Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2025:39
Faculty of Veterinary Medicine and Animal Sciences

Early supplementation with probiotics and prebiotics to pigs

Effects on gut microbiota, metabolite production, and behavioural outcomes

Lidija Arapovic

Early supplementation with probiotics and prebiotics to pigs

Effects on gut microbiota, metabolite production, and behavioural outcomes

Lidija Arapovic
Faculty of Veterinary Medicine and Animal Sciences
Department of Applied Animal Science and Welfare
Uppsala

SLU
SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

DOCTORAL THESIS
Uppsala 2025

Acta Universitatis Agriculturae Sueciae
2025:39

Cover: A continuing puzzle (The image was created using a combination of ChatGPT OpenAI and Midjourney)

ISSN 1652-6880
ISBN (print version) 978-91-8046-474-1
ISBN (electronic version) 978-91-8046-524-3
https://doi.org/10.54612/a.6qf8a3d955

Swedish University of Agricultural Sciences, Department of Department of Applied Animal Science and Welfare, Uppsala, Sweden
Print: SLU Grafisk service, Uppsala 2025

Early supplementation with probiotics and prebiotics to pigs-Effects on gut microbiota, metabolite production and behavioural outcomes

Abstract

The early establishment of intestinal microbiota is essential for gut health in pigs. Nutritional interventions that target the microbiota during the first weeks of life can positively influence microbiota development and thereby impact gut health, immune function, growth, as well as behavioural development. This thesis aimed to evaluate the effects of early dietary interventions by supplementing piglets' oat β-glucan or two lactobacilli strains during the suckling period. The oat β-glucan supplement was examined for its effects on gut microbiota development, short chain fatty acid (SCFA) production, growth, gut histology, and behaviour. The supplement with lactobacilli, Limosilactobacillus reuteri, and Lactiplantibacillus plantarum focused on effects on microbiota composition, SCFA, gut histology, and growth performance. The initial study, predominantly showed age-related effects, with limited oat β-glucan supplement specific impacts. However, analysis of microbiota composition and behaviour in the follow-up study revealed significant correlations between certain bacterial taxa and specific behavioural traits. Oat β-glucan negatively affected reversal learning. The supplement with the two lactobacilli did not significantly alter microbiota composition rectal swabs of piglets, but induced localised changes in the ileum and colon, including an increased abundance of Prevotella 2 in ileum mucosa and an increased abundance of Lachnospiraceae_NK4136 in ileum mucosa, colon mucosa, and colon digesta. The supplements did not affect SCFA production, gut histology, or growth performance compared to controls. Taken together, the results revealed certain, albeit limited, impacts of prebiotic and probiotics for gut health in piglets during suckling. Additional studies are needed to clarify these findings and unravel the complexity of their interactions.

Keywords: early interventions, β-glucan, SCFA, behaviour, Limosilactobacillus reuteri and Lactiplantibacillus plantarum, Prevotella 2 and Lachnospiraceae_NK4136

Tidigt tillskott av probiotika och prebiotika till grisar - Effekter på tarm mikrobiota, metabolitproduktion och beteende

Sammanfattning

Den tidiga etableringen av mikrobiotan i tarmen är viktig för grisens tarmhälsa. Dietinterventioner med syfte att påverka mikrobiotan under de fösta veckorna i livet har potential att ge en positiv utveckling på tarmmikrobiotan vilket i sin tur kan påverka tarmhälsa, immunfunktion, tillväxt, men också grisens kognitiva utveckling. Syftet med avhandlingen var att utvärdera effekterna av tidiga kostinterventioner till diande grisar, antingen genom tillskodd av β-glukaner från havre, eller via tillskott av två mjölksyrabakterier under diperioden. I studierna med β-glukanerna studerades effekter på tarmmikrobiota, kortkedjiga fettsyror, tillväxt, tarmhistologi och beteende. I studien med mjölksyrabakterierna användes en blandning av *Limosilactobacillus reuteri* och *Lactiplantibacillus plantarum*, och fokuserade på tarmmikrobiotans sammansättning, korta fettsyror, tarmhistologi och viktutveckling. Resultaten från studierna visade på tydliga åldersrelaterade effekter men supplementet av β-glukanerna visade inte några tydliga effekter. Däremot, sågs en koppling mellan mikrobiotans sammansättning och beteendevariabler med signifikanta samband mellan specifika bakteriegrupper och beteendemönster. Tillskott av β-glukaner hade dessutom en negativ inverkan på grisens förmåga att bryta ett inlärt beteende. Tillskott av mjölksyrabakterierna förändrade inte mikrobiotans sammansättning i rektalsvabbar hos grisar, men orsakade lokala förändringar i tunntarmen (ileum) och tjocktarmen (kolon), med ökad förekomst av bakteriegruppen *Prevotella 2* i tarmslemhinnan i ileum och ökad förekomst av *Lachnospiraceae_NK4136* i tarmslemhinnan i både ileum och kolon, samt i tarminnehållet som fanns i kolon. Tillskotten påverkade dock inte mängd kortkedjiga fettsyror, tarmhistologi eller tillväxt jämfört med kontrollgruppen. Sammantaget visade resultaten från interventionen på begränsade effekter, men uppföljande studier behöver genomföras inom för att klarlägga potentialen med tidiga kostinterventioner.

Nyckelord: Kostintervention, β-glukan, kostkedjuga fettsyror, beteende, *Limosilactobacillus reuteri*, *Lactiplantibacillus plantarum*, *Prevotella 2*, *Lachnospiraceae_NK4136*

Dedication

To my beloved family

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Arapovic L., Huang Y., Manell E., Verbeek E., Keeling L., Sun L., Landberg R., Lundh T., Lindberg JK., Dicksved J. (2023). Age rather than supplementation with oat β-glucan influences development of the intestinal microbiota and SCFA concentrations in suckling piglets. MDPI Animals, 13,1349

II. Arapovic L., Verbeek E., Manell E., Keeling L., Landberg R., Lundh T., Lindberg JK., Dicksved J. Early oral administration of *Limosilactobacillus reuteri* and *Lactiplantibacillus plantarum* and the effects on intestinal microbiota and SCFA profiles in piglets. (manuscript)

III. Verbeek E., Arapovic L., Lindberg JE, Landberg R., Keeling L., Dicksved J. The influence of β-glucan suplementation on behaviour and spatial learning and memory in piglets. (manuscript)

Paper I is reproduced with the permission of the publishers.

The contribution of Lidija Arapovic to the papers included in this thesis was as follows:

I. Planned the experiment with the supervisors, organised and carried out the animal trial, including supplement formulation and administration as well as sample collection (rectal swabs, plasma, intestinal tissues). Conducted parts of the laboratory analysis, mainly the microbiota analysis. Interpretation of result and wrote the first draft of the paper. Responsible for revision of the paper based on feedback from co-authors.

II. Planned the experiment with the supervisors, organised and carried out the animal trial, including supplement formulation and administration as well as sample collection (rectal swabs, plasma, intestinal tissues). Performed the statistical analysis and interpretation of the results. Composed and edited the manuscript in collaboration with supervisors. Wrote the first draft of the paper and handled revision of the paper based on feedback from co-authors.

III. Performed the parts of animal trial, including supplement formulation and administration as well as sample collection (rectal swabs and plasma). Conducted parts of the laboratory analysis (microbiota analysis). Interpreted parts of the results and participated in writing of discussion of the manuscript. Read and commented on the manuscript.

List of tables

List of figures

Abbreviations

ACTH	Adenocorticotropic hormone
ADG	Average daily weight gain
AGP	Antibiotics as growth promoters
AMR	Antimicrobial-resistant bacteria
ANOSIM	Analysis on similarity
BG	A group of animals supplemented with oat β-glucan
CFU	Colony forming units
CNS	Central nervous system
CON	Control group of animals
CRH	Corticotropin-releasing hormone
DF	Dietary fiber
ETEC	Enterotoxigenic Escherichia coli
GABA	Gamma-aminobutyric acid
GBA	Gut-brain axis
GI	Gastrointestinal
GIT	Gastrointestinal tract
GPCRs	G-protein-coupled receptors
HPA	Hypothalamic-pituitary-adrenal
LAB	Lactic acid bacteria
MGBA	Microbiota-gut-brain axis
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PRO	A group of animals supplpemented with a probiotic mixture
PDW	Post-weaning diarrhoea
SCFA	Short chain fatty acids

SLU	Swedish University of Agricultural Sciences
SPF	Specific Patogen Free
ZnO	Zink oxide

1. Introduction

1.1 The pig-weaning challenges and pre-weaning opportunities

The pig (*Sus scrofa*) is an important species within agriculture and the second most terrestrial-farmed animal after chickens, with a global population of almost one billion (Kim *et al.* 2024). Pork meat consumption is estimated to be 36% of the world's meat intake, on average 11.4 kg/capita/year (Mateos *et al.* 2024). Worldwide, pig production represents 42% of total meat production, with an estimated growth of 11% by 2032 (OECD-FAO, Agricultural Outlook 2021). China, the world's largest producer, is responsible for 50% of global pig production, followed by Europe with 21% production (OECD, 2023). Pork production contributes significantly to global agriculture and the overall economy, supporting various jobs and sectors.

Throughout the past decades, the pig industry has changed considerably. Indeed, the focus on fertility and advanced programmes in breeding have resulted in increased litter sizes (Harper *et al.* 2024), which is key for high profitability in pig production. Currently, the average litter size is over 19.8 live-born piglets in Western Europe with Denmark as number one (Hansen *et al*. 2022; Theil *et al.* 2023).

Defining the appropriate weaning age is critical for promoting and ensuring the welfare of nursery pigs. This has been widely researched and plays a crucial role in determining the health and performance of piglets during the post-weaning phase of life. The weaning age has drastically changed in modern pig production; what was once a process that took between 12-18

weeks is now just 3 to 4 weeks in most production systems. In the pig's natural setting, development is gradual; as they grow and mature they begin to explore and consume solid feed, whilst the sow gradually reduces the duration of suckling bouts, encouraging independence. Piglets have a longer period to progressively transition from an exclusively milk-based diet to solid feed. In modern pig farming, weaning is abrupt, with an average standard of 3-4 weeks of age, with little to no gradual transition, sharply contrasting to the natural weaning process. In Sweden, the average weaning age is 5 weeks.

Various arguments have been voiced regarding different weaning ages, focusing on different aspects: gut and immune development and health, growth, feed intake and efficiency, survival and mortality rate, and behavioural adaptation and welfare (Faccin *et al.* 2020; Huting *et al.* 2019; Van Kerschaver *et al.* 2023; Lyderik *et al.* 2023). To this day, no "ideal" age has been determined as the most economically effective for the industry, as numerous factors affect weaning. However, the entire pig industry is unified in recognising that weaning is one of the vital periods in a pig's life. Piglets are confronted with a range of stressors at weaning. The social and physical environments, management, and nutrition are changed. Weaning occurs abruptly, in a couple of days, resulting in tremendous stress, followed by changes in gastrointestinal physiology, microbiology, and immunology, generating a dramatic shift in the microbiota (Li *et al.* 2018). Unbalanced intestinal health will predispose piglets to diseases and the proliferation of pathogenic bacteria such as enterotoxigenic *Escherichia coli* (ETEC) or diarrhoea viruses that can cause post-weaning diarrhoea (PWD). Disease is a significant threat to the swine industry and one of the largest sources of economic loss for pig farmers. Post-weaning diarrhoea can be financially devastating for farmers, notably large-scale pig farms which can be affected by PWD by more than 50% and experience a mortality rate of 15-20 % (Tang *et al.* 2024). Diarrhoea at weaning and adjusting to the new diet after weaning often results in growth depression, villous atrophy in the small intestine, and, therefore, impaired absorption and digestion of feed, resulting in decreased growth performance and profitability of pig meat production (Cornelison *et al.* 2018). Antibiotics have been a means of prevention against subclinical and clinical diseases for decades. However, this drives the development of antimicrobial-resistant bacteria (AMR), which are imminent threats to global animal and human health. Consequently, in January 2006 the European

Union banned the use of antibiotics as growth promoters (AGP) in the livestock industry (Salam *et al.* 2023). Sweden was the first country in the world to ban all use of AGP in 1986 (Wierup, 2001) and was followed by other Scandinavian countries about ten years later (Grave *et al*. 2006). Several other countries have since followed the ban. Replacements for antibiotics for many years among pig farmers were zinc oxide (ZnO) and copper sulphate, added to the feed in high doses in the first two weeks after weaning. Studies have shown that minerals such as copper and zinc can be highly harmful to the environment when used long-term, especially for aquatic species (Jensen *et al.* 2018). Although ZnO was well-known for its antimicrobial properties, it also had a negative impact on public health due to an increase in AMR, resulting in another ban by the European Commission in June 2022 for all veterinary medical products containing ZnO administrated orally to food-producing species (Ortiz *et al.* 2024). Resultingly, the need to find alternatives to prevent infections and increase robustness in production has increased exponentially. Various nutritional strategies have been proposed as an effective alternative known to improve the function and structure of the gastrointestinal tract (GI tract) and promote post-weaning growth to avoid PWD in piglets. A wide range of nutritional strategies have been studied over the years; feed additives such as prebiotics and probiotics seem promising, although they have not been conclusively proven effective. There is some evidence that probiotics and prebiotics may improve the establishment of robust gut microbiota, reduce the growth of harmful pathogenic bacteria, promote beneficial gut microbiota and boost immune responses and protection in the host (Maftei *et al.* 2024). Studies have shown that probiotics may enhance the intestinal barrier function in pigs (Zheng *et al*. 2023b), whilst both probiotics and prebiotics appear to increase the number of desirable microorganisms in the intestine (Liao *et al.* 2017; Kiernan *et al.* 2023).

Although multiple factors predispose piglets to PWD, one of the most significant is an abrupt change in feed at weaning. The abrupt post-weaning transition from highly digestible sow milk rich in bioactive compounds to dry plant-based feed often results in diarrhoea, a period characterised with low feed intake and decreased growth rate (Canibe *et al.* 2022). Despite the presumption that offering creep feed during the suckling period will prepare and train piglets' guts for an abrupt diet change, research results are

conflicting and still unclear (Muro *et al.* 2023). In addition, studies have shown that piglets actively discover and consume creep feed in larger amounts at around three to four weeks of age. (Bruininx *et al.* 2004; Middelkoop *et al*. 2018). Piglets generally only eat small amounts of creep feed before four weeks of age but there is a large individual variation in intake. In countries where the weaning age is 5 weeks of age, such as Sweden, the consumption of creep feed in the final weeks before weaning could be beneficial for piglets. However, in most commercial pig productions worldwide, weaning occurs at around 2 to 4 weeks of age, often before piglets have the opportunity to discover creep feed (Faccin *et al.* 2020). Consumption of creep feed has been correlated to both digestive maturation and lower milk yield of the sow before weaning (Pluske *et al.* 2018; de Greeff *et al.* 2016). A certain proportion of piglets in every litter (ca 30%) do not consume creep feed in significant amounts even at the age of four weeks and thereby possibly miss an opportunity to accelerate the maturation of the gut microbiota and better prepare themselves for weaning (Muro *et al.* 2023). Thus, specific bioactive components found in different prebiotics and probiotics that are given to piglets as a supplement early in life may stimulate the consumption of solid feed pre-weaning. Prebiotics are known to help balance the gut microbiota, improve the digestive process, enhance the breakdown of feed components, make nutrients more available for absorption, alter the appetite-related hormones (such as ghrelin and leptin), and make animals consume more feed. Probiotics can help decrease inflammation, boost the growth of certain bacteria and contribute to maturation of the gut microbiota, and mitigate the abrupt microbial changes at weaning. Enhanced microbial development and gut enzyme production optimise the gut for easier absorption of solid feed earlier in life (Liu *et al.* 2014). Thus, these supplements can offer the bridge that piglets need to successfully overcome the sudden loss of bioactive compounds in sow milk by consuming more solid feed during suckling. This process better reflects how piglets gradually increase their intake of solid feed in nature. A smooth weaning transition could therefore be an alternative to prevent PWD without medication.

1.2 Microbiota-gut brain axis

Weaning, being a highly stressful period for piglets, can, aside from causing gastrointestinal issues, lead to psychological stress and behavioural issues. Recently, greater focus has been placed on the microbiota-gut-brain axis (MGBA) in piglets, a bi-directional biochemical signalling pathway that represents the interaction and communication between the gut and the brain (Figure 1). Studies have discovered that the intestinal microbiota is an important regulator and modulator of the central nervous system (CNS), influencing brain physiology and behaviour (Collins *et al.* 2012; Sherwin *et al.* 2019). Although the exact communication pathways are not yet fully elucidated, they may occur through endocrine, neural, and immune pathways that can be affected and modulated by the gut microbiota and/or gut microbiota metabolites (Liu *et al.* 2015). The hypothalamic-pituitary-adrenal axis (HPA-axis) plays a primary role in regulating stress responses as well as the interaction between the gut microbiota and the brain. During environmental challenges such as weaning, the hypothalamus releases corticotropin-releasing hormone (CRH), leading to the release of adenocorticotropic hormone (ACTH) from the pituitary which consequently leads to the release of cortisol from the adrenal glands (Neuman, 2022). Therefore, alterations in the gut microbiome may influence concentrations of the stress hormones cortisol and catecholamines (adrenaline, noradrenaline, and dopamine) (Rusch *et al.* 2023; Paudel *et al*. 2022).

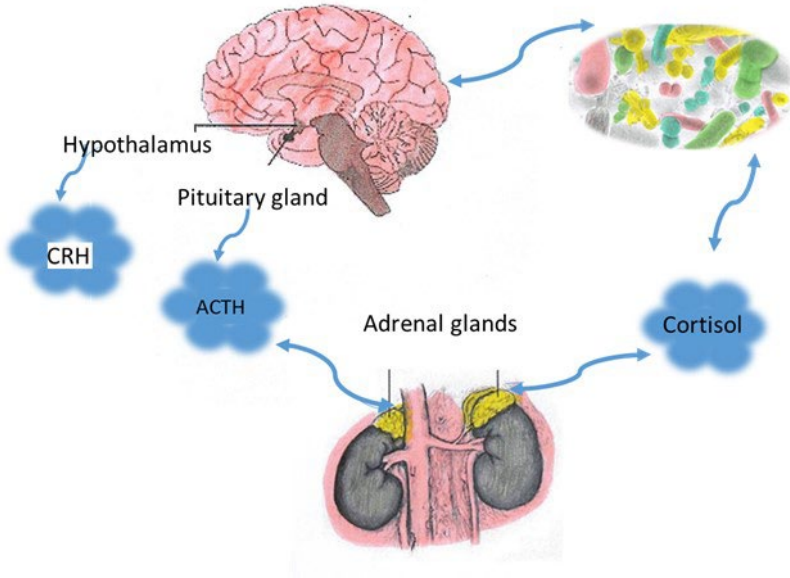

Figure 1. Regulation of stress response by gut-brain axis; corticotropin-releasing hormone (CRH), adenocorticotropic hormone (ACTH).

The study by Sudo *et al.* (2004) is a landmark paper that demonstrates how microbiota early in life can programme the HPA axis and stress responses in mice. The researchers compared germ-free mice (raised in sterile conditions without any gut microbes) to specific pathogen-free mice (with normal gut microbiota) in a short-term restrain test. Germ-free mice displayed significantly higher corticosterone and adrenocorticotropic hormone concentrations when compared to pathogen free mice. However, when *Bifidobacterium infantis* was introduced to germ-free mice early in life, their stress responses normalised and were similar to specific pathogen-free mice responses. If this microbial colonisation occurred later in life, stress responses persisted, suggesting a critical early window for microbial programming of the HPA axis (Sudo *et al.* 2004). Another study conducted on pigs also showed a link between gut microbiota changes, cortisol fluctuations, and stress responses. In this study, weaning was used as a

stressor. When cortisol concentrations were compared between suckling and weaned piglets, cortisol concentrations were elevated in weaned piglets. Concurrently, notable shifts in colonic microbiota were observed, including a higher abundance of the genus *Prevotellaceae*-NK3B31 which was also positively correlated to cortisol concentrations (Jiang *et al.* 2020).
Thus, the bi-directional relationship and communication between the co-development of microbiota and the neuroendocrine system early in life may be critical in preventing intestinal diseases and a range of behavioural and emotional issues that can be problematic later in life. This may be the best period in a piglet's life for desirable modulation of both the gut and the HPA-axis resulting in healthier piglets that are more tolerable to weaning stressors. Pigs possess significant cognitive abilities, and enhancing these through probiotic and prebiotic supplements may decrease stress and anxiety. Increased playing behaviour can make piglets more curious, social, and explorative. Early interventions together with environmental enrichments may be the solution that would positively influence their tolerance to stress and improve their overall well-being (Zebunke *et al.* 2013; Vanheukelom *et al*. 2012). However, studies directly addressing the possibilities of different dietary interventions to enhance cognitive abilities in pigs are limited. From a production perspective, attending to a piglet's cognitive needs early in life can lead to better health and productivity whilst also addressing ethical considerations (Nawroth *et al.* 2019). Nevertheless, only a handful of studies are based on early life pig development of the gut and the brain, evaluating the two-way influence that gut microbiota and behaviour have. (Fleming *et al.* 2017; Verbeek *et al.* 2021; Parois *et al*. 2021; Choudhury *et al.* 2022). Current evidence suggests that both strategies individually may positively contribute to piglets' health and behaviour. Further research is needed to confirm the synergic effects of combining environmental enrichment with prebiotic and probiotic supplementation.

In certain gut-bran axis studies, pigs were used as a translational model for humans (Mudd *et al.* 2017). However, most studies examining the possible effects of different interventions that potentially impact the gut-brain axis were performed on mice or germ-free rodents that have quite a different developmental physiology from humans and pigs (Cryan *et al.* 2019). For example, it has been shown that mice given prebiotics had improved recognition memory (Griffin *et al.* 2022) and social behaviour, as well as

reduced anxiety (Savignac *et al.* 2016), neuroinflammation (Zhang *et al.* 2023), and stress responses (Dinan *et al*. 2012). A potential cognitional improvement was uncovered in pigs supplemented with a combined probiotic and prebiotic known as symbiotic (Parois *et al*. 2021). In humans, prebiotics improved cognitive performance among both young and older individuals (Fekete *et al.* 2024).

There is a scarcity of information regarding the impact that prebiotics have on the GI tract, microbiota, and brain early in life before weaning, necessitating more research to expand the knowledge and find successful strategies that will improve piglet's health and wellbeing.

1.3 Prebiotics and probiotics and gut health

1.3.1 Prebiotics definition and mode of action

In 1995, Gibson and Roberfroid defined a prebiotic as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson *et al.* 1995; Hutkins *et al.* 2016). A recently upgraded and expanded definition has been provided by The International Scientific Association for Probiotic and Prebiotics: "a substrate that is selectively utilized by host microorganisms conferring a health benefit". Not all fermentable carbohydrates are prebiotics, only those with documented microbial and beneficial health effects (Gibson *et al.* 2017). Several dietary fibres (DF) and their by-products have interesting bioactive properties but are not classified as prebiotics (Nunez-Gomez *et al.* 2023). The implementation of DF in pig nutrition has increased due to the potential beneficial effects on gut health, boosted immunity, and increased growth performance, together with favourable economic reasons such as lowering the cost of diet (Molist *et al.* 2014; Atta *et al.* 2017). Fibres are known to enhance the intestinal barrier function and alleviate inflammatory responses (Slavin 2013; Jha *et al.* 2019). Fermentation of fibres in the hindgut produce short chain fatty acids (SCFA) that can be utilised by epithelial cells and play different roles in modulatory processes of GIT (gastrointestinal tract) and immunity. There are two major SCFA signalling pathways involving G-

protein-coupled receptors (GPCRs) and histone deacetylases. SCFA bind to cell membrane receptors that activate different signalling pathways in inflammation, glucose, and lipid metabolism or it directly enters the cell to regulate histone deacetylation. (He *et al.* 2020). Polysaccharide and bioactive DF cereal β-glucan has demonstrated prebiotic properties by passing the GIT undigested and acting as a substrate for microbes, stimulating the growth and activity of beneficial bacteria (Lam *et al.* 2013). A slow fermentation of cereal β-glucan also exhibited prebiotic properties by stimulating beneficial metabolite production of the SCFA (Shoukat *et al.* 2021). Cereal β-glucan is a well-researched novel prebiotic that provides health benefits to both humans and animals (Shoukat *et al.* 2021; Lante *et al.* 2023; Choi *et al.* 2023). β-glucans are located in the cell walls of the endosperm of cereals such as oat, barley, wheat, and rice. Regardless of the source, cereal β-glucan share the same molecular structure whilst other characteristics, such as molar ratio, molar mass, solubility, viscosity, and content of β-glucans differ between different cereal β-glucans (Lante *et al.* 2023). Viscose fibre such as oat bran is a rich source of β-glucan, containing between 3% to 7% of soluble β-glucan (Jurkaninova *et al.* 2024). Nutrient digestibility and its effect on the gut microbiota is directly correlated with increased viscosity of DF modulating the digestive physiology by affecting the passage of digesta (Kim *et al.* 2013; Hung *et al.* 2022). Research on humans and pigs has found positive correlations between decreased passage time of nutrients and more active and diverse gut microbiota composition, leading to an increased SCFA production and reduced proliferation of pathogens in the small intestine (Hung *et al.* 2022; Minnebo *et al.* 2023). β-glucan from oat bran has a high fermentation rate and may increase the production of butyric acid, provide energy to epithelial cells, and increase gut tissue development, immune modulation, and inflammation reduction (Jha *et al.* 2015; Bedford *et al.* 2018; Gonçalves *et al.* 2018). Oat β-glucan has also been reported to promote the growth of beneficial bacteria in the gut and reduce and prevent the binding and colonisation of harmful pathogenic bacteria in the GI tract (Fabiano *et al.* 2023).

1.3.2 Probiotics definition and mode of action

The International Scientific Association for Probiotics and Prebiotics defines probiotics as “live microorganisms that, when administered in adequate

amounts, confer a health benefit on the host" (Hill *et. al.* 2014). Probiotics can be bacteria or yeast. Certain bacteria used as probiotics are spore-forming bacteria such as *Bacillus* spp. and *Clostridium* spp. whereas others are non-spore forming. All probiotic additives must be toxic-free, non-pathogenic, and lack any transmissible antibiotic resistant genes (Vidal *et al.* 2022). Additionally, probiotics must be able to resist gastric acid, bile salts, and enzymes in the pancreas to survive and grow in the GI-tract (Goderska *et al.* 2024). Probiotics have various benefits to the host. These include altering the gastrointestinal microbiota population, producing antimicrobial substances such as organic acids, proteins, and peptides, as well as competing for nutrients and epithelial binding sites with pathogenic bacteria. Probiotics can enhance and maintain intestinal barrier function, thereby improving growth and immunity by reducing the migration of pathogenic bacteria from gut lumen through the intestinal mucosa (Ma *et al.* 2018).

In general, the most common probiotics on the market are lactic acid bacteria (LAB) and bifidobacteria. However, many of the probiotics used within research are not commercially available. Specific strains are developed in labs and tested for particular experiments targeting specific health effects.

LAB strains have good adhesive abilities and can bind to the intestinal mucosa and colonise it rapidly. They excel in competing for food sources and produce organic acids that will inhibit the growth of pathogenic bacteria such as *E. coli* and *Salmonella* (Aleman *et al.* 2024). On the other hand, oral administration of probiotics does not appear to establish permanent residence on the intestinal mucosal surface and they cannot compete with the indigenous microbiota and are excreted from the colon shortly after administration (Han *et al.* 2021). Changes in the gut microbiota with an increased microbial diversity and richness have also been observed with a supplement containing *Lactobacillus* strains as well as an increased number of mucin-producing goblet cells, all resulting in an improved intestinal barrier function and gut health (Huang *et al.* 2022a). Additionally, *Lactobacillus* spp. may support absorption of nutrients by increasing villus height and crypt depth within the mucosa (Shin *et al.* 2019; Zhu *et al.* 2022). One of the dominant species in the GI-tract of pigs is *Limosilactobacillus reuteri* and is therefore often used as a suitable probiotic in pigs (Valeriano *et al.* 2017; Wang *et al.* 2020a). It has a known role in modulation of gut microbiota and elevation of SCFA, protection against stress, and regulation of immune responses (Yu *et al.* 2023). Another beneficial strain known to be

a promotor of intestinal barrier function with a robust repair effect of epithelial cells and the possibility to modulate gut microbiota is *Lactiplantibacillus plantarum*. This species has been studied to effectively prevent diarrhoea via microbiota modulation and regulation of pro-inflammatory cytokines in humans and pigs infected with *E. coli* (Yang *et al.* 2021a; Wang *et al.* 2018).

2. Background

2.1 The digestive tract of pigs

The functional maturation of the digestive system i.e. maturation of enzymes responsible for the breakdown of food in pigs commences before birth and grows exponentially in the first month to meet all of the challenges that occur in that period. In comparison, for humans a similar maturation takes more than 6 months. The GI tract serves as an interface between the pigs' physical body and the outer environment. The gastrointestinal mucosa constitutes a barrier from harmful components and it is the main absorbent of nutrients (carbohydrate, protein, and fat), the source of vital energy. GIT includes five main parts, the mouth, oesophagus, stomach, and small and large intestines (Figure 2). Food is broken down mechanically and enzymatically through the digestion process, to be absorbed by intestinal epithelium, enters the bloodstream and becomes the energy that fuels metabolism and contributes to physical performance. In mechanical digestion, food is broken down into smaller particles that digestive enzymes can further degrade into a form that can be absorbed into the bloodstream. Digestible carbohydrates such as starch and disaccharides are hydrolysed to monosaccharides and absorbed in the small intestine, whilst non-digestible carbohydrates such as oligosaccharides, resistant starch, and non-starch polysaccharides may be digested by microbial enzymes in the large intestine. The remaining undigested food components leave the body via faeces. (Lærke, *et al.* 2012; Navarro *et al.* 2019).

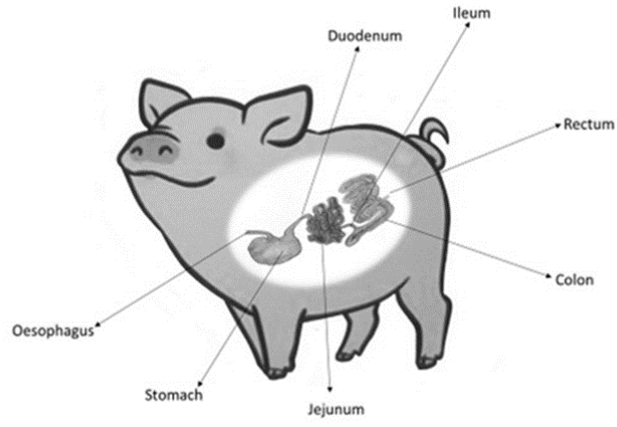

Figure 2. The digestive tract of a piglet.

2.1.1 Maturation of GI-tract in the first weeks of life

Although piglets are capable of suckling within half an hour after birth, the ability to chew and ingest feed requires more complex motoric activity and mouth and dentition maturation takes several weeks. To be able to masticate feed, piglets must develop coordinated action of the tongue and jaw, facial muscles, and the teeth. Research suggests that piglets can consume solid feed after just 3 weeks of age. However, in certain production systems, piglets are weaned from as early as 2 weeks of age (Faccin *et al.* 2020). Before 3 weeks of age piglets are clumsy, drop much of the feed, and seem to experience dental discomfort, inflammation, and bleeding, as the first premolars erupt (Tucker *et al.* 2009; Depalle *et al.* 2023). The stomach capacity and weight increase with age and appears to adapts to a feeding regime around the third week of age. The secretion of hydrochloric acid follows the same pattern. With an increased intake of solid feed at week three of age, stomach acids increase as well as the digestive enzyme capacity (Cranwell *et al.* 1995). The small intestine grows tremendously in the first two weeks of a piglet's life, representing up to 4% of their body weight. Supplemental feeding with milk and creep feed revealed a positive correlation in the maturation of the small

intestine and nutrient intake. This indicates the capacity of the small intestine to adjust early in life as it prepares to accommodate to the increased feed intake (Adeola *et al.* 2006; De Greeff *et al.* 2016; Wang *et al.* 2020b; Chordhury *et al.* 2021a). Within the small intestine, the cells (enterocytes) that line the intestinal wall form the brush border and produce key enzymes for carbohydrate digestion. Secretion of the enzyme lactase, responsible for breakdown of lactose, seems to be highest up to three weeks of age in suckling pigs regardless of solid feed intake. However, after 21 days the access to solid feed decreased the secretion of lactase and increased secretion of the enzyme sucrose that breaks down disaccharides and oligosaccharides to absorbable monosaccharides (Rabb *et al.* 2007; Marion *et al.* 2005). Intestinal barrier maturation was previously considered to be solely dependent on age. Studies found an increase in intestinal permeability to micro-molecules with age. Post-weaning stressors often result in an increase in small intestinal permeability and lower feed intake. However, recent studies hint toward a possibly earlier maturation and better response to post-weaning stress with different nutritional approaches early in life (Upandhaya *et al.* 2021). Development and maturation of the large intestine is moderate and slower in first weeks of a piglet's life, compared to small intestine. The transition at weaning to solid feed seems to stimulate the growth of the large intestine (Pluske *et al.* 2018).

2.1 Gut health in pigs, microbiota, and SCFA

2.1.1 Gut health and its importance in pig production

The health of the gastrointestinal tract, known as "gut health", involves a broad range of factors, both physiological and functional. Although, a clear definition of gut health is still not comprehensibly defined within scientific literature, gut health can be explained as not just the absence of disease but also the prevention or avoidance of disease (Bischoff, 2011). A healthy GIT includes a successful nutrient digestion and absorption, a balanced, active, and diverse microbiota and homeostatic host metabolism. In addition, the epithelial barrier function and immune function should be active but balanced. Moreover, there should be an efficient interaction between all of these components (Kogut *et al.* 2016; Celi *et al.* 2017; Pluske *et al.* 2018). In

modern and intensive pig farming systems, many challenging factors can impair gut health. This is especially important early in piglets' life when GI maturation is dynamic and influenced by both intrinsic and extrinsic factors. It is crucial to establish and maintain beneficial gut microbiota in those initial weeks of life by utilising a certain degree of plasticity of GIT in young animals in response to bioactivities of supplied nutrients. Throughout this period, rapid gut development is occurring, meaning there is increasing enzyme production, microbiota establishment, and immune system maturation. Interventions during this time can have lasting effects on digestive efficiency and overall health. Thus, by leveraging this window of opportunity, it may be possible to decrease antibiotic usage, and increase survival rate and growth performance resulting in higher returns of investment and successful pig farming business. (Bischoff, 2011; Guevarra *et al.* 2019; Szabo *et al.* 2023).

2.1.2 The gut microbiota and its function in health

The gut microbiota can be defined as a community of microorganisms, encompassing bacteria, viruses, fungi, archaea, and protozoa, that adheres to the intestinal mucosa and also appears more loosely in the intestinal lumen. Bacteria is the most abundant and studied component of the gut microbiota and, thus, the only domain of focus in this thesis.

The bacterial part of the microbiota, hereinafter referred to as the microbiota, has a symbiotic relationship with the host. It regulates gut tissue structure, gut health and functionality, produces SCFA, protects against pathogens, and promotes immune homeostasis (Rinninella *et al.* 2018). Nutrients largely define the number, diversity, and composition of bacteria in different locations in the gut. The resident bacteria are more adapted to the environment and have co-evolved with the host, becoming an important and beneficial part in the metabolism of the ingested food that is otherwise indigestible for the host such as milk oligosaccharides and dietary fibre (Broom *et al.* 2018). Regulation of gut mucosa and permeability is also one of the microbial mechanisms which help the host to absorb nutrients. The host interacts with microbes directly via food metabolism or indirectly through the immune system as well as via the microbial metabolites that can

regulate or alter the permeability, thus affecting the nutrient transfer, microbiota metabolism, and immune system. (Aurora, 2015). Certain bacteria produce substances such as SCFA, hydrogen peroxide, and bacteriocins with antimicrobial activity that prevent other harmful bacteria from flourishing in the gut. Bacteria also boast a range of adhesins, and some of these facilitate binding to the intestinal mucosa. Thus, bacteria with these adhesins can persist in the gut mucus layer. Other strains rely on another bacteria's metabolism for metabolic cross-feeding, meaning that different bacterial strains have different metabolic activities, and they help each other to further metabolise the available nutrient source (Dunne 2002; Smith *et al.* 2019). Thus, to obtain a healthy balance of that complex ecosystem, diversity and richness of each microbial community is paramount.

The microbiota composition of a healthy pig is challenging to define as many factors can influence the gut microbiota. At the phylum level, the most dominant bacteria found in the swine gut belongs to the Bacillota (formerly Firmicutes), Bacteroidota (formerly Bacteroidetes), and Pseudomonadota (formerly Proteobacteria) phyla. The pig microbiota is influenced by genetics, breed, nutrition, life stage, level of feed intake, digestion and absorption efficiency, environment, extent of stress, and medication intake along with individual differences (Kubasova *et al.* 2018; Szabo *et al.* 2023). However, studies primarily based on pig fecal microbiota established that the structure of microbial communities differ more at early ages, becoming more unified with age. They can be roughly categorised in these several phases; birth to weaning phase, post-weaning phase and growing, and finishing phase, with an additional gestation phase for female pigs (Dong *et al.* 2023; Pantazi *et al.* 2023; Liao *et al.* 2024).

2.1.3 Gut microbiota pre-weaning

Possible *in utero* microbial colonisation during gestation has not been investigated in pigs and appears to stay contradictory across species. The gut microbiota in new-born piglets, apart from genetics and individual variations, are acquired mostly from the mother, possibly from exposure to the microbiome through the fetal membranes, amniotic fluid, and placenta before birth, traveling through the birth canal during the birth, and after birth from the farrowing environment. The colostrum additionally builds the

piglets gut microbiota and their immune system development via colostrum immunoglobulins. Microbiota during the suckling period is likely affected by the combination of many factors such as the bacterial communities and nutrients from sow milk (Jost *et al.* 2014), social contact with mother and siblings', their nasal and oral cavity and skin, their faeces, and the pen environment. Additional factors that impact microbiota development is if the pig consumes milk replacement and creep or sow feed, medication, genetics, and exposure to stressors (Chen *et al.* 2018; Lim *et al.* 2023; Mahmud *et al.* 2023). The "suckling microbiome" are most abundant during the first weeks of life when piglets suckle sow milk containing the genera *Bacteroides*, *Escherichia*, *Lactobacillus*, *Clostridium*, *Prevotella,* and *Fusobacterium*. (Luo *et al.* 2022). Gastrointestinal infections in the first weeks of a piglet's life cause diarrhoea and gut microbiota disturbances with reduced species richness and diversity. There is also a shift in community composition with decreased abundances of *Prevotellaceae*, *Ruminococcaceae*, *Rikenellaceae,* and *Lachnospiraceae* and an accumulation of *Enterobacteriaceae* such as *Escherichia*-like species (Huang *at al.* 2019; Shrestha *et al.* 2020). Studies have found that healthy pigs that did not develop diarrhoea early in life had a higher abundance of *Bacteroides* within their faeces. Other taxa with a higher abundance found in their faeces are *Prevotellaceae*, *Lachnospiraceae*, and *Lactobacillaceae* (Yang *et al.* 2021; Kong *et al.* 2022). Another study refuted previous reports and did not find any significant statistical differences in microbiota between healthy piglets and those with diarrhoea (Gryaznova *et al.* 2023). The discrepancy between these studies nessitates further research within this area, as numerous factors can affect this complex and diverse ecosystem. What may be a healthy microbiota for one group of pigs may not be for another group, considering various environments and circumstances with different feed sources, housing, hygienic status, genetics, etc. Another reason that may explain differences is the selected research methodology and the small sample size in many studies.

The genus *Prevotella* is often associated with the post-weaning period. *Prevotella* produce enzymes that utilise plant-based complex polysaccharides, thus it is more abundant when the appropriate nutrient source appears (Guevarra *et al.* 2019; Liao *et al.* 2024). Studies have found an opportunity to build-up the gastrointestinal microbiota to a more mature composition in the suckling phase through an earlier introduction to solid feed in the pre-weaning phase. The increased abundance of *Prevotella* could

improve fibre digestibility and feed efficiency (Choudhury *et al.* 2021a and Choudhury *et al.* 2021b). Possible advances in optimising gut health with dietary interventions in the initial weeks of life are becoming increasingly important and recognised.

Even less is known about microbiota in the first weeks of a piglet's life in different intestinal segments. From the available few studies it appears that the microbiota from digesta in the small intestine, ileum, and jejunum differ from that of the colon in the early stages of life. Microbial composition from colon digesta at 4 weeks of age was similar to faecal microbiota with a higher abundance of *Bacteroides*, *Campylobacter*, *Helicobacter*, *Clostridium*, *Prevotella,* and *Streptococcus*, whilst ileal and jejunal digesta consisted of predominantly Proteobacteria, *Prevotella,* and *Lactobacillus*. Additionally, differences were found between digesta and mucosa in same region; *Bacteroides*, *Campylobacter*, *Helicobacter*, *Prevotella,* and *Roseburia*, were found to be higher in mucosa compared to digesta. However, *Lactobacillus*, *Clostridium*, and *Streptococcus* were found to be higher in digesta compared to mucosa. (Mu *et al.* 2017; Adhikari *et al.* 2019; Arnaud *et al.* 2020; Shrestha *et al.* 2020; Gryaznova *et al.* 2022).This may suggest that microbiota in every niche have a different role and function, however, more research and knowledge is needed to make any final conclusions.

2.1.4 Gut microbiota at weaning and early post-weaning stage

In most counties weaning occurs at around 3 to 4 weeks of age, when piglets are still in a development phase and thus vulnerable to stressors that suddenly take place at weaning. Disruption in gut microbial diversity due to the abrupt absence of sow milk as the main energy source and radical diet change can have a negative long-term effect setting the foundation for adult microbiota development. The pre-weaning microbial population, adapted to a nutrient source primarily from sow milk is not well suited to utilise polysaccharides and other plant-based substrates which become the only available nutrient source at weaning. Post-weaning is often followed by a low voluntary intake of feed, reducing the microbial diversity and opening the door for opportunistic pathogens to proliferate, which can lead to diseases such as PWD (St-Pierre *et al.* 2023).

Recently gained knowledge around plasticity of gut microbiota in young piglets and the possibility of early colonisation with beneficial microbial species open the gates for further research focusing on gut microbiota around weaning. Some of the taxa present in faecal samples at weaning are *Prevotella,* the most predominant genera, followed by *Ruminococcaeae*, *Lactobacillus*, *Alloprevotella*, *Bacteroides*, *Echerichia-Shigella,* and *Fusobacterium*. Firmicutes and Bacteroidetes increase successively with age (Chen *et al.* 2017; Cremonesi *et al.* 2022). Diarrhoea at weaning is associated with a dysbiotic microbiota.
Piglets with diarrhoea post-weaning have been shown to have an increased abundance of *Campylobacter* and *E. coli*, *Fusobacteriaceae*, *Prevotella* spp, and *Bacteroides fragilis* whilst the abundance of *Lactobacillus* and *Muribaculaceae*, (previously known as S24-7), *Lachnospiraceae*, and *Strepotococcaceae* were decreased (Gryaznova *et al.* 2023; Zheng *et al.* 2023a). Despite some studies not finding any difference in microbiota between piglets with diarrhoea and healthy piglets pre-weaning (Rydal *et al.* 2023) others found an increased abundance of *Fusobacterium* and decreased abundance of *Enterococcus faecium* and *Bacteroides fragilis* in suckling piglets with diarrhoea (Herman-Bank *et al.* 2015; De-Witte *et al.* 2017; Yang *et al.* 2021a).

2.1.5 Short-chain fatty acids in pigs and its function in health

One of the functions of the gut microbiota that benefits the host is the production of SCFA as a result of microbial metabolism. SCFA are recognised as potential mediators between gut microbiota and gut tissue, and are thus potentially crucial for intestinal immune function. Receptors involved in the regulation and immune response to an inflammation are the GPCRs, specifically GPR43 that is expressed by enteroendocrine L cells and activated by acetate and propionate (Macia *et al.* 2015). SCFA are also involved in several cellular processes such as cell proliferation, cell differentiation, gene expression, and chemotaxis. Moreover, SCFA have been linked to the regulation of several biological functions that maintain intestinal health through preservation of barrier integrity, mucus production, and protection against inflammation and regulation of microbial structure in the gut. The colon has the highest microbial density. Therefore, the highest concentration of SCFA is also located in the proximal colon where they are

utilised by enterocytes or transported across the gut epithelium into the bloodstream. The concentration of SCFA increases with age and is relatively stable during the early growing stages of life.

SCFA predominantly exist in the intestine as acetate, propionate, and butyrate in the approximate ratio of 60:20:20, respectively (den Besten *et al.* 2013). The proportion of these acids depends on catabolism of different SCFA producing bacteria. In pigs, acetic acid is mainly produced by anaerobic bacteria that digest DF, such as *Bacteroides* spp. and several taxa within the Firmicutes phylum. It is absorbed by intestinal epithelial cells and transported via the portal vein to the liver where it is either metabolised or further released to the peripheral venous system. Among the SCFA, acetic acid is the most abundant not just in gut lumen but also in the peripheral circulation. Both animal and human studies have found that acetate has different effects on tissue, and it is involved in lipid metabolism; it maintains energy balance, and affects appetite, and immunity. In the brain, acetate is thought to have an effect on microglial maturation and play a role in the regulation of metabolic homeostasis (den Besten *et al.* 2013). Acetate is also able to pass the blood-brain barrier and can be found in different concentrations in almost every tissue and fluid in the body. Together with butyrate it is impertive for the energy supply in colonocytes as well (Frost *et al.* 2014). Propionate is another organic acid produced from carbohydrate metabolism by bacteria such as *Bacteroides*, *Clostridium,* and *Roseburia*. It benefits the host by inhibiting the growth of harmful bacteria, and is involved in lipid metabolism and gluconeogenesis (the process that generates glucose from non-carbohydrate sources) and has a neuroprotective effect. However, an excessive concentration of propionate has been reported to potentially lead to dementia and Alzheimer's disease (Killingsworth *et al.* 2021) Propionate can be absorbed by epithelial cells and is further transported via the portal vein to the liver where it is absorbed by hepatocytes. After acetate, propionate is the most abundant SCFA found in the portal circulation (Zhang *et al.* 2023b). Butyrate is mainly produced by members of the Firmicutes phylum from *Ruminococcaceae* and *Lachnospiraceae* families, and it is mostly utilised in the intestinal epithelium by colonocytes. It plays an important role in the maintenance of energy supply in colon cells, restores the epithelial barrier, and locally suppresses intestinal inflammatory responses. Butyrate together with propionate regulate division and proliferation of the large intestinal mucosa (Lange *et al.* 2023). In

homeostatic conditions, the colonocyte metabolism consume much oxygen. Consequently, epithelial hypoxia in colon forms an ideal environment for fermentation and beneficial anaerobic microbial communities, whilst in dysbiosis, colon epithelial oxygenation is increased making the environment more suitable for pathogenic facultative anaerobic bacteria. Butyrate is an important substrate that helps colonocytes to maintain homeostasis (Litvak *et al.* 2018). A low concentration of propionate and butyrate is connected to certain bowel diseases such as irritable bowel syndrome (IBS) (Pozuelo *et al.* 2015). The production of propionate and butyrate by the same bacteria is not common. Only a few bacteria such as *Roseburia inulinivorans* and *Coprococcus catus* have the ability to produce both propionic and butyric acid (Louis *et al.* 2014). There is no "perfect" method or site to measure gut SCFA concentration, they all only partially provide information. Most of the SCFA produced in the colon are rapidly absorbed by colonocytes whilst the remaining part is secreted in faeces. It is estimated that only 5% of the produced SCFA are left unabsorbed and excreted in faeces (Sakata, 2019). Some researchers found that the circulating concentration of SCFA best reflects the consumption of dietary fibres (DF) (Mueller *et al.* 2020) whereas others found the faecal concentration to be more accurate (den Besten *et al.* 2013). Interpretations of the findings in both faeces and blood have their limits. The process of DF fermentation and absorption is dynamic and dependent on, among other things, the occurrence of the fermentation, as different parts of the colon may give different results in faeces. Different fibre types ferment in different parts of the colon, slow fermentable in the distal part whilst rapidly fermented DF are fermented in the proximal colon. Gut transit time along the colon is another factor that can affect the fermentation and thus also the concentration of SCFA in faeces (Gill *et al.* 2018).

2.1.6 Short-chain fatty acids and their function in health early in life

Research on microbiota and SCFA and its importance early in life has gained interest but clear knowledge about microbiota development, SCFA production, and metabolism in young animals is still largely unclear.

The solid diet has a more prominent role once the suckling period ends. From the few studies that have been researching SCFA and its effect on pigs early in life a similar pattern was found (Beaumont *et al.* 2021, Nakatani *et al.* 2018; Li *et al.* 2018; Lerch *et al.* 2023).

Both human and pig studies have reported that during breastfeeding/suckling, concentrations of acetate, propionate, and butyrate in circulating blood is lower but increased after or at the end of the lactation period. A possible explanation may be that during intensive development early in life enterocytes, as well as other cell tissue and organs, use organic acids as a source of energy which limits the hepatocytic metabolism. Conversely, at weaning and post-weaning, the concentration of SCFA increase in the circulation due to the involvement of SCFA in cell reinforcement and protection of the gut health. The gut microbial density, composition, and diversity is lower early in life also directly affects the microbial production of SCFA. Ultimately, different intestinal microbes will produce different amounts of SCFAs (van Beets-Schreurs *et al.* 1998; Nakatani *et al.* 2018; Tsukuda *et al.* 2021).

2.2 Gut-brain axis

Over the past decade, researchers have uncovered evidence of the gut microbiota not only being the crucial player in the intestinal ecosystem and digestive health but also playing an important role in shaping the host's cognitive processes, mood, and behaviour. It has become evident that host behaviour can be influenced by the gut microbiota through bidirectional communication with the brain, a pathway commonly referred to as the microbiota-gut-brain axis. Research found a clear connection between normal microbiota and normal brain development and behaviour when comparing mice with intact microbiota and germ free mice (without any microbiota brought up in sterile conditions). Germ free mice were more open to risky behaviour, hyperactivity and anxiety, had social deficits and had greater difficulty learning and remembering how to navigate different obstacles than mice with a normal microbiota (Diaz Heijtz *et al.* 2011; Luczynski *et al.* 2016). The MGBA involves close communication between the central and the enteric nervous systems, the emotional and cognitive centres of the brain which are connected to peripheral intestinal functions (Carabotti *et al.* 2015). A global research trend in the last decade has remarkably advanced our understanding of the importance of gut microbiota in gut-brain axis. Studying the communication mode as well as signalling pathways and the structure is crucial for understanding its role in diseases.

The production of stress hormones is regulated via the neuroendocrine system activating two central axis hypothalamus-pituitary-adrenal axis (HPA) and the sympathetic-adrenal-medullary axis (SAM) (Kanczkowski *et al.* 2017). Later studies suggested one additional axis, the gut-brain axis (GBA) (Carabotti *et al.* 2015; Foster *et al.* 2017). That changed the outlook on the intestinal microbiota and increased interest regarding the role it plays in control of GBA. Further research established that the microbiota plays one of the key roles during environmental challenges and the body's stress responses. The routes of communication between the gut microbiota and the brain include gut hormone signalling, the immune system, vagus nerve, tryptophan metabolism, and microbial metabolites such as SCFA but the mechanisms are not yet fully elucidated (Tremblay *et al.* 2021). It is believed that the gut microbiota contributes to this communication route via hormone-like metabolites such as stress hormones and neurotransmitters that host cells use in systemic responses. The same metabolites have an effect on the proliferation of certain microbes, such as gram-negative pathogens, resulting in gut microbial dysbiosis (Clarke *et al.* 2014; Ahmed *et al.* 2022). It is a bidirectional process of communication between the GIT and the brain. On one side, the brain is sending neural, hormonal, and enzymatic signals to the GIT lumen to regulate secretion, movement, and transmission. On the other, signals are being sent from the GIT to the brain, thereby impacting brain function and regulation. (Ansari *et al.* 2023). The importance of microbiota in regulating the development of the brain and behaviour was prominent in studies on germ-free mice. Maladaptive stress responses were much more common with behaviour involving self-harm, persistent chronic stress with high blood cortisol, aggression, fear, or depression compared to normal mice (Luczynski *et al.* 2016; Foster *et al.* 2017). In pigs, studies found an association between tail biting and microbiota, another abnormal behaviour that has severe welfare and financial consequences in the pig industry due to pain and distress leading to both appetite and weight loss and subsequently more veterinary treatments or even culling and damaged carcasses. (Rabhi *et al.* 2020; Verbeek *et al.* 2021; Kobek-Kjeldagen *et al.* 2022). Other studies linked a disrupted microbiota in pregnant animals to higher aggression levels in their offspring as well as the possibility of intervention during the early development and suppressing the aggression level in young animals (Mikami *et al.* 2023).

2.3 The specific prebiotics and probiotics impact on the gut-brain axis

Data from different studies have indicated beneficial modulation of the brain by different probiotics and prebiotics via the gut-brain axis. The impact of prebiotics and probiotics on the brain seems to be of a dual nature, both directly and by balancing the gut microbiota. The mode of action may be by directly influencing the gut epithelium and signalling via the enteric nerve system and the vagus nerve. It can also be through modification of the gut microbiota leading to a shift in metabolites, for example, SCFA or other metabolites linked to tryptophan metabolism, gamma-aminobutyric acid (GABA), serotonin, and dopamine (Liu *et al.* 2015; Ansari *et al.* 2023).
Dietary fibres may impact brain function via the gut microbiota composition and SCFA. In human studies, a clear relationship between the consumption of DFs and better cognitive performance has been detected (Smith *et al.* 2010; Defeyter *et al.* 2013; Kennedy *et al.* 2020; Hu *et al.* 2022). Similar results were detected in studies on mice (Shi *et al.* 2020; Zhang *et al.* 2023). SCFA produced by the microbiota fermentation of DF appears to have a role in the gut-brain axis by impacting the blood-brain barrier integrity, regulating neurotransmission, and improving brain memory (Silva *et al.* 2020; Hart *et al.* 2024).

Certain lactobacilli may also influence the brain and regulate behaviour (Bravo *et al.* 2011; Casertano *et al.* 2024). Some probiotic strains such as *L. plantarum* and *L. reuteri* have been found capable of decreasing the amount of stress hormones such a cortisol and reducing the levels of pro-inflammatory cytokines, via the gut-brain axis, thereby reducing stress and anxiety in humans (Lew *et al.* 2019; Xie *et al.* 2020). Similar results were reported in studies on pigs (König *et al.* 2024; Verbeek *et al.* 2021). Other studies found a connection between the abundance of different bacteria within the faeces, such as Acidaminococcaceae, Fusobacteriaceae, Porphyromonadaceae, Rikenellaceae , Enterobacteriaceae , *Lactobacillus* and stress-induced behaviour, mental disorders, and cognitive abilities (Jiang *et al.* 2015; Lew *et al.* 2019; König *et al.* 2024).

2.3.1 Microbiota gut-brain axis, interventions early in life

The gut microbiota may play a key role in immune activation, metabolism, brain development, and behaviour via the gut-brain axis early in life, opening up the possibility for better-developed postnatal MGBA. Early life interventions have the prospect of boosting the rapid parallel developmental maturation of the brain at an early age to get ensure established communication paths later in life. This may be essential during periods when different stressors, such as weaning, perturb microbial composition and challenge immune responses. A study on mice found abnormal brain development later in life when young animals experienced stress. Elevated stress caused behavioural abnormalities, anxiety, decreased memory, and increased corticosterone secretion (Ueno *et al.* 2018). Another study on mice showed the possibility of changing and modulating the behaviour early in life when germ-free mice were exposed to the microbiota of conventional mice early in life which reversed the behaviour of germ-free mice via the modulation of gut microbiota (Diaz Heijtz *et al.* 2011).
Research from the last decade has revealed the ability of probiotics to influence central neuronal processes such as neurogenesis, neuro-inflammation, and expression of neuropeptides, as well as neuro-transmission and behaviour. This has opened the possibility to treat various disorders by targeting the gut microbiota at early age (Jena *et al.* 2020). Microbiota interventions during this early stage of development may help ease stress-related behaviours that cause injury and pain in pigs. Along with environmental improvements more adequately meet piglets' needs, microbiota modulations can contribute to lowering overall stress levels. The potential of probiotic supplementation with different *Lactobacillus* strains has been detected in different studies in young humans, mice, and pigs. The impact on the brain and behaviour has been observed during the first weeks (or months in humans) of life whilst the gut microbes were improving barrier function and stress induced inflammation demonstrating the bidirectional function of the MGBA (Verbeek *et al.* 2021; Zhou *et al.* 2022a; Sabit *et al.* 2023; Ansari *et al.* 2023; Mikami *et al.* 2023). At present, most research on MGBA has been performed on rodent animal models with a different developmental trajectory than pigs. Pigs are also a more suitable animal model for humans due to their more comparable anatomy and physiology (Walters *et al.* 2013).

Gaining a better understanding of the MGBA and its pathways may be another tool to tackle challenges within pigs' production that impact animal welfare and health.

3. Aim and Objectives

Dietary interventions early in a piglet's life became a focus of research interest following the issues related to overuse of antibiotics, the ban of antibiotics as growth promotors, and the ban for all veterinary medical products containing zinc oxide. Certain feed supplements have the potential to improve gut health and stimulate early development of piglets' microbiota. This can lead to a richer, diverse, and mature microbiota that is sufficiently prepared for utilisation of a post-weaning diet and stronger and more resilient piglets. Thus, different early dietary interventions may be crucial in preventing diseases such as PWD, one of the major economic and welfare problems that affects pig production.

The overall aim of this thesis was to evaluate the possible effects of early nutritional interventions in piglets during the suckling period and its impact on the development of microbiota and short-chain fatty acids, along with the role it plays in the microbiota-gut-brain axis.

Specific objectives of the work in Papers I-III were to:

- Determine the effects of β-glucan supplementation during the suckling period on microbiota development and SCFA production (Paper I)
- Evaluate the effects of a mixture of two lactobacilli, *L. reuteri* and *L. plantarum,* orally supplemented to piglets during the suckling period on gut microbiota and SCFA production at the end of suckling period and shortly after weaning (Paper II)
- Investigate the association between the microbiota and SCFA and how these link to pig behaviour i.e. the microbiota-gut-brain axis in piglets supplemented with β-glucan during the suckling period (Paper III)

4. Material and methods

Detailed information on the materials and methods applied in Papers I-III can be found in the individual papers. All animals were housed at the Swedish Livestock Research Centre at Lövsta in Uppsala, Sweden.

4.1 Experimental design

The studies reported in Papers I-III were all performed with piglets supplemented with β-glucan or *L. reuteri* and *L. plantarum* and control animals.
Throughout the studies,blood and rectal swab samples were obtained together with different behavioural observations and tests. Pre-post-mortem sampling of blood and intestinal content was performed for selected animals from both the treated and control groups. An overview of the study design is illustrated in figures 3 and 4. The behaviours registered in the study are summarised in Table 1.

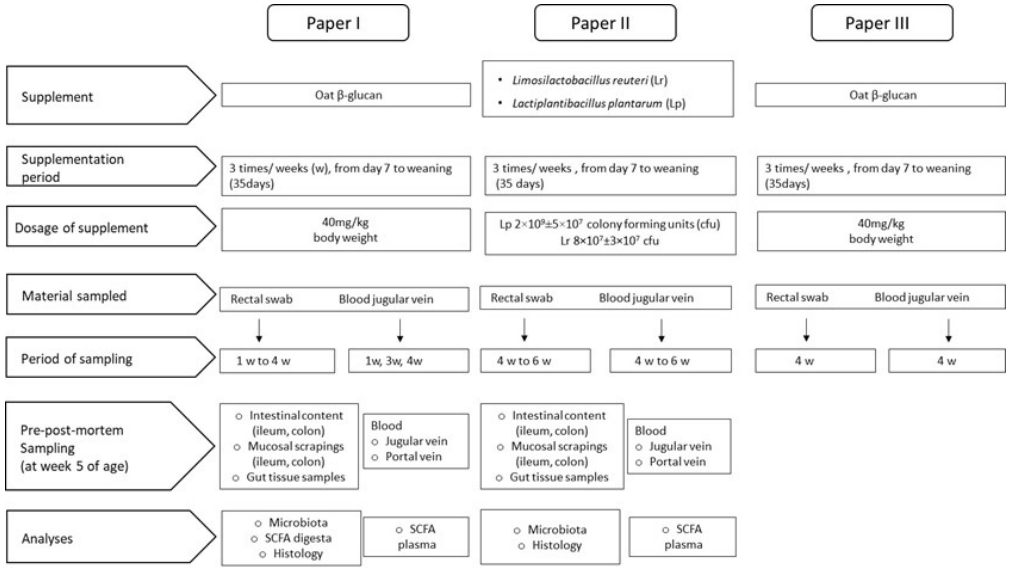

Figure 3. The schematic diagram of the experimental design in Papers I-III

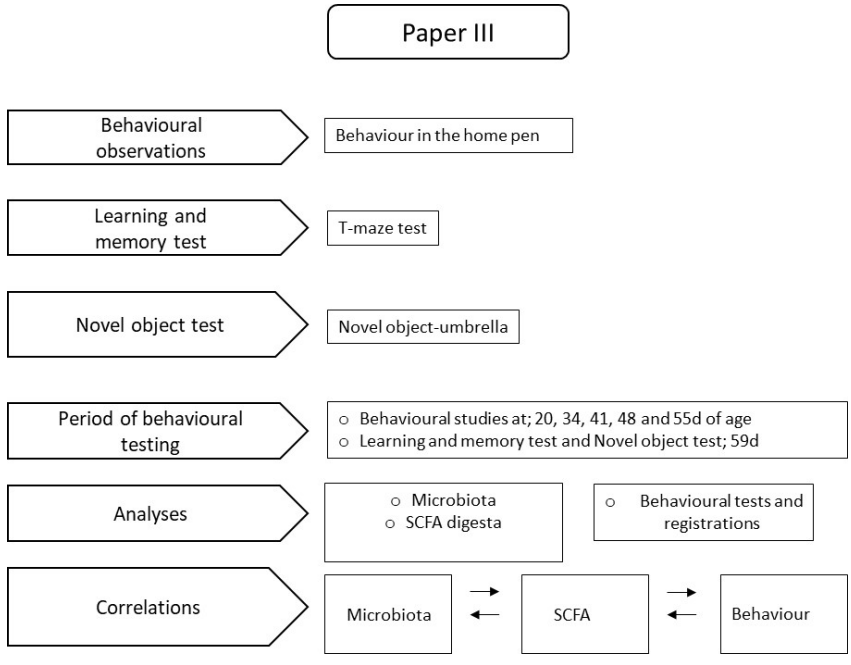

Figure 4. The schematic diagram of the experimental design of the behavioural part of the study in Paper III

Table 1. Ethogram used for scan sampling outside the home pen.

Behaviour	**Definition**
Eating concentrates	Pig's mouth inside the feeder
Exploring	Snout movements along the floor and into the substrate, often making chewing movements
Affiliate behaviours	Sum of touch, play, and social play
Agonistic behaviours	Sum of pushing, head knocks, levering, parallel pressing, fighting, tail-related behaviour, ear biting, other biting, belly nosing
Nursing behaviour	Sum of suckling and teat directed behaviour
Locomotion	Sum of running and walking
Standing	All four hooves are on the pen floor, no movement

4.2 The piglets included in experiments

The piglets used in all experiments (Papers I-III) were of Hampshire x Yorkshire breed. A total number of 50 animals (Paper I) from five litters, 70 (Paper II) from seven litters, and 60 (Paper III) from six litters, respectively, were included in the experiments. In Papers I and III, the same 50 animals were included in both experiments; however, in Paper III an additional litter of 10 piglets was included. All piglets were healthy, with no record of severe diarrhoea that required treatment. In the case of infectious arthritis during suckling, animals treated with antimicrobial drugs were excluded from the experiment. Animals selected for each respective experiment were balanced for the body weight and sex in each treatment group. Mean birth body weight at the start of the experimentin Paper I was 1.85 (β-glucan) and 1.86 kg (control), in Paper II 1.62 (probiotic) and 1.67 (control), and lastly in Paper III 1.83 kg (β-glucan) and 1.83 kg (control). Aside from the provided supplementation, the piglets were housed and managed according to regular routines and conditions at the farm. All piglets had *ad libitum* access to water. Creep feed was offered during the suckling period, from 5 days of age. The experiments were conducted at the Swedish Livestock Research Centre Lövsta, Uppsala. The farm is certified as Specific Pathogen Free (SPF).

4.3 The piglets included in experiments

The dietary supplements used in all three papers were formulated and produced at Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden.

Papers I and III

The oat bran BG28 used in the first and third experiment (Papers I and III) contained 28% of soluble β- glucan with a molecular weight 2000 kDa and a total of 52 g/100 g dietary fibre. Five piglets from each litter were fed with freshly prepared oat β- glucan paste (oat β- glucan powder mixed with water) orally via disposable syringes (Figure 5). The remaining five piglets in each

litter received water as a control, supplemented in the same manner as described above. Administration of β-glucans to piglets commenced at seven days of age, thrice a week until weaning at day 35 of age. Each supplement dose contained 40mg of β- glucan per kg body weight.

Paper II

The supplement applied in the second experiment was a mixture of two bacterial strains: *L. reuteri* ATCC-PTA-6475 and *L. plantarum* L1-6. Each supplement contained a dose of $8\times10^7\pm3\times10^7$ colony forming units (CFU) of *L. reuteri* and $2\times10^9\pm5\times10^7$ CFU of L. *plantarum*. The mixture was made of an aliquot of 100 µl of each bacterial strain mixed with 20 µl caramel colour and 80 µl water (Figure 5). Syringes filled with 300 µl of a freshly made mixture of bacteria (described above) were retained on ice until administration. Ten piglets were selected from each litter and five were supplemented orally with the mixture of Lactic Acid Bacteria (LAB) probiotic, whist the remaining five received an oral control supplement consisting of the same liquid formula but without any bacteria. Supplementation of piglets started from day three of age until weaning at 35 days of age.

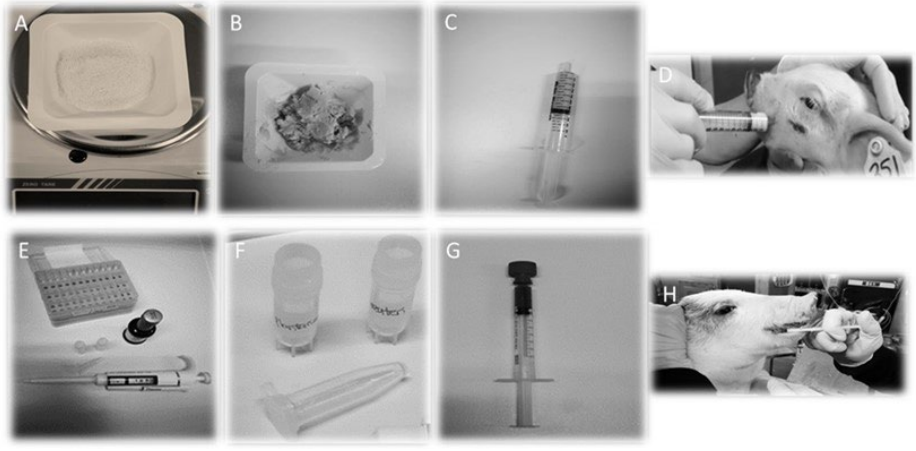

Figure 5. Illustration of the preparation and administration of the supplement used in Papers I-III. Letters correspond to different steps performed to prepare supplements in all three papers. Papers I and III: The oat β- glucan supplement was prepared by weighing (A), making a paste by mixing it with water (B), filling the syringe with the paste (C), and the paste was then orally administrated (D). The probiotic supplement in Paper II was prepared by mixing caramel colour and water (E) with aliquots of L. reuteri and L. plantarum (F), filling syringes with the mixture (G), and orally administering it to the piglets (H).

4.4 Sampling

Sampling for both the supplemented piglets and the controls was conducted at approximately the same time.

4.4.1 Rectal swab sampling (Papers I-III)

In Paper I, rectal swab samples for microbiota composition were collected weekly from week 1 to 4 from 20 piglets, four piglets per litter and two from each treatment group, balanced as much as possible for sex and birth weight. In Paper II, rectal swab samples were collected from 46 animals, 23 from the probiotic group and 23 from the control group at week 4, during suckling, and at week 6, in the early post-weaning phase. In Paper III rectal swab samples were collected at 4 weeks of age from 16 animals supplemented with oat β- glucan and 20 controls. All three studies' samples were kept on ice and

stored shortly after arriving at SLU in a freezer at -80°C until further analysis.

Although most studies on pigs use faecal samples for gut microbiota analyses, in the present experiments rectal swab samples were obtained instead. Due to a limited time for sampling, considering that blood sampling was completed at the same time, rectal swabs were more suitable. Rectal swabs are a good alternative to faecal samples and no significant differences were found in alpha and β- diversity when swab and faecal samples were compared (Radhakrishnan *et al.* 2023). Moreover, studies have concluded that storage conditions did not alter the gut microbiota results when microbiota from stool samples was compared between samples stored at 4 °C or -20 °C and those immediately stored at -80 °C (Bassis *et al*. 2017).

4.4.2 Post-mortem intestinal sampling (Papers I-II)

In Paper I, one piglet from each treatment and litter was euthanised one day before weaning for post-mortem sampling, meaning that a total of 10 piglets were euthanised. Digesta and mucosal scraping samples were obtained from the central colon, and mucosal scraping were also obtained from the distal part of the ileum. The collected samples were immediately snap-frozen in liquid nitrogen and stored at -80 °C until analysis. Additionally, tissue samples were obtained from the ileum and colon, and fixed in 10% formalin for further histology analyses. A similar post-mortem sampling was performed in Paper II, with sampling from one pig per treatment and litter, in total 14 piglets. Intestinal samples were collected from the distal ileum and proximal colon, approximately 50 cm, before and after cecum. The tissue samples were fixed for 24 hours in Carnoy's solution. Both of the fixatives used in the two studies are suitable for preservation of intestinal tissue for histology analysis. However, Carnoys's solution was more suitable for the preparation of the mucus layer and analysis of the barrier function (Blick *et al.* 2019) which was initally planned at the time of sampling but ultimately not included in Paper II. Animals in both experiments were balanced for sex and body weight as much as possible.

4.4.3 Blood sampling

To avoid placing any additional unnecessary stress on the animals, blood sampling was usually collected in connection to the the rectal swabs. In the first experiment (Paper I) blood samples from the jugular vein were obtained in weeks 1, 3, and 4. In the second experiment (Paper II) blood samples were taken at week 4 and 6 of age. In Paper III, blood samples were obtained at 4 weeks of age. All samples were placed on ice immediately after sampling and kept on ice until arriving at SLU where they were centrifuged for collection of blood plasma, which was then aliquot, and subsequently stored in a freezer at -80°C. Studies have concluded that the condition of blood samples cooled on ice before centrifuging will not significantly change the test results for the analyses performed in Papers I-III (Tobias *et al.* 2016).

4.4.4 Pre-mortem blood sampling Papers I and II

In Papers I and II blood samples were obtained from the jugular vein from all 10 animals , respectively, from 14 animals under anesthesia. Access to the portal vein blood was not always achievable due to a rupture of the vein causing a high tendency of quick blood coagulation. Portal vein blood samples were successfully obtained from 9 animals in Paper I, 5 from the β-glucan group and 4 from the control group. In Paper II, portal vein samples were obtained from 10 animals, 6 from the control group and 4 from the probiotic treatment group. The blood samples were kept on ice until the sampling procedure was completed, and the samples were then centrifuged and plasma was stored in a freezer at -80°C until analyses.

4.4.5 Behavioural observations

In Paper III, several behavioural observations were performed. Behaviour in the home pen was observed as a baseline behaviour at 20, 34, 41, 48, and 55 days of age. A t-maze test, assessing spatial learning, memory, and decision-making, was performed between day 59 and 84 on 20 piglets from the β-glucan supplemented group and 24 piglets from the control group. Piglets were trained to find a food reward in one of the arms and were given 60 seconds to choose between the east and west arms (Figure 6A). After, they

were assessed on a reversal learning test. In the novel object test piglets were tested for fear responses after the t-maze test was finished, at around day 86 of age (Figure 6B).

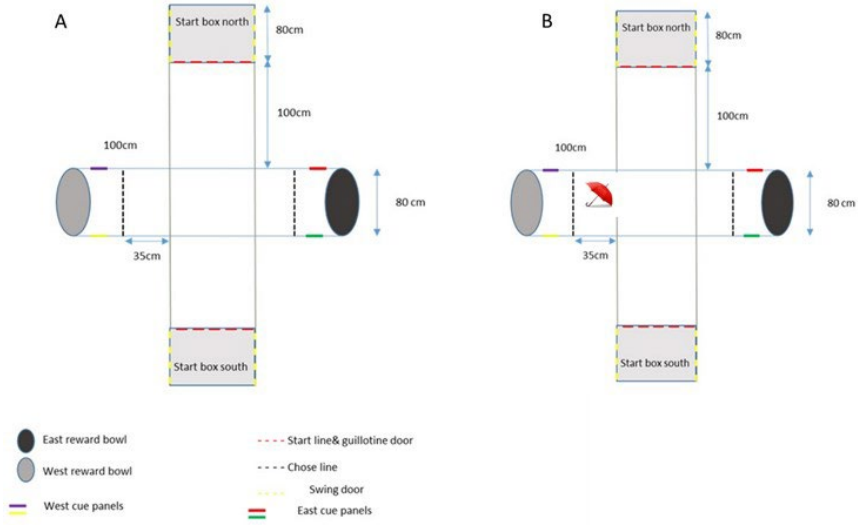

Figure 6. Schematic diagram showing the T-maze used to examine learning and memory in pigs supplemented with oat β- glucan (A), schematic diagram of the T-maze with umbrella used in a novel object test to examine fear responses (B).

4.5 Analyses

4.5.1 Microbiota

In Papers I and III, DNA extraction and PCR amplification of the V3-V4 regions of the 16S rRNA gene were performed at SLU. Samples were pooled in equimolar amounts and sent to SciLifeLab in Stockholm, Sweden for Illumina MiSeq sequencing. In Paper II, the V3-V4 16S rRNA gene PCR

amplicons were generated and sequenced on the Illumina platform using a NovaSeq 6000 system at Novogene in Beijing, China.

4.5.2 Short-chain fatty acids

In Paper I, SCFAs were analysed in both plasma and digesta, whereas in Papers II and III only SCFA in plasma was analysed. The concentration of SCFAs was determined using a liquid chromatography-mass spectrometry method (Fristedt *et al.* 2024). This method is accurate and highly sensitive, and can detect a wide range of concentrations (0.2-200μM) making it suitable for both plasma and rectal swab samples (Qian *et al.* 2006; Mayo-Martínez *et al.* 2024).

4.5.3 Weight gain and intestinal tissue histology analysis

In Papers I-III, animal growth was analysed from birth to nine weeks of age. This was completed by weighing the animals on a weekly basis. Additionally, the average daily weight gain (ADG) was calculated for all animals in Papers I and II. For histological analyses, microscopy image analysis was used to view and measure gut and mucosal thickness, villi heights, crypt depths, and thickness of muscularis externa in Papers I and II.

4.5.4 Behavioural observations, home pen, T-maze test, and novel object test

In Paper III, different behavioural tests were analysed. Live observations were conducted from outside the home pen, using the behavioural recording program Mangold Interact. Scan sampling was conducted for six hours at 5 dates, 60 scans per piglet, two dates before weaning and three dates after weaning. Additionally, certain behaviours were summed into a final ethogram for easier categorisation and inventory.
The T-maze test analysed learning and memory, whilst the novel object test assessed fear responses using an ethogram. The time spent in the arms

finding the reward food, time spent exploring the umbrella, and the occurrence and frequency of behavioural elements were analysed.

4.5.5 Statistical analysis

Univariate and multivariate approaches were used to assess the effects of treatments. Data was analysed using statistical software PAST and R as well as R studio version 4.2.1. In Paper I, multivariate analyses were performed with PAST software, version 4.04, to determine the microbial composition and relative abundance data from all OTUs using principal coordinate analysis (PCoA) based on the Bray-Curtis dissimilarity index. Clustering patterns were validated by analysis on similarity (ANOSIM) based on 9999 permutations. The linear mixed model package lme4 and lmerTest were used (Bates *et al.* 2015, Kuznetsova *et al*. 2017) with treatment, sex, age, and their interactions as fixed effects and piglet ID nested within litter as a random effect to analyse the relative abundance of the five most abundant phyla and 10 most abundant genera, alpha diversity, SCFAs, and weight data. All data met normality assumptions after applying a log transformation and removing outliers greater than 2.5 units. Post-hoc Turkey HSD test was used for pairwise comparison, and correlations between microbiota and SCFA plasma concentration were analysed with linear Pearson correlations. A T-test was performed to determine the effect of supplements on samples taken from euthanised animals.

In Paper II, a similar approach was used but all data was analysed with R, version 4.2.1. Multivariate analysis detected microbial composition and relative abundance from OTU data with PCoA based on Bray-Curtis distance. ANOSIM test based on 9999 permutations analysed clustering patterns. A linear mixed model approach was performed to assess the effect of treatments on the ten most abundant genera, alpha diversity, SCFAs, weight, and ADG data and samples from euthanised animals.

In Paper III, PAST software performed a principal component analysis (PCA) to explore the effects of the supplement on microbiota, SCFA, and their correlation with the behaviour data. Correlations were further assessed with Spearman rank correlation coefficient. Scan samples, learning and memory, and novel object data were analysed by linear mixed model in R.

5. Main Results

The main results obtained from Papers I-III are described briefly below. A more detailed description of the results can be found in the individual papers.

5.1 General outcome of supplementing piglets with specific pre-probiotics

The dietary supplements did not lead to any significant changes in overall gut microbiota, SCFA production, weight, or histological measurement. In Paper I, an age effect was prominent for all evaluated parameters. However, certain effects of treatment were detected in Paper II. Microbiota composition in the ileum and colon was affected by the probiotic supplement. Several correlations between microbiota and SCFAs were also found in Papers I-II, as well as correlations between microbiota, SCFA, and behaviour data in Paper III. Sex and litter effects were significant across all three papers.

5.2 Gut microbiota in Papers I and II

In Paper I, supplementation with oat β- glucan did not impact microbiota development. However, a change in gut microbiota composition with age was present in all treatments. Development of microbiota (swab samples) gradually changed to more a "mature" composition in the third and fourth week, i.e. more similar to composition known from literature after weaning, with a higher relative abundance of *Prevotella* spp. (Amat *et al.* 2020). *Escherichia*, *Lactobacillus*, and *Bacteroides* were dominating taxa throughout the experiment but *Lactobacillus* and *Bacteroides* decreased significantly with age whilst *Prevotella* gradually increased throughout the suckling period in Paper I. In Paper II, the rectal swab samples taken at 4 and 6 weeks of age did not show any treatment effects. The difference in the microbiota composition was predominantly age-related. *Escherichia* dominated, followed by *Bacteroides*, *Lactobacillus,* and *Prevotella*. *Prevotella* spp. in Paper II followed a similar pattern to Paper I with an

increased abundance with higher age. *Lactobacillus* also increased with age and was higher in week 6 compared to week 4 of age but with no difference in abundance linked to the treatment. The microbial composition differed significantly between colon and ileum in Paper II. The Shannon diversity index was lower in ileum mucosa than in colon digesta, but no differences were found between colon mucosa and ileum mucosa. However, the species richness was higher in ileum mucosa compared to both colon digesta and mucosa. The probiotic supplement had an effect on the abundance of some microbial taxa in ileum. The abundance of *Bacteroides* was lower in ileum mucosa in the probiotic group than ileum mucosa in the control group. In the probiotic group, there was also a difference in the abundance of *Bacteroides* between sample types (mucosa vs digesta). Moreover, the relative abundance was higher in ileum mucosa compared to both colon mucosa and digesta in probiotic group. The abundance of *Prevotella* 2 was higher in ileum mucosa in the probiotic group of piglets when compared to the control group. *Lachnospiraceae*_NK4136 was more abundant in the probiotic group than the control group in all three sample types (ileum mucosa, colon mucosa, and digesta).

Although individual differences were present in microbiota composition between animals in both Papers I and II, a clear clustering pattern based on litter was observed in both studies (Figure 7).

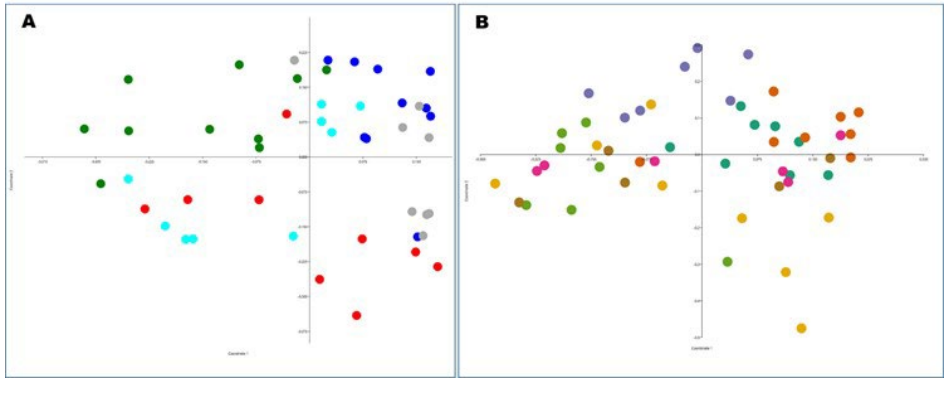

Figure 7. Principal coordinate analysis (PCoA) plots based on Bray-Curtis dissimilarities showing the clustering pattern of the microbiota. Panel A shows the plot with a separation in microbiota composition in different litters at 4 weeks of age in piglets in β- glucan (Papers I and III) (A) and the probiotic study (Paper II) (B). Each colour represents a different litter.

To assess any possible differences between the two studied treatments on microbiota development, the relative abundance of the most dominating taxa from rectal swabs at four weeks of age was compared in this thesis (Figure 8). Although large individual differences were present in both studies, when relative abundance was compared between the two studies, microbial taxa were similar, quite consistent, and not supplement-dependent. However, *Escherichia* was more abundant in the probiotic study, whilst *Prevotella* was more dominant in the β-glucan study.

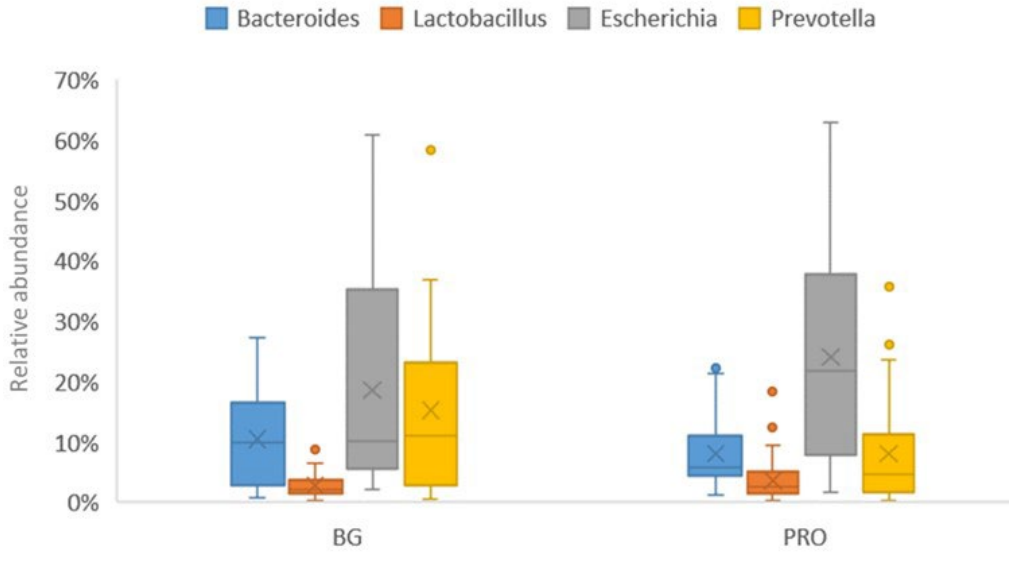

Figure 8. The relative abundance of the 4 most abundant genera in rectal swabs in both the β- glucan (BG) and probiotic (PRO) experiment at 4 weeks of age. Line within boxes marks the median and X marks the mean. Whiskers show samples within the 1.5 interquartile range whilst dots outside whiskers represent outliers.

5.3 Short-chain fatty acids in Papers I and II

SCFA concentration was measured in both plasma and colon digesta in Paper I. Acetic acid concentration in plasma was lower in pigs supplemented with β- glucan ($p < 0.05$) than pigs in the control group. All SCFA were influenced by age and gradually increased, except for succinic and formic acids. Propionic acid had the highest concentration in week 3 of the 4 week experiment. The concentration of SCFA in colon digesta and plasma samples taken from euthanised animals showed no effect of the treatment. The SCFA concentrations were higher in plasma taken from portal vein blood samples compared to jugular vein blood samples and colon digesta samples. Portal vein blood samples and digested samples were more comparable than jugular vein samples, indicating that SCFA were likely metabolised in the liver. The SCFA concentrations were lower in the jugular vein. (Figure 9).

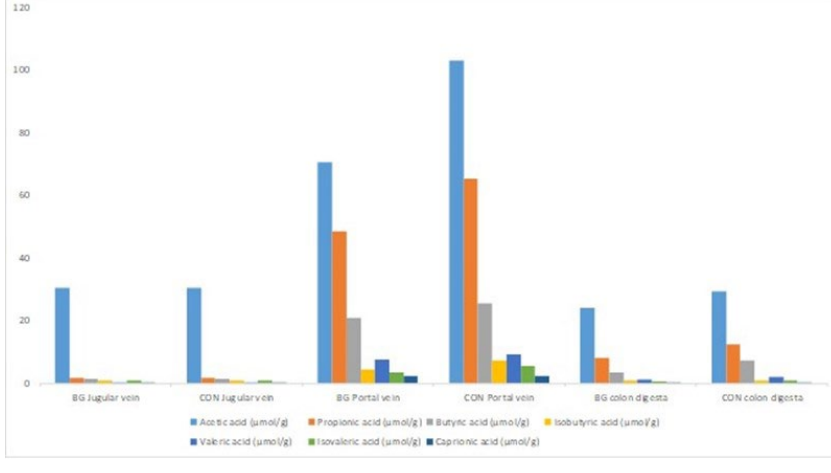

Figure 9. Mean concentration (µmol/L) of SCFAs from plasma and digesta from five piglets in the supplemented group and five piglets in the control group in Paper I, BG indicates β- glucan supplemented group, CON indicates control.

In Paper II, SCFA concentration was measured in plasma and displayed findings similar to Paper I, as it was mainly affected by age rather than treatment. Propionic acid showed an interaction between the probiotic supplement and age, where the concentration was higher in the probiotic group ($p < 0.05$) of animals in week 4 when compared with the control group. All SCFA increased with age with higher concentrations after weaning compared to before weaning. In week 5, at weaning, plasma samples from the jugular vein were taken from euthanised animals, and the probiotic supplement was associated with lower concentration of caproic acid.

The concentration of acetic, propionic, and butyric acid comparison was examined from jugular vein samples at 4 weeks of age to investigate a possible influence of the supplements on the SCFA concentrations (Figure 10). Treatment comparisons revealed certain numerical differences in the SCFA concentrations. However, no statistical analysis was conducted to determine if this difference was significant. Butyric acid was highest in the BG supplemented group compared to the other groups. Propionic acid was lower in the probiotic group compared to the β-glucan group, whereas the opposite pattern was observed for acetic acid, which was somewhat higher in the probiotic group. The SCFA concentration was higher in the portal vein than in the jugular vein in both experiments (Figure 11).

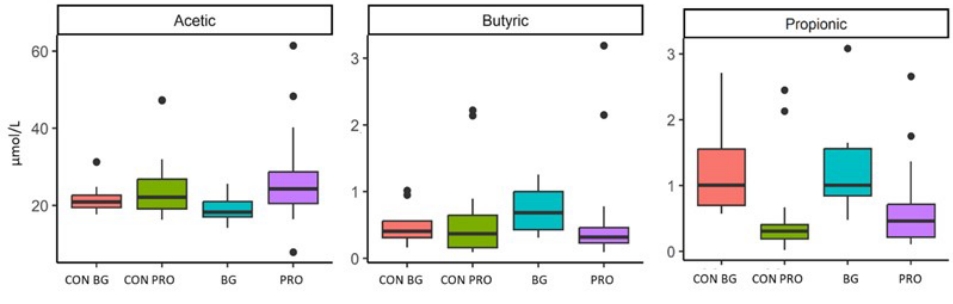

Figure 10. Boxplot showing the concentration (µmol/L) of acetic, propionic, and butyric acid taken from jugular vein samples at 4 weeks of age from piglets supplemented with β- glucan and probiotic treatment. BG indicates β- glucan supplemented group, CON is for control, and PRO indicates probiotic supplemented group. The line within the boxes marks the median. Whiskers show samples within the 1.5 interquartile range, whilst dots outside whiskers represent outliers.

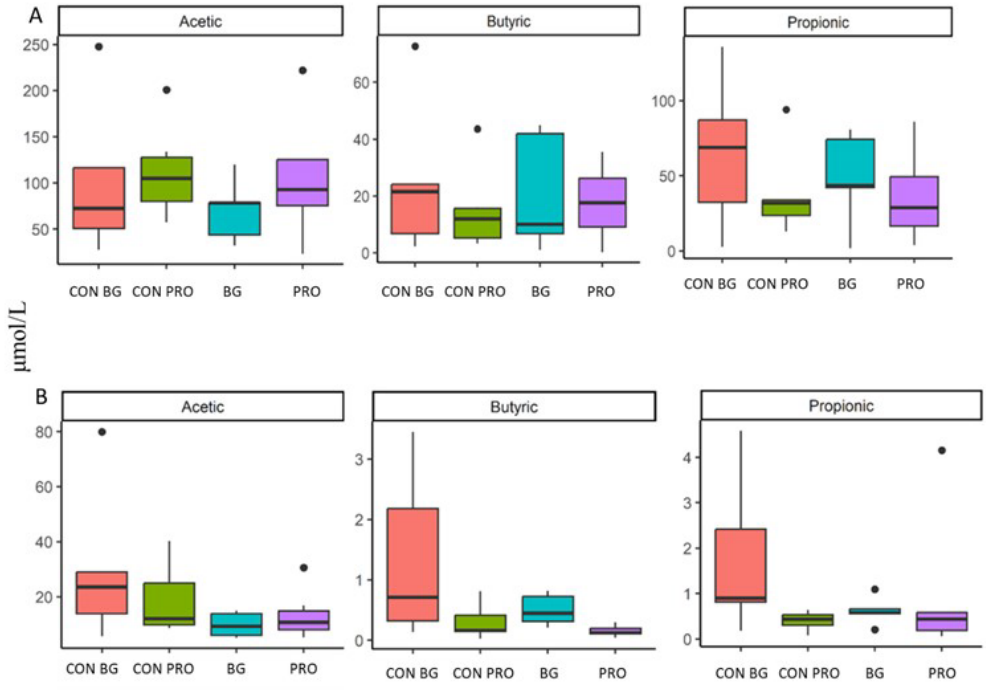

Figure 11. The concentration (µmol/L) of acetic, butyric, and propionic acid from portal vein (A) and from jugular vein (B) taken in both experiments from euthanised animals. BG indicates β- glucan supplemented group, CON is for control, and PRO indicates probiotic supplemented group. The line within the boxes marks the median. Whiskers show samples within the 1.5 interquartile range whilst dots outside whiskers represent outliers.

5.3.1 Correlations between bacterial taxa and SCFA in Papers I and II

There were correlations among the 10 most abundant bacterial taxa and SCFA in plasma in both Papers I and II. In Paper I, correlations followed the microbiota age development. *Lactobacillus* and *Bacteroides* decreased in abundance as age increased and were thus negatively correlated with acetic and propionic acid that had increased concentrations in plasma with older age. On the contrary, *Prevotella* increased with age and was positively correlated with acetic, butyric and valeric acid. In Paper II, correlations were different at week 4 before weaning and week 6 after weaning. Before weaning the strongest correlations were found between *Prevotella* 2 and

several SCFA, however, after weaning a strong positive correlation was found between *Bacteroides* and formic acid, as well as both positive and negative correlations between SCFA and different *Prevotella* species.

To observe the possible influence of two different supplements on correlations between microbiota and SCFA, and identify possible generalisable patterns, a comparison was made for correlating SCFA and bacteria at week 4 of age. *Prevotella* spp. was positively correlated in both studies with acetic acid. The supplement containing β-glucan revealed several positive and negative correlations between different SCFA and bacteria at week 4 of age (Figure 12). However, the probiotic supplement showed positive correlations with several SCFAs at week 4 with just one bacteria, *Prevotella* 2 (Figure 12).

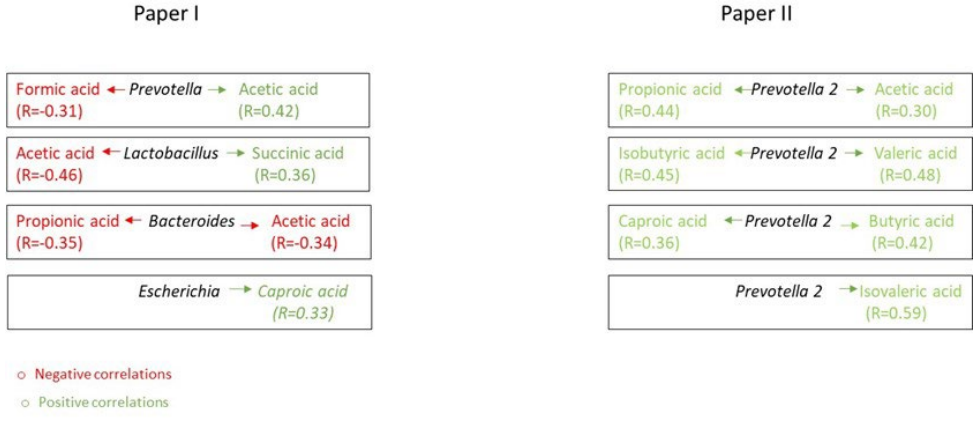

Figure 12. The correlations between the most abundant bacterial taxa from rectal swab samples and SCFA in plasma samples at 4 weeks of age in Papers I and II.

5.4 Growth performance and histology of intestinal tissue samples

In studies I and II, no differences were found in weight gain between supplemented piglets and controls. ADG did not reveal statistical differences in any of the studies between the two groups linked to the supplement.

However, certain numerical differences in ADG measurements were found in Paper II from week 6 to week 9 where the probiotic group had higher ADG compared to controls.
In Paper I, histological analysis of ileum and colon tissue samples did not reveal any effects of treatment on any of the measured parameters; however, in Paper II, total gut thickness in ileum was higher in the control group than in the probiotic group.

5.5 Associations between the gut microbiota and behaviour

In Paper III, the β-glucan supplement did not affect behaviours such as eating, exploring, nursing, learning, and memory. The novel object test for assessing fear did not reveal any differences between the treatment and control piglets. However, affiliative behaviours including playfulness, social bonding, and touching each other playfully was noted less in the β-glucan supplemented group than the control group. Additionally, the β-group of piglets demonstrated lower performance in reversal learning compared to the control group.
Furthermore, several correlations were found between microbiota, SCFA, and behaviours. Exploring, standing, and locomotion displayed several significant correlations to microbiota abundance and the SCFA concentrations. For example, *Ruminococcaceae* spp. was negatively correlated to exploring behaviour and standing in week 5, whilst a negative correlation was found with locomotion and standing in week 7. *Fusobacterium* showed a positive correlation with locomotion and standing at week 7 and a positive correlation with exploring at week 5. *Lachnospiraceae* was negatively correlated with exploring behaviour at week 5 and standing behaviour at both weeks 5 and 7. Further, Chao-1 community richness index was negatively correlated to behavioural variables, exploring at week 5, and locomotion and standing at week 7. Concentration of formic acid revealed a negative correlation to locomotor behaviour and exploring at week 7 of age.

6. General discussion

This thesis explored the influence of early-life supplementation of specific probiotics and prebiotics on intestinal microbiota and SCFA in piglets. Moreover, it studied the impact of several behavioural parameters and examined if there were associations between the microbiota, SCFA, and behaviours that could indicate an impact via the gut-brain axis.

Neither the β-glucan nor the probiotic supplement significantly impacted overall gut microbiota, SCFA production, weight, or histological measurements. However, specific gut segments showed microbial changes in Papers II and III, with correlations found between specific bacteria and behaviours. Additionally, the β-glucan supplement had an effect on cognition.

It is important to highlight the challenges and difficulties among the results in this thesis compared to previous studies and the inconsistencies within the literature. Firstly, there is a lack of knowledge as a limited number of studies have focused on the effects of supplements during the suckling period on the GIT, and the gut microbiota and its metabolites. Additionally, there are minimal studies on how prebiotic supplements influence early-life behaviour in pigs. Although more studies have addressed the topic in the last couple of years, many research gaps remain. General knowledge of the developmental stages of the GIT, morphology, and the piglet's ability to utilise feed in those first weeks of life is not consistent within the literature, and data is, at times, conflicting, likely because of differences in age, breed, and diets, housing conditions, and analytical and statistical methodologies. Secondly, different weaning ages are used in different research studies, ranging from 21-28 in some studies to 35 days in the present studies. The comparison was primarily made, especially in Papers I and II, with published papers that used already weaned piglets that were around the same age as the piglets in the present studies. The differences in outcome between the studies and other published papers may be explained by the intensive development of the GIT microbiota and the plasticity of the developing brain in the first weeks of a piglet's life. Further, the differences in microbiota between suckling piglets and post-weaned piglets fed solid plant-based feed may also explain some of the differences. There were also some general differences in the methodology of obtained SCFAs. In the present studies, SCFA concentration was obtained

from blood plasma samples, whereas the vast majority of other studies analysed only the concentration of SCFA from faecal samples. Comparing these is challenging as they represent different biological contexts and serve various purposes.

6.1 Gut microbiota

6.1.1 Oat β-glucan effects on the gut microbiota

The role of oat β- glucan on the gut health of suckling piglets has not been investigated to a large extent. In Paper I, the results showed that intestinal microbiota composition was not affected by the oat β- glucan supplement. Other published studies reported significant variabilities in the results of the effects of oat β- glucans on the bacterial composition both pre- and post-weaning. One study reported an increase in the abundance of *Bifidobacterium* spp and *Lactobacillus* spp in the colon and stomach (Choi *et al.* 2023). Another study reported a decreased number of *Lactobacillus* and an increased total number of γ-Proteobacteria in colon digesta when suckling piglets were fed oat hulls (van Hess *et al.* 2023). A different study found that oat β- glucan in weaned piglets decreased the abundance of *Bifidobacterium* in ileum digesta (Wu *et al.* 2018). Conversely, findings from another study showed that the number of *Lactobacillus* spp. and *Bifidobacterium* spp. increased in colon digesta, whereas the abundance of clostridia cluster XIVa in colon digesta decreased in weaned piglets fed with oat β-glucan enriched with calcium phosphate (Metzler-Zebeli *et al.* 2011). These inconsistencies make it difficult to assess the impact of oat β- glucan on microbiota composition and draw any final conclusions. Interestingly, the study by Metzler-Zebeli (2011) showed an increased number of several bacteria in digesta from the stomach such as *Lactobacillus* spp., *Streptococcus* spp., clostridia clusters I and XIVa, the *Bacteroides–Prevotella– Porphyromonas* group, and *Enterobacteriaceae.* These findings highlight that β-glucan supplement may enhance microbiota already present in the stomach of weaned pigs (Metzler-Zebeli *et al.* 2011). In comparison, the samples in Paper I were not collected from the stomach, therefore no

information and insights can be provided regarding the impact of oat β-glucans on the microbiota in the stomach. However, the ileum and colon digesta samples did not show similar differences. The oat β- glucans supplement did not seem to have a significant and measurable impact on microbiota development, nor did the rectal swab samples show any direct effects of the supplement on the microbiota composition.

A reason for the inconsistency in results between studies may be due to the purity of the offered product, and the variation in processing methods and extraction techniques that effect the size of polysaccharide chains and thus the molecular weight of β-glucans and the functionality of oat β-glucans (Hakkola *et al.* 2021). The molecular weight of oat β-glucan is reported to be between 65-3100kDa (Du *et al.* 2019). A lower molecular weight of β-glucans makes them more fermentable by the gut microbiota. Indeed, studies on mice have shown an enhanced enrichment of bacteria such as *Bacteroides*, *Prevotella,* and *Bifidobacterium* that thrive on more soluble fibres with lower molecular weight (Luo *et al.* 2017; Cronin *et al.* 2021). This may provide short-term benefits in modulating microbiota but may also reduce microbial diversity over time due to the domination of bacterial species that have easy access to rapid and easily metabolised substrate. However, oat β-glucans with lower molecular weight did not show consistent responses on the gut microbiota in pigs in previously published studies. In one study, the *Bacteroides* decreased in faecal samples (Metzler-Zebeli *et al.* 2010) and *Bifidobacterium* increased in the ileum and colon in the early growing phasein pigs (Reilly *et al.* 2010) and decreased in the ileum in weaned pigs (Wu *et al.* 2018). This highlights that soluble dietary fibres with low molecular weight, although important nutrients, may have different implications for gastrointestinal health. However, it is also important to consider that during the early period of a piglet's life soluble fibre with low molecular weight may not be the optimal choice due to insufficient support for fibre degrading bacteria that are essential for the transition towards a mature gut microbiota (Mahmud *et al.* 2023). It may also exacerbate PWD in piglets due to the limiting water absorption, which can lead to increased water content in the intestinal lumen and cause higher diarrhoea rates and impaired feed digestibility (Canibe *et al.* 2022; Huang *et al.* 2022c).

In contrast, the higher molecular weight of β-glucans that was utilised in Paper I (2000 kDa) is expected to increase the viscosity of digesta and contribute to slowing down the gastric emptying, fermentation, and nutrient

absorption. Together, this is suggested to positively impact long-term gut health, providing a gradual and sustained energy source for a wider range of fibre-degrading bacteria and increasing the microbial diversity in pigs (Guang *et al.* 2022; Sushytskyi *et al.* 2023). Several mouse studies have found associations between improved gut health and enhanced gut barrier protection and an increased abundance of bacteria such as *Ruminococcaceae* and *Lachnospiraceae* when oat β- glucans with higher molecular weights were supplemented (Jaeger *et al.* 2024; Cheng *et al.* 2021; Guzowska *et al.* 2024). In weaned pigs, some studies found an enrichment of *Ruminococcaceae* (Murphy *et al.* 2012), whilst others found that the abundance of *Faecalibacterium* had decreased after oat β-glucan supplement was administered (Korczak *et al.* 2020).

The gut microbiota composition in rectal swabs in both supplemented and un-supplemented groups in Paper I was dominated by *Escherichia*, *Bacteroides*, *Prevotella,* and *Lactobacillus*. These particular microbiota members are known as bacterial genera which are typically associated with suckling pigs (Lue *et al.* 2022b; Floes *et al.* 2024). The taxa *Lactobacillus* decreased from 10% in the first two weeks to under 3% at 4 weeks of age. This is in agreement with findings from studies on suckling piglets (Guevarra *et al.* 2019; Amat *et al.* 2020). *Lactobacillus* are usually a predominant bacteria in the first weeks of a piglet's life due to the usage of the oligosaccharides within the milk as a primary energy source (Nowland *et al.* 2022). Compared to the results in Paper I, *Prevotella* is generally less abundant in other studies (Mach *et al.* 2015; Wang *et al.* 2019c) during the initial weeks of life. Although no differences were found in connection with the treatment, *Prevotella* taxa continually increased throughout the suckling period but were more dominant in the third and fourth weeks of the experiment. In swab samples, the relative abundance accumulated to 10% at week 3 of age and 15% at week 4. After weaning, the abundance of *Prevotella* generally rises due to changes in diet and has been reported to comprise around 25% of the overall gut microbiota community (Chen *et al.* 2017). Findings from several other studies have shown effects of solid feed and a fibrous enriched creep feed on the gut microbiota in suckling piglets similar to the one found in Paper I (Choudhury *et al.* 2020; Choudhury *et al.* 2021a; Choudhury *et al.* 2021b, Van Hess *et al.* 2019). This may indicate that the piglets in Paper I were exposed to a source of more soluble fibre as

a similar development of microbiota was observed when piglets were offered different fibre-enriched diets during suckling in different studies. Diet plays a role in modulating the abundance of *Prevotella*, Clostridia cluster XIVa, and *Eubacterium* (Zhang *et al.* 2016, Mu *et al.* 2017a). Other studies found an association between higher abundance of the taxa *Prevotella*, *Roseburia*, *Eubacterium*, *Lachnospira,* and *Ruminococcus* with consumption of dietary fibres (Gardiner *et al.* 2020) as well as a reduction in the abundance of *Escherichia-Shigella* (Van Hess *et al.* 2019).

Noticeably, the relative abundance of *Prevotella* was high in Paper I and increased in all pigs regardless of the treatment throughout the suckling period. This contrasts to Choudhury's studies wherein the abundance of *Prevotella* was above 1% in the control group of pigs throughout the suckling period. Unfortunately, intake of creep feed was not recorded in Paper I, therefore, no solid conclusion can be made regarding the increased abundance of *Prevotella*. Nevertheless, the data from studies by Choudhury and co-workers suggests that the suckling piglets in Paper I were consuming more creep feed, especially in the last two weeks before weaning. Previous studies (Choudhury *et al.* 2021a; Middelkoop *et al.* 2020) observed a prominent increase in eating behaviour with time, specifically in the final weeks of the suckling period, at weeks 3 and 4 of age.

However, another plausible explanation for the higher abundance of *Prevotella* in piglet swab samples in Paper I that should not be overlooked is the possibility of the consumption of maternal faeces by piglets. This behaviour in piglets may have impacted microbiota establishment in the first weeks of life and elevation in abundance of *Prevotella*. Recent research has not precisely quantified the amount of consumed faeces for young pigs. The specific amount of 20g/day that is referred to in several studies originates from a study conducted in 1981, which focused on the suckling piglets' reliance on maternal faeces for essential nutrients and minerals (Sansom *et al.* 1981). However, little research in that area has been conducted on pigs since 1981. The nutritional composition of sow feed, creep feed, and milk replacement has changed significantly since the 1980s. Nonetheless, maternal faeces could still play a key role in seeding piglets' gut microbiota with beneficial bacteria as it is not solely about nutrition; maternal faeces may contain important immunological components and eating faeces may be a natural behavioural trait. Several studies have explored this behaviour under modern farm conditions. One study found it to be significant for

weight gain and immune development (Aviles-Rosa *et al.* 2019). Other studies indicated that maternal faeces are partially responsible for early microbiota colonisation in piglets (Li *et al.* 2022; Nowland *et al.* 2021). A different study found the consumption of maternal faeces to be an innate behaviour in pigs (Aviles-Rosa *et al.* 2020). Although this behaviour was not documented in the study in Paper I, it still deserves recognition as a possible factor that may have influenced microbiota development.

Additionally, the amount of the supplement of β- glucan may not have been sufficient to have any additional effect on the gut microbiota already modulated by available feed. The amount of β- glucan in Paper I was 40mg/kg body weight and was based on data from metabolic effects in human studies. Based on experience from conducting the trial, it was concluded that suckling piglets would not be able to consume more than 40mg/kg body weight per one oral supplement due to the bulky nature of β-glucans and young piglets' limiting capacity to ferment fibres from oats (Knudsen *et al.* 2012). Other studies on weaned pigs that had measurable modulatory effects on microbiota were supplementing higher dosages of β-glucan from 50-200mg/kg body weight (Tiwari *et al.* 2019). The dosage was higher when pigs were challenged with *Escherichia*, 500 mg/kg BW (Zhou *et al.* 2022b), or *Salmonella enterica*, in feed amended diet with 500g β-glucan additive per ton feed (Bearson *et al.* 2023).

However, the suckling piglets in the first weeks of age may not have had enough developed microbiota to start digesting the high molecular weight oat β-glucans in Paper I. The intestinal tract needs time to adapt to diet and modulation and change of the gut microbiota can take time, even in older piglets (Gao *et al.* 2023). The suckling piglets likely lacked the ability to efficiently digest and metabolise complex carbohydrates from β-glucans due to undeveloped microbiota in the early stages of life and this may have compromised the effectiveness of the supplement.

All these various factors, whether separately or in different combinations, may have influenced the results, highlighting the complexity of the gut microbiota and the digestion in those first weeks of a piglet's life.

6.1.2 Probiotic supplementation effects on the gut microbiota

Lactobacillus is a common genus in the colon of growing pigs and one of the most abundant genus in the colonic microbiota of young piglets during suckling (Li *et al.* 2019). Paper II demonstrated that a supplement with two different strains of LAB did not increase the relative abundance of *Lactobacillus* in rectal swab samples at either week 4 or 6 of age. Certain studies investigating weaning pigs found an increase in the relative abundance of *Lactobacillus* in faecal samples with the supplementation of *Lactobacillus* strains (Lee *et al.* 2022; Yu *et al.* 2024). Another study on suckling pigs did not find any microbiota alterations in faecal samples after probiotic administration (Chen *et al.* 2025). To ensure that all samples for all of piglets were synchronised, a sampling method with rectal swabs was selected in the study in Paper II. Rectal swab samples, although found to be a viable alternative for faecal samples, may influence the detected bacterial composition. Rectal swab samples tend to collect more bacteria adhering to the intestinal mucosa, whilst faecal samples capture luminal bacteria. (Dell'Anno *et al.* 2021a). The method of sample collection with rectal swabs may have overrepresented bacteria that adhere well to the gut lining and affected the results of gut microbiota composition. This makes it challenging to directly compare to microbiota composition in other studies where samples were collected via faecal sampling. However, a difference between the mucosa and digesta was present in samples of euthanised animals in Paper II.The mean relative abundance of *Lactobacillus* in the colon digesta of supplemented piglets was around 5% whereas the abundance was 2% in samples from the colon mucosa. It has been established that most probiotics do not stay in the colon for long and are unable to compete with the host colonic microbiota (Wang *et al.* 2019b). A recent study found a significant daily fluctuation of *Lactobacillus* in growing pigs' colon. There was a particularly strong oscillation in *L. reuteri* strains during the day, with a lower abundance at both night and morning and a higher abundance in the afternoon, demonstrating the importance of time of sampling (Xu *et al.* 2024). In Paper II, the sampling was not conducted at exactly the same time for all piglets, but was performed within a few hours of each other and at the

same time for all piglets within a litter. Furthermore, the age of sampling may have impacted *Lactobacillus* abundance as it has been shown both in rats (Wenzl *et al.* 2001; Fåk *et al.* 2008) and pigs (Nowland *et al.* 2022), that the amount of *Lactobacillus* in the intestine is higher during the first weeks of life than closer to weaning. Although increased numbers of *Lactobacillus* may be beneficial for piglets, natural colonisation of the microbiota and higher natural occurrence/abundance of LAB in the first weeks may possibly limit potential effects of additional supplemented LAB. Indeed, studies have found that higher supplemented doses may cause an imbalance in bacterial structure and result in a higher excreted rate in faeces (Guerra *et al.* 2007).

The LAB probiotic supplement did not exhibit any effects on the gut microbiota in rectal swabs, however, microbiota modulation was found to be different depending on the gut segment. Changes in the relative abundance of several microbial genera were detected in the ileal mucosa in piglets supplemented with probiotic compared to control in Paper II. Foremost, a decreased abundance of *Bacteroides* and *Lactobacillus* were found in ileum mucosa compared to colon mucosa in the probiotic group of piglets, whilst the *Prevotella* 2 had higher abundance in the probiotic group in ileum mucosa compared to the control group. Supplement of *L. plantarum* has been reported to increase the abundance of *Prevotella* in weaned pigs (Park *et al.* 2024). *Lachnospiraceae* _NK41136 were also found in higher abundance in the probiotic group than in control group. Similar findings were observed in weaned pigs feed a fibre rich diet (Petry *et al.* 2021), indicating the possible enhanced maturation of the microbiota composition in ileum in suckling piglets supplemented with LAB. An earlier study showed that piglets younger than three weeks of age harbour a low microbiota diversity in the gut and are more susceptible to infection and pathogen adhesion to mucosal surface (Han *et al.* 2021). However, this also presents a good prospect for early modulations that appear to be different depending on the intestinal segments and may plausibly explain the differential effects on microbial modulation of colon and ileum in supplemented piglets in Paper II. Intestinal diseases such as PWD primarily affect the composition of the microbiota in the small intestine, despite the majority of research being focused on occurrences in the colon. *Escherichia coli* attach to the small intestine and initiate the colonisation and infection (Kim *et al.* 2022). The ileum plays an important role for the evaluation of probiotic effects on the microbiota. This is because of the close interplay between the host and the ileum microbiota

due to many immune cells, lower mucus secretion, and looser junctions compared to the colon and a more direct interaction with epithelium, opening the possibilities for modulations of microbiota (Duarte *et al.* 2022; Jensen *et al.* 2023). *Lactobacillus* have good binding ability to the intestinal mucosa, can protect and support the intestinal epithelial cells, and exclude pathogenic bacteria either by occupying binding sites on intestinal mucosa or by competing for nutrients with pathogenic bacteria (Dowarah *et al.* 2017). However, *Lactobacillus* can also have a perfect mutual cooperation with other beneficial bacteria that can utilise available nutrients and by-products (Aleman *et al.* 2024). The transient time of LAB probiotics may have been short and therefore not found in increased numbers in ileum mucosa in Paper II, but the supplement with *Lactobacillus* could still have had a positive influence on the gut microbiota. Microbes such as *Prevotella* and *Lachnospiraceae* can degrade complex carbohydrates and provide energy whilst *Lactobacillus* can produce microbicidal substances thereby inhibiting pathogenic bacteria that may compete for the binding site on the intestinal epithelial cell surface with *Prevotella* and *Lachnospiraceae*. Furthermore, because of the ability to break down mucin *Lachnospiraceae* can promote the turnover of the mucus layer and help *Lactobacillus* to protect the gut from pathogens (Vacca *et al.* 2020). Similar cooperative interactions were found in previous studies where *Lactobacillus* was in a synergic relationship with *Lachnospiraceae* and *Ruminococcaceae.* They cooperatively treated colitis and worked together with other bacteria such as *Bifidobacterium* to influence the overall gut health and maintain the stability and balance of the gut microbiota (Li *et al.* 2023). However, it is still unclear how developed the ileum microbiota is early in life, as well as the ability of ileum to digest available nutrients and how this complicated cooperation between the microbiota and epithelial cells works as the research is insufficient. Still, the available data shows that increased depth of the crypts in ileum, usually associated with maturation and post-weaning, was positively correlated with the abundance of *Prevotella* and *Lachnospiraceae* (Lu *et al.* 2021). This may indicate a possible accelerated maturation of the ileum physiology and its microbiota in Paper II in supplemented piglets, however, the histological analysis of tissue samples did not reveal any detectable differences in villi height and crypt depth between the probiotic and control groups.

Assessing the impact of *Lactobacillus* strains studied so far is challenging, due to numerous variables in different published studies (Zhu *et al.* 2022b;

Hou *et al.* 2015: Wang *et al.* 2021b), including variations in dosage, age, and health status of piglets. Moreover, a combination of two bacterial strains does not necessarily have a good synergy, as sometimes they appear to compete for nutrients and adhesion to intestinal cells, and therefore may reduce the efficacy of the mixture (Chapman *et al.* 2012). Both *L. reuteri* and *L. plantarum* produce exopolysaccharides to increase intestinal adhesion and have ability to co-regulate anti-inflammatory cytokines and promote immunity as well as intestinal morphology thus improving growth performance in pigs (Hou *et al.* 2015, Lee *et al.* 2012). It has been shown that a combination of *Lactobacillus* strains can cause a stronger inhibition of photogenic bacteria such as *E. coli*, *Salmonella enteritidis,* and *Enterobacter sakazakii* (Drago *et al.* 1997; Collado *et al.* 2008). However, in other studies it appears that *L. plantarum* was more efficient when piglets were exposed to photogenic *E. coli* compared to *L. reuteri* (Lee *et al.* 2012; Yang *et al.* 2014). Tang and co-authors (Tang *et al.* 2021) have observed an inefficiency in a study on effects from supplementing *L. reuteri* and *L. plantarum* to weaned piglets, where they hypothesised that *L. plantarum* was unable to excrete a significant amount of bacteriocins without being challenged. This may be relevant to the study in Paper II as all animals in the experiment were healthy and the environment on the research farm met high hygiene standards that may have restricted the potential probiotic efficiency on the gut health.

Another important factor to consider is how different strains of *L. reuteri* and *L. plantarum* can have varied effects on gut microbiota composition due to their unique genetic profiles and functional properties, which may provide another explanation for the variations in results between studies. The strains of *L. reuteri* and *L. plantarum* that were utilised in the present study have been identified for their potential to influence and modulate the behaviour via the gut-brain axis.

6.1.3 Other confounding factors in Papers I and II

Experiments in this thesis were conducted at the Swedish Livestock Research Centre, which is a farm containing Specific Pathogen Free (SPF) pigs. This means that the animals have high growth rates, high health levels, a significantly lower medicine consumption, and high hygiene standards compared to facilities/countries without a SPF-system or many other

countries that are also connected to the SPF-system but with a lower SPF grade (Swedres-Svarm, 2023). A lower usage of medicine in sows during gestation results in piglets with a higher body mass and more developed intestines compared to piglets from mothers that were administrated more medicine during gestation (de Greeff *et al.* 2020). Those piglets may cope better with weaning stress compared to piglets with a lower body weight (Declerck *et al.* 2016; Winter *et al.* 2023). Studies also found a correlation between the abundance of microbes and birth weight. A lower birth weight resulted in a lower abundance of the genus *Lactobacillus* spp., *Prevotella* spp., and *Faecalibacterium* spp. in ileum and colon (Li *et al.* 2019). *Lactobacillus* strains seem to be adaptable to the host and have a different competing potential depending on the host microbiota. They are found to be more active in an environment with a presence of harmful bacteria compared to a disease-free environment (Huang *et al.* 2022b). Therefore, effects of the supplements used in Paper II could have potentially been more pronounced on farms with lower hygiene standards, higher disease incidents, and in piglets with a lower growth rate.

Considering that no effect of treatment was found in microbiota from swab samples in any of the studies and all animals came from the same environment, no major differences were expected between the experimental trials when the microbiota in the rectal swabs were compared at 4 weeks of age. Still, some differences were found, such as the difference in the abundance of *Prevotella* spp (Figure 8). This may be due to the maternal influence on piglets microbiota as we observed significant litter effects (Figure 7), but it could have also been due to methodological differences. For example, different bioinformatics approaches for generation of the 16S rRNA gene datasets. It is also worth noting that a high individual variation was present throughout the suckling period in both Papers I and II, as well as strong effects of sex. The microbial variations between sexes are yet to be extensively researched in pigs, especially in young animals (Pluske *et al.* 2019). However, the overall gut microbiota composition in Paper II had a higher abundance of *Enterobacteriaceae* during the suckling period when compared to the microbiota in Paper I, regardless of treatment, sex, or litter. Furthermore, results on the abundance of microbiota community may diverge from other studies due to differences in management practice. The piglets in the present experiments (Papers I-III) had access to creep feed

during the suckling period, from 5 days of age , whilst piglets in some of the other published studies were only suckling sow milk or had access to creep feed with different fibre compositions. Microbiota was shown to be modulated by creep feed when compared to piglets fed solely sow milk (Lerch *et al.* 2023). Creep feed in all experiments presented in this thesis contained both soluble and insoluble fibres. Although the amount of consumed feed was not recorded in experiments, it may have influenced the results and potentially masked certain effects of the supplements.

Another important factor that may have had an impact was the selected study design of the present studies. Piglets were provided with supplements in all three studies within the same litter. The split-litter design separating the effects of the sow and the effects of the diet was shown to be significant in another study in young pigs (Craig *et al.* 2019). However, within-litter supplemented piglets had several advantages, for example, the same genetics and early life litter exposure, maternal influence, and early life environment. Additionally, all animals in both the control and treated group of piglets were sampled within a short time period, reducing the bias and avoiding confounding factors that could mask the true effects of a treatment. However, due to suckling and exploring habits, piglets are influenced by their mother's and environmental microbes. One negative aspect of this design is the risk of cross-contamination between supplemented piglets and controls, and this may have impacted the results in the present studies. Although plausible across all studies, this may be particularly important in a probiotic study, where a possible microbial cross-contamination may cover and/or reduce the potential effects of the probiotic supplement. For example, the abundance of *Lactobacillus* in the probiotic study in Paper II was higher than the β-glucan study but did not differ between the control and supplemented groups. This may be due to the occurrence of allocoprophagy i.e. feeding on faeces from other piglets.

Although no such observations were recorded in the present studies to be able to fully assess the importance of coprophagy and make any final conclusions about the possible influence on the results, it must be considered. Furthermore, it should be noted that there was a high within-group (treatment) variation present in all experiments and in many variables, which may have obscured treatment effects.

Together, these factors, as well as their possible interactions, may have been the reason for the limited impact of supplements and should be taken into account when assessing the results of studies.

6.2 Short-chain fatty acids

Paper I

The results of the short-chain fatty acids analysis in Paper I showed, for the most part, differences linked to age. Both plasma and digesta samples were collected for analysis. The concentration in plasma samples only differed between β- glucan supplemented animals and controls for acetic acid, where the concentration was lower in the supplemented group of piglets compared to the control group piglets. Still, it is important to note that the concentration was already higher in the control group of piglets at the beginning of the experiment and may simply reflect individual differences unrelated to the treatment. All SCFA in plasma, except for formic and succinic acid, increased with age. Despite knowledge about the possibility of young animals to utilise a fibre rich diet being limited one study found that pigs at the beginning of the growing stage (weighing around 20kg) supplemented with fibre showed an increased total SCFA concentration in faecal samples (Gao *et al.* 2022). However, reported results seem to vary between studies. In one study, an oat bran supplement given to pigs had a less prominent effect on the concentration of SCFA when compared to other fibres containing the same fibre content such as wheat bran, corn bran, and rice bran when samples were taken from ileal content in cannulated pigs and from faecal inoculum-based fermentation (Bai *et al.* 2021). A supplement containing oat was found to enrich levels of butyric acid in pigs in the stomach, caecum, and colon digesta (Metzler-Zebeli *et al.* 2011), whereas in other studies butyric acid in caecal and colonic digesta was not affected by oat bran supplement (He *et al.* 2018). A clear difference was found in the concentration of SCFA between the colon digesta, portal, and jugular vein in Paper I (Figure 9). The reduced proportion of propionic and butyric acid in the jugular vein

compared to the portal vein suggests that those acids were metabolised in the liver.
Differences between studies could be linked to the high fermentability rate of oat bran that may have been digested differently along the gastrointestinal tract, starting already in the stomach or/and in the proximal part of the intestine. Although less fermentable, complex carbohydrates mainly affect the microbiota in the colon, some studies have found that metabolic activity already starts in the stomach of weaned piglets (Leterme *et al.* 2000). Production of SCFA, especially butyric acid in the upper part, may be more important in young animals due to mucosal maturation of the stomach and small intestine (Tsukahara *et al.* 2003; Claus *et al.* 2007; Mazzoni *et al.* 2008). An increased concentration of SCFA in colon digesta samples was found when young animals were supplemented with less digestible oat hull (Van Hees, 2023). The β-glucans with the lower molecular weight are more fermentable by gut microbiota. The fermentation has been shown to be higher and production of SCFA faster in digesta of pigs, notably acetic and propionic acid due to the dominance of bacteria such as *Prevotella* and *Bacteroides* (Lambo *et al.* 2005, Holtekjølen *et al.* 2014a). The rapid fermentation that possibly occurs in the proximal colon leads to an early release of metabolites and may lower the total concentration of SCFA in faecal samples. The higher molecular weight of β-glucans obtained in the study in Paper I were found to be more slowly fermented and enhanced the production of butyric acid (Volman *et al.* 2008). However, an increase in the butyric acid was present in all animals with no difference between the supplemented pigs and the controls.

Paper II

The concentration of SCFA in Paper II was analysed from blood samples from both living and euthanised animals. *L. plantarum* and *L. reuteri* are known to produce organic acids, but whilst *L. plantarum* have been reported to produce lactic acid, *L. reuteri* can produce both lactic and acetic acid (Shah *et al.* 2024). A recent study reported the ability of *L. plantarum* to produce other SCFA as well, such as formic acid, acetic acid, propionic acid, and butyric acid *in vitro* in MRS broth, highlighting the potential of *L. plantarum* strains to provide an acidic environment unsuitable for many pathogenic microorganisms (Jang *et al.* 2021). Previous studies have reported that an

oral intake of *L. plantarum* increased the faecal SCFA concentration of post-weaned piglets (Thu *et al.* 2011) and SCFA in colon digesta at weaning (Wang *et al.* 2019). *L. reuteri* was found to increase the concentration of butyrate in colonic digesta during suckling (Liu *et al.* 2014) and in the faeces of weaned piglets (Zhang *et al.* 2019c). No differences were found between piglets in the probiotic and control group in blood plasma samples at weeks 4 and 6. Isobutyric, succinic, and caproic acid were higher at week 4 than week 6 of age in Paper II, whilst all of the other SCFA concentrations increased with age but with no detectable connection to the treatment. This progression and increase of SCFA with age is in line with results from other studies where SCFA concentation was taken from both blood and faeces, transiting from the fermentation of disaccharides and oligosaccharides from maternal milk to the fermentation of fibre-rich solid feed (Nakatani *et al.* 2018; Qi *et al.* 2021a; Beaumont *et al.* 2021). Aside from the available nutrients, SCFA production is affected by the age of the animals, organ size, and amount and composition of the intestinal bacteria. Depending on the substrate, both acetate (Amat *et al.* 2020) and propionate (Poeker *et al.* 2018) can be produced by *Prevotella* spp. Further, acetate may be used by taxa such as *Ruminococcaceae* and *Lachnospiraceae* to produce butyrate (Macfarlane and Macfarlane, 2012; Lan *et al*. 2023). Although both *Prevotella* and *Lachnospiraceae* were more abundant in ileum digesta in probiotic supplemented piglets, changes in SCFA concentration were not detected between the treatment and control group in either jugular or portal vein plasma. This could be due to the small sample size and individual differences but also the fact that plasma SCFA may not have reflected the actual production and absorption of the SCFAs (Nakatani, 2018).

In young animals, SCFA may be utilised and metabolised differently due to the ongoing development of the intestinal tract. Expression of SCFA transporters into epithelial cells or bloodstream may be lower in young animals, thereby limiting the absorption of SCFA. In young animals, para-cellular transport is more prominent due to immature and looser tight junctions and higher intestinal permeability (Metzer-Zebeli *et al.* 2022). Colonocytes may not have the capacity to metabolise butyric acid and may use other energy sources in the first weeks of life (Tanel *et al.* 2010). However, the lack of clarity hinders any final conclusions as the specific knowledge of the absorption, transportation, and metabolism of SCFA in suckling piglets is not yet fully researched and established.

6.2.1 Comparison between SCFA concentrations in Papers I and II

Comparisons of the concentrations of acetic, propionic, and butyric acid from plasma samples taken in experiments from pigs supplemented with specific prebiotics or probiotics during suckling at 4 weeks of age revealed differences between the studies (Figure 10). Butyric and propionic acid were numerically higher in the prebiotic group when compared to the probiotic group of piglets, but with large individual variations. Maternal influence may be another reason. SCFA from milk from different mothers contains various SCFA concentrations and bacterial diversity and compositions that may have had some direct influence on the SCFA concentrations or/and influence on faecal microbiota composition in piglets and consequently the SCFA concentrations. This correlation has been confirmed in a recent human study (XI *et al.* 2024) and may be relevant in pigs as well.

Plasma samples taken from euthanised animals revealed considerably higher SCFA concentrations in plasma samples taken from the portal vein compared with the jugular vein (Figure 11) regardless of the treatment, indicating that SCFA were metabolised in the liver or directly in the gut mucosa. However, due to considerable individual variations and the limitation of a small sample size, it is hard to know if that is the correct conclusion. It is worth noting another recent human study that found strong correlations between SCFA concentrations in infants' plasma and SCFA concentrations in maternal plasma but weak correlations between SCFA in mothers' milk and SCFA concentrations in infants' blood plasma (Barman *et al.* 2024). The researchers hypothesised that infants use SCFA in mothers' milk to fulfil the needs of the upper gastro-intestinal epithelia or/and SCFA in mothers' milk, which is converted into glucose or other acids in the liver when it arrives via the portal vein. All piglets in the present studies were suckling sow milk at week 4 of age. This may partially explain the higher SCFA concentrations in the portal vein than in both the jugular vein (Papers I and II) and digesta (Paper I).

However, at present we lack any experimental evidence that would support all these possible correlations in pigs and, if so, to what extent. These

findings simply confirm the complexity of the physiology and multiplexity that gut microbiota and SCFA may have early in a piglet's life.

6.2.2 Correlations

The correlation analysis was performed between certain bacterial taxa from rectal swabs and the concentration of SCFA in blood, in both Papers I and II at 4 weeks of age with the aim of searching for associations between microbiota and SCFA data (Figure 12). In Paper I, several bacterial taxa were both positively and negatively correlated to SCFA concentrations in blood plasma. Correlations followed and reflected the microbiota development, where the decreased abundance of *Lactobacillus* and *Bacteroides* resulted in a negative correlation with acetic and propionic acid. In contrast, the increased abundance of *Prevotella* was positively correlated to acetic, butyric, and valeric acid. *Lactobacillus* spp., *Bacteroides* spp., *Bifidobacterium* spp., *Prevotella* spp., and *Ruminococcus* spp. are the most important producers of acetate in the gastrointestinal tract. Propionate producing bacteria are *Bacteroides* spp. and *Bifidobacterium* spp., (Koh *et al.* 2016; Feng *et al.* 2018; Vasgues *et al.* 2022). The shift in bacterial population may explain the negative correlation between acetic and propionic acid and *Lactobacillus* and *Bacteroides* in Paper I. The production of acetic and propionic acid was not affected due to the microbiota succession influenced by age; a higher abundance of another bacterial group replaced the *Lactobacillus* spp., *Bacteroides* in the production of acetic and propionic acid at 4 weeks of age. At week four, *Prevotella* was one of the most abundant bacteria in all piglets; unsurprisingly, both treatments showed a positive correlation between *Prevotella* spp. and acetic acid. However, prominent individual variations in both studies may have obscured certain correlations in complex correlational patterns across treatments. Another fact to consider is that the correlations were between blood plasma samples and rectal swab samples. Rectal swab samples may not always be the most accurate proxies for microbiota in the cecum and proximal colon, where the majority of SCFA production occurs in addition to the already mentioned process of SCFA before reaching the systemic circulation. All of these factors may have influenced correlation results either individually or cumulatively.

6.2.3 Other factors possibly affecting SCFA production

The production of SCFA early in life has not been investigated much. However, based on the available data, it appears that it is dependent on a combination of factors. Besides the gut microbiota composition and the available nutrients, both the size of the gastrointestinal tract and the site to measure gut SCFA concentration plays a role and may affect the results. The concentration and proportion of SCFA vary in different regions of the gut. Some studies found that the proximal colon showed a higher concentration than the distal colon, whilst the distal colon had a similar concentration to the rectum content (Lange *et al.* 2023). Moreover, notable differences in the concentration among individuals have been reported (McOrist *et al.* 2011; Beaumont *et al.* 2021) and are also found in all experiments in this thesis.
Additionally, it is important to note that SCFA production in the experiments in this thesis may have originated from the fermentation of fibre ingredients in available feed. In most of the other studies examining the effects of different prebiotics and probiotics, piglets did not have access to anything but milk. Piglets in all experiments in this thesis had access to creep feed during the suckling, from 5 days of age, which may have influenced the results.

6.3 The role of β-glucan supplement on cognition in suckling piglets and the potential link between gut microbiota and behaviour

Paper III

The study in Paper III investigated the effects of oat β- glucan on behaviour and its potential links to gut microbiota composition and SCFA. Overall, the supplement did not significantly impact the evaluated behavioural observations. However, the result in which the β- glucan supplemented group exhibited poor reversal learning was intriguing, as β- glucan is generally associated with cognitive benefits, induced levels of brain- derived neurotrophic factor expressed in the brain, and neuroprotective effects (Huang *et al.* 2011; Gomez-Pinilla *et al.* 2012; Zhou *et al.* 2019). A plausible explanation may be due to the modification of the brain signalling of serotonin and dopamine by β- glucan. Studies have found an association between low serotonin concentrations and impairments in reversal learning (Clarke *et al.* 2004; Lapiz-Bluhm *et al.* 2009). A study on ducks found that a low dosage of β- glucan reduced levels of serotonin and increased dopamine levels in plasma samples, whereas the opposite effect was recorded when a high dose was supplemented (Mahmoud *et al.* 2021). Another study with a rat model supplemented with oat extract in doses of 100, 200, and 400mg/kg diet/day for 5 weeks found a significant increased dopaminergic signalling in the group that received the lower dosage and an increase in serotonin levels with higher dosages (Ehab *et al.* 2025). This suggests that in Paper III, the dosage of 40g/kg body weight of β- glucan three times per week may not have been sufficient to produce cognitive benefits in piglets. Stressful conditions and social dynamics can affect neurotransmitter levels. For instance, pigs subjected to stressors such as transportation or social hierarchy changes exhibit alterations in serotonin and dopamine systems. One study found that pigs experiencing aggression had reduced serotonin and dopamine in mixed animals (Shen *et al.* 2020)
The microbiota composition and the microbial metabolites were not affected by the β- glucan supplement. Nevertheless, there were a number of correlations between certain behaviours and gut microbiota in Paper III, with no connection to the treatment. Gut microbiota have been shown to play an

important role in shaping behaviour during the early development of brain structure and function (Dash *et al.* 2022). Previous studies have identified specific bacteria that appear to be influential in this partnership. For instance, the abundance of *Lachnospiraceae*, a common butyrate producing group of bacteria in the gut, are found to be linked to various behaviours across species, potentially through mechanisms involving metabolic pathways that produce neuroactive compounds. Members of this taxa were identified as key bacteria that can benefit infant neurodevelopment early in life (Oliphant *et al.* 2021). Studies have also found associations with feeding behaviour, where *Lachnospiraceae* have been positively correlated to food intake in pigs (Luo *et al.* 2022a), humans, and mice (Samulenaite *et al.* 2024). Another reported association was connected to aggressive behaviour among rats where more aggressive rats had a higher abundance of *Lachnospiraceae* in their gut microbiome (Voulgari-Kokota *et al.* 2024). In addition, an association was also linked to tail biting behaviour in pigs, where the biters exhibited a higher abundance of *Lachnospiraceae* (Verbeek *et al.* 2021). Heat stressed pigs were also shown to have an increased abundance of *Lachnospiraceae* (Ringseis *et al.* 2022). This is consistent with another study on mice where the lower abundance of *Lachnospiraceae* was found to be potentially associated with reduced early life stress (Otaru *et al.* 2024). The results in Paper III found *Lachnospiraceae* bacteria to be negatively correlated with different behaviours, such as standing at weeks 5 and 7 as well as exploring at week 5. This relationship may imply a direct association of stress and aggression to physical activity and *Lachnospiraceae* may play a role in this dynamic.

Another bacteria that correlated negatively with exploration, locomotion, and standing at weeks 5 and 7 in Paper III was *Ruminococcaceae*. Previous studies found that anxiety and depression were positively correlated with the abundance of *Ruminococcaceae* in mice (Kang *et al.* 2014; Jin *et al.* 2021) as well as tail biting in pigs (Verbeek *et al.* 2021). Another study found that greater depression and anxiety in humans were linked with a decreased abundance of *Ruminococcaceae* (Humbel *et al.* 2020). In a different study, the voluntary exercise behaviour among rats in a wheel running activity was found to be positively influenced by an increased abundance of *Ruminococcus bromii,* suggesting its potential role in promoting exercise behaviour (Rusling *et al.* 2024). The differences between studies highlights that the effects of *Ruminococaceae* on cognition and behaviour are complex, linked to both beneficial and detrimental outcomes, and may be dependent on the specific species and their metabolic products produced in the gut microbiome. Further research is necessary to delineate the specific

mechanism by which individual strains influence the gut-brain axis to develop targeted therapeutic strategies.

Negative correlations were found between *Treponema* and exploring at week 5 and 7, and also standing at both weeks 5 and 7. Several studies found associations between the abundance of *Treponema* species and behaviour. Research focused on pigs in relation to *Treponema* is mainly focused on a skin infection that can cause shoulder ulcers, ear necrosis, and, consequently, additional stress and damaging behaviour (Boyle *et al.* 2021). However, some studies on pigs investigated the impact of social stress (mixing with unfamiliar piglets and reduced space) and found that stress led to an increased abundance of *Treponema* in faecal samples (Nguyen *et al.* 2023; Clavell-Sansalvador *et al.* 2024). Further, several studies on humans and animals linked a *Treponema pallidium* infection to microbiota alterations, behavioural changes, cognitive impairments, and learning difficulties, along with reduced motivation and decreased interest in new activities (Roy *et al.* 2016; Wang *et al.* 2022b; Fadel *et al.* 2024). A bacterial group that had a significant positive correlation with behaviours was *Fusobacterium*. At week 5, exploring was positively correlated to *Fusobacterium* and at week 7 both locomotion and standing were positively correlated to *Fusobacterium*. Certain studies reported a relationship between stress, depression, and an increased *Fusobacterium* abundance in faecal samples (Paudel *et al.* 2022; Dong *et al.* 2021). However, a review article by Hantsoo *et al*. (2021) emphasises the complexity of the interplay between stress and the gut microbiome. Their findings revealed a decreased abundance of *Fusobacterium* in Caucasian women exposed to stress, whilst Black women exposed to stress had an increased abundance of *Fusobacteium*, suggesting that stress may influence *Fusobacterium* levels differently across diverse populations.

The negative correlations that were found between several bacteria and active behaviour in pigs in Paper III, although unrelated to the treatment, may be of interest as it may imply a connection between certain bacteria and stress. This offers a possible preventive strategy against post-weaning stress disorders, which would improve resilience early in life and revolutionise modern farming, by making it both more sustainable and profitable. The statistical correlations found in the current study suggest a relationship, but only experimental research can verify the cause and effect. Nevertheless, statistically identifying bacteria capable of modulating stress responses early in life is valuable information due to the gut microbiome's significant role in brain development and long-term health.

6.3.1 Other possible factors which potentially explain the poor reversal learning and correlations in Paper III

The supplementation with yeast β- glucan alleviated cognitive and brain function (Zhang *et al.* 2023) but β- glucan derived from different sources may have different effects on cognition. The results from a study indicated that although all three types of beta-glucans (mushrooms, curdlan, and oats bran) enhanced recognition memory, mushroom-derived β-glucan significantly increased synaptic function and the plasticity responsible for learning and decision-making, whilst the other two sources did not display these effects. The same study also found that different sources affected the gut microbiota composition and brain inflammation differently and that the cognitive effects of β-glucan may differ based on the source of the β-glucans and its molecular structure (Hu *et al.* 2022). Solubility, complexity, and degree of polymerisation directly affect the microbiota composition, which in turn influences brain function via the gut-brain axis (Hu *et al.* 2022; Meng *et al.* 2022; Martin *et al.* 2018). Furthermore, variations in the preparation of β-glucan are attributed to differences in sources and extraction procedures, leading to significant differences in their effects (Murphy *et al.* 2020; Cognigni *et al.* 2021).

The effects may depend on the duration of supplementation. For example, long-term oat β- glucan supplementation resulted in improved cognitive performance in mice, whilst short-time supplementation of β- glucan in mice failed to produce beneficial cognitive effects (Shi *et al.* 2020).

Individual differences and piglets' baseline health status may influence the response to different β- glucan supplementation. Several studies on other animals suggest that healthier animals may receive different benefits from the supplement compared to those who were under pathogenic challenges or/and under stress. Piglets that were not subjected to the *E. coli* challenge showed less pronounced benefits from the β- glucan supplementation compared to those that were challenged (Zhou *et al.* 2022b). Studies on broiler chickens exposed to heat stress also found that β- glucan supplementation was more beneficial in animals that were under stress than those kept under normal conditions (Zhang *et al.* 2020).

When comparing the study results in Paper III to those mentioned above, all piglets, regardless of the treatment, were handled in approximately the same way and exposed to the same new environment. However, piglets fed with β- glucan may have been exposed to certain stress levels due to the oral supplement's unfamiliar taste, palatability, and texture, which could have induced greater stress on those animals than the control group.

Weaning is a stressful event and all piglets were weaned before the cognitive test was performed. However, both rectal swab and jugular blood plasma samples were collected before weaning. The combination of pre-weaning stressors and the abrupt changes introduced during weaning could have led to compounded stress responses.

Stress and elevated cortisol levels have been associated with impairment in cognitive flexibility. One human study found that acute stress exposure and elevated cortisol levels impaired both working memory and inhibition, functions that are essential for task requiring cognitive flexibility, including reversal learning (Shield *et al.* 2016).

When experiencing chronic stress, the body's HPA axis can adopt or become dysregulated, which may lead to a blunted cortisol response to familiar stressors and unchanged or lower baseline cortisol concentrations. This adaptation does not mean that the animals are free from stress; it may simply mask the hormonal indicators whilst still allowing chronic stress to negatively affect functions such as learning and memory (McEwen *et al.* 1993). It is important to add that although the gut microbiota communicates with the brain, no differences were detected between the control and treated groups of piglets regarding microbiota composition. Some microbiota disturbance in piglets that were possibly exposed to stressful situations may be expected. Still, one should also consider that in healthy pigs, gut microbiota may have recovered quickly after a stressful event depending on the specific microbial communities involved. Both *Lachnospiraceae* and *Ruminococaceae*, which were, according to the literature, associated with stress-related behaviours, are part of the normal gut microbiota and play important roles in gut health and stability, potentially adding to quicker normalisation after stress. A disturbance in these populations may induce stress, but their robust presence in the gut microbiota could suggest that they facilitate a faster return to baseline microbiota after exposure to stress. The relation between microbiota recovery and the restoration of cognitive function following stress is multifaceted, and timelines may not be synchronised. Cognitive recovery may involve various brain regions and mechanisms beyond microbiota normalisation. Early life microbiome may have "set the stage" for post-weaning brain function. The current understanding of this process is not yet clear enough, therefore any conclusions at this stage can only be speculative. Piglets in the β-glucan group also showed fewer affiliative interactions than the controls and were less interested in exploring the T-maze.

Another factor that may impact behaviour and microbiota correlations is immune system development before weaning and the reorganisation after weaning. Immune modulations, which in turn affected the brain, may have

been affected by *Fusobacterium* and *Treponema* (Kaminiow *et al.* 2024; Lee *et al.* 2014). Systematic immune responses may have long-term effects on brain function and development (Bilbo *et al.* 2012). Contrarily, *Lachnospiraceae* and *Ruminococcaceae* are linked to maintaining and balancing immune responses (Zhao *et al.* 2018; Vacca *et al.* 2020). Whilst this complex interplay between systematic immune responses, brain development, behaviour, and microbiota falls outside the scope of this thesis, it is important to mention as a potential factor that may have influenced the results.

7. Conclusions

Pre-weaning dietary interventions could play an important role in shaping piglets' microbiota and improving their gut health, ultimately enhancing their overall robustness.

The general conclusion of this thesis is that early supplementation with β-glucan and a mixture of *L. reuteri* and *L. plantarum* did not significantly alter the overall composition of microbiota or the production of SCFA in piglets throughout the suckling period. However, local effects on the microbiota in specific segments and the influence of treatment on reversal learning, together with correlations between specific bacterial taxa and behaviour, indicate the potential of the treatments early in life.

Specific conclusions:

- Supplementation with β-glucan did not affect microbiota development. Differences were primarily associated with age-related microbiota development and the production of SCFAs. The abundance of *Prevotella* increased across all piglets throughout the suckling period, irrespective of the treatment. This suggests that factors such as age, rather than specific interventions with oat β-glucan, play a significant role in shaping *Prevotella* populations during early development.
- Supplementation with *L. reuteri* and *L. plantarum* had some localised effects on the microbiota composition. The abundance of *Prevotella 2* increased in the ileum mucosa of probiotic piglets. At the same time, the proportion of *Lachnospiraceae_NK4136* in the probiotic group was enriched in the ileum mucosa, colon mucosa, and colon digesta during the suckling period. These results suggest that the probiotic supplement may stimulate the microbiota development in the ileum early in life.
- No detectable impact on gut microbiota composition was observed following the β-glucan supplementation, suggesting that behavioural effects were not mediated through microbiota modulation.
- The implications of the β-glucan supplement on reversal learning suggest that cognitive modulation may occur through pathways not directly linked to microbiota changes. These results emphasise the need for additional research that can build on these

findings to explore the broader implications of microbiota in cognitive functions and behavioural regulations.

- The observed significant correlations between particular bacterial taxa and specific behaviours point to possible indirect involvement in behavioural traits. Determining which bacteria play direct or indirect roles in particular behaviours is still in its early stage and remains an area requiring further research.

8. Future perspectives

Early nutritional interventions in pigs hold significant potential for improving gut health, and establishing a balanced gut microbiome and SCFA crucial for digestion, immunity, and overall growth of pigs, as well as regulation of early brain development. These interventions may reduce the occurrence of PWD and mitigate negative behaviours in pigs, improving welfare and enhancing overall productivity and profitability in the pig industry.

The results in this thesis indicate possibilities for both the prebiotic and probiotic in the early stages of piglets' life. However, the results also highlight the importance of several factors and the complex interactions between the different measured parameters which should be carefully considered when implementing early-life interventions in research. Methodologies, measurements, and reporting practices should be more standardised to allow for easier comparisons of studies and results and improve the reliability and reproduction of studies. This would also minimise bias between studies, provide better data integration, and ensure consistency in result interpretation (de Vries *et al.* 2018; Amos *et al.* 2020). Seeing more multicentre research across multiple locations, institutions, or countries among different research teams would be interesting and beneficial, offering valuable insights. This approach would enhance scientific findings' reliability, generalisability, and impact by increasing sample sizes, reducing biases, and ensuring diverse representation. Multicentre studies would open the possibilities for more global research on early gut challenges and how early nutrition may affect gut health differently in various regions. It may bridge of gaps in knowledge more rapidly by allowing expertise from different disciplines and locations to collaborate (Hunniford *et al*. 2023;

Drude *et al.* 2021). Tailored individual approaches to early-life challenges around weaning in pigs is another futuristic idea that would utilise modern technologies and provide meaningful insights. Despite efforts to standardise and control for various variables, individual differences were evident across the studies in this thesis. However, it is important not to over-standardise either and to recognise individual differences and tailor interventions accordingly. Future research may improve that through precise monitoring with specific electronic identification tags that would track individual pigs' weight gain, feeding behaviour, health status, etc. Cameras and AI-based data collection and analysis would enable new insights into adaptive nutritional strategies on individual levels (Pineiro *et al.* 2014; Ghosh *et al.* 2024). More comprehensive information would provide further details about customised probiotic approaches, taking the piglet's unique microbiota composition and health status into account. It would enable a tailored plan to select targeted strains of bacteria that would have distinct effects on every individual piglet's needs and goals. Certain strains are more effective for boosting immunity, whilst others may improve gut health and digestion or brain development, decrease stress, and boost wanted behaviour. A customised approach would involve ongoing feedback, provide greater possibilities for adjustments, and offer more precise and effective solutions. Along with probiotics and prebiotics, symbiotics (a combination of both probiotics and prebiotics) may offer a more comprehensive approach to better gut health in young animals than just prebiotics and probiotics. The synergistic effects of symbiotics are when prebiotics nourish the probiotics, helping them to colonise the gut more effectively and leading to a more stable and resilient gut microbiome which is crucial to establish early in life (Quintero *et al.* 2022). Symbiotics may be a more comprehensive solution and more beneficial for underweight piglets with severe diarrhoea and unbalanced microbiota. However, given the high cost and complexity of some of these approaches, near-term strategies may involve methods that are already available but not yet fully exploited. Maternal probiotic supplementation may be one promising strategy. Administration of probiotics such as different *Lactobacillus* and *Bifidobacterium* strains to sows during late gestation and lactation can potentially influence the gut microbiota composition in piglets from birth. This may help piglets to establish a more balanced and robust microbiota (Wang *et al.* 2022a) and enhance gut health, immunity, and growth (Wang *et al.* 2021a). Studies on

mice have indicated that maternal probiotic supplementation could positively influence early brain development in offspring (Lopez-Tello *et al.* 2024).
Another solution is the routine usage of a capsule endoscopy called the Capsule for Sampling (CapSa), where a small capsule collects small intestine content in post-weaning pigs (Garcia Vinado *et al*. 2024). This non-invasive tool could open a new world of possibilities in gut health assessment. It represents a significant advancement, eliminating the necessity for surgical interventions or euthanasia. The application of an improved enhanced capsule design in dietary intervention studies early in life may allow for ethical research that can simplify the sample collection process, improve data accuracy, and enhance pigs' welfare.
Overall, the results in this thesis point to the need for further research. However, to understand the complex interplay between gut microbiota, SCFA, and behavioural outcomes early in piglets' lives and the potential of early nutritional intervention, some of these new and exciting approaches may significantly accelerate research and close the current knowledge gaps. A systems perspective approach in research may broaden the scope of inquiry, challenge existing assumptions, foster innovations, and open new research paths.
There is one thing we can say with certainty: the research on supplementation in piglets early in life and its role on cognition and the potential link between gut microbiota and behaviour is still in its infancy. The specific mechanisms underlying β- glucans effects in that early stage are not fully understood. Further research is necessary to elucidate the precise pathways through which β- glucans affect cognitive flexibility and reversal learning.
That being said, when looking at the results in Paper III and the correlations between certain bacteria and locomotor behaviours, together with impaired reversal learning, it is important to maintain a careful balance in modulating the gut microbiota early in life. Indeed, many gut bacteria exert a dual influence, affecting both feeding behaviour and stress-related responses, therefore, their effects are not unidirectional. This complexity means that efforts to modulate the gut microbiota early in life must be carefully balanced. Whilst certain microbial profiles may enhance feed intake and growth, they could also predispose animals to stress-related behaviours such as increased physical activity, aggression, or tail biting. Thus, the key lies in fine-tuning the microbiota to harness its benefits without triggering adverse behavioural outcomes.
This includes dietary interventions such as prebiotics and probiotics that may balance the gut microbiota, in addition to environmental enrichment such as

toys or objects to root that may help to reduce stress and aggression. Finding the right mix would minimise negative behaviours whilst maximising productivity. In the last decade, significant progress has been made in identifying strategies that can balance welfare, productivity, and practical application. However, further research is needed to uncover strategies that would be most effective, accessible, and beneficial for both animals and farmers.

References

Abe F, Ishibashi N, Shimamura S. Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. Journal of Dairy Science. 1995 Dec;78(12):2838-46.

Adeola, O.; King, D.E. Developmental changes in morphometry of the small intestine and jejunal sucrase activity during the first nine weeks of postnatal growth in pigs. Journal of Animal Science 2006, 84, 112-118.

Adhikari, B.; Kim, S.W.; Kwon, Y.M. Characterization of Microbiota Associated with Digesta and Mucosa in Different Regions of Gastrointestinal Tract of Nursery Pigs. International Journal of Molecular Sciences 2019, 20.

Ahmed, H.; Leyrolle, Q.; Koistinen, V.; Kärkkäinen, O.; Layé, S.; Delzenne, N.; Hanhineva, K. Microbiota-derived metabolites as drivers of gut-brain communication. Gut Microbes 2022, 14, 2102878.

Aleman, R.S.; Yadav, A. Systematic Review of Probiotics and Their Potential for Developing Functional Non-dairy Foods. Applied Microbiology 2024, 4, 47-69.

Amat, S.; Lantz, H.; Munyaka, P.M.; Willing, B.P. Prevotella in Pigs: The Positive and Negative Associations with Production and Health. Microorganisms 2020, 8.

Amos, G.C.A.; Logan, A.; Anwar, S.; Fritzsche, M.; Mate, R.; Bleazard, T.; Rijpkema, S. Developing standards for the microbiome field. Microbiome 2020, 8, 98.

Ansari, F.; Neshat, M.; Pourjafar, H.; Jafari, S.M.; Samakkhah, S.A.; Mirzakhani, E. The role of probiotics and prebiotics in modulating of the gut-brain axis. Frontiers in Nutrition 2023, 10.

Arnaud, A.P.; Rome, V.; Richard, M.; Formal, M.; David-Le Gall, S.; Boudry, G. Post-natal co-development of the microbiota and gut barrier function follows different paths in the small and large intestine in piglets. FASEB journal 2020, 34, 1430-1446.

Atta, K.A.; Nyachoti, C.M. Nutritional and Metabolic Consequences of Feeding High-Fiber Diets to Swine: A Review in Engineering 2017, 3, 716-725.

Aurora, R.; Sanford, T. Host Microbiota Contributes to Health and Response to Disease. Missouri Medicine 2015, 112, 317-322.

Aviles-Rosa, E.O.; Rakhshandeh, A.; McGlone, J.J. Preliminary Study: Depriving Piglets of Maternal Feces for the First Seven Days Post-Partum Changes Piglet Physiology and Performance before and after Weaning. Animals (Basel) 2019, 9.

Aviles-Rosa, E.O.; Surowiec, K.; McGlone, J. Identification of Faecal Maternal Semiochemicals in Swine (Sus scrofa) and their Effects on Weaned Piglets. Scientific Reports 2020, 10, 5349.

Bai, Y.; Zhou, X.; Zhao, J.; Wang, Z.; Ye, H.; Pi, Y.; Che, D.; Han, D.; Zhang, S.; Wang, J. Sources of Dietary Fiber Affect the SCFA Production and Absorption in the Hindgut of Growing Pigs. Frontiers in Nutrition 2021, 8, 719935.

Barman, M.; Gio-Batta, M.; Andrieux, L.; Stråvik, M.; Saalman, R.; Fristedt, R.; Rabe, H.; Sandin, A.; Wold, A.E.; Sandberg, A.S. Short-chain fatty acids (SCFA) in infants' plasma and corresponding mother's milk and plasma in relation to subsequent sensitisation and atopic disease. EBioMedicine 2024, 101, 104999.

Bassis, C.M.; Moore, N.M.; Lolans, K.; Seekatz, A.M.; Weinstein, R.A.; Young, V.B.; Hayden, M.K.; for the, C.D.C.P.E.P. Comparison of stool versus rectal swab samples and storage conditions on bacterial community profiles. BMC Microbiology 2017, 17, 78.

Bates, D.; Mächler, M.; Bolker, B.; Walker, S. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 2015, 67, 1 - 48.

Bearson, S.M.D.; Trachsel, J.M.; Bearson, B.L.; Loving, C.L.; Kerr, B.J.; Shippy, D.C.; Kiros, T.G. Effects of β-glucan on Salmonella enterica serovar Typhimurium swine colonization and microbiota alterations. Porcine Health Management 2023, 9, 7.

Beaumont, M.; Cauquil, L.; Bertide, A.; Ahn, I.; Barilly, C.; Gil, L.; Canlet, C.; Zemb, O.; Pascal, G.; Samson, A.; et al. Gut Microbiota-Derived Metabolite Signature in Suckling and Weaned Piglets. Journal of Proteome Research 2021, 20, 982-994.

Bedford, A.; Gong, J. Implications of butyrate and its derivatives for gut health and animal production. Animal Nutrition 2018, 4, 151-159.

Bilbo, S.D.; Schwarz, J.M. The immune system and developmental programming of brain and behavior. Frontiers in Neuroendocrinology 2012, 33, 267-286.

Bischoff, S.C. 'Gut health': a new objective in medicine? BMC Medicine 2011, 9, 24.

Blick, A.K.; Giaretta, P.R.; Sprayberry, S.; Bush-Vadala, C.; Paulk, C.B.; Boeckman, J.; Callaway, T.R.; Gill, J.J.; Rech, R.R. Comparison of 2 fixatives in the porcine colon for in situ microbiota studies. Journal of Animal Science 2019, 97, 4803-4809.

Boyle Laura A. , Edwards Sandra A. , Bolhuis J. Elizabeth , Pol Françoise , Šemrov Manja Zupan , Schütze Sabine , Nordgreen Janicke , Bozakova Nadya , Sossidou Evangelia N. , Valros Anna ,The Evidence for a Causal Link Between Disease and Damaging Behavior in Pigs, Frontiers in Veterinary Science, volume 8-2021

Bravo, J.A.; Forsythe, P.; Chew, M.V.; Escaravage, E.; Savignac, H.M.; Dinan, T.G.; Bienenstock, J.; Cryan, J.F. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proceedings of National Academy Sciences U S A 2011, 108, 16050-16055.

Broom, L.J.; Kogut, M.H. Gut immunity: its development and reasons and opportunities for modulation in monogastric production animals. Animal Health Research Review 2018, 19, 46-52.

Canibe, N.; Højberg, O.; Kongsted, H.; Vodolazska, D.; Lauridsen, C.; Nielsen, T.S.; Schönherz, A.A. Review on Preventive Measures to Reduce Post-Weaning Diarrhoea in Piglets. Animals 2022, 12, 2585.

Carabotti, M.; Scirocco, A.; Maselli, M.A.; Severi, C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Annals of Gastroenterology 2015, 28, 203-209.

Casertano M., Dekker M.,, Valentino V., De Filippis F., Fogliano V.,Ercolini D., Gaba-producing lactobacilli boost cognitive reactivity to negative mood without improving cognitive performance: A human Double-Blind Placebo-Controlled Cross-Over study. Brain, Behavior, and Immunity 2024, 122, 256-265.

Celi, P.; Cowieson, A.J.; Fru-Nji, F.; Steinert, R.E.; Kluenter, A.M.; Verlhac, V. Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production. Animal Feed Science and Technology 2017, 234, 88-100.

Chapman, C.; Gibson, G.; Rowland, I. In vitro evaluation of single- and multi-strain probiotics: Inter-species inhibition between probiotic strains, and inhibition of pathogens. Anaerobe 2012, 18, 405–413.

Chen, T.; Long, W.; Zhang, C.; Liu, S.; Zhao, L.; Hamaker, B.R. Fiber-utilizing capacity varies in Prevotella- versus Bacteroides-dominated gut microbiota. Scientific Reports 2017, 7.

Chen, W.; Mi, J.; Lv, N.; Gao, J.; Cheng, J.; Wu, R.; Ma, J.; Lan, T.; Liao, X. Lactation Stage-Dependency of the Sow Milk Microbiota. Frontiers of Microbiology 2018, 9, 945.

Chen, Z.; Liu, Z.; Chen, C.; Fu, Y.; Wang, H.; Zhao, C.; Zhang, J.; Liu, L. The Effect of an Early-Life Lactiplantibacillus plantarum LPJZ-658 Intervention on Performance and Gut Microbiota in Suckling Piglets. Microbiology Research 2025, 16, 41.

Cheng, W.Y.; Lam, K.L.; Li, X.; Kong, A.P.; Cheung, P.C. Circadian disruption-induced metabolic syndrome in mice is ameliorated by oat β-glucan mediated by gut microbiota. Carbohydrate Polymers 2021, 267, 118216.

Choi, H.; Kim, S.W. Characterization of β-Glucans from Cereal and Microbial Sources and Their Roles in Feeds for Intestinal Health and Growth of Nursery Pigs. Animals (Basel) 2023, 13.

Choudhury, R.; Middelkoop, A.; Boekhorst, J.; Gerrits, W.J.J.; Kemp, B.; Bolhuis, J.E.; Kleerebezem, M. Early life feeding accelerates gut microbiome maturation and suppresses acute post-weaning stress in piglets. Environmental Microbiology 2021b, 23, 7201-7213.

Choudhury, R.; Middelkoop, A.; Bolhuis, J.E.; Kleerebezem, M. Exploring the association between microbiota and behaviour in suckling piglets. Scientific Reports 2022, 12, 12322.

Choudhury, R.; Middelkoop, A.; de Souza, J.G.; van Veen, L.A.; Gerrits, W.J.J.; Kemp, B.; Bolhuis, J.E.; Kleerebezem, M. Impact of early-life feeding on local intestinal microbiota and digestive system development in piglets. Scientific Reports 2021a, 11, 4213.

Clarke, H.F.; Dalley, J.W.; Crofts, H.S.; Robbins, T.W.; Roberts, A.C. Cognitive inflexibility after prefrontal serotonin depletion. Science 2004, 304, 878-880.

Clavell-Sansalvador, A.; Río-López, R.; González-Rodríguez, O.; García-Gil, L.J.; Xifró, X.; Zigovski, G.; Ochoteco-Asensio, J.; Ballester, M.; Dalmau, A.; Ramayo-Caldas, Y. Effect of Group Mixing and Available Space on Performance, Feeding Behavior, and Fecal Microbiota Composition during the Growth Period of Pigs. Animals 2024, 14, 2704.

Cognigni, V.; Ranallo, N.; Tronconi, F.; Morgese, F.; Berardi, R. Potential benefit of β-glucans as adjuvant therapy in immuno-oncology: a review. Exploration of Targeted Antitumor Therapy 2021, 2, 122-138.

Collado MC, Isolauri E, Salminen S. Specific probiotic strains and their combinations counteract adhesion of Enterobacter sakazakii to intestinal mucus. FEMS Microbiology Letters 2008; 285(1):58e64.

Cornelison, A.S.; Karriker, L.A.; Williams, N.H.; Haberl, B.J.; Stalder, K.J.; Schulz, L.L.; Patience, J.F. Impact of health challenges on pig growth performance, carcass characteristics, and net returns under commercial conditions. Translational Animal Science 2018, 2, 50-61.

Craig JR, Dunshea FR, Cottrell JJ, Furness JB, Wijesiriwardana UA, Pluske JR.A comparison of the anatomical and gastrointestinal functional development between gilt and sow progeny around birth and weaning. Journal of Animal Science 2019.

Cranwell PD:; Tarvid I., Harrison D, Cambell RG, (1995), Weight at weaning, causes and consequences in manipulating pig production, Australasian Pis Science Association, Werribee, Australia p.174

Cremonesi P, Biscarini F, Castiglioni B, Sgoifo CA, Compiani R, Moroni P. Gut microbiome modifications over time when removing in-feed antibiotics from the prophylaxis of post-weaning diarrhea in piglets. PLoS One. 2022 Mar 7;17(3)

Cronin P, Joyce SA, O'Toole PW, O'Connor EM. Dietary Fibre Modulates the Gut Microbiota. Nutrients. 2021 May 13;13(5):1655.

Cryan, J.F.; O'Riordan, K.J.; Cowan, C.S.M.; Sandhu, K.V.; Bastiaanssen, T.F.S.; Boehme, M.; Codagnone, M.G.; Cussotto, S.; Fulling, C.; Golubeva, A.V.; et al. The Microbiota-Gut-Brain Axis. Physiological Review 2019, 99, 1877-2013.

Dash, S.; Syed, Y.A.; Khan, M.R. Understanding the Role of the Gut Microbiome in Brain Development and Its Association With Neurodevelopmental Psychiatric Disorders. Frontiers in Cell and Developmental Biology 2022, 10, 880544.

de Greeff, A.; Resink, J.W.; van Hees, H.M.J.; Ruuls, L.; Klaassen, G.J.; Rouwers, S.M.G.; Stockhofe-Zurwieden, N. Supplementation of piglets with nutrient-dense complex milk replacer improves intestinal development and microbial fermentation1. Journal of Animal Science 2016, 94, 1012-1019.

de Greeff, A.; Schokker, D.; Roubos-van den Hil, P.; Ramaekers, P.; Vastenhouw, S.A.; Harders, F.; Bossers, A.; Smits, M.A.; Rebel, J.M.J. The effect of maternal antibiotic use in sows on intestinal development in offspring. Journal of Animal Science 2020, 98.

de Vries, H.; Jakobs, K.; Egyedi, T.M.; Eto, M.; Fertig, S.; Kanevskaia, O.; Klintner, L.; Koch, C.; Mijatovic, I.; Mirtsch, M.; et al. Standardization: Towards an Agenda for Research. International Journal of Standardization Research (IJSR) 2018, 16, 52-59.

De Witte, C.; Flahou, B.; Ducatelle, R.; Smet, A.; De Bruyne, E.; Cnockaert, M.; Taminiau, B.; Daube, G.; Vandamme, P.; Haesebrouck, F. Detection, isolation and characterization of Fusobacterium gastrosuis sp. nov. colonizing the stomach of pigs. Systematic and Applied Microbiology 2017, 40, 42-50.

Declerck, I., Dewulf, J., Sarrazin, S., Maes, D., 2016. Long-term effects of colostrum intake in piglet mortality and performance. Journal of Animal Science 94, 1633–1643.

Defeyter, M.A.; Russo, R. The effect of breakfast cereal consumption on adolescents' cognitive performance and mood. Frontiers in Human Neuroscience 2013, 7, 789.

Dell'Anno, M.; Callegari, M.L.; Reggi, S.; Caprarulo, V.; Giromini, C.; Spalletta, A.; Coranelli, S.; Sgoifo Rossi, C.A.; Rossi, L. Lactobacillus plantarum and Lactobacillus reuteri as Functional Feed Additives to Prevent Diarrhoea in Weaned Piglets. Animals (Basel) 2021a, 11.

den Besten, G.; van Eunen, K.; Groen, A.K.; Venema, K.; Reijngoud, D.-J.; Bakker, B.M. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. Journal of lipid research 2013, 54, 2325-2340.

Depalle, B.; Karaaslan, H.; Obtel, N.; Gil-Bona, A.; Teichmann, M.; Mascarin, G.; Pugach-Gordon, M.; Bidlack, F.B. Rapid post-eruptive maturation of porcine enamel. Frontiers in Physiology 2023, 14, 1099645

Diaz Heijtz, R.; Wang, S.; Anuar, F.; Qian, Y.; Björkholm, B.; Samuelsson, A.; Hibberd, M.L.; Forssberg, H.; Pettersson, S. Normal gut microbiota modulates brain development and behavior. Proceedings of the National Academy of Sciences of the U S A 2011, 108, 3047-3052.

Dinan, T.G.; Cryan, J.F. Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology. Psychoneuroendocrinology 2012, 37, 1369-1378.

Dong, W.; Ricker, N.; Holman, D.B.; Johnson, T.A. Meta-analysis reveals the predictable dynamic development of the gut microbiota in commercial pigs. Microbiology Spectrum 2023, 11, e01722-01723.

Dong Zaiquan , Shen Xiaoling , Hao Yanni , Li Jin , Li Haoran , Xu Haizheng , Yin Li , Kuang Weihong , A Potential Indicator for Differential Diagnosis of Major Depressive Disorder and General Anxiety Disorder, Frontiers in Psychiatry, volume 12-2021

Dowarah, R.; Verma, A.K.; Agarwal, N. The use of Lactobacillus as an alternative of antibiotic growth promoters in pigs: A review. Anim Nutr 2017, 3, 1-6.

Drago L, Gismondo MR, Lombardi A, de Haen C, Gozzini L. Inhibition of in vitro growth of enteropathogens by new Lactobacillus isolates of human intestinal origin. FEMS Microbiology Letters 1997; 153(2):455e63.

Drude, N.I.; Martinez Gamboa, L.; Danziger, M.; Dirnagl, U.; Toelch, U. Improving preclinical studies through replications. Elife 2021, 10.

Du, B.; Meenu, M.; Liu, H.; Xu, B. A Concise Review on the Molecular Structure and Function Relationship of β-Glucan. International Journal of Molecular Sciences 2019, 20.

Duarte ME, Kim SW, Intestinal microbiota and its interaction to intestinal health in nursery pigs, Animal Nutrition, Volume 8, 2022, pages 169-184.

Dunne, W.M., Jr. Bacterial adhesion: seen any good biofilms lately? Clinical Microbiology Reviews 2002, 15, 155-166.

Ehab, M.; Omran, N.; Handoussa, H. The modulatory effect of oat on brain-derived neurotrophic factor, orexigenic neuropeptides, and dopaminergic signaling in obesity-induced rat model: a comparative study to orlistat. Journal of Science of Food and Agriculture 2025, 105, 1251-1262.

Fabiano, G.A.; Shinn, L.M.; Antunes, A.E.C. Relationship between Oat Consumption, Gut Microbiota Modulation, and Short-Chain Fatty Acid Synthesis: An Integrative Review. Nutrients 2023, 15.

Faccin, J.E.G.; Laskoski, F.; Hernig, L.F.; Kummer, R.; Lima, G.F.R.; Orlando, U.A.D.; Gonçalves, M.A.D.; Mellagi, A.P.G.; Ulguim, R.R.; Bortolozzo, F.P. Impact of increasing weaning age on pig performance and belly nosing prevalence in a commercial multisite production system. Journal of Animal Science 2020, 98.

Fadel, A.; Hussain, H.; Hernandez, R.J.; Clichy Silva, A.M.; Estil-las, A.A.; Hamad, M.; Saadoon, Z.F.; Naseer, L.; Sultan, W.C.; Sultan, C.; et al. Mechanisms

of Neurosyphilis-Induced Dementia: Insights into Pathophysiology. Neurology International 2024, 16, 1653-1665.
Fåk, F.; Ahrné, S.; Linderoth, A.; Molin, G.; Jeppsson, B.; Weström, B. Age-related effects of the probiotic bacterium Lactobacillus plantarum 299v on gastrointestinal function in suckling rats. Digestive Diseases and Science 2008, 53, 664-671.
Fekete, M.; Lehoczki, A.; Major, D.; Fazekas-Pongor, V.; Csípő, T.; Tarantini, S.; Csizmadia, Z.; Varga, J.T. Exploring the Influence of Gut-Brain Axis Modulation on Cognitive Health: A Comprehensive Review of Prebiotics, Probiotics, and Symbiotics. Nutrients 2024, 16.
Feng, W.; Ao, H.; Peng, C. Gut Microbiota, Short-Chain Fatty Acids, and Herbal Medicines. Frontiers in Pharmacology. 2018, 9, 1354.
Fleming, S.A.; Monaikul, S.; Patsavas, A.J.; Berg, B.M.; Waworuntu, R.V.; Dilger, R.N. Early-Life Prebiotics Alter Exploratory Behavior, Recognition Memory, and Brain Serotonin in the Neonatal Piglet. The FASEB Journal 2017, 31, 650.622-650.622.
Flores, J.N.; Lubin, J.B.; Silverman, M.A. The case for microbial intervention at weaning. Gut Microbes 2024, 16, 2414798.
Foster, J.A.; Rinaman, L.; Cryan, J.F. Stress & the gut-brain axis: Regulation by the microbiome. Neurobiology of Stress 2017, 7, 124-136.
Fristedt, R.; Ruppert, V.; Trower, T.; Cooney, J.; Landberg, R. Quantitation of circulating short-chain fatty acids in small volume blood samples from animals and humans. Talanta 2024, 272, 125743.
Frost, G.; Sleeth, M.L.; Sahuri-Arisoylu, M.; Lizarbe, B.; Cerdan, S.; Brody, L.; Anastasovska, J.; Ghourab, S.; Hankir, M.; Zhang, S.; et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. Nature Communications 2014, 5, 3611.
Gao Q, Liu Z, Li K, Bai G, Liu L, Zhong R, Chen L, Zhang H. Time-course effects of different fiber-rich ingredients on energy values, microbiota composition and SCFA profile in growing pigs. Animal Nutrition 2022 Nov 2;12:263-275.
García Viñado, I., Correa, F., Trevisi, P. et al. A non-invasive tool to collect small intestine content in post weaning pigs: validation study. Scientific Reports 14, 9964 (2024).
Gardiner, G.E.; Metzler-Zebeli, B.U.; Lawlor, P.G. Impact of Intestinal Microbiota on Growth and Feed Efficiency in Pigs: A Review. Microorganisms 2020, 8, 1886.
Ghosh, A.; Choudhary, G.; Medhi, B. The pivotal role of artificial intelligence in enhancing experimental animal model research: A machine learning perspective. Indian Journal of Pharmacology 2024, 56, 1-3.

Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Jornal of Nutrition 1995 Jun;125(6):1401-12.

Gibson, G.R.; Hutkins, R.; Sanders, M.E.; Prescott, S.L.; Reimer, R.A.; Salminen, S.J.; Scott, K.; Stanton, C.; Swanson, K.S.; Cani, P.D.; et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology & Hepatology 2017, 14, 491-502.

Gill, P.A.; van Zelm, M.C.; Muir, J.G.; Gibson, P.R. Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. Alimentary Pharmacology & Therapeutics 2018, 48, 15-34.

Goderska K, Agudo Pena S. An in vitro gastrointestinal model to evaluate the tolerance of encapsulated Lactobacillus and Lactococcus strains with synbiotic containing lactobionic acid via lyophilization technique to harsh gastric conditions during storage time. Europian Journal of Pharmaceutics Biopharmaceutics 2024 Apr; 197:114147.

Gomez-Pinilla F and Nguyen TTJ, Natural mood foods: the actions of polyphenols against psychiatric and cognitive disorders. Nutritional Neuroscience 15:127–133 (2012).

Gonçalves, P.; Araújo, J.R.; Di Santo, J.P. A Cross-Talk Between Microbiota-Derived Short-Chain Fatty Acids and the Host Mucosal Immune System Regulates Intestinal Homeostasis and Inflammatory Bowel Disease. Inflammatory Bowel Diseases 2018, 24, 558-572.

Griffin, S. M., Lehtinen, M. J., Meunier, J., Ceolin, L., Roman, F. J., & Patterson, E. (2022). Restorative effects of probiotics on memory impairment in sleep-deprived mice. Nutritional Neuroscience, 26(3), 254–264.

Gryaznova, M.; Smirnova, Y.; Burakova, I.; Morozova, P.; Nesterova, E.; Gladkikh, M.; Mikhaylov, E.; Syromyatnikov, M. Characteristics of the Fecal Microbiome of Piglets with Diarrhea Identified Using Shotgun Metagenomics Sequencing. Animals (Basel) 2023, 13.

Gryaznova, M.V.; Dvoretskaya, Y.D.; Syromyatnikov, M.Y.; Shabunin, S.V.; Parshin, P.A.; Mikhaylov, E.V.; Strelnikov, N.A.; Popov, V.N. Changes in the Microbiome Profile in Different Parts of the Intestine in Piglets with Diarrhea. Animals (Basel) 2022, 12.

Guerra NP, Bernárdez PF, Méndez J, Cachaldora P, Castro LP, Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets, Animal Feed Science and Technology, Volume 134, Issues 1–2, 2007 Pages 89-107.

Guevarra, R.B.; Lee, J.H.; Lee, S.H.; Seok, M.-J.; Kim, D.W.; Kang, B.N.; Johnson, T.J.; Isaacson, R.E.; Kim, H.B. Piglet gut microbial shifts early in life:

causes and effects. Journal of animal science and biotechnology 2019, 10, 1-1.
Guzowska, M.; Dziendzikowska, K.; Kopiasz, Ł.; Gajewska, M.; Wilczak, J.; Harasym, J.; Czerwińska, M.; Gromadzka-Ostrowska, J. Oat Beta-Glucans Modulate the Gut Microbiome, Barrier Function, and Immune Responses in an In Vivo Model of Early-Stage Colorectal Cancer. International Journal of Molecular Sciences 2024, 25, 13586.
Han, S.; Lu, Y.; Xie, J.; Fei, Y.; Zheng, G.; Wang, Z.; Liu, J.; Lv, L.; Ling, Z.; Berglund, B.; et al. Probiotic Gastrointestinal Transit and Colonization After Oral Administration: A Long Journey. Frontiers in Cellular and Infection Microbiology 2021, 11, 609722.
Hansen C 2022. National average productivity of danish pig farms 2021. SEGES. Available:https://www.landbrugsinfo.dk//media/landbrugsinfo/public/e/2/c/notat_2204_average_productivity_danish_pig_farms_2021.
Hantsoo, L.; Zemel, B.S. Stress gets into the belly: Early life stress and the gut microbiome. Behavioural Brain Research 2021, 414, 113474.
Harper, J.; Bunter, K.L. Review: Improving pig survival with a focus on birthweight: a practical breeding perspective. Animal 2024, 18, 100914.
Hart JB, Poon RK, Ward RE, Hintze KJ, Freeman SM. Effects of Dietary Fiber on Short Chain Fatty Acid Receptor mRNA in Microglia and Serotonergic Neurons in the Mouse Brain. Neuroscience. 2024 Apr 19;544:88-101.
He, B.; Bai, Y.; Jiang, L.; Wang, W.; Li, T.; Liu, P.; Tao, S.; Zhao, J.; Han, D.; Wang, J. Effects of Oat Bran on Nutrient Digestibility, Intestinal Microbiota, and Inflammatory Responses in the Hindgut of Growing Pigs. International Journal of Molecular Sciences 2018, 19, 2407.
He, J.; Zhang, P.; Shen, L.; Niu, L.; Tan, Y.; Chen, L.; Zhao, Y.; Bai, L.; Hao, X.; Li, X.; et al. Short-Chain Fatty Acids and Their Association with Signalling Pathways in Inflammation, Glucose and Lipid Metabolism. International Journal of Molecular Sciences 2020, 21.
Hermann-Bank, M.L.; Skovgaard, K.; Stockmarr, A.; Strube, M.L.; Larsen, N.; Kongsted, H.; Ingerslev, H.C.; Mølbak, L.; Boye, M. Characterization of the bacterial gut microbiota of piglets suffering from new neonatal porcine diarrhoea. BMC Veterinary Research 2015, 11, 139.
Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S.; et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology & Hepatology 2014, 11, 506-514.
Holtekjølen, A.K.; Vhile, S.G.; Sahlstrøm, S.; Knutsen, S.H.; Uhlen, A.K.; Åssveen, M.; Kjos, N.P. Changes in relative molecular weight distribution of soluble barley beta-glucan during passage through the small intestine of pigs. Livestock Science 2014a, 168, 102–108.

Hou, C.; Zeng, X.; Yang, F.; Liu, H.; Qiao, S. Study and use of the probiotic Lactobacillus reuteri in pigs: a review. J Anim Sci Biotechnol 2015, 6, 14.

Hu, M.; Zhang, P.; Wang, R.; Zhou, M.; Pang, N.; Cui, X.; Ge, X.; Liu, X.; Huang, X.F.; Yu, Y. Three Different Types of β-Glucans Enhance Cognition: The Role of the Gut-Brain Axis. Frontiers in Nutrition 2022, 9, 848930.

Huang XF, Yu Y, Beck EJ, South T, Li Y, Batterham MJ et al., Diet high in oat β-glucan activates the gut-hypothalamic (PYY3-36-NPY) axis and increases satiety in diet-induced obesity in mice. Molecular Nutrition and Food Research 55:1118–1121 (2011).

Huang, R.; Wu, F.; Zhou, Q.; Wei, W.; Yue, J.; Xiao, B.; Luo, Z. Lactobacillus and intestinal diseases: Mechanisms of action and clinical applications. Microbiology Research 2022b, 260, 127019.

Huang, R.; Wu, F.; Zhou, Q.; Wei, W.; Yue, J.; Xiao, B.; Luo, Z. Lactobacillus and intestinal diseases: Mechanisms of action and clinical applications. Microbiology Research 2022a, 260, 127019.

Huang, S.; Cui, Z.; Hao, X.; Cheng, C.; Chen, J.; Wu, D.; Luo, H.; Deng, J.; Tan, C. Dietary fibers with low hydration properties exacerbate diarrhea and impair intestinal health and nutrient digestibility in weaned piglets. Journal of Animal Science and Biotechnology 2022c, 13, 142.

Humbel, F.; Rieder, J.H.; Franc, Y.; Juillerat, P.; Scharl, M.; Misselwitz, B.; Schreiner, P.; Begré, S.; Rogler, G.; von Känel, R.; et al. Association of Alterations in Intestinal Microbiota With Impaired Psychological Function in Patients With Inflammatory Bowel Diseases in Remission. Clinical Gastroenterology and Hepatology 2020, 18, 2019-2029.e2011.

Hung, Y.T.; Zhu, J.; Shurson, G.C.; Urriola, P.E.; Saqui-Salces, M. Decreased nutrient digestibility due to viscosity is independent of the amount of dietary fibre fed to growing pigs. The British Journal of Nutrition 2022, 127, 177-187.

Hunniford, V.T.; Grudniewicz, A.; Fergusson, D.A.; Montroy, J.; Grigor, E.; Lansdell, C.; Lalu, M.M. A systematic assessment of preclinical multilaboratory studies and a comparison to single laboratory studies. eLife 2023, 12, e76300.

Huting, A.M.S.; Wellock, I.; Tuer, S.; Kyriazakis, I. Weaning age and post-weaning nursery feeding regime are important in improving the performance of lightweight pigs. Journal of Animal Science 2019, 97, 4834-4844.

Hutkins RW, Krumbeck JA, Bindels LB, Cani PD, Fahey G Jr, Goh YJ, Hamaker B, Martens EC, Mills DA, Rastal RA, Vaughan E, Sanders ME. Prebiotics: why definitions matter. Current Opinion in Biotechnology 2016 Feb;37:1-7.

Jaeger, J.W.; Brandt, A.; Gui, W.; Yergaliyev, T.; Hernández-Arriaga, A.; Muthu, M.M.; Edlund, K.; Elashy, A.; Molinaro, A.; Möckel, D.; et al. Microbiota

modulation by dietary oat beta-glucan prevents steatotic liver disease progression. JHEP Reports 2024, 6, 100987.
Jang HJ, Lee NK, Paik HD. Lactobacillus plantarum G72 Showing Production of Folate and Short-chain Fatty Acids. Microbiology and Biotechnology Letters 2021; 49:1823.
Jena, A.; Montoya, C.A.; Mullaney, J.A.; Dilger, R.N.; Young, W.; McNabb, W.C.; Roy, N.C. Gut-Brain Axis in the Early Postnatal Years of Life: A Developmental Perspective. Frontiers in Integrative Neuroscience 2020, 14, 44.
Jensen, B.A.H.; Heyndrickx, M.; Jonkers, D.; Mackie, A.; Millet, S.; Naghibi, M.; Pærregaard, S.I.; Pot, B.; Saulnier, D.; Sina, C.; et al. Small intestine vs. colon ecology and physiology: Why it matters in probiotic administration. Cell Reports Medicine 2023, 4, 101190.
Jensen, J.; Kyvsgaard, N.C.; Battisti, A.; Baptiste, K.E. Environmental and public health related risk of veterinary zinc in pig production - Using Denmark as an example. Environment International 2018, 114, 181-190.
Jha, R.; Berrocoso, J.D. Review: Dietary fiber utilization and its effects on swine's physiological functions and gut health. Animal 2015, 9, 1441-1452.
Jha, R.; Fouhse, J.M.; Tiwari, U.P.; Li, L.; Willing, B.P. Dietary Fiber and Intestinal Health of Monogastric Animals. Frontiers of Veterinary Science 2019, 6, 48.
Jiang, H.Y.; Zhang, X.; Yu, Z.H.; Zhang, Z.; Deng, M.; Zhao, J.H.; Ruan, B. Altered gut microbiota profile in patients with generalized anxiety disorder. Journal of Psychiatric Research 2018, 104, 130-136.
Jiang, X.; Lu, N.; Zhao, H.; Yuan, H.; Xia, D.; Lei, H. The Microbiome–Metabolome Response in the Colon of Piglets Under the Status of Weaning Stress. Frontiers in Microbiology 2020, 11.
Jost, T.; Lacroix, C.; Braegger, C.P.; Rochat, F.; Chassard, C. Vertical mother-neonate transfer of maternal gut bacteria via breastfeeding. Environmental Microbiology 2014, 16, 2891-2904.
Jurkaninová, L.; Dvořáček, V.; Gregusová, V.; Havrlentová, M. Cereal β-d-Glucans in Food Processing Applications and Nanotechnology Research. Foods 2024, 13, 500.
Kaminiów, K.; Kiołbasa, M.; Pastuszczak, M. The Significance of the Cell-Mediated Host Immune Response in Syphilis. Microorganisms 2024, 12.
Kang SS, Jeraldo PR, Kurti A, Miller ME, Cook MD, Whitlock K, Goldenfeld N, Woods JA, White BA, Chia N, Fryer JD. Diet and exercise orthogonally alter the gut microbiome and reveal independent associations with anxiety and cognition. Molecular Neurodegeneration. 2014 Sep 13;9:36.
Kanczkowski W, Sue M, Bornstein SR. The adrenal gland microenvironment in health, disease and during regeneration. Hormones (Athens). 2017 Jul;16(3):251-265.

Kennedy, D.O.; Bonnländer, B.; Lang, S.C.; Pischel, I.; Forster, J.; Khan, J.; Jackson, P.A.; Wightman, E.L. Acute and Chronic Effects of Green Oat (Avena sativa) Extract on Cognitive Function and Mood during a Laboratory Stressor in Healthy Adults: A Randomised, Double-Blind, Placebo-Controlled Study in Healthy Humans. Nutrients 2020, 12.

Killingsworth Jessica , Sawmiller Darrell , Shytle R. Douglas; Propionate and Alzheimer's Disease, Frontiers in Aging Neuroscience Volume 12 – 2021

Kim, H.J.; White, P.J. Impact of the molecular weight, viscosity, and solubility of β-glucan on in vitro oat starch digestibility. Journal of Agricultural and Food Chemistry 2013, 61, 3270-3277.

Kim, K.; Song, M.; Liu, Y.; Ji, P. Enterotoxigenic Escherichia coli infection of weaned pigs: Intestinal challenges and nutritional intervention to enhance disease resistance. Frontiers in Immunology 2022, 13, 885253.

Kim, S.W.; Gormley, A.; Jang, K.B.; Duarte, M.E. - Invited Review - Current status of global pig production: an overview and research trends. Animal Bioscience 2024, 37, 719-729.

Knudsen, E.; Mette Skou, H.; Helle Nygaard, L. The role of carbohydrates in intestinal health of pigs. Animal Feed Science and Technology 2012, 173, 41-53.

Kobek-Kjeldager, , Anna A. Schönherz, Nuria Canibe, Lene Juul Pedersen,Diet and microbiota-gut-brain axis in relation to tail biting in pigs: A review, Applied Animal Behaviour Science, Volume 246, 2022

Kogut, M.H.; Arsenault, R.J. Editorial: Gut Health: The New Paradigm in Food Animal Production. Front Vet Sci 2016, 3, 71.

Koh, A.; De Vadder, F.; Kovatcheva-Datchary, P.; Bäckhed, F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. Cell 2016, 165, 1332–1345.

Kong, Q.; Zhang, W.; An, M.; Kulyar, M.F.; Shang, Z.; Tan, Z.; Xu, Y.; Li, J.; Liu, S. Characterization of Bacterial Microbiota Composition in Healthy and Diarrheal Early-Weaned Tibetan Piglets. Frontiers in Veterinary Science 2022, 9, 799862.

König, E.; Heponiemi, P.; Kivinen, S.; Räkköläinen, J.; Beasley, S.; Borman, T.; Collado, M.C.; Hukkinen, V.; Junnila, J.; Lahti, L.; et al. Fewer culturable Lactobacillaceae species identified in faecal samples of pigs performing manipulative behaviour. Scientific Reports 2024, 14, 132.

Korczak, R.; Kocher, M.; Swanson, K.S. Effects of oats on gastrointestinal health as assessed by in vitro, animal, and human studies. Nutrition Reviews 2019, 78, 343-363.

Kubasova, T.; Davidova-Gerzova, L.; Babak, V.; Cejkova, D.; Montagne, L.; Le-Floc'h, N.; Rychlik, I. Effects of host genetics and environmental conditions on fecal microbiota composition of pigs. PLoS One 2018, 13.

Kuznetsova, A.; Brockhoff, P.B.; Christensen, R.H.B. lmerTest Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software 2017, 82, 1 - 26.

Lærke, Helle Nygaard; Hedemann, Mette Skou, The digestive system of the pig;Nutritional physiology of pigs - Online Publication. ed. / Knud Erik Bach Knudsen; Niels Jørgen Kjeldsen; Hanne Damgaard Poulsen; Bent Borg Jensen. Foulum: Videncenter for Svineproduktion, 2012.

Lange O, Proczko-Stepaniak M, Mika A. Short-Chain Fatty Acids-A Product of the Microbiome and Its Participation in Two-Way Communication on the Microbiome-Host Mammal Line. Curr Obes Rep. 2023 Jun;12(2):108-126.

Lam, Ka-Lung and Peter Chi-Keung Cheung. "Non-digestible long chain beta-glucans as novel prebiotics." Bioactive Carbohydrates and Dietary Fibre 2 (2013): 45-64.

Lambo, A.M.; Öste, R.; Nyman, M.E.L. Dietary fibre in fermented oat and barley _-glucan rich concentrates. Food Chemistry. 2005, 89, 283–293.

Lan Q, Lian Y, Peng P, Yang L, Zhao H, Huang P, Ma H, Wei H, Yin Y, Liu M. Association of gut microbiota and SCFAs with finishing weight of Diannan small ear pigs. Frontiers in Microbiology. 2023 Jan 27;14:1117965.

Lante, A.; Canazza, E.; Tessari, P. Beta-Glucans of Cereals: Functional and Technological Properties. Nutrients 2023, 15.

Lapiz-Bluhm, M.D.; Soto-Piña, A.E.; Hensler, J.G.; Morilak, D.A. Chronic intermittent cold stress and serotonin depletion induce deficits of reversal learning in an attentional set-shifting test in rats. Psychopharmacology (Berl) 2009, 202, 329-341.

Lee JS, Awji EG, Lee SJ, Tassew DD, Park YB, Park KS, et al. Effect of Lactobacillus plantarum CJLP243 on the growth performance and cytokine response of weaning pigs challenged with enterotoxigenic Escherichia coli. Journal of Animal Science (2012) 90:3709–17.

Lee, P.; Tan, K.S. Fusobacterium nucleatum activates the immune response through retinoic acid-inducible gene, Journal of Dental Research 2014, 93, 162-168.

Lerch, F.; Yosi, F.; Vötterl, J.C.; Koger, S.; Ehmig, J.; Sharma, S.; Verhovsek, D.; Metzler-Zebeli, B.U. An insight into the temporal dynamics in the gut microbiome, metabolite signaling, immune response, and barrier function in suckling and weaned piglets under production conditions. Frontiers in Veterinary Science 2023, 10.

Leterme P., Souffrant WB, Théwis A.,Effect of Barley Fibres and Barley Intake on the Ileal Endogenous Nitrogen Losses in Piglets, Journal of Cereal ScienceVolume 31, Issue 3, 2000,Pages 229-239.

Lew, L.C.; Hor, Y.Y.; Yusoff, N.A.A.; Choi, S.B.; Yusoff, M.S.B.; Roslan, N.S.; Ahmad, A.; Mohammad, J.A.M.; Abdullah, M.; Zakaria, N.; et al. Probiotic Lactobacillus plantarum P8 alleviated stress and anxiety while enhancing

memory and cognition in stressed adults: A randomised, double-blind, placebo-controlled study. Clinical Nutrition 2019, 38, 2053-2064.
Li, N.; Huang, S.; Jiang, L.; Dai, Z.; Li, T.; Han, D.; Wang, J. Characterization of the Early Life Microbiota Development and Predominant Lactobacillus Species at Distinct Gut Segments of Low- and Normal-Birth-Weight Piglets. Frontiers in Microbiology 2019, 10, 797.
Li, Y.; Guo, Y.; Wen, Z.; Jiang, X.; Ma, X.; Han, X. Weaning Stress Perturbs Gut Microbiome and Its Metabolic Profile in Piglets. Scientific Reports 2018, 8, 18068.
Li, Y.; Liu, Y.; Ma, Y.; Ge, X.; Zhang, X.; Cai, C.; Yang, Y.; Lu, C.; Liang, G.; Guo, X.; et al. Effects of Maternal Factors and Postpartum Environment on Early Colonization of Intestinal Microbiota in Piglets. Frontiers in Veterinary Science 2022, 9.
Liao, S.F.; Ji, F.; Fan, P.; Denryter, K. Swine Gastrointestinal Microbiota and the Effects of Dietary Amino Acids on Its Composition and Metabolism. International Journal of Molecular Sciences 2024, 25, 1237.
Lim, J.A.; Cha, J.; Choi, S.; Kim, J.H.; Kim, D. Early Colonization of the Intestinal Microbiome of Neonatal Piglets Is Influenced by the Maternal Microbiome. Animals (Basel) 2023, 13.
Litvak, Y.; Byndloss, M.X.; Bäumler, A.J. Colonocyte metabolism shapes the gut microbiota. Science 2018, 362.
Liu, H.; Zhang, J.; Zhang, S.; Yang, F.; Thacker, P.A.; Zhang, G.; Qiao, S.; Ma, X. Oral Administration of Lactobacillus fermentum I5007 Favors Intestinal Development and Alters the Intestinal Microbiota in Formula-Fed Piglets. Journal of Agricultural and Food Chemistry 2014, 62, 860-866.
Liu, X.; Cao, S.; Zhang, X. Modulation of Gut Microbiota-Brain Axis by Probiotics, Prebiotics, and Diet. Journal of Agricultural and Food Chemistry 2015, 63, 7885-7895.
Lopez-Tello, J.; Kiu, R.; Schofield, Z.; Zhang, C.X.W.; van Sinderen, D.; Le Gall, G.; Hall, L.J.; Sferruzzi-Perri, A.N. Maternal gut Bifidobacterium breve modifies fetal brain metabolism in germ-free mice. Molecular Metabolism 2024, 88, 102004.
Louis, P.; Hold, G.L.; Flint, H.J. The gut microbiota, bacterial metabolites and colorectal cancer. Nature Reviews Microbiology 2014, 12, 661-672.
Lu, C.; Liu, Y.; Ma, Y.; Wang, S.; Cai, C.; Yang, Y.; Zhao, Y.; Liang, G.; Cao, G.; Li, B.; et al. Comparative Evaluation of the Ileum Microbiota Composition in Piglets at Different Growth Stages. Frontiers in Microbiology 2021, 12, 765691.
Luczynski, P.; McVey Neufeld, K.A.; Oriach, C.S.; Clarke, G.; Dinan, T.G.; Cryan, J.F. Growing up in a Bubble: Using Germ-Free Animals to Assess the Influence of the Gut Microbiota on Brain and Behavior. International Journal of Neuropsychopharmacology 2016, 19.

Luo, Y.; Liu, Y.; Li, H.; Zhao, Y.; Wright, A.-D.G.; Cai, J.; Tian, G.; Mao, X. Differential Effect of Dietary Fibers in Intestinal Health of Growing Pigs: Outcomes in the Gut Microbiota and Immune-Related Indexes. Frontiers in Microbiology 2022a, 13

Lyderik, K.K.; Madsen, J.G.; Larsen, C.; Pedersen, M.L.M.; Kjeldsen, N.J.; Williams, A.R.; Hedemann, M.S.; Amdi, C. An increased weaning age and liquid feed enhances weight gain compared to piglets fed dry feed pre-weaning. Animal 2023, 17, 100801.

Ma, T.; Suzuki, Y.; Guan, L.L. Dissect the mode of action of probiotics in affecting host-microbial interactions and immunity in food producing animals. Veterinary Immunology and Immunopathology 2018, 205, 35-48.

Macfarlane GT, Macfarlane S. Bacteria, colonic fermentation, and gastrointestinal health. Journal of AOAC International. 2012 Jan-Feb;95(1):50-60.

Mach, N.; Berri, M.; Estellé, J.; Levenez, F.; Lemonnier, G.; Denis, C.; Leplat, J.-J.; Chevaleyre, C.; Billon, Y.; Doré, J.; Early-life establishment of the swine gut microbiome and impact on host phenotypes. Environmental Microbiology Reports 2015, 7, 554-569.

Macia, L., Tan, J., Vieira, A. et al. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. Nature Communications 6, 6734 (2015).

Maftei, N.-M.; Raileanu, C.R.; Balta, A.A.; Ambrose, L.; Boev, M.; Marin, D.B.; Lisa, E.L. The Potential Impact of Probiotics on Human Health: An Update on Their Health-Promoting Properties. Microorganisms 2024, 12, 234.

Mahmoud, U.T.; Mahmoud, M.A.M.; Abd-Elkareem, M.; Ahmed, F.A.; Khalil, N.S.A. Prebiotics reduce feather pecking behavior, and improve trace element profile and redox balance in Mule ducks. Journal of Veterinary Behavior 2021.

Mahmud, M.R.; Jian, C.; Uddin, M.K.; Huhtinen, M.; Salonen, A.; Peltoniemi, O.; Venhoranta, H.; Oliviero, C. Impact of Intestinal Microbiota on Growth Performance of Suckling and Weaned Piglets. Microbiol Spectr 2023, 11.

Marion, J.; Petersen, Y.M.; Romé, V.; Thomas, F.; Sangild, P.T.; Le Dividich, J.; Le Huërou-Luron, I. Early weaning stimulates intestinal brush border enzyme activities in piglets, mainly at the posttranscriptional level. Jornal of Pediatric Gastroenterology and Nutrition 2005, 41, 401-410.

Martin, C.R.; Osadchiy, V.; Kalani, A.; Mayer, E.A. The Brain-Gut-Microbiome Axis. Cellular and Molecular Gastroenterology and Hepatology 2018, 6, 133-148.

Mateos, G.G.; Corrales, N.L.; Talegón, G.; Aguirre, L. - Invited Review - Pig meat production in the European Union-27: current status, challenges, and future trends. Animal Bioscience 2024, 37, 755-774.

Mayo-Martínez, Lorenzo, Martos-Moreno, Graell, Barbas, J. Rupérez, Argente, García, Short-chain fatty acids in plasma and feces: An optimized and validated LC-QqQ-MS method applied to study anorexia nervosa. Microchemical Journal 2024, 200, 110255.

Mazzoni, Le Gall, De Filippi, Minieri, Trevisi, Wolinski, Lalatta-Costerbosa, Lallès, PGuilloteau, Bosi,Supplemental Sodium Butyrate Stimulates Different Gastric Cells in Weaned pigs, The journal of Nutrition, Volume 138, 8, 2008, pages 1426-1431

McEwen, B.S.; Stellar, E. Stress and the individual. Mechanisms leading to disease. Archives of Internal Medicine 1993, 153, 2093-2101.

McOrist AL, Miller RB, Bird AR, Keogh JB, Noakes M, Topping DL, Conlon MA. Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. Jornal of Nutrition. 2011 May;141(5):883-9.

Meng, X.; Zheng, J.; Wang, F.; Zheng, J.; Yang, D. Dietary fiber chemical structure determined gut microbiota dynamics. iMeta 2022, 1, e64.

Metzler-Zebeli, B., Hooda, S., Pieper, R., Zijlstra, R., van Kessel, A., Mosenthin, R., and Gänzle, M., Nonstarch polysaccharides modulate bacterial microbiota, pathways for butyrate production, and abundance of pathogenic Escherichia coli in the pig gastrointestinal tract, Appled Environtal Microbiology 76 (2010) 3692–3701.

Metzler-Zebeli, B.U. Porcine Gut Microbiota and Host Interactions During the Transition from the Suckling to Postweaning Phase. In Gut Microbiota, Immunity, and Health in Production Animals, Kogut, M.H., Zhang, G., Eds.; Springer International Publishing: Cham, 2022; pp. 147-178.

Metzler-Zebeli, B.U.; Zijlstra, R.T.; Mosenthin, R.; Gänzle, M.G. Dietary calcium phosphate content and oat β-glucan influence gastrointestinal microbiota, butyrate-producing bacteria and butyrate fermentation in weaned pigs. FEMS Microbiology and Ecology 2011, 75, 402-413.

Middelkoop, A. & Choudhury, R. & Gerrits, Walter & Kemp, Bas & Kleerebezem, Michiel & Bolhuis, J.. (2018). Dietary diversity affects feeding behaviour of suckling piglets. Applied Animal Behaviour Science. 205. 10.1016.

Mikami, K.; Watanabe, N.; Tochio, T.; Kimoto, K.; Akama, F.; Yamamoto, K. Impact of Gut Microbiota on Host Aggression: Potential Applications for Therapeutic Interventions Early in Development. Microorganisms 2023, 11.

Minnebo, Y.; Delbaere, K.; Goethals, V.; Raes, J.; Van de Wiele, T.; De Paepe, K. Gut microbiota response to in vitro transit time variation is mediated by microbial growth rates, nutrient use efficiency and adaptation to in vivo transit time. Microbiome 2023, 11, 240.

Molist, F.; M; Pérez, J.F.; Mateos, G.G.; Nyachoti, C.M.; P.J. Relevance of functional properties of dietary fibre in diets for weanling pigs. Animal Feed Science and Technology 2014, 189, 1-10.

Mu, C.; Zhang, L.; He, X.; Smidt, H.; Zhu, W. Dietary fibres modulate the composition and activity of butyrate-producing bacteria in the large intestine of suckling piglets. Antonie Van Leeuwenhoek 2017a, 110, 687-696.

Mudd, A.T.; Dilger, R.N. Early-Life Nutrition and Neurodevelopment: Use of the Piglet as a Translational Model. Advances in Nutrition 2017, 8, 92-104.

Mueller NT, Zhang M, Juraschek SP, Miller ER, Appel LJ. Effects of high-fiber diets enriched with carbohydrate, protein, or unsaturated fat on circulating short chain fatty acids: results from the OmniHeart randomized trial. The American Journal of Clinical Nutrition 2020 Mar 1;111(3):545-554.

Murphy P, Bello FD, O'Doherty JV, Arendt EK, Sweeney T, Coffey A. Effects of cereal β-glucans and enzyme inclusion on the porcine gastrointestinal tract microbiota. Anaerobe. 2012;18(6):557–65.

Murphy, E.J.; Rezoagli, E.; Major, I.; Rowan, N.J.; Laffey, J.G. β-Glucan Metabolic and Immunomodulatory Properties and Potential for Clinical Application. J Fungi (Basel) 2020, 6.

Nakatani, M.; Inoue, R.; Tomonaga, S.; Fukuta, K.; Tsukahara, T. Production, Absorption, and Blood Flow Dynamics of Short-Chain Fatty Acids Produced by Fermentation in Piglet Hindgut during the Suckling–Weaning Period. Nutrients 2018, 10, 1220.

Navarro, D.; Abelilla, J.J.; Stein, H.H. Structures and characteristics of carbohydrates in diets fed to pigs: a review. J Animal Science and Biotechnology 2019, 10, 39.

Nawroth C. , Langbein J. , Coulon M. , Gabor V. , Oesterwind S. , Benz-Schwarzburg J., von Borell E. Farm Animal Cognition—Linking Behavior, Welfare and Ethics, Frontiers in Veterinary Science, Volume 6, 2019

Nguyen, T.Q.; Martínez-Álvaro, M.; Lima, J.; Auffret, M.D.; Rutherford, K.M.D.; Simm, G.; Dewhurst, R.J.; Baima, E.T.; Roehe, R. Identification of intestinal and fecal microbial biomarkers using a porcine social stress model. Frontiers in Microbiology 2023, 14.

Nowland, T.L.; Kirkwood, R.N.; Pluske, J.R. Review: Can early-life establishment of the piglet intestinal microbiota influence production outcomes? Animal 2022, 16, 100368.

Núñez-Gómez, V.; González-Barrio, R.; Periago, M.J. Interaction between Dietary Fibre and Bioactive Compounds in Plant By-Products: Impact on Bioaccessibility and Bioavailability. Antioxidants (Basel) 2023, 12.

OECD/FAO (2021), OECD-FAO Agricultural Outlook 2021-2030, OECD Publishing, Paris, https://doi.org/10.1787/19428846-en. Corrigenda to publications may be found on line at: www.oecd.org/about/publishing/corrigenda.htm.; 2021.

Oliphant, K.; Ali, M.; D'Souza, M.; Hughes, P.D.; Sulakhe, D.; Wang, A.Z.; Xie, B.; Yeasin, R.; Msall, M.E.; Andrews, B.; et al. Bacteroidota and Lachnospiraceae integration into the gut microbiome at key time points in early life are linked to infant neurodevelopment. Gut Microbes 2021, 13, 1997560.

Ortiz Sanjuán, J.M.; Argüello, H.; Cabrera-Rubio, R.; Crispie, F.; Cotter, P.D.; Garrido, J.J.; Ekhlas, D.; Burgess, C.M.; Manzanilla, E.G. Effects of removing in-feed antibiotics and zinc oxide on the taxonomy and functionality of the microbiota in post weaning pigs. Animal Microbiome 2024, 6, 18.

Otaru, N.; Kourouma, L.; Pugin, B.; Constancias, F.; Braegger, C.; Mansuy, I.M.; Lacroix, C. Transgenerational effects of early life stress on the fecal microbiota in mice. Communications Biology 2024, 7, 670.

Pantazi, A.C.; Balasa, A.L.; Mihai, C.M.; Chisnoiu, T.; Lupu, V.V.; Kassim, M.A.K.; Mihai, L.; Frecus, C.E.; Chirila, S.I.; Lupu, A.; et al. Development of Gut Microbiota in the First 1000 Days after Birth and Potential Interventions. Nutrients 2023, 15.

Park, S.-Y.; Kim, Y.-H.; Kim, S.-J.; Han, J.-H. Impact of Long-Term Supplementation with Probiotics on Gut Microbiota and Growth Performance in Post-Weaned Piglets. Animals 2024, 14, 1652.

Parois, S.P.; Eicher, S.D.; Lindemann, S.R.; Marchant, J.N. Potential improvements of the cognition of piglets through a synbiotic supplementation from 1 to 28 days via the gut microbiota. Scientific Reports 2021, 11, 24113.

Paudel, D.; Uehara, O.; Giri, S.; Yoshida, K.; Morikawa, T.; Kitagawa, T.; Matsuoka, H.; Miura, H.; Toyofuku, A.; Kuramitsu, Y.; et al. Effect of psychological stress on the oral-gut microbiota and the potential oral-gut-brain axis. The Japanese dental science review 2022, 58, 365-375.

Petry, A.L.; Patience, J.F.; Koester, L.R.; Huntley, N.F.; Bedford, M.R.; Schmitz-Esser, S. Xylanase modulates the microbiota of ileal mucosa and digesta of pigs fed corn-based arabinoxylans likely through both a stimbiotic and prebiotic mechanism. PLoS One 2021, 16.

Piñeiro, C.; Morales, J.; Doncecchi, P.; Dereu, A.; Wuyts, N.; Vizcaíno, E.; Azlor-Marsinach, O. Individual Pig Care improves productive performance and animal health in nursery-growing pigs. Journal of Swine Health and Production 2014, 2014, 296-299.

Pluske J., Turpin DL., Kim JC., Gastrointestinal tract (gut) health in the young pig. Animal Nutrition 2018, 4, 187-196.

Pluske, J.R.; Miller, D.W.; Sterndale, S.O.; Turpin, D.L. Associations between gastrointestinal-tract function and the stress response after weaning in pigs. Animal Production Science 2019, 59, 2015-2022.

Poeker, S.A.; Geirnaert, A.; Berchtold, L.; Greppi, A.; Krych, L.; Steinert, R.E.; de Wouters, T.; Lacroix, C. Understanding the prebiotic potential of different

dietary fibers using an in vitro continuous adult fermentation model (PolyFermS). Scientific Reports 2018, 8, 4318.
Pozuelo, M.; Panda, S.; Santiago, A.; Mendez, S.; Accarino, A.; Santos, J.; Guarner, F.; Azpiroz, F.; Manichanh, C. Reduction of butyrate- and methane-producing microorganisms in patients with Irritable Bowel Syndrome. Scientific Reports 2015, 5, 12693.
Qian, W.J.; Jacobs, J.M.; Liu, T.; Camp, D.G., 2nd; Smith, R.D. Advances and challenges in liquid chromatography-mass spectrometry-based proteomics profiling for clinical applications. Mol Cell Proteomics 2006, 5, 1727-1744.
Quintero GDF, Kok CR and Hutkins R (2022) The Future of Synbiotics: Rational Formulation and Design. Frontiers in Microbiology 13:919725.
Raab S. , Leiser R , Kemmer H, A review on early gut maturation and colonization in pigs, including biological and dietary factors affecting gut homeostasis. Animal Feed Science and Technology 2017, 233, 89-103
Rabhi Nassima , Thibodeau Alexandre , Côté Jean-Charles , Devillers Nicolas , Laplante Benoit , Fravalo Philippe , Larivière-Gauthier Guillaume , Thériault William P. , Faucitano Luigi , Beauchamp Guy , Quessy Sylvain, Association Between Tail-Biting and Intestinal Microbiota Composition in Pigs, Frontiers in Veterinary Science,Volume 7 - 2020
Radhakrishnan, S.T.; Gallagher, K.I.; Mullish, B.H.; Serrano-Contreras, J.I.; Alexander, J.L.; Miguens Blanco, J.; Danckert, N.P.; Valdivia-Garcia, M.; Hopkins, B.J.; Ghai, A.; et al. Rectal swabs as a viable alternative to faecal sampling for the analysis of gut microbiota functionality and composition. Scientific Reports 2023, 13, 493.
Reilly, P.; Sweeney, T.; Smith, A.G.; Pierce, K.M.; Gahan, D.A.; Callan, J.J.; Doherty, J.V.O. The effects of cereal-derived β-glucans and enzyme supplementation on intestinal microbiota, nutrient digestibility and mineral metabolism in pigs. Livestock Science 2010, 133, 144-147.
Ringseis, R.; Eder, K. Heat stress in pigs and broilers: role of gut dysbiosis in the impairment of the gut-liver axis and restoration of these effects by probiotics, prebiotics and synbiotics. Journal of Animal Science and Biotechnology 2022, 13, 126.
Rinninella, E.; Raoul, P.; Cintoni, M.; Franceschi, F.; Miggiano, G.A.D.; Gasbarrini, A.; Mele, M.C. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms 2018, 7.
Roy M, Roy AK, Farrell JJ., Ocular syphilis in an immunocompetent host, IDCases, volume 19, 2020
Rusch, J.A.; Layden, B.T.; Dugas, L.R. Signalling cognition: the gut microbiota and hypothalamic-pituitary-adrenal axis. Frontiers in Endocrinology (Lausanne) 2023, 14, 1130689.

Rusling, M.; Karim, A.; Kaye, A.; Lee, C.-M.J.; Wegman−Points, L.; Mathis, V.; Lampeter, T.; Yuan, L.-L. Influences of Ruminococcus bromii and Peptostreptococcaceae on voluntary exercise behavior in a rodent model. Frontiers in Microbiomes 2024, 3.

Rydal, M.P.; Gambino, M.; Castro-Mejia, J.L.; Poulsen, L.L.; Jørgensen, C.B.; Nielsen, J.P. Post-weaning diarrhea in pigs from a single Danish production herd was not associated with the pre-weaning fecal microbiota composition and diversity. Frontiers in Microbiology 2023, 14.

Sabit, H.; Kassab, A.; Alaa, D.; Mohamed, S.; Abdel-Ghany, S.; Mansy, M.; Said, O.A.; Khalifa, M.A.; Hafiz, H.; Abushady, A.M. The Effect of Probiotic Supplementation on the Gut–Brain Axis in Psychiatric Patients. Current Issues in Molecular Biology. 2023, 45, 4080-4099.

Sakata, T. Pitfalls in short-chain fatty acid research: A methodological review. Animal Science Journal 2019, 90, 3-13.

Salam, M.A.; Al-Amin, M.Y.; Salam, M.T.; Pawar, J.S.; Akhter, N.; Rabaan, A.A.; Alqumber, M.A.A. Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. Healthcare (Basel) 2023, 11.

Samulėnaitė, S.; García-Blanco, A.; Mayneris-Perxachs, J.; Domingo-Rodríguez, L.; Cabana-Domínguez, J.; Fernàndez-Castillo, N.; Gago-García, E.; Pineda-Cirera, L.; Burokas, A.; Espinosa-Carrasco, J.; et al. Gut microbiota signatures of vulnerability to food addiction in mice and humans. Gut 2024, 73, 1799-1815.

Sansom, B. F. & Gleed, P. T. The ingestion of sow's faeces by suckling piglets. Br. Journal of Nutrition. 46, 451–456. 1981.

Savignac HM., Couch Y., Michael Stratford, David M. Bannerman, George Tzortzis, Daniel C. Anthony, Philip W.J. Burnet, Prebiotic administration normalizes lipopolysaccharide (LPS)-induced anxiety and cortical 5-HT2A receptor and IL1-β levels in male mice, Brain, Behavior, and Immunity, Volume 52, 2016, Pages 120-131.

Shah, A.B.; Baiseitova, A.; Zahoor, M.; Ahmad, I.; Ikram, M.; Bakhsh, A.; Shah, M.A.; Ali, I.; Idress, M.; Ullah, R.; et al. Probiotic significance of Lactobacillus strains: a comprehensive review on health impacts, research gaps, and future prospects. Gut Microbes 2024, 16, 2431643.

Shen C. , Tong X, Chen R, Gao S., Liu X, Schinckel AP, Li Y, Xu F, Zhou B., Identifying blood-based biomarkers associated with aggression in weaned pigs after mixing. Applied Animal Behaviour Science 2020, 224, 104927.

Shi, H.; Yu, Y.; Lin, D.; Zheng, P.; Zhang, P.; Hu, M.; Wang, Q.; Pan, W.; Yang, X.; Hu, T.; et al. β-glucan attenuates cognitive impairment via the gut-brain axis in diet-induced obese mice. Microbiome 2020, 8, 143.

Shields, G.S.; Sazma, M.A.; Yonelinas, A.P. The effects of acute stress on core executive functions: A meta-analysis and comparison with cortisol. Neuroscience and Biobehavioural Reviews 2016, 68, 651-668.

Shin, D.; Chang, S.Y.; Bogere, P.; Won, K.; Choi, J.Y.; Choi, Y.J.; Lee, H.K.; Hur, J.; Park, B.Y.; Kim, Y.; et al. Beneficial roles of probiotics on the modulation of gut microbiota and immune response in pigs. PLoS One 2019, 14, e0220843.

Shoukat, M.; Sorrentino, A. Cereal β-glucan: a promising prebiotic polysaccharide and its impact on the gut health. International Journal of Food Science & Technology 2021, 56, 2088-2097.

Shrestha, A.; Metzler-Zebeli, B.U.; Karembe, H.; Sperling, D.; Koger, S.; Joachim, A. Shifts in the Fecal Microbial Community of Cystoisospora suis Infected Piglets in Response to Toltrazuril. Frontiers in Microbiology 2020, 11.

Silva YP, Bernardi A, Frozza RL. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. Frontiers in Endocrinology (Lausanne). 2020 Jan 31;11:25.

Slavin, J. Fiber and prebiotics: mechanisms and health benefits. Nutrients 2013, 5, 1417-1435

Smith AP. An investigation of the effects of breakfast cereals on alertness, cognitive function and other aspects of the reported well-being of children. Nutritional Neuroscience 2010, 13, 230—236.

Smith, N.W.; Shorten, P.R.; Altermann, E.; Roy, N.C.; McNabb, W.C. The Classification and Evolution of Bacterial Cross-Feeding. Frontiers in Ecology and Evolution 2019, 7.

St-Pierre, B.; Perez Palencia, J.Y.; Samuel, R.S. Impact of Early Weaning on Development of the Swine Gut Microbiome. Microorganisms 2023, 11.

Sudo, N.; Chida, Y.; Aiba, Y.; Sonoda, J.; Oyama, N.; Yu, X.N.; Kubo, C.; Koga, Y. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J Physiol 2004, 558, 263-275.

Sushytskyi, L.; Synytsya, A.; Čopíková, J.; Lukáč, P.; Rajsiglová, L.; Tenti, P.; Vannucci, L.E. Perspectives in the Application of High, Medium, and Low Molecular Weight Oat β-d-Glucans in Dietary Nutrition and Food Technology—A Short Overview. Foods 2023, 12, 1121.

Swedres –Svarm, 2023, reports available for download at www.folkhalsomyndigheten.se/ publicerat-material/ or at www.sva.se/swedres-svarm/

Szabó, C.; Kachungwa Lugata, J.; Ortega, A.D.S.V. Gut Health and Influencing Factors in Pigs. Animals 2023, 13, 1350.

Tang, Q.; Yi, H.; Hong, W.; Wu, Q.; Yang, X.; Hu, S.; Xiong, Y.; Wang, L.; Jiang, Z. Comparative Effects of L. plantarum CGMCC 1258 and L. reuteri LR1 on Growth Performance, Antioxidant Function, and Intestinal Immunity in Weaned Pigs. Frontiers in Veterinary Science 2021, 8.

Tang Q., Lan T., Zhou C., Gao J, Wu L., Wei H., Wenxue Li, Zhiru Tang, Wenjie Tang, Hui Diao, Yetong Xu, Xie Peng, Jiaman Pang, Xuan Zhao, Zhihong

Sun (2024), Nutrition strategies to control post-weaning diarrhea of piglets: From the perspective of feeds,Animal Nutrition,Volume 17, 2024, 297-311.
Theil, P.K.; Krogh, U.; Bruun, T.S.; Feyera, T. Feeding the modern sow to sustain high productivity. Molecular Reproduction and Development 2023, 90, 517-532.
Thu TV, Loh TC, Foo HL, Yaakub H, Bejo MH. Effects of liquid metabolite combinations produced by Lactobacillus plantarum on growth performance, faeces characteristics, intestinal morphology and diarrhoea incidence in postweaning piglets. Tropical Animal Health and Production. 2011 Jan;43(1):69-75.
Tiwari, U.P.; Singh, A.K.; Jha, R. Fermentation characteristics of resistant starch, arabinoxylan, and β-glucan and their effects on the gut microbial ecology of pigs: A review. Animal Nutrition 2019, 5, 217-226.
Tobias, K.M.; Serrano, L.; Sun, X.; Flatland, B. The effectiveness of cooling conditions on temperature of canine EDTA whole blood samples. PeerJ 2016, 4.
Tonel, I.; Pinho, M.; Lordelo, M.M.; Cunha, L.F.; Garres, P.; Freire, J.P.B. Effect of butyrate on gut development and intestinal mucosa morphology of piglets. Livestock Science 2010, 133, 222-224.
Tremblay A, Lingrand L, Maillard M, Feuz B, Tompkins TA. The effects of psychobiotics on the microbiota-gut-brain axis in early-life stress and neuropsychiatric disorders. Progress in Neuropsychopharmacology and Biological Psychiatry. 2021 Mar 8;105:110142.
Tsukuda, N.; Yahagi, K.; Hara, T.; Watanabe, Y.; Matsumoto, H.; Mori, H.; Higashi, K.; Tsuji, H.; Matsumoto, S.; Kurokawa, K.; et al. Key bacterial taxa and metabolic pathways affecting gut short-chain fatty acid profiles in early life. The ISME Journal 2021, 15, 2574-2590.
Tucker, A.L.; Widowski, T.M. Normal profiles for deciduous dental eruption in domestic piglets: effect of sow, litter, and piglet characteristics. Journal of Animal Science 2009, 87, 2274-2281.
Tsukahara T, Koyama H. Masaaki O. Ushida K. Stimulation of Butyrate Production by Gluconic Acid in Batch Culture of Pig Cecal Digesta and Identification of Butyrate-Producing Bacteria,The Journal of Nutrition, Volume 132, Issue 8,2002, Pages 2229-2234,
Ueno, H.; Suemitsu, S.; Murakami, S.; Kitamura, N.; Wani, K.; Matsumoto, Y.; Okamoto, M.; Aoki, S.; Ishihara, T. Juvenile stress induces behavioral change and affects perineuronal net formation in juvenile mice. BMC Neuroscience 2018, 19, 41.
Upadhaya, S.-D.; Kim, I.-H. The Impact of Weaning Stress on Gut Health and the Mechanistic Aspects of Several Feed Additives Contributing to Improved Gut Health Function in Weanling Piglets—A Review. Animals 2021, 11, 2418.

Vacca, M.; Celano, G.; Calabrese, F.M.; Portincasa, P.; Gobbetti, M.; De Angelis, M. The Controversial Role of Human Gut Lachnospiraceae. Microorganisms 2020, 8.

Valeriano, V.D.; Balolong, M.P.; Kang, D.K. Probiotic roles of Lactobacillus sp. in swine: insights from gut microbiota. J Appl Microbiol 2017, 122, 554-567.

van Beers-Schreurs HM, Nabuurs MJ, Vellenga L, Kalsbeek-van der Valk HJ, Wensing T, Breukink HJ. Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. Journal of Nutrition 1998 Jun;128(6):947-53.

van Hees, Chiers, Hartog, van Kempen, Maes, Millet, Janssens. Supplementing oat hulls to the diet of suckling piglets altered their intestinal tract and colonic microbiota development. Animal Nutrition 2023, 12, 284-296.

Van Kerschaver, C.; Turpin, D.; Michiels, J.; Pluske, J. Reducing Weaning Stress in Piglets by Pre-Weaning Socialization and Gradual Separation from the Sow: A Review. Animals 2023, 13, 1644.

Vanheukelom, Driessen, Geers,The effects of environmental enrichment on the behaviour of suckling piglets and lactating sows: A review, Livestock Science, Volume 143, Issues 2–3,2012, Pages 116-131, ISSN 1871-1413.

Vasquez, R.; Oh, J.K.; Song, J.H.; Kang, D.K. Gut microbiome-produced metabolites in pigs: a review on their biological functions and the influence of probiotics. Journal of Animal Science and Technology 2022, 64, 671-695.

Verbeek, E.; Keeling, L.; Landberg, R.; Lindberg, J.E.; Dicksved, J. The gut microbiota and microbial metabolites are associated with tail biting in pigs. Scientific Reports 2021, 11, 20547.

Vidal EB, Martín-Orúe SM, Castillejos L,Vinelli, V.; Biscotti, P.; Martini, D.; Del Bo, C.; Marino, M.; Meroño, T.; Nikoloudaki, O.; Calabrese, F.M.; Turroni, S.; Taverniti, V.; et al. Effects of Dietary Fibers on Short-Chain Fatty Acids and Gut Microbiota Composition in Healthy Adults: A Systematic Review. Nutrients 2022, 14.

Volman JJ, Ramakers JD, Plat J, Dietary modulation of immune function by β-glucans,Physiology & Behavior,Volume 94, 2, 2008, pages 276-284

Voulgari-Kokota, A.; Falcao Salles, J.; Schoemaker, R.G. Aggression shapes the gut microbiome; a study in rats. PLOS ONE 2024, 19.

Walters EM, Prather RS. Advancing swine models for human health and diseases. Missouri Medicine 2013 May-Jun;110(3):212-5.

Wang, G.; Wang, X.; Ma, Y.; Cai, S.; Yang, L.; Fan, Y.; Zeng, X.; Qiao, S. Lactobacillus reuteri improves the development and maturation of fecal microbiota in piglets through mother-to-infant microbe and metabolite vertical transmission. Microbiome 2022a, 10, 211.

Wang, G.; Zou, D.; Lu, X.; Gu, X.; Cheng, Y.; Qi, T.; Cheng, Y.; Yu, J.; Ye, M.; Zhou, P. Gut Microbiota Alternation in Disease Progression of Neurosyphilis. Infection and Drug Resistance 2022b, 15.

Wang, H.; Kim, I.H. Evaluation of Dietary Probiotic (Lactobacillus plantarum BG0001) Supplementation on the Growth Performance, Nutrient Digestibility, Blood Profile, Fecal Gas Emission, and Fecal Microbiota in Weaning Pigs. Animals (Basel) 2021a, 11.

Wang, J.; Zeng, Y.; Wang, S.; Liu, H.; Zhang, D.; Zhang, W.; Wang, Y.; Ji, H. Swine-Derived Probiotic Lactobacillus plantarum Inhibits Growth and Adhesion of Enterotoxigenic Escherichia coli and Mediates Host Defense. Frontiers in Microbiology 2018, 9, 1364.

Wang, K.; Hu, C.; Tang, W.; Azad, M.A.K.; Zhu, Q.; He, Q.; Kong, X. The Enhancement of Intestinal Immunity in Offspring Piglets by Maternal Probiotic or Synbiotic Supplementation Is Associated With the Alteration of Gut Microbiota. Frontiers in Nutrition 2021b, 8, 686053.

Wang, M.; Wu, H.; Lu, L.; Jiang, L.; Yu, Q. Lactobacillus reuteri Promotes Intestinal Development and Regulates Mucosal Immune Function in Newborn Piglets. Frontiers in Veterinary Science 2020a, 7, 42.

Wang, M.; Yang, C.; Wang, Q.Y.; Li, J.Z.; Li, Y.L.; Ding, X.Q.; Yin, J.; Yang, H.S.; Yin, Y.L. The growth performance, intestinal digestive and absorptive capabilities in piglets with different lengths of small intestines. Animal 2020b, 14, 1196-1203.

Wang, T.; Teng, K.; Liu, Y.; Shi, W.; Zhang, J.; Dong, E.; Zhang, X.; Tao, Y.; Zhong, J. Lactobacillus plantarum PFM 105 Promotes Intestinal Development Through Modulation of Gut Microbiota in Weaning Piglets. Frontiers in Microbiology 2019b, 10.

Wang, X.; Tsai, T.; Deng, F.; Wei, X.; Chai, J.; Knapp, J.; Apple, J.; Maxwell, C.V.; Lee, J.A.; Li, Y.; et al. Longitudinal investigation of the swine gut microbiome from birth to market reveals stage and growth performance associated bacteria. Microbiome 2019c, 7, 109.

Wenzl, H.H.; Schimpl, G.; Feierl, G.; Steinwender, G. Time course of spontaneous bacterial translocation from gastrointestinal tract and its relationship to intestinal microflora in conventionally reared infant rats. Digestive Diseases and Sciences 2001, 46, 1120-1126.

Wierup, M. The Swedish experience of the 1986 year ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. Microbial Drug Resistance 2001, 7, 183-190.

Winters, Kobek-Kjeldager, Foldager, Tecles, Pedersen Stress responses in pigs postweaning: Effect of heavier hybrid and weaning intact litters. Applied Animal Behaviour Science 2023, 269, 106106.

Wu, D. Chen, B. Yu, Y. Luo, P. Zheng, X.Mao, J. Yu, J. He, Effect of different dietary non-starch fiber fractions on growth performance, nutrient digestibility, and intestinal development in weaned pigs, Nutrition 51-52 (2018) 20–28.

Xi, M.; Yan, Y.; Duan, S.; Li, T.; Szeto, I.M.; Zhao, A. Short-chain fatty acids in breast milk and their relationship with the infant gut microbiota. Frontiers in Microbiology 2024, 15, 1356462.

Xie, R.; Jiang, P.; Lin, L.; Jiang, J.; Yu, B.; Rao, J.; Liu, H.; Wei, W.; Qiao, Y. Oral treatment with Lactobacillus reuteri attenuates depressive-like behaviors and serotonin metabolism alterations induced by chronic social defeat stress. Journal of Psychiatric Reseaarch 2020, 122, 70-78.

Xu J, Wang H, Xu R, Li Q, Li L, Su Y, Liu J, Zhu W. Daily fluctuation of Lactobacillus species and their antibiotic resistome in the colon of growing pigs. The Science of the Total Environment. 2024 Mar 25; 918:170821.

Yang KM, Jiang ZY, Zheng CT, Wang L, Yang XF. Effect of Lactobacillus plantarum on diarrhea and intestinal barrier function of young piglets challenged with enterotoxigenic Escherichia coli K88. Journal of Animal Science (2014) 92:1496–503.

Yang, B.; Yue, Y.; Chen, Y.; Ding, M.; Li, B.; Wang, L.; Wang, Q.; Stanton, C.; Ross, R.P.; Zhao, J.; et al. Lactobacillus plantarum CCFM1143 Alleviates Chronic Diarrhea via Inflammation Regulation and Gut Microbiota Modulation: A Double-Blind, Randomized, Placebo-Controlled Study. Frontiers in Immunology 2021a, 12.

Yu, Z.; Chen, J.; Liu, Y.; Meng, Q.; Liu, H.; Yao, Q.; Song, W.; Ren, X.; Chen, X. The role of potential probiotic strains Lactobacillus reuteri in various intestinal diseases: New roles for an old player. Frontiers in Microbiology 2023, 14, 1095555.

Zebunke, M.; Puppe, B.; Langbein, J. Effects of cognitive enrichment on behavioural and physiological reactions of pigs. Physiol Behav 2013, 118, 70-79.

Zhang, D.; Jian, Y.-P.; Zhang, Y.-N.; Li, Y.; Gu, L.-T.; Sun, H.-H.; Liu, M.-D.; Zhou, H.-L.; Wang, Y.-S.; Xu, Z.-X. Short-chain fatty acids in diseases. Cell Communication and Signaling 2023, 21, 212.

Zhang, L.; Mu, C.; He, X.; Su, Y.; Mao, S.; Zhang, J.; Smidt, H.; Zhu, W. Effects of dietary fibre source on microbiota composition in the large intestine of suckling piglets. FEMS Microbiology Letters 2016, 363.

Zhang, Q.; Zhao, W.; Hou, Y.; Song, X.; Yu, H.; Tan, J.; Zhou, Y.; Zhang, H.-T. β-Glucan attenuates cognitive impairment of APP/PS1 mice via regulating intestinal flora and its metabolites. CNS Neuroscience & Therapeutics 2023c, 29, 1690-1704.

Zhang, S.; Ou, J.; Luo, Z.; Kim, I.H. Effect of dietary β-1,3-glucan supplementation and heat stress on growth performance, nutrient digestibility, meat quality,

organ weight, ileum microbiota, and immunity in broilers. Poultry Science 2020, 99, 4969-4977.

Zhang D., Wang S., Li G., Chen M, Liu H., Ji H,Two doses of Lactobacillus induced different microbiota profiles and serum immune indices in pigs, Journal of Functional Foods,volume 102, 2023b, 105405

Zhao, L.; Zhang, F.; Ding, X.; Wu, G.; Lam, Y.Y.; Wang, X.; Fu, H.; Xue, X.; Lu, C.; Ma, J.; et al. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. Science 2018, 359, 1151-1156.

Zheng, Y.; Zhang, Z.; Tang, P.; Wu, Y.; Zhang, A.; Li, D.; Wang, C.Z.; Wan, J.Y.; Yao, H.; Yuan, C.S. Probiotics fortify intestinal barrier function: a systematic review and meta-analysis of randomized trials. Frontiers in Immunology 2023b, 14, 1143548.

Zhou WB, Miao ZN, Zhang B, Long W, Zheng FX, Kong J et al., Luteolin induces hippocampal neurogenesis in the Ts65Dn mouse model of down syndrome. Neural Regeneration Research 14:613–620 (2019).

Zhou, B.; Jin, G.; Pang, X.; Mo, Q.; Bao, J.; Liu, T.; Wu, J.; Xie, R.; Liu, X.; Liu, J.; et al. Lactobacillus rhamnosus GG colonization in early life regulates gut-brain axis and relieves anxiety-like behavior in adulthood. Pharmacology Research 2022a, 177, 106090.

Zhou, Y.; Luo, Y.; Yu, B.; Zheng, P.; Yu, J.; Huang, Z.; Mao, X.; Luo, J.; Yan, H.; He, J. Effect of β-Glucan Supplementation on Growth Performance and Intestinal Epithelium Functions in Weaned Pigs Challenged by Enterotoxigenic Escherichia coli. Antibiotics (Basel) 2022b, 11.

Zhu, C.; Yao, J.; Zhu, M.; Zhu, C.; Yuan, L.; Li, Z.; Cai, D.; Chen, S.; Hu, P.; Liu, H.Y. A meta-analysis of Lactobacillus-based probiotics for growth performance and intestinal morphology in piglets. Frontiers in Veterinary Science 2022a, 9, 1045965.

Zhu, C.; Yao, J.; Zhu, M.; Zhu, C.; Yuan, L.; Li, Z.; Cai, D.; Chen, S.; Hu, P.; Liu, H.-Y. A meta-analysis of Lactobacillus-based probiotics for growth performance and intestinal morphology in piglets. Frontiers in Veterinary Science 2022b, 9.

Populärvetenskaplig sammanfattning

Inom slaktsvinsproduktion har man gjort flera framsteg de senaste decennierna, bland annat inom reproduktionsteknik, genetik och foder, vilket bidragit till större griskullar, förbättrad tillväxt och produktivitet. En kvarstående utmaning är dock stress kopplat till perioden runt avvänjning, där smågrisen abrupt får sluta dia och istället övergå till fast föda. Detta kan leda till flera störningar, bland annat en rubbad balans av tarmmikrobiotan, försämrad tillväxt och ökad sjukdomsbenägenhet, vilket påverkar grisarnas hälsa och prestation negativt. Det finns därför ett växande behov av att hitta lösningar som förbättrar grisar motståndskraft mot störningar vid avvänjning, vilket har blivit ännu mer angeläget under det senaste decenniet. Tarmmikrobiotan har på senare år varit i fokus då den sammankopplas med både hälsa och sjukdom. Sammansättningen på tarmmikrobiotan har visats viktig för bland annat att kunna skydda mot infektioner, bidra till tarmens immunologiska utmognad, men även kan påverka en individs beteende, då den kan påverka kommunikationen mellan tarmen och hjärnan.

En tidigare mognad av tarmmikrobiotan före avvänjningen skulle kunna vara en effektiv strategi för att förbättra tarmens robusthet och förhindra mottaglighet för patogena infektioner och därmed minska problematik med diarré.

Ett sätt att påverka tarmhälsa är via kosten. Prebiotika är fibrer som bidrar till en bra tarmmikrobiota och god tarmhälsa och probiotika är nyttiga mikroorganismer som bidrar till god hälsa. Både prebiotika och probiotika har potentialen att stärka tarmmikrobiotan, immunförsvaret och tillväxten, vilket förbättrar den övergripande tarmhälsan och stresståligheten tidigt i grisarnas liv. Ett kosttillskott som använts till både människor och djur är havre beta-glukaner, som har prebiotiska egenskaper och är mycket fermenterbar av tarmbakterier, vilket leder till produktion av fördelaktiga

kortkedjiga fettsyror (SCFA). Tidigt tillskott av beta glukaner kan stimulera tarmmikrobiotan och förbättra den mikrobiella etableringen i tarmen genom att främja fördelaktiga bakterier, samt potentiellt även stödja både tarmens- och hjärnans utveckling. På samma sätt kan probiotika, exempelvis mjölksyrabakterier, hjälpa till att kolonisera tarmen med fördelaktiga bakterier under utvecklingsfasen och konkurrera ut skadliga bakterier och minska risken för infektioner och diarré i tidig ålder. Probiotika har också visats kunna förbättra tarmens barriärfunktion, tillväxt och immunförsvar.
Att stimulera ätbeteendet och bidra till en tidig etablering av tarmmikrobiota skulle kunna ge en solid grund för smågrisars tillväxt och hälsa. Dock finns det få studier som fokuserat på hur fodertillskott under diperioden kan bidra till att modulera mikrobiotan och tarmhälsan. Därför var det huvudsakliga syften att utvärdera effekterna av tidiga kostinterventioner till diande grisar, antingen genom tillskott av β-glukaner från havre, eller via tillskott av två mjölksyrabakterier (*Limosilactobacillus reuteri* och *Lactiplantibacillus plantarum*) under diperioden. I studierna utvärderades effekter på tarmmikrobiota, kortkedjiga fettsyror, påverkan på vikt och tarmslemhinnan. Dessutom studerades effekter på beteende och möjliga korrelationer mellan beteende, tarmmikrobiota och kortkedjiga fettsyror.
Resultaten från studierna visade på tydliga åldersrelaterade effekter men supplementet av beta-glukanerna visade inte några tydliga effekter på vare sig tarmmikrobiotan, koncentration av kortkedjiga fettsyror eller vikt. Däremot, sågs en koppling mellan mikrobiotans sammansättning och beteendevariabler med signifikanta samband mellan specifika bakteriegrupper och beteendemönster. Tillskott av β-glukaner hade dessutom en negativ inverkan på grisens förmåga att bryta ett inlärt beteende. Tillskott av mjölksyrabakterierna förändrade inte mikrobiotans sammansättning i rektalsvabbar hos grisar, men orsakade lokala förändringar i tunntarmen (ileum) och tjocktarmen (kolon), med ökad förekomst av bakteriegruppen *Prevotella 2* i tarmslemhinnan i ileum och ökad förekomst av *Lachnospiraceae_NK4136* i tarmslemhinnan i både ileum och kolon, samt i tarminnehållet som fanns i kolon. Tillskotten påverkade dock inte mängd kortkedjiga fettsyror, tarmhistologi eller tillväxt jämfört med kontrollgruppen. Dessa resultat tyder på att tillskott av mjölksyrabakterier har potential att påverka utvecklingen av mikrobiota lokalt i olika delar av tarmen.

Sammantaget visade resultaten från interventionen på begränsade effekter, men uppföljande studier behöver genomföras inom för att klarlägga potentialen med tidiga kostinterventioner.

Acknowledgements

"Success is not final; failure is not fatal: it is the courage to continue that count."
— Winston S. Churchill

The time I spent at HUV, today called THV, before and after my PhD, was a formative experience.
So many memories were made here, so much happiness, life experience and learned lessons. I learned much about science, research, nutrition, gut health, monogastric animals and suckling piglets. I was introduced and fell in love with the marvels of the multitude of gut microbes. An entirely new world of wonders.
New expanded knowledge and many learning curves that rewrote a little bit of my code. My subjective experience, but now my whole world is entirely transformed, and THE world is precisely the same. From Snowball to Boxer, then Clover, and finally Benjamin. The entire game changed, and that is powerful.

I could not possibly name all of the people I owe great thanks to, but I will try to mention some of them.
I want to thank **PhD students** and **colleagues** for our memorable times together. I appreciate every one of you!
Especially **Malin**, **Anna**, **Aprajita**, **Eliška**, **Katrin** and **Hanna**. We have known each other for some years, and you have made my days at the department much happier. I want to thank you for all those fikas talks and

walks, messages and the pep talks! I am happy I have such lovely friends like you guys!
Then, all my people in the **lab** and at **Lövsta**, retired and still working, **technicians**, **assistants**, **researchers** and **engineers**, you are all much appreciated! We had so many laughs together, and I learned so much from all of you. Thanks to our **administration** for making my life bearable when I got stuck in many bureaucratic tangles over the years.
Carlos H., thanks for introducing this wild world of science to me!
Margareta E., thank you for allowing me to push myself forward. You recognised my dreams and urged me to engage in different research projects, learn and grow.
Mikaela L., I genuinely appreciate your trusting me and allowing me to prove myself and work with you. It was so refreshing, and I learned so much from you. I am incredibly grateful for that opportunity.
I owe many thanks to **Bengt-Ove R.**, you offered me so much warmth, support, guidance and wisdom. And without our many talks, your fatherly approach, support and understanding, I wouldn't be here today. I never found the right words to tell you how much I appreciate everything you did for me, but I do! At the same time, I would like to say that I truly enjoyed working alongside you; it was a privilege to learn from you. I am grateful to have experienced both your professional excellence and the warm guidance of a great mentor!
There is a place of pride in this world for people like **Sara Ö.** Gutsy women like you deserve all the appreciation and love, and we all know that and miss you! I learned so much from you about leadership. Balancing warmth and firmness, you showed me what strength is with compassion and confidence without arrogance and how respect isn't demanded but earned through integrity, consistency and action. Authentic leadership is not just about giving direction but sparking growth in others. Thank you for reminding me that I earned my seat when you invited me to the table, and I shall not allow anyone to take it away from me. You will always have a special place in my heart!
Magdalena Å., you stepped in and changed things, showing everyone that it can be done; if there's a will, there is a way. You set an example for me, proving that everything is achievable with effort. Although we didn't have much time to work together, I am happy our paths crossed and grateful for everything you did for me!

Maria Å., you are one of the most interesting, funny and clever people I have met at HUV, although you are actually from Växa. Your uniqueness is your magic power, and I am so grateful I had the opportunity to spend time with you and get to know you a bit! Thank you for being you around me!
I thought I would start this chapter with the famous Charles Dickens opening of The Tale of Two Cities, but then I was afraid our **Ewa W.** would say, "Your time at HUV lasted almost as long as the French Revolution" So I changed my mind 😊 Thank you, Ewa, for all your support and for helping me accomplish my goals; I sincerely appreciate everything you have done for me this last year! You offered me important insights into leadership, decision-making, and taking initiative when necessary.

I came here not expecting much, but I am leaving with the most incredible group of friends! Words may never be enough to show my gratitude, but I will try my best.
My **Hanna P.**, my colleague and my friend for life! Thank you for your unwavering support, warmth, and love you generously shared! I am truly grateful for your safe space, which allows me to vent freely. It has been invaluable in helping me find clarity and understanding in moments of uncertainty. Thank you for all the small family moments you offered; your kindness has been a guiding light, and you have given me comfort and strength when I needed it most! Bamsekram!
Aleksandar, we drank a lot of coffee together from my first week at HUV, and I truly enjoyed both the caffeine and the conversations! Thanks for sticking around and tolerating me despite my naïve optimism and not disowning me…yet, it is not an easy task (I KNOW!). Thanks for being my friend, even when my wide-eyed stupidity and stubbornness probably made you want to facepalm daily, just occasionally losing your cool and rolling your eyes.
And our **Dragana**, the wise one with a clever mind and compassionate soul, speaks with the Dalai Lama's calm depth. I cherish you both a lot!

Abby and **Ewa**, my "statgeniueses"! You had so much understanding, and you were so patient with me. I learned so much from you guys; without your help, I couldn't finish my thesis. Thank you!
My beautiful **Anna M.**, my world would be so small without you! Thank you for never letting me down, for always being by my side, cheering me

and from time to time giving me the cruel truth I needed to hear! Your boys and princess are bringing me so much happiness and laughter, and I love you all!
I always firmly believed that women should support and hype other women. My magical five just reaffirmed that belief. My friends, I can't thank you enough! **Bojana, Limara, Maria, Sanam and Lilibeth**, you have the peace of my heart forever.
Special thanks to Bojana for always being my Jamie! And your princesses- you guys are my definition of girl power! I love you a bunch!
Thanks to all of my people who stood by me, especially those who believed in me when I hadn't believed in myself and reminded me what is essential and what I truly deserve: my **Ivana K, John T, Hanna B, Yi H, Olivier P.**
My beloved **Haru J,** thanks for broadening my horizons and teaching me about wabi-sabi and kintsugi. I'm glad I was blessed with so many wonderful people!
Special thanks to my **Edita** for always making me smile; even when I cried, you always found a way to make me laugh! Moja najdraža "vrećica mlijeka", uvijek i zauvijek! One day, when the time comes for us to be in a nursing home, I do not doubt that you will make it the most joyful place on earth, making it the best place to be, and we will have so much fun together!
My **Sam**, my person. I don't have to pretend to be anyone or anything when I am with you, and I will forever be grateful you came into my life with your unlimited kindness and loyalty. The world wouldn't be the same without your undeniable humour and loving heart!
Vanna, my Swedish bonus mother. You were the one who opened your home and your heart for me and gave me so much love, support and kindness. No words can describe how much you mean to me and how deep my connection is to you. You accidentally came into my life, but you stayed on purpose! My Agathos kai sophos!
Harald and Ulla, my extended family! I love and cherish all our memorable talks and our laughs! I learned so much about Sweden, Swedish people, culture, politics, and literature from the two of you! Thank you for all your support and love; you will always be a massive part of my life!
My beloved family, my **mother** and my **father**, taught me that a rising tide lifts all boats, and at the end of the path, it will be known by those who matter. I was raised with the mantras to work hard until the job is finished, to show up and fight, but not forget to enjoy the journey. I admit, sometimes I forgot

parts of the mantras 😊 Thank you for never giving up on me and supporting and loving me all these years! You always said I can achieve anything I set my mind to. As my fathers and my favourite Frank said, I know that sometimes I bite off more than I can chew 😊
But then again, I faced it all, and I stood tall and carved my path, even when it meant crawling on my knees, pushing forward through the storm, forging my way with grit and resilience, step by step. After all, as my mother always said, their whispers have nothing on you.
Hvala mama i tata što ste mi svojim primjerom pokazali što je u životu vrijedno truda i kako se uvijek na kraju isplati biti čovjek! Volim vas do neba i natrag!
Many thanks to my beloved **sister**, her husband and nephews; without your love and support, I wouldn't be the person I am today! You made my life richer and happier in every possible way. Moj **T**. moj **K**. tetka vas beskrajno voli! Family is about the past we share and the moments we continue to create. I look forward to the moments ahead and many more unforgettable memories!

Thanks to all my beloved friends worldwide, whom my tired brain didn't remember to mention by name, but you know how much I appreciate and love you. You all have had such a significant impact on my life, making it so much more fun and better. You know you are all in my heart!

And, of course, last but not least, my supervisors!

Johan D. Through your guidance, insightful feedback, and remarkable ability to break down complex topics and make them easy to grasp, I have gained invaluable knowledge and broadened my understanding of microbiology, physiology, biochemistry and its intricate interplay with the gut and the brain. On a personal note, I sincerely appreciate the experience and the valuable insights you provided regarding mentorship. It gave me a deeper understanding of different leadership and decision-making approaches, even when perspectives diverged. While there were challenges, they ultimately contributed to the growth and refinement of my future professional approach. Some lessons arrived unexpectedly, shaping my perspective in ways I could not have foreseen and offering truly

transformative insights. You left me with wisdom that continues shaping my path, and I am genuinely grateful.

Torbjörn L. It has been a pleasure working and engaging in insightful conversations with you. Your mentorship taught me much valuable knowledge; your clever observations helped streamline my PhD path and made my journey easier. And on a more personal level, you gave me a profound understanding of the intricate dance of decision-making and hierarchy and how individuals navigate. You taught me how important it is to take initiative when necessary, when to push through and pivot, and when not to seize the opportunity but to let things unfold naturally. It was a mirror, reflecting more profound lessons of choices' quiet yet powerful influence on one's journey. I sincerely appreciate it.

Emma I. Your guidance in a friendly manner and the insights you provided when I was writing my thesis have been a true gift. From a personal perspective, thank you for imparting a valuable lesson on staying within one's designated role and not overstepping. It served as a reminder that there is strength in focus and wisdom in knowing when to hold ground, allowing others to walk their path regardless of external circumstances. I am grateful for your shared wisdom, which resonated deeply and left a lasting impact.

Else V. Though resolute in your expectations, you always treated me with fairness and integrity. Gifted teacher, weaving clarity into every explanation and with every word, you inspire the courage to try on my own, nurturing my growth with encouragement that lifts me higher. Your voice was one of both kindness and wisdom, providing advice with both warmth and clarity. Thanks for always understanding the challenges I faced. With your guidance, I found both understanding and the confidence to explore. I genuinely appreciate your support and help throughout this time; your assistance has been invaluable. Your help has been a quiet yet profound source of strength and a gesture I hold in high regard.

I am also grateful for all the disappointments and failures that have crossed my path, for they have been the silent teacher, guiding me with their lessons. In their shadows, I have found strength and resilience; through them, I have discovered the depth of my own growth. Letting go of what was not for me and moving on. One chapter ended, clearing out the old to make room for the new.

I

animals

MDPI

Article

Age Rather Than Supplementation with Oat β-Glucan Influences Development of the Intestinal Microbiota and SCFA Concentrations in Suckling Piglets

Lidija Arapovic [1], Yi Huang [1,2], Elin Manell [3], Else Verbeek [4], Linda Keeling [4], Li Sun [1,5], Rikard Landberg [6], Torbjörn Lundh [1], Jan Erik Lindberg [1] and Johan Dicksved [1,*]

[1] Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden
[2] Department of Animal Science and Technology, Guangxi University, Nanning 530004, China
[3] Department of Clinical Sciences, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden
[4] Department of Animal Environment and Health, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden
[5] Department of Molecular Sciences, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden
[6] Department of Biology and Biological Engineering, Division of Food and Nutrition Science, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden
* Correspondence: johan.dicksved@slu.se; Tel.: +46-18-672810

Simple Summary: Early development of the intestinal microbiota is considered critical for enteric health in pigs. Dietary strategies that improve gut microbiota development can thus help prevent disturbances associated with weaning. This study evaluated the effects of a soluble oat β-glucan supplement, compared with a placebo, in five litters of piglets during the suckling period. The supplement was introduced at week 1 of age and was provided three times per week until weaning. The effects of the supplement were evaluated in terms of weight development, concentrations of short-chain fatty acids in plasma and intestinal contents, and microbiota composition in rectal swabs and intestinal contents. The results showed that microbiota composition and short-chain fatty acid concentration changed with piglet age, but with no clear effects of the β-glucan supplement. There were no differences in body weight gain between β-glucan-supplemented and control piglets. Several correlations between specific microbes and short-chain fatty acids were identified. Significant litter effects were also found, confirming the importance of genetics and pen environment in suckling piglets.

Abstract: The effects of early supplementation with oat β-glucan during the suckling period on piglet gut microbiota composition, concentrations of short-chain fatty acids, and gut physiological markers were assessed. Fifty piglets from five litters, balanced for sex and birth weight, were divided within litters into two treatment groups: β-glucan and control. Piglets in the β-glucan group received the supplement three times/week from day 7 of age until weaning. Rectal swab samples were collected from 10 piglets per treatment group (balanced across litters) from week 1 to week 4, and plasma samples were collected at 1, 3, and 4 weeks of age. Additional samples of intestinal tissues and jugular and portal vein plasma were collected from 10 animals at weaning (one per treatment group and litter). The concentrations of short-chain fatty acids in plasma and the microbiota composition in rectal swabs were mainly influenced by piglet age, rather than the supplement. There were significant differences in microbiota composition between litters and several correlations between concentrations of short-chain fatty acids in plasma and specific microbial taxa in rectal swabs. Overall, β-glucan supplementation did not have any clear impact on the gut environment in suckling piglets, whereas a clear age-related pattern emerged.

Keywords: β-glucan; microbiota; SCFA; suckling piglets

Citation: Arapovic, L.; Huang, Y.; Manell, E.; Verbeek, E.; Keeling, L.; Sun, L.; Landberg, R.; Lundh, T.; Lindberg, J.E.; Dicksved, J. Age Rather Than Supplementation with Oat β-Glucan Influences Development of the Intestinal Microbiota and SCFA Concentrations in Suckling Piglets. *Animals* **2023**, *13*, 1349. https://doi.org/10.3390/ani13081349

Academic Editors: Guillermo Ramis and Livia Mendonça Pascoal

Received: 14 March 2023
Revised: 6 April 2023
Accepted: 10 April 2023
Published: 14 April 2023

1. Introduction

A range of factors influence the composition of microbiota in the gastrointestinal tract of piglets, including genetics, housing conditions, use of medication and diet [1,2]. The weaning period is known to be a very stressful time in the life of piglets, because weaning is associated with abrupt separation from the sow, major dietary changes, and mixing with unfamiliar piglets. Stress can lead to increased incidence of enteric infections and intestinal inflammation [3,4]. Overgrowth of harmful bacteria is a major risk factor in the development of enteric diseases such as post-weaning diarrhoea, which can lead to financial losses due to decreased growth rate, mortality, and the extra cost of medication. Although the use of antibiotics as growth promotors has been banned in the European Union for almost two decades, antibiotics are still extensively used for therapeutic purposes, e.g., to treat gastrointestinal infections. Antimicrobial use and associated antimicrobial resistance can affect animal and also human health [5]. Thus, it is important to find alternatives to antibiotics to promote enteric health in piglets. One option is diet manipulation, targeting fibre content and quality, to stimulate microbial fermentation, feed intake, and weight gain [6]. Dietary fibre is used as a biological response modifier because it has beneficial effects on the intestinal tract and stimulates the growth of beneficial bacteria. Bacterial fermentation in the small and large intestines produces metabolites such as short-chain fatty acids (SCFA) that have been shown to have positive effects on energy metabolism, intestinal barrier, and immune system in both animals and humans [6–8].

Dietary fibre, in particular oat bran, contains large amounts of β-glucans, which are almost fully fermented by bacteria in the large intestine [9]. Fermentation of β-glucans has been shown to increase the concentrations of lactic acid and SCFA, reduce intestinal ammonia production, and stimulate growth of a diverse, beneficial intestinal microbiota in adult pigs [10,11]. β-glucans of microbial origin have also been shown to have immunostimulatory and anti-inflammatory properties and may promote survival in experimental animals infected with different microbes [12]. Fermentation of β-glucans is believed to increase the production of butyrate [10] and may be beneficial for intestinal development in young piglets because it provides an energy source for colonocytes and stimulates mucus production [13]. Butyrate has also been shown to lower the number of harmful enterobacteria and reduce inflammation in the gut [14]. Therefore, concentrations of faecal SCFA are considered a marker of gut health status [15]. However, a large fraction of the SCFA produced in the gut is absorbed, so faecal levels of SCFA do not necessarily reflect the amount of SCFA produced in the gut [16]. To obtain more comprehensive data, SCFA excreted in faeces and SCFA absorbed and circulating in blood should both be analysed. In piglets, only a few studies have measured both faecal and circulating levels of SCFA [17,18]. SCFA from plasma appears to be linked to metabolic health and is considered a good biomarker of the effects of prebiotic interventions [19]. Even fewer studies have examined prebiotic fibre supplements and their influence on circulating levels of SCFAs during the suckling period of piglets. Previous studies in piglets have mainly focused on the post-weaning period, with few studies focusing on the early-life establishment of the gut microbiota, its contribution to individual piglet performance, and whether or not it can be modified by nutritional treatments [20,21].

The aim of this study was to evaluate growth performance, gut microbiota composition, SCFA concentration in faeces and blood, and histological development of the gut in suckling piglets provided with an oat β-glucan supplement. The starting hypothesis was that the β-glucan supplementation leads to earlier development of a mature microbiota and higher SCFA production.

2. Materials and Methods

2.1. Ethics Statement

This study was approved by the Ethics Committee for the Uppsala region, with reference number DNR C 1054/16. The study was conducted in accordance with the ARRIVE guidelines [22].

2.2. Animals

The experiment involved 50 Hampshire x Yorkshire pathogen-free piglets from five litters [23]. Ten piglets from each litter were allocated to two equal-sized treatment groups that were balanced for sex and birth weight. In litters with more than 10 piglets, the remaining piglets were allowed to stay with the litter but were not included in the study. These selection criteria were set before the experiment.

2.3. Housing and Management

The experiment was carried out at the Swedish Livestock Research Centre at Lövsta, Uppsala. Pregnant sows were moved to the farrowing unit one week before expected farrowing and stayed with their piglets until weaning at 35 days of age. The farrowing pens (3.35 × 2.0 m) consisted of a heated concrete floor as the lying and feeding area (2.1 m × 2. 0 m), a slatted dung area (1.25 m × 2.0 m), and a heated corner that was only accessible to the piglets. The sows were given 15–20 kg of chopped straw two days prior to the expected farrowing date. An additional small amount of straw (0.5–1 kg/day) was given daily as enrichment after the pens were manually cleaned. Piglets were weighed, marked with an individual number within one day after birth (ear-tattoo), and ear-tagged with their individual number at five days of age. A 1-mL intramuscular injection of an iron supplement (Uniferon, 200 mg/mL) was given at five days and again at two weeks of age. All piglets had ad libitum access to creep feed (Gottfrid, Lantmännen, Sweden, Supplementary Table S1) and water during the suckling period. At weaning, the sow was removed, and the piglets remained in their pen until nine weeks of age, when they were handled according to regular routines at the Research Centre.

2.4. Treatments

Five suckling piglets per litter received an oral supplement of oat β-glucan (40 mg/kg body weight) (BG group) and five piglets per litter received an oral supplement of water (CON group). Disposable syringes were prefilled with a paste form of β-glucan dissolved in water, which was supplemented directly into the mouth of each pig in the BG group. The piglets were assigned to the treatment groups prior to supplementation, which started at seven days of age and was continued three times per week until weaning at five weeks of age. The pigs in the CON group were handled in the same way but received water by syringe, instead of the BG supplement. The dietary supplement used in the experiment was SweOat bran BG28 (Swedish oat fibre, Gothenburg, Sweden), which contained 28% soluble β-glucan with molecular weight 2000 kDa. The total dietary fibre content of BG28 was 52 g/100 g.

2.5. Experimental Procedures

An overview of the experimental set-up is shown in Figure 1. Weight gain was recorded for all piglets on a weekly basis from birth until nine weeks of age, with an extra weight measurement on the day of weaning. Four piglets per litter, two from each treatment, balanced for sex and weight between treatments, were selected for collection of faecal microbiota samples using rectal swabs (E-Swabs, Copan Diagnostics) at 1, 2, 3, and 4 weeks of age. The swabs were immediately placed on ice after sampling and within 2 h were transferred to a freezer and stored at −80 °C until analysis. Blood samples from the jugular vein of the same piglets were collected at 1, 3, and 4 weeks of age, using a 21G vacutainer needle and 4 mL VenoSafe Vacutainer EDTA-tubes. After centrifugation for 20 min at 4 °C and 1500× g, plasma was collected and stored at −80 °C until analysis. The oral supplementation and sampling were performed consecutively. Due to obvious differences in the characteristics of the supplements, the individual collecting the samples was not blind to the treatment. However, the individual performing the laboratory analyses was blind to the treatments, reducing any potential bias in outcome for the two treatment groups. Piglets that needed treatment with antimicrobial drugs were excluded from the study.

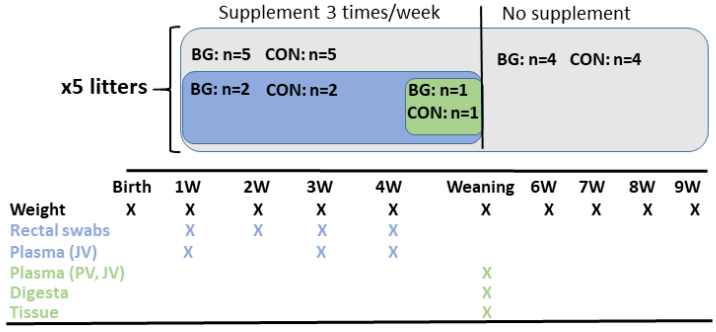

Figure 1. Overview of the experimental set-up. BG = β-glucan, CON = control, JV = jugular vein, PV = portal vein.

2.6. Post-Mortem Sampling Procedures

On the day before weaning, one piglet per treatment group and litter was euthanized for collection of blood and intestinal samples as described below, while the remaining piglets were weaned and handled according to existing farm routines. The five BG and five CON piglets used for post-mortem sampling were selected so that sex and body weight were balanced between the two diet treatments. The selected piglets were anaesthetized with a combination of medetomidine (Domitor® vet, 1 mg/mL; Orion Pharma Animal Health, Espoo, Finland) at a dose of 0.05 mg/kg BW, and tiletamine and zolazepam (Zoletil® 50 mg + 50 mg/mL; Virbac, Carros, France) at a dose of 2.5 + 2.5 mg/kg BW injected intramuscularly (i.m.). Buprenorphine (Vetergesic® vet, 0.3 mg/mL; Orion Pharma Animal Health, Espoo, Finland) was given at a dose of 0.01 mg/kg BW i.m. for additional analgesia and a 22G cannula (BD™ Venflon; BD, Franklin Lakes, NJ, USA) was placed in the auricular vein. Throughout the sampling procedure, anaesthesia was maintained with intravenous (i.v.) bolus doses of the pharmaceuticals listed above. Blood samples were collected from the jugular vein with a 20G vacutainer needle into EDTA vacutainer tubes. For blood collection from the portal vein, the abdomen was opened with an incision in linea alba and along the last rib. The portal vein was reached by blunt dissection, and blood was collected with a 20G needle and a 10 cc syringe and immediately transferred to an EDTA vacutainer tube. At the end of the procedure, the pigs were euthanized with pentobarbital sodium (Allfatal vet, 100 mg/mL; Omnidea, Stockholm, Sweden) i.v. while still under general anaesthesia. The blood samples were kept on ice and centrifuged and stored as described above. After euthanasia, the gastrointestinal tract was removed. A vertical incision was made immediately on the distal part of the ileum (approximately 15 cm from the caecum) and the central part of the colon, for the collection of intestinal samples. Intestinal contents collected from the colon were snap-frozen in liquid nitrogen and stored at −80 °C until analysis. In addition, one segment of tissue each from the distal ileum and central colon were taken and fixed in 10% formalin. All samples were individually re-coded and labelled before analysis, to avoid any bias in data analysis.

2.7. Histology of Intestinal Tissue

Ileal and colon tissue were stored in formalin with 10% neutral phosphate buffer (Merck KGaA, Darmstadt, Germany) for 48 h. After fixation, three samples per tissue and piglet were trimmed, dehydrated, cleaned, and embedded in paraffin. The paraffin-embedded tissue blocks were then cut into 4 mm thick sections, which were placed on a slide and stained with haematoxylin and eosin. A light microscope (Nikon ECLIPSE 80i, BergmanLabora AB, Danderyd, Sweden), equipped with image analysis software (NIS-Elements D 5.20.02, Nikon Instruments, Melville, NY, USA) was used for exami-

nation of gut thickness, mucosal thickness, villus height, crypt depth, and thickness of muscularis externa.

2.8. DNA Extraction, PCR Conditions and Sequence Analysis

DNA was extracted from rectal swabs and intestinal contents using QIAamp DNA Stool Minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, but with the addition of an extra mechanical lysis step using 0.1 mm zirconium/silica beads (Biospec Products, Bartlesville, OK, USA) and 2 × 1 min at 6000 rpm with a Precellys evolution (Bertin Instruments, Montigny-le-Bretonneaux, France). The isolated DNA was stored at −20 °C until analysis. PCR amplicons were generated from 16S rRNA genes using the primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC). PCR amplification used DreamTaq PCR chemistry with the following PCR conditions: initial denaturation at 94 °C for 3 min, followed by 25 cycles with 94 °C for 40 s, 58 °C for 40 s, and 72 °C for 60 s, and finally elongation at 72 °C for 7 min. Positive PCR reactions were confirmed with gel electrophoresis (1% agarose) using a Thermo Fisher Scientific Gene Ruler 1 kB DNA ladder as a size marker. The PCR product was purified using Agencourt AMPure purification (Beckmann Coulter, Indianapolis, IN, USA). A second PCR step was then applied to attach sample-specific barcodes and adaptor sequences. Cycling conditions were as follows: initial denaturation at 94 °C for 3 min, 8 cycles of 94 °C for 40 s, 58 °C for 40 s, and 72 °C for 60 s. PCR amplification ended with a final elongation step at 72 °C for 7 min. After a second cleaning step, all samples were quantified using a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples were pooled in equimolar amounts and sent for sequencing at SciLifeLab in Stockholm, Sweden, using Illumina MiSeq.

The sequencing data generated were processed according to the procedure described by Müller et al. [24]. The Quantitative Insights into Microbial Ecology (QIIME) version 1.8.0 pipeline was used for the quality filtering of data and to generate operational taxonomic units (OTUs) using the open reference OTU picking strategy at a threshold of 97%, with U-CLUST against a Greengenes core set (gg_13_8) [25]. Representative sequences were aligned against the Greengenes core set using PyNAST software (chimeric sequences were removed by ChimeraSlayer [26]). Taxonomy was assigned to each OTU using the Ribosomal Database Project (RDP) classifier with a minimum confidence threshold of 80% [27]. The final OTU table was filtered based on two criteria: an OTU had to be observed in three samples to be retained and an OTU had to contain 87 reads (0.001% of total reads). The sequence dataset was normalised so that each sample contained 14,900 sequences prior to analysis of microbiota. The raw sequencing data have been deposited at the National Center for Biotechnology Information database (NCBI), under accession number PRJNA940420.

2.9. Determination of SCFAs in Plasma and Digesta

Concentrations of SCFAs and caproic acid in EDTA plasma and digesta were analysed by liquid chromatography-mass spectrometry (LC-MS) according to a method described previously [28], with some modifications (for details, see Supplementary File S1).

2.10. Statistical Analysis

Microbiota composition was analysed using both univariate and multivariate approaches. All multivariate statistical analyses were performed using the statistical software PAST, version 4.04 [29]. A multivariate approach involving principal coordinate analysis (PCoA) based on Bray–Curtis distance was performed on OTU data. Clustering patterns identified in the PCoA were validated by analysis of similarity (ANOSIM) based on 9999 permutations.

Relative abundance of the five most abundant phyla and the 10 most abundant genera, alpha diversity index values, plasma concentrations of SCFA and caproic acid, and body weight were analysed in r software (version 4.0.2.). Model assumptions of normality and homoscedasticity of the data were visually checked by QQ-plots (LMERConvenienceFunc-

tions package) [30]. Relative abundance, alpha diversity, SCFA, caproic acid, and weight data were analysed by mixed models (packages lme4 [31] and lmerTest [32]), with treatment, sex, age, and their interactions as fixed effects and with piglet ID nested within sow ID as a random effect. If the data did not meet the normality assumptions, data transformation was applied. Data on acetate and butyrate concentrations and on phylum Proteobacteria remained untransformed, but data on valeric acid, Fusobacteria, S24_7:g, and [*Prevotella*] were square-root transformed, and all other variables were log-transformed. If the variable still did not meet normality assumption, any outliers with residuals larger than 2.5 units were excluded from the analysis (Supplementary Table S2). After data transformation and exclusion of the outliers, all variables met the normality assumption.

Stepwise backward selection was used for model selection. Post-hoc Tukey HSD tests were performed using the emmeans package (R Foundation for Statistical Computing, Vienna, Austria) [33]. Correlations between the top 10 genera and plasma concentrations of SCFA and caproic acid were analysed with linear correlations (Pearson) on samples from animals for which data on both microbiota and SCFA were available. Effects of treatment on samples collected from the euthanized animals (tissues, jugular, and portal vein plasma) were analysed with *t*-tests. To correct for multiple analyses in the correlation analysis, the Benjamini–Hochberg procedure was used with a false discovery rate (FDR) of 5% [34].

3. Results

3.1. Animal Growth and Performance

Mean birth weight of the piglets was 1.85 ± 0.04 kg (BG) and 1.86 ± 0.04 kg (CON). The animals grew well in both treatment groups, and there were no differences in growth (Figure 2) or in average daily weight gain (Supplementary Figure S1) between BG and CON piglets. The piglets were healthy during the experiment, none died, and there were no cases of diarrhoea that needed treatment, although loose stools were prevalent within both groups during the experimental period.

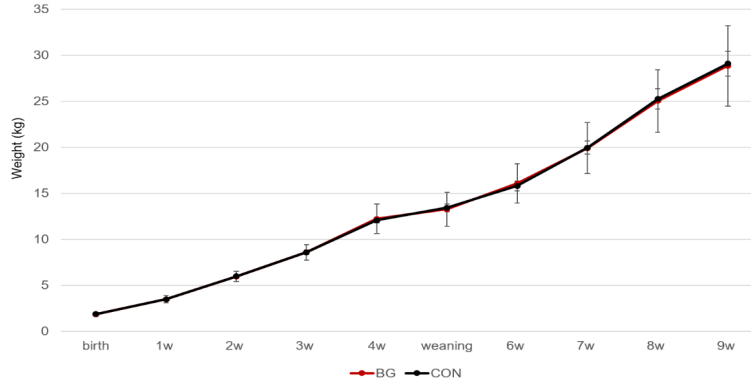

Figure 2. Weight gain (mean ± SE) of piglets in the β-glucan supplemented group (BG) and control group (CON) from birth to nine weeks of age.

3.2. Development of the Microbiota

Analysis of microbial diversity revealed a significant increase with age in both Shannon index and Chao-1 index, but with no differences between BG and CON piglets (Table 1). Shannon index was influenced by a sex by age interaction ($F_{(3,52.5)} = 9.3$, $p < 0.001$), with female piglets showing higher microbiota diversity than males at 4 weeks of age ($p < 0.001$). Chao-1 index was also influenced by a sex by age interaction ($F_{(3,54)} = 3.8$, $p < 0.05$), with females having higher species richness than males at 4 weeks of age ($p < 0.05$).

Table 1. Microbial diversity (mean ± SE) determined based on Illumina sequencing data in samples collected from piglets receiving a β-glucan supplement (BG) and control (CON) piglets.

	BG				CON				T	A	A:S	T:A
	1w	2w	3w	4w	1w	2w	3w	4w				
Shannon index	3.26 ± 0.13	3.59 ± 0.19	3.63 ± 0.14	4.19 ± 0.21	3.13 ± 0.17	3.68 ± 0.14	3.73 ± 0.19	4.21 ± 0.26 [a]	ns	*	*	ns
Chao-1	538 ± 38	803 ± 35	910 ± 40	980 ± 50	535 ± 30	798 ± 39	939 ± 38	977 ± 62 [a]	ns	*	*	ns

* indicates $p < 0.05$, ns = not significant. T = treatment, A = age, A:S = sex:age interaction, T:A = treatment:age interaction; w = weeks, [a] = significant difference at 4 weeks of age.

At phylum level, the microbiota was primarily dominated by Firmicutes, Bacteroidetes, and Proteobacteria (Figure 3A). At genus level, *Escherichia*, *Bacteroides*, *Prevotella*, and *Lactobacillus* were most dominant, comprising more than 50% of relative abundance in swab samples (Figure 3B).

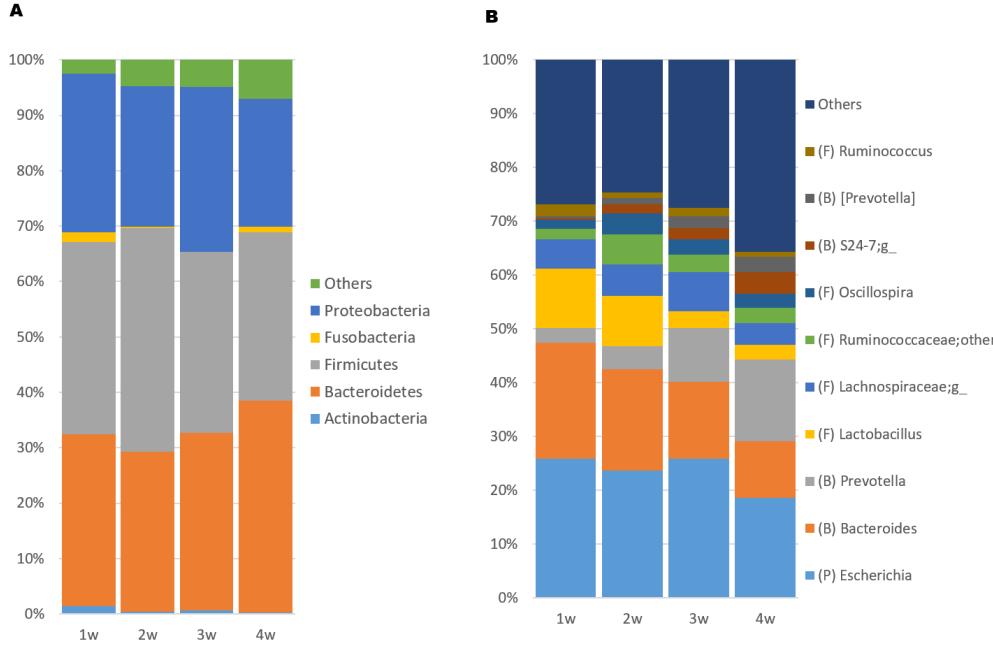

Figure 3. Stacked bar plot showing relative abundance of (**A**) the five most abundant bacterial phyla and (**B**) the 10 most abundant genera in rectal swab samples collected from piglets over the whole suckling period. Others = remaining accumulated abundance of bacterial taxa not among the top 10 in abundance in the samples, B = Bacteroidetes, F = Firmicutes, P = Proteobacteria.

To further assess microbial community composition, a multivariate statistical approach was applied. The ordination with PCoA revealed a clustering pattern that was linked to piglet age but did not indicate any difference in clustering pattern between BG and CON pigs (Figure 4A). This was confirmed in two-way ANOSIM, where age was a significant factor ($p = 0.0001$, R = 0.24) but not treatment ($p = 0.32$, R = 0.013). The PCoA and ANOSIM results were also used to evaluate the effect of litter on microbiota composition and revealed an evident effect of litter (Figure 4B), with a significant difference between both age and litter in the two-way ANOSIM (age: $p = 0.0001$, R = 0.43; litter: $p = 0.0001$, R = 0.45).

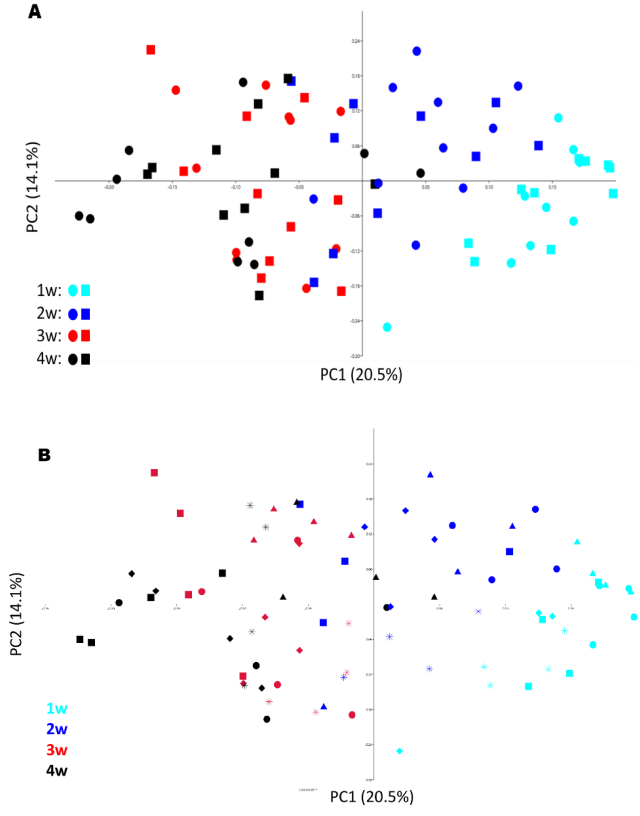

Figure 4. (**A**,**B**) Principal coordinates analysis (PCoA) plots with BrayCurtis distances showing microbiota development in 20 piglets (swab samples) from 1–4 weeks of age (10 receiving a β-glucan (BG) supplement and 10 from the control group (CON)). Symbols in plot (**A**) represent different treatments (circles = BG, squares = CON) and symbols (squares, diamonds, circles, triangles and stars) in plot (**B**) different litters.

A univariate statistical approach was also applied to evaluate the effect of treatment and age on the five most abundant phyla and the 10 most abundant genera (Table 2). At phylum level, there was a tendency for a treatment x age interaction for Actinobacteria ($F_{(3,53.4)}$ = 2.5, p = 0.07), with BG piglets tending to have higher relative abundance than CON piglets at week 1 (p = 0.07) (Table 2). Relative abundance of Actinobacteria decreased with age ($F_{(3,53.4)}$ = 8.24, $p < 0.001$) (Table 2). Males (0.9 ± 0.2%) had a higher relative abundance of Actinobacteria than females (0.4 ± 0.1%), $F_{(1,13.3)}$ = 5.9, $p < 0.05$) (Table 2). A tendency for a treatment x age interaction was found for Bacteroidetes ($F_{(3.68)}$ =2.4, p = 0.07), with higher relative abundance at week 1 in pigs from the BG group (p = 0.007) (Table 2). Relative abundance of Firmicutes was also influenced by age ($F_{(3,72.0)}$ = 5.1, $p < 0.01$, with a significant decrease in abundance at week 4 compared with week 2 ($p < 0.05$), and week 3 compared with week 4 ($p < 0.01$) (Table 2). Moreover, Fusobacteria was influenced by age ($F_{(3,55,9)}$ = 34.4, $p < 0.0001$, with abundance decreasing significantly from week 1 to week 2 ($p < 0.001$), week 3 ($p < 0.001$) and week 4 ($p < 0.001$) (Table 2). There was a tendency for a sex effect on Proteobacteria ($F_{(1,14.3)}$ = 4.2, p = 0.06), where male piglets had a higher abundance of Proteobacteria than females (Table 2).

Table 2. Relative abundance (mean percentage ± SE) on phylum and genus level of microbial taxa in swab samples collected from piglets receiving a β-glucan supplement (BG) and control (CON) piglets during the suckling period.

Bacteria	**BG**				**CON**				**T**	**A**	**T:A**
	1w	2w	3w	4w	1w	2w	3w	4w			
					Phylum						
Actinobacteria	0.88 ± 0.40^{a}	0.35 ± 0.08^{b}	1.01 ± 0.35^{b}	0.35 ± 0.13^{b}	2.01 ± 0.66^{a}	0.41 ± 0.19^{b}	0.43 ± 0.15^{b}	0.30 ± 0.19^{b}	ns	*	ns
Bacteroidetes	40.1 ± 4.67^{a}	26.3 ± 3.07	30.8 ± 4.47	33.7 ± 4.16	27.6 ± 6.38^{a}	31.6 ± 3.59	33.2 ± 4.58	42.6 ± 3.79	ns	ns	ns
Firmicutes	34.6 ± 3.32	41.7 ± 3.71^{a}	33.3 ± 2.10^{b}	32.3 ± 3.62^{b}	34.8 ± 3.20	39.1 ± 2.84^{a}	32.0 ± 2.83^{b}	28.5 ± 2.77^{b}	ns	*	ns
Fusobacteria	1.55 ± 0.34^{a}	0.11 ± 0.05^{b}	0.01 ± 0.01^{b}	0.08 ± 0.03^{b}	1.29 ± 0.33^{a}	0.21 ± 0.11^{b}	0.03 ± 0.01^{b}	0.42 ± 0.30^{b}	ns	*	ns
Proteobacteria	22.9 ± 5.08	27.7 ± 5.23	30.1 ± 4.45	27.6 ± 5.59	34.3 ± 6.51	23.2 ± 3.26	29.4 ± 6.09	18.5 ± 4.93	ns	ns	ns
					Genera						
Escherichia	19.9 ± 5.15	25.6 ± 5.27	30.2 ± 3.75	23.7 ± 5.79^{A}	32.0 ± 6.46	21.6 ± 3.22	24.5 ± 5.35	13.5 ± 4.86^{A}	ns	ns	*
Bacteroides	28.4 ± 4.75^{A}	13.9 ± 3.24^{B}	14.4 ± 3.03	10.6 ± 2.06	17.4 ± 4.27^{A}	23.9 ± 3.57^{B}	14.1 ± 1.97	11.4 ± 3.24	ns	ns	*
Prevotella	3.64 ± 1.60^{a}	$7.31 \pm 2.28^{b,c,A}$	$9.46 \pm 3.46^{b,d}$	$12.1 \pm 3.70^{b,d}$	2.0 ± 0.61^{a}	$1.99 \pm 0.43^{b,c,A}$	$10.6 \pm 3.10^{b,d}$	$18.3 \pm 5.65^{b,d}$	ns	*	*
Lactobacillus	9.44 ± 1.95^{a}	8.45 ± 1.82^{c}	$4.65 \pm 1.53^{b,d,e}$	$2.63 \pm 0.79^{b,d,f}$	12.5 ± 3.12^{a}	12.1 ± 2.60^{c}	$1.67 \pm 0.37^{b,d,e}$	$2.95 \pm 0.60^{b,d,f}$	ns	*	ns
Lachnospiraceae	7.73 ± 2.64	7.44 ± 2.96	7.33 ± 2.60	4.45 ± 1.67	3.10 ± 1.56	4.33 ± 1.36	7.05 ± 3.50	3.49 ± 0.99	ns	ns	ns
Ruminococcaceae; other	1.93 ± 0.37^{a}	$1.53 \pm 0.70^{b,c}$	1.00 ± 0.15	0.97 ± 0.21^{d}	2.39 ± 0.51^{a}	$0.63 \pm 0.09^{b,c}$	2.03 ± 0.82	0.99 ± 0.36^{d}	ns	*	ns
Oscillospira	1.78 ± 0.37^{a}	3.82 ± 0.74^{b}	2.74 ± 0.47^{b}	3.43 ± 0.84	1.46 ± 0.45^{a}	4.03 ± 0.64^{b}	3.21 ± 0.79^{b}	1.89 ± 0.28	ns	*	ns
S24_7:g	0.27 ± 0.12^{a}	$2.11 \pm 0.68^{b,c}$	1.94 ± 0.41^{b}	$3.11 \pm 0.91^{b,d}$	0.29 ± 0.13^{a}	$1.31 \pm 0.36^{b,c}$	2.00 ± 0.48^{b}	$2.72 \pm 0.55^{b,d}$	ns	*	ns
[*Prevotella*]	0.53 ± 0.44^{a}	1.21 ± 0.48	$0.88 \pm 0.40^{b,c}$	$1.93 \pm 0.44^{b,d}$	0.29 ± 0.16^{a}	0.94 ± 0.34	$2.56 \pm 1.06^{b,c}$	$3.66 \pm 1.14^{b,d}$	ns	*	ns
Ruminococcus	1.93 ± 0.37^{a}	1.53 ± 0.70^{b}	1.00 ± 0.15^{c}	0.97 ± 0.21^{d}	2.39 ± 0.51^{a}	0.63 ± 0.09^{b}	2.03 ± 0.82^{c}	0.99 ± 0.36^{d}	ns	*	ns

* Indicates $p < 0.05$, ns = not significant, T = treatment, A = age, T:A = treatment:age interaction, w = weeks, ^{a,b,c,d,e,f} = significant difference within weeks of age, ^{A,B} = significant difference within weeks of age:treatment.

At genus level, relative abundance of *Escherichia* was influenced by a treatment x age interaction ($F_{(3,63)}$ = 3.5, $p < 0.05$), with higher relative abundance in the BG group at 4 weeks of age ($p = 0.003$) (Table 2). An age x sex interaction was also found ($F_{(3,63)}$ = 4.7, $p < 0.01$), where female piglets had higher relative abundance of *Escherichia* than male piglets at week 4 ($p < 0.001$). Relative abundance of *Bacteroides* was influenced by a treatment x age interaction ($F_{(3,62)}$ = 3.6, $p < 0.05$), with higher abundance in the BG group in week 1 ($p < 0.05$), but higher abundance in the CON group in week 2 ($p < 0.05$). In addition, an age × sex interaction was found ($F_{(3,62)}$ = 4.0, $p < 0.05$), with higher relative abundance in male piglets compared with females ($p < 0.01$) (Table 2). Relative abundance of *Prevotella* was influenced by a treatment x sex x age interaction ($F_{(3,46.5)}$ = 6.6, $p < 0.001$), where in week 1 ($p < 0.01$), week 2 ($p < 0.05$), and week 3 ($p < 0.05$), female BG piglets had a higher relative abundance of *Prevotella* than female CON piglets (Table 2). Relative abundance of *Lactobacillus* was influenced by age ($F_{(3,56)}$ = 18.6, $p < 0.001$) and was higher in weeks 1 and 2 compared with weeks 3 and 4 ($p < 0.001$) (Table 2). The relative abundance of *Ruminococcus* was influenced by age ($F_{(3,72.2)}$ = 8.9, $p < 0.001$), with abundance increasing from week 1 to week 2 ($p = 0.001$) and decreasing from week 2 to week 4 ($p < 0.001$) (Table 2). The relative abundance of *Oscillospira* was influenced by age ($F_{(3,71.9)}$ = 6.8, $p < 0.001$), with lower abundance in week 1 compared with week 2 ($p = <0.001$), week 3 ($p < 0.05$), and week 4 ($p < 0.05$) (Table 2). The abundance of S24_7:g was influenced by an age x sex interaction ($F_{(3,52.9)}$ = 3.3, $p < 0.05$), where female piglets had higher abundance at week 4 ($p < 0.05$) than male piglets (Table 2). Relative abundance of [*Prevotella*] was influenced by age ($F_{(3,56.7)}$ = 8.5, $p < 0.001$), with a higher abundance at week 3 ($p < 0.05$) and week 4 ($p < 0.001$) compared with week 1, and a higher abundance in week 4 ($p < 0.05$) compared with week 2 (Table 2). *Ruminococcus* was influenced by age ($F_{(3,72.2)}$ = 8.9, $p < 0.001$), with abundance decreasing from week 1 to week 2 ($p < 0.001$) and week 3 ($p < 0.05$), and from week 2 to week 4 ($p < 0.05$) (Table 2).

The microbiota in colon digesta from euthanized piglets from the BG and CON groups were also compared, but PCoA did not reveal any separate clustering of these samples according to treatment or any significant effects of the supplement (Supplementary Figure S2).

3.3. SCFA and Caproic Acid Concentrations in Plasma and Colon Digesta

Analysis of SCFA and caproic acid levels in plasma samples collected from the jugular vein at 1, 3, and 4 weeks of age revealed that the concentration of SCFA was mainly affected by age. The only observed effect of treatment was on acetic acid concentration, which was lower in the BG group than in the CON group ($F_{(1,18)}$ = 6.1, $p < 0.05$) (Table 3). Acetic acid concentration was also influenced by age ($F_{(2,38)}$ = 39.8, $p < 0.001$), with an increase from week 1 to week 3 ($p < 0.001$) and week 4 ($p < 0.001$). The concentration of propionic acid was influenced by age ($F_{(2,38)}$ = 3.3, $p < 0.05$), with the highest concentration at week 3 ($p < 0.05$) (Table 3). Plasma concentration of butyric acid was influenced by an age x sex interaction ($F_{(2,34.6)}$ = 4.5, $p < 0.05$), with higher concentrations in females than in males in week 4 ($p < 0.05$). Formic acid concentration was influenced by age ($F_{(2,38)}$ = 25.7, $p < 0.001$), decreasing from week 1 to week 3 ($p < 0.01$) and week 4 ($p < 0.001$), and from week 3 to week 4 ($p < 0.01$) (Table 3). Iso-butyric acid concentration was influenced by age ($F_{(2,38)}$ = 6.3, $p < 0.05$), and was higher in week 3 ($p < 0.05$) and week 4 ($p < 0.05$) than in week 1 (Table 3). Valeric acid concentration tended to be higher in the BG than in the CON group ($F_{(1,18)}$ = 4.0, $p = 0.06$) and was influenced by age ($F_{(2,38)}$ = 9.3, $p < 0.001$), with an increase from week 1 to week 3 ($p < 0.05$) and week 4 ($p < 0.001$). Succinic acid concentration was influenced by age ($F_{(2,38)}$ = 5.4, $p < 0.05$) and decreased from week 1 to week 4 ($p < 0.05$) (Table 3). Caproic acid concentration tended to be higher in BG piglets than in CON piglets ($F_{(1,12)}$ = 3.5, $p = 0.08$) (Table 3).

Table 3. Concentrations (μM) of different short-chain fatty acids (SCFA) and caproic acid in plasma collected from piglets receiving a β-glucan supplement (BG) and control (CON) piglets during the suckling period. Mean ± SE.

SCFA	BG			CON			T	A	T:A
	1w	3w	4w	1w	3w	4w			
Acetic acid	10.9 ± 0.69 ^{a}	17.1 ± 0.85 ^{b,c}	18.9 ± 1.10 ^{b,d}	13.0 ± 0.76 ^{a}	18.4 ± 1.44 ^{b,c}	21.7 ± 1.27 ^{b,d}	*	*	ns
Propionic acid	0.88 ± 0.12	1.19 ± 0.20 ^{a}	1.27 ± 0.23	1.03 ± 0.09	1.82 ± 0.34 ^{a}	1.21 ± 0.21	ns	*	ns
Butyric acid	0.15 ± 0.30	0.38 ± 0.71 ^{a}	0.68 ± 0.10 ^{a}	0.21 ± 0.05	0.34 ± 0.11 ^{a}	.045 ± 0.08 ^{a}	ns	*	ns
Formic acid	576 ± 20.3 ^{a}	447 ± 34.7 ^{b,c}	346 ± 30.1 ^{b,d}	546 ± 17.6 ^{a}	494 ± 53.4 ^{b,c}	419 ± 50.5 ^{b,d}	ns	*	ns
Iso-butyric acid	0.42 ± 0.04 ^{a}	0.69 ± 0.16 ^{b}	0.81 ± 0.12 ^{b}	0.43 ± 0.06 ^{a}	0.83 ± 0.13 ^{b}	0.57 ± 0.08 ^{b}	ns	*	ns
Valeric acid	0.08 ± 0.00 ^{a}	0.14 ± 0.03 ^{b}	0.16 ± 0.03 ^{b}	0.06 ± 0.02 ^{a}	0.08 ± 0.02 ^{b}	0.11 ± 0.01 ^{b}	ns	*	ns
Succinic acid	9.07 ± 1.66 ^{a}	7.62 ± 0.94	7.28 ± 1.36 ^{b}	11.89 ± 1.80 ^{a}	9.34 ± 1.38	7.52 ± 0.96 ^{b}	ns	*	ns
Caproic acid	0.27 ± 0.02	0.29 ± 0.02	0.24 ± 0.03	0.23 ± 0.03	0.23 ± 0.04	0.20 ± 0.03	ns	ns	ns

* Indicates $p < 0.05$, ns = not significant, T = treatment, A = age, T:A = treatment:age interaction, w = weeks, ^{a,b,c,d} = significant difference within weeks.

Jugular vein and portal vein plasma and colon digesta taken from 10 euthanized animals were evaluated for the possible influence of the BG supplement on SCFA concentrations. SCFA concentration varied between individual pigs and was considerably higher in plasma samples from the portal vein than in samples from the jugular vein. However, no significant effects linked to the treatment were found for colon digesta or plasma samples (Supplementary Tables S3 and S4). The molar proportions of SCFA differed between plasma and colon digesta, with higher similarity in portal vein and colon digesta than in jugular vein and colon digesta (Figure 5). However, correlation analysis of individual SCFA in the different sample types did not reveal any significant correlations.

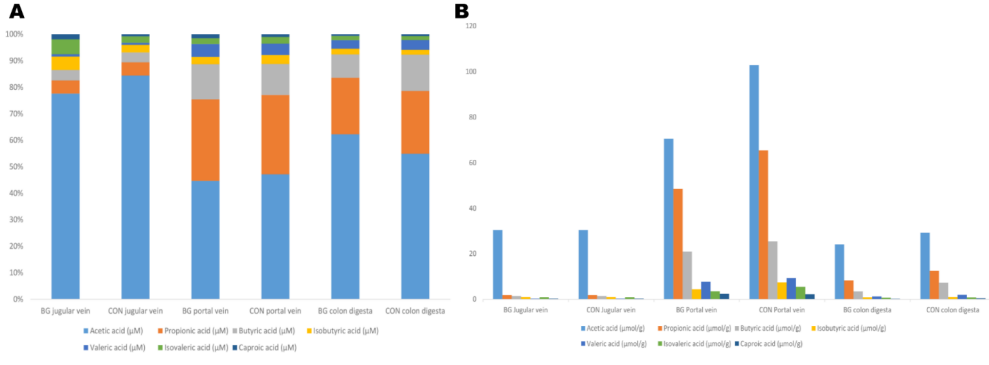

Figure 5. (**A**) Relative concentrations (% of total) and (**B**) mean absolute concentrations (μmol/g) of SCFA and caproic acid in jugular vein and portal vein plasma and colon digesta from 10 euthanized piglets (five piglets in a group receiving a β-glucan supplement and five control (CON) piglets.

3.4. Gut Histological Measurements

Morphological parameters measured in tissue samples are shown in Table 4. No significant differences were detected between BG and CON for any of the measured parameters.

Table 4. Villus height (μm), crypt depth (μm), and thickness of mucosa, total gut (μm), and muscularis externa of the ileum and colon in piglets receiving a β-glucan supplement (BG) and in control (CON) piglets during the suckling period. Mean ± SE.

	N [(1)]	CON	BG	*p*-Value
Ileum (μm)				
Villus height	10	346 ± 19.6	360 ± 25.1	0.58
Crypt depth	5–9	234 ± 15.3	238 ± 21.9	0.82
Thickness mucosa	10	598 ± 30.7	619 ± 36.0	0.62
Thickness muscularis externa	10	543 ± 33.3	480± 24.5	0.34
Thickness total gut	5	1925 ± 70.1	2199 ± 274	0.65
Colon (μm)				
Crypt depth	10	410 ± 13.0	376 ± 17.2	0.42
Thickness mucosa	10	483 ± 14.0	445 ± 9.4	0.61
Thickness muscularis externa	10	400 ± 20.5	366 ± 20.4	0.57
Thickness total gut	5–6	1152 ± 80.0	1042 ± 76.5	0.57

[(1)] Number of measurements per pig.

3.5. Correlations

Relative abundance data from the 10 most abundant genera were analysed for correlations with SCFA in plasma (Supplementary Figure S2). Among the genera classified to the phylum Bacteroidetes, *Bacteroides* was negatively correlated with acetic acid (r = −0.34, $p = 0.008$) and propionic acid (r = −0.35, $p = 0.004$), while *Prevotella* was positively correlated with acetic acid (r = 0.42, $p = 0.0008$), but negatively correlated with formic acid (r = −0.31, $p = 0.016$). The taxon S24_7:g was negatively correlated with formic acid (r =−0.48, $p = 0.0001$), but positively correlated with acetic acid (r = 0.55, $p = 0.000005$), propionic acid (r = 0.34, $p = 0.007$), butyric acid (r = 0.49, $p = 0.00005$), iso-butyric acid (r = 0.34, $p = 0.009$), and valeric acid (r = 0.33, $p = 0.01$). The taxon [*Prevotella*] was positively correlated with acetic acid (r = 0.51, $p = 0.00003$), propionic acid (r = 0.37, $p = 0.003$), and butyric acid (r = 0.39, $p = 0.002$). Among the dominating genera within the phylum Firmicutes, *Lactobacillus* was positively correlated with succinic acid (r = 0.36, $p = 0.005$) but negatively correlated with acetic acid (r = −0.46, $p = 0.0002$). Lachnospiraceae;g was positively correlated with valeric acid (r = 0.31, $p = 0.016$), Ruminococcaceae;g_other was positively correlated with propionic acid (r = 0.36, $p = 0.004$) and iso-butyric acid (r = 0.31, $p = 0.015$), and *Oscillospira* was positively correlated with iso-butyric acid (r = 0.33, $p = 0.011$). Moreover, *Ruminococcus* showed a positive correlation with formic acid (r = 0.42, $p = 0.0008$) and a negative correlation with acetic acid (r = −0.39, $p = 0.002$). *Escherichia,* belonging to Proteobacteria, was positively correlated with caproic acid (r = 0.33, $p = 0.009$). There were also several correlations between individual bacterial taxa (Supplementary Figure S3).

4. Discussion

This study investigated the effect of early dietary supplementation with β-glucan on the development of the gut microbiota, concentrations of SCFA in plasma and digesta, and the morphological structure of the gut in piglets. The results showed mainly age-related effects, with maturation of microbiota composition and higher concentrations of certain SCFA in plasma at older age. Several correlations were found between certain microbial taxa and concentrations of SCFA and caproic acid in plasma samples.

Early in the piglets' life, *Escherichia*, *Lactobacillus*, and *Bacteroides* were dominating taxa, but with older age both *Lactobacillus* and *Bacteroides* showed a reduction in relative abundance. The relative abundance of *Lactobacillus* decreased from 9% initially to 2% by the end of the suckling period. Some previous studies have found a similar trend, with a significant decrease in the abundance of *Lactobacillus* from newly weaned piglets compared to piglets at the end of the suckling period [35,36]. In contrast, Frese et al. [37] found an increase in the proportion of *Lactobacillus* directly after weaning. In the present study, we

observed a decrease in the relative abundance of *Bacteroides* with older age, which is in agreement with findings in other studies [37,38].

A gradual increase in the relative abundance of *Prevotella* was observed during the suckling period, which is in agreement with findings in earlier studies on gut microbiome sampled at different ages [38–42]. *Prevotella* is known to harbour specific enzymes that can utilise plant polysaccharides [43]. Thus, it is commonly present in low abundance in nursing animals and usually increases in abundance when pigs are introduced to solid food [38]. Interestingly, in the present study, *Prevotella* showed a gradual increase over the whole suckling period, possibly due to increasing intake of creep feed with age during the suckling period [44]. Consumption of creep feed has been shown to contribute to microbiota development [20,21]. Creep feed consumption was not recorded in the present study, so no conclusions can be drawn on whether it contributed to the observed increase in the abundance of *Prevotella*.

Several differences in microbial composition correlated with piglet sex were observed. Relative abundance of the phylum Proteobacteria was higher in male piglets, while at genus level *Escherichia* showed higher abundance in female piglets and *Bacteroides* showed higher abundance in male piglets. However, previous studies have found conflicting results, e.g., Zhou et al. [45] found a higher abundance of Proteobacteria in female pigs, and He et al. [46] found a higher abundance of *Escherichia* in males and *Bacteroides* in females. The reason for the discrepancies between studies is unknown, but one possible explanation is age differences, as the pigs in those studies were older than the piglets in our study.

It is uncertain to what extent the immature gut microbiota in young piglets can utilise dietary fibre [47]. Microbial metabolism of dietary fibre leads to various end-products [48], and the production of SCFA is considered to be a key factor influencing overall gut health. A substantial part of the energy contribution from the gut in weaned pigs is related to SCFA, with intestinal production of SCFA depending on the size of the gastrointestinal tract and the population and composition of bacteria in the gut [49]. In the present study, changes in SCFA concentrations in plasma were linked more strongly to age than to treatment. The only observed effect linked to the treatment was for acetic acid. However, the acetic acid concentration in plasma was higher in CON piglets at the start of the study, so this difference likely reflected individual differences rather than being an effect of the supplementation.

Formic and succinic acid concentrations in plasma decreased with age, whereas the concentrations of acetic, propionic, butyric, iso-butyric, and valeric acid all increased with age. As the abundance of *Bacteroides* and *Lactobacillus* decreased with age, while the levels of acetic and propionic concentration increased, it was not surprising that these variables were negatively correlated. In contrast, *Prevotella* increased with age and was positively correlated with concentrations of acetic, butyric, and valeric acid. Previous studies have found that *Lactobacillus* spp., *Bacteroides* spp., *Bifidobacterium* spp., *Streptococcus* spp., *Prevotella* spp., and *Ruminococcus* spp. are the most important producers of acetate in the gastrointestinal tract [50,51]. Propionate is mainly produced by *Bacteroides* spp., *Megasphaera elsdenii*, *Roseburia inulinivorans*, *Dialister* spp., *Ruminococcus obeum*, *Veillonella* spp., *Coprococcus catus*, and *Phascolarctobacterium succinatutens* [52]. In the present study, *Prevotella* aligned with prior knowledge of the specific gut bacteria producers of acetate, but *Lactobacillus* spp. and *Bacteroides* spp. were negatively correlated with the concentrations of acetic and propionic acid. This could be due to the fact that SCFA were measured in plasma and microbiota taxa in rectal swabs. It has been reported in several previous studies that the concentrations and proportions of circulating SCFA and SCFA in faeces samples are not well correlated [53–55]. There was a clear difference in the concentrations of SCFA and caproic acid between portal and jugular vein plasma, with higher concentrations in the portal vein samples (even higher than found in the colon digesta). The observed difference in molar proportions between the portal and jugular vein samples, with reduced proportions of both propionic and butyric acid, suggests that they were metabolised in the liver.

Previous studies on weaned pigs have found that inclusion of oat or barley β-glucan or oat products in the diet is associated with an increased proportion of specific microbial taxa in the colon and ileum, i.e., *Lactobacillus* spp., *Bifidobacterium* spp., and *Prevotella* [56–58]. Other studies have found no changes in the gut microbial community, plasma SCFA, or immune function on adding oat β-glucan to the diet of pigs [59,60]. In the present study, there were no significant differences in microbiota, SCFA concentrations, or gut morphological structure that could be linked to the BG treatment alone. The β-glucan supplement was introduced from seven days of age, with three doses per week until weaning. It is possible that more frequent doses or a higher dose than 40 mg/kg body weight could have resulted in an effect of the treatment on the gut microbiota, and hence increased SCFA production. Previous studies on weaned pigs receiving β-glucan from cereals and microbial origin have used doses ranging from 50 to 200 mg/kg body weight [61]. A recent study on weaned pigs receiving a dietary β-glucan supplement and challenged with *Escherichia coli* used a high dose of β-glucan (500 mg/kg BW) and observed an increased abundance of *Lactobacillus* in the supplemented group compared with the control group [62].

Apart from most previous studies being performed post-weaning, on older pigs with a more developed microbiota that can utilise β-glucan more effectively, other possible reasons for the differences in response between studies could be related to fermentation rate in the digestive tract, which depends on the chemical composition and physiochemical properties of different types of β-glucans [63,64]. β-glucans can differ widely in both concentration and solubility, depending on the source [65]. For instance, β-glucan from fungi and yeasts is known to have immuno-modulating effects [66,67]. Yeast-derived β-glucan has high bioactivity [68], and effects on the immune system have been demonstrated in vitro [69,70] and in a recent in vivo study [71]. Although barley has a higher β-glucan content than oats [72], β-glucan from oats has been shown to be better for gut health than barley β-glucan when fed to rats at the same dosage [73].

The lack of differences between the treatment groups in the present study could potentially also be related to microbial cross-contamination by allocoprophagy [74], as we provided the CON and BG supplement to individuals within the same litters. However, this study design had many advantages, e.g., siblings from the BG and CON groups had a shared mother, genetic predisposition, and early-life litter exposure. We observed clear litter effects on gut microbial community clusters in our study. The univariate statistical analyses were litter-adjusted, so these effects were accounted for in the statistical model.

Previous studies on weaned pigs fed β-glucan derived from cereal and microbial sources have reported increased levels of butyrate and butyrate-producing bacteria [56,75]. Butyrate can act as an inhibitor of pathogenic microorganisms by reinforcing the mucus layer, enhancing immunological development, and acting as a nutrient source for the colon epithelium [76,77]. In the present study, the concentration of butyric acid was numerically, but not statistically significantly, higher in plasma samples from the BG group, but not in digesta samples, indicating that BG supplementation contributed to higher concentrations of butyrate and butyrate-producing bacteria. Families such as Lachnospiraceae and Ruminococcaceae, which contain several genera known to produce butyrate, were found in the samples, but did not differ in relative abundance between the treatments.

It has been shown that transition to solid feed can cause short-term villus and crypt disorders in piglets [78]. Moreover, it is suggested that 14–21 days of life is a crucial period for maturation of the mucosal structure and mucosal development [79]. However, no measurable changes in intestinal mucosa were seen in BG piglets in our study, and we found no indications of local inflammation in villi and crypts in any of the piglets. The effects of dietary fibre on gut morphology in piglets cannot be assessed based on the literature, as some studies report changes in morphology in piglets fed solid feed before weaning [80] and others report no changes [81].

Comparison of the results from this and previous studies is challenging, due to wide variation in the data obtained, differences in supplement dose and timing, and lack of studies on β-glucan in young piglets during the suckling period.

5. Conclusions

Early supplementation of piglets with oat β-glucan during the suckling period had no obvious or possible long-lasting effects, with piglet age and litter rather than dietary β-glucan supplementation having the most effect on gut microbiota development and on SCFA concentrations. This suggests that cereal β-glucan has a limited capacity to contribute to a beneficial gut microbiota development during the suckling period.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani13081349/s1, Supplementary Figure S1: Average daily weight gain of piglets included in the study; Supplementary Figure S2: PCoA plot of colon digesta from euthanized piglets; Supplementary Figure S3: Correlation between the top 10 genera and SCFA concentrations; Supplementary Table S1: Dietary composition of creep feed for suckling piglets; Supplementary Table S2: Excluded outliers; Supplementary Table S3: SCFA and caproic acid concentrations in colon digesta from euthanized piglets; Supplementary Table S4: SCFA and caproic acid concentrations in plasma from euthanized piglets; Supplementary File S1: Analysis of plasma SCFA.

Author Contributions: Conceptualization, J.E.L. and J.D.; methodology, J.E.L., R.L. and J.D.; software, L.S.; formal analysis, J.D., E.V. and L.A.; data interpretation, J.E.L., L.K. and T.L.; investigation, J.D., E.M., Y.H. and L.A.; resources, J.D.; data curation, L.S. and J.D.; writing—original draft preparation, L.A. and J.D.; writing, review and editing, J.E.L., T.L., E.V., L.K., L.S., R.L., Y.H. and E.M.; supervision, J.D.; project administration, J.D. and L.A.; funding acquisition, J.E.L., J.D. and L.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Swedish research council FORMAS (contracts: DNR 942-2015-630 and DNR 2017-01128). The funder did not have any influence on the experimental design, data analysis, interpretation of the results, or the decision to publish.

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the Uppsala region, Sweden (DNR C 1054/16.).

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw sequencing data have been deposited at the National Center for Biotechnology Information database (NCBI), under accession number PRJNA940420. Other data from this research study are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Aluthge, N.D.; Van Sambeek, D.M.; Carney-Hinkle, E.E.; Li, Y.S.; Fernando, S.C.; Burkey, T.E. The pig microbiota and the potential for harnessing the power of the microbiome to improve growth and health1. *J. Anim. Sci.* **2019**, *97*, 3741–3757. [CrossRef] [PubMed]
2. Gresse, R.; Chaucheyras-Durand, F.; Fleury, M.A.; Van de Wiele, T.; Forano, E.; Blanquet-Diot, S. Gut Microbiota Dysbiosis in Postweaning Piglets: Understanding the Keys to Health. *Trends Microbiol.* **2017**, *25*, 851–873. [CrossRef] [PubMed]
3. Lee, I.K.; Kye, Y.C.; Kim, G.; Kim, H.W.; Gu, M.J.; Umboh, J.; Maaruf, K.; Kim, S.W.; Yun, C.H. Stress, Nutrition, and Intestinal Immune Responses in Pigs—A Review. *Asian-Australas J. Anim. Sci.* **2016**, *29*, 1075–1082. [CrossRef] [PubMed]
4. Tang, X.; Xiong, K.; Fang, R.; Li, M. Weaning stress and intestinal health of piglets: A review. *Front. Immunol.* **2022**, *13*, 1042778. [CrossRef]
5. Rhouma, M.; Fairbrother, J.M.; Beaudry, F.; Letellier, A. Post weaning diarrhea in pigs: Risk factors and non-colistin-based control strategies. *Acta Vet. Scand.* **2017**, *59*, 31. [CrossRef]
6. Van Hees, H.M.J.; Davids, M.; Maes, D.; Millet, S.; Possemiers, S.; den Hartog, L.A.; van Kempen, T.; Janssens, G.P.J. Dietary fibre enrichment of supplemental feed modulates the development of the intestinal tract in suckling piglets. *J. Anim. Sci. Biotechnol.* **2019**, *10*, 83. [CrossRef]
7. Vetvicka, V.; Vannucci, L.; Sima, P.; Richter, J. Beta Glucan: Supplement or Drug? From Laboratory to Clinical Trials. *Molecules* **2019**, *24*, 1251. [CrossRef]
8. Jayachandran, M.; Chen, J.; Chung, S.S.M.; Xu, B. A critical review on the impacts of β-glucans on gut microbiota and human health. *J. Nutr. Biochem.* **2018**, *61*, 101–110. [CrossRef]
9. Jha, R.; Fouhse, J.M.; Tiwari, U.P.; Li, L.; Willing, B.P. Dietary Fiber and Intestinal Health of Monogastric Animals. *Front. Vet. Sci.* **2019**, *6*, 48. [CrossRef]

10. Jha, R.; Berrocoso, J.D. Review: Dietary fiber utilization and its effects on physiological functions and gut health of swine. *Animal* **2015**, *9*, 1441–1452. [CrossRef]
11. Tian, G.; Wu, X.; Chen, D.; Yu, B.; He, J. Adaptation of gut microbiome to different dietary nonstarch polysaccharide fractions in a porcine model. *Mol. Nutr. Food Res.* **2017**, *61*, 10. [CrossRef] [PubMed]
12. Jin, Y.; Li, P.; Wang, F. β-glucans as potential immunoadjuvants: A review on the adjuvanticity, structure-activity relationship and receptor recognition properties. *Vaccine* **2018**, *36*, 5235–5244. [CrossRef] [PubMed]
13. Bedford, A.; Gong, J. Implications of butyrate and its derivatives for gut health and animal production. *Anim. Nutr.* **2018**, *4*, 151–159. [CrossRef] [PubMed]
14. Gonçalves, P.; Araújo, J.R.; Di Santo, J.P. A Cross-Talk Between Microbiota-Derived Short-Chain Fatty Acids and the Host Mucosal Immune System Regulates Intestinal Homeostasis and Inflammatory Bowel Disease. *Inflamm. Bowel Dis.* **2018**, *24*, 558–572. [CrossRef]
15. Ríos-Covián, D.; Ruas-Madiedo, P.; Margolles, A.; Gueimonde, M.; de Los Reyes-Gavilán, C.G.; Salazar, N. Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. *Front. Microbiol.* **2016**, *7*, 185. [CrossRef]
16. Calderón-Pérez, L.; Gosalbes, M.J.; Yuste, S.; Valls, R.M.; Pedret, A.; Llauradó, E.; Jimenez-Hernandez, N.; Artacho, A.; Pla-Pagà, L.; Companys, J.; et al. Gut metagenomic and short chain fatty acids signature in hypertension: A cross-sectional study. *Sci. Rep.* **2020**, *10*, 6436. [CrossRef]
17. Knudsen, K.E.B.; Jørgensen, H.; Theil, P.K. Changes in short-chain fatty acid plasma profile incurred by dietary fiber composition. *J. Anim. Sci.* **2016**, *94*, 476–479. [CrossRef]
18. Verbeek, E.; Keeling, L.; Landberg, R.; Lindberg, J.E.; Dicksved, J. The gut microbiota and microbial metabolites are associated with tail biting in pigs. *Sci. Rep.* **2021**, *11*, 20547. [CrossRef]
19. Muller, M.; Hernandez, M.A.G.; Goossens, G.H.; Reijnders, D.; Holst, J.J.; Jocken, J.W.E.; van Eijk, H.; Canfora, E.E.; Blaak, E.E. Circulating but not faecal short-chain fatty acids are related to insulin sensitivity, lipolysis and GLP-1 concentrations in humans. *Sci. Rep.* **2019**, *9*, 12515. [CrossRef]
20. Choudhury, R.; Middelkoop, A.; Boekhorst, J.; Gerrits, W.J.J.; Kemp, B.; Bolhuis, J.E.; Kleerebezem, M. Early life feeding accelerates gut microbiome maturation and suppresses acute post-weaning stress in piglets. *Environ. Microbiol.* **2021**, *23*, 7201–7213. [CrossRef]
21. Choudhury, R.; Middelkoop, A.; De Souza, J.G.; Van Veen, L.A.; Gerrits, W.J.; Kemp, B.; Bolhuis, J.E.; Kleerebezem, M. Impact of early-life feeding on local intestinal microbiota and digestive system development in piglets. *Sci. Rep.* **2021**, *11*, 1–16.
22. du Sert, N.P.; Hurst, V.; Ahluwalia, A.; Alam, S.; Avey, M.T.; Baker, M.; Browne, W.J.; Clark, A.; Cuthill, I.C.; Dirnagl, U.; et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol.* **2020**, *18*, e3000410.
23. Wallgren, P.; Vallgårda, J. Seroproduktion—Presentation, definition och kravlista. *Sven. Vet. Tidn.* **1993**, *45*, 733–735.
24. Müller, B.; Sun, L.; Westerholm, M.; Schnürer, A. Bacterial community composition and fhs profiles of low- and high-ammonia biogas digesters reveal novel syntrophic acetate-oxidising bacteria. *Biotechnol. Biofuels* **2016**, *9*, 48. [CrossRef] [PubMed]
25. Caporaso, J.G.; Bittinger, K.; Bushman, F.D.; DeSantis, T.Z.; Andersen, G.L.; Knight, R. PyNAST: A flexible tool for aligning sequences to a template alignment. *Bioinformatics* **2010**, *26*, 266–267. [CrossRef]
26. Haas, B.J.; Gevers, D.; Earl, A.M.; Feldgarden, M.; Ward, D.V.; Giannoukos, G.; Ciulla, D.; Tabbaa, D.; Highlander, S.K.; Sodergren, E.; et al. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res.* **2011**, *21*, 494–504. [CrossRef]
27. Wang, Q.; Garrity, G.M.; Tiedje, J.M.; Cole, J.R. Naive Bayesian classsifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **2007**, *73*, 5261–5267. [CrossRef]
28. Han, J.; Lin, K.; Sequeira, C.; Borchers, C.H. An isotope-labeled chemical derivatization method for the quantitation of short-chain fatty acids in human feces by liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* **2015**, *854*, 86–94. [CrossRef]
29. Hammer, Ø.; Harper, D.A.; Ryan, P.D. PAST: Paleontological Software Package for Education and Data Analysis. *Paleontol. Electron.* **2001**, *4*, 1–9.
30. Tremblay Antoine, R.J. *LMER Convenience Functions: Model Selection and Post-Hoc Analysis for (G)LMER Models, R Package Version 3.0*; R Foundation for Statistical Computing: Vienna, Austria, 2020.
31. Bates, D.; Mächler, M.; Bolker, B.; Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [CrossRef]
32. Kuznetsova, A.; Brockhoff, P.B.; Christensen, R.H.B. lmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* **2017**, *82*, 1–26. [CrossRef]
33. Lenth, R.V. *Estimated Marginal Means, aka Least-Squares Means, R Package Version 1.5.1*; R Foundation for Statistical Computing: Vienna, Austria, 2020.
34. Benjamini, Y.; Hochberg, Y. Controlling the false discovery rate: A practical and powerful approach to multiple hypothesis testing. *J. Roy. Statist. Soc. Ser. B* **1995**, *57*, 289–300. [CrossRef]
35. Qi, R.; Qiu, X.; Du, L.; Wang, J.; Wang, Q.; Huang, J.; Liu, Z. Changes of Gut Microbiota and Its Correlation With Short Chain Fatty Acids and Bioamine in Piglets at the Early Growth Stage. *Front. Vet. Sci.* **2020**, *7*, 617259. [CrossRef] [PubMed]

36. Bian, G.; Ma, S.; Zhu, Z.; Su, Y.; Zoetendal, E.G.; Mackie, R.; Liu, J.; Mu, C.; Huang, R.; Smidt, H.; et al. Age, Introduction of Solid Feed and Weaning Are More Important Determinants of Gut Bacterial Succession in Piglets than Breed and Nursing Mother as Revealed by a Reciprocal Cross-Fostering Model: Gut Bacterial Succession in Piglets. *Environ. Microbiol.* **2016**, *18*, 1566. [CrossRef] [PubMed]
37. Frese, S.A.; Parker, K.; Calvert, C.C.; Mills, D.A. Diet shapes the gut microbiome of pigs during nursing and weaning. *Microbiome* **2015**, *3*, 28. [CrossRef]
38. Mach, N.; Berri, M.; Estellé, J.; Levenez, F.; Lemonnier, G.; Denis, C.; Leplat, J.-J.; Chevaleyre, C.; Billon, Y.; Doré, J.; et al. Early-life establishment of the swine gut microbiome and impact on host phenotypes. *Environ. Microbiol. Rep.* **2015**, *7*, 554–569. [CrossRef]
39. Wang, X.; Tsai, T.; Deng, F.; Wei, X.; Chai, J.; Knapp, J.; Apple, J.; Maxwell, C.V.; Lee, J.A.; Li, Y.; et al. Longitudinal investigation of the swine gut microbiome from birth to market reveals stage and growth performance associated bacteria. *Microbiome* **2019**, *7*, 109. [CrossRef]
40. Ke, S.; Fang, S.; He, M.; Huang, X.; Yang, H.; Yang, B.; Chen, C.; Huang, L. Age-based dynamic changes of phylogenetic composition and interaction networks of health pig gut microbiome feeding in a uniformed condition. *BMC Vet. Res.* **2019**, *15*, 172. [CrossRef]
41. Guevarra, R.B.; Hong, S.H.; Cho, J.H.; Kim, B.-R.; Shin, J.; Lee, J.H.; Kang, B.N.; Kim, Y.H.; Wattanaphansak, S.; Isaacson, R.E.; et al. The dynamics of the piglet gut microbiome during the weaning transition in association with health and nutrition. *J. Anim. Sci. Biotechnol.* **2018**, *9*, 54. [CrossRef]
42. Guevarra, R.B.; Lee, J.H.; Lee, S.H.; Seok, M.-J.; Kim, D.W.; Kang, B.N.; Johnson, T.J.; Isaacson, R.E.; Kim, H.B. Piglet gut microbial shifts early in life: Causes and effects. *J. Anim. Sci. Biotechnol.* **2019**, *10*, 1. [CrossRef]
43. Ivarsson, E.; Roos, S.; Liu, H.Y.; Lindberg, J.E. Fermentable non-starch polysaccharides increases the abundance of Bacteroides–Prevotella–Porphyromonas in ileal microbial community of growing pigs. *Animal* **2014**, *8*, 1777–1787. [CrossRef]
44. Fraser, D.; Pajor, E.A.; Feddes, J.J.R. The relationship between creep feeding behavior of piglets and adaptation to weaning: Effect of diet quality. *Can. J. Anim. Sci.* **1994**, *74*, 1–6. [CrossRef]
45. Zhou, Z.; Zheng, W.; Shang, W.; Du, H.; Li, G.; Yao, W. How host gender affects the bacterial community in pig feces and its correlation to skatole production. *Ann. Microbiol.* **2015**, *65*, 2379–2386. [CrossRef]
46. He, M.; Gao, J.; Wu, J.; Zhou, Y.; Fu, H.; Ke, S.; Yang, H.; Chen, C.; Huang, L. Host Gender and Androgen Levels Regulate Gut Bacterial Taxa in Pigs Leading to Sex-Biased Serum Metabolite Profiles. *Front. Microbiol.* **2019**, *10*, 1359. [CrossRef] [PubMed]
47. Li, H.; Yin, J.; Tan, B.; Chen, J.; Zhang, H.; Li, Z.; Ma, X. Physiological function and application of dietary fiber in pig nutrition: A review. *Anim. Nutr.* **2021**, *7*, 259–267. [CrossRef] [PubMed]
48. Wegh, C.A.M.; Geerlings, S.Y.; Knol, J.; Roeselers, G.; Belzer, C. Postbiotics and Their Potential Applications in Early Life Nutrition and Beyond. *Int. J. Mol. Sci.* **2019**, *20*, 4673. [CrossRef] [PubMed]
49. Hume, I.D. Flow dynamics of digesta and colonic fermentation. In *Physiological and Clinical Aspects of Short-Chain Fatty Acids*; Cummings, J.H., Rombeau, J.L., Sakata, T., Eds.; Cambridge University Press: Cambridge, UK, 1995; pp. 119–132.
50. Fernández, J.; Redondo-Blanco, S.; Gutiérrez-del-Río, I.; Miguélez, E.M.; Villar, C.J.; Lombo, F. Colon microbiota fermentation of dietary prebiotics towards short-chain fatty acids and their roles as anti-inflammatory and antitumour agents: A review. *J. Func. Foods* **2016**, *25*, 511–522. [CrossRef]
51. Feng, W.; Ao, H.; Peng, C. Gut Microbiota, Short-Chain Fatty Acids, and Herbal Medicines. *Front. Pharmacol.* **2018**, *9*, 1354. [CrossRef]
52. Koh, A.; De Vadder, F.; Kovatcheva-Datchary, P.; Bäckhed, F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* **2016**, *165*, 1332–1345. [CrossRef]
53. van Beers-Schreurs, H.M.; Nabuurs, M.J.; Vellenga, L.; Kalsbeek-van der Valk, H.J.; Wensing, T.; Breukink, H.J. Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. *J. Nutr.* **1998**, *128*, 947–953. [CrossRef]
54. Müller, M.; Hermes, G.D.A.; Canfora, E.E.; Smidt, H.; Masclee, A.A.M.; Zoetendal, E.G.; Blaak, E.E. Distal colonic transit is linked to gut microbiota diversity and microbial fermentation in humans with slow colonic transit. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2020**, *318*, 361–369. [CrossRef]
55. Nakatani, M.; Inoue, R.; Tomonaga, S.; Fukuta, K.; Tsukahara, T. Production, Absorption, and Blood Flow Dynamics of Short-Chain Fatty Acids Produced by Fermentation in Piglet Hindgut during the Suckling-Weaning Period. *Nutrients* **2018**, *10*, 1220. [CrossRef]
56. Metzler-Zebeli, B.U.; Zijlstra, R.T.; Mosenthin, R.; Gänzle, M.G. Dietary calcium phosphate content and oat β-glucan influence gastrointestinal microbiota, butyrate-producing bacteria and butyrate fermentation in weaned pigs. *FEMS Microbiol. Ecol.* **2011**, *75*, 402–413. [CrossRef]
57. He, B.; Bai, Y.; Jiang, L.; Wang, W.; Li, T.; Liu, P.; Tao, S.; Zhao, J.; Han, D.; Wang, J. Effects of Oat Bran on Nutrient Digestibility, Intestinal Microbiota, and Inflammatory Responses in the Hindgut of Growing Pigs. *Int. J. Mol. Sci.* **2018**, *19*, 2407. [CrossRef]
58. Pieper, R.; Jha, R.; Rossnagel, B.; Van Kessel, A.G.; Souffrant, W.B.; Leterme, P. Effect of barley and oat cultivars with different carbohydrate compositions on the intestinal bacterial communities in weaned piglets. *FEMS Microbiol. Ecol.* **2008**, *66*, 556–566. [CrossRef] [PubMed]
59. Hiss, S.; Sauerwein, H. Influence of dietary ß-glucan on growth performance, lymphocyte proliferation, specific immune response and haptoglobin plasma concentrations in pigs. *J. Anim. Physiol. Anim. Nutr.* **2003**, *87*, 2–11. [CrossRef] [PubMed]

60. Xu, D.; Feng, M.; Chu, Y.; Wang, S.; Shete, V.; Tuohy, K.M.; Liu, F.; Zhou, X.; Kamil, A.; Pan, D.; et al. The Prebiotic Effects of Oats on Blood Lipids, Gut Microbiota, and Short-Chain Fatty Acids in Mildly Hypercholesterolemic Subjects Compared With Rice: A Randomized, Controlled Trial. *Front. Immunol.* **2021**, *12*, 787797. [CrossRef] [PubMed]
61. Tiwari, U.P.; Singh, A.K.; Jha, R. Fermentation characteristics of resistant starch, arabinoxylan, and β-glucan and their effects on the gut microbial ecology of pigs: A review. *Anim. Nutr.* **2019**, *5*, 217–226. [CrossRef]
62. Zhou, Y.; Luo, Y.; Yu, B.; Zheng, P.; Yu, J.; Huang, Z.; Mao, X.; Luo, J.; Yan, H.; He, J. Effect of β-Glucan Supplementation on Growth Performance and Intestinal Epithelium Functions in Weaned Pigs Challenged by Enterotoxigenic *Escherichia coli*. *Antibiotics* **2022**, *11*, 519. [CrossRef]
63. Vetvicka, V.; Vetvickova, J. Physiological Effects of Different Types of Beta-Glucan. *Biomed. Pap.* **2007**, *151*, 225–231.
64. Dong, J.L.; Yu, X.; Dong, L.E.; Shen, R.L. In vitro fermentation of oat [beta]-glucan and hydrolysates by fecal microbiota and selected probiotic strains. *J. Sci. Food Agric.* **2017**, *97*, 4198. [CrossRef]
65. Gong, L.; Cao, W.; Chi, H.; Wang, J.; Zhang, H.; Liu, J.; Sun, B. Whole cereal grains and potential health effects: Involvement of the gut microbiota. *Food Res. Int.* **2018**, *103*, 84–102. [CrossRef] [PubMed]
66. Volman, J.J.; Ramakers, J.D.; Plat, J. Dietary modulation of immune function by beta-glucans. *Physiol. Behav.* **2008**, *94*, 276–284. [CrossRef]
67. Stier, H.; Ebbeskotte, V.; Gruenwald, J. Immune-modulatory effects of dietary Yeast Beta-1,3/1,6-D-glucan. *Nutr. J.* **2014**, *13*, 38. [CrossRef] [PubMed]
68. Atanasov, J.; Schloermann, W.; Trautvetter, U.; Glei, M. The effects of β-glucans on intestinal health. *Ernahr. Umsch.* **2020**, *67*, 52–59.
69. Geervliet, M.; Lute, L.C.; Jansen, C.A.; Rutten, V.P.; Savelkoul, H.F.; Tijhaar, E. Differential immunomodulation of porcine bone marrow derived dendritic cells by E. coli Nissle 1917 and β-glucans. *PLoS ONE* **2020**, *15*, e0233773. [CrossRef] [PubMed]
70. Sonck, E.; Devriendt, B.; Goddeeris, B.; Cox, E. Varying effects of different β-glucans on the maturation of porcine monocyte-derived dendritic cells. *Clin. Vacc. Immunol.* **2011**, *18*, 1441–1446. [CrossRef]
71. de Vries, H.; Geervliet, M.; Jansen, C.A.; Rutten, V.; van Hees, H.; Groothuis, N.; Wells, J.M.; Savelkoul, H.F.J.; Tijhaar, E.; Smidt, H. Impact of Yeast-Derived β-Glucans on the Porcine Gut Microbiota and Immune System in Early Life. *Microorganisms* **2020**, *8*, 157. [CrossRef] [PubMed]
72. Mejía, S.M.V.; de Francisco, A.; Bohrer, B. A comprehensive review on cereal β-glucan: Extraction, characterization, causes of degradation, and food application. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 3693–3704. [CrossRef]
73. Shen, R.L.; Dang, X.Y.; Dong, J.L.; Hu, X.Z. Effects of oat β-glucan and barley β-glucan on fecal characteristics, intestinal microflora, and intestinal bacterial metabolites in rats. *J. Agric. Food Chem.* **2012**, *60*, 11301–11308. [CrossRef]
74. Boes, J.; Johansen, M.V.; Eriksen, L.; Bogh, H.O.; Nansen, P.; Stephenson, L.S. False-positive Trichuris suis egg counts in pigs in relation to coprophagia. *Parasite* **1998**, *5*, 91–93. [CrossRef]
75. Luo, J.; Chen, D.; Mao, X.; He, J.; Yu, B.; Cheng, L.; Zeng, D. Purified β-glucans of Different Molecular Weights Enhance Growth Performance of LPS-challenged Piglets via Improved Gut Barrier Function and Microbiota. *Animals* **2019**, *9*, 602. [CrossRef] [PubMed]
76. Huang, C.; Song, P.; Fan, P.; Hou, C.; Thacker, P.; Ma, X. Dietary Sodium Butyrate Decreases Postweaning Diarrhea by Modulating Intestinal Permeability and Changing the Bacterial Communities in Weaned Piglets. *J. Nutr.* **2015**, *145*, 2774–2780. [CrossRef]
77. Guilloteau, P.; Martin, L.; Eeckhaut, V.; Ducatelle, R.; Zabielski, R.; Van Immerseel, F. From the gut to the peripheral tissues: The multiple effects of butyrate. *Nutr. Res. Rev.* **2010**, *23*, 366–384. [CrossRef] [PubMed]
78. Lalles, J.P.; Boudry, G.; Favier, C.; Le Floc'H, N.; Luron, I.; Montagne, L.; Oswald, I.P.; Pié, S.; Piel, C.; Sève, B. Gut function and dysfunction in young pigs: Physiology. *Anim. Res.* **2004**, *53*, 301–316. [CrossRef]
79. Inoue, R.; Tsukahara, T.; Nakatani, M.; Okutani, M.; Nishibayashi, R.; Ogawa, S.; Harayama, T.; Nagino, T.; Hatanaka, H.; Fukuta, K.; et al. Weaning Markedly Affects Transcriptome Profiles and Peyer's Patch Development in Piglet Ileum. *Front. Immunol.* **2015**, *6*, 630. [CrossRef]
80. Alizadeh, A.; Akbari, P.; Difilippo, E.; Schols, H.A.; Ulfman, L.H.; Schoterman, M.H.C.; Garssen, J.; Fink-Gremmels, J.; Braber, S. The piglet as a model for studying dietary components in infant diets: Effects of galacto-oligosaccharides on intestinal functions. *Br. J. Nutr.* **2016**, *115*, 605–618. [CrossRef]
81. Bruininx, E.M.; Binnendijk, G.P.; van der Peet-Schwering, C.M.; Schrama, J.W.; den Hartog, L.A.; Everts, H.; Beynen, A.C. Effect of creep feed consumption on individual feed intake characteristics and performance of group-housed weanling pigs. *J. Anim. Sci.* **2002**, *80*, 1413–1418. [CrossRef]

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2025:39

This thesis investigated the effects of prebiotic and probiotic supplements on gut microbiota, short-chain fatty acids, growth and behaviour in suckling piglets. Findings showed primarily age related effects, but indicated that administration of probiotics can influence gut microbiota in ileum and colon early in life. The prebiotic supplement was associated with impairment in reversal learning and alteration in social behaviour. Significant correlations were also identified between microbiota and behavioural data in piglets.

Lidija Arapovic received her doctoral education at the Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences (SLU). She obtained her Master of Science degree from the Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences, Croatia.

Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

ISSN 1652-6880
ISBN (print version) 978-91-8046-474-1
ISBN (electronic version) 978-91-8046-524-3