmer et al., 2015). Therefore, the main limiting factor for colostrum and milk production is the availability of glucose, amino acids, and fatty acids from both the gestation and lactation diets (Farmer et al., 2008). First, the main precursor for the synthesis of lactose is glucose, up to 70% of which is used by the mammary epithelial cells for the synthesis of lactose (Theil et al., 2012). Subsequently, the amino acids supplied by the bloodstream account for up to 95% of the protein in the colostrum and milk whereas the remaining part could be derived from non-protein nitrogen. The extraction efficiency from the bloodstream varies from 16.8% for non-essential amino acids up to 20.5% for essential ones (Boyd and Kensinger, 1998) Finally, the last group of nutrients of quantitative importance for the

chemical composition of colostrum and milk are the fatty acids. Triglycerides from circulating blood act as the major precursors for the synthesis of colostrum and milk fat (Farmer et al., 2008). Triacylglycerol stored in lipoproteins is hydrolysed by the lipoprotein lipase so that fatty acids can be absorbed by the mammary epithelial cells either by passive diffusion or a facilitated transport mechanism driven by the plasma concentration gradient (Theil et al., 2012).

1.3 Gross chemical composition of sow colostrum and milk

1.3.1 Protein

The protein component in colostrum and milk represents not only a source of amino acids for the newborn piglets but is also a source of several bioactive compounds, such as immunoglobulins (Igs), growth factors and hormones (Theil and Hurley, 2016). Caseins and whey proteins represent the major milk proteins found in sow milk. Caseins include αS1-casein, αS2-casein, β-casein and κ-casein. The percentage of caseins in the total protein content of colostrum ranges from 9 to 32% after farrowing. In the following 24 hours, it then reaches approximately 30 to 45% while during the rest of the lactation period, it ranges from 50 to 55% (Csapó et al, 1996). The whey proteins include a wide range of proteins, such as immunoglobulins, β-lactoglobulin, α-lactoalbumin and lactoferrin. The percentage of whey proteins in the colostrum represents up to 90% of the total protein, mainly due to the high concentration of immunoglobulins, while they decrease until reaching 70% 24 hours after the onset of farrowing, and it then remains stable at 47 to 50% in mature milk (Klobasa et al, 1987). Of further interest, immunoglobulins represent the primary protein components of colostrum and the primary source of passive immunity for the piglets. Immunoglobulins include IgG, IgA and IgM isotypes (Hurley and Theil, 2013). The IgG isotypes are the most prevalent in the colostrum (81%), while the IgAs are

the most abundant isotypes in milk (70%) (Butler et al, 1974). As sow Igs cannot pass the placental barrier during gestation, piglets are born agammaglobulinemic. Therefore, colostrum plays a vital role in reaching adequate immunity at birth. In particular, IgGs are absorbed intact before gut closure. However, as lactation proceeds, IgG decreases and IgA becomes more abundant, playing a major role in intestinal health, preventing diarrhoea during the suckling period (Le Dividich et al, 2005; Devillers et al, 2011). Finally, growth factors include insulin-like growth factor, transforming growth factor-β2, and some members of the epidermal growth factor. However, their biological function is still unclear in pigs. Many studies on cow milk have shown that the epidermal growth factor stimulates the proliferation of epidermal, epithelial, and embryonic cells, inhibits the secretion of gastric acid, and promotes wound healing, and bone resorption. Transforming growth factor-β2 stimulates the proliferation of some cells, especially in connective tissue, whereas it acts as a growth inhibitor of some other cells, such as lymphocytes and epithelial cells. Insulin-like growth factor stimulates cellular growth, development, and differentiation, glucose uptake, and the synthesis of glycogen (Gauthier et al, 2006).

1.3.2 Lipids

The fat proportion in colostrum and milk is crucial for increasing piglet survival, especially in the first hours of life. As piglets are born with no energy reserves, lipids from the colostrum and transient milk represent the first source of energy which can aid the thermoregulation process of the offspring. The consequence of underfeeding is weakness which can cause crushing (Theil et al., 2014). However, regardless of their biological functions, lipids are the most variable compounds in colostrum and milk composition. Their composition and levels are extensively affected by the stage of lactation, diet, breed, and parity order (Hurley, 2015; Luise et al. 2018). Moreover, it is widely known that sow milk is mainly characterised by a predominance of oleic acid followed by palmitic, linoleic and

palmitoleic acids. Nevertheless, the levels of palmitic and palmitoleic acids are considerably lower in the colostrum than in mature milk while the level of linoleic acid is correspondingly higher (Csapó et al, 1996; Klobasa et al, 1987). The latter nutrient, as part of the polyunsaturated fatty acids is well recognised for its beneficial effect regarding piglet development and health (Bontempo et al., 2004).

1.3.3 Carbohydrates

1.3.3.1 Lactose

Lactose represents the most abundant sugar found in mammal milk; lactose is mainly composed of galactose and glucose by a β-glycosidic linkage. Due to its osmotic effect, lactose can regulate the volume of water absorbed in the alveoli and, therefore, the amount of milk produced (Costa et al., 2019). Lactose can be directly used as an energy source or indirectly for amino acid metabolism (Jang et al., 2022). Indeed, a previous study showed that dietary lactose intake in neonatal piglet can increase tissue protein synthesis (Frank et al., 2006). Moreover, it can be important for intestinal health, especially during the first days of life when the piglets are susceptible to several enteric diseases or conditions of stress (Jang et al., 2022). In fact, bacteria such as Lactobacillus, can ferment part of the lactose ingested during the suckling period in the stomach. The main products of fermentation are lactate and acetate which can help to maintain gastric acidity, activating endogenous enzymes and shaping the gut microbiota which could ultimately lead to a favourable environment for preventing the attachment of pathogenic bacteria to the gut epithelia (Zhao et al., 2021).

1.3.3.2 Oligosaccharides

Apart from lactose, oligosaccharides are the second most abundant carbohydrates in porcine milk (Jang et al., 2022). More than 30 oligosaccharides have been identified in porcine milk, with 3′-sialyllactose, lacto-N-tetraose, α1–3,β1–4-d-galactotriose, 2′-fucosyllactose, and 6′-sialyllactose, making up the major part of the total oligosaccharides. Although for a long time they were considered to have no relevant biological effects, in recent years oligosaccharides have begun to be considered for their "prebiotic" effects on gut health (Salcedo et al., 2016). As piglets do not have specific enzymes to digest milk oligosaccharides, the latter are not absorbed but instead pass through the small intestine and are then fermented by microbes in the large intestine, resulting in an increased proportion of potentially beneficial bacteria, including *Lactobacilllus* (Jang et al, 2022; Tian et al., 2019). Therefore, there is growing interest in them, especially regarding their potential beneficial effects in the first stages of life. In fact, as the intial colonisation in the gut is of vital importance for the maturation of the intestinal function and as oligosaccharide content is, for the most part, higher in colostrum than in mature milk (Salcedo et al., 2016), it is possible that these carbohydrates could be effective in early life nutrition intervention by shaping the intestinal microbiota of suckling piglets, thereby improving their development and health (Tian et al., 2019; Trevisi et al., 2020).

1.4 Microbiological profile

During lactation, colostrum and mature milk represent a key factor in shaping the intestinal microbiota of suckling piglets as they can be considered to be a source of bacteria which can colonise the piglet intestine during the first days of life (Trevisi et al., 2021). Liu et al. (2019) observed that vertical transmission from the mother by means of milk can occur and affect the small intestine microbiota of the offspring. Using next-generation sequencing technologies, Chen et al. (2018a) have recently identified the most predominant bacteria phyla found in porcine milk throughout the entire lactation period.

Firmicutes and Proteobacteria accounted for approximately 53 and 25.4% of the relative abundance, respectively while the remaining part was, for the most part, composed of Bacteroidetes, Actinobacteria, Fusobacteria and Tenericutes **(Figure 1).** Moreover, at the genus level, they found more than 212 genera in sow milk. In particular, the most abundant genera were those belonging to the Firmicutes phylum, including *unclassified Ruminococcaceae*, *Streptococcus*, *Lactobacillus* and *Lachnospiraceae*. The other predominant genera belonging to the Proteobacteria phylum included *Acinetobacter*, *Moraxella*, and unclassified *Neisseria* **(Figure 2).** Similar to the gross chemical composition, the microbial composition changes during lactation. Recent studies have shown that the stage of lactation can affect the relative abundance of predominant phyla and genera. Chen et al. (2018a) observed that, during the colostral period, *Streptococcus* was the most abundant genus while unclassified *Ruminococcaceae*, *Bifidobacterium*, *Staphylococcus* and *Acinetobacter* were predominant in transient milk and mature milk. Moreover, Chen et al. (2018b) showed that the *Lactobacillus* genus increased, following the same trend as lactose throughout lactation.

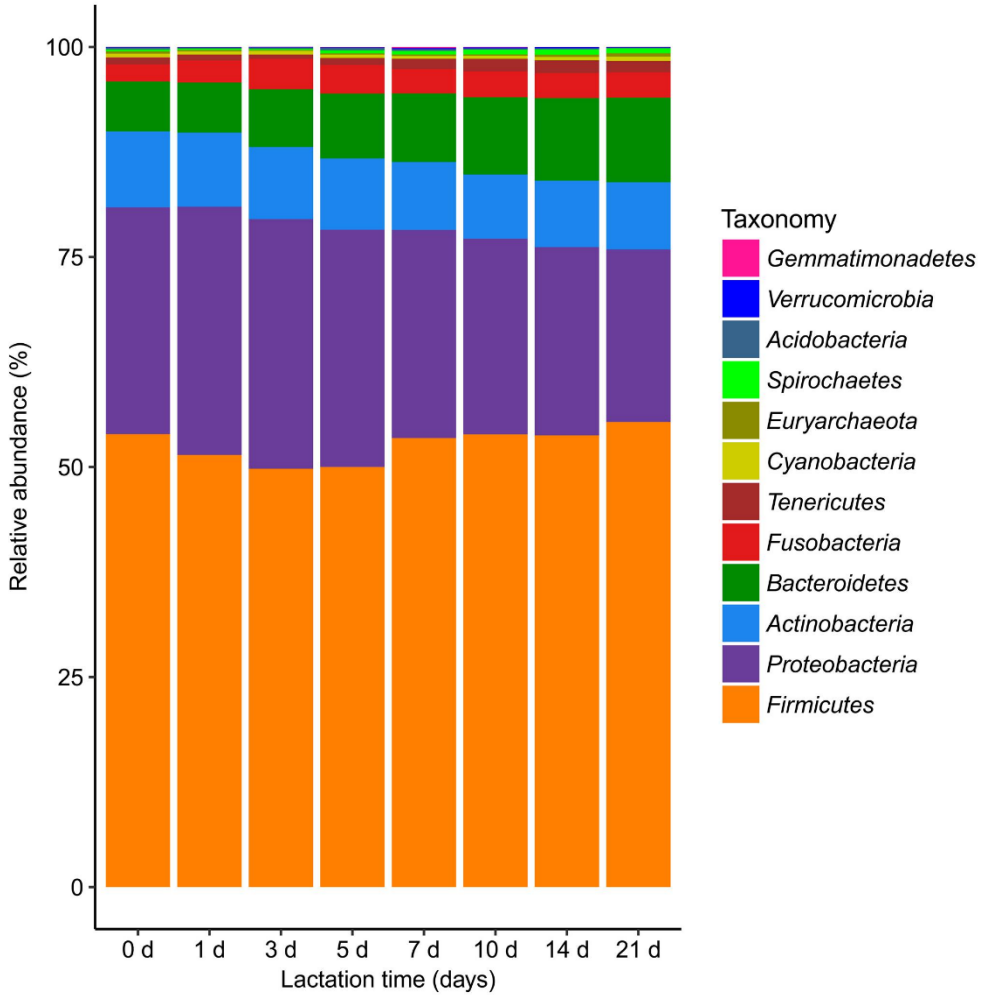

Figure 1. Bacterial taxonomic composition at phylum level in sow milk samples (*n* = 130) (Chen et al. 2018a)

Data are expressed as relative abundances produced by polymerase chain reaction amplification and pyrosequencing of 16S rRNA.

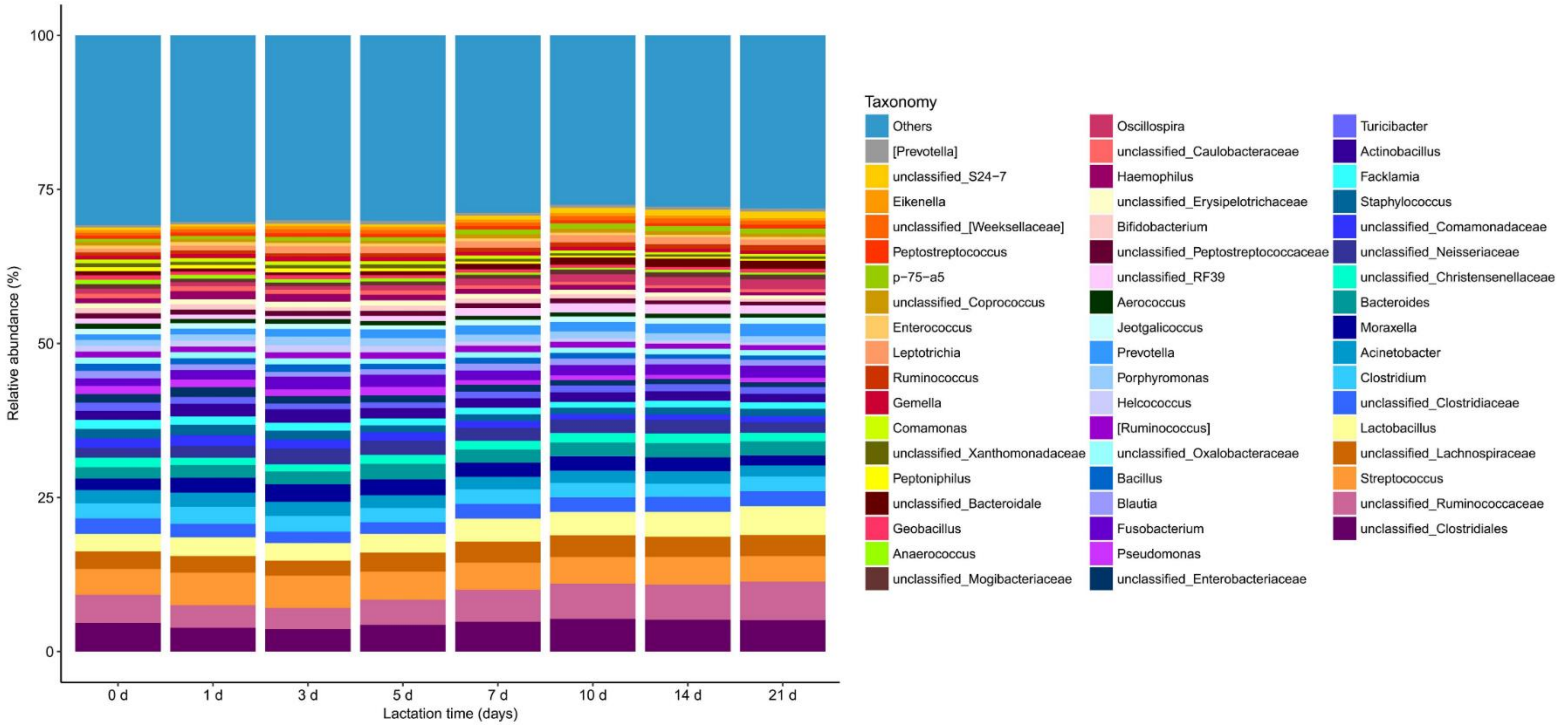

Figure 2. Bacterial taxonomic composition at the genus level in sow milk samples (*n* = 130) (Chen et al. 2018a)

Data are expressed as: relative abundances produced by polymerase chain reaction amplification and pyrosequencing of 16S rRNA

2 Sow hyperprolificacy and nutritional strategies to enhance the performance of the offspring.

2.1 Problems related to sow hyperprolificacy

During the last two decades, genetic selection for sow prolificacy increased the total number of piglets born per sow (Quesnel et al., 2008). Nowadays, it is common for pig producers to deal with a litter size of between 16 and 21 newborn piglets (Björkman et al., 2017). However, this improvement has caused several problems regarding the health and the growth performance of the offspring, mainly during the pre-weaning period; this has subsequent effects which can influence the production outcomes during the post-weaning period (Blavi et al., 2021). Moreover, the average litter birth weight has strongly decreased, and therefore, the inter-litter and within-litter weight heterogeneity has increased as well as the inter-litter and within-litter average daily gain heterogeneity (Quiniou et al., 2002). Compared with heavier litter mates, piglets weighing less than 5 kg at weaning will have slower growth, occupy pen space longer and thus reach slaughter weight later, making them economically inefficient. Therefore, it is very important to reach a high body weight at weaning as, from this point onwards, the opportunity of implementing strategies so that lighter pigs can successfully catch up to their heavier pen mates decreases dramatically (López-Vergé et al., 2018). It is well known that early-life gut colonisation plays an important role in the development and maturation of the gut, thereby improving the growth and disease resistance of piglets (Nowland et al., 2021). Therefore, understanding and improving gut health through different nutritional strategies is becoming a reality in the pig industry.

2.2 Early life colonisation of the piglet intestinal environment

Several studies have shown that the modulation of gut microbiota using probiotics and prebiotics early in life could represent a promising strategy for improving the energy supply of the piglets, and for avoiding or reducing the detrimental effects of the post-weaning syndrome (Williams et al 2016). In addition to direct interventions on piglets, the modulation of the piglet microbiota may be achieved by means of the sow diet. Both in gestation and lactation, the maternal diet has been shown to modify colostrum and milk composition and to affect the intestinal microbiota of newborn piglets, favouring the development of beneficial bacteria, including Lactobacillus and unclassified Lactobacillaceae and reducing those belonging to Clostridium sensu stricto and Escherichia (Tian et al., 2020). Both colostrum and maternal milk represent a source of vertical microbial transmission from the mother to the offspring in which the immune status and the gut microbiota of the sow can affect colostrum composition and the immunoglobulin profile, improving the development and the intestinal health of the offspring (Hasan et al., 2018). However, colostrum and milk are not the only important factors in intestinal colonisation early in life. A recent study has shown that the vaginal and faecal microbiota could significantly affect the faecal microbiota of the offspring, without, however having a long-term effect (Chen et al., 2018b). In particular, for the most part, the first bacterial colonisation of the offspring gastrointestinal tract seems to occur when the newborn is passing through the birth canal, via contact with the vaginal microbiota (Mackie et al., 1999). In addition, due to their exploratory behaviour, piglets are always in contact with sow faeces; they may acquire the faecal microbiota of their mother and use it for the establishment of the gut microbiota (Aviles-Rosa et al., 2019). In the following paragraphs, the most studied feeding strategies which shape the sow microbiota using probiotics or

prebiotics will be introduced. Probiotics can be defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Hill et al., 2014). A prebiotic can be defined as “A non-digestible compound that, through its fermentation by specific microorganisms in the gut, can promote the abundance of specific bacteria and therefore modulate the composition and the activity of the gut microbiota, thus, conferring a beneficial physiological effect on the host” (Bindels et al., 2015).

2.2.1 Probiotics

The aim of using probiotics in the swine industry is to colonise the gastrointestinal tract with beneficial living microorganisms and by that improve the well-being of the pigs benefit the intestinal microbiota, and, therefore, to enhance the health and well-being of the animals (Kenny et al., 2011). To be used for pig production, these compounds must have several properties. First, they must have the capacity of settling or being metabolically active in the intestine; thus, they must be resistant to the gastric environment and to the process of digestion in order to interact with the intestinal environment of the host. Thus, they should promote gut health, either directly by means of direct stimulation of the animal's immunological response or indirectly by lowering the number of pathogenic microorganisms. In addition, industrial suitability is crucial; for instance, allowing high-scale production, long stability during storage or under on-farm conditions and good organoleptic properties for being fed to livestock. Finally, safety is also an important aspect, not only in terms of the health of the animal, but also regarding consumer health, such as the absence of transmissible antibiotic resistance genes and/or zoonotic pathogens (Musa et al., 2009; Gaggìa et al., 2010). Feeding sows during gestation and lactation with probiotic supplementation has been shown to have an impact on the intestinal microbiota of the offspring (Taras et al., 2005). A possible reason for these findings might be related to early exposure to the surroundings and the ingestion of microorganisms excreted with the sow

faeces. Nevertheless, another possibility might be an altered compositional change in the milk of the sow (Scharek-Tedin et al., 2015). A variety of approaches have been suggested for the early administration of probiotics by means of the sow-piglet axis. First, one should strongly consider the entero-mammary pathway of the probiotics by means of the colostrum. Human research has shown that bacteria belonging to *Lactobacillus* and *Bifidobacterium* genera are efficiently supplied through this pathway (Jiménez et al., 2008, Jost et al., 2014). As is currently known, the delivery of probiotics directly using the entero-mammary pathway from the sow to the piglets has not yet been described. In addition, the constituents of the colostrum or milk, namely, fat and proteins, can also be influenced by probiotic feeding supplementation, leading to an improvement in piglet health and performance (Alexopoulos et al., 2004). In conclusion, probiotic supplementation to sows has also demonstrated beneficial effects for the piglets. Nevertheless, a major limitation for probiotics is the fact that the effects are dependent on the strain, the dosage, and the rearing context (Bosi and Trevisi, 2010). Furthermore, most studies have examined the supplementation effect on only one production cycle, even though there is proof that probiotic supplementation for more than one cycle is beneficial (Kritas et al., 2015).

2.2.2 Prebiotics

As well as probiotics, prebiotics play a significant role in animal nutrition. Although, all prebiotics are fibres, not all fibres are prebiotics (Slavin, 2013). The benefits provided by prebiotics are linked to the fact that they are not degraded in the small intestine and are then fermented by specific bacteria in the large intestine. Fermentation is likely to result in changes in the metabolic processes and better immune system functioning, thereby having a beneficial effect on the host health. In addition, prebiotics can increase the growth of specific probiotic bacteria (Gibson and Roberfroid., 1995; Gibson 2004; Wang et al., 2009). Non digestible carbohydrates, such as oligosaccharides and polysaccharides, are

the major sources of prebiotic compounds. The oligosaccharides most used in animal nutrition are fructo-oligosaccharides, inulin, arabinoxylo-oligosaccharides, xylo-oligosaccharides, and chito-oligosaccharides (Olveira et al., 2016). Supplementing the diet of lactating sows with fructo-oligosaccharides could improve the intestinal health of their offspring (Le Bourgot et al., 2017). The latter effect could be due to a modulation of the oligosaccharide composition of sow milk as recent literature has suggested that supplementing lactating sows with chito-oligosaccharides significantly alters the oligosaccharide composition of milk (Cheng et al., 2015). Moreover, another term of classification of prebiotics consists of dividing them into two categories: DFs and functional fibres (Slavin, 2013). DFs consist of non-digestible carbohydrates which are intrinsic and intact in plants, while functional fibres are part of DFs which have been isolated using industrial chemical and enzymatic methods and are able to exert beneficial physiological effects on the host.The latter compounds are a central component of sow's gestation diet and their inclusion increase satiety, reduce negative social interactions and overall increase the welfare of gestating sows (Noblet and Le Goff, 2001). In addition, the interaction of DFs and gut microbes can produce bioactive metabolites, and regulate sow gut microbial diversity, the intestinal immune system, and lactation performance (Tian et al., 2020). In the following paragraphs, the definition of DFs, its role in sow nutrition and their contribution to offspring gut health and development will be discussed.

3 Dietary fibres

3.1 Definition, characteristics, and classification

Carbohydrates derived from or contained in plants which are not digested by endogenous enzymes in the small intestine and become available as substrates of fermentation for bacteria populating the large intestine are referred to as DFs (Jha and Berrocoso, 2015). These carbohydrates include components of the plant cell wall, such as cellulose, hemicelluloses, lignin, mixed linked β-glucan, pectins, gums and mucilages (Davidson and McDonald, 1998) as well as oligosaccharides, pectins, resistant starches, resistant proteins, and associated compounds, such as polyphenols (Jiménez-Escrig and Sánchez-Muniz, 2000). Their mechanism of action and their degree of fermentability in the large intestine is mainly related to their physiochemical properties, such as water holding capacity, water solubility, viscosity, binding ability, absorptive capacity, and faecal bulking capacity (Mudgil and Barak, 2013). Water holding capacity and viscosity affect gastric emptying and, therefore, the digestion of several nutrients, such as proteins and lipids. Faecal bulking capacity can influence gastric distension and subsequently decrease feed intake. The most common method of classifying DFs is to divide them into “soluble” and “insoluble” DFs. This characteristic is mainly related to their capability of being fully dissolved in water. Examples of soluble DFs include hemicelluloses, pectins, gums and mucilages while insoluble DFs includes cellulose and lignin (Mudgil and Barak, 2013; Jiménez-Escrig and Sánchez-Muniz, 2000). The inclusion of soluble DFs in the diet increases the viscosity of the digesta content which promotes the development of an impermeable layer of non-mixed water on the gut surface, thereby forming a physical barrier which reduces nutrient absorption and digestion (Goff et al., 2018). Insoluble DFs are resistant to microbial degradation in the small intestine, and their dietary inclusion

results in enhanced faecal dry matter and bulkiness (Mudgil and Barak, 2013). Soluble and insoluble DFs share several physical properties, such as water and mineral binding capacity (Jiménez-Escrig and Sánchez-Muniz, 2000). However, their fermentability can vary according to the physicochemical properties of each component. Marked differences exist in the soluble portion of DFs in terms of fermentability, with many of them enhancing the proliferation of health-promoting bacteria genera, such as *Bifidobacterium*, *Lactobacillus* and *Eubacterium* (Flint and Bayer., 2008). Purified soluble oligosaccharides are currently very commonly used as potential prebiotics, in part because they do not affect the viscosity or the consistency of feeds owing to their relatively low molecular weight, and their relatively high fermentability (Gibson, 2004). Oligosaccharides are normally encountered in many plant tissues in the form of fructans. Some plant sources known for their fructan content are cereals, onions, chicory, and Jerusalem artichokes (Lovegrove et al., 2017). They may possibly be so easily fermented that they are completely degraded at the end of the small intestine (Houdijk et al., 2002). Supplying them in conjunction with slower-fermentable DFs, which may allow continuous fermentation in the large intestine has therefore been suggested (Williams et al., 2001). It is frequently assumed that the insoluble fraction of DFs is slowly fermented in the large intestine to a lesser extent than soluble DFs (Williams et al., 2019). However, the study of Comino et al. (2018) has shown that soluble and insoluble DFs have an essentially identical in vitro pattern of fermentation with a porcine faecal inoculum. Therefore, equating insoluble fibre with non-fermentable fibre is no longer a valid premise. As the large intestine is the main site of fermentation in monogastric species, insoluble DFs, as they are not degraded in the small intestine, may arrive to a greater extent in the large intestine and, depending on the source of plant, might offer a greater substrate of fermentation for the bacteria (Williams et al., 2001; Graham and Aman, 1987).

3.2 Dietary fibres and gut microbiota

The microbial communities and their activities in the intestine are affected by different factors, the most important being diet (Trevisi et al., 2021). Of the various constituents of diets, DFs have consistently been reported to exert an effect on the intestinal environment (Williams et al., 2017). As the main microbial “fuel”, an increase in the level of DFs in the large intestine leads to an increase in the activity of the entire microbial community (Gorham et al., 2017). Moreover, DF affects fermentation in the gut by promoting the abundance of cellulolytic bacteria species or their metabolism (Williams et al., 2001). These bacteria promote the production of VFAs in the large intestine, reducing the pH of the intestinal contents. A decrease in the pH enhances the growth of beneficial bacteria species such as Bifidobacteria and Lactobacilli, at the expense of disease-causing ones, such as Clostridium or Salmonella (Slavin, 2013). This effect is known as the 'prebiotic effect', which has been studied in various monogastric species, including pigs. However, the latter effect on microbial diversity may vary, depending on the type of substrate available for fermentation and the gut environment of the host. Therefore, certain sources or types of DFs might have a specific effect and selectively promote the growth of specific microorganism niches. In line with this, Wu et al. (2018) showed that xylan from birch included in a weaning diet enhanced the proliferation of Bifidobacterium in the ileum, while glucan from oat inhibited it. In addition, Chen et al. (2014) reported that feed based on pea fibre significantly increased the number of Lactobacillus in the large intestine of finishing pigs, while feed based on soybean fibre significantly increased the amount of Escherichia coli. Likewise, Owusu-Asiedu et al. (2006) found an increased number of Bifidobacteria and Enterobacteria species in the ileum of growing pigs fed diets supplemented with either guar gum or cellulose compared to a standard diet.

3.3 Dietary fibres and products of fermentation

In the small intestine, host endogenous enzymes are not able to digest DFs which escape and become a substrate of fermentation for microorganisms residing in the large intestine (Jha and Berrocoso, 2015). The large intestine acts as a fermentation gut district, and bacteria produce gases and organic acids (McNeil, 1988; Varel, 1987). The gases obtained by the fermentation of DFs include, for the most part, hydrogen, methane and carbon dioxide while the organic acids produced are mainly lactic acid and VFAs (Wenk, 2001). In particularly, these VFAs are the main products of DF fermentation and consist for the most part of acetate, propionate and butyrate (Williams et al., 2001). The most abundant VFA produced in the large intestine is acetate, approximately 60.0%, while propionate and butyrate are produced in smaller quantities (Lunn and Buttriss, 2000). In the large intestine, those VFAs (approximately 90%) are absorbed by means of a mechanism called “passive diffusion” by the cells in the gut and used as a source of energy by the bacteria (Jørgensen et al., 1997; Knudsen et al., 2001). However, VFAs are also utilised as an important “fuel” by other tissues (Lindberg, 2014). In fact, absorbed VFAs are basically metabolised at three main sites: in the caecum-colonic epithelial cells (which use butyrate in their pathway for energy production), in the hepatic cells (which metabolise up to 70% of the acetate, and the remaining butyrate and propionate for gluconeogenesis), and in the muscle cells (primarily in skeletal and cardiac muscles, which oxidise the remaining acetate) (Jha et al., 2019) **(Figure 3.)**. The amount of energy provided by VFAs may contribute up to 15% of the requirement for maintenance energy in growing pigs and even up to 28% in gestating sows (Dierick et al., 1989; Le Goff and Noblet, 2001). Depending either on the type or the source of the DF, different effects have been observed on the production and the profile of VFAs. For instance, a previous study reported the effects of including 20% wheat bran, sugar beet pulp, soybean hulls or alfalfa

meal in the weaning diet on the total VFA concentration in the large intestine (Freire et al. 2000). The inclusion of soybean hulls increased the concentration of total VFAs by 11.2%, 30.5% and 27.2% as compared to weaning diets with wheat bran, sugar beet pulp and alfalfa meal, respectively. A greater production of acetate and a lower production of butyrate in the large intestine has been reported when wheat bran was replaced by maize cobs in the diet of weaned piglets (Lordelo et al., 2008).

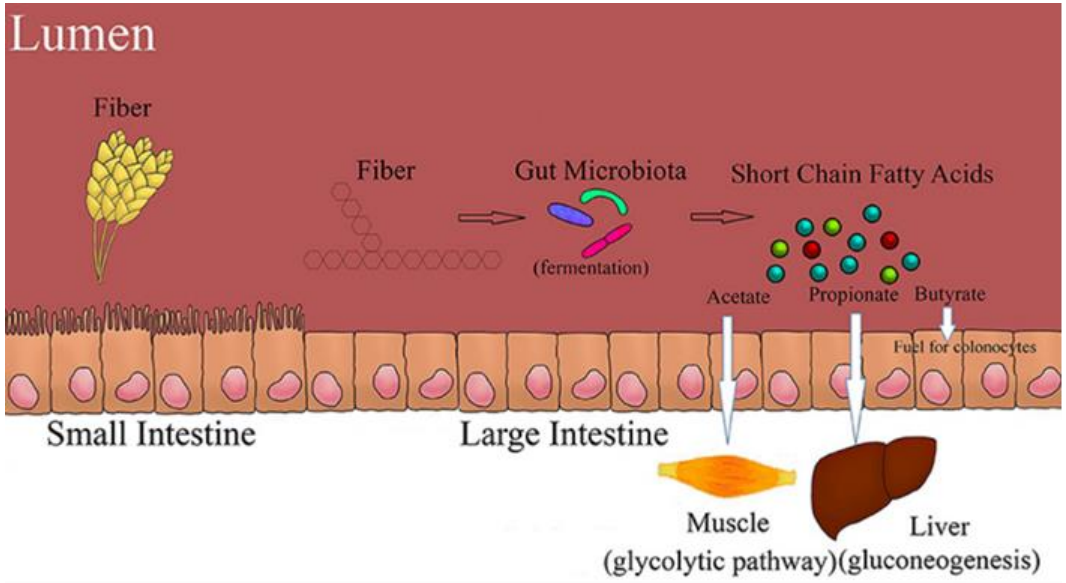

Figure 3. Dietary fibre fermentation and its primary utilization pathways (Jha, et al., 2019).

4 The use of dietary fibres in gestating and lactating sows

4.1 The effects of dietary fibre levels and sources on behaviour traits and reproductive performance in sows

Feeding limited amounts of feed is a standard practice in gestating sows in order to avoid excess body weight gain and impairment of the locomotor and reproductive functions (Quesnel et al., 2009). However, this dietary approach supplies enough nutrients to satisfy the sow reproductive and maintenance demands; however, it does not provide enough feed to reach sow satiety. A lack of satiety due to restricted feeding can lead to aggressiveness and stereotypic behaviour which is a major concern for sow welfare (Meunier-Salaün et al., 2001). Thus, the aim of including DFs during pregnancy is to reduce the feeling of hunger, and to alleviate behavioural problems and aggressiveness caused by starvation without increasing the energy intake due to their low energy density (Meunier-Salaün and Bolhuis, 2015). Nevertheless, the effectiveness of including an elevated quantity of DFs on sow satiety and behaviour depends on the source and their physicochemical properties. For example, Ramonet et al. (2000) showed that including sugar beet pulp as the main source of DFs during gestation increased mastication time, and delayed glucose and insulin peaks as compared with wheat bran. Several studies have also reported the beneficial effects of including a greater quantity of DFs during pregnancy on reproductive performance during lactation. Interestingly, Quesnel et al. (2009) reported increased average daily feed intake during the first weeks of lactation due to the increased size and capacity of the digestive tract induced by including a greater quantity of DFs during gestation. In addition, it has also been stated that a greater intake of DFs during the prenatal period could decrease the duration of farrowing and therefore reduce the number of stillborn piglets (Feyera et al., 2018; Feyera et al., 2017). Both these

effects could be explained by a lesser probability of being constipated prior to farrowing due to the increased capacity of DFs to retain water which reduces the hardness of the faeces (Oliviero et al., 2009). A lower constipation rate can help to prevent physical obstruction in the birth canal, enabling the prompt transit of the piglets during farrowing (Feyera et al., 2017). Moreover, the other potential beneficial effect of increasing DF intake during late gestation could be related to energy uptake from the production of VFAs in the large intestine; these could provide additional energy to alleviate prolonged farrowing duration and ultimately reduce the stillbirth rate. (Serena et al., 2007; Zhuo et al., 2020). Therefore, it seems that increasing the level of DFs during gestation enhances the reproductive performance of the sow, although, the source of the DFs calls for special attention. Tan et al. (2015) reported that supplementing gestating sow withs 2% konjac flour, a source of soluble DFs, increased the average daily feed intake during lactation as compared to a standard diet which contained wheat bran as a source of insoluble DFs. Sun et al. (2014) also reported that increasing the intake of soluble DFs throughout pregnancy, linearly increased the average daily feed intake during lactation. Apart from the role of DFs in gestation, its inclusion in the lactating sow diet has been less studied as the lactation period requires feeds with high protein and energy contents. Nevertheless, increased inclusion of DFs in the lactating sow's diet could help sows reduce constipation, thereby decreasing the bacterial toxin absorbed and consequently the potential risk of mastitis (Smith, 1985; Hermansson et al., 1978). Only a few studies have analysed the effects of supplementing the sow diet with DFs during lactation. Yan et al. (2016) reported no detrimental effects on sow feed intake during lactation when adding up to 28% of resistant starches. However, Renaudeau et al., 2003 reported that adding up to 20% of DFs during lactation increased sow body weight loss at the end of lactation.

4.2 The effects of dietary fibre levels and sources on colostrum and milk chemical composition and the microbiota

During the last month of gestation and then during lactation, the mammary glands are among the most active tissues. To provide adequate nutritional substances to the offspring, a large quantity of nutrients absorbed from the intestine is transferred to the mammary glands through the blood stream for the synthesis of mammary secretions (Tian et al., 2020). Thus, the nutrients ingested during those periods, can affect colostrum and milk composition. As previously mentioned, providing DFs to sows enhances microbial fermentation and ultimately VFA production which can be used as a source of energy for the synthesis of nutrients in colostrum and milk. In fact, increasing the level of DFs during gestation has been shown to enhance the content of fat in colostrum as well as the concentration of IgA (Loisel et al., 2013; Feyera et al., 2019; Liu et al., 2020; Shang et al., 2019). Yan et al. (2016) reported that, during lactation, a higher level of DFs in the sow diet increased milk dry matter and tended to increase milk fat content. In addition, their inclusion level, there is more and more evidence that the water solubility of DFs may affect colostrum and milk quality. Liu et al. (2020) reported that a greater quantity of soluble rather than insoluble DFs in the late gestation diet tended to increase the fat content in the milk. As well as nutrients, colostrum and milk also contain numerous microorganisms. Previous studies which have investigated the effect of DFs on colostrum and milk quality never examined whether the microbiota of the colostrum and milk could also be affected. Given that DFs affect gut microbiota, one can question whether a modification of the intestinal microbiota influences the milk microbiota **(Figure 4)** (Tian et al., 2020). Colostrum and milk also contain numerous microorganisms; it has been suggested that part of the microorganisms in colostrum and milk may originate from the intestinal environment. Human research studies have hypothesised that microbes from the gut can

be transferred to the lymph nodes with the help of dendritic cells and then reach the mammary glands by means of the intestinal-lymphatic circulation system (Rodriguez, 2014). In support of this hypothesis, Chen et al. (2018a) found bacteria belonging to the *Streptococcus* genera, *Ruminococcacae* and *Lactobacillus* in both colostrum and milk. These bacteria are widely distributed in the large intestine of sows, with the latter two being very important in the fermentation process of DFs. This study gave some preliminary indication that the maternal diet could change the microbial profile of mammary secretions. In addition to microorganisms, milk oligosaccharides, as they are not digested by the host, could supply a substrate of fermentation for the gut-colonising bacteria. Although, to the best of the Author's knowledge, no studies have explored the effects of DFs in the sow diet on sow colostrum and milk microbiota and the oligosaccharide profile.

4.3 The effects of dietary fibre levels and sources on offspring microbiota and development

At birth, piglets are exposed to a considerable microorganism load from the birth channel and the faeces of the sow. Commercially bred suckling piglets are kept in the same crate as their mother and are in permanent contact with faeces, mucous surfaces, skin, and mammary gland secretions until weaning (Nowland et al., 2021). As a result, the microbiota of the sow contributes considerably to the development of the microbiota of the progeny in the postpartum period (Chen et al., 2018b). Therefore, the positive effects of the interaction between DFs and the sow gut microbes can directly influence the intestinal microbiota of her offspring (Tian et al., 2020). For instance, Li et al. (2019) observed that the ratio between insoluble and soluble DFs in the gestation diet changed both the composition of the faecal microbiota of the sow and that of the piglets. In line with this, previous studies showed that the inclusion of wheat bran, a source of insoluble DFs, or inulin, a soluble DFs, in the maternal diet affected the piglet intestinal microbiota and its

fermentation products (Leblois et al., 2017; Paßlack et al., 2015). More specifically, Paßlack et al. (2015) reported that supplementing the sow diet with 3% inulin during late gestation and lactation, improved piglet gut health by reducing Enterobacteriaceae populations. As the gestation diet could affect the colonisation of the intestine of the suckling piglet, Leblois et al. (2017) hypothesised that the umbilical cord blood is not sterile, suggesting that a transfer of maternal microbiota could potentially take place during gestation. In their study, several bacteria genera belonging to the intestinal environment, such as *Corynebacterium, Prevotella* and *Lactobacillus*, were detected in the microbial profile of the umbilical cord. However, supplementing the sow diet with up to 25% wheat bran during late gestation resulted in no significant differences regarding the relative abundance of the aforementioned genera. Nevertheless, the maternal diet affected the microbial composition of the piglet colon during the suckling period. These results indicated that modulation of the piglet microbiota could begin during gestation and lactation by shaping the sow gut microbiota and by modifying milk composition, having an effect which might also affect their development. For instance, several studies have consistently reported that the supplementation of DFs throughout the gestation period increased the body weight of weaned piglets (Peltoniemi et al., 2010; Veum et al., 2009; Matte et al., 1994). It has also, been reported that litter development might also be affected by the source of the DF supplementation during pregnancy or lactation. In fact, piglets reared on sows fed konjac flour during gestation grew faster during the first and third weeks of lactation (Tan et al., 2015) and increasing the intake of soluble DFs during gestation linearly enhanced the piglet pre-weaning survival rate (Sun et al., 2004).

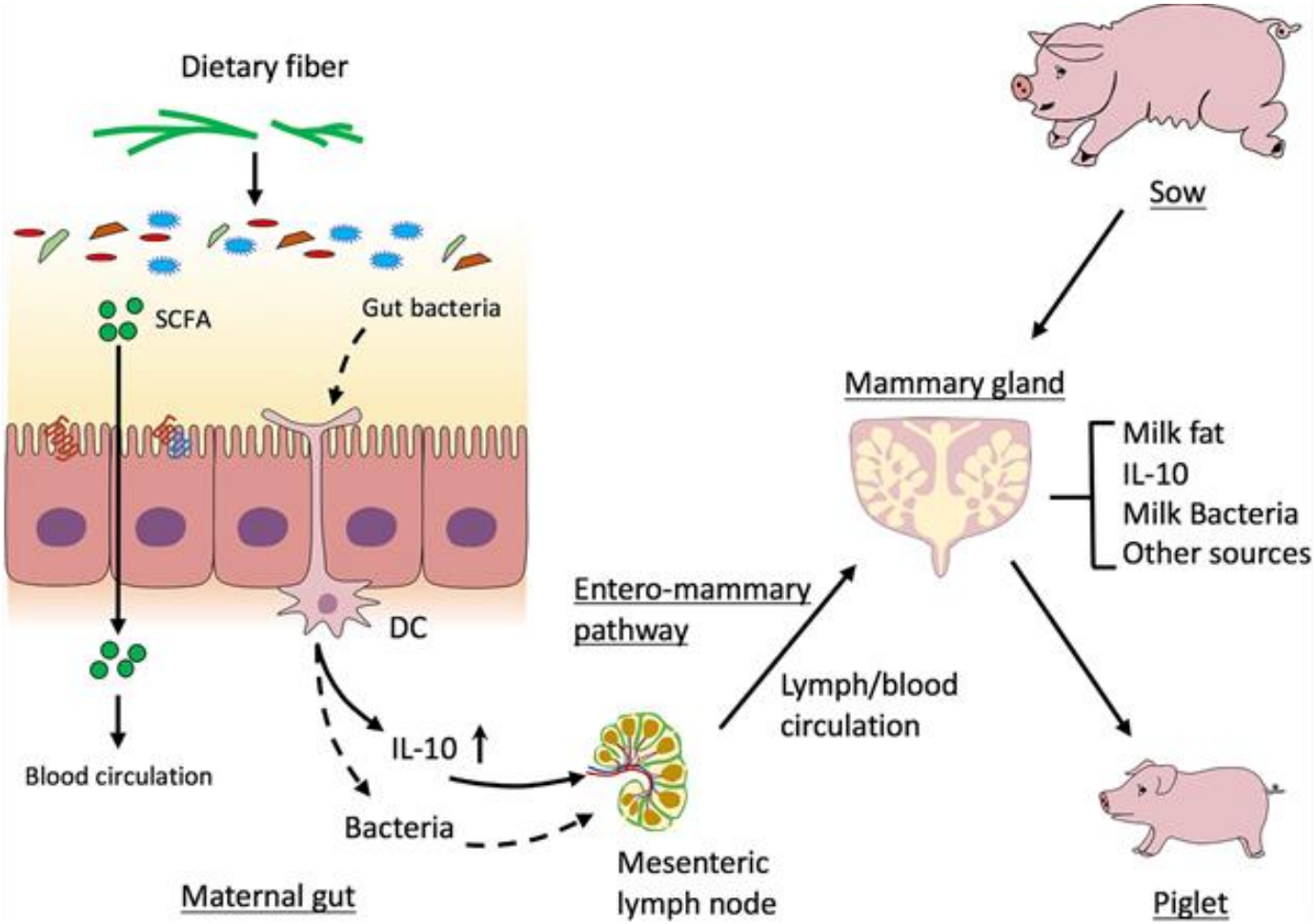

Figure 4. Dietary fibres and gut microbe interaction changes the composition of milk (Tian, et al., 2020).

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Aim of the thesis

The main objective of this PhD thesis was to investigate nutritional strategies to modulate piglet's intestinal microbiota during early life to improve its growth performance and intestinal health. Our focus was mainly on the indirect or direct transmission of bacteria, nutrients, and microbe-derived metabolites such as volatile fatty acids (VFAs) that might be derived from the mother during gestation and lactation. In this context, dietary fibres (DFs) are key components and could play a crucial role in colostrum and milk production and composition, offspring's 'initial microorganism colonization of the gut and their growth as outlined in the introduction (Chapter 4). However, regardless of their level of inclusion in diet, their effects on sow's intestinal environment might be also related to their physiochemical properties such as the ability to be completely dissolved in water. Therefore, our hypothesis was that modifying the ratio of insoluble to soluble DFs while maintaining a similar level of DFs in sow's diet would impact intestinal fermentation mainly in the large intestine, modifying colostrum and milk composition and having a subsequent positive effect on offspring performance. In the first two studies the ratio of insoluble to soluble DFs was changed by modifying the level of hemicelluloses in the lactation diet, while in the third study the abovementioned ratio was modified by substituting a source of soluble DFs by a source of insoluble DFs both in gestation and lactation diets. More specifically, the first study aimed to explore the effect of different levels of hemicelluloses in sow lactation diet on reproductive performance, milk quality and piglet growth. In addition, it was also paid particular attention on the effects provided by the maternal diet on the offspring according to their birthweight (BtW), differentiating the piglets into normal (BtW > 1.20 kg) or low (BtW ≤ 1.20 kg). Based on the findings obtained from the study cited above and using the same dietary treatments, the second study was designed to better understand if the observed effects may be driven by the gut microbiota of the sow,

which in turn may influence the one of the offspring. Therefore, the aim of this study was to evaluate effect of the lactation diet on sow apparent total tract digestibility of ADF, NDF, crude protein and gross energy and on the faecal VFA profile and microbiota of the sow and the piglet. Moreover, based on litter's average daily gain (ADG) from birth to 16 days post-farrowing, piglets were selected and divided into slow (ADG=167 ± 10.1 g/day) or fast (ADG=280 ± 10.1 g/day) growing, thus, the possible connection between gut microbiota and growth during suckling period was also investigated. Then, the third study was mainly focused on the effects of substituting 5% of chicory roots extract, a source of soluble DFs, by oat hulls, a source of insoluble DFs in sow's gestation and lactation diet on reproductive performance, colostrum quality and offspring development. Finally, in the fourth study we furnish a workflow from sample collection until data analysis for low microbial biomass data from umbilical blood cord.

Experimental studies

Manuscript 1

Decreasing the level of hemicelluloses in sow's lactation diet affects the milk composition and post-weaning performance of low birthweight piglets.

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Decreasing the level of hemicelluloses in sow's lactation diet affects the milk composition and post-weaning performance of low birthweight piglets.

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Abstract

Hemicelluloses (HC) are polysaccharides constituents of the cell walls of plants. They are fermented in the gut to produce volatile fatty acids (VFA). The present study investigated the effects of decreasing HC level in sow's lactation diet on sow performances, offspring development and milk composition. From 110 days (d) of gestation until weaning (26±0.4 d post-farrowing), 40 Swiss Large White sows were assigned to one of the four dietary treatments: (1) T13 (HC: 127g/kg), (2) T11 (HC: 114g/kg), (3) T9 (HC: 94g/kg) and (4) T8 (HC: 80g/kg). Milk was collected at 3 and 17d of lactation. At birth, piglets were divided into two groups according to their birthweight (BtW): normal (N-BtW; BtW > 1.20 kg) or low (L-BtW; BtW ≤ 1.20 kg). Decreased HC levels in the maternal diet linearly increased ($P \leq 0.05$) the body weight of L-BtW piglets at two weeks post-weaning and linearly decreased ($P \leq 0.05$) diarrhoea incidence and duration in this category. The concentrations of copper, threonine and VFA, as well as the proportion of butyrate, in milk linearly increased ($P \leq 0.05$), whereas lactose content linearly decreased ($P \leq 0.05$) with decreased HC in the maternal diet. The present study provides evidence that decreasing HC level in sow's

lactation diet can positively affect the composition and VFA profile of milk and ultimately favour the growth and health of L-BtW piglets.

Keywords: Dietary fibres, lactose, pigs, volatile fatty acids, butyrate

Highlights

- The results of this study showed that decreasing the level of hemicelluloses in sow's lactation diet modified milk composition and had positive effects on the post-weaning performance of low birthweight piglets.
- This study highlighted the role of dietary fibres in the maternal diet to alleviate body weight variations at two weeks post-weaning.
- Nevertheless, before advising pig producers, further research should elucidate the optimal level of hemicelluloses for lactating sows.

Introduction

Hemicelluloses (HC) represent a complex group of polysaccharides present in the cell walls of all plants, consisting mainly of pentoses (D-xylose and D-arabinose), hexoses (D-galactose, D-glucose and D-mannose) and uronic acids that can be estimated as the difference between NDF and ADF (Huang et al., 2021; Van Soest et al., 1991). As part of dietary fibres (DF), they can resist digestion by endogenous enzymes of the gut. Thus, they can reach the large intestine and promote the growth and activity of beneficial bacteria that produce volatile fatty acids (VFA) (Lattimer and Haub, 2010). These latter, namely acetate, propionate and butyrate, provide up to 28% of the energy requirements in growing pigs and even more in sows, where they can be absorbed and transferred to the milk and serve as an energy source for milk synthesis (Noblet and Le Goff, 2001; Tian et al., 2020). A previous study focused on increasing the level of DF in sow's gestation diet

showed that adding up to 20% DF increases colostrum fat content, as well as colostrum intake, of low birthweight (BtW) piglets (0.6 kg q BtW <0.9 kg) and decreases litter mortality during the suckling period (Loisel et al., 2013). These positive effects on growth and survival are in line with findings of Paßlack et al. (2015) who reported that inclusion of 3% inulin, a source of DF offered to lactating sows positively affected the development and gut health of their litters.

Apart from the quantity of DF provided, the beneficial effects of DF is also related to their physiochemical properties such as their solubility in water and their intestinal fermentability. For instance, due to a slower fermentability compared to soluble dietary fibres (SDF), the majority of the insoluble dietary fibres (IDF) reach the large intestine and stimulate the growth of commensal and probiotic bacteria such as Ruminococcus, Faecalibacterium, Lactobacillus and Bifidobacterium (LeBlanc et al, 2017). The large intestine acts then as a fermentation chamber producing VFA, CO2, H2 and other carboxylic acids (Lattimer and Haub, 2010). Conversely, SDF are easily fermented and may be completely degraded at the end of the small intestine (Houdijk et al., 2002). Depending on the plants, HC might be considered as a source of SDF (Jiménez-Escrig et al., 2000). A previous study in growing pigs reported that decreasing HC level increased VFA produced in the ileum (Zhao et al., 2019). However, to our knowledge, little is known about the effects of HC level in lactating sows. Therefore, the present study aims to fill this gap by comparing four diets characterised by similar total DF content but different HC levels by varying the sources of DF. We hypothesised that decreasing the level of HC while maintaining a similar total DF level in sow's lactation diet would affect the IDF to SDF ratio and by that impact gut fermentation particularly in the large intestine and ultimately modify milk composition.

Material and methods

Animals, housing and treatments

The experiment was conducted during late gestation and lactation of 40 Swiss Large White sows from five farrowing batches. Approximately 10 days before the expected time of farrowing, sows were moved to farrowing rooms arranged with individual 7.1 m2 farrowing crates, consisting of a 5.89 m2 concrete solid floor and a 1.21 m2 concrete slatted floor. Each crate was equipped with an electronic sow feeder (Schauer Spotmix, Schauer Agrotronic GmbH, Austria), nipple drinker and a heated covered area for piglets. The ambient temperature was maintained at 24 °C, and artificial lights were on from 0800 h to 1700 h. On day 110 of gestation, the sows were randomly allocated to one of the four experimental lactation diets based on parity (mean ± SEM: 3.5 ± 0.7) and BW (mean ± SEM: 286.5 ± 13.6 kg). Parturition was induced when gestation period exceeded 116 days with an intramuscular injection of 1 ml (0.25 g/ml) of cloprostenol (Estrumate®, MSD Animal Health GmbH, Luzern, Switzerland). Within the first 24 h following birth, piglets were identified by an individual ear tag and received iron injection (Feridex® 10%, AMAG Pharmaceuticals, Inc., Waltham, USA). Piglets weighing less than 800 g at birth were excluded from the experiment. To adjust litter size to an average of 12 piglets per sow, cross-fostering was carried out within the same dietary treatment and only on male piglets 24 h post-farrowing. After anaesthetisation, the male piglets were castrated in the second week. Piglets were weaned on day 25.7 ± 0.44 (mean ± SEM) of age but were kept in their respective farrowing crates until 2 weeks post-weaning. The heating nest temperature was set at 40 °C following birth and then gradually decreased by 0.5 °C per day to reach a final temperature of 32 °C.

Diets and feeding

The experimental diets were formulated to be isonitrogenous and isocaloric (Table 1) and to differ in DF sources and HC content: (1) T13 (HC: 127 g/kg), (2) T11 (HC: 114 g/kg), (3) T9 (HC: 94 g/kg) and (4) T8 (HC: 80g/kg). The daily feed allowance was calculated according to the current Swiss feeding recommendations for pigs (Agroscope, 2018). Sows had ad libitum access to water and were provided with moderate quantities of straw bedding, as required by the Swiss legislation. During the end of gestation, feed allowance was on average 3.04 ± 0.16 kg (mean ± SEM). While, during lactation, the feed allowance was gradually increased by 0.5 kg/day until ad libitum feeding on day 12 of lactation approximatively. All diets were delivered in pelleted form three times per day in three equal meals using a computerised feed delivery system (Schauer Spotmix, Schauer Agrotronic GmbH, Austria). Throughout the experiment, the feed refusals of the sows were weighed daily to calculate actual feed intake. From day 18.7 ± 0.44 of age (mean ± SEM) to 2 weeks post-weaning (mean ± SEM: day 39.7 ± 0.44 of age), piglets had ad libitum access to a post-weaning standard starter diet and water. The post-weaning starter diet contained 170 g/kg crude protein, 58 g/kg fat, 50 g/kg crude fibre and 14 MJ/kg digestible energy.

Sow and piglet performance

The BW of the sows, body condition score (BCS) and backfat thickness were recorded at the 110th day of gestation and on the day of farrowing and weaning. Weight loss during lactation was calculated as the weight difference between farrowing and weaning. Based on visual observation and palpations, BCS was determined according to a scale ranging from 1 (very thin) to 6 (obese) points (Dourmad et al., 2001), including intermediate values of 0.33 points. Briefly, the trained personnel assessed sows by palpating the shoulders, ribs, backbone and hips, followed by a visual observation. Backfat thickness was measured on each side at 65 mm of the dorsal midline at the level of the last rib (P2) using a digital ultrasound back-fat indicator (Renco Lean Meter Digital Backfat Indicator, Renco

Corporation, Minneapolis, Minnesota, USA). Backfat thickness loss during lactation was then calculated as the difference between backfat thickness measurements during farrowing and weaning. At farrowing, the number of born alive, stillborn and mummified piglets were recorded within each litter. Farrowing was recorded using a digital video recorder to estimate the farrowing duration, which is defined as the time span between the time of birth of the first and last piglet of the litter. At birth, the piglets were individually weighed, and crown-to-rump length and body circumference were recorded. Piglets were then individually weighed 5 and 16 days postpartum, during weaning (mean ± SEM: 25.7 ± 0.44 days of age) and at 1 (mean ± SEM: 32.7 ± 0.44 days of age) and 2 weeks post-weaning (mean ± SEM: 39.7 ± 0.44 days of age). The average daily gain (ADG) and litter weight during birth and weaning were calculated from these data. Milk yield was calculated as the individual piglet gain summed in the same litter multiplied by a numerical coefficient of 4.2 (Van der Peet-Schwering et al., 1998). The indices of body conformation were calculated based on the measurements of the individual BtW and the crown-to-rump length. The body mass index was calculated as the ratio of BtW to the squared value of the crown-to-rump length, and the ponderal index was calculated as the ratio of BtW to the cubic value of the crown-to-rump length (Hales et al., 2013). In addition, piglets were divided into two BtW groups: normal (N-BtW; BtW > 1.20 kg) or low (L-BtW; BtW ≤ 1.20 kg). From 1 week before weaning onwards, feed intake and refusals (including feed waste) per pen as well as the occurrence of diarrhoea were recorded daily. Diarrhoea incidence was determined according to a daily faecal score assessed using a scale from 0 = no diarrhoea to 1 = diarrhoea. The percentage of diarrhoea per group was calculated as the sum of piglets with a faecal score of one divided by the total number of piglets.

Sample collection

Within each farrowing series, feed samples of the four diets were collected weekly and pooled over the experimental period to determine the chemical composition. On days 3 and 17 of lactation, milk samples were manually collected from all functional teats after an intramuscular injection of 2 ml of oxytocin (Intertocine-S, MSD Animal Health GmbH, Luzern, Switzerland). Before milking, the piglets were temporarily isolated from the sow for 2 h, and the teats were cleaned with humid wipes. One aliquot of milk was refrigerated at 5 °C with 4 mg of bronopol to determine somatic cell concentration, and three aliquots were immediately stored at −20 °C for further analysis.

Analytical Methods

Feed Analysis

After being ground to pass a 1-mm screen (Brabender rotary mill; Brabender GmbH & Co. KG, Duisburg, Germany), feed samples were analysed for dry matter content by heating at 105˚C for 3h followed by incineration at 550°C until a stable mass was reached to determine the ash content according to ISO 5984:2002 (prepASH, Precisa Gravimetrics AG, Dietikon, Switzerland). An inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 7300 DV; Perkin-Elmer, Schwerzenbach, Switzerland) was used to measure mineral content (European Standard EN 15510:2008). The CP content was calculated as nitrogen (N) content multiplied by a coefficient of 6.25, where N was determined with the Dumas method (ISO 16634–1:2008). Fat content was extracted with petrol ether after acid hydrolysis (ISO 6492:1999). Different categories of fibres were analysed by standard protocols. Crude fibre content was determined gravimetrically (ISO 6865:2000) by incineration of residual ash after acid and alkaline digestions using a fibre analyser (Fibretherm Gerhardt FT-12, C. Gerhardt GmbH & Co. KG, Königswinter, Germany). The NDF and ADF contents (ISO 16472:2006 for NDF and ISO 13906:2008 for

ADF) were analysed with the same fibre analyser (Fibretherm Gerhard FT-12, C. Gerhardt GmbH & Co. KG, Konigswinter, Germany) and were expressed without residual ash. NDF determination was evaluated with heat stable amylase and sodium sulfite and expressed without residual ash after incineration at 600°C for 3 h. The contents of SDF, IDF and low-molecular-weight DF were measured according to AOAC Method 2011.25, and the total DF content was calculated as the sum of the three aforementioned types of DFs.

Milk Analysis

The dry matter of the frozen milk samples was determined after freeze-drying (Christ DELTA 2-24 LSC, Kühner AG, Birsfelden, Switzerland) for 70 hours. Subsequently, freeze-dried samples were milled with a mortar. Residual dry matter, ash, mineral and nitrogen contents were analysed as previously described for the feed chemical analysis, except that CP was expressed as N x 6.38. Except for tryptophan, all amino acids were determined as described in ISO 13903:2005. Briefly, after oxidation, 24 h of acid hydrolysis occurred with 6M HCl and derivatization with AccQ-Tag Ultra reagent (Waters corporation, Milford, USA USA), the amino acid profile was determined by ultra-high-performance liquid chromatography (UHPLC) coupled with a UV detector (Vanquish, Thermo Scientific, Reinach, Switzerland. Tryptophan content was quantified by HPLC (LC 1290 Infinity II LC System, Agilent Technologies, USA) according to ISO 13904:2016. Gross energy content was determined by combustion in a calorimetric vessel under pure oxygen condition using an adiabatic bomb calorimeter (AC600 Semi-Automatic Calorimeter, Leco Corporation, USA) (ISO 9831:1998). Lactose content was determined by enzymatic testing with β-galactosidase and galactose dehydrogenase (Enzytec TM Liquid Lactose/D-Galactose Ref. No. E8110, R-Biopharm AG, Darmstadt, Germany). Somatic cells count (ISO 13366-2) was determined by flow cytometry (Somacount FC, Bentley Instruments Inc., USA). Fatty acid methyl esters, as described by Kragten et al.

(2014), and the VFA profile (ISO 15884:2002) (ISO 15885:2002) were determined by gas-liquid chromatography (Gaschromatograph Series II Agilent 6850, Agilent Technologies 2000, USA and Gaschromatograph Serie Agilent 6890, Agilent Technologies 2000, USA, respectively). Fat content was determined as total fatty acids multiplied by a coefficient of 1.05.

Statistical Analysis

Due to health problems that could not be related to the dietary treatment, one T9 sow was excluded from the experiment. Data were analysed by ANOVA using the 'lme' and the 'glmmPQL' function of the nlme package of R Studio (version 4.0.2 for Windows). Regarding sow performance, milk composition and VFA profile, the sow was the experimental unit; the pen was the experimental unit regarding piglet feed intake and litter performance; and the piglet was the experimental unit of piglet's individual performance, days and percentage of diarrhoea. Linear regression models, including the treatment and the farrowing batch as fixed effects, were used to fit data related to sow performance, litter performance, piglet feed intake and days with diarrhoea. Data related to piglets' individual performance were analysed using a linear mixed-effects model, including the treatment and the farrowing batch as fixed effects and the sow as random effects. Milk composition and VFA profile were analysed with a linear mixed-effects model and fitted in repeated measurements, including the treatment, the farrowing batch, the sampling day, and the interaction between the treatment and sampling day as fixed effects and the sow as random effect. Before analysis, logarithmic transformation was applied to the milk fatty acid and milk VFA data due to the non-normality of the residuals. The percentage of diarrhoea was analysed using a generalised linear mixed model using Penalized Quasi-Likelihood, including the treatment, the farrowing batch and the day as fixed effects and the piglet as a random factor. Orthogonal polynomial contrasts were implemented to

evaluate the linear or quadratic effects of decreasing HC level. The results are expressed as the least square means ± SEM. Linear and quadratic effects were considered significant at $P \leq 0.05$.

Results

Sows' performance

The sow BW, BCS and backfat thickness on day 110 of gestation and during farrowing and weaning were not influenced by the dietary treatment, resulting in similar weight and backfat thickness losses during the lactation period (Table 2). Daily feed intake in the pre-farrowing period and during lactation did not differ between treatments. Fibre intake was partially influenced by dietary treatments. In both the pre-farrowing and lactation periods, the NDF, HC, (linear effects; $P < 0.01$), low-molecular-weight DF and SDF intake decreased (linear and quadratic effects; $P < 0.01$), and the ADF intake increased (linear effect; $P < 0.01$) with decreasing HC levels in the diet. A quadratic effect ($P \leq 0.04$) of the HC level was found in the diets on the intake of total DFs in the pre-farrowing and lactation periods (Supplementary Table 1). At birth, litter traits, such as total born, born alive and stillborn piglets, did not differ, leading to comparable litter weights in the four treatments. Likewise, the dietary treatments had no effect on the total number of piglets weaned and, consequently, on litter weight at weaning. Farrowing duration was not influenced by dietary treatments. During the entire lactation period, milk yield was not influenced by the dietary treatments, with an average estimated production of 10.38 kg/day per sow (Table 2).

Piglets' individual performance

Body characteristics, such as body circumference, crown-to-rump length, body mass index and ponderal index, were not affected by the lactation diet of the sows (Supplementary

Table 2). Similarly, piglet BW development, ADG and feed intake were not affected by the dietary treatments. During the first week post-weaning, the incidence of diarrhoea and the number of days with diarrhoea were similar among the treatments. By contrast, during the second week post-weaning, a quadratic increase ($P \leq 0.05$) in the incidence of diarrhoea and the number of days with diarrhoea was observed with decreasing HC level. When focusing on the two BtW categories, the effect of the sow diets in the L-BtW group showed interesting observations (Table 3). The BtW, the BWs until one week post-weaning and in accordance the ADG in this period were similar among the experimental treatments for L-BtW piglets. By contrast, the decrease in HC level in the sow diets increased (linear effect; $P \leq 0.04$) the BW and the ADG in the second week post-weaning and the overall ADG from birth to two weeks post-weaning of L-BtW piglets. In the first week post-weaning, the dietary treatments did not affect either the incidence of diarrhoea or the days with diarrhoea of L-BtW piglets. In the second week post-weaning, the incidence of diarrhoea and days with diarrhoea linearly decreased ($P < 0.01$) with decreased HC level in the maternal diet. Except for the linear increase in the incidence of diarrhoea and increase in the number of days with diarrhoea in the second week post-weaning ($P < 0.01$) with decreasing HC level, no dietary effects on growth traits were observed in N-BtW pigs (Supplementary Table 3).

Milk Composition

Throughout lactation, no dietary treatment and sampling day interaction was found (data not shown). At days 3 and 17 of lactation, DM, ash, protein and somatic cell count, as well as milk yield estimated from farrowing to day 3 and from day 4 to day 17 of lactation, were similar among dietary treatments (Table 4). With a decreasing HC level, milk lactose content linearly decreased ($P < 0.01$). Regarding mineral levels in the sow milk, calcium, phosphorus, sodium, magnesium and zinc contents remained similar among experimental

treatments, whereas the copper content linearly increased (P = 0.02) with decreasing HC content in the maternal diet. Excluding the linear increase (P = 0.04) in the threonine level and the quadratic increase (P = 0.04) in the monounsaturated fatty acid portion, decreasing HC level in the maternal diet had no impact on the amino acid and fatty acid profiles. Regardless of the dietary treatments, somatic cell counts did not differ between the sampling days. However, the sampling day influenced protein, mineral and lactose contents, as well as milk yield. Between days 3 and 17 of lactation, protein, phosphorus, potassium and zinc contents decreased (P ≤ 0.05), whereas lactose and calcium contents and milk yield increased (P ≤ 0.05). Furthermore, histidine, leucine, isoleucine, phenylalanine, threonine, tryptophan, tyrosine, valine, alanine, aspartic acid and serine decreased (P ≤ 0.05), whereas glutamate and proline increased (P ≤ 0.05) between days 3 and 17. The fatty acid profile in milk changed during lactation. Monounsaturated and polyunsaturated fatty acid portions decreased (P ≤ 0.05) and saturated fatty acid content increased (P ≤ 0.05) from day 3 to day 17. More precisely, the portions of C18:0, C18:1n-9, C18:2n-6, C18:3n-6, C18:3n-3, C20:4n-6, C20:5n-3 and C22:5n-3 decreased (P ≤ 0.05), whereas C16:0 level increased (P ≤ 0.05) between days 3 and 17.

Volatile fatty acid concentrations in milk

The VFA concentration and the proportion of butyrate linearly increased (P < 0.01; Table 5) with decreased HC content in the maternal diet, resulting in an increased in total VFA by 25% and butyrate proportion by 60%. Regardless of the dietary treatment, total VFA concentration decreased (P ≤ 0.05) by 71% between days 3 and 17. The proportion of methanoate increased (P < 0.01), and the proportion of acetate decreased (P < 0.01) between days 3 and 17, whereas the levels of propionate, isobutyrate, butyrate and isovalerate remained unchanged.

Discussion

Effect of decreasing the level of hemicelluloses on sows' performance

In the present study, providing straw as enrichment was mandatory for the sow welfare. One cannot exclude that straw consumption may have attenuated the potential impact of the diets. Nevertheless, as the quantity of straw provided was the same for all the sows, a similar straw consumption may be assumed regardless of the dietary treatment. Excluding fibre intake, the sow's performances were not affected by dietary HC. In the present study, one goal was to have a similar total DF intake among the sows in the four treatments but different intakes of IDF and SDF. This objective was only partially achieved as there was no linear effect but a quadratic effect for total DF intake. Nonetheless, due to similar feed intake during the pre-farrowing and lactation periods, decreasing the level of HC also reduced the intake of the low-molecular-weight DF and SDF fractions. Similar to the present study, Shang et al. (2021) found no effect either on sow's BW or backfat thickness at farrowing and weaning when the dietary SDF level was decreased from 40.6 g/kg to 13.9 g/kg in the late gestation and from 27.2 g/kg to 14.3 g/kg during lactation. In addition, considerably high SDF intake can negatively affect litter performance. Indeed, Liu et al. (2020) reported that from day 90 of gestation to farrowing, a daily intake of 215 g of SDF (SDF: 45.7 g/kg as fed), compared with 138 g/day (29.7 g/kg as fed) and 96 g/day (17.8 g/kg), decreases the number of piglets and litter weight at weaning. In the present study, sows received between 133 and 83 g/day of SDF according to the diets, from day 110 of gestation to farrowing. Therefore, compared to the study of Liu et al. (2020), the SDF intake during this period for the four treatment groups was not sufficiently elevated to negatively impact litter performances.

Effect of decreasing hemicelluloses levels on milk composition and milk VFA profile

Milk yield and composition play a crucial role in the growth of suckling piglets to reach an adequate weaning weight. In the present study, decreasing the level of HC in the maternal diet affected milk composition but did not affect milk yield. Furthermore, lactose content decreased, whereas copper and threonine proportions increased with decreased HC level. A previous study showed that glucose, glycerol and other glucose precursors play an important role in the synthesis of lactose in sow's milk (Boyd et al., 1995). Houdijk et al. (2002) reported that the fermentation of SDF occurs already at the end of the ileum. As decreasing the level of HC also decreased the intake of SDF, one can hypothesize that lowering the HC supply reduced the absorbed HC fermentation products available for lactose synthesis. Moreover, due to the osmotic power of lactose (Costa et al., 2019), milk yield may drop together with lactose as the HC level decreases. Surprisingly, milk yield only decreased numerically, and this result could be due to the differences in lactose concentration between the experimental groups, which were not sufficiently large to affect milk yield. A further interest in the present study is the linear increase in copper in milk with a decreased HC level. Copper is an essential microelement for animals, with many biological functions, including iron metabolism, immunity, protection from oxidative stress and improvement in the activity of digestive enzymes (Huang et al., 2015). The milk concentration of copper is affected by the source of the micromineral (Peters et al., 2010). However, as the same micromineral source was used among the four dietary treatments, the mechanism underlying the increase in copper concentration remains unclear. Similarly, with decreased HC levels in the diet, the proportion of threonine in the milk increased. This effect remains unclear, as the calculated digestible threonine levels were similar between the T13 and T8 diets. In addition, in the present study, a quadratic increase of mono-unsaturated fatty acid portion in milk was also observed as the HC level decreases, with the T11 sows exhibiting the greatest MUFA percentage in milk. It is well known that the fat level and the fatty acid composition in sow milk reflect the level and the sources of fat

included in sow's diet (Lauridsen and Danielsen, 2004). As the fat source and the fat level in the dietary treatments were the same, no plausible explanation was found. Besides a similar DF content, hypothetically, decreasing the HC level using several DF sources may affect the fermentation patterns in the gut, namely, the concentration and proportion of VFA. As VFA can be absorbed, transported through the blood and finally reach the mammary glands, modifications in the milk composition are expected (Tian et al., 2020). Decrease in HC level increased total VFA concentration and butyrate proportion in milk. Zhao et al. (2019) showed a positive correlation between VFA concentration in pig's ileum and decreased HC level. Given that sows can ferment DFs better than growing pigs, a similar phenomenon may have occurred in the ileum of sows fed with a low HC level (Noblet and Le Goff, 2001). Furthermore, this effect on VFA in milk may also be due to differences in the intake of other DF fractions. As previously mentioned, decreasing HC level concomitantly increased ADF intake and decreased SDF intake. A positive correlation was reported between the ADF level in pig's diet and butyrate concentration in the faeces (Zhao et al., 2019). In the present study, hypothetically, increased ADF intake in sows fed with decreasing level of HC might have increased the butyrate proportion in the faeces and then in the milk. Compared with IDF, SDF is rapidly fermented by bacteria, thereby enhancing the production of VFA (Jha and Berrocoso, 2015). Therefore, with decreased SDF intake, VFA production should be lowered. However, the present study showed that this concept was not evident and confirmed the importance of the source of DF, as reported by some authors (Theil et al., 2014). Therefore, to understand the effects of DF on milk composition, different fractions of DF, including HC and ADF contents, must be considered.

Effects of the lactation diet on piglets' performance

In the present study, modifying the level of HC in the maternal diet did not enhance litter performance. This result is consistent with the results of Loisel et al. (2013), which showed that modifying the maternal diet is easier to positively affect the performances of L-BtW piglets than the performance of the litter overall. Therefore, decreasing the HC level improved post-weaning performance and reduced the occurrence of diarrhoea in the L-BtW piglets. By contrast, the reason for the quadratic effect observed on the occurrence and the number of days in diarrhoea in the second week post-weaning remains unknown. Nevertheless, these effects were not severe enough to affect the growth of the litter overall. In addition, the L-BtW piglets usually exhibit poor performances, such as a high mortality rate and low ADG, which represents high economic costs for farmers due to reduced slaughter weight and increased occupancy of the stables (López-Vergé et al., 2018). Previous studies highlighted the importance of early-life interventions to improve the post-weaning development and health of piglets and more particularly of this sub-population of piglets (Girard et al., 2021; Girard et al., 2020). In the present study, the beneficial effects observed in L-BtW piglets during post-weaning period like the improved growth performance and the lower incidence of diarrhoea may be related to the combination of an increased relative abundance of butyrate, threonine and copper and to an increased concentration of total VFA in milk. Given that piglets are highly susceptible to intestinal bacterial disorders during the post-weaning period, butyrate, due to its recognised role in gut health, could have been useful in increasing gut impermeability, alleviating diarrhoea in L-BtW piglets during the second week post-weaning (Feng et al., 2018). In addition, increasing threonine and copper proportions in the milk in the pre-weaning period can help accelerate the gut maturation of those piglets (Lalles et al., 2009). Threonine plays a critical role in the regulation of intestinal mucosal integrity, as it is required for the production of mucins and immunoglobulins, improving the physical protection from the attachment of microbes to the mucosal surface (Van Klinken et al.,

1995). By contrast, copper can help against pathogenic bacteria because of its bacteriostatic properties, which affect the community structure of microorganisms in the caecum and colon (Højberg et al., 2005). A lower relative abundance of *Alistipes,* *Lachnospiraceae*, *Ruminococcaceae* and *Prevotellaceae* has been reported in the colon and ileum of L-BtW piglets compared with N-BtW piglets (Li et al., 2019). These genera enhance gut health and immune functions in the host (Den Besten et al., 2013). Given that, colostrum and mature milk are key components in shaping piglet microbiota (Trevisi et al., 2021), the modification of milk composition induced by decreased HC level in the sow diet might have changed the gut microbiota of L-BtW piglets and improved their health and growth.

Effect of lactation stage on milk composition

Sow's milk composition is strongly affected by changes throughout the lactation period. Transitional milk (48–72 h after parturition) contain higher amounts of lipids, protein and dry matter compared with mature milk (from day 10 of lactation) (Csapó et al., 1996). In the present study, the passage from transitional milk to mature milk was characterised by a decrease in protein and ash contents and an increase in lactose content. Nevertheless, the contents of fat, dry matter and gross energy decreased only numerically from day 3 to day 17. Indeed, in the present experiment, the lack of statistical differences on those traits is in disagreement with the study of Csapó et al., (1996), where differences between the sampling days were reported. This might be related to differences in sow genotypes, and litter size between the present study and the previous ones. Different genotypes such as Danish Duroc, Danish Large White and Norwegian Landrace with an average litter size of 9.9 piglets were reported in the study of Csapó et al. (1996) whereas the present study was conducted on Swiss Large White sows with an average litter size of 11.5 piglets after cross-fostering. Similarly, the decrease in amino acid proportion follows the same trend as

protein content, except for glycine, lysine and methionine, which remained stable over lactation, and for glutamate and proline, which increased from day 3 to day 17. Therefore, the high level of amino acids in transitional milk reflects the protein level, mainly because of the high content of immunoglobulins (Klobasa et al., 1987). The mineral content was also affected by the stage of lactation with an increase in the calcium level and a decrease in the potassium and zinc levels from transitional milk to mature milk in agreement with Csapó et al. (1996). Moreover, the phosphorus content decreased between days 3 and 17. The reason for this decrease over lactation remains unclear but might be related to a dilution effect, as it follows the numerical decrease in dry matter. When expressed per kilogram of dry matter, the phosphorus concentration was similar between days 3 and 17. Moreover, from transitional milk to mature milk, the decrease in the proportion of mono- and polyunsaturated fatty acids and the increase in the proportion of saturated fatty acids are related to changes in the proportion of individual fatty acids. The increase in C16:0 proportion and decrease in the proportions of C18:0, C18:1n-9, C18:2n-6, C18:3n-6, C18:3n-3, C20:4n-6 and C20:5n-3 observed in the present study have already been described in a previous study (Hu et al., 2019). Furthermore, Hu et al. (2019) reported a positive correlation between calcium and C16:0 fatty acid.

Conclusions

In conclusion, when the DF level is the same, feeding lactating sows with a lower HC level can positively affect the milk composition and the development of L-BtW piglets. As HC content decreased, the growth performance of the L-BtW piglets improved after weaning, and the occurrence of diarrhoea decreased, particularly in the second week post-weaning. Moreover, it increased the proportion of butyrate, copper and threonine and increased the VFA concentration in the milk. The characterization of DF components and their role during lactation may also have promising implications in shaping the offspring's microbiota,

controlling the colonization and the spread of pathogens, and reducing the risk of diseases. Furthermore, improving the development and health of L-BtW piglets in the post-weaning period through the maternal diet may offer an interesting approach to help these piglets to cope with their heavier litter mates in the post-weaning period and to homogenize litter weight. Whether these improvements have long-term effects deserve further investigation

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Disclosure statement

The authors declare they have no conflict of interest relating to the content of this article.

Ethics approval

The experiment was conducted in accordance with the Swiss Guidelines for Animal Welfare, and the Swiss Cantonal Committee for Animal Care and Use approved all procedures involving animals (approval number: 2019_25_FR).

Data availability statement

The data that support the findings of this study are publicly available in Zenodo (https://doi.org/10.5281/zenodo.5814624).

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Table 1. Ingredients and composition of the sow's lactation diet

Item	Dietary Treatments[1]			
	T13	T11	T9	T8
Ingredients (%)				
Barley, ground	54.4	38.7		4.7
Oat flakes			4.0	18.2
Corn, ground	10.3		26.9	16.0
Rye		25.0	10.0	
Wheat, ground			13.1	15.0
Wheat starch	4.0	4.0	4.0	4.0
Molasses				4.0
Animal fat RS 65	2.4	2.4	3.0	3.8
Potato protein	10.0	10.0	10.0	10.0
Soybean meal	10.0	10.0	10.0	10.0
Flaxseed Meal	0.6			
Rapeseed meal		0.4		1.7
Oat hulls			4.0	8.0
Lupin			2.5	
Wheat bran			4.0	
Beet pulp	3.0	5.0	4.0	
L-lysine-HCL	0.070	0.057	0.057	0.056
DL-methionine	0.200			
L-threonine	0.050			0.050
L-tryptophan	0.020	0.006	0.013	0.003
Dicalcium phosphate	0.94	0.70	0.82	0.85
Calcium carbonate	1.57	1.38	1.39	1.47
Salt	0.59	0.52	0.42	0.41
Pellan[2]	0.40	0.40	0.40	0.40
Celite	1.00	1.00	1.00	1.00
Premix[3]	0.40	0.40	0.40	0.40
Natuphos 5000 G[4]	0.01	0.01	0.01	0.01
Gross chemical composition analysed (g/kg as fed)				
Dry matter	900	894	897	900
Crude protein	193	191	192	196
Fat	51	46	57	60
Crude fibre	43	43	47	46
Ash	63	61	60	63
NDF	184	174	163	154
ADF	57	60	69	79

Hemicelluloses[5]	127	114	94	80
Total dietary fibres	210	227	220	203
Low-molecular-weight dietary fibres	18	23	18	14
Soluble dietary fibres	43	44	35	28
Insoluble dietary fibres	149	160	167	161
IDF/SDF[6]	3.46	3.63	4.77	5.75
Calcium	9.4	9.4	9.3	8.7
Phosphorus	5.0	4.6	5.0	4.7
Gross chemical composition calculated				
Digestible energy (MJ/kg)	14.1	14.1	14.1	14.1
Digestible phosphorus (g/kg as fed)	3.1	2.8	2.8	2.8
Digestible essential amino acids (g/kg as fed)				
Lysine	9.6	9.6	9.6	9.6
Methionine	4.9	2.9	3.0	3.0
Threonine	6.9	6.3	6.4	6.9
Tryptophan	2.0	1.8	1.8	1.8

[1]T13= Sow's lactation diet containing 13% of hemicelluloses; T11 = Sow's lactation diet containing 11% of hemicelluloses; T9 = Sow's lactation diet containing 9% of hemicelluloses; T8 = Sow's lactation diet containing 8% of hemicelluloses.

[2]Pellet binding aid: Pellan, Mikro-Technik, Bürgstadt, Germany.

[3]Supplied per kg of diet: vitamin A, 8000 IU; vitamin D3, 800 IU; vitamin E, 40 mg; menadione, 2 mg; thiamine, 2 mg; riboflavin, 5 mg; biotin, 0.1 mg; niacin, 20 mg; pantothenic acid, 20 mg; iodine (as calcium iodate), 0.55 mg; copper (as copper sulphate), 7 mg; manganese (as manganese oxide), 20 mg; zinc (as zinc oxide), 55 mg; selenium (as sodium selenite), 0.2 mg.

[4]Phytase supplemented with 500 units of *Aspergillus niger* phytase/kg diet.

[5]Hemicellulose: calculated as the difference between NDF and ADF.

[6]Ratio of insoluble to soluble dietary fibre

Table 2. Effect of decreasing hemicelluloses level in lactation diet on sow's performance

	[1]Dietary Treatments					[2]Contrasts	
Item	T13	T11	T9	T8	SEM	*L*	*Q*
Sows							
Number of sows, *n*	10	10	9	10			
Range of parity, *n*	3.8	3.8	3.5	3.5	0.69	0.51	0.99
Farrowing duration, min	308	337	321	262	70.7	0.54	0.44
Body weight, kg							
D110	284	291	284	287	13.6	0.71	0.89
Farrowing	264	267	269	272	14.1	0.67	0.99
Weaning	233	238	248	246	12.5	0.39	0.78
Weight loss in lactation, kg	30.6	28.7	20.9	26.1	2.58	0.19	0.30
BCS, *n*							
D110	4.09	4.10	4.03	3.83	0.129	0.79	0.18
Farrowing	3.58	3.59	3.40	3.64	0.148	0.96	0.40
Weaning	2.71	2.62	2.81	2.94	0.246	0.42	0.62
Backfat thickness, mm							
D110	13.8	14.8	12.7	15.8	0.88	0.31	0.23
Farrowing	13.7	14.6	12.7	15.6	0.88	0.34	0.24
Weaning	11.3	11.9	11.5	12.9	0.71	0.18	0.52
Backfat thickness loss in lactation, mm	2.38	2.66	1.25	2.67	0.505	0.82	0.24
Milk yield, kg/day	10.61	10.85	10.09	9.97	0.720	0.41	0.79
Feed intake, kg/day							
Pre-farrowing	2.93	3.03	3.03	3.00	0.155	0.75	0.68
Lactation	5.67	5.93	5.77	5.87	0.237	0.69	0.73
Suckling piglets							
Number of piglets per litter, *n*							
Total born[3]	13.5	13.7	13.5	14.3	1.12	0.65	0.76
Born alive[3]	12.8	12.4	11.4	12.7	1.27	0.82	0.49
Stillborn	0.7	1.3	2.1	1.6	0.65	0.22	0.40
After cross-fostering	11.4	11.4	11.5	11.6	0.79	0.85	0.91
Weaned	10.7	10.9	11.3	10.7	0.76	0.94	0.60
Litter weight, kg							
At birth	20.5	20.5	21.1	20.3	1.63	0.99	0.81
At weaning	81.9	83.9	78.0	79.4	5.50	0.59	0.96

[1]T13 = Sow's lactation diet containing 13% of hemicelluloses; T11 = Sow's lactation diet containing 11% of hemicelluloses; T9 = Sow's lactation diet containing 9% of hemicelluloses; T8 = Sow's lactation diet containing 8% of hemicelluloses.

[2]Contrasts: L = Linear; Q = Quadratic.

[3] including piglets weighing less than 800g at birth

Table 3. Effect of decreasing hemicelluloses level in maternal diet on the performance of low birthweight piglets

	[1]Dietary Treatments					[2]Contrasts	
	T13	T11	T9	T8	SEM	*L*	*Q*
Number of piglets, *n*	25	23	15	20			
Body measurements at birth, cm							
Crown-to-rump length	25.0	25.4	25.5	26.0	0.56	0.22	0.97
Body circumference	22.3	21.8	21.9	22.3	0.42	0.98	0.16
Body mass index, kg/m^2	16.6	15.5	16.1	15.8	0.65	0.51	0.43
Ponderal index, kg/m^3	66.6	61.4	63.6	61.7	3.22	0.37	0.54
Body weight, kg							
At birth	1.04	1.01	1.04	1.06	0.047	0.64	0.46
5 days post-farrowing	1.61	1.59	1.58	1.54	0.095	0.53	0.86
16 days post-farrowing	3.94	3.78	3.59	3.85	0.287	0.70	0.39
Weaning	5.86	5.73	5.42	6.55	0.468	0.38	0.12
1 week post-weaning	5.92	5.96	5.56	6.95	0.498	0.20	0.12
2 week post-weaning	6.55	6.66	6.43	8.35	0.545	0.02	0.06
ADG, g/day							
Birth to 5 days post-farrowing	113	118	104	91	14.1	0.14	0.43
Birth to16 days post-farrowing	181	173	158	173	16.7	0.57	0.41
Birth to weaning	192	184	171	201	16.3	0.83	0.18
Weaning to 2 weeks post-weaning	50	62	74	113	27.0	0.09	0.56
1 week to 2 weeks post-weaning	91	103	125	187	27.0	0.01	0.25
Birth to 2 weeks post-weaning	141	143	135	177	11.5	0.04	0.05
Post-weaning diarrhoea,%							
1 week post-weaning	19.8	34.1	16.4	20.8	10.50	0.69	0.50
2 weeks post-weaning	36.4	16.7	6.5	5.2	8.31	<0.01	0.35
Days in diarrhoea, days							
1 week post-weaning	1.66	2.26	1.32	1.63	0.502	0.56	0.71
2 weeks post-weaning	2.36	1.22	0.55	0.87	0.512	<0.01	0.07

[1]T13 = Sow's lactation diet containing 13% of hemicelluloses; T11 = Sow's lactation diet containing 11% of hemicelluloses; T9 = Sow's lactation diet containing 9% of hemicelluloses; T8 = Sow's lactation diet containing 8% hemicellulose.

[2]Contrasts: *L* = Linear; *Q* = Quadratic.

Table 4. Effect of decreasing hemicellulose level in sow's lactation diet on gross composition, mineral content, amino acid profile and fatty acid profile of milk

	[1]Dietary Treatments					[2]Contrasts		[3]Stage of lactation			
Item	T13	T11	T9	T8	SEM	*L*	*Q*	d3	d17	SEM	*P*-value
Milk yield, kg/day	9.66	9.90	9.39	8.80	0.76	0.81	0.76	7.03	11.85	0.41	<0.01
Gross chemical composition											
Dry matter, %	19.5	20.7	19.9	20.6	0.60	0.25	0.49	20.7	19.7	0.40	0.06
Total protein, %	5.86	5.82	5.84	6.07	0.153	0.48	0.34	6.40	5.40	0.091	<0.01
Fat, %	7.50	8.65	8.07	8.69	0.533	0.15	0.38	8.50	7.96	0.364	0.27
Lactose, %	5.17	4.99	4.92	4.77	0.110	0.01	0.76	4.56	5.37	0.068	<0.01
Ash,%	0.86	0.86	0.88	0.85	0.150	0.29	0.89	0.89	0.83	0.098	<0.01
Somatic cells, log 10^3 cells/ml	6.99	6.92	7.40	7.71	0.325	0.18	0.41	7.40	7.11	0.248	0.93
Gross energy, MJ/kg	5.14	5.70	5.43	5.70	0.230	0.10	0.30	5.65	5.34	0.162	0.72
Minerals											
Calcium, g/kg	1.91	1.98	2.02	1.99	0.051	0.97	0.94	1.88	2.07	0.033	<0.01
Phosphorus, g/kg	1.57	1.58	1.57	1.53	0.026	0.09	0.63	1.61	1.52	0.017	<0.01
Potassium, g/kg	1.11	1.10	1.11	1.05	0.028	0.07	0.24	1.29	0.90	0.019	<0.01
Sodium, g/kg	0.37	0.35	0.35	0.34	0.016	0.21	0.89	0.36	0.34	0.011	0.93
Magnesium, g/kg	0.10	0.11	0.11	0.11	0.003	0.42	0.09	0.11	0.11	0.002	0.57
Copper, mg/kg	1.37	1.45	1.51	1.76	0.135	0.02	0.28	1.68	1.37	0.085	0.67
Zinc, mg/kg	6.04	6.64	6.02	5.44	0.363	0.16	0.07	6.38	5.69	0.213	<0.01
Amino acids, % of total protein											
Alanine	3.28	3.29	3.33	3.35	0.025	0.21	0.46	3.41	3.21	0.017	<0.01
Arginine	4.57	4.62	4.68	4.67	0.029	0.13	0.99	4.72	4.55	0.020	<0.01
Aspartic acid	7.70	7.68	7.75	7.74	0.035	0.78	0.29	7.83	7.61	0.025	<0.01
Cysteine	1.40	1.39	1.39	1.42	0.015	0.15	0.10	1.44	1.36	0.010	<0.01
Glutamate	17.8	17.6	17.8	17.6	0.16	0.29	0.99	17.5	17.9	0.11	<0.01
Glycine	2.98	3.02	3.12	3.05	0.030	0.10	0.40	3.06	3.03	0.019	0.15
Histidine	2.53	2.53	2.53	2.56	0.015	0.85	0.31	2.56	2.51	0.009	<0.01

Isoleucine	3.85	3.80	3.80	3.83	0.039	0.36	0.53	3.84	3.80	0.022	0.05
Leucine	8.03	8.12	8.02	8.15	0.049	0.74	0.83	8.18	7.99	0.030	<0.01
Lysine	6.86	6.79	6.82	6.86	0.049	0.61	0.36	6.85	6.82	0.029	0.22
Methionine	1.74	1.72	1.71	1.71	0.014	0.10	0.68	1.72	1.72	0.008	0.52
Phenylalanine	3.86	3.85	3.87	3.92	0.026	0.17	0.13	3.92	3.83	0.017	<0.01
Proline	10.2	10.3	10.4	10.2	0.11	0.37	0.15	10.1	10.5	0.07	<0.01
Serine	4.70	4.66	4.73	4.76	0.047	0.15	0.39	4.75	4.67	0.030	0.02
Threonine	3.88	3.88	3.90	3.98	0.036	0.04	0.19	3.99	3.83	0.023	<0.01
Tryptophan	1.18	1.18	1.21	1.20	0.017	0.17	0.94	1.23	1.15	0.011	<0.01
Tyrosine	4.02	3.97	3.99	4.05	0.050	0.43	0.20	4.05	3.96	0.028	<0.01
Valine	5.16	5.21	5.20	5.26	0.039	0.17	0.98	5.30	5.12	0.025	<0.01
Fatty acids, % of total fatty acids											
C16:0	27.2	27.4	26.4	27.8	0.70	0.52	0.38	24.9	29.5	0.48	<0.01
C18:0	4.29	4.43	4.33	4.41	0.143	0.70	0.70	4.78	3.95	0.089	<0.01
C18:1n-9	35.3	36.1	35.8	34.8	0.83	0.51	0.38	37.2	33.8	0.57	<0.01
C18:2n-6	11.45	9.63	12.08	11.74	0.412	0.09	0.09	12.20	10.30	0.245	<0.01
C18:3n-6	0.14	0.12	0.15	0.13	0.012	0.93	0.87	0.20	0.08	0.008	<0.01
C18:3n-3	1.08	1.12	1.16	1.32	0.057	0.06	0.53	1.25	1.09	0.036	<0.01
C20:3n-3	0.11	0.11	0.11	0.09	0.010	0.76	0.48	0.11	0.10	0.006	0.06
C20:4n-6	0.52	0.50	0.55	0.55	0.022	0.16	0.53	0.65	0.41	0.014	<0.01
C20:5n-3	0.09	0.09	0.08	0.08	0.006	0.55	0.74	0.09	0.07	0.003	<0.01
C22:5n-3	0.23	0.22	0.21	0.22	0.018	0.77	0.61	0.26	0.18	0.010	<0.01
n-3[4]	1.75	1.59	1.50	1.48	0.082	0.19	0.60	1.72	1.44	0.051	<0.01
n-6[5]	12.1	10.3	12.8	12.4	0.43	0.09	0.10	13.0	10.8	0.26	<0.01
Saturated	36.1	36.4	35.3	37.1	0.76	0.57	0.40	33.8	38.6	0.52	<0.01
Mono-unsaturated	49.1	50.8	49.5	48.1	0.61	0.71	0.04	50.4	48.3	0.42	<0.01
Poly-unsaturated	14.8	12.8	15.2	14.9	0.53	0.22	0.14	15.7	13.1	0.32	<0.01

[1]T13 = Sow's lactation diet containing 13% of hemicelluloses; T11 = Sow's lactation diet containing 11% of hemicelluloses; T9 = Sow's lactation diet containing 9% of hemicelluloses; T8 = Sow's lactation diet containing 8% of hemicelluloses.

[2]Contrasts: *L* = Linear; *Q* = Quadratic.

[3] Days: d3 = Day 3 of lactation; d17 = Day 17 of lactation;

[4] *n*-3: sum of C18:3n-3, C20:3n-3, C20:5n-3, C22:5n-3.

[5] *n*-6: sum of C18:2n-6, C18:3n-6 and C20:4n-6

Table 5. Effect of decreasing hemicellulose levels in sow's lactation diet on the volatile fatty acid profile of milk

	[1]Dietary Treatments					[2]Contrasts		[3]Stage of lactation			
Item	T13	T11	T9	T8	SEM	*L*	*Q*	d3	d17	SEM	*P*-value
Total volatile fatty acids, mmol/kg	3.07	3.58	3.60	3.86	0.28	0.03	0.60	4.12	2.94	0.19	<0.01
Proportion of individual VFA, %											
Methanoate	9.41	9.50	9.38	9.93	0.287	0.94	0.28	9.16	9.95	0.187	<0.01
Acetate	88.90	89.00	88.90	88.30	0.353	0.31	0.17	89.21	88.36	0.220	<0.01
Propionate	0.30	0.30	0.25	0.20	0.041	0.19	0.84	0.25	0.28	0.026	0.29
Isobutyrate	0.04	0.04	0.05	0.03	0.007	0.86	0.79	0.04	0.05	0.004	0.17
Butyrate	0.53	0.60	0.75	0.86	0.153	<0.01	0.64	0.68	0.69	0.104	0.29
Isovalerate	0.76	0.55	0.57	0.57	0.080	0.80	0.21	0.61	0.61	0.043	0.81

[1]T13 = Sow's lactation diet containing 13% of hemicelluloses; T11 = Sow's lactation diet containing 11% of hemicelluloses; T9 = Sow's lactation diet containing 9% of hemicelluloses; T8 = Sow's lactation diet containing 7% of hemicelluloses.

[2]Contrasts: *L* = Linear; *Q* = Quadratic

[3]d3 = Day 3 of lactation; d17 = Day 17 of lactation

Supplementary Table S1. Effect of decreasing hemicelluloses level in lactation diet on sow' fibre intake

Item	[1]Dietary Treatments				SEM	[2]Contrasts	
	T13	T11	T9	T8		*L*	*Q*
Fibre intake, g/day							
Pre-farrowing							
Crude fibre	127	129	141	139	7.0	0.13	0.74
NDF	538	527	492	461	26.1	0.03	0.69
ADF	168	182	208	224	10.3	<0.01	0.90
Hemicelluloses	370	345	284	237	16.1	<0.01	0.48
Total dietary fibres	614	688	667	610	33.1	0.82	0.04
Low-molecular-weight dietary fibre	53	70	55	42	2.8	<0.01	<0.01
Soluble dietary fibres	133	126	106	83	5.8	<0.01	<0.01
Insoluble dietary fibres	436	485	506	484	24.8	0.14	0.14
Lactation							
Crude fibre	246	254	269	272	10.4	0.06	0.81
NDF	1043	1033	937	903	41.3	<0.01	0.75
ADF	325	356	396	438	14.8	<0.01	0.71
Hemicelluloses	718	677	541	465	26.8	<0.01	0.49
Total dietary fibres	1190	1350	1270	1190	51.1	0.76	0.02
Low-molecular-weight dietary fibre	102	137	104	82	4.6	<0.01	<0.01
Soluble dietary fibres	244	261	202	163	9.6	<0.01	<0.01
Insoluble dietary fibres	845	950	964	947	37.1	0.06	0.09

[1]T13 = Sow's lactation diet containing 13% of hemicelluloses; T11 = Sow's lactation diet containing 11% of hemicelluloses; T9 = Sow's lactation diet containing 9% of hemicelluloses; T8 = Sow's lactation diet containing 8% of hemicelluloses.

[2]Contrasts: *L* = Linear; *Q* = Quadratic.

Supplementary Table S2. Effect of decreasing hemicelluloses level in the maternal diet on the performance of piglets

	[1]Dietary Treatments					[2]Contrasts	
	T13	T11	T9	T8	SEM	*L*	*Q*
Body measurements at birth, cm							
Crown-to-rump length	28.7	28.9	28.8	28.4	0.53	0.60	0.56
Body circumference	25.5	25.6	25.8	25.3	0.49	0.81	0.57
Body mass index, kg/m^2	19.2	18.8	19.5	18.7	0.55	0.78	0.73
Ponderal index, kg/m^3	67.2	65.1	67.9	66.3	2.03	0.99	0.88
Body weight, kg							
At birth	1.61	1.60	1.63	1.52	0.083	0.55	0.50
5 days post-farrowing	2.38	2.37	2.45	2.22	0.126	0.49	0.36
16 days post-farrowing	5.36	5.28	5.25	4.94	0.272	0.29	0.65
Weaning	7.69	7.54	7.26	7.36	0.348	0.41	0.71
1 week post-weaning	7.82	7.69	7.42	7.48	0.371	0.55	0.92
2 week post-weaning	8.93	9.17	8.71	8.93	0.453	0.82	0.99
ADG, g/day							
Birth to 5 days post-farrowing	154	154	160	137	12.6	0.44	0.34
Birth to 16 days post-farrowing	235	230	225	212	13.9	0.25	0.74
Birth to weaning	237	232	222	222	11.5	0.29	0.82
Weaning to 2 weeks post-weaning	86	116	103	113	17.3	0.38	0.52
1 week to 2 weeks post-weaning	172	194	184	207	20.8	0.31	0.98
Birth-2 week post-weaning	185	191	180	184	9.9	0.73	0.90
Feed intake, g/piglet							
1 week pre-weaning	182	186	157	189	24.5	0.95	0.55
1 week post-weaning	753	883	760	786	121.0	0.96	0.65
2 weeks post-weaning	1428	1638	1436	1600	144.0	0.63	0.87
Post-weaning diarrhoea,%							
1 week post-weaning	26.1	29.3	27.0	29.6	2.47	0.47	0.77
2 weeks post-weaning	17.4	17.2	12.8	22.2	2.82	0.44	0.05
Days with diarrhoea, days							
1 week post-weaning	1.89	2.09	1.85	2.10	0.171	0.61	0.90
2 weeks post-weaning	1.45	1.40	1.11	1.80	0.172	0.34	0.02

[1]T13= Sow's lactation diet containing 13% of hemicelluloses; T11 = Sow's lactation diet containing 11% of hemicelluloses; T9 = Sow's lactation diet containing 9% of hemicelluloses; T8 = Sow's lactation diet containing 8% of hemicelluloses.

[2]Contrasts: *L* = Linear; *Q* = Quadratic.

Supplementary Table S3. Effect of decreasing hemicelluloses levels in maternal diet on the performances of normal birthweight piglets

	[1]Dietary Treatments					[2]Contrasts	
	T13	T11	T9	T8	SEM	*L*	*Q*
Number of piglets, *n*	90	93	90	98			
Body measurements at birth, cm							
Crown to rump length	29.4	29.5	29.1	28.8	0.46	0.28	0.60
Body circumference	26.1	26.1	26.2	25.7	0.41	0.53	0.48
Body mass index, kg/m^2	19.7	19.4	20.0	19.2	0.54	0.69	0.63
Ponderal index, kg/m^3	67.6	65.8	69.0	66.9	2.16	0.90	0.93
Body weight, kg							
At birth	1.70	1.69	1.70	1.59	0.070	0.29	0.44
5 days post-farrowing	2.52	2.49	2.52	2.32	0.112	0.26	0.39
16 days post-farrowing	5.64	5.53	5.38	5.06	0.252	0.10	0.65
Weaning	8.08	7.86	7.43	7.45	0.320	0.12	0.72
1 week post-weaning	8.13	8.04	7.61	7.55	0.341	0.21	0.81
2 weeks post-weaning	9.41	9.56	8.91	8.99	0.422	0.32	0.94
ADG, g/d							
Birth-5 days post-farrowing	162	160	164	144	12.5	0.38	0.44
Birth-16 days post-farrowing	246	240	230	216	13.5	0.11	0.76
Birth- Weaning	248	240	227	224	11.1	0.10	0.83
Weaning-2 weeks post-weaning	90	122	105	111	17.1	0.56	0.41
Birth-2 week post-weaning	194	198	183	184	9.6	0.29	0.85
Post-weaning diarrhoea,%							
1 week post-weaning	26.1	28.4	27.4	30.7	2.82	0.32	0.86
2 weeks post-weaning	13.1	17.2	13.5	24.6	3.15	0.02	0.23
Days in diarrhoea, d							
1 week post-weaning	1.92	2.05	1.96	2.17	0.189	0.44	0.81
2 weeks post-weaning	1.16	1.43	1.20	1.96	0.187	0.01	0.17

[1]T13= Sow's lactation diet containing 13% of hemicelluloses; T11 = Sow's lactation diet containing 11% of hemicelluloses; T9 = Sow's lactation diet containing 9% of hemicelluloses; T8 = Sow's lactation diet containing 8% of hemicelluloses.

[2]Contrasts: *L* = Linear; *Q* = Quadratic.

Manuscript 2

The level of hemicelluloses in lactation diet affects faecal microbiota of sows and their piglets differing for slow and fast growth during suckling period.

The level of hemicelluloses in lactation diet affects faecal microbiota of sows and their piglets differing for slow and fast growth during suckling period.

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Abstract

The level of hemicelluloses (HCs) in sow lactation diet can affect milk composition and the post-weaning performance of low birthweight piglets. It was hypothesised that these effects might be driven by the intestinal microbiota of the sow, which in turn can affect the one of the piglets. The objectives of this study were to determine the effect of increasing the level of HCs in sow's lactation diet on the nutrient apparent total tract digestibility (ATTD) of the sow, the performance of the offspring, the faecal volatile fatty acid (VFA) profile and the microbiota of the sow and the piglets. A total number of 35 Swiss Large White sows received the lactation diets from 110 days (d) of gestation until weaning (25±0.4 d post-farrowing). Each sow was assigned to one of the four experimental groups: (1) HC8 (HC: 8.0%), (2) HC9 (HC: 9.4 %), (3) HC11 (HC: 11.4 %), and (4) HC13 (HC: 12.7 %). According to their average daily gain (ADG) at 16 days of age, two piglets per litter were selected and allocated into two growth categories: slow growing (SG; 167±10.1 g/day) and fast growing (FG; 280±10.1 g/day). Faeces were collected on day 110 of gestation, day 3 and 17 of lactation from the sows and at day 16 of age from the piglets. The

concentration of VFAs was quantified on both the sow and piglet faecal samples at each time point. After bacterial DNA extraction, sequencing was performed on the faeces of sows collected on day 17 of lactation and on those of piglets collected at day 16 of age. In addition, at the same time point, for the sows, an aliquot of faeces was also collected to determine the ATTD of ADF, NDF, gross energy and crude protein. Increased HC levels in the maternal diet decreased the ATTD of crude protein (P<0.02) and linearly increased the ATTD of ADF (P=0.02), NDF (P<0.01) and gross energy (P=0.04). As well as the proportions of butyrate and valerate linearly increased (P<0.05) in sow faeces at day 3 of lactation, whereas the proportion of propionate linearly decreased on day 17 of lactation (P=0.04). The dietary treatments affected (r^2=0.11; P=0.02) the beta diversity and revealed eleven common genera that differ in sow's faecal microbiota (P<0.05). The comparison of the faecal microbiota of piglets born from sow feed the HC8 diet with HC9, HC11 and HC13 diets revealed five common genera that differ (P<0.05). Regardless of the dietary treatments, FG piglets had a greater proportion of isobutyrate and isovalerate (P<0.05) in faeces compared to SG piglets at day 16 of age. At the genus level, *Lachnospiraceae_XPB1014_group*, *Enterococcus* and *Succinovibrio* were more abundant (P<0.05) and *Olsenella* was less abundant (P<0.05) in FG than SG piglets. The present findings provide evidence that increasing HC level in sow's lactation diet can exert an effect on the ATTD and VFA profile of sow's faeces and ultimately affect its faecal microbiota but with limited effects on slow and fast growing piglet faecal microbiota, and no effects on the performance and the faecal volatile fatty acid profile of these piglets. Additional studies are needed to better understand the association between the differences in ADG within litter before weaning and the faecal microbiota profile.

Keywords

Dietary Fibres, Gut, Swine, Volatile Fatty Acids, Butyrate, Bacteria

Introduction

Due to the genetic selection for sow prolificacy, the litter size has been increasing during the end of last century (Quesnel et al., 2008). Although this commercial choice has brought economic benefits to the pig industry, it has over the years negatively affected the development and health of the offspring (Baxter et al., 2020). Indeed, the average litter birth weight has been dramatically decreasing, and the birth weight variability between and within litters has been increasing, as well as the proportion of low birthweight piglets (Le Cozler et al., 2004). Considering that birthweight is one of the main factors affecting pre and post- weaning average daily gain (ADG), these piglets are more prone to a slow growth (Quiniou et al., 2002). Slow growing (SG) piglets are defined as piglets lighter than the rest of the litter and who require additional time to reach the targeted slaughter weight (Camp Montoro et al., 2020). They represent a management challenge and an additional cost for the farmers since they may have a higher mortality and morbidity risk compared to their fast growing (FG) siblings (López-Vergé et al., 2018). Therefore, it is essential to find several strategies to ensure that lighter piglets can successfully catch up their heavier litter mates. In this context, nutritional interventions during the perinatal period might be key factors contributing to piglet's growth and health. Since piglets are strictly in contact with faeces, skin and mucosal and environmental surfaces of the sow during the suckling period, shaping sow's gut microbiota may be an effective way to influence the one of its offspring. The modulation of sow gut microbiota can be achieved by using dietary fibres (DFs) that include components of the cell wall of the

plants such as cellulose, hemicelluloses (HCs), lignin, mixed linked β-glucan, pectins, gums and mucilages (Davidson and McDonald, 1998). In the large intestine, beneficial bacteria such as Lactobacilli and Bifidobacteria ferment DFs and produce volatile fatty acids (VFAs) which decrease the pH of the gut content reducing thereby the development of potential pathogens bacteria such as Clostridium and Salmonella (Bouhnik et al., 2004). The use of specific DF sources in sow gestation and lactation diets such as inulin or wheat bran has been showed to affect both the sow and its litter health by modulating the piglet's intestinal microbial population (Paßlack et al., 2015; Leblois et al., 2017). However, to our best knowledge, little is known about the effects of some specific fractions of the cell wall of the plants in sow lactation diet. A previous study showed that increasing the level of HCs in sow's lactation diet affected the post-weaning performance of low birthweight piglets and increased the post-weaning diarrhoea during the second week after weaning (Palumbo et al., 2022). We hypothesized that those changes, are mainly driven by the microbiota of the sow that in turn can affect the one of low birthweight piglets. Therefore, the aim of the present study was to compare the effect of increasing the level of HCs in sow's lactation diet on the performance of the offspring, faecal VFA profile and microbiota of the sow, SG and FG piglets during suckling period. In addition, the apparent total tract digestibility (ATTD) of ADF, NDF, gross energy and crude protein were compared for the sows with the aim of better understand if increasing the level of HCs in sow lactation diet would affect the ATTD of these nutrients. Finally, to our best knowledge just few studies investigated the possible connection between piglet's gut microbiota and growth during suckling period (Gaukroger et al., 2020; Li et al., 2018; Li et al., 2019). Therefore, the present study was also designed to fill this

gap and compare the faecal microbiota and VFA profile of SG and FG piglets during the suckling period.

Material and methods

Diets and feeding

The four experimental diets were previously reported in the study of Palumbo et al. (2022) (Supplementary table 1). They were isocaloric and isonitrogeous and differ in DF sources and HC content: (1) HC8 (HC: 8.0%), (2) HC9 (HC: 9.4%), (3) HC11 (HC: 11.4%), and (4) HC13 (HC: 12.7%). Each diet contained 1% (10 g/kg) of Celite® used as indigestible marker and were delivered in pelleted form. The daily feed allowance for each sow was calculated according to the current Swiss feeding recommendations for pigs to cover sow's requirement based on their weight and litter size (Agroscope, 2018). In lactation, feed allowance was settled at 3 kg after farrowing and was gradually increased by 0.3 kg/day and 0.5 kg/day for primiparous and multiparous sows, respectively, until reaching a plateau close to the *ad libitum* feeding after 12 days of lactation approximately. Diets were provided to the sows in three equal meals via a computerised feed delivery system (Schauer Spotmix, Schauer Agrotronic GmbH, Austria). From day 18±0.4 of age (mean ± SEM) to 39±0.4 days of age (2 weeks after the weaning), creep feed was provided to piglets *ad libitum* with free access to water. The composition of the creep feed was 14 MJ/kg digestible energy, 17% crude protein, 5.8 % fat, 5% crude fibre and 0.99% digestible lysine.

Animals, housing and experimental design

The present study was carried out between October 2019 to March 2020 and was performed on 35 Swiss Large White sows, divided in five farrowing batches, from 110 days of gestation until weaning. Sows were individually housed in farrowing crates.

They had free access to water and daily moderate quantities of straw bedding were provided. The environmental temperature was kept at 24°C and each crate had a total surface of 7.1 m^2, and was furnished with an electronic feeder (Schauer Spotmix, Schauer Agrotronic GmbH, Austria), nipple drinker and a heated covered area for piglets. Artificial light has been kept on from 08.00h to 17.00h. On day 110 of gestation, sows were assigned to one of the four experimental feeding groups as described above, based on parity (3.4±0.69) and body weight (BW) (286±14.2): (1) HC8 (n=9), (2) HC9 (n=8), (3) HC11 (n=10), and (4) HC13 (n=8). When the gestation time exceeded 115 and 116 days for primiparous and multiparous sows, respectively, two intramuscular injections of 0.5 ml (0.25 g/ml) at 24 hours interval of cloprostenol (Estrumate®, MSD Animal Health GmbH, Luzern, Switzerland) were administered to induce the parturition. At birth, the temperature of the heating nests was settled at 40°C and gradually decreased each day by 0.5°C until to reach 32°C.During the first 24 h of life, each piglet was identified by an individual ear tag and an injection of iron was administered (Feridex®, AMAG Pharmaceuticals, Inc., Waltham, USA). Piglets below 800g were excluded from the experimental trial. Litter size was adjusted to an average of 12 piglets per sow and cross-fostering was performed only on male piglets within the first 48h of life. At the same time point, male piglets were castrated after anaesthesia. At 16 days of age, per litter and included in the study. Within each litter, two female piglets, a SG and a FG, were selected piglets based on the average daily gain (ADG) from birth to 16 days of lactation. Piglets were weaned at 25±0.4 days and kept in their respective crates until 2 weeks after the weaning.

Measurements and sampling

Feed samples for each farrowing batch were collected weekly and pooled throughout the study period to determine the chemical composition. Faeces were collected on day 110 of gestation, days 3 and 17 of lactation from the sows and at day 16 from the piglets. They were sampled directly from the rectum and defecation in piglets was briefly induced by stimulation with a cotton swab. The concentration of VFAs was quantified on both the sow and piglet faecal samples at each time point: Prior freezing at −20 °C with 1 mL of phosphoric acid (25%, w/v), those faecal samples were weighed A second aliquot of faeces of sows collected on day 17 of lactation and those of piglets collected at day 16 of age was immediately frozen at -80°C for later bacterial DNA extraction. Finally, a third aliquot of faeces collected at the same time point for the sows, was weighed, and stored at −20 °C for determination of the chemical composition. The selected female piglets were individually weighed at birth, at 5 and 16 days of age, at weaning and at 1 week (32±0.4 days post-farrowing) and 2 weeks after the weaning. The ADG was calculated for each piglet as the difference of BW between two experimental time points. From the weaning until 2 weeks post-weaning, diarrhoea incidence was assessed daily with a score from 0 (no diarrhoea) to 1 (diarrhoea) and the weekly percentage of diarrhoea was calculated from those data.

Laboratory analysis

Chemical analysis

Feed samples were grounded to pass a 1-mm screen (Brabender rotary mill; Brabender GmbH & Co. KG, Duisburg, Germany) and then analysed for dry matter by heating at 105 ˚C for 3h. After, incineration at 550°C was performed to reach a stable mass and by that to determine the ash content according to standard method

(ISO 5984:2002; prepASH, Precisa Gravimetrics AG, Dietikon, Switzerland). The concentration of acid insoluble ashes was quantified gravimetrically (ISO 5985:2002) by incineration at 550°C followed by digestion in hydrochloric acid. Nitrogen content was quantified using the Dumas method (ISO 16634–1:2008), and subsequently, crude protein content was calculated as 6.25 × N. The crude fibre content was determined gravimetrically (ISO 6865:2000) by acid and alkaline digestions followed by the incineration of residual ashes using a fibre analyser (Fibretherm Gerhardt FT-12, C. Gerhardt GmbH & Co. KG, Königswinter, Germany). Cell wall components NDF and ADF were analysed using the same fibre analyser cited above (ISO 16472:2006 for NDF and ISO 13906:2008 for ADF). The NDF determination was performed with heat stable amylase and sodium sulfite and expressed without residual ash after incineration at 600°C for 3 h. Soluble, insoluble and low-molecular-weight DF contents were measured according to AOAC Method 2011.25, and the total DF content was calculated as the sum of the three types of DFs. Regarding the faeces, before analysis faecal samples were freeze dried to determine the DM the content, subsequently the acid insoluble ash concentration, NDF, ADF, gross energy and crude protein content were analysed as previously described for the feed chemical analysis.

Volatile fatty acid profile and bacterial DNA extraction

The VFA profile in the faeces was determined using high-performance liquid chromatography (HPLC) (Htoo et al.,2007). Faeces samples were unfrozen and mixed with 1 mL of internal standard (pivalic acid at 1%, w/v) and 18 mL of distilled water. Subsequently, samples were stirred for 3 h at room temperature and then centrifuged for 5 min at 4000 g. Finally, after filtering the supernatants, the VFA concentration was analysed by HPLC (Ultimate 3000, Thermo Fisher Scientific,

Reinach, Switzerland) with an exchange ion column (Nucleogel ION 300 OA 300 × 7.8 mm, Marcherey-Nagel AG, Oensingen, Switzerland) and equipped with a refractive index detector (RefractoMax 521, Thermo Fisher Scientific, Reinach, Switzerland). To isolate and extract total bacterial DNA from sow and piglet faeces, a FastDNA SPIN kit (MP Biomedicals, Santa Ana, Ca, USA) was used following manufacturer's instructions. The concentration and the purity of isolated bacterial DNA (ratio of absorbance 260/280 and 260/230) were checked using the NanoDrop spectrophotometer (Fisher Scientific, 13 Schwerte, Germany). The V3-V4 region of the 16S rRNA gene (~460 bp) was amplified, amplicons were produced using the universal primers Pro341F: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNBGCASCAG-3' and Pro805R: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACNVGGGTATCTAATCC-3' (Takahashi et al., 2014) using the Platinum™ Taq DNA Polymerase High Fidelity (Termo Fisher Scientific, Monza, Italy) and sequenced using the Illumina MiSeq platform 300x2bp. The libraries were prepared using the standard protocol for MiSeq Reagent Kit V3 and sequenced on MiSeq platform (Illumina Inc., San Diego, Ca, USA).

Bioinformatic analysis

Amplicon sequence variants (ASVs) were generated using DADA2 1.14.0 (Callahan et al., 2016) running on R 4.0.2, for the taxonomic assignment the Silva database release 138 (Quast et al., 2013) was used as reference. Briefly, the sequences were filtered for removing low-quality sequence, primers were trimmed to a consistent length to remove low quality sequence, forward reads were truncated at position 290, and reverse reads at position 220. Four sow faeces samples and one piglet's faeces

sample did not produce enough reads, and thus were removed from the analysis. As a sanity check, the rarefaction curves were plotted and presented in Supplementary Figure 1. In total, the number of subjects in the analysis were 31 for the sows (7 HC13, 9 HC11, 8 HC9 and 7 HC8) and 69 for the piglets (15 HC13, 20 HC11, 16 HC9 and 18 HC8; 34 SG and 35 FG). The sequencing process produced a total of 16,949,584 reads (54,676 on average) and a total of 1684 ASVs were identified. The ASVs identified for the sows belong to 19 different Phyla (Firmicutes 77.72% and Bacteroidetes 16.11%), 70 families (Lactobacillaceae 13.06% and Clostridiaceae 11.89%) and 178 genera (*Lactobacillus* 13.06% and *Clostridium_sensu_stricto*_1 10.89%). For the piglets, the ASVs identified belong to 19 different Phyla (Firmicutes 59.42% and Bacteroidetes 28.71%), 83 families (Lactobacillaceae 17.43% and Oscillospiraceae 11.49%) and 209 genera (*Lactobacillus* 17.97% and *Bacteroides* 8.79%).

Calculation and statistical analysis

The acid insoluble ash concentrations of the dietary treatments and of the faeces were used to calculate the apparent total tract digestibility (ATTD), using the following equation from Jang et al. (2014):

$$ATTD_N = 100 - \left[100 \times \frac{IM\ in\ feed}{IM\ in\ faeces} \times \frac{N\ in\ faeces}{N\ in\ feed}\right]$$

Where IM is the indigestible marker (Celite ®) and N is the nutrient of interest, i.e. ADF, NDF, gross energy and crude protein in this experiment. Both IM and N are expressed in g/kg.

All the statistical analysis were carried out in R (version 4.0.2 for Windows). Data related to VFA profile in sow and piglet faeces, digestibility of diets and performance

of the selected piglets were analysed with the nlme package considering either the sow or the piglet as experimental unit. Linear regression models, using the 'lm' function, were used to fit data related to days in diarrhoea, VFA profile in sow's faeces and the digestibility of the diets. The statistical model related to VFA profile in sow's faeces and digestibility of the diets included the treatment and the farrowing batch as fixed effect. As faeces collected on day 110 were used as a baseline, day 110 was included as a covariate in the statistical model. The statistical model for days in diarrhoea in piglet included the treatment, the growth category, the farrowing batch and the interaction treatment × growth category. For data related to piglets (performance and faecal VFA), linear mixed-effects models ("lme" function) were used where the treatment, the growth category, the farrowing batch, the interaction treatment ×growth category were considered as fixed effects and the sow as random effect. Finally, the percentage of diarrhoea was analysed using a generalised linear mixed model using Penalized Quasi-Likelihood, using the "glmmPQL" function and the statistical model included the maternal diet, the farrowing batch, the growth category and the day as fixed effects and the piglet as a random effect. The statistical analysis, on Alpha diversity, Beta diversity and taxonomic composition were carried out, using phyloseq (McMurdie and Holmes, 2013), Vegan (Dixon, 2003) and DESeq2 (Love et al., 2014) packages. For the alpha diversity, the Shannon, Simpson and Chao1 indices were calculated, and a Multifactorial ANOVA (MANOVA) model was fitted to test the differences between the treatments. The effect of the treatment, the farrowing batch and the parity were considered for sows. For piglets, the following factor were included in the model: the sequencing depth, the treatment, the farrowing batch, the growth category, the interaction treatment ×growth category as fixed effects and the sow as random effect. For the Beta

diversity, samples abundances were normalized using variance stabilizing transformation provided by DESeq2 package and the results were plotted using a principle coordinate analysis plot (PCoA). The Euclidian distance matrix was calculated, and the differences between treatments and growth categories were tested using a non-parametric PERMANOVA (Adonis) model, with 999 permutations. For piglet and sow, the same factors as for the alpha diversity were included in the model. Pairwise contrast were made using the pairwise Adonis function provided by the pairwise Adonis R package (Arbizu, 2020). In addition, to tests the homogeneity of dispersion among them a PERMDISP test was used (Anderson, 2001). Differences for the taxonomic composition between treatments were tested using DESeq2 aggregating the data at Genus level. The P-value (*P*) was adjusted for multiple comparison using the False Discovery Rate method (*P*-adj). Significance was declared if $P \leq 0.05$. Orthogonal polynomial contrasts were implemented to evaluate the linear (*L*) or quadratic (*Q*) effects of increasing HC level on the Shannon, Simpson and Chao1 indices of alpha diversity, VFA profile in sow and piglet faeces, digestibility of the diets and selected piglet performance. The results are expressed as the least square means ± SEM. *L* and *Q* effects were considered significant at $P \leq 0.05$.

Results

Apparent total tract digestibility of NDF, ADF, gross energy and crude protein

Increasing the level of HCs in sow's lactation linearly increased the ATTD of ADF (*L*=0.02), NDF (*L*<0.01) and gross energy (*L*=0.04) and decreased the ATTD of crude protein (*L*=0.02; *Q*<0.01) (Table 1). In particular, the ATTD of NDF was greater (*P*<0.01) in HC13 and HC11 groups than the HC9 and the HC8 groups, and in the HC9 compared to the HC8 group. The ATTD of crude protein was greater (*P*<0.01) in HC9 and HC8 compared to HC11, with intermediate values for HC13.

Volatile fatty acid profile in sow faeces

On day 110 of gestation, total VFA concentration was higher (*P*=0.03) in HC11 than the HC13 group, with intermediate values for the HC8 and HC9 group and a quadratic effect was also observed (*Q*=0.01) (Table 2). On day 3 of lactation, the dietary treatment affected (*P*=0.05) total VFA concentration. However, the pairwise comparisons showed no significant differences between the dietary treatments. Moreover, on the same time point, increasing the level of HCs in sow's lactation diet linearly increased the proportion of butyrate and valerate (*L*<0.05): In particular, the butyrate proportion was higher (*P*=0.02) in HC13 and HC11 group than the HC8 group, with intermediate values for the HC9 group. Finally, on day 17 of lactation, increasing the level of HC in sow's lactation diet linearly decreased the proportion of propionate (L=0.04).

A-diversity indices, β-diversity and taxonomical differences at genus level in sow faecal microbiota

Increasing the level of HCs in sow's lactation diet had no significant effects on Chao1, Shannon, and Simpson alpha diversity indexes in sow's faeces (Figure 1). The dietary treatments did affect (r^2=0.11; *P*=0.02) the beta diversity (Figure 2). However, the pairwise comparisons showed no significant effect between the four dietary treatments. The comparison of HC8 with HC9, HC11 and HC13 revealed eleven common genera that differ in sow's faeces: *Turicibacter, Terrisporobacter, Sutterella, Pyramidobacter, Parasutterella, Parabacteroides, Hungatella, Faecalicoccus and Erysipelotrichaceae_UCG-006* were more abundant (*P*-adj<0.05) while *Angelakisella* and *Lachnospiraceae_UCG-008* were less abundant (*P*-adj<0.05) in HC8 compared to HC9, HC11 and HC13 (Table 3).

Volatile fatty acid profile in piglet faeces

Increasing the level of HCs in the maternal diet had no effect either on the total VFA content or the proportion of each VFA in piglet's faeces (Table 4). Regardless of the maternal diet, besides a similar total VFA concentration, the proportion of isobutyrate (4.1 ± 0.17 % and 4.8 ± 0.17 %, respectively for SG and FG piglets) and isovalerate (5.7 ± 0.26 % and 7.1 ± 0.26 %, respectively for SG and FG piglets) were 0.7 and 1.4%-units greater (*P*<0.01), respectively in FG than SG piglets. The interaction between treatment and growth category was never significant.

A-diversity indices, β-diversity and taxonomical differences at genus level in piglet faecal microbiota

Increasing the level of HCs in the maternal diet did not affect either alpha diversity or beta diversity (Figure 3 and Figure 4, respectively). The comparison of HC8 with HC9, HC11 and HC13 revealed five common genera that differ in piglet faeces: *Paludibacteraceae*_H1 was more abundant (*P*-adj<0.05) while *Catenibacterium*,

Lachnospiraceae_CAG-56, *Lachnospiraceae_UCG-002* and *Succinivibrio* were less abundant (*P*-adj<0.05) (Table 5). Regardless of the maternal diet, the growth category had no effect either on alpha diversity or beta diversity. Taxonomical differences at the genus level showed that *Lachnospiraceae_XPB1014_group*, *Enterococcus* and *Succinovibrio* were less abundant (*P*-adj<0.01) and *Olsenella* was more abundant (*P*-adj<0.01) in SG compared to FG piglets (Table 6). No significant interaction was found between treatment and growth category.

Selected piglet performance

Increasing the level of HCs in the maternal diet had no effect either on body weight, ADG or the occurrence of diarrhoea (Table 7). However, these measurements were affected by the growth category during the pre-weaning and post-weaning periods. Indeed, the weight at birth (1.37 ± 0.055 and 1.72 ± 0.055, respectively for SG and FG piglets), at 5 days of age (1.91 ± 0.080 and 2.68 ± 0.080, respectively for SG and FG piglets), at 16 days of age (4.04 ± 0.193 and 6.20 ± 0.193, respectively for SG and FG piglets), at weaning (5.75 ± 0.289 and 8.61 ± 0.289, respectively for SG and FG piglets), 1 week post-weaning (6.06 ± 0.294 and 8.63 ± 0.294, respectively for SG and FG piglets) and 2 weeks post-weaning (7.49 ± 0.349 and 10.00 ± 0.344, respectively for SG and FG piglets) were greater (*P*<0.01), in the FG group than the SG group. Consequently, from birth to 5 days of age (108 ± 9.4 and 192 ± 9.4, respectively for SG and FG piglets), from birth to 16 days of age (167 ± 10.1 and 280 ± 10.1, respectively for SG and FG piglets), from birth to weaning (171 ± 10.2 and 268 ± 10.2, respectively for SG and FG piglets) and from birth to the second week post-weaning (154 ± 8.1 and 208 ± 8.0, respectively for SG and FG piglets), the ADG was greater (P< 0.01) in the FG group than the SG group. Nonetheless, the ADG was greater (*P*<0.01) in the SG group than the FG group from weaning to the first

week post-weaning (45 ± 16.3 and 2 ± 16.3, respectively for SG and FG piglets). The interaction between treatment and growth category was never significant (Table 7).

Discussion

Effect of increasing the level of hemicelluloses on sows' faecal apparent total tract digestibility, volatile fatty acid profile and microbial composition

In the present experiment, increasing the level of HCs in sow's lactation diet increased the ATTD of gross energy, ADF and NDF. Indeed, DFs can have an impact on the digestive process even before reaching the large intestine (Lenis et al., 1996). This effect may be related to the type of DFs included in the lactation diet and to their physiochemical properties such as water solubility. Water solubility is defined as the capability of DFs to be fully dispersed in water (Mudgil et al., 2013). Regarding HCs, it is known that these compounds can be both soluble and insoluble (Bendahou et al., 2007). However, Palumbo et al. (2022), using the same diets as in the present study, observed that increasing the level of HCs in sow's lactation diet also increased the intake of soluble DFs. Soluble DFs compared to insoluble DFs can be easily fermented at the end of the small intestine (Houdijk et al., 2002). Therefore, one can hypothesise that the greater soluble DF intake might be responsible for the observed effects on the digestibility of the ATTD of gross energy, ADF and NDF. Renteria-Flores et al. (2008) similarly reported a positive correlation between the intake of soluble DFs and energy digestibility, but no differences were observed in N digestibility. Conversely, the present study reported that increasing the level of HCs in sow's lactation diet decreased the crude protein digestibility. In a growing pig model, it has been already reported that increasing the intake of soluble DFs can decrease the crude protein digestibility (Schulze et al., 1994). This negative effect might be caused by the ability of certain sources of DFs such as sugar beet pulp in forming polysaccharides gel that increase the viscosity of the small intestine,

reducing the absorbed amino acids (Mosenthin et al.,1994). However, to evaluate the latter parameter, it would have been better to evaluate ileal digestibility rather than ATTD, since a certain proportion of amino acids could also be derived from bacterial origin (Dai et al., 2015). Moreover, because increasing the level of HCs in sow's diet during lactation, improved the degradation of ADF and NDF in the small intestine, it would have been also reasonable to find a lower concentration of VFAs in the large intestine due mainly to a lower substrate of fermentation for cellulolytic bacteria in the large intestine (Lindberg, 2014). Palumbo et al. (2022) showed that increasing the level of HCs in sow lactation diet decreased the proportion of butyrate and the total concentration of VFAs in sow milk. As VFAs are absorbed from large intestine to the blood circulation, they might arrive to the mammary glands where they can be used as source of energy for milk production (Tian et al., 2020). Therefore, due to the results of the previous study, one can expect a lower proportion of butyrate and a lower concentration of VFAs in sow's hindgut. Surprisingly, the present experiment showed that increasing the level of HCs in sow's lactation diet had an opposite trend regarding the butyrate proportion and slightly affected total VFA concentration on day 3 of lactation. Nonetheless, attention must be paid when comparing these results, as the time after the meal was not considered in either the aforementioned or the current study. Indeed, this latter parameter might have affected the kinetics of fermentation in the large intestine and by that also the time interval that VFAs need to be transferred to the mammary glands (Bach Knudsen et al., 2016). Therefore, the mechanism underlying the passage of VFAs from the large intestine to the mammary glands should be better understood for further studies. In addition, it was observed that increasing the level of HCs in sow lactation diet increased the proportion of valerate on day 3 of lactation, while it decreased the proportion of propionate on day

17 in faeces. Zhao et al. (2019) observed no correlations between the intake of HCs and any proportion of VFAs in growing pig faeces. However, in the present study, it is was not a surprise to expect different pattern of fermentations due to better ability of sows to degrade DFs (Noblet and Le Goff, 2001). A decreased level of propionate is in contrast of what observed by Tan et al. (2016) that reported that increasing the intake of soluble DFs increased level of propionate in sow's faeces on day 3 of lactation. Those discrepancies could be explained by the different sources of DFs included in the diet and by the sampling day as it is known that microbial composition during lactation is varying and by that its products of fermentation (Leblois et al., 2017). However, since different pattern of fermentation were found in sow faeces during lactation, it is plausible to expect different microbial composition. Interestingly, a positive correlation between genus *Parabacteroides* and propionate concentration has been reported in human faeces (Medawar et al., 2021). Therefore, the greater *Parabacteroides* abundance in the HC8 group may explain the greater propionate proportion in this group. A lower *Lachnospiraceae_UCG-008* abundance was reported in the HC8 group. As the family of Lachnospiraceae has been showed to be positively correlated with butyrate production in the large intestine, it could explain the increase in butyrate proportion in the faeces when the level of HCs increased (Medvecky et al., 2018).

Effects of the maternal diet on piglet' faecal volatile fatty acid profile, microbial composition, growth performance and intestinal health

The effects of DF sources in sow gestation and lactation diet on the microbiota of the piglets are well known (Paßlack et al., 2015; Leblois et al., 2017). However, to our best knowledge, the present study is the first one that investigates the effect of increasing the level of HCs in sow lactation diet on offspring microbiota. According to

the present findings, increasing the level of HCs in sow's lactation diet induced modifications in the faecal microbiota of the sow and their piglets before weaning. The maternal diet affected certain genera in piglet faecal microbial composition. When HC8 diet was compared to increasing levels of HCs, a higher abundance of *Paludibacteraceae_H1*, and a lower abundance of *Catenibacterium*, *Lachnospiraceae_CAG-56*, *Lachnospiraceae_UCG-002* and *Succinivibrio* were observed. Bacteria from the family of Paludibacteraceae and Catenibacterium are considered potential pathogens. Simultaneously, certain genera of Succinovibrionaceae family like *Succinovibrio* and Lachnospiraceae family like *Lachnospiraceae_CAG-56 and Lachnospiraceae_UCG-002* are well-known as fibre degrading commensal bacteria (Luo et al., 2018). As already discussed above, genera belonging to the Lachnospiraceae family are also butyrate-producing bacteria. A similar effect was also observed in the faecal microbiota of the sow, where a higher *Lachnospiraceae_UCG-008* abundance was reported when the level of HCs increased. Therefore, the faecal microbiota of the sow may act as a microbial reservoir for vertical transmission of this family of bacteria to the piglets (Trevisi et al., 2021). By eating or being in contact with the faeces of the mother, piglets may have acquired the faecal microbiota of the mother (Nowland et al., 2019). However, these modifications, were neither associated with changes in piglet faecal butyrate proportion, nor in intestinal health. In addition, the maternal diet did not have any effect on the overall growth performance of the piglets before and after weaning in agreement the study of Palumbo et al. (2022) in which, using the same diets, the pre and post-weaning growth were not affected by increasing the level of HCs in sow's lactation diet.

Effects of the growth rate on piglet performance, faecal microbiota and fermentations

Modern sow breeds are characterized by hyper-prolificacy that has caused wide variations in birthweight (Quesnel et al., 2008). Piglets with a lower birthweight are more prone to diseases, slow growth and to a higher risk of mortality compared to the heavier littermates (López-Vergé et al., 2018). In this context, Panzardi et al. (2013) showed that piglets born below 1.5 kg have higher odds of a low BW at weaning compared to their heavier littermates. In the present study, SG piglets with a mean birthweight of 1.4 kg were 2 kg-lighter at weaning compared to their FG siblings with a mean birthweight of 1.8 kg. After weaning, the first week is crucial to achieve an adequate subsequent growth (Tokach et al., 1992). In the present study, during the first week post-weaning, SG piglets showed even a greater ADG compared to FG piglets. This phenomenon might be related to the fact that ADG during suckling period is affected by milk intake rather than creep feed (Hojgaard et al., 2020). Therefore, it can be assumed that FG piglets that might have preferred to consume more milk than creep feed during pre-weaning period are less prone to a switch to solid feed compared to their SG siblings (Pajor et al., 1991). Although no growth differences were observed in the post-weaning period between the two growth categories and the difference in BW was still up to 2.5 kg. Differences in the early establishment of gut microbiota between different birthweight categories and growth rates have been already investigated (Gaukroger et al., 2020). The current study showed that regardless of the maternal diet, growth rate slightly affected faecal microbiota before weaning. Genera within the family of Lachnospiraceae like Lachnospiraceae*_XPB1014_group* and Succinovibrionaceae like *Succinovibrio* were greater in FG piglets compared to SG piglets. The bacteria belonging to these genera

have great abilities to degrade starches and to produce VFAs (Zhang et al., 2018). In fact, these characteristics, during the post-weaning period, might be the driver of a better feed conversion and by that also a better ADG until slaughter (Varel, 1987). Of further interest, genera like *Enterococcus* have been widely associated with improved performance of piglets at weaning and during the growing period (Zhang et al., 2014). Moreover, bacteria belonging to this genera are also characterised by proteolytic activities (Suzzi et al., 2000). Indeed, isobutyrate and isovalerate originate exclusively from valine and leucine fermentation by gut microbiota, respectively, and can serve as marker of protein fermentation in the large intestine (Dai et al., 2011). Therefore, the greater abundance of *Enterococcus* in FG piglets' faeces may have increased the proportions of isobutyrate and isovalerate (Duarte and Kim, 2022), which in turn has improved the performance of FG piglets, in agreement with what observed Girard et al. (2021).

In conclusion, feeding lactating sows with a similar DF level and increasing the level of HCs affects their faecal microbiota and VFA profile but with limited effects on SG and FG piglet's faecal microbiota, and no impact on faecal VFA profile and growth performance. For future studies we will aim to compare the effect of the maternal diet on vaginal, colostrum and milk microbiota to understand the role that these sources of bacteria might play to colonize the gastrointestinal tract of piglets during suckling period. In addition, we will aim to further explore the relation between gut microbiota and growth during suckling period, thus, comparing the faecal microbiota and VFA profile in SG and FG piglets characterised by a similar birthweight.

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Table 1. Apparent total tract digestibility of ADF, NDF, gross energy and crude protein of sow diet with increasing level of hemicelluloses during lactation period.

	[1]Dietary Treatments					[2]P-values		
	HC8	HC9	HC11	HC13	SEM	T	*L*	*Q*
Apparent total tract digestibility, %								
ADF	40.3	46.8	49.9	51.7	3.29	0.09	0.02	0.46
NDF	44.5[a]	54.6[b]	63.0[c]	67.9[c]	2.28	<0.01	<0.01	0.23
Gross energy	81.9	82.5	82.0	84.5	0.74	0.07	0.04	0.18
Crude Protein	88.1[b]	86.6[b]	83.3[a]	86.2[ab]	0.82	<0.01	0.02	<0.01

[1]Dietary Treatments: HC13=Sow's lactation diet containing 13% of hemicelluloses; HC11=Sow's lactation diet containing 11% of hemicelluloses; HC9=Sow's lactation diet containing 9% of hemicelluloses; HC8=Sow's lactation diet containing 8% of hemicelluloses.

[2]Contrasts: *L*=Linear; *Q*=Quadratic

[2]P-values: The presented P-values depict the overall sow dietary treatment (T), linear (L) and quadratic (Q) effects.

Table 2. Volatile fatty acid profile in the faeces of sows feed with sows feed with increasing level of hemicelluloses during lactation period on day 110 of gestation, day 3 and 17 of lactation.

	[1]Dietary Treatments					[2]P-values		
	HC8	HC9	HC11	HC13	SEM	T	*L*	*Q*
Day 110 of gestation								
Total volatile fatty acids, µmol/g	90.7[ab]	98.0[ab]	108.7[b]	89.5[a]	5.25	0.03	0.77	0.01
Individual VFA, %								
Acetate	60.6	60.1	61.5	62.0	1.47	0.81	0.42	0.73
Propionate	24.3	25.4	23.1	22.7	1.20	0.40	0.21	0.53
Isobutyrate	2.3	2.1	2.0	2.2	0.18	0.59	0.83	0.21
Butyrate	8.5	8.3	9.6	9.0	0.51	0.23	0.27	0.73
Isovalerate	2.8	2.7	2.4	2.6	0.26	0.74	0.52	0.54
Valerate	1.5	1.5	1.5	1.5	0.14	0.96	0.76	0.85
Day 3 of lactation								
Total volatile fatty acids, µmol/g	79.3	115.4	114.9	108.8	10.50	0.05	0.07	0.06
Individual VFA, %								
Acetate	60.8	59.4	58.3	58.5	1.18	0.38	0.14	0.48
Propionate	25.0	25.4	25.0	24.2	1.18	0.92	0.61	0.60
Isobutyrate	2.8	2.4	2.7	2.9	0.20	0.34	0.52	0.10
Butyrate	5.8[a]	7.8[ab]	8.5[b]	8.4[b]	0.65	0.02	<0.01	0.10
Isovalerate	3.6	3.2	3.4	3.7	0.23	0.38	0.65	0.10
Valerate	1.9	1.8	2.2	2.3	0.14	0.08	0.03	0.34
Day 17 of lactation								
Total volatile fatty acids, µmol/g	112	120	132	137	12.9	0.50	0.14	0.94
Individual VFA, %								
Acetate	56.9	56.7	57.6	57.6	1.24	0.92	0.59	0.94
Propionate	25.0	23.4	22.1	22.0	1.05	0.15	0.04	0.45
Isobutyrate	3.1	2.9	2.9	3.4	0.22	0.32	0.35	0.12
Butyrate	9.3	10.9	11.6	10.5	1.02	0.35	0.33	0.16
Isovalerate	3.6	4.0	3.6	4.1	0.24	0.24	0.38	0.68

Valerate	2.4	2.2	2.2	2.1	0.13	0.38	0.14	0.36

[1]Dietary Treatments: HC13=Sow's lactation diet containing 13% of hemicelluloses; HC11=Sow's lactation diet containing 11% of hemicelluloses; HC9=Sow's lactation diet containing 9% of hemicelluloses; HC8=Sow's lactation diet containing 8% of hemicelluloses

[2]P-values: The presented P-values depict the overall sow dietary treatment (T), linear (L) and quadratic (Q) effects.

Table 3 Common taxonomic genera differing on day 17 of lactation in the faeces of sows fed with 8% of hemicelluloses compared with 9, 11 and 13% of hemicelluloses during lactation period.

[1]Dietary treatments	log2FC[2]	lfcSE[3]	*P*-adj[4]
HC8 vs HC9			
Angelakisella	-21.93	4.373	<0.01
Lachnospiraceae_UCG-008	-14.37	4.373	0.01
Erysipelotrichaceae_UCG-006	23.95	4.368	<0.01
Faecalicoccus	23.74	4.367	<0.01
Hungatella	23.95	4.368	<0.01
Parabacteroides	3.02	1.016	0.02
Parasutterella	22.17	4.368	<0.01
Pyramidobacter	30.34	4.367	<0.01
Sutterella	24.20	4.368	<0.01
Terrisporobacter	1.56	0.561	0.04
Turicibacter	2.46	0.883	0.04
HC8 vs HC11			
Angelakisella	-19.47	4.331	<0.01
Lachnospiraceae_UCG-008	-27.48	4.314	<0.01
Erysipelotrichaceae_UCG-006	26.09	4.328	<0.01
Faecalicoccus	25.21	4.328	<0.01
Hungatella	26.09	4.328	<0.01
Parabacteroides	3.74	0.999	<0.01
Parasutterella	25.54	4.329	<0.01
Pyramidobacter	32.76	4.328	<0.01
Sutterella	26.87	4.328	<0.01
Terrisporobacter	1.53	0.556	0.04
Turicibacter	3.51	0.875	<0.01
HC8 vs HC13			
Angelakisella	-30.00	4.868	<0.01
Lachnospiraceae_UCG-008	-19.49	4.892	<0.01
Erysipelotrichaceae_UCG-006	15.13	4.887	0.01
Faecalicoccus	16.07	4.887	0.01
Hungatella	15.13	4.887	0.01
Parabacteroides	3.20	1.129	0.03
Parasutterella	14.51	4.887	0.02
Pyramidobacter	18.32	4.887	<0.01
Sutterella	16.03	4.887	0.01
Terrisporobacter	1.95	0.628	0.01
Turicibacter	3.66	0.988	<0.01

[1]Dietary treatments : HC13=Sow's lactation diet containing 13% of hemicelluloses; HC11=Sow's lactation diet containing 11% of hemicelluloses; HC9=Sow's lactation diet containing 9% of hemicelluloses; HC8=Sow's lactation diet containing 8% of hemicelluloses.

[2]log2FC:log2 fold change is the effect size estimate

[3]lfcSE:standard error for the log2 fold change estimate.

[4]*P*-adj:P-value adjusted for multiple comparison using the False Discovery Rate method

Table 4. Volatile fatty acid (VFA) profile in the faeces of slow and fast growing piglets originating from sows fed increasing levels of hemicelluloses during the lactation period.

	[1]Dietary Treatments x Growth Categories								SEM	[2]P-values				
	HC8		HC9		HC11		HC13			T	G	T x G	*L*	*Q*
	SG	FG	SG	FG	SG	FG	SG	FG						
Total volatile fatty acids,µmol/g	46.1	47.4	51.7	40.2	41.5	58.8	38.6	42.1	6.70	0.13	0.55	0.12	0.93	0.46
Individual VFA, % of total VFAs														
Acetate	54.9	51.7	54.3	54.3	58.2	55.2	56.6	52.6	3.21	0.83	0.22	0.92	0.81	0.40
Propionate	19.7	19.6	19.8	15.6	17.4	15.2	16.3	17.9	2.01	0.29	0.32	0.39	0.56	0.09
Isobutyrate	3.7	4.8	3.9	4.5	4.5	4.7	4.3	5.3	0.35	0.41	<0.01	0.36	0.30	0.17
Butyrate	12.5	12.8	12.6	15.2	11.3	14.2	13.0	13.2	1.81	0.75	0.19	0.76	0.96	0.32
Isovalerate	5.2	7.2	5.9	6.8	5.8	7.04	6.0	7.4	0.52	0.84	<0.01	0.69	0.72	0.42
Valerate	4.0	3.9	3.4	3.7	2.8	3.6	3.9	3.6	0.42	0.94	0.58	0.49	0.57	0.82

[1]Dietary treatments: HC13=Sow's lactation diet containing 13% of hemicelluloses; HC11=Sow's lactation diet containing 11% of hemicelluloses; HC9=Sow's lactation diet containing 9% of hemicelluloses; HC8=Sow's lactation diet containing 8% of hemicelluloses; Growth categories: SG = piglets displaying slow growth (average daily gain: 167±10.1 g/d) from 0 to 16 days of age; FG = piglets displaying fast growth (average daily gain: 280±10.1 g/d) from 0 to 16 days of age

[2]P-values: The presented P-values depict the overall sow dietary treatment (T), growth category (G), the interaction between sow dietary treatment and growth category (T x G), linear (L) and quadratic (Q) effects.

Table 5. Common taxonomic genera differing on day 16 in the faeces of piglets originating from sows fed increasing levels of hemicelluloses during the lactation period.

[1]Dietary treatments	log2FC[2]	lfcSE[3]	*P*-adj[4]
HC8 vs HC9			
Catenibacterium	-38.48	5.955	<0.01
Lachnospiraceae_CAG-56	-15.65	4.566	0.01
Lachnospiraceae_UCG-002	-18.53	5.944	0.02
Succinivibrio	-45.72	5.930	<0.01
Paludibacteraceae_H1	27.41	5.963	<0.01
HC8 vs HC11			
Catenibacterium	-44.99	5.660	<0.01
Lachnospiraceae_CAG-56	-17.22	4.343	<0.01
Lachnospiraceae_UCG-002	-21.77	5.653	<0.01
Succinivibrio	-34.72	5.663	<0.01
Paludibacteraceae_H1	27.97	5.672	<0.01
HC8 vs HC13			
Catenibacterium	-25.83	6.168	<0.01
Lachnospiraceae_CAG-56	-17.91	4.700	<0.01
Lachnospiraceae_UCG-002	-21.23	6.144	0.01
Succinivibrio	-27.45	6.166	<0.01
Paludibacteraceae_H1	21.59	6.163	0.01

[1]Dietary Treatments: HC13=Sow's lactation diet containing 13% of hemicelluloses; HC11=Sow's lactation diet containing 11% of hemicelluloses; HC9=Sow's lactation diet containing 9% of hemicelluloses; HC8=Sow's lactation diet containing 8% of hemicelluloses.

[2]log2FC:log2 fold change is the effect size estimate

[3]lfcSE: standard error for the log2 fold change estimate

[4]*P*-adj: P-value adjusted for multiple comparison using the False Discovery Rate method

Table 6. Taxonomic differences at genus level of piglets characterised by slow and fast growth

[1]Growth Categories	log2FC[2]	lfcSE[3]	*P*-adj[4]
SG vs FG			
Lachnospiraceae_XPB1014_group	-28.00	6.302	<0.01
Enterococcus	-6.92	1.774	<0.01
Olsenella	19.74	3.561	<0.01
Succinivibrio	-27.20	6.299	<0.01

[1]Growth categories: SG = piglets displaying slow growth (average daily gain: 167±10.1 g/d) from 0 to 16 days of age; FG = piglets displaying fast growth (average daily gain: 280±10.1 g/d) from 0 to 16 days of age

[2]log2FC=log2 fold change is the effect size estimate

[3]lfcSE=standard error for the log2 fold change estimate

[4]*P*-adj=P-value adjusted for multiple comparison using the False Discovery Rate method

Table 7. Growth performance and occurrence of diarrhoea in piglets characterised by slow and fast growth and born from sows fed with increasing level of hemicelluloses during the lactation period.

	[1]Dietary Treatments x Growth Categories								SEM	[2]P-values				
	HC8		HC9		HC11		HC13			T	G	T x G	*L*	*Q*
	SG	FG	SG	FG	SG	FG	SG	FG						
Body weight, kg														
At birth	1.21	1.54	1.48	1.81	1.37	1.79	1.43	1.72	0.111	0.24	<0.01	0.92	0.30	0.12
5 days post-farrowing	1.79	2.44	2.07	2.82	1.94	2.76	1.85	2.68	0.162	0.29	<0.01	0.89	0.37	0.14
16 days post-farrowing	3.79	5.67	4.35	6.30	3.99	6.42	4.03	6.41	0.387	0.41	<0.01	0.68	0.19	0.39
Weaning	5.71	8.20	5.84	8.48	5.69	9.20	5.76	8.57	0.580	0.58	<0.01	0.65	0.48	0.41
1 week post-weaning	5.90	8.12	6.36	8.42	6.23	9.22	5.76	8.76	0.590	0.52	<0.01	0.65	0.31	0.50
2 week post-weaning	7.44	9.70	7.53	9.54	7.51	10.64	7.48	10.13	0.693	0.62	<0.01	0.76	0.44	0.79
ADG, g/d														
Birth-5 days post-farrowing	116	179	117	202	114	195	83	191	18.9	0.83	<0.01	0.52	0.73	0.46
Birth-16 days post-farrowing	161	258	179	280	164	289	162	293	20.2	0.58	<0.01	0.58	0.22	0.62
Birth- Weaning	171	252	174	266	169	289	169	265	20.5	0.56	<0.01	0.66	0.50	0.34
Weaning-1 week post-weaning	27	-11	74	-8	78	2	0	26	32.5	0.87	<0.01	0.11	0.49	0.60
Weaning-2 weeks post-weaning	124	107	120	75	132	106	82	114	30.2	0.77	0.38	0.40	0.44	0.78
Birth-2 week post-weaning	154	201	154	197	156	224	153	211	17.0	0.60	<0.01	0.77	0.44	0.78
Post-weaning diarrhoea,%														
1 week post-weaning	23.3	30.7	30.6	19.5	32.0	32.1	33.7	25.4	10.64	0.89	0.70	0.74	0.67	0.98
2 weeks post-weaning	13.1	15.1	10.2	22.3	18.9	22.6	23.8	0.05	10.78	0.81	0.92	0.26	0.94	0.44
Days in diarrhoea, d														
1 week post-weaning	1.77	2.22	2.17	1.42	2.30	2.30	2.43	1.93	0.690	0.88	0.70	0.80	0.63	0.92
2 weeks post-weaning	1.22	1.45	0.89	1.76	1.46	1.66	2.01	0.64	0.638	0.97	1.00	0.32	0.92	0.72

[1]Dietary Treatments x Growth Categories : HC13=Sow's lactation diet containing 13% of hemicelluloses; HC11=Sow's lactation diet containing 11% of hemicelluloses; HC9=Sow's lactation diet containing 9% of hemicelluloses; HC8=Sow's lactation diet containing 8% of hemicelluloses; Growth categories: SG = piglets displaying slow growth (average daily gain: 167±10.1 g/d) from 0 to 16 days of age; FG = piglets displaying fast growth (average daily gain: 280±10.1 g/d) from 0 to 16 days of age

[2]P-values: The presented P-values depict the overall sow dietary treatment (T), growth category (G), the interaction between sow dietary treatment and growth category (T x G), linear (L) and quadratic (Q) effects

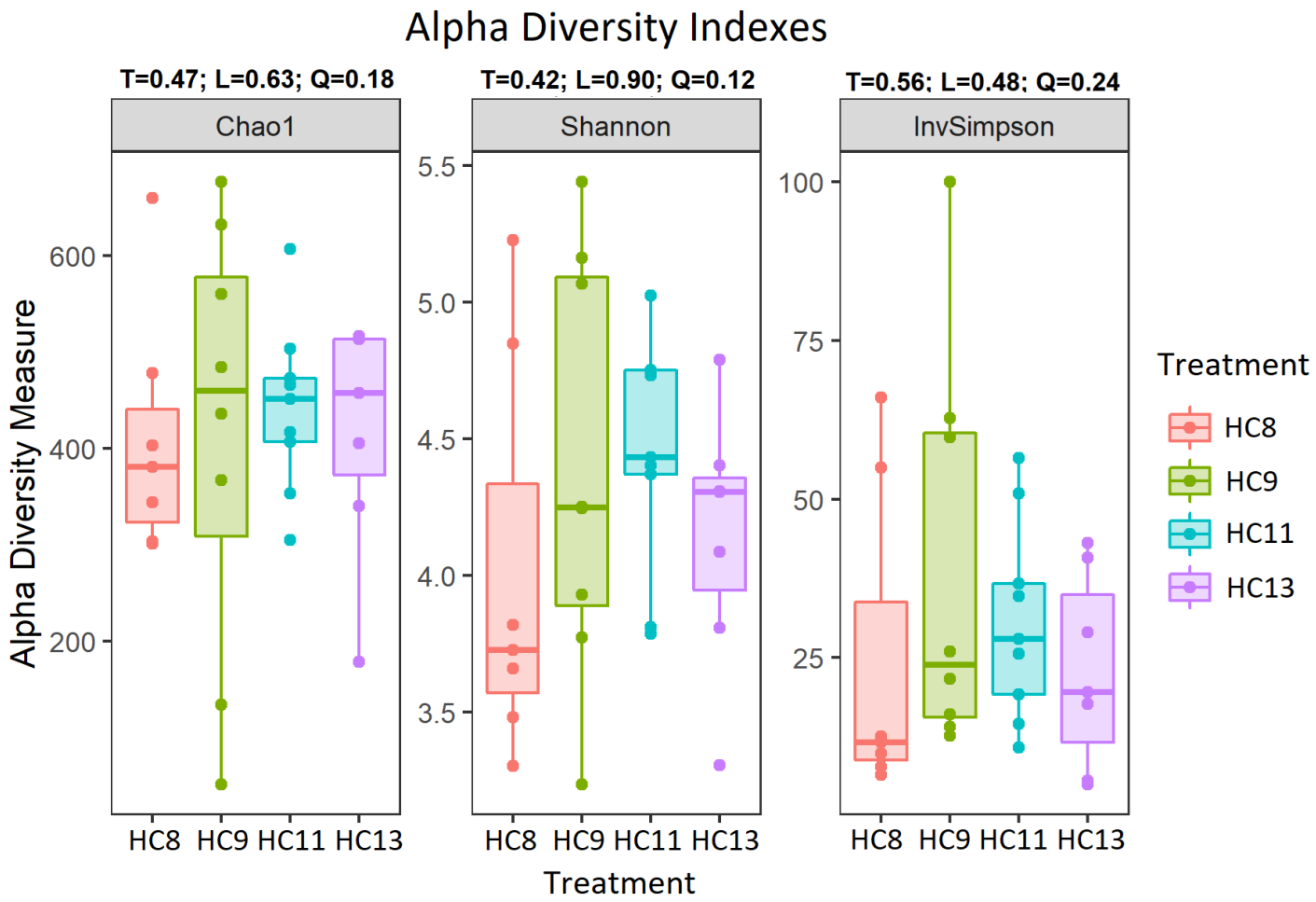


Figure 1. Box plot showing alpha diversity indexes (Chao1, Shannon and InvSimpson) in faecal microbiota at day 17 of lactation of sows fed increasing dietary levels of hemicelluloses (HC13 = 13%, HC11 = 11%; HC9 = 9%; HC8 = 8%) during lactation period. The presented P-values depict the overall sow dietary treatment (T), linear (L) and quadratic (Q) effects.

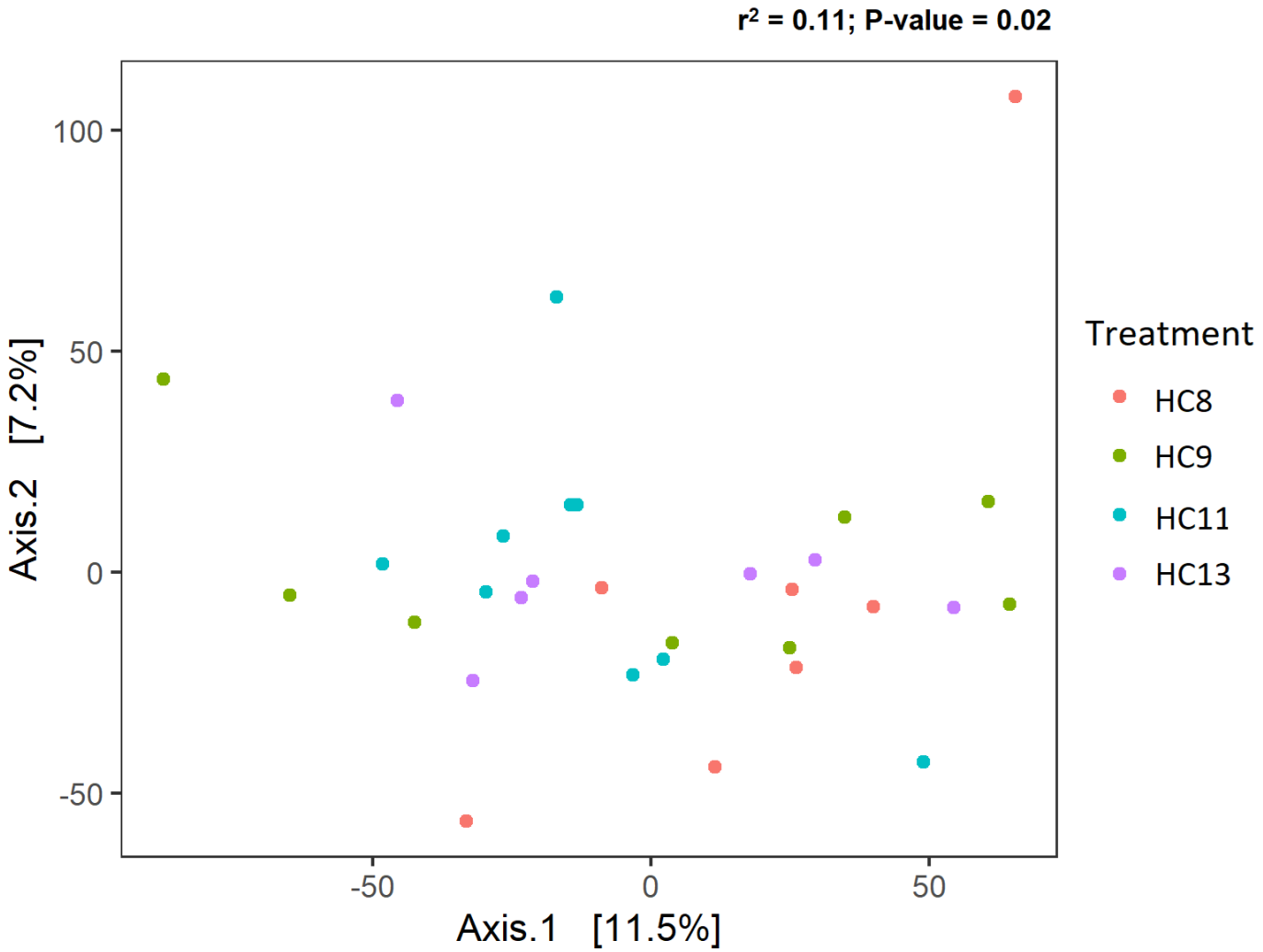


Figure 2. Principal coordinate analysis plot (PCoA) using Euclidean distance matrix at the amplicon sequence variant level in faecal microbiota at day 17 of lactation of sows fed increasing levels of hemicelluloses (HC13 = 13%, HC11 = 11%; HC9 = 9%; HC8 = 8%) during lactation period. Axis 1 and Axis 2 respectively explained 11.5 % and 7.2 % of the variance of the abundance of gut microbiota at the amplicon sequence variant level. The present r-square (r^2) and P-value depict the overall sow dietary treatment effect.

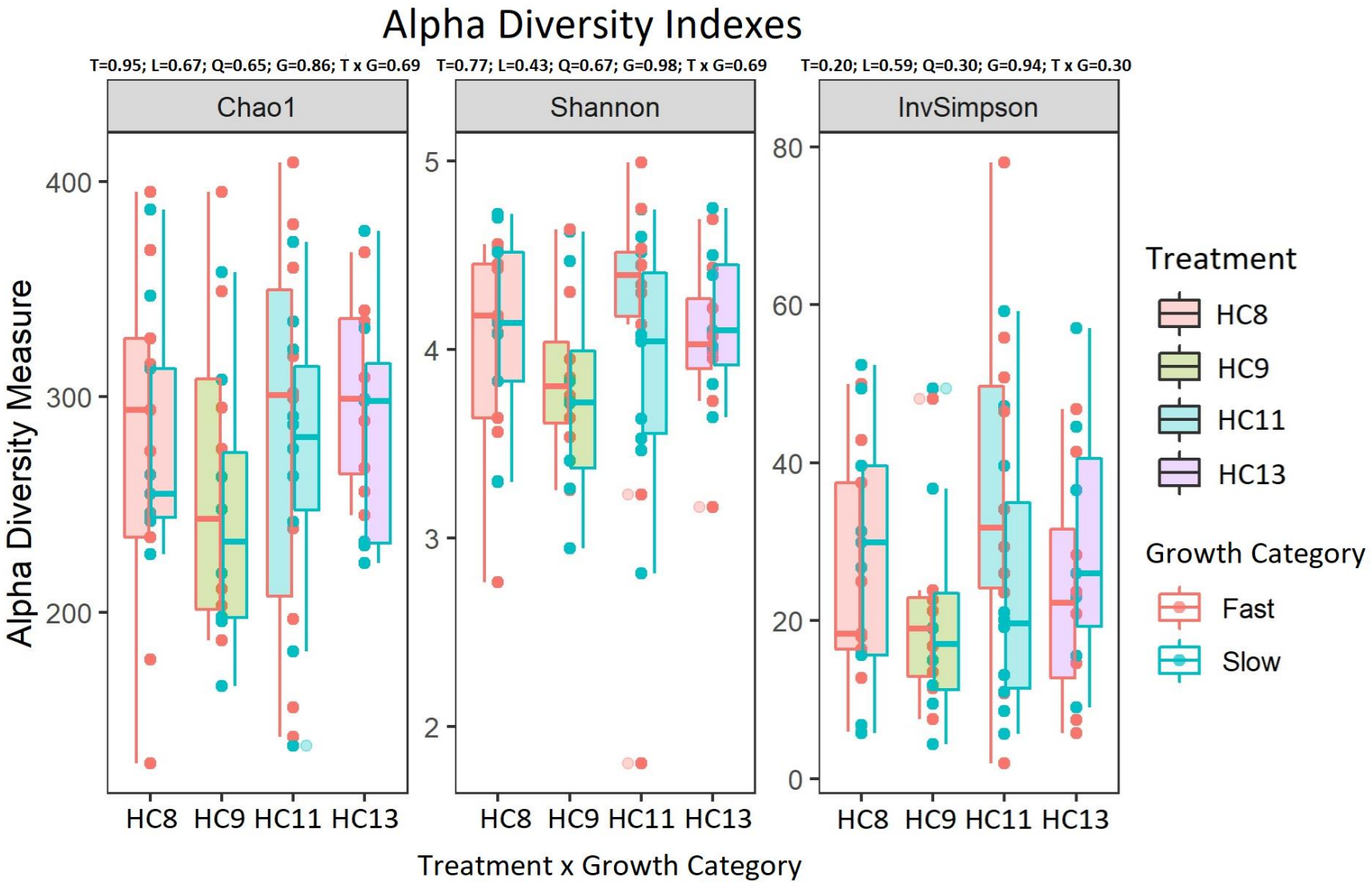


Figure 3. Box plot showing alpha diversity indexes (Chao1, Shannon and InvSimpson) in faecal microbiota at day 16 of life of piglets displaying slow growth (average daily gain: 167±10.1 g/d) and fast growth (average daily gain: 280±10.1 g/d) from 0 to 16 days of age and originating from sows fed increasing levels of hemicelluloses (HC13 = 13%, HC11 = 11%; HC9 = 9%; HC8 = 8%) during the lactation period. The presented P-

values depict the overall sow dietary treatment (T), growth category (G), the interaction between sow dietary treatment and growth category (T x G), linear (L) and quadratic (Q) effects.

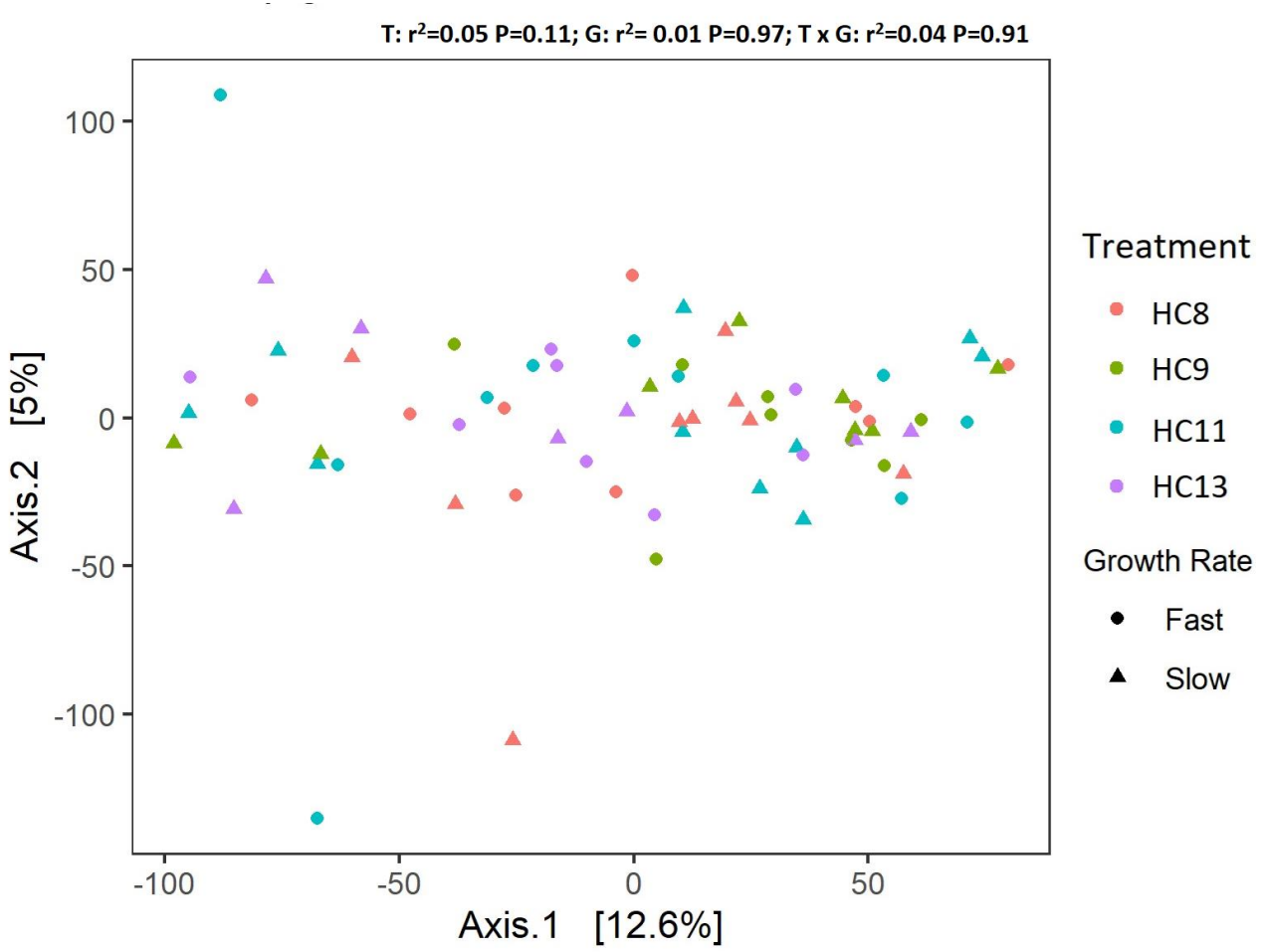


Figure 4 Principal coordinate analysis plot (PCoA) using Euclidean distance matrix at the amplicon sequence variant level in faecal microbiota at at day 16 of life of piglets= piglets displaying slow growth (average daily gain: 167±10.1 g/d) and fast growth (average daily gain: 280±10.1 g/d) from 0 to 16 days of age and originating from sows fed increasing levels of hemicelluloses (HC13 = 13%, HC11 = 11%; HC9 = 9%; HC8 = 8%) during the lactation period.. Axis 1 and Axis 2 respectively explained 12.6 % and 5 % of the variance of the abundance of gut microbiota at the

amplicon sequence variant level. The present r-square (r^2) and P-value depict the overall sow dietary treatment effect (T) growth category (G), the interaction between sow dietary treatment and growth category (T x G) effects.

Supplementary Table 1. Ingredients and composition of the sow's lactation diet

	[1]Dietary Treatments			
	HC8	HC9	HC11	HC13
Compounds (g/kg as fed)				
Barley, ground	47		387	544
Oat Flakes	182	40		
Corn, ground	160	269		103
Rye		100	250	
Wheat, ground	150	131		
Wheat Starch	40	40	40	40
Molasses	40			
Animal Fat RS 65	38	30	24	24
Potato protein	100	100	100	100
Soybean meal	100	100	100	100
Flaxseed Meal				6
Rapeseed meal	17		4	
Oat Hulls	80	40		
Lupin		25		
Wheat Bran		40		
Beet pulp		40	50	30
L-Lysine-HCL	0.56	0.57	0.57	0.70
DL-Methionine				2
L-Threonine	0.5			0.5
L-Tryptophan	0.03	0.13	0.06	0.20
Dicalcium phosphate	8.5	8.2	7.0	9.4
Calcium carbonate	14.7	13.9	13.8	15.7
Salt	4.1	4.2	5.2	5.9
[2]Pellan	4.0	4.0	4.0	4.0
Celite	10.0	10.0	10.0	10.0
[3]Premix	4.0	4.0	4.0	4.0
[4]Natuphos 5000 G	0.1	0.1	0.1	0.1
Gross chemical composition analysed (%)				
Dry Matter	90.0	89.7	89.4	90.0
Crude Protein	19.6	19.2	19.1	19.3
Fat	6.0	5.7	4.6	5.1
Crude fibre	4.6	4.7	4.3	4.3
Ash	6.3	6.0	6.1	6.3
NDF	15.4	16.3	17.4	18.4
ADF	7.4	6.9	6.0	5.7

[5]Hemicelluloses	8.0	9.4	11.4	12.7
Total dietary fibres	20.3	22.0	22.7	21.0
Low molecular weight dietary fibres	1.4	1.8	2.3	1.8
Soluble dietary fibres	2.8	3.5	4.4	4.3
Insoluble dietary fibres	16.1	16.7	16.0	14.9
[6]IDF/SDF	5.75	4.77	3.63	3.46
Calcium	0.87	0.93	0.94	0.94
Phosphorus	0.47	0.50	0.46	0.50
Gross chemical composition calculated				
Digestible energy (MJ/kg)	14.1	14.1	14.1	14.1
Digestible phosphorus (%)	0.3	0.3	0.3	0.3
Digestible essential amino acids (%)				
Lysine	0.96	0.96	0.96	0.96
Methionine	0.30	0.30	0.29	0.49
Threonine	0.69	0.64	0.63	0.69
Tryptophan	0.18	0.18	0.18	0.20

[1]Dietary Treatments: HC13=Sow's lactation diet containing 13% of hemicelluloses; HC11=Sow's lactation diet containing 11% of hemicelluloses; HC9=Sow's lactation diet containing 9% of hemicelluloses; HC8=Sow's lactation diet containing 8% of hemicelluloses.

[2]Pellan=pellet binding aid: Pellan, Mikro-Technik, Bürgstadt, Germany.

[3]Premix=supplied per kg of diet: vitamin A, 8000 IU; vitamin D3, 800 IU; vitamin E, 40mg; menadione, 2 mg; thiamine, 2 mg; riboflavin, 5 mg; biotin, 0.1 mg; niacin, 20 mg; pantothenic acid, 20 mg; iodine, 0.55 mg as calcium iodate; copper, 7mg as copper sulfate; manganese, 20 mg as manganese oxide; zinc, 55 mg as zinc oxide; selenium, 0.2 mg assodium selenite.

[4]Natuphos 5000 G=phytase supplemented at 500 units of *aspergillus niger* phytase/kg diet.

[5]Hemicelluloses=calculated as the difference between NDF and ADF

[6]IDF/SDF=ratio of insoluble to soluble dietary fibres

Supplementary Table 2. Taxonomic differences at the genus level on faecal microbiota at day 17 of lactation of sows fed increasing dietary levels of hemicelluloses (HC13 = 13%, HC11 = 11%; HC9 = 9%; HC8 = 8%) during lactation period.

Dietary treatments	log2FC[1]	lfcSE[2]	*P*-adj[3]
HC8 vs HC9			
Angelakisella	-21.93	4.373	<0.01
Lachnospiraceae_UCG-008	-14.37	4.373	0.01
Shuttleworthia	-5.78	1.750	0.01
Anaerovoracaceae_Family_XIII_AD3011_group	1.17	0.433	0.04
Terrisporobacter	1.56	0.561	0.04
Roseburia	2.10	0.747	0.04
Turicibacter	2.46	0.883	0.04
Parabacteroides	3.02	1.016	0.02
Streptococcus	4.15	1.033	<0.01
Butyricicoccus	5.80	2.155	0.04
Pygmaiobacter	7.81	1.944	<0.01
Erysipelatoclostridiaceae_UCG-004	13.73	4.345	0.01
Eisenbergiella	18.88	4.366	<0.01
Negativibacillus	21.03	2.875	<0.01
Mycoplasma	21.19	3.134	<0.01
Parasutterella	22.17	4.368	<0.01
Faecalicoccus	23.74	4.367	<0.01
Erysipelotrichaceae_UCG-006	23.95	4.368	<0.01
Hungatella	23.95	4.368	<0.01
Sutterella	24.20	4.368	<0.01
Pyramidobacter	30.34	4.367	<0.01
Defluviitaleaceae_UCG-011	34.31	4.368	<0.01
Candidatus_Stoquefichus	35.34	4.343	<0.01
HC8 vs HC11			
Lachnospiraceae_UCG-008	-27.48	4.314	<0.01
Amnipila	-26.72	4.324	<0.01
Angelakisella	-19.47	4.331	<0.01
Shuttleworthia	-4.70	1.725	0.04
Terrisporobacter	1.53	0.556	0.04
Bacteroides	2.96	0.961	0.01
Escherichia/Shigella	3.03	1.093	0.04
Turicibacter	3.51	0.875	<0.01
Parabacteroides	3.74	0.999	<0.01
Phocea	4.10	1.291	0.01
Lachnospiraceae_UCG-006	22.42	4.324	<0.01
Oscillospiraceae_V9D2013_group	23.78	4.336	<0.01
Faecalicoccus	25.21	4.328	<0.01
Parasutterella	25.54	4.329	<0.01
Erysipelotrichaceae_UCG-006	26.09	4.328	<0.01
Hungatella	26.09	4.328	<0.01

Sutterella	26.87	4.328	<0.01
Lachnospiraceae_possible_genus_Sk018	27.78	4.329	<0.01
Pyramidobacter	32.76	4.328	<0.01
Tuzzerella	35.91	4.312	<0.01
Muribaculaceae_CAG-873	50.00	4.295	<0.01
Lachnospiraceae_CAG-56	56.09	4.300	<0.01
HC8 vs HC13			
Angelakisella	-30.00	4.868	<0.01
Lachnospiraceae_UCG-008	-19.49	4.892	<0.01
Phascolarctobacterium	1.89	0.681	0.03
Terrisporobacter	1.95	0.628	0.01
Catenisphaera	2.72	0.857	0.01
Parabacteroides	3.20	1.129	0.03
Escherichia/Shigella	3.58	1.277	0.03
Turicibacter	3.66	0.988	<0.01
Denitrobacterium	4.13	0.953	<0.01
Pygmaiobacter	6.87	2.198	0.01
Lachnoclostridium	7.18	1.842	<0.01
Parasutterella	14.51	4.887	0.02
Erysipelotrichaceae_UCG-006	15.13	4.887	0.01
Hungatella	15.13	4.887	0.01
Cloacibacillus	15.44	4.885	0.01
Tuzzerella	15.99	4.854	0.01
Sutterella	16.03	4.887	0.01
Faecalicoccus	16.07	4.887	0.01
Butyricimonas	16.75	4.885	0.01
Pyramidobacter	18.32	4.887	<0.01
Defluviitaleaceae_UCG-011	18.58	4.887	<0.01
Fusobacterium	21.50	4.853	<0.01
Muribaculaceae_CAG-873	21.98	4.845	<0.01
Lachnospiraceae_CAG-56	29.34	4.849	<0.01
HC9 vs HC11			
Defluviitaleaceae_UCG-011	-32.56	4.125	<0.01
Amnipila	-26.99	4.112	<0.01
Candidatus_Stoquefichus	-25.95	4.123	<0.01
Eisenbergiella	-22.29	4.122	<0.01
Mycoplasma	-19.47	2.976	<0.01
Negativibacillus	-16.24	2.752	<0.01
Lachnospiraceae_UCG-008	-13.11	4.094	0.01
Fusicatenibacter	-5.66	1.947	0.03
Streptococcus	-3.07	0.975	0.01
Subdoligranulum	-3.07	0.970	0.01
Ruminococcaceae_Incertae_Sedis	-3.02	0.753	<0.01
Roseburia	-2.35	0.686	0.01
Coprococcus	-1.53	0.560	0.04
Succinivibrio	3.42	1.273	0.05
Phocea	4.09	1.245	0.01

Lachnospiraceae_UCG-006	19.42	4.124	<0.01
Lachnospiraceae_possible_genus_Sk018	22.80	4.124	<0.01
Oscillospiraceae_V9D2013_group	25.30	4.116	<0.01
Tuzzerella	29.29	4.112	<0.01
Muribaculaceae_CAG-873	50.64	4.095	<0.01
Lachnospiraceae_CAG-56	53.44	4.100	<0.01
HC9 vs HC13			
Eisenbergiella	-29.57	4.663	<0.01
Mycoplasma	-19.84	3.398	<0.01
Negativibacillus	-16.64	3.139	<0.01
Streptococcus	-4.94	1.112	<0.01
Denitrobacterium	3.05	0.922	0.01
Ruminiclostridium	15.72	4.674	0.01
Butyricimonas	16.37	4.682	0.01
Cloacibacillus	16.85	4.683	0.01
Fusobacterium	18.86	4.677	<0.01
Muribaculaceae_CAG-873	22.62	4.669	<0.01
Lachnospiraceae_CAG-56	26.68	4.681	<0.01
HC11 vs HC13			
Lachnospiraceae_UCG-006	-28.61	4.316	<0.01
Oscillospiraceae_V9D2013_group	-27.29	4.331	<0.01
Lachnospiraceae_possible_genus_Sk018	-24.30	4.315	<0.01
Tuzzerella	-19.92	4.330	<0.01
Phocea	-4.14	1.327	0.02
Lachnospiraceae_NC2004_group	1.61	0.565	0.04
Catenisphaera	2.24	0.752	0.03
Denitrobacterium	3.40	0.851	<0.01
Fusobacterium	12.92	4.330	0.03
Amnipila	13.68	4.324	0.02
Cloacibacillus	15.55	4.331	<0.01
Papillibacter	15.57	4.318	<0.01
Butyricimonas	16.04	4.331	<0.01
Ruminiclostridium	16.40	4.310	<0.01
Defluviitaleaceae_UCG-011	16.83	4.332	<0.01

[1]log2FC=log2 fold change is the effect size estimate

[2]lfcSE=standard error for the log2 fold change estimate

[3]*P*-adj=P-value adjusted for multiple comparison using the False Discovery Rate method.

Supplementary Table 3. Taxonomic differences at genus level in faecal microbiota at day 16 of life of piglets displaying slow growth (average daily gain: 167±10.1 g/d) and fast growth (average daily gain: 280±10.1 g/d) from 0 to 16 days of age and originating from sows fed increasing levels of hemicelluloses (HC13 = 13%, HC11 = 11%; HC9 = 9%; HC8 = 8%) during the lactation period.

Dietary treatments	log2FC[1]	lfcSE[2]	*P*-adj[3]
HC8 vs HC9			
Succinivibrio	-45.72	5.930	<0.01
Catenibacterium	-38.48	5.955	<0.01
Eggerthellaceae_DNF00809	-36.29	5.950	<0.01
Eubacterium	-23.15	5.941	<0.01
Lachnospiraceae_UCG-002	-18.53	5.944	0.02
Lachnospiraceae_CAG-56	-15.65	4.566	0.01
Subdoligranulum	-6.17	2.032	0.02
Prevotella	-2.82	0.985	0.04
Methanobrevibacter	2.09	0.745	0.04
Denitrobacterium	2.93	0.968	0.02
Oscillospiraceae_UCG-003	7.42	2.221	0.01
Lachnospiraceae_UCG-010	17.03	5.964	0.04
Epulopiscium	19.12	5.961	0.01
Butyricicoccaceae_UCG-009	21.33	2.900	<0.01
Akkermansia	21.84	3.825	<0.01
Solobacterium	22.5	5.950	<0.01
Citrobacter	26.71	5.954	<0.01
Paludibacteraceae_H1	27.41	5.963	<0.01
Butyricicoccaceae_UCG-008	30.36	5.959	<0.01
Muribaculaceae_CAG-873	30.51	5.484	<0.01
Bifidobacterium	38.07	5.949	<0.01
HC8 vs HC11			
Catenibacterium	-44.99	5.660	<0.01
Eggerthellaceae_DNF00809	-39.43	5.654	<0.01
Succinivibrio	-34.72	5.663	<0.01
Lachnospiraceae_UCG-002	-21.77	5.653	<0.01
Acidaminococcus	-20.73	5.663	<0.01
Clostridioides	-20.52	5.669	<0.01
Lachnospiraceae_CAG-56	-17.22	4.343	<0.01
Clostridium_sensu_stricto_2	-17.12	5.678	0.03
Erysipelotrichaceae_UCG-006	14.99	3.289	<0.01
Frisingicoccus	16.12	2.758	<0.01
Lachnospiraceae_UCG-010	21.25	5.673	<0.01
Citrobacter	24.48	5.666	<0.01
Erysipelotrichaceae_UCG-009	27.83	5.660	<0.01
Paludibacteraceae_H1	27.97	5.672	<0.01
Fibrobacter	28.41	5.654	<0.01

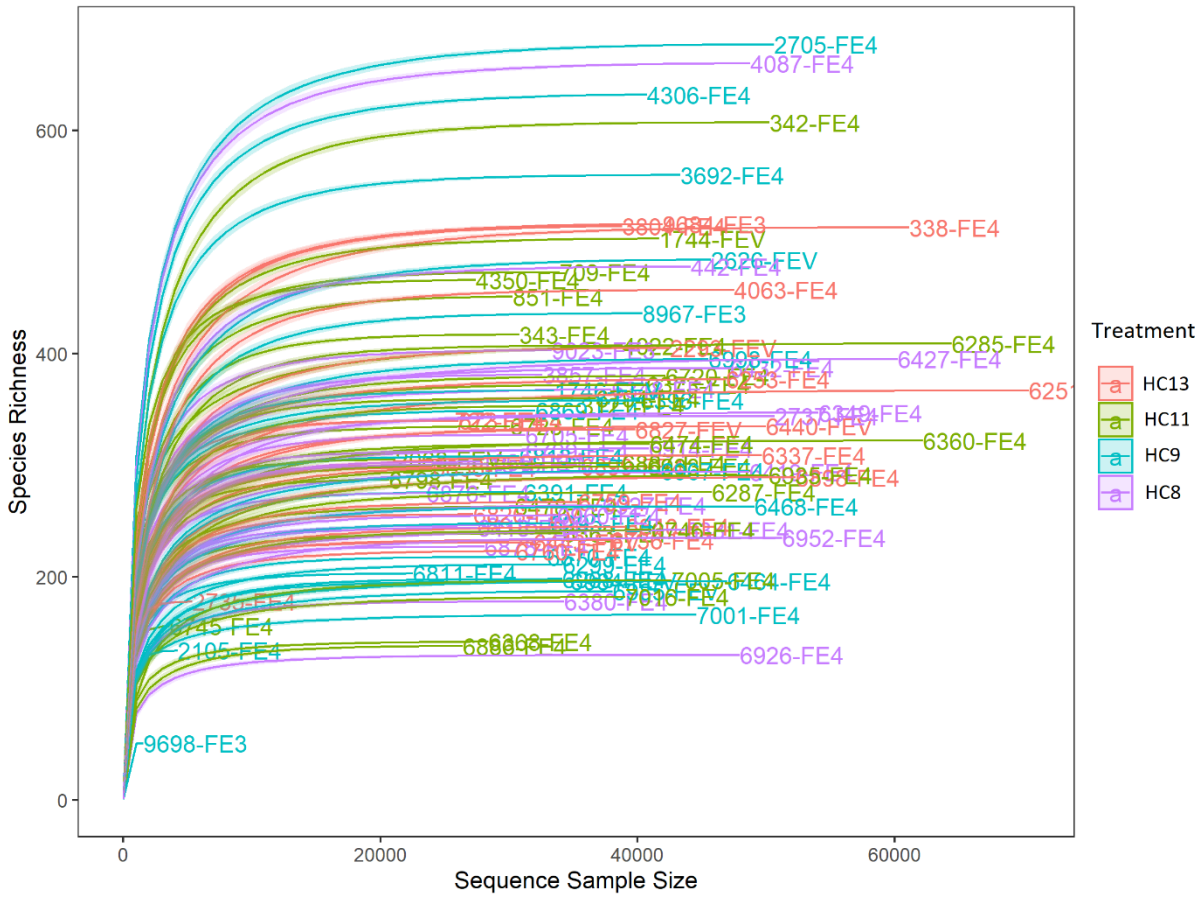
Butyricicoccaceae_UCG-008	33.63	5.667	<0.01
HC8 vs HC13			
Eubacterium	-28.20	6.141	<0.01
Succinivibrio	-27.45	6.166	<0.01
Catenibacterium	-25.83	6.168	<0.01
Fusicatenibacter	-21.76	6.169	0.01
Lachnospiraceae_UCG-002	-21.23	6.144	0.01
Dialister	-19.53	6.155	0.02
Lachnospiraceae_CAG-56	-17.91	4.700	<0.01
Faecalicoccus	-9.27	3.171	0.03
Subdoligranulum	-6.05	2.100	0.03
Faecalibacterium	-5.77	1.778	0.01
Anaerovibrio	7.82	2.452	0.02
Cerasicoccus	10.28	3.141	0.01
Olsenella	10.51	3.526	0.03
Akkermansia	17.03	3.954	<0.01
Solobacterium	17.83	6.152	0.03
Parasutterella	19.73	6.148	0.02
Bifidobacterium	20.05	6.152	0.01
Paludibacteraceae_H1	21.59	6.163	0.01
Lachnospiraceae_XPB1014_group	25.40	6.157	<0.01
Muribaculaceae_CAG-873	30.00	5.670	<0.01
HC9 vs HC11			
Solobacterium	-34.17	5.788	<0.01
Clostridioides	-29.83	5.814	<0.01
Bifidobacterium	-25.16	5.814	<0.01
Epulopiscium	-24.97	5.800	<0.01
Akkermansia	-22.72	3.731	<0.01
Muribaculaceae_CAG-873	-22.16	5.367	<0.01
Butyricicoccaceae_UCG-009	-21.47	2.829	<0.01
Acidaminococcus	-21.32	5.807	<0.01
Olsenella	-17.31	3.284	<0.01
Peptostreptococcus	-13.47	3.714	<0.01
Negativibacillus	-9.17	2.493	<0.01
Actinomyces	-7.74	2.612	0.03
Lachnospiraceae_AC2044_group	-6.33	1.900	0.01
Oscillospiraceae_UCG-003	-6.29	2.178	0.03
Fusobacterium	-4.24	1.301	0.01
Frisingicoccus	15.67	2.828	<0.01
Eubacterium	18.04	5.798	0.02
Erysipelotrichaceae_UCG-009	20.43	5.812	<0.01
Pelistega	27.81	5.819	<0.01
Fibrobacter	31.00	5.792	<0.01
HC9 vs HC13			
Facklamia	-21.56	6.273	0.02
Butyricicoccaceae_UCG-008	-20.62	6.268	0.02
Dialister	-20.39	6.265	0.02

Fusicatenibacter	-20.32	6.277	0.02
Epulopiscium	-19.98	6.261	0.02
Butyricicoccaceae_UCG-009	-16.66	3.079	<0.01
Peptostreptococcus	-15.88	3.988	<0.01
Negativibacillus	-10.46	2.669	<0.01
Faecalibacterium	-5.59	1.814	0.03
Prevotella	3.1	1.036	0.04
Prevotellaceae_NK3B31_group	3.5	1.081	0.02
Succinivibrio	18.27	6.239	0.04
Parasutterella	20.63	6.251	0.02
HC11 vs HC13			
Fibrobacter	-28.22	5.833	<0.01
Butyricicoccaceae_UCG-008	-23.88	5.836	<0.01
Eubacterium	-23.09	5.817	<0.01
Lachnospiraceae_UCG-010	-20.97	5.834	<0.01
Erysipelotrichaceae_UCG-009	-20.92	5.844	<0.01
Dialister	-20.75	5.832	<0.01
Frisingicoccus	-18.59	2.798	<0.01
Erysipelotrichaceae_UCG-006	-12.92	3.384	<0.01
Faecalicoccus	-9.12	3.006	0.02
Faecalibacterium	-5.74	1.699	0.01
Prevotella	2.63	0.965	0.04
Prevotellaceae_NK3B31_group	2.88	1.007	0.03
Alloprevotella	3.47	1	0.01
Fusobacterium	4.42	1.306	0.01
Christensenella	8.3	2.834	0.03
Cerasicoccus	8.35	2.983	0.04
Lachnospiraceae_XPB1014_group	15.93	5.84	0.04
Eggerthellaceae_DNF00809	17.47	5.824	0.02
Akkermansia	17.92	3.746	<0.01
Catenibacterium	19.16	5.833	0.01
Olsenella	19.17	3.322	<0.01
Muribaculaceae_CAG-873	21.65	5.384	<0.01
Parasutterella	21.95	5.828	<0.01
Solobacterium	29.49	5.81	<0.01

[1]log2FC=log2 fold change is the effect size estimate

[2]lfcSE=standard error for the log2 fold change estimate.

[3]*P*-adj=P-value adjusted for multiple comparison using the False Discovery Rate method.



Supplementary Figure 1. Rarefaction curve of sow and piglet faecal microbiota samples by sequencing the V3-V4 region of the 16S rRNA gene on the Illumina MiSeq platform (Illumina Inc., San Diego, Ca, USA). HC13=Sow's lactation diet containing 13% of hemicelluloses; HC11=Sow's lactation diet containing 11% of hemicelluloses; HC9=Sow's lactation diet containing 9% of hemicelluloses; HC8=Sow's lactation diet containing 8% of hemicelluloses

Manuscript 3

Increasing the proportion of insoluble dietary fibres in sow's late gestation and lactation diet reduce the farrowing duration, affects the colostrum composition, and enhance the performance of the piglets.

Increasing the proportion of insoluble dietary fibres in sow's late gestation and lactation diet reduce the farrowing duration, affects the colostrum composition, and enhance the performance of the piglets.

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Abstract

Increasing the level of dietary fibres (DFs) in sow diet can reduce the farrowing duration, improve the litter weight at weaning and enhance colostrum quality. However, the beneficial effects may be due to DF physiochemical properties, in particular the water solubility of DFs. This study aims at evaluating the effects of substituting 5% of a source of soluble DFs (SOL) by a source of insoluble DFs (INS) on sow reproductive performance, colostrum quality and offspring development. The SOL diet contained a commercial product of purified chicory roots containing inulin (Orafti®SIPX, Inulin, Beneo-Orafti SA, Belgium) as source of soluble DFs, while the INS diet contained oat hulls as source of insoluble DFs (INS). A total of 40 sows had access to the late gestation diet from 85 ± 0.6 days (d) of gestation to the farrowing and to the lactation diet from 1 d after birth to the weaning. The SOL and INS diets had a similar total DF level during gestation (32.5 % and 33.1 %, respectively for SOL and INS diets) and lactation (21.9 % and 22.0 %, respectively for SOL and INS diets). Farrowing was visually monitored. Colostrum was collected two hours after the onset of farrowing. Piglet body weight was recorded at birth, 1d of lactation,

at weaning (26 ± 1.1 d of lactation) and then weekly until 6 weeks post-weaning (68 ± 1.1 d of age). Despite to a similar number of stillborn and born alive piglets between the experimental groups, the farrowing duration and the mean birth interval of the litter were shorter ($P < 0.01$) in INS sows than in SOL sows. Colostrum chemical composition and immunoglobulins contents were not affected by the dietary treatments, while the concentration of lactoferrin was greater ($P < 0.01$) in INS group than SOL group. The total VFA concentration tended to be higher ($P = 0.07$), while the proportion of isobutyrate was lower ($P=0.05$) and the proportion of methanoate tended to be lower ($P=0.09$) in the colostrum of INS sows compared to the colostrum of SOL sows. Compared to SOL diet, the INS diet enhanced ($P=0.05$) the piglet growth from birth to 1d of lactation, tended to improve ($P = 0.09$) the growth in the suckling period and improved ($P \leq 0.05$) the post-weaning growth and then throughout the study. Moreover, piglets born from INS sows were 1.2 kg heavier ($P < 0.01$) body weight on average at 6 weeks post-weaning than piglets born from SOL sows. Finally, compared to the SOL diet, the INS diet reduced the incidence of diarrhoea ($P < 0.05$) and days in diarrhoea during the first week post-weaning. In conclusion, the present findings highlighted the importance of oat hulls as source of insoluble DFs in the maternal diet to improve farrowing process, colostrum quality and thereby to increase piglet's body weight at weaning and throughout the post-weaning period.

Introduction

Dietary fibres (DFs) are plant polysaccharides that can resist enzymatic digestion and are partially or fully fermented by microorganisms in the hind part of the small intestine and in the large intestine (Lattimer and Haub, 2010). Their fermentation produces volatile fatty acids (VFAs) such as such as acetate, butyrate, and propionate, that can be used as source of energy from the animal (Bergman, 1990). Moreover, DFs can have beneficial

effects in gestating sows, which may affect piglet development. Previous studies showed that supplementing sows up to 20% of total DFs during gestation reduced farrowing duration, increased the feed intake during lactation, increased the content of fat in colostrum and enhanced the litter weight at weaning (Moturi et al., 2022; Loisel et al., 2013;). However, other studies reported detrimental effects on piglet growth and sow reproductive performance or no effects on colostrum quality (Van der Peet-Schwering et al., 2003; Li et al., 2021; Krogh et al., 2017). These discrepancies may be related to the physiochemical properties of DFs included in sow's diet and in particular their water solubility. Soluble DFs include gum, pectin, and inulin; while insoluble DFs are mainly represented by lignin and cellulose (Chawla et al., 2010). Mainly, the beneficial effects of DFs are depending on their degree of fermentability (Zhang et al., 2018). It is largely believed that soluble DFs are highly fermented, producing more VFAs than insoluble DFs by promoting the growth of specific lactic acid bacteria belonging to *Lactobacillus* and *Bifidobacterium* genera at the expense of pathogenic ones such as *Listeria monocytogenes*, *Clostridium perfringens*, and *Escherichia coli* (Jha and Berrocoso, 2015). However, depending on the source of DFs, soluble DFs might be completely degraded at the end of the small intestine, without reaching the hindgut, where the greatest production of VFAs occurs (Houdijk et al., 2002). Indeed, part of these VFAs might be absorbed, transferred to the mammary glands, and serve as source of energy for colostrum and milk synthesis (Tian et al., 2020). Moreover, it has also been shown that the ratio of insoluble and soluble DFs in the sow diets had an impact both on sow reproductive performance and the growth of the litter (Moturi et al., 2022; Li et al 2019). In this context, Palumbo et al. (2022) observed that increasing the ratio of insoluble to soluble DFs from 3.5 to 5.8 with similar levels of total DFs in sow's lactation diet linearly increased the concentration of total VFAs in milk. Therefore, the aim of this study is to understand the effects of substituting 5% of chicory roots extract by oat hulls in sow's late gestation and lactation diet on sow

reproductive performance, colostrum quality and offspring development. Our hypothesis is that, with similar levels of total DF, replacing chicory roots extract with oat hulls might increase the portion of DFs available for bacterial fermentations in the hindgut, increasing the concentration of VFAs in colostrum without any detrimental effect on the sow and thus improving offspring performance. In this study was chosen to compare inulin, purified form chicory roots, recognized for its high degree of fermentability and its ability to increase the population of lactic acid bacteria and oat hulls a co-product that primarily contain cellulose and lignin, which are recognized for their low degree of fermentability and for their reduced nutritional value (Van Loo et al., 1995; Schmitz et al., 2020)

Material and methods

Diets and experimental design

The experiment was performed on 40 primiparous and multiparous (ranging from 2 to 6 parities) Swiss Large White sows originated from the Agroscope herd from 85 days of gestation until the end of lactation and divided into four farrowing batches. The first two farrowing batches were carried out between January 2021 to March 2021 and the second two farrowing batches from August 2021 to October 2021. Sows were weighed at 78 ± 6.0 (mean ± SD) days (d) of gestation and allocated to one of the two experimental diets according to their BW (240 ± 53.8 kg) and parity (2.6 ± 1.89). From 85 ± 0.6 d of gestation to farrowing, sows had access to the gestation diet and from 1-day post-farrowing to weaning (26 ± 1.1 d of lactation), they were fed with the lactation diet. Dietary treatments consisted of one basal gestation diet and one basal lactation diet, containing either 5% of oat hulls rich in insoluble (INS) DFs or a commercial product of purified chicory roots (Orafti®SIPX, Inulin, Beneo-Orafti SA, Belgium) rich in soluble (SOL) DFs. During gestation, sows had free access to the experimental diets through electronic automatic

feeder stations with individual sow recognition system (Schauer Agrotronic GmbH, Prambachkirchen, Austria). Subsequently, from 106 d of gestation until the end of lactation, the experimental diets were offered three times per day through electronic sow feeders (Schauer Spotmix, Schauer Agrotronic GmbH, Austria). The components and chemical analysis of the experimental diets are reported in Table 1. Feed allowances during gestation were adjusted according to the current Swiss feeding recommendations for pigs to cover sow's requirement based on their BW (Agroscope, 2020), while the lactation diets were offered *ad libitum*. No creep feed was offered to the suckling piglets, and from weaning to 6 weeks post-weaning (68 ± 1.1 d of age), a post-weaning standard starter diet was provided *ad libitum* to the piglets. The post-weaning standard starter diet was mainly composed by 16.9% crude protein, 5.2% fat, 5.1% crude fibre,14 MJ/kg digestible energy and 9.9 g/kg digestible lysine.

Animals and housing

During gestation, sows were housed in group and approximately one week before the expected day of parturition, sows were moved to individual farrowing crates. Each crate consisted of a 5.89 m2 concrete solid floor and a 1.21 m2 concrete slatted floor and were equipped with a heated covered nest area for the piglets. The temperature in each nest area was set at 40°C following birth and decreased daily by 0.5°C to reach a temperature of 32°C. Room temperature was set to 24°C and artificial lights were kept on from 8.00 to 17.00. When the gestation length exceeded 115 and 116 days for primiparous and multiparous sows, respectively, parturition was induced through two intramuscular doses (0.5 ml each) of cloprostenol (0.25 g/ml) (Estrumate, MSD Animal Health GmbH, Luzern, Switzerland) at 24-hour intervals. Following birth, after one day, piglets received an individual ear tag and an iron injection (Feridex® 10%, AMAG Pharmaceuticals, Inc, Waltham, USA). Thereafter, cross-fostering was implemented to balance litters size to an

average of 13 piglets per sow within the same treatment group. In addition, male piglets were also castrated during the first 10 days of life. During lactation, according to the Swiss legislation, sows were provided with moderate quantities of straw bedding. After weaning, the sows returned to the herd of Agroscope while the piglets remained in the farrowing crate until 2 weeks post-weaning. From 3 to 6 weeks post-weaning, the piglets were allocated in new rearing pens of 6 or 7 piglets based on their weaning weights, the maternal diet and the space available in the experimental barn. All the animals had free access to water that was supplied through nipple drinkers or drinking water bowls.

Sow and piglet performance

Each farrowing was visually monitored by at least two experimenters. For each litter, the farrowing duration was defined as the time interval between the birth of the first and last piglet and the birth interval as the length of time elapsed between two consecutive piglets (live or stillborn). The BW of the sow was recorded on day 110 of gestation, after farrowing and weaning. Backfat thickness was measured at farrowing and weaning using a digital ultrasound back-fat indicator (Renco Lean Meter Digital Backfat Indicator, Renco Corporation, Minneapolis, Minnesota, USA) at 10^{th} rib (P2), 6.5 cm from each side of the dorsal midline. The BW and backfat thickness loss during lactation was then calculated as the difference in BW and backfat thickness measurements at farrowing and weaning. Feed intake and refusals of the sows were recorded daily. At birth, the number of born alive piglets and stillborn piglets were recorded. For each piglet, the BW, the crown to rump length and the body circumference were recorded immediately at birth. From these data were also calculated the body mass index, which is the ratio of the birthweight to the squared value of the crown-to-rump length, and the ponderal index, which is the ratio of birthweight to the cubic value of the crown-to-rump length. In addition, piglet BW was also measured on d 1 after birth (24 h after the expulsion of the first piglet), at weaning and

then weekly until 6 weeks post-weaning. The average daily (ADG) and the litter weight were calculated from these data. Colostrum intake was estimated according to the equation proposed by Devillers et al. (2004a). The yield of colostrum for each sow was then calculated as the sum of each piglet individual colostrum intake. The feed provided and refusals were measured daily per pen from the weaning day to 2 weeks post-weaning to determine the daily feed intake per pen. From 3 weeks post-weaning to the end of the experiment, the feed provided was recorded daily, while the refusals were weighed once on the last day of the experiment to assess the total feed intake per pen *(n=39)* during these four weeks. The incidence of diarrhoea was assessed daily based on faeces physical appearance (0: no diarrhea; 1: diarrhea) during the first 2 weeks post-weaning.

Sample collection

During each farrowing batch, feed samples of the gestation and the lactation diets were collected weekly and pooled over the experimental period to determine the chemical composition. From the first two farrowing batches, colostrum samples were collected on 10 sows per treatment two hours after the birth of the first piglet. The nipple and the surrounding area were cleaned with a sponge and soap and rinsed with hot water. The samples were then collected manually from each functional teat. Subsequently, each sample was divided in three aliquots that were placed on ice immediately and then stored at either -20°C or -80°C until further analysis.

Analytical Methods

Feed Analysis

Before analysis, feed samples were ground finely to pass through a 1-mm sieve (Brabender rotary mill; Brabender GmbH & Co. KG, Duisburg, Germany). Dry matter was

determined by heating at 105˚C for 3h followed by incineration at 550°C by using thermogravimetry (prepASH, Precisa Gravimetrics AG, Dietikon, Switzerland) to determine the total ash content (ISO 5984:2002) until a stable mass is achieved. The content of nitrogen was measured using the Dumas method (ISO 16634-1:2008), thereafter the crude protein content was calculated by multiplying the nitrogen content by a coefficient of 6.25. Fat content was extracted with petrol ether after acid hydrolysis (ISO 6492:1999). Calcium and phosphorus contents were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 7300 DV; Perkin-Elmer, Schwerzenbach, Switzerland). Using a fiber analyser, the content of crude fibre was determined gravimetrically (ISO 6865:2000) by an acid and alkaline digestion followed by incineration of residual ashes (Fibretherm Gerhardt FT-12, C. Gerhardt GmbH & Co. KG, Königswinter, Germany). The content of NDF and ADF were also measured using the same fibre analyser (ISO 16472:2006 for NDF and ISO 13906:2008 for ADF). The content of NDF was determined with heat stable amylase and sodium sulphite and expressed without residual ash after incineration at 600°C for 3 h. The contents of soluble, insoluble, and soluble low-molecular-weight DFs were measured according to AOAC Method 2011.25, subsequently the sum of the three aforementioned types of DFs was calculated to determine the total amount of DFs.

Colostrum analysis

Frozen colostrum samples were freeze-dried (Christ DELTA 2-24 LSC, Kühner AG, Birsfelden, Switzerland) for 70 hours to determine the content of dry matter. Nitrogen content was obtained by block-digestion method (ISO 8968-3:2007) and subsequently it was multiplied by a coefficient of 6.38 to calculate the level of crude protein. Lactose was determined by an enzymatic testing analysis with β-galactosidase and galactose dehydrogenase (Enzytec TM Liquid Lactose/D-Galactose Ref. No. E8110, R-Biopharm

AG, Darmstadt, Germany). The total VFA concentration and the proportion of each VFA (ISO 15884:2002;ISO 15885:2002) were obtained by gas-liquid chromatography (Gaschromatograph Series II Agilent 6850, Agilent Technologies 2000, USA and Gaschromatograph Serie Agilent 6890, Agilent Technologies 2000, USA, respectively). The content of fat was measured gravimetrically (ISO 1211:2010). To quantify the concentrations of immunoglobulin A (IgA) and G (IgG) concentrations, two commercially available double antibody sandwich ELISA kits were used (IgG Pig and IgA Pig ELISA Kit, Abnova, Lucerna-Chem AG, Luzern, Switzerland) according to the instructions of the manufacturers. Briefly, using a tube rotator (PTR-60, Grant Instruments, Cambridgeshire, UK), each sample was thawed and rotated for 5 min at room temperature. For IgA determination, a 1/100,000 dilution of samples was made with diluent buffer, while a 1/10,000 dilution was prepared for the analysis of IgG. The IgA concentrations in the standard solutions were 400, 200, 100, 50, 25, 12.5, and 0 ng/ml, while IgG ones were 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 0 ng/ml. The concentrations of IgG in the standard solutions were 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 0 ng/ml while those of IgA were 400, 200, 100, 50, 25, 12.5, and 0 ng/ml. All samples and standards were tested in duplicate. Colostrum and 100 µl of standard solution were added to pre-designated wells. The prepared micro titer plate was placed in an automatic ELISA miniWorkstation (Crocodile, Berthold Technologies GmbH, Bad Wildbad, Germany) and incubated at room temperature for 45 minutes for IgA and 30 minutes for IgG, then a diluted washing buffer was used to wash the wells and then aspirated four times. Then, each well was loaded with 100 µl of either diluted anti-IgA or –IgG conjugated solution. The steps of incubation and washing were repeated as aforementioned, except that the time of for IgA incubation was 15 minutes. Subsequently, 100 µl of chromogen substrate solution (3,3’,5,5’-tetramethylbenzidine and hydrogen peroxide in citric acid buffer at pH 3.3) was added to the plate and incubated in the dark for 10 minutes before adding 100 µl

of 0.3 M of sulfuric acid to terminate the reaction. Finally, the plates were read on a microplate reader (Asys UVM 340, Biochrom, Cambridge, UK) at an absorbance of 450 nm. The results were obtained after appropriate dilution factor correction by using a program MikroWin 2000 (v4.43, Mikrotek Laborsysteme GmbH, Overath, Germany). A competitive ELISA kit specific for pig (Cusabio, Houston, USA) was used to measure lactoferrin content. Samples were diluted 1:1000 (v/v) with the dilution buffer provided and further processed following manufacturer instructions. ELISA plates were processed with a Crocodile 4-in-one assay mini workstation (Berthold GmbH, Bad Wildbad, Germany). Absorbance was read with an Asys UVM340 microplate reader in combination with MikroWin 2000 software (Biochrom Ltd, Cambridge, UK).

Statistical analysis

All data except days in diarrhoea and the percentage of diarrhoea were analysed using the MIXED procedure of SAS (v9.4, SAS Institute Inc., Cary, NC, USA) with. Days in diarrhoea and the percentage of diarrhoea were analysed by ANOVA using the ‘lme’ and the ‘glmmPQL’ function of the nlme package of R Studio (version 4.0.2 for Windows), respectively. When the sow is considered as experimental unit, a linear regression model was fitted, including the dietary treatment, the farrowing batch, the parity and the interaction between the dietary treatment and the parity. For data related to piglet individual performance, the experimental unit was the litter A linear mixed-effects model was fitted to include the treatment, the parity, the farrowing batch and the interaction between the dietary treatment and the parity as fixed effects and the sow nested within treatment × parity × farrowing batch as random effects. The percentage of diarrhoea was analysed using a generalised linear mixed model using Penalized Quasi-Likelihood, including the treatment, the farrowing batch and the day as fixed effects and the piglet as a random factor, while the days in diarrhoea were fitted in a linear regression model

including the treatment and the farrowing batch as fixed effects. All the results are presented as least squares mean ± SEM. Differences with p-values (P) ≤ 0.05 were considered significant and 0.05 < P < 0.10 were considered as a tendency. When an effect was considered significant, the PDIFF (p-values for differences of the LS-means) option with an adjustment for the Tukey–Kramer test was used to differentiate least squares means.

Results

Sow performance

Farrowing duration as well as the mean birth interval was lower (P < 0.01) in INS sows than SOL sows (Table 2). The colostrum yield was greater (P < 0.01) in INS sows than SOL sows, resulting in a 1 kg greater estimated production in INS sows. The sow BW on day 110 of gestation, at farrowing and weaning were not affected by the dietary treatment, showing a similar weight loss during lactation. The sow backfat thickness was similar after farrowing, while at weaning INS sows tended to have a lower (P = 0.08) backfat thickness than SOL sows. Nevertheless, no differences were observed in backfat thickness loss during lactation. Feed intake, as well as crude protein and digestible energy intake were not affected by the dietary treatment either during the gestation or the lactation period. Regarding the litter characteristics, no significant differences between the dietary treatments were observed either at birth or at weaning. Regardless of the dietary treatments, compared to multiparous sows, primiparous sows, had a lower (P < 0.01) BW at 110 d of gestation (230.1 ± 6.90 kg and 314.1 ± 5.33 kg, respectively for primiparous and multiparous sows), at farrowing (P < 0.01; 207.3 ± 7.09 kg and 295.5 ± 5.54 kg, respectively for primiparous and multiparous sows), at weaning (191.8 ± 7.62 kg and 270.6 ± 5.89 kg, respectively for primiparous and multiparous sows) and tended to have a lower

(P=0.06) BW loss during lactation (15.4 ± 3.70 kg and 25.0 ± 2.86 kg, respectively for primiparous and multiparous sows). Similarly, primiparous sows had also a lower (P < 0.01) backfat thickness at farrowing (12.7 ± 0.94 mm and 16.54 ± 0.72 mm, respectively for primiparous and multiparous sows) and at weaning (10.6 ± 1.03 mm and 14.48 ± 0.80 mm, respectively for primiparous and multiparous sows). In addition, since feed allowance during gestation was based on sow BW, primiparous sows had a lower (P < 0.01) daily feed intake (2.8 ± 0.08 kg and 3.4 ± 0.06 kg, respectively for primiparous and multiparous sows), and by that also a lower (P < 0.01) crude protein (396 ± 8.2 g/day and 471 ± 10.6 g/day, respectively for primiparous and multiparous sows) and digestible energy intake (35 ± 1.0 MJ/day and 42 ± 0.7 MJ/day, respectively for primiparous and multiparous sows) than multiparous sows. However, during lactation, no differences (P>0.10) were observed on the aforementioned parameters (data not shown). No significant interactions were found between dietary treatment and the parity (data not shown).

Colostrum composition and VFA profile

The colostrum concentration of lactoferrin was greater (P < 0.01) in INS sows than SOL sows, while no other effects were observed on gross chemical composition or IgA and IgG concentrations. Regarding the VFA profile, the total VFA concentration tended to be greater (P=0.07), while the proportion of isobutyrate was lower (P=0.05) and the proportion of methaonate tended to be lower (P=0.09) in the colostrum produced by INS sows than SOL sows (Table 3). Regardless of the dietary treatments, the parity affected both gross chemical composition and VFA profile. Indeed, DM (27.6 ± 0.92 % and 24.8 ± 0.88 %, respectively for primiparous and multiparous sows) and fat proportion (7.7 ± 0.58 % and 4.2 ± 0.55 %, respectively for primiparous and multiparous sows) as well as the concentration of estimated energy (7.7 ± 0.33 MJ and 4.2 ± 0.31 MJ, respectively for primiparous and multiparous sows) were greater (P < 0.05), while the proportion of

isovalerate (0.050 ± 0.0462 % and 0.333 ± 0.0486 %, respectively for primiparous and multiparous sows) was lower ($P < 0.01$) in the colostrum produced by primiparous than multiparous sows. No significant interaction was found between dietary treatment and the parity (data not shown).

Piglet performance

The maternal diet had no effect on piglet body measurements at birth, such as body circumference, crown-to-rump length, body mass index and ponderal index. Within the first 24 hours post-farrowing, piglets born from INS sows tended to have a 17%-greater ($P=0.06$) estimated colostrum intake than piglets born from SOL sows. The BW was similar at birth, while BW from weaning to 6 weeks post-weaning was greater ($P < 0.05$) in piglet born from INS sows than SOL sows (Figure 1). Before weaning, piglets born from INS sows had a greater ADG from birth to 1 day post-farrowing ($P =0.03$) and tended to have a greater ($P=0.09$) ADG from birth to weaning compared to piglets born from SOL sows. From weaning to 2 weeks post-weaning, piglets born from INS sows had a greater ($P < 0.01$) ADG than piglets born from SOL sows, as well as from weaning to 6 weeks post-weaning. Throughout the experimental period, piglets born from INS sows had a 6 %-greater ($P < 0.01$) ADG than piglets born from SOL sows. After weaning, feed intake, incidence of diarrhoea and days in diarrhoea were also partially influenced by the maternal diet. During the first 4 weeks post-weaning, feed intake of piglets born from INS sows was greater ($P < 0.05$) than piglets born SOL sows, while no differences were observed in the fifth and sixth weeks post-weaning. (Figure 2). Unlike in the second week post-weaning where no differences were observed, in the first week post-weaning, the incidence of diarrhoea and the number of days in diarrhoea were lower ($P < 0.05$) in piglets born from INS sows than in those born from SOL sows (Table 4). Regardless of the maternal diet, piglets born from multiparous sows had a greater ($P < 0.05$) crown-to-rump length (27.4 ±

0.80 cm and 29.33 ± 0.41 cm, respectively for primiparous and multiparous sows), BW at weaning (7.5 ± 0.24 kg and 6.8 ± 0.29 kg, respectively for primiparous and multiparous sows) and ADG (303 ± 5.2 g/day and 278 ± 9.7 g/day, respectively for primiparous and multiparous sows) throughout all the experimental period than piglets born from multiparous sows.

There were several treatment × parity interactions regarding piglet growth performance. Indeed, BW at five (18.0 ± 0.81 kg, 17.6 ± 0.40, 14.7 ± 0.66 and 17.1 ± 0.42, respectively for primiparous INS sows, multiparous INS sows, primiparous SOL sows and multiparous SOL sows) and six weeks post-weaning (22.6 ± 1.07 kg, 22.2 ± 0.50, 18.8 ± 0.82 and 21.9 ± 0.52, respectively for primiparous INS sows, multiparous INS sows, primiparous SOL sows and multiparous SOL sows) was lower ($P < 0.05$) in piglets born from primiparous SOL sows than those born from the three other groups. Similarly, piglets born from primiparous SOL sows had a lower ($P < 0.05$) ADG in the post-weaning period (366 ± 19 g/day, 345 ± 10 g/day, 294 ± 16 g/day and 345 ± 10 g/day, respectively for primiparous INS sows, multiparous INS sows, primiparous SOL sows and multiparous SOL sows) and throughout all the experimental period (303 ± 14 g/day, 306 ± 7 g/day, 252 ± 12 g/day and 300 ± 7 g/day, respectively for primiparous INS sows, multiparous INS sows, primiparous SOL sows and multiparous SOL sows) compared to the piglets born from the three other groups.

Discussion

Taken all together, the results of the present study demonstrated the substitution of DFs from chicory roots by DFs from oat hulls during late gestation and lactation decreased the farrowing duration, improved the growth of the offspring, and reduced the incidence of post-weaning diarrhoea during the first week post-weaning.

Conversely to the results of Li et al. (2020), replacing soluble DFs with insoluble DFs during late gestation had no negative effect on the reproductive performance of sows, particularly on litter birth weight and average daily feed intake during lactation. In fact, the authors observed that replacing 1.6 % inulin with rice bran and soybean hull decreased litter weight at birth and reduced average daily feed intake during lactation. In the present study, sows fed the INS diet during late gestation had also a reduced duration of farrowing due to a shorter average birth interval between piglets. This effect might be explained by the slower fermentability of insoluble than soluble DFs (Houdijk et al., 2002). Indeed, it might be due to the greater proportion of DFs that reached the large intestine, becoming a substrate of fermentation for the microorganisms in the hindgut, producing a greater amount of VFAs. Those VFAs might be used as source energy that helped sows fed INS diet to accelerate the farrowing process compared to the sows fed the SOL diet (Feyera et al., 2018). Recently, a study by Hasan et al. (2019) negatively correlated farrowing duration with colostrum yield. In line with this, the present study also showed that feeding sows with the INS diet enhanced the colostrum yield during the first 24 hours compared to the sows fed the SOL diet. Altogether, these findings showed that the source of DFs is playing an important role regarding farrowing duration and colostrum yield when the level of DFs is the same between the dietary treatments. Quesnel et al. (2012) have reported that a colostrum intake of about 200 g during the first day post-farrowing is the minimum amount able to significantly decrease mortality pre-weaning rate, as well as provide passive immunity and enable slight increase in weight gain. In the present study, piglets were able to ingest more than 250 g of colostrum on average during the first 24 hours after birth that is the amount required to have an optimal ADG both during pre- and post-weaning period (Quesnel et al., 2012). Nevertheless, piglets born from sows fed the INS diet tended to have even a greater colostrum intake compared to those born from sows fed the SOL diet. Therefore, the latter effect might also explain the greater ADG in the first day

post-farrowing observed in INS piglets. Moreover, colostrum intake might also have a long-term effect on the growth performances of the piglets even after weaning (Devillers et al., 2011). In agreement with Devillers (2004b), in the present study, INS piglets, that had a higher colostrum intake after 1d post-farrowing, had also a higher ADG during the post-weaning period and throughout the experimental period, and thereby a higher BW in the post-weaning period. The greater colostrum intake (tendency) may have also improved the ingestion capacity of these piglets (Le Dividich et al., 2005) as piglets born from INS sows had a higher feed intake compared to the piglets born from SOL sows during the first four weeks post-weaning.

Besides the amount of colostrum ingested, colostrum nutrient concentration, especially immunoglobulin contents, plays a role in piglet performance, due to their effects on intestinal health (Hasan et al., 2019). Because IgG in colostrum originates exclusively from the maternal blood, their concentration in colostrum is positively correlated with the IgG concentration in the maternal blood during late gestation (Bourne and Curtis, 1973; Quesnel et al., 2011). Therefore, increasing the concentration of IgG in maternal blood may improve the acquisition of passive immunity by piglets. Immunoglobulins are very sensitive to nutritional changes (Krakowski et al., 2002). However, no effect on immunoglobulin concentration in colostrum was observed in the present study. Similarly, a recent study observed no differences on immunoglobulin concentration when replacing 15% sugar beet pulp (rich in soluble DF) with a lignocellulose mixture (rich in insoluble DF) with a similar level of DFs in gestation (Grześkowiak et al., 2022). Another study showed that increasing the level of DFs in the diet of gestating sows can increase the concentration of immunoglobulins in colostrum (Gao et al., 2022). Therefore, it might be plausible to think that, according to the aforementioned results, DF level has a greater impact on IgG and IgA concentrations than DF sources. As in the present study, the level of DF was similar in sow's gestation diets, no effects were observed on immunoglobulin

concentrations. Finally, the higher concentrations of lactoferrin and VFA (tendency) in the colostrum of INS sows may have improved piglet immunological functions and inhibited the growth of pathogenic bacteria, which in turn have helped them to cope with post-weaning diarrhoea, having a beneficial effect on piglet's gut microbiota during early life. A recent investigation reported that lactoferrin, owing to its bacteriostatic properties, increased the abundance of beneficial bacteria such as *Lactobacillus* and reduce the abundance of pathogenic ones such as *Escherichia-Shigella* and *Veillonella* in the jejunum of suckling piglet (Hu et al., 2019). At the same time, the higher concentration of VFAs in colostrum might have lowered the pH of the small intestine of piglets born from INS sows, helping them also to enhance the population lactic acid bacteria and reducing pathogenic ones such as *Clostridium* or *Salmonella* during suckling period (Prohaszka et al., 1990). Altogether, these latter effects might partially explain the reduced post-weaning diarrhoea in the first week post-weaning and by that the improved growth performance during the first 2 weeks post-weaning and Indeed, it might be possible that a beneficial manipulation of piglet's intestinal microbiota during early life was capable to improve their performance even during post-weaning period in agreement of what has been observed by Luise et al. (2021). However, further investigations are needed to confirm this hypothesis. Regarding the proportion of each VFA, sows fed the SOL diet had a greater proportion of isobutyrate and tended to have a greater proportion of methanoate in colostrum compared to the sows fed the INS diet. Isobutyrate is a branched chain fatty acid that is mainly produced by leucine fermentation. It is well known that increasing the intake of soluble DFs can decrease the crude protein digestibility in the small intestine (Schulze et al., 1994). This effect might be caused by the ability of soluble DFs in forming polysaccharides gel that increases the viscosity of the small intestine, withholding amino acids and peptides from absorption and making them available for fermentation in the large intestine (Mosenthin et al.,1994). Similarly, methanoate might also be derived by a greater intake of soluble DFs,

in agreement with what Pi et al. (2021) observed. Compared to lignocellulose (rich in insoluble DFs), the authors reported that konjac flour (rich in soluble DFs) increased VFA and methanoate concentration during *in vitro* fermentation with faecal inoculum of lactating sow.

In conclusion, the present findings showed that feeding sows during late gestation and lactation with similar levels of DFs, but different proportions of insoluble and soluble DFs can affect the reproductive performance of sows and the colostrum composition as well as the growth and the diarrhoea incidence of the piglets. It would be intriguing for further studies to understand if the beneficial effects observed in the present study could also be driven by the gut microbiota of the sow, which could also exert an effect on the microbiota of the offspring. Moreover, it would be interesting to understand whether a passage of intestinal bacteria from the sow's gut environment occurs through colostrum and/or milk as has already been demonstrated in a in a mice model (Perez et al., 2007). Therefore, additional studies are necessary to identify the aforementioned underlying mechanisms and the optimal ratio of insoluble and soluble DFs for gestating and lactating sows to improve piglet growth performance on long-term.

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Table 1.

Gross chemical composition of sow late gestation and lactation diet.

	Dietary Treatments[1]			
	Gestation		Lactation	
Item	SOL	INS	SOL	INS
Ingredients (g/kg as fed)				
Barley, ground	236	236	-	-
Oat, ground	95	95	-	-
Corn, ground	178	178	-	-
Wheat, ground	-	-	554	554
Rapeseed oil	-	-	20	20
Animal fat RS 65	18	18	23	23
Soybean meal	72	72	127	127
Sunflower pressure meal	133	133	150	150
Dried beet pulp	133	133	26	26
Apple pomace	44	44	-	-
L-Lysine-HCL	0.8	0.8	2.8	2.8
L-Threonine	0.3	0.3	0.7	0.7
Dicalcium phosphate	9.4	9.4	8.5	8.5
Calcium carbonate	2.8	2.8	11	11
Salt	3.2	3.2	4.1	4.1
Celite	20	20	20	20
Vitamin-mineral premix[2]	4	4	4	4
Oat hulls	-	51	-	51
Orafti SIPX (Inulin)	51	-	51	-
Gross chemical composition analysed, % as fed				
Dry Matter	90.7	90.6	90.7	90.6
Crude Protein	13.6	14.3	19.3	19.3
Fat	5.3	5.4	6.8	6.9
Crude Fibre	8.1	9.9	3.7	5.2
Ash	7.0	7.0	7.3	7.2
NDF	19.9	24.4	12.4	15.9
ADF	10.3	12.9	6.3	8.1
Calcium	0.9	0.8	1.0	1.0
Phosphorus	0.5	0.5	0.7	0.7
Total dietary fibres	32.5	33.1	21.9	22.0
Proportion of total dietary fibre types, %				
Low-molecular weight soluble dietary fibres	18.0	4.8	28.8	11.6
Soluble dietary fibres	14.8	14.4	12.8	11.6
Insoluble dietary fibres	67.2	80.8	58.4	76.8
Gross chemical composition calculated (as fed)				
Digestible energy, MJ/kg	12.5	12.2	14.4	14.1
Lysine, %	0.5	0.5	1.0	1.0

[1]Dietary treatments: INS= gestation and lactation diets containing 5% of oat hulls as source of insoluble dietary fibre; SOL= gestation and lactation diets containing 5% inuln (Orafti SIPX, Chicory roots) as source of soluble dietary fibre.

[2]Vitamin-mineral premix supplied per kg of diet: vitamin A, 8000 IU; vitamin D3, 800 IU; vitamin E, 40 mg; menadione, 2 mg; thiamine, 2 mg; riboflavin, 5 mg; biotin, 0.1 mg; niacin, 20 mg; pantothenic acid, 20 mg; iodine (as calcium iodate), 0.55 mg; copper (as copper sulphate), 7 mg; manganese (as manganese oxide), 20 mg; zinc (as zinc oxide), 55 mg; selenium (as sodium selenite), 0.2 mg.

Table 2.

Effect of degree of solubility of dietary fibres include in late gestation and lactation diets on sow reproductive performance

Item	Dietary Treatments[1]		SEM	P-value
	SOL	INS		
Sows				
Number of sows, *n*	20	19		
Farrowing duration, h	8.5	3.3	1.14	<0.01
Mean interval, min	41.0	15.4	5.96	<0.01
Colostrum yield, kg	3.4	4.4	0.25	<0.01
Body weight, kg				
D110*	277.3	266.9	6.04	0.21
Farrowing*	256.8	246.0	6.20	0.20
Weaning*	238.5	223.9	6.67	0.11
Weight loss in lactation, kg†	18.3	22.1	3.23	0.40
Backfat thickness, mm				
Farrowing*	15.5	13.8	0.82	0.13
Weaning*	13.7	11.4	0.90	0.08
Backfat thickness loss in lactation, mm	1.8	2.3	0.51	0.46
Feed intake, kg/day				
Gestation*	3.1	3.1	0.07	0.87
Lactation	6.4	6.6	0.26	0.37
Crude protein intake, g/day				
Gestation*	424	443	9.3	0.12
Lactation	1242	1300	49.5	0.39
Digestible energy intake, MJ/day				
Gestation*	39	38	0.8	0.32
Lactation	93	95	3.7	0.64
Litter				
Number of piglets per litter, *n*				
Total born	14.2	15.0	0.84	0.50
Born alive	12.7	14.0	0.83	0.25
Stillborn	1.5	1.0	0.43	0.42
After cross-fostering	12.4	12.8	0.40	0.52
Weaned	12.3	12.2	0.39	0.80
Litter weight, kg				
Total born	20.1	21.3	0.96	0.36
Born Alive	18.3	20.0	0.99	0.18
At 1 day of lactation	18.5	20.8	1.01	0.10
After cross-fostering	19.1	20.2	0.84	0.33
At weaning	85.1	92.2	3.37	0.12

[1]Dietary treatments: INS= gestation and lactation diets containing 5% of oat hulls as source of insoluble dietary fibre; SOL= gestation and lactation diets containing 5% inuln (Orafti SIPX, Chicory roots) as source of soluble dietary fibre.

*Parity effect: p-value ≤ 0.05; †Parity effect: 0.05 < p-value < 0.10

Table 3.

Effect of degree of solubility of dietary fibres include in late gestation and lactation diets on the nutrient composition, the estimated energy content, the immunoglobulin levels and the total and relative volatile fatty acid content of sow colostrum

	Dietary Treatments[1]			
Item	SOL	INS	SEM	P-value
Gross chemical composition				
Dry Matter, %*	25.9	26.5	0.92	0.66
Total Protein, %	16.2	15.5	0.81	0.53
Fat, %*	5.4	6.5	0.58	0.19
Lactose, %	2.9	2.7	0.25	0.47
Estimated energy, * MJ/kg	6.5	6.7	0.33	0.65
Immunoglobulins, mg/ml				
IgG	65.96	63.89	7.814	0.85
IgA	8.74	8.17	2.050	0.84
Lactoferrin, mg/ml	0.51	0.64	0.016	<0.01
VFA Profile				
Total volatile fatty acids, mmol/kg	10.99	13.03	0.765	0.07
Proportion of individual VFA, %				
Methanoate	3.850	3.382	0.1868	0.09
Acetate	95.087	95.982	0.4347	0.16
Propionate	0.081	0.068	0.0106	0.37
Isobutyrate	0.014	0.005	0.0030	0.05
Butyrate	0.734	0.323	0.2658	0.28
Isovalerate*	0.166	0.218	0.0485	0.45

[1]Dietary treatments: INS= Sow's gestation and lactation diets containing 5% of oat hulls as source of insoluble dietary fibre; SOL= Sow's gestation and lactation diets containing 5% inuln (Orafti SIPX, Chicory roots) as source of soluble dietary fibre

*Parity effect: p-value ≤ 0.05;

Table 4.

Effect of degree of solubility of dietary fibres include in late gestation and lactation diets on piglet performance

	Dietary Treatments[1]			
Item	SOL	INS	SEM	P-value
Body measurements at birth, cm				
Crown-to-rump length*	28.3	28.4	0.62	0.99
Body circumference	24.3	24.6	0.33	0.46
Body mass index, kg/m^2	17.8	18.3	0.83	0.65
Ponderal index, kg/m^3	64.1	65.9	4.07	0.73
Colostrum intake, g	280	345	27.4	0.06
ADG, g/day				
Birth to 1 day post-farrowing	74	115	14.1	0.03
Birth to weaning	205	224	7.3	0.09
Weaning to 2 weeks post-weaning	51	101	14.0	<0.01
Weaning to 6 weeks post-weaning‡	319	356	10.3	<0.01
Birth-6 weeks post-weaning*‡	279	298	7.3	<0.01
Post-weaning diarrhoea,%				
1 week post-weaning	35.7	28.7	2.11	0.02
2 weeks post-weaning	58.0	55.1	1.70	0.20
Days with diarrhoea, days				
1 week post-weaning	2.2	1.7	0.12	<0.01
2 weeks post-weaning	4.5	4.2	0.14	0.14

[1]Dietary treatments: INS= Sow's gestation and lactation diets containing 5% of oat hulls as source of insoluble dietary fibre SOL= Sow's gestation and lactation diets containing 5% inuln (Orafti SIPX, Chicory roots) as source of soluble dietary fibre

*Parity effect: p-value ≤ 0.05; †Parity effect: 0.05 < p-value < 0.10

‡Dietary treatment x parity effect: p-value ≤ 0.0

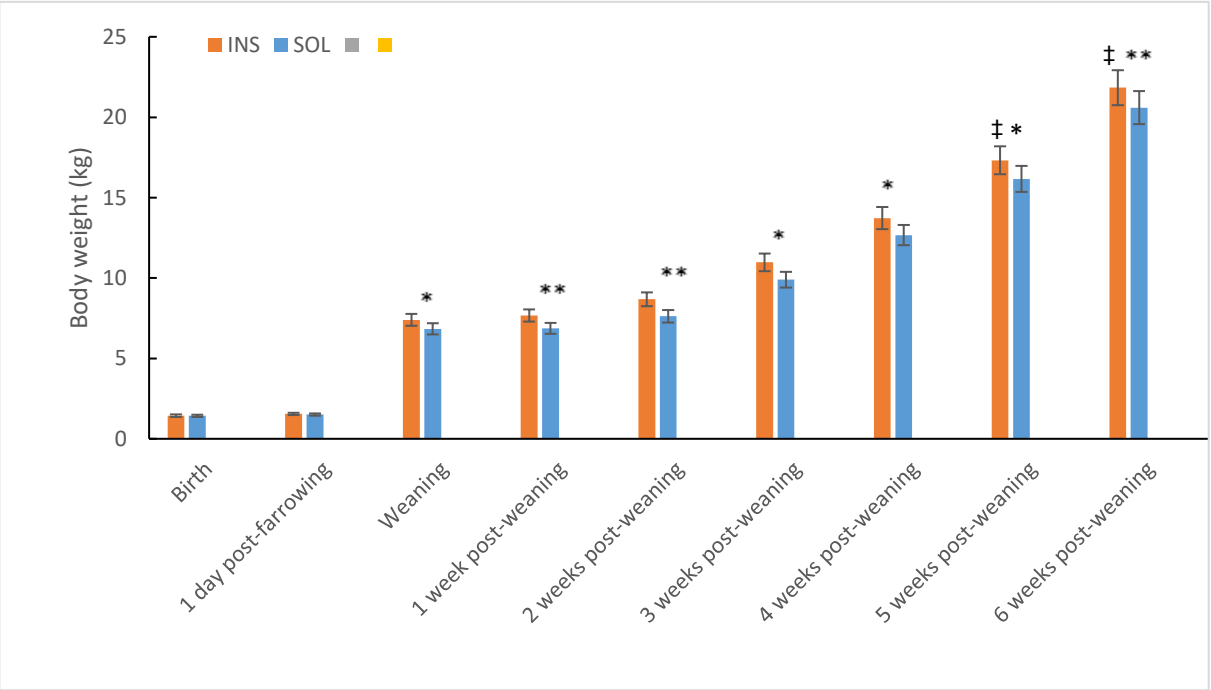

Figure 1 Effect of degree of solubility of dietary fibres included in the late gestation and lactation diets on piglet body weight up to 68 ± 1.1 d of age. Dietary treatments: INS= Sow's gestation and lactation diets containing 5% of oat hulls as source of insoluble dietary fibre; SOL= Sow's gestation and lactation diets containing 5% of Orafti SIPX (Inulin) as source of soluble dietary fibre. Maternal diet effect: * p-value ≤ 0.05; **: p-value < 0.01; Dietary treatment x parity effect: ‡ p-value ≤ 0.05

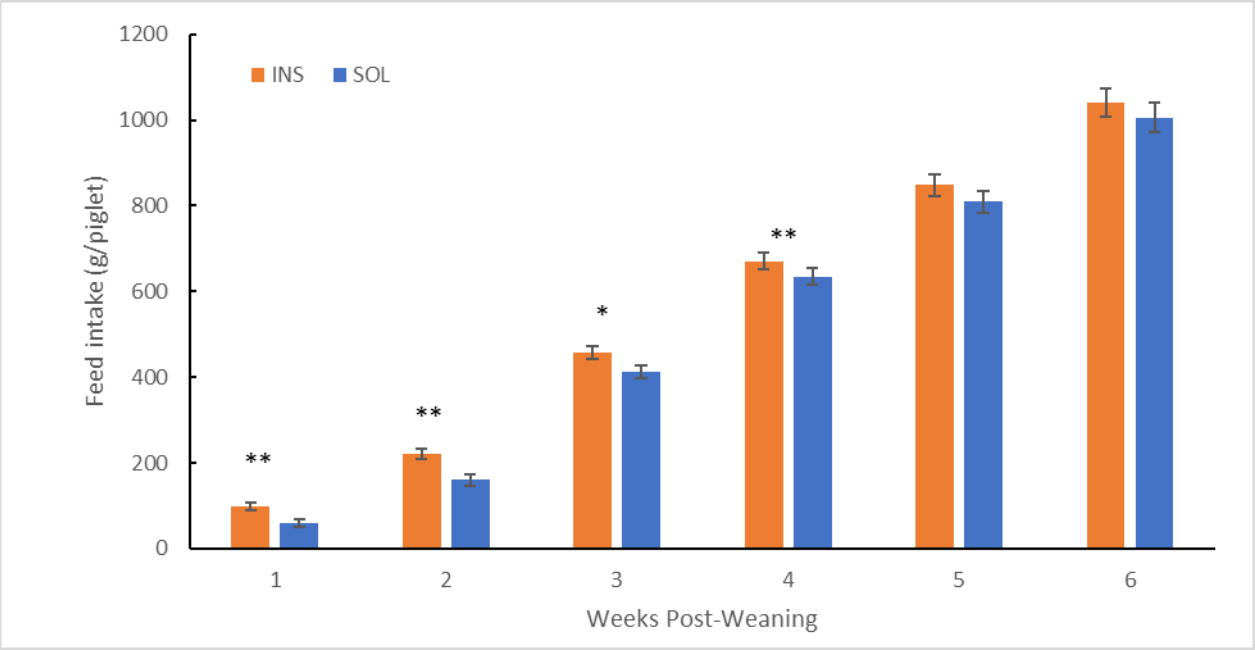

Figure 2 Effect of degree of solubility of dietary fibres included in the late gestation and lactation diets on piglet body weight up to 68 ± 1.1 d of age. Dietary treatments: INS= Sow's gestation and lactation diets containing 5% of oat hulls as source of insoluble dietary fibre; SOL= Sow's gestation and lactation diets containing 5% of Orafti SIPX (Inulin) as source of soluble dietary fibre. Maternal diet effect: * p-value ≤ 0.05 ; ** : p-value < 0.01

Manuscript 4

A workflow to study the microbiota profile of piglet's umbilical cord blood: from sampling to data analysis.

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A workflow to study the microbiota profile of piglet's umbilical cord blood: from sampling to data analysis.

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Abstract

The possibility of pre-birth microbiota colonisation remains controversial in the scientific community. Due to the placenta's characteristics in pigs, the umbilical cord is the sole way for mother-foetus microbial transmission to occur. Studies on this topic have demonstrated conflicting results; some of these discrepancies might be due to differences during sampling, DNA extraction, bioinformatics and data analysis. The aim of this study is to assess a workflow for characterising the umbilical cord blood microbial profile by adjusting for the contaminating sources of bacterial DNA during the extraction procedure. The results show that among 735 amplicon sequence variants (ASVs), 568 ASVs were contaminants, while 165 ASVs were true samples. Using this workflow, we could distinguish the contaminant ASVs introduced during bacterial DNA extraction and amplification. With the results of the present study, however, we cannot confirm the pre-birth bacterial transfer by the umbilical cord blood due to the lack of samples representative of the contaminants in the surrounding sampling environment. Nevertheless, the present study can be used as a reference to address low microbial biomass, particularly with umbilical cord blood.

Keywords: Bacteria, sow, low microbial biomass, 16S rRNA gene, farrowing

Implications

Conducting analyses of low microbial mass, such as with umbilical cord blood, can be challenging because of the presence of contaminants in the surrounding sampling environment and in the laboratory. Such challenges can lead to the misinterpretation of results. The present study proposes a workflow – from sampling methods to DNA extraction, bioinformatics and data analysis – that characterises the bacterial profile of umbilical cord blood samples, taking into account the contaminants found throughout the procedure of bacterial DNA extraction and amplification.

Specifications table

Subject	*Physiology and Functional Biology*
Type of data	Boxplots, R code (version 4.0.2)
How samples were acquired	Umbilical cord blood samples were collected with a 3 ml disposable sterile syringe (Covetrus BV, Cuijk, Netherlands) and a 21 G × $^{5}/_{8}$" (0.8 x 16 mm) sterile injection needle (Kruuse, Marslev, Denmark) and transferred into a 4 ml BD Vacutainer K2E (BD Vacutainer Systems, Plymouth, UK); bacterial DNA was extracted using the HostZero Microbial DNA Kit (Zymo Research, Irvine, CA, USA).
Data format	Raw data, analysed data and output from RStudio (version 4.0.2)
Parameters for sample collection	Data were collected from piglets' umbilical cords at birth; during each farrowing, one piglet per litter of medium visual weight was randomly selected.
Description of data collection	Amplicon sequence variants (ASVs) were generated using DADA2 1.14.0 (Callahan et al., 2016), running on R 4.0.2; for taxonomic assignment, the Silva database, release 138 (Quast et al., 2012, was used as reference. The V3-V4 region of the 16S rRNA gene (~460 bp) was then amplified; amplicons were produced using the universal primers Pro341F: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNBGCASCAG-3' and Pro805R: 5'-

	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACNVGGGTATCTAATCC-3' (Takahashi et al., 2014) using the Platinum Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific, Monza, Italy). Sequencing was performed using the Illumina MiSeq platform 300 x 2bp. The libraries were prepared using the standard protocol for MiSeq Reagent Kit V3 and sequenced on the MiSeq platform (Illumina Inc., San Diego, CA, USA).
Data source location	Institution: Agroscope City/Town/Region: Posieux, Fribourg Canton Country: Switzerland Latitude and longitude (and GPS coordinates, if possible) for collected samples/data: 46°46'07.5"N, 7°06'17.9"E
Data accessibility	Repository name: *Sequence Read Archive (SRA)* Data identification number: *PRJNA880850* The DADA2 phyloseq object and R scripts used for analysis are available at: https://doi.org/10.5281/zenodo.7112497

Introduction

The uterus is generally accepted to represent a sterile environment for the foetus (Tissier, 1900). The first bacterial colonisation of the offspring gastrointestinal tract seems to occur mainly when the newborn is passing through the birth canal, via contact with the vaginal microbiota (Mackie et al., 1999). However, Walker et al. (2017) hypothesised that in humans, the establishment of the intestinal microbiota starts before birth via the passage of bacteria through the placental barrier or through the ingestion of amniotic fluids. Several studies using 16S rRNA sequencing agreed on the hypothesis that a vertical transfer from mother to foetus already occurs before birth. One finding that could confirm this hypothesis is the umbilical cord blood microbial profile reported in human and pig studies (Jiménez et al., 2005; Leblois et al., 2017).

Nonetheless, the existence of pre-birth microbiota is still questioned, as reported by Gschwind et al. (2020), who found that bacterial DNA extracted from the in utero environment might be the result of samples exposed to contaminant bacteria during farrowing. The discrepancies between studies might be the result of different methods of sampling and handling as well as the absence of true negative controls, such as blank samples for estimating the bias that may occur in every step of the analysis. Especially when considering low microbial biomass samples, estimating the amount of contaminant bacteria introduced during each step of the analysis (from DNA extraction to the library preparation) is crucial (Glassing et al., 2016). In this sense, tools that can recognise, remove and classify contaminant amplicon sequence variants (ASVs), such as the “Decontam” R package developed by Davis et al. (2018), have been successfully applied in different studies (Karstens et al., 2019; Claassen-Weitz et al., 2020). The aim of the present work was to define a detailed workflow – from sampling methods to DNA extraction, bioinformatics and data analysis – to allow for investigating pre-birth microbiota transfer through umbilical cord blood analysis.

Material and methods

Animal housing

The experiment was performed on 13 Swiss Large White sows originating from the Agroscope herd, divided into two farrowing batches separated by three weeks. The first farrowing batch was composed of seven animals, while the second farrowing batch was composed of six animals. The sows were individually housed in pens of 7 m^2 and bedded with straw. Room temperature was maintained at 24°C, and artificial lights were kept on from 08:00 h to 17:00 h. Feed was provided three times per day (at 07:00, 12:00 and 17:00), and they had free access to water. Farrowing was induced once the gestation period exceeded 115 and 116 days for primiparous and multiparous sows, respectively. Sows received two intramuscular doses (0.5 ml each) of cloprostenol (0.25 g/ml) (Estrumate, MSD Animal Health GmbH, Luzern, Switzerland) at 24-hour intervals. Two people were present during the whole time of farrowing.

Material preparation and sampling procedures

The sampling methods and materials used in the present study followed the procedure described by Leblois et al. (2017). During each farrowing, one piglet per litter of medium visual weight (total 13 piglets) was randomly selected. The sampling procedure required two people. When a piglet was expulsed, one person held the newborn and took care that it would not touch the floor and then clamped the cord. The umbilical cord surface was then disinfected with 70% ethanol by the other person. After disinfection, the second person collected blood while wearing sterile gloves, using a 21 G × $^5/_8$” (0.8 × 16 mm) sterile injection needle (Kruuse, Marslev, Denmark) and a 3 ml disposable sterile syringe (Covetrus BV, Cuijk, Netherlands). Blood was immediately transferred into a 4 ml BD Vacutainer K2E tube (BD Vacutainer Systems, Plymouth, UK) and mixed thoroughly.

Samples were snap-frozen in liquid nitrogen and stored at −80°C. Sterile gloves were worn during the whole sampling process.

Analytical methods

Bacterial DNA extraction and sequencing

The DNA of the umbilical cord blood samples was extracted in two extraction batches using a HostZero Microbial DNA Kit (Zymo Research, Irvine, CA, USA) following the manufacturer's instructions. The DNA extraction of the 13 samples was carried out on the same day. Seven samples were extracted in the morning from 09:00 to 12:00 h and six samples were extracted in the afternoon from 14:00 to 17:00 h. To determine possible reagent and laboratory contamination, in both the morning and afternoon extraction series, the same DNA procedure as for the umbilical cord blood was used for the nuclease-free water provided with the extraction kit (negative control). The yield and the purity (ratio of absorbance 260/280 and 260/230) of the extracted DNA were measured using a NanoDrop spectrophotometer (Fisher Scientific, Schwerte, Germany) and by a 1% (w/v) agarose gel (1%). The V3-V4 region of the 16S rRNA gene (~460 bp) was amplified, and amplicons were produced using the universal primers Pro341F: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNBGCASCAG-3' and Pro805R: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACNVGGGTATCTAATCC-3' (Takahashi et al., 2014) using Platinum Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific, Monza, Italy). The DNA samples were stored at –20°C until sequencing, which was performed using the Illumina MiSeq platform 300 x 2bp. The libraries were prepared using the standard protocol for MiSeq Reagent Kit V3 and sequenced on the MiSeq platform (Illumina Inc., San Diego, CA, USA).

Bioinformatics analysis

The ASVs were generated using DADA2 1.14.0 (Callahan et al., 2016) running on R 4.0.2; the Silva database, release 138 (Quast et al., 2012), was used as reference for the taxonomic assignment. The Decontam R package was used to identify contaminant ASVs using the prevalence method and a false discovery rate (FDR) threshold of 0.5 (Davis et al., 2018). Because the umbilical cord is generally considered a low microbial biomass (Glassing et al., 2016), and according to the observations of Davis et al. (2018), the *isNotContaminant* function was also implemented to select non-contaminant candidate ASVs. This function identifies non-contaminant sequences based on the prevalence of each ASV in the input feature table across true samples and negative controls (rdrr.io/bioc/decontam/man/isNotContaminant.html). Results about taxonomical composition are expressed as mean ± SD. When a specific taxon is present in only one sample, the calculation of the SD was not possible and was defined as not applicable (NA).

Results

A total of 57,347 reads were attributed to 735 ASVs for the 13 umbilical cord blood samples and the two negative control samples. Before the application of the Decontam R package, 17 phyla (mainly Firmicutes 61 ± 4.1% and Proteobacteria 26 ± 4.5%), 99 families (mainly Lactobacillaceae 44 ± 8.7%, Pseudomonadaceae 16 ± 10.6% and Muribaculaceae 5 ± 3.7%), and 196 genera (mainly *Lactobacillus* 44 ± 8.7%, *Pseudomonas* 16 ± 10.6% and *Muribaculaceae_CAG-873* 5 ± 4.5%) were identified in the umbilical blood cord samples (Figure 1). In the negative control samples, two phyla (mainly Proteobacteria 50 ± NA% and Firmicutes 5 ± NA%), two families (mainly Pseudomonadaceae 50 ± NA% and Lactobacillaceae 5 ± NA%) and two genera (mainly *Pseudomonas* 50 ± NA% and *Lactobacillus* 5 ± NA%) were identified. Using the Decontam

R package, 568 ASVs were identified as contaminants, representing 77% of the previously identified ASVs.

After the application of the Decontam R package, two ASVs were identified as belonging to the Eukaryote kingdom and removed. Only 165 ASVs of the 735 ASVs we detected (22%) were thus identified as characterising the umbilical cord blood samples. The latter resulted in 10 phyla (Firmicutes 63 ± 6.2% and Proteobacteria 27 ± 6.3%, composing the major part), 58 families (Lactobacillaceae 50 ± 10.5%, Pseudomonadaceae 19 ± 13.0% and Muribaculaceae 6 ± 7.8%, composing the major part) and 89 genera (*Lactobacillus* 50 ± 10.5%, *Pseudomonas* 19.4 ± 13.0% and *Muribaculaceae_CAG-873* 6 ± 8.9%, composing the major part); see Figure 2. Table 1 summarises the major bacterial profiles detected in the umbilical cord blood samples at the phylum, family and genus levels; before and after that, microbial sequences were processed using the Decontam R package.

Authors' points of view

The present study showed how most of the ASVs isolated from samples with low microbial biomass (such as umbilical cord blood) were derived from the negative control samples. Almost 78% of the total ASVs were considered contaminants, while only the remainder may be considered a picture of the microbial composition of the true samples. Similar observations were reported by Lauder et al. (2016), who sequenced the 16S rRNA gene on samples of placenta biopsy and on several negative control samples from the sampling environment, the reagents of two different DNA extraction kits and the laboratory where the bacterial DNA extraction was performed. The authors showed that most of the sequences observed in the placenta samples originated from sample contamination during the sampling and extraction procedures. Using Lauder et al.'s (2016) dataset, Davis et al. (2018) implemented the same bioinformatics analysis method as the one described in the

present study. Using the function *IsNotContaminant* of the Decontam R package and a FDR threshold of 0.5, the authors observed that more than 93% of the 810 ASVs were identified as contaminants.

The main limitation of the present study is that, in contrast with Lauder et al.'s (2016) experiment, no negative control samples from the sampling area or from the farm environment were collected during the sampling procedure. Indeed, our results after the application of the Decontam R package showed the presence of bacteria belonging to the *Pseudomonas* genus. These bacteria are generally considered environmental contaminants, especially in water and soil, and can survive to common disinfectants and adapt to a wide range of environments (Kerr et al., 2009). Similarly, a relatively high abundance of bacteria belonging to the genus *Lactobacillus* were isolated in the present samples. These bacteria are mostly found in the intestinal microflora of humans and animals; they are also common in environments contaminated by human and animal faecal material (Kagkli et al., 2007). The samples in the present study were collected in an experimental farm, and despite careful sampling precautions, faecal contamination cannot be excluded. Part of the 165 ASVs considered to not be contaminants from the extraction process thus might be contaminants from the sampling process. Leblois et al.'s (2017) hypothesis about maternal microbial transfer during gestation through the umbilical cord blood thus cannot be fully supported by the present study. In future studies aiming at investigating the microbial profile of the umbilical cord blood, one may consider sampling the vaginal mucus layer and the environmental area of the sow like the floor, the trough and the pen wall, and characterize their microbial profile to use them as negative controls. Vaginal mucosa can be easily sampled before farrowing using a sterile cotton swab (Wang et al., 2017). Similarly, Chen et al. (2018) described a procedure to sample the environmental area by scrubbing the slatted floor of the nursing pen with sterile water. One cannot exclude the possibility, that a small portion of the bacteria population isolated in the

present samples (such as those belonging to the *Lactobacillus* genus) originated from live bacteria or DNA fragments of the intestinal environment and transported through the bloodstream of the sow. In a study performed on mice, Macpherson and Uhr (2004) showed that intestinal dendritic cells could retain and transport a limited number of commensal bacteria for several days, although the mechanism underlying a possible interaction between dendritic cells from the intestinal environment of the mother and a pre-birth transfer of microbiota in the offspring requires further investigation.

In conclusion, the present study has highlighted the complexity of analysing microbial taxonomy data on low microbial biomass, where the concentrations of the contaminants may be higher than that of the target DNA. A few amendments are still needed, however, such as the use of negative control samples from the surroundings of the samplings and the farm environment, such as vaginal and/or environmental swabs (floor, faeces, etc.) of the farm, respectively. Although, the present workflow can be used as reference to deal with low microbial biomass and in particular with the blood from umbilical cord.

Ethics approval

This experiment was conducted in accordance with the Swiss Guidelines for Animal Welfare, and the Swiss Cantonal Committee for Animal Care and Use approved all procedures involving animals (approval number 2020_46_FR).

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Author contributions

FP and FC validated the data and conducted the main statistical and bioinformatics analyses. MG conceived the study, and MG and GB secured substantial funding. FP, FC and MG performed the animal experiments, recorded the data, and collected and processed the umbilical cord blood samples. MG, GB and PT supervised the analyses. FP and FC drafted the manuscript, and MG, GB and PT critically reviewed the manuscript. All authors read and approved the final manuscript.

Declaration of interest

The authors declare they have no conflict of interest relating to the content of this article.

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Table 1

Major bacterial profiles detected in umbilical cord blood samples at the phylum, family and genus levels; before and after that, microbial sequences were processed using Davis et al. (2018)'s Decontam R package

	The Decontam R package			
	Before[1]		After[2]	
	Mean	SD	Mean	SD
Number of				
Phyla	17	-	10	-
Families	99	-	58	-
Genera	196	-	89	-
Relative abundance;%				
Phyla				
Firmicutes	61	4.1	63	6.2
Proteobacteria	26	4.5	27	6.3
Families				
Lactobacillaceae	44	8.7	50	10.5
Pseudomonadaceae	16	10.6	19	13.0
Muribaculaceae	5	3.7	6	7.8
Genera				
Lactobacillus	44	8.7	50	10.5
Pseudomonas	16	10.6	19	13.0
*Muribaculaceae*_CAG-873	5	4.5	6	8.9

[1] The major bacterial profile detected in umbilical cord blood samples of piglets at the phylum, family and genus levels; before that, microbial sequences were processed using Davis et al.'s (2018) Decontam R package.

[2] The major bacterial profile detected in umbilical cord blood samples of piglets at the phylum, family and genus levels; after that, microbial sequences were processed using Davis et al.'s (2018) Decontam R package.

Figure 1

Boxplot illustrating the major bacterial profile detected in each piglet's umbilical cord blood sample at the phylum, family and genus levels; before that, microbial sequences were processed using Davis et al.'s (2018) Decontam R package

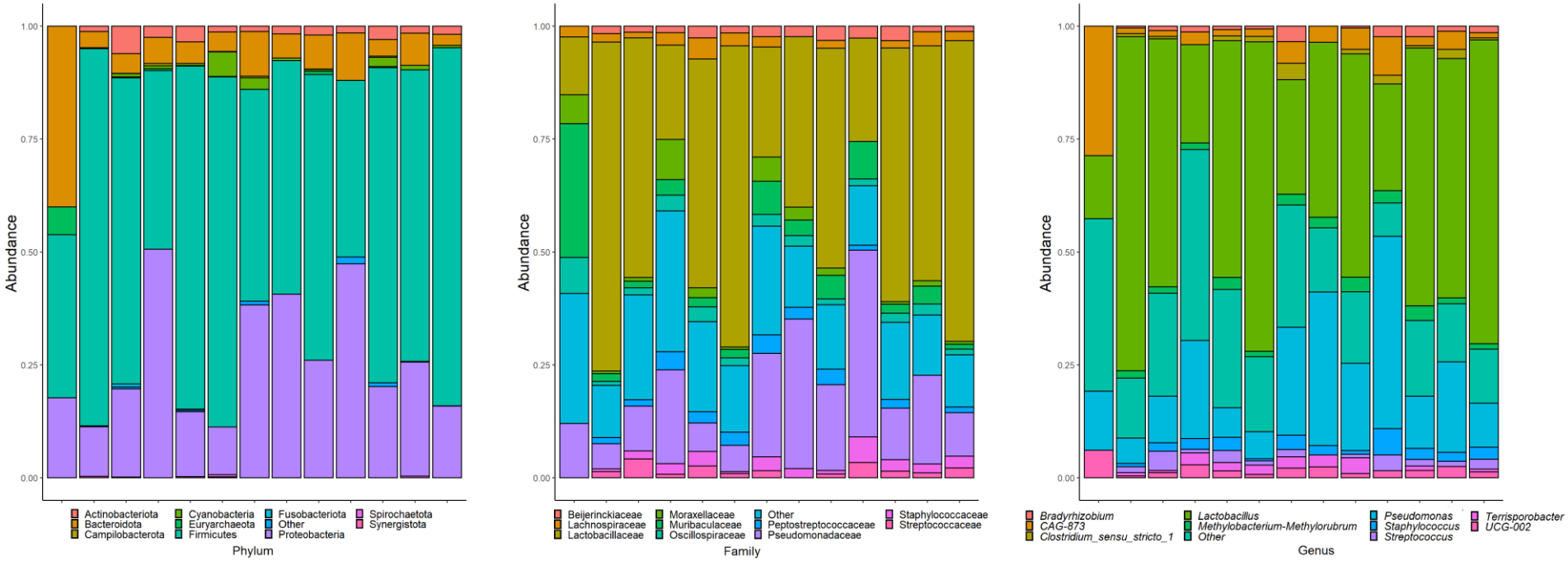
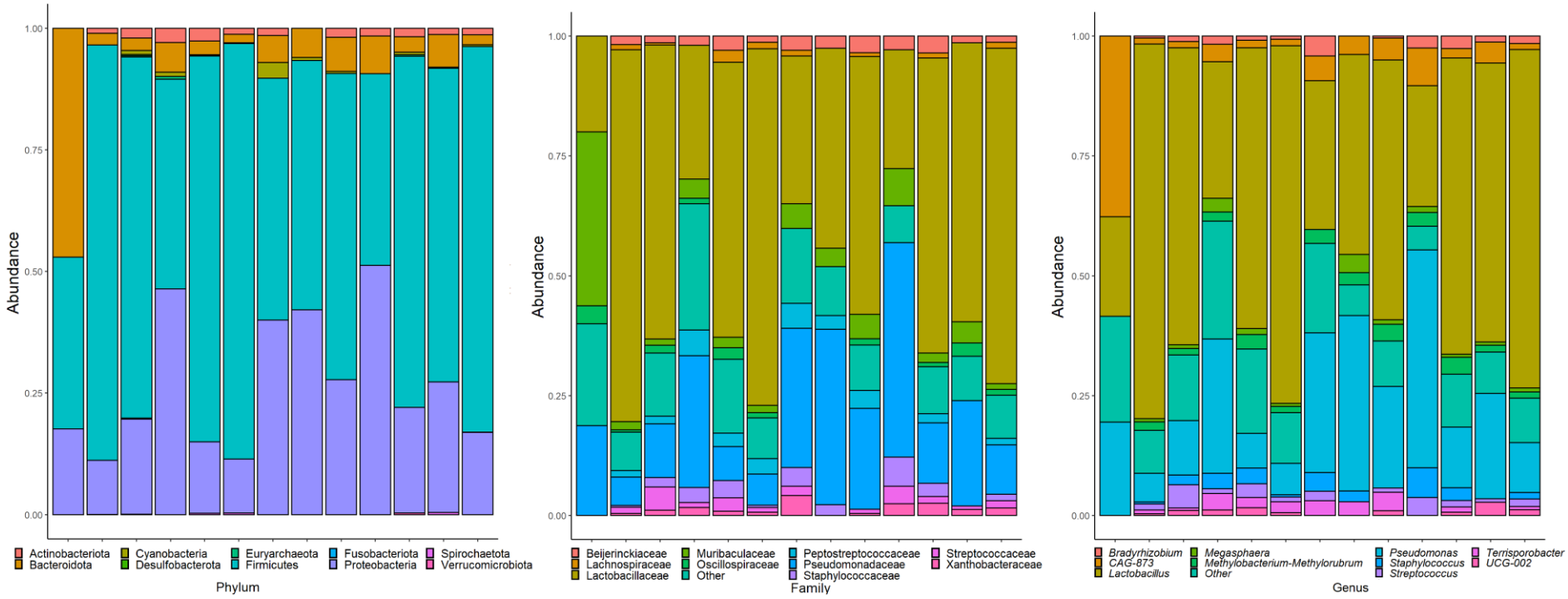


Figure 2

Boxplot illustrating the major non-contaminant bacterial profile detected in each piglet's umbilical cord blood sample at the phylum, family and genus levels; after that, microbial sequences were processed using Davis et al.'s (2018) Decontam R package



General Discussion

The association between sow and piglets plays a key role in gut health and offspring development. In fact, during lactation, piglets are exposed to different bacterial species that progressively colonize their gastrointestinal tract (Van de Vliet et al., 2022). Moreover, nutrients, bacteria and other bioactive compounds present in colostrum and milk might be a resource to enhance piglets' resilience to pathogen infections and limit early post-weaning induced impaired growth (Huting et al., 2021). Shaping the sows' microbiota through the diet seems to be an attractive way to influence piglet's microbiota during early life with long-lasting effects on the animal (Nowland et al., 2021). Furthermore, both gestation and lactation periods seem to positively affect the development of the offspring (Shang et al., 2019). In this context, dietary fibres (DFs) are interesting feed ingredients that can influence the intestinal ecosystem of the sows, and by that modify colostrum and milk composition, and affect the development of the offspring (Tian et al., 2020). However, limited knowledge is currently available on the effect of maternal DFs sources or types in diet on colostrum and milk composition, offspring growth and intestinal microbiota. Moreover, the physiological effects of DFs on sow's gut microbiota might vary widely due to their different physicochemical characteristics (Chen et al., 2013; Mudgil and Barak, 2013).

Therefore, this doctoral dissertation explored the effects of different sources or types of DFs in the maternal diet, regardless of the total DF level. The objective was to improve offspring development and health by exploring the mechanisms underlying the composition of the maternal gut microbiota, the passage of bacteria from the sow to the piglets, and the production of colostrum and milk from the sow.

The results presented in **Manuscript I** demonstrated that decreasing hemicellulose (HC) levels by varying the sources of DFs in sow's lactation diet increased the body weight of low birthweight piglets at two weeks post-weaning and decreased the incidence and the duration of diarrhoea in this category. In addition, the concentrations of copper, threonine, and volatile fatty acids (VFAs), as well as the proportion of butyrate, in milk increased, whereas lactose content linearly decreased with decreased HC in the maternal diet.

Based on the results obtained from the first study, in the **Manuscript II** were reported data referred to a trial designed to evaluate whether the observed beneficial effects on the development of low birthweight piglets are mainly determined by the sow microbiota. Therefore, using the same diets, we compared the effect of different level of HC in the diet (8%; 9%; 11% and 13%) on the sow faecal VFA profile and microbiota. In addition, we evaluated these parameters in fast and slow growing piglets to determine whether they could be affected by maternal diets. Thus, by increasing HC levels in the maternal diet, the proportions of butyrate and valerate increased in sow faeces at day 3 of lactation, while the proportion of propionate decreased at day 17 of lactation. Dietary treatments influenced beta diversity and comparing HC8 with HC9, HC11 and HC13 revealed eleven differing genera in common in sow's faeces: *Turicibacter, Terrisporobacter, Sutterella, Pyramidobacter, Parasutterella, Parabacteroides, Hungatella, Faecalicoccus and Erysipelotrichaceae_UCG-006* were more abundant while *Angelakisella* and *Lachnospiraceae_UCG-008*, while comparing the faecal microbiota of piglets born from sows fed HC8 diet with HC9, HC11 and HC13 diets revealed five different genera in common: *Paludibacteraceae_H1* was more abundant while

Catenibacterium, *Lachnospiraceae_CAG-56*, *Lachnospiraceae_UCG-002* and *Succinivibrio* were less abundant.

Manuscript III evaluated the effects of substituting 5% extract of chicory roots, a source of soluble (SOL) DFs, with oat hulls, a source of insoluble (INS) DFs in sow's diet during the last month of gestation and throughout the lactation period on sow reproductive performance, colostrum quality and offspring development. Our results showed that increasing the proportion of insoluble DFs in sow's diet reduced the farrowing duration, while it increased the production of colostrum. Regarding the quality of colostrum, the INS diet had no effect on chemical composition and immunoglobulin concentrations, but it increased the concentration of total VFA (tendency) and lactoferrin and reduced the proportion of isobutyrate and methanoate (tendency) compared to the SOL diet. Finally, piglets born from sows fed the INS diet tended to have a greater colostrum intake, had a reduced incidence of diarrhoea during the first week post-weaning, a greater average daily gain throughout all the experimental period and by that, they were 1.2 kg heavier compared to the piglets born from sows fed the SOL diet.

Manuscript IV tested the hypothesis of a possible existence of a pre-birth microbiota. Therefore, a workflow was assessed to characterize the microbial profile of umbilical cord blood by adjusting the contaminating sources of bacterial DNA during the extraction procedure. The results showed that nearly 78% of the amplicon sequence variants (ASVs) isolated were contaminants, while the remainder were considered as true ASVs. However, from the latter ASVs, we were unable to distinguish contaminant ASVs introduced during the sampling procedure due to the lack of samples from the surrounding sampling environment. Therefore, prenatal bacterial transfer through umbilical cord blood was impossible to confirm.

Merging the results obtained with the first three manuscripts we can, therefore, assert that besides the level of DF inclusion in sow's diet, different sources of DFs can shape the microbiota of the sow, modify the colostrum and milk composition, and affect the development of the offspring. Moreover, they can also play an important role regarding the reproductive performance of the sow in agreement as what has been observed in several studies (Li et al., 2019; Shang et al., 2019). More specifically, **Manuscript III** reported a significative difference regarding farrowing duration, while **Manuscript I** reported no differences on this parameter. Mainly, the beneficial effect observed in **Manuscript III** has been related to a greater intake of insoluble DFs included from sows fed the INS diet, which might increase the substrate of fermentation for the bacteria in the hindgut, enhancing the production of VFAs and supplying those latter to the sow as source of energy during the farrowing process (Houdijk et al., 2002; Feyera et al., 2018). Similarly, in **Manuscript I** decreasing the level of HCs in sow's lactation diet increased the proportion of insoluble DFs from 70 to 80%. However, with just numerical differences in the intake of this portion of DFs during pre-farrowing period. In addition, the absence of differences could be also related to the timing of the distribution of the experimental diets, as in **Manuscript III** the diets were offered during the last month of gestation, while in **Manuscript I** only 5 days before the expected date of farrowing. In fact, Sappok et al. (2015) pointed out the requirement of more than 19 days to adapt and stabilize the sow's gut microbial environment. In addition, **Manuscript III** reported a greater estimated colostrum yield in sows fed the INS diet compared to the sows fed the SOL diet, while this parameter was not measured in **Manuscript I**

Both manuscripts also reported an effect on the composition of colostrum or milk. The nutritional components of colostrum and milk play a key role in the development

and immunological status of piglets (Hurley, 2015). Therefore, changing the composition of colostrum and milk is crucial to improve the growth of piglets during the lactation period and could have lasting effects on their performance even several weeks after weaning (Theil et al., 2014). Regarding the chemical composition, **Manuscript I** showed that decreasing the HC level in sows' lactation reduced the lactose content of milk, while **Manuscript III** reported no differences on this parameter in colostrum. The lack of difference in the lactose content of colostrum observed in **Manuscript III** could be related to the characteristics of colostrum itself. In fact, lactose is the least variable component of colostrum and varies only within a restricted range (Declerck et al., 2015). Previous studies have shown the difficulty of influencing the lactose content of colostrum (Theil et al., 2014). Lactose synthesis is largely influenced by glucose, which is the main nutrient precursor, and the gestation diet has been shown to have no impact on its synthesis (Boyd, 1995; Quesnel et al., 2009). In contrast, the proportion of lactose in milk is more variable, and its synthesis may be influenced by the gestation diet (Quesnel et al., 2009). Interestingly, as parity effect on colostrum was reported in **Manuscript III,** However, both manuscripts reported different effects on the VFA profile of colostrum and milk. On the one hand, **Manuscript III** reported that increasing the percentage of insoluble DF during gestation tended to increase the total VFA concentration while reducing the percentage of isobutyrate and tending to reduce the percentage of methanoate in colostrum. On the other hand, **Manuscript I** observed that a decrease in HC level during sow lactation increased total VFA concentration and the percentage of butyrate in milk. The effects on milk lactose content and total VFA concentration in colostrum and milk could both be related, as described above, to a higher proportion of insoluble DF and, consequently, a lower proportion of soluble DF in the sow's diet.

In fact, the decreased proportion of soluble DF in the sow's diet might have reduced the nutrients absorbed, including glucose, in the small intestine, and consequently the availability of substrates for bacterial fermentation might have increased in the large intestine, where the greatest production of VFA occurs (Houdijk et al., 2002; Jarrett et al., 2018). Subsequently, these VFAs are absorbed and through the bloodstream should reach the mammary gland, where they can be used as an energy substrate for colostrum and milk production (Tian et al., 2016). Since an effect of parity was observed in Manuscript III for the DM and fat of colostrum and for the proportion of isobutyrate, attention should be paid in future to performing experiments with sows of the same parity to reduce variability between milk components.

However, the mechanism underlying the passage of VFAs from the intestinal environment to colostrum and/or milk needs to be further elucidated in additional studies. Indeed, **Manuscript II** reports the interactions between the level of HCs in the sow's diet and the profile of VFAs and the sow's faecal microbiota. Specifically, with the same dietary treatments as in **Manuscript I**, the proportion of butyrate in faeces showed an opposite trend from the proportion of butyrate in milk. Furthermore, no differences in total faecal VFA concentration were found throughout the experimental period. However, care must be taken when comparing these results, as the time after the meal was not considered in both manuscripts. In fact, the latter parameter might have influenced the products of fermentation in the large intestine and, consequently, also the time interval in which VFAs should be transferred to the mammary glands (Bach Knudsen et al., 2016). However, the level of HCs in the lactating sow's diet was able to influence her faecal microbiota and affect barely the one of the piglets, with no impact on the faecal VFA profile. In the present doctoral thesis with **Manuscript IV**, the possible passage of bacteria from the

mother's intestinal environment to piglets during gestation was also tested. This hypothesis was tested through an experimental workflow according to the results of Leblois et al. (2017), in which the presence of intestinal bacteria was detected in umbilical cord blood samples. As mentioned above, our workflow was unable to distinguish the bacteria from contamination during the sampling procedure. Therefore, although the cord blood samples in the present study were mainly composed of bacteria belonging to the genera *Pseudomonas* and *Lactobacillus*, it is still unclear whether these bacteria came from the maternal intestinal environment or the sampling environment.

Finally, the effects of the maternal diet on piglet growth and intestinal health (incidence and days of diarrhoea) were addressed in **Manuscripts I, II** and **III**. However, the beneficial effects of the maternal diet on piglet development, incidence, and days in diarrhoea were found only in **Manuscripts I** and **III**. Notably, both studies showed a lasting effect of maternal diet throughout the experimental period: in **Manuscript I**, decreasing the level of HC in the sow lactation diet improved the average daily gain of low birth weight piglets up to two weeks after weaning and reduced the incidence and days of diarrhoea during the second week after weaning in this subcategory of piglets, while in **Manuscript III**, increasing the proportion of insoluble DFs in sow gestation and lactation diets improved overall piglet growth from birth to six weeks after weaning and reduced the incidence of diarrhoea during the first week after weaning. Therefore, the observed beneficial effects could be determined by an underlying change that occurred in colostrum or milk (Quesnel et al., 2012). Regardless of the other colostrum or milk nutrients that were affected either by sow's gestation or lactation diet, a higher or a trend toward a higher concentration of total VFAs in milk or colostrum was observed in both **Manuscript I**

and **III**. These VFAs could arrive in the digestive tract of piglets, through colostrum or milk and lower the pH of the piglets' small intestine, increasing the abundance of beneficial bacteria such as those belonging to the Lactobacillaceae family and decreasing the harmful ones such as those belonging to the Entorobacteriaceae family (Diao et al., 2019). Therefore, exerting a beneficial effect on the piglets' microbiota during the first days of life with consequent positive effects in the post-weaning period.

Conclusions

In conclusion, although varying the source of DF in the sow's diet showed a limited effect on the microbiota of piglets during suckling, it shaped the sow's microbiota and influenced the biochemical composition of colostrum and milk. Exploring the different effects of DF sources differing in their fractions may provide evidence that allows us to select the best source or the best ratio of insoluble to soluble DFs to include in sow’s diet to optimize their reproductive performance, to ameliorate the composition of colostrum and milk and by that to improve the growth and the intestinal health of the piglets. Although the maternal transfer of the microbiota during gestation has not been completely proof in the present PhD thesis, it is important to continue to pursue further research in this direction, using more caution regarding sample contamination. Moreover, for further studies it would be interesting to understand whether a passage of intestinal bacteria from the sow's gut environment occurs during lactation through colostrum and/or milk.

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