

**Aus dem Institut für Tierernährung  
des Fachbereichs Veterinärmedizin  
der Freien Universität Berlin**

**Effects of dietary rye and rapeseed on growth performance, nutrient  
digestibility, digesta characteristics and the intestinal microbiome of weaner  
piglets**

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## List of abbreviations

AA	Amino acids
ADF	Acid detergent fibre
ADL	Acid detergent lignin
AID	Apparent ileal digestibility
ANF	Anti-nutritional factor
ATTD	Apparent total tract digestibility
AX	Arabinoxylans
BW	Body weight
CP	Crude protein
DF	Dietary fibre
DM	Dry matter
ETEC	Enterotoxigenic <i>Escherichia coli</i>
GIT	Gastrointestinal tract
IDF	Insoluble dietary fibre
ME	Metabolizable energy
NDF	Neutral detergent fibre
NH <sub>3</sub>	Ammonia
NSP	Non-starch polysaccharides
PWD	Post-weaning diarrhoea
RS	Resistant starch
RSM	Rapeseed meal
SBM	Soybean meal
SCFA	Short-chain fatty acids
SDF	Soluble dietary fibre

## 1. General introduction

Currently, a major issue in pig production is the demand for more sustainability along the entire production chain. The cultivation of feed crops and feed production as part of this chain are facing increasing extreme weather conditions due to climate change and challenges associated with the globalized trading of feed components. Moreover, a decreased use of antibiotics in pig husbandry is necessary. To address these difficulties the feeding of rye and rapeseed as an alternative to commonly used wheat and soybean is an interesting approach in pig nutrition. Rye is a very robust cereal crop with lower requirements with respect to soil quality and climate than wheat (Kamphues et al. 2019). Rapeseed is a high-protein oil seed crop which is better adapted to European climate than soybean (Miedaner 2014a). However, in the past the inclusion of rye and rapeseed into diets for pigs, especially for weaner pigs, was limited primarily due to characteristics such as anti-nutritional factors (ANF) and a high content of fibre, considered to be detrimental to palatability, nutrient digestibility or animal health and growth (Mejicanos et al. 2016; Kamphues et al. 2019). Nonetheless, plant breeding succeeded in reducing the concentration of ANF and the high content of dietary fibre (DF) is considered not only a negative feature anymore (Mejicanos et al. 2016; Kamphues et al. 2019). In fact, fermentation of complex fibre fractions mainly in the large intestine provides several benefits for the pig (Jha et al. 2019; Yang and Zhao 2021). Recent research indicates that the feeding of rye and rapeseed meal (RSM) instead of wheat and soybean meal (SBM) does not have any negative effects on health, feed intake and growth performance of grower pigs (Wilke 2020). Nevertheless, research on the combined feeding of rye and RSM is scarce, especially with respect to effects on digestive physiology and microbial fermentation processes in weaner pigs.

Therefore, this study focussed on comparing effects of the cereals rye and wheat in combination with the protein meals RSM and SBM when fed to weaner piglets. A feeding trial arranged in a 2x2 factorial design was conducted with 88 piglets weaned at 28 days. Piglets were allotted to four groups, two piglets per pen ( $n = 11$ ), and fed one of the following isonitrogenous diets: wheat/ SBM (W-SBM), wheat/ RSM (W-RSM), rye/ SBM (R-SBM) and rye/ RSM (R-RSM). The cereals were included at high inclusion levels of 48 %, SBM at 25 % and RSM at 30 % into the diets. Data of growth performance and faecal consistency were assessed during the trial. After 33 days, dissection of one piglet per pen served for sampling of digesta and the assessment of the morphology of the intestine. Feed was analysed for nutrient composition and digesta samples were subjected analysis of nutrient content (protein, non-starch-polysaccharides (NSP)), physico-chemical parameters (pH, viscosity) and the



relative abundance and metabolic activity (short-chain fatty acids (SCFA), lactic acid, ammonia (NH<sub>3</sub>)) of microbiota.

### **Aims and objectives of the thesis**

The aim of this thesis was to determine the effects of dietary rye and rapeseed in comparison to wheat and soybean on

1. growth performance, apparent ileal protein digestibility and intestinal characteristics
2. fibre digestibility and composition and metabolic activity of the intestinal microbiota

in weaner piglets.

To achieve these aims, an *in vivo* experiment was carried out, and results were published in two scientific articles.

### **Hypothesis**

It was hypothesised that dietary rye and rapeseed instead of wheat and soybean lead to changes of digestive physiology and more fermentative activity of the intestinal microbiota in the sense of improving gut and animal health of weaner pigs. Therefore, comparable results of growth performance and intestinal development were expected.

## 2. Literature review

### 2.1. Effects of weaning on health and growth performance of piglets

Piglets raised under commercial conditions are commonly weaned at 21-28 days of age (Worobec et al. 1999). Many factors such as the new environment and the change from suckling milk to a solid diet present a challenge to piglets at this age (Upadhaya and Kim 2021). The gastrointestinal tract (GIT) as well as the immune system are not fully developed, hence health and growth of piglets may be affected by weaning related stress factors (Upadhaya and Kim 2021). This can lead to increased piglet mortality and economic losses. Therefore, the main aim in the post-weaning period is the reduction of negative effects to maintain health and achieve good growth performance. This includes efforts to improve gut health, which may be defined by six criteria: 1) *effective digestion and absorption of food and excretion of wastes*, 2) *a functional and protective gut barrier*, 3) *a stable and appropriate microbial population*, 4) *effective functioning of the gut immune system*, 5) *minimal activation/stimulation of stress/neural pathways*, and 6) *the absence of disease(s)* (Pluske et al. 2018a). Moreover, with increasing prevalence of antimicrobial resistant bacteria and the ban of antibiotics as growth promoters, alternative strategies are required to meet these criteria. In this sense, many factors should be considered such as an adequate management of housing conditions and effective post-weaning feeding concepts (Pluske et al. 2018a; Upadhaya and Kim 2021).

#### 2.1.1. Stress factors of weaning

In nature, weaning is a gradual process until the age of 10 to 12 weeks (Worobec et al. 1999). In contrast, weaning in commercial production systems is an abrupt change for the piglets accompanied with many stress factors. At three to four weeks of age, the GIT and the immune system are still immature and not fully developed (Modina et al. 2019). Secretion of gastric acid and digestive enzymes is insufficient for the digestion of solid feed (Campbell et al. 2013). One of the most important stressors is the change from the palatable milk to solid creep feed (Modina et al. 2019). Moreover, the passive immunity derived from the sow's milk declines with the stop of suckling (Stokes 2017). Psychosocial stress factors are the separation from the sow, transportation as well as the mixing of new, unfamiliar piglets resulting in fights and the establishment of a group hierarchy (Campbell et al. 2013; Khafipour et al. 2014). Stressors with respect to the new environment in nursery pens may be restricted space allowance, lower ambient temperature, and poor sanitary conditions (Pastorelli et al. 2012; Jayaraman and Nyachoti 2017). In reaction to additive effects of these stress factors, the piglets' digestive and immune functions must adapt fast to re-establish homeostasis (Hyun et al. 1998; Jayaraman

and Nyachoti 2017). Consequences of weaning stress are a lower feed intake, alterations of gut structure, reduced intestinal barrier function, decreased nutrient digestion and adsorption, an impaired function of the immune system, oxidative stress and a shift of the intestinal microbiome (Hampson 1986; Spreeuwenberg et al. 2001; Dong and Pluske 2007; Wijtten et al. 2011; Guevarra et al. 2019; Ji et al. 2019). This may lead to post-weaning diarrhoea (PWD), a higher susceptibility to pathogens and finally a reduced growth performance (Hyun et al. 1998; Khafipour et al. 2014; Gao et al. 2019).

### 2.1.2. Feed intake and body weight gain

Weaning is often accompanied by an immediate reduction of feed intake or anorexia for several days (McCracken et al. 1999; Dong and Pluske 2007). This reduction may be caused by the exposure to social and environmental stress as well as by the change of diet (Dong and Pluske 2007; Jayaraman and Nyachoti 2017). Taste and physical form of solid feed are unfamiliar and palatability may be low, especially compared to sow's milk (Dong and Pluske 2007). Especially protein meals from soybean or rapeseed contain anti-nutritional factors (ANF) such as sinapine which can reduce palatability (Dong and Pluske 2007; Mejicanos et al. 2016). In consequence of the reduced feed intake the intake and digestion of nutrients and metabolizable energy (ME) is lower (Le Dividich and Sève 2000). Moreover, it leads to intestinal inflammation which can impair intestinal barrier function and gut architecture (McCracken et al. 1999; Spreeuwenberg et al. 2001). Finally, the lower feed intake reduces growth performance until it may recover after a few days of adaptation to the post-weaning conditions (Le Dividich and Sève 2000). Post-weaning body weight (BW) and weight gain are correlated to the final body weight of fattening pigs, which has implications regarding economic aspects of pig production (Tokach et al. 1992). Taken together, it is essential for animal health and productivity to maximize feed intake after weaning.

### 2.1.3. Post-weaning diarrhoea

PWD is considered a major challenge in pig production and leads to impaired growth performance, increased morbidity, and mortality (Gao et al. 2019). Therefore, the management of PWD is essential for animal welfare and economic success. Diarrhoea frequently occurs in the post-weaning period because the piglets' immune system and intestinal microbial composition are still immature (Hampson 1986; Gresse et al. 2017; Guevarra et al. 2019). Weaning stress may lead to a transient inflammation in the gut, the loss of mucosal surface and an increased permeability of the intestinal epithelium making the piglets more susceptible to infections (McCracken et al. 1999; Wijtten et al. 2011; Al Masri et al. 2015). The resulting

reduction of absorptive capacity in the intestine and increased intestinal secretion produce an excess of faecal water (Kunzelmann and Mall 2002; Wijtten et al. 2011). Predisposing factors to the development of PWD are the genetic background, inadequate housing conditions and diets high in protein (Meijerink et al. 1997; Wellock et al. 2008; Dou et al. 2017). As a response to weaning stress, PWD may be provoked by a perturbation of the commensal microbiota as well as by an infection with specific pathogens. Healthy piglets weaned in low sanitary conditions showed higher relative abundance of *Prevotellaceae*, *Lachnospiraceae*, *Ruminococcaceae* and *Lactobacillaceae* than pigs with PWD (Dou et al. 2017). Moreover, a loss of microbial diversity was associated with PWD (Dou et al. 2017). The reduced feed intake and resulting inflammatory conditions may further enhance the microbial imbalance (Zeng et al. 2017). Reactive oxygen species provide a nitrate-rich milieu favourable for the growth of potential pathogens belonging to *Enterobacteriaceae* such as *Salmonella enterica* serovar Typhimurium and enterotoxigenic *E. coli* (ETEC) (Winter et al. 2013; Zeng et al. 2017). ETEC is one of the major causes for PWD (Fairbrother et al. 2005; Gebhardt et al. 2020). Among several strategies controlling PWD such as antibiotics, the optimization of housing conditions, and genetic selection, a very efficient measure is the feeding of a diet with adequate proportions of protein, fibre, starch, and electrolytes (Meijerink et al. 1997; Fairbrother et al. 2005; Dou et al. 2017; Gao et al. 2019).

### 2.1.4. Post-weaning feeding strategies

Facing the increased development of antibiotic resistances and the public demand for minimal use of antibiotics (Xiong et al. 2018), effective post-weaning feeding strategies have become an important tool in pig production (Pluske 2013). Different strategies aim to improve gut health and growth performance of the piglets and to prevent PWD by increasing palatability, optimizing nutrient digestion and absorption, balancing gut microbiota in the sense of eubiosis and stabilizing the local immune system (Wijtten et al. 2011; Pluske et al. 2018a; Gao et al. 2019; Modina et al. 2019). Key aspects that are considered in this respect are the control of dietary fibre (DF), protein, starch, and electrolytes within the diet (Pluske et al. 2018a; Gao et al. 2019). Additionally, there is a broad range of feed additives developed to support piglets in the stressful post-weaning period (Modina et al. 2019; Upadhaya and Kim 2021). Different sources of DF can influence intestinal barrier function and the resident microbiota of weaner pigs and will be discussed in detail further below. The concentration and digestibility of crude protein (CP) and individual amino acids (AA) should be considered as well as the AA pattern and the inclusion of essential AA (Goodband et al. 2014; Wu et al. 2014; Capozzalo et al. 2017). A low-protein diet prevents growth of potential pathogens, the microbial degradation of undigested protein and the resulting formation of harmful metabolites such as NH<sub>3</sub> and amines

(Pieper et al. 2016). These metabolites may increase the incidence of PWD by impairing epithelial integrity and disturbing resident microbiota (Pieper et al. 2016). Ratio of sulfuric amino acids to lysine should be 60-65 %, tryptophan to lysine 21 % and threonine to lysine 70 % (Goodband et al. 2014; Capozzalo et al. 2017). Starch is the main source of carbohydrates and energy for pigs (Wiseman 2006). It is composed of easily and slowly digestible starch absorbed in the small intestine but also contains resistant starch (RS) that cannot be degraded enzymatically and reaches the large intestine (Bach Knudsen et al. 2016b). There, RS can be fermented by resident microbiota to useful metabolites such as short-chain fatty acids (SCFA) (Haenen et al. 2013) and promote growth of potentially beneficial bacteria such as Bifidobacteria (Tiwari et al. 2019). Effects of SCFA on gut health will be discussed below. Dietary content of minerals should meet the nutritional requirements and be balanced regarding large intestinal water absorption, intracellular pH, and cell volume (Kato and Romero 2011).

Available feed additives for weaner piglets comprise probiotics, prebiotics, phytogetic compounds, fatty acids, organic acids, functional amino acids, and nucleotides (Modina et al. 2019; Upadhaya and Kim 2021). Probiotics are defined by FAO and WHO as live microorganisms administered in sufficient amounts to confer health benefits to the host (Hill et al. 2014), e.g. *Bacillus subtilis* and *Lactobacillus plantarum* (Heo et al. 2018; Park et al. 2020). Together with prebiotics such as fructo-oligosaccharides they are considered to beneficially influence intestinal barrier function (Xu et al. 2005; Wang et al. 2018a; Park et al. 2020; Upadhaya and Kim 2021), inhibit growth of bacterial pathogens (Wang et al. 2018b), reduce incidence and severity of PWD (Taras et al. 2005; Büsing and Zeyner 2015), and promote the development of the immune system (Lewis et al. 2013).

### **2.2. Intestinal microbiome of pigs**

The intestinal microbiome represents the genomic information of the microbial community in the gut (Pluske et al. 2018a). Due to the fast development of molecular technologies in the past decade and similarities between pig and human GIT physiology research on the pig intestinal microbiome has increased (Kim and Isaacson 2015; Ke et al. 2019). The intestinal microbiota is essential for the health of the host (Guevarra et al. 2019; Ke et al. 2019). It can influence nutrient digestibility, produce beneficial metabolites such as SCFA, help preventing the colonization of pathogens and interact with the hosts' immune system (Isaacson and Kim 2012; Stokes 2017). The composition of the intestinal microbiome is influenced by host genetics, environmental factors, age, and diet (Guevarra et al. 2019; Ke et al. 2019).

### 2.2.1. Characterization of the intestinal microbiome along the gastrointestinal tract of pigs

The intestinal microbiota differ in each GIT segment (Holman et al. 2017; Fan et al. 2021) and with respect to luminal or mucosal localization (Zhang et al. 2018). Differences occur regarding the composition and diversity of the microbial population (Holman et al. 2017). The varying physico-chemical conditions, digesta passage rate and nutrient availability from the upper to the lower GIT are factors influencing the microbiome (Holman et al. 2017; Fan et al. 2021). Due to the lower gastric pH acid tolerant *Lactobacillus* spp. may be enriched in the stomach compared to caudal intestinal segments (van Winsen et al. 2001). Alpha-diversity, richness and evenness are increased in caudal direction of the intestine which may be related to the longer transit time and less competition for nutrients with the host in the large intestine compared to the small intestine (Holman et al. 2017). The phyla *Firmicutes* and *Bacteroidetes* represented almost 85% of the sequenced microbiota in digesta samples from ileum, caecum, colon, and faeces analysed in a meta-analysis on the pig microbiome (Holman et al. 2017). *Prevotella* was the most abundant genus and was determined along with *Clostridium*, *Blautia*, *Lactobacillus*, *Ruminococcus*, and *Roseburia*, the RC9 gut group, and *Subdoligranulum* as core microbiota being present in more than 90 % of the samples (Holman et al. 2017).

In the small intestine, bacterial growth is limited due to the presence of bile acids and antimicrobial peptides (Donaldson et al. 2016). However, in the distal ileum pH is neutral and oxygen availability reduced which enables growth of mainly *Firmicutes* and *Proteobacteria* (Zhang et al. 2018). Moreover, *Clostridium* seems to be a core genus of ileal microbiota (Holman et al. 2017). Predominant phyla in the large intestinal digesta and faeces are *Firmicutes* and *Bacteroidetes* (Holman et al. 2017; Zhang et al. 2018). Caecal luminal microbiota mainly consist of anaerobic *Prevotellaceae*, *Lachnospiraceae*, *Ruminococcaceae*, and *Veillonellaceae* (Zhang et al. 2018). Core genera in the caecum are *Anaerovibrio*, *Clostridium*, *Phascolarctobacterium*, *Ruminococcus*, *Sarcina*, and *Streptococcus* (Holman et al. 2017). In the colon, *Prevotella* is the predominant genus and belongs to the colonic core genera together with *Blautia* (Holman et al. 2017; Zhang et al. 2018). Faecal core genera are *Prevotella*, *Clostridium*, *Alloprevotella*, *Ruminococcus*, and the RC9 gut group (Holman et al. 2017).

### 2.2.2. Effects of weaning on the intestinal microbial community

The change from sow's milk to a plant-based diet induces a shift from microbiota capable to mainly metabolize lactate to microbiota which can ferment complex plant polysaccharides (Alain et al. 2014). This is reflected in a shift from *Bacteroides* to *Prevotella* and *Lactobacillus*,

with *Bacteroides* degrading milk monosaccharides and oligosaccharides and *Prevotella* hemicelluloses and xylans (Alain et al. 2014; Guevarra et al. 2018). Additionally, weaning may induce an increase of alpha-diversity of the intestinal microbiome (Guevarra et al. 2019).

### 2.2.3. Effects of bacterial metabolites on gut and animal health

The intestinal microbiome is considered to play an important role regarding gut health, growth performance, and well-being of piglets (McCormack et al. 2017; Stokes 2017). The exact mechanisms and pathways are still not completely known, but the maintenance of gut health seems to depend rather on a balanced bacterial population than on the absence of specific pathogens or the presence of specific commensals (Pluske et al. 2018b). Important microbial functions in this respect are the production of metabolites from dietary and endogenous substrates such as DF and the interaction with the immune system (Schnupf et al. 2017; Jha et al. 2019; Trachsel et al. 2019; Peng et al. 2021).

Fermentation of carbohydrates results in metabolites such as SCFA and lactic acid considered beneficial for the host and occurs mainly in the large intestine (Jha and Berrocoso 2015; Tiwari et al. 2019). Microbes prefer saccharolytic fermentation over the degradation of undigested dietary or endogenous protein (Tiwari et al. 2019). These proteolytic processes result in de novo proteins but also in potentially harmful metabolites such as NH<sub>3</sub>, indoles and phenols (Williams et al. 2019).

Acetate, propionate, and butyrate are the major SCFA, and the production amount and ratio depend on the available substrate reaching the large intestine (Bach Knudsen 2015). *Prevotella* are major fermenters of plant carbohydrates such as non-starch polysaccharides (NSP) and produce mainly acetate (Amat et al. 2020). *Clostridium*, *Blautia*, *Ruminococcus* can use acetate to generate butyrate via the butyryl-coenzyme A (CoA):acetate CoA-transferase pathway (Vital et al. 2014). SCFA are also produced by *Lactobacilli* from oligosaccharides and starch (Gänzle and Follador 2012). SCFA are rapidly absorbed by the intestinal epithelium or taken up by resident microbiota and only a minor amount is excreted via faeces (Topping and Clifton 2001). There are many effects of SCFA contributing to animal and gut health. An important factor is the use of SCFA as an energy source for the entire animal, for the colonocytes as well as for other bacteria in the sense of cross-feeding (Hijova and Chmelarova 2007; Guilloteau et al. 2010; den Besten et al. 2013). Other functions of SCFA are a decrease of the gut pH, increase of nutrient and electrolyte absorption and the stimulation of cell division and cell proliferation (Herrmann et al. 2011; den Besten et al. 2013). Moreover, SCFA may regulate the secretion of adipokines in the intestinal epithelium and promote intestinal barrier

function by stimulating the secretion of mucus, antimicrobial peptides, and IgA (Montagne et al. 2004; Xiong et al. 2016; Trachsel et al. 2019). Acetate is the predominant SCFA in venous blood circulation and can be used in peripheral tissues (Williams et al. 2019). In the liver it is involved in the synthesis of new fatty acids (Tremaroli and Bäckhed 2012; Williams et al. 2017). Propionate is used for gluconeogenesis, influences cholesterol synthesis and may stimulate satiety (Al-Lahham et al. 2010; Williams et al. 2017). Moreover, propionate was shown to interact with the immune system (Brown et al. 2003; Williams et al. 2019). Being metabolised rapidly and to a high extent in the colonocytes butyrate is the main energy fuel for the gut epithelium (Guilloteau et al. 2010). Butyrate can stimulate epithelial development and health by the modification of gene expression for epidermal growth factor and by repairing damaged cells (Hamer et al. 2008; Liu et al. 2018; Yang and Zhao 2021).

Potentially harmful bacterial metabolites result from the degradation of undigested dietary or endogenous protein, especially in the absence of fermentable carbohydrates (Bikker et al. 2006; Pieper et al. 2012; Pieper et al. 2014). Many putative pathogens such as *E. coli*, *Klebsiella* spp., *Campylobacter* spp., *Streptococcus* spp., *C. perfringens*, *C. difficile* or *Bacteroides fragilis* are proteolytic bacteria (Opapeju et al. 2009; Pieper et al. 2016). However, also commensal members of *Fusobacteria*, *Firmicutes* (*Streptococcaceae*, *Veillonellaceae*, *Megasphera*, *Selenomonas*), *Proteobacteria* and *Bacteroidetes* species are known to produce NH<sub>3</sub>, amines, phenols, and indoles from protein (Dai et al. 2011; Rist et al. 2013; Pieper et al. 2016). These substances can directly damage the epithelial cells and cause inflammation (Rist et al. 2013; Pieper et al. 2016). Moreover, many colonic diseases including colon cancer were associated with toxic protein degradation products (Williams et al. 2019). Consequently, pigs' growth performance may be reduced in case of a lack of fermentable substrate and an increased bacterial protein degradation (Bikker et al. 2006; Rist et al. 2013).

### **2.3. The use of dietary fibre in pig nutrition**

The term dietary fibre (DF) describes plant-derived carbohydrate polymers and lignin which are resistant to hydrolyzation by digestive enzymes in the small intestine and which contribute to maintaining physiological functioning and health of the host (Cummings and Stephen 2007; Jones 2014). Plant non-digestible carbohydrates include RS and non-digestible oligosaccharides (e.g., inulin and fructo-oligosaccharides) from plant cell content and NSP (e.g.,  $\beta$ -glucans and arabinoxylans (AX)) from the plant cell wall (Stephen et al. 2017; Williams et al. 2017). In contrast to DF, monosaccharides, disaccharides, and most starches are digestible and absorbable in the small intestine (Cummings and Stephen 2007). In the past, DF was considered as anti-nutritional factor mainly because it may reduce nutrient digestibility,



dietary energy density and consequently growth performance (Jha and Berrocoso 2015; Yang and Zhao 2021). However, the feeding of DF may hold benefits for the pig, especially its fermentation in the large intestine. The dietary inclusion level as well as the chemical composition and structure of DF influences its effects on gut health and growth performance, its fermentability and shifts of the microbial population (Chen et al. 2013; Ingerslev et al. 2014; Weiss et al. 2016; Agyekum and Nyachoti 2017; Lee and Woyengo 2018). Known beneficial effects of DF are the stimulation gut motility, growth of potentially beneficial microbiota, reduced growth of potential pathogens, a reduction of diarrhoea and other post-weaning GIT disorders, and as discussed above an increased production of SCFA instead of proteolytic processes (Wilfart et al. 2007; Molist et al. 2012; Awad et al. 2013; Ingerslev et al. 2014; Umu et al. 2018; Gao et al. 2019; Chuppava et al. 2020). Moreover, DF may improve intestinal barrier function, e.g., by increasing number and mucin secretion of goblet cells and expression of tight junction proteins (Chen et al. 2013; Zhou et al. 2017).

### 2.3.1. Classification of dietary fibre

The molecular structure and chemical properties of DF influencing its solubility and viscosity in water as well as its fermentability differ between plants and plant tissues (Glitsso et al. 1998; Nielsen et al. 2014; Stephen et al. 2017). Often, DF is described by a given analytical method or by solubility, both associated with the chemical structure of the respective fibres. Common fibre analysis methods are the Van Soest detergent method resulting in neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL), and the enzymatic-gravimetric method measuring total dietary fibre (TDF) which comprises insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) (Van Soest et al. 1991; McCleary 2014; Li et al. 2021). The TDF method measures soluble and insoluble fractions of DF including NSP, RS and lignin, whilst the NDF method lacks a proportion of the soluble fibre components (Agyekum and Nyachoti 2017; Li et al. 2021). The term solubility refers to the capability of DF to be fully dispersed in water (Williams et al. 2019). It increases with more branched and with a higher diversity of linkages of fibre molecules, but also with smaller molecule size (Lovegrove et al. 2017). Generally, more soluble fibre is fermented more readily. However, some polymers defined as soluble tend to aggregate or phase-separate which may hamper water solubility and may influence fermentability (Lovegrove et al. 2017). Moreover, soluble high-molecular weight DF polymers increase viscosity of solutions (Lovegrove et al. 2017). Examples of typical SDF are hemicelluloses, pectins, and gums, typical IDF are cellulose, lignin, and RS (Williams et al. 2019). Chemically, DF varies in monosaccharide composition, glycosidic linkages, and backbone structure. Non-digestible oligosaccharides such as inulin are composed of 3-9 monosaccharides connected with either  $\alpha$ -1:4 or  $\alpha$ -1:6 glycosidic bonds (Stephen et al. 2017).

DF polysaccharides such as NSP and RS consist of a minimum of 10 monosaccharides (Stephen et al. 2017).

Major NSP in cereals used in pig nutrition are AX, cellulose, and 1,3-1,4- $\beta$ -D-glucans (mixed linked  $\beta$ -D-glucans) which are mainly present in plant cell walls (Bach Knudsen et al. 2016b; Rodehutsord et al. 2016). Depending on the botanical origin, composition and structure of AX and  $\beta$ -glucans varies (Rodehutsord et al. 2016). AX are hemicelluloses composed of a xylan backbone substituted with arabinose by  $\beta$ -linkages. Arabinose and xylose monomers can be detected by chromatography from soluble and insoluble TDF fractions (Englyst et al. 2007). Arabinose residues form heterogenous, slowly fermentable molecular complexes by binding to ferulic acid (Tiwari et al. 2019). A lower degree of substitution described by the ratio of arabinose to xylose may indicate a higher degradability of AX (Tiwari et al. 2019). However, other factors such as double substitutions must be considered regarding AX fermentability (Nielsen et al. 2014). Mixed linked  $\beta$ -D-glucans are non-cellulosic branched polysaccharides consisting of glucose and bonded by  $\beta$ -1:3 and  $\beta$ -1:4 linkages (Tiwari et al. 2019). Generally,  $\beta$ -glucans are considered highly soluble, and fermentation may take place already in the small intestine (Wood 2010; Metzler-Zebeli and Zebeli 2013). Molecular weight and ratio of  $\beta$ -1:3 and  $\beta$ -1:4 linkages of  $\beta$ -glucans may vary depending on cereal type (Wood 2010). Cellulose is a highly insoluble, linear polymer of glucose monomers linked by  $\beta$ -1:4 linkages (Stephen et al. 2017; Williams et al. 2019). It is the major component of plant cell walls and crosslinking to soluble pectin or hemicelluloses can make those components insoluble (Williams et al. 2019). Pectins are a very heterogenic group of  $\beta$ -1:4 galacturonic acid polymers substituted with arabinans, galactans or arabinogalactans (Stephen et al. 2017). They are highly soluble and viscous polysaccharides and were shown to be highly fermentable *in vitro* (Bauer et al. 2001; Bauer et al. 2010). Fructans are polymers composed of sucrose disaccharides which contain fructose and glucose (Ritsema and Smeekens 2003). Fructose molecules in cereal fructans are linked through either  $\beta$ -2:1 or  $\beta$ -2:6 fructose-fructosyl linkages, while inulin-type fructans are mainly built of  $\beta$ -2:1 fructose-fructosyl linkages (Abdi and Joye 2021). Fructans are present in the cereal bran and endosperm (Adebowale et al. 2019; Abdi and Joye 2021). Lignin is a non-carbohydrate phenolic highly branched biopolymer with a hydrophobic surface (Davin et al. 2008; Bach Knudsen 2014). Building cross-linkages with cell wall NSP it forms rigid structures hampering the access of microbiota (Davin et al. 2008; Bach Knudsen 2014; Terrett and Dupree 2019). Lignin content may indicate insolubility and indigestibility of feed components and can be quantified as acid detergent lignin (ADL) (Van Soest et al. 2020). RS are resistant to degradation by amylases and are grouped into five types according to digestion kinetics (Birt et al. 2013): Type 1 are starch granules encapsulated by proteins and cell wall

matrix of cereal grains or seeds, Type 2 is raw or high-amylose starch, e.g., from maize or potato which can get highly digestible by thermal processing, Type 3 is retrograded amylose and starch, Type 4 is chemically modified starch and Type 5 is starch linked to lipids, amylose, and amylopectin-helices (Birt et al. 2013).

### 2.3.2. Effects of dietary fibre on digestive physiology: physico-chemical parameters of digesta and nutrient digestibility

The feeding of DF may influence the digestive physiology by altering digesta pH and viscosity as well as nutrient digestibility. Fermentation products SCFA and lactic acid decrease digesta pH which may improve mineral absorption and stimulate growth of potentially beneficial microbiota (Gorham et al. 2017; Bournazel et al. 2018). In piglets fed rye instead of wheat which contains less NSP and AX, small intestinal and colonic pH was decreased, and lactic acid concentration increased (Wilke et al. 2021). Moreover, compared to a wheat-based diet, diets high in RS and AX decreased pH in the caecum and proximal colon and increased large intestinal SCFA concentrations in growing pigs with the AX-rich diet causing more pronounced effects compared to RS (Nielsen et al. 2014). Additionally, AX and  $\beta$ -glucans caused lower pH values in the caecum and distal colon than a diet based on wheat starch, again with AX showing stronger effects than  $\beta$ -glucans (Gorham et al. 2017). In contrast, rapeseed meal (RSM) richer in IDF than soybean meal (SBM) did not change pH of ileal, caecal, and colonic digesta of weaner pigs (Hong et al. 2021a). Viscosity is a rheological parameter describing increased thickening of a solution and increases in digesta when soluble fibre dissolves in the liquid phase of digesta (Bach Knudsen and Laerke 2010; Brachet et al. 2015). An increased digesta viscosity may result in a decrease of nutrient digestibility and growth performance (Cervantes-Pahm et al. 2014; McGhee and Stein 2018). These effects on nutrient digestibility are associated with alterations of the mucosal histomorphological structure and a reduced mixing of digesta hampering the access and activity of digestive enzymes (McDonald et al. 2001; Ratanpaul et al. 2019). However, these detrimental effects might be attenuated by a lower rate of gastric emptying and a slower passage rate of viscous digesta (Hooda et al. 2010). In fact, a higher viscosity might lead to a delayed glucose absorption and a moderate insulin response (Ellis et al. 1995). SDF known to increase digesta viscosity are AX,  $\beta$ -glucans and pectins (Bach Knudsen et al. 2005; Dikeman and Fahey 2006; Laerke et al. 2008; Le Gall et al. 2009). Small intestinal digesta viscosity linearly increased with increasing levels of rye replacing wheat and therefore with increasing levels of soluble AX (Wilke et al. 2021). In addition to an increased digesta viscosity induced by SDF, other factors related to DF may reduce nutrient digestibility such as increased endogenous nitrogen losses, an increased passage rate and alterations of gut motility (Bartelt et al. 2002; Wilfart et al. 2007; Swiech et

al. 2012). Especially IDF e.g., derived from wheat bran, is associated with abrasive effects on the intestinal mucosa, the stimulation of the secretion of endogenous nitrogen and a faster transit of digesta through the GIT (Wilfart et al. 2007). Moreover, the rigid cell wall structure of IDF may hinder the access of digestive enzymes on nutrients such as starch granules, lipids, or protein (Tiwari et al. 2019; Williams et al. 2019). However, compared to wheat, rye and pectin both richer in SDF also lowered apparent ileal digestibility (AID) of protein in growing pigs without increasing digesta viscosity (Swiech et al. 2012).

### 2.3.3. Effects of dietary fibre on composition and diversity of the intestinal microbiome

Depending on the type and inclusion level of DF growth and diversity of intestinal microbiota may shift. Providing distinct DF sources might therefore contribute to intestinal eubiosis and animal health. Generally, IDF is expected to be fermented mainly in the distal segments of the large intestine, while fermentation of SDF may start already in the distal jejunum and ileum (Bach Knudsen et al. 2016b; Jaworski and Stein 2017). Common cereals used in pig nutrition are barley, wheat and oats containing varying amounts of AX, 1,3-1,4- $\beta$ -D-glucans, and RS (Rodehutsord et al. 2016; Agyekum and Nyachoti 2017). Moreover, protein meals such as SBM and RSM show a different fibre composition (Mejicanos et al. 2016). Other fibre sources are by-products from food and biofuel industries such as wheat bran, sugar beet pulp, soybean hulls and distillers dried grains with solubles (Li et al. 2021). Within different sources of SDF and IDF, varying effects on the microbiome may occur. The feeding of sugar beet pulp high in SDF from mainly pectin may increase growth of *Lactobacilli*, *Bifidobacteria* and *Prevotella* and reduce pathogenic *E. coli* and *Campylobacter* (Laitat et al. 2015; Li et al. 2020; Hong et al. 2021b). However, no difference of alpha-diversity was observed between sugar beet pulp and distillers dried grains with solubles or soybean hulls, both IDF sources (Li et al. 2020; Hong et al. 2021b). SDF-rich  $\beta$ -glucans from oats and barley increased *Prevotella*, *Lactobacillus*, *Mitsuokella*, *Ruminococcaceae* and *Lachnospiraceae* as well as alpha-diversity and decreased relative abundance of *Clostridium* and *Streptococcus* in the large intestine (Pieper et al. 2008; Gorham et al. 2017). AX are partially soluble and feeding of rye-based diets rich in AX resulted in increased relative abundance of ileal *Lactobacillus*, *Streptococcus* and *Bifidobacterium* and of faecal *Prevotella*, *Lactobacillus*, *Bifidobacterium* and *Olsenella* (Bach Knudsen 2014; Nielsen et al. 2014; Burbach et al. 2017). Moreover, rye containing more AX than wheat reduced the shedding of *Salmonella* in weaner pigs (Chuppava et al. 2020). The feeding of RS type 3 from tapioca increased the relative abundance of faecal *Lachnospiraceae* and *Ruminococcaceae* but decreased diversity indices (Umu Ö et al. 2015). Within IDF sources, wheat bran and pea hull fibre were shown to increase ileal *Lactobacillus* and colonic

*Bifidobacterium* counts compared to soybean hulls (Chen et al. 2013). Moreover, wheat bran reduced intestinal *E. coli* (Chen et al. 2013; Chen et al. 2017). Relative abundance of colonic *Campylobacter* was increased in pigs fed soybean hulls compared to sugar beet pulp which is considered a potential risk to human health (Hong et al. 2021b). Increased faecal microbial diversity and growth of *Bacteroidetes*, *Prevotella* and *Fibrobacter* was observed upon the feeding of IDF-rich corn bran (Liu et al. 2017).

### **2.4. Comparison of rye and rapeseed meal to wheat and soybean meal as feed ingredients**

#### 2.4.1. Growing conditions: climate, nutrient requirements, and developments in plant breeding

Rye (*Secale cereale*) is a cereal crop adapted to grow on dry, low nutrient soil and is also known for its resistance against temperatures far below 0 degrees (Miedaner 2014b). This is mainly possible due to its root mass which grows relatively deep and intense (Hoffmann 2008; Kamphues et al. 2019). Therefore, rye is more efficient in the use of water and nitrogen which is interesting with respect to increasing periods of drought and the demand for a reduced nitrogen input into the soil (Hoffmann 2008; Kamphues et al. 2019; Dougherty et al. 2020). Notably, there are also efforts to adapt wheat varieties (*Triticum aestivum*) to heat and drought stress (Semenov et al. 2014). Compared to wheat, traditional rye varieties have a lower crop yield (Michel et al. 2007; Kamphues et al. 2019). However, hybrid rye varieties yield even more than wheat (Michel et al. 2007; Kamphues et al. 2019). Shedding less pollen than population varieties, common hybrid varieties may be more susceptible to the infection with *Claviceps purpurea* which contains toxic ergot alkaloids (Miedaner and Geiger 2015; Kamphues et al. 2019). This risk was addressed by the selection for resistance genes and by the development of new hybrid rye varieties with increased pollen shedding and therefore lower risk of ergot contamination (Mirdita 2006; Miedaner and Geiger 2015). Another advantage of rye over wheat is the lower contamination level with the mycotoxins deoxynivalenol and zearalenone (Kamphues et al. 2019).

Rapeseed (*Brassica napus*) is an oil seed crop which is more adapted to European climate than soybean (*Glycine max*). In Europe, mainly the winter cultivar is grown as an annual break crop in rotation with cereals and legumes (Alford 2003). Yield losses may be caused by pests such as the cabbage root fly or foliar diseases (Alford 2003). Plant breeding has reduced the content of anti-nutritional factors and double-low-varieties containing less than 2 % erucic acid and glucosinolates below 30  $\mu\text{mol/g}$  are widely available for cultivation (Chmielewska et al. 2021).

### 2.4.2. Cultivation in Germany: acreage, yield, and import

Due to adverse weather conditions, yield of cereal crops in Germany in 2021 was considerably lower than the average per year from 2015 to 2020 (BMEL 2021). Despite being the most important crop with the highest yield per ha and covering 48 % of the total cereal acreage all over Germany, winter wheat acreage declined by 4.9 % compared to the average from 2015 to 2020 (BMEL 2021). In total, yield of winter wheat was 21 Mio. t equalling 9.0 % less than the 6-year-average (BMEL 2021). Yield per ha was lower for rye than for wheat in 2021 being 52.9 vs. 73.3 dt/ha, respectively (BMEL 2021). The acreage of rye was substantially lower compared to wheat until 2020 with the average from 2015 to 2020 being 3.11 Mio. ha for wheat and only 0.58 Mio. ha for rye (BMEL 2021). Nevertheless, the acreage and yield per ha of rye increased by 7.8 % and 1.1 %, respectively, compared to the 6-year-average (BMEL 2021). Moreover, total yield of rye was 3.3 Mio. t in 2021 which is 8.9 % more than the average (BMEL 2021). In total, the use of rye (0.7 Mio. t) as ingredient in compound feed was still low compared to wheat (4.4 Mio. t), corn (3.6 Mio. t) and barley (2.2 Mio. t) in 2018/19 (BMEL 2020).

Rapeseed is the second most important oil seed crop in Europe next to soybean (BMEL 2021). It is mainly cultivated to produce oil and acreage is constantly increasing in Europe. In Germany however, compared to the average from 2015-2020 acreage has decreased by 13.5 % and yield by 11.3 % which was mainly due to effects of drought and heavy rainfall (BMEL 2021). European acreage of soybeans is also increasing, but is still comparably low (BMEL 2021). However, the increase was lower than expected due to unfavourable climate conditions (BMEL 2021). RSM is a by-product of oil extraction and is used as a high-quality protein source in animal feed. In 2020, German production of RSM exceeded production of SBM (5.2 Mio. t vs. 2.8 Mio. t) but import of SBM was higher than of RSM (2.5 Mio. t vs. 0.9 t) (OVID 2021). Both were exported at around 2 Mio. T (OVID 2021). Import of soybeans into the European Union was stable at 14-16 Mio. t since 2005 (OVID 2021). Moreover, consumption of SBM decreased whilst consumption of RSM increased in Germany in the last decade from 4.7 to 3.3 Mio. t of SBM and 3.1 to 4.2 Mio. t RSM in 2020 (OVID 2021).

### 2.4.3. Nutritional value of rye, wheat, RSM, and SBM

With respect to the nutritional value, dry matter (DM) content and gross energy of rye and wheat are similar, whereas starch content is lower in rye (Rodehutscord et al. 2016). ME is similar in rye (13.7 MJ/kg as-fed) compared to wheat (13.8 MJ/kg as-fed) (Stein et al. 2016; McGhee and Stein 2020). Further differences occur regarding fibre composition, protein content and AA composition (Table 1 and 2, respectively). Rye contains more fibre with a

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higher proportion of the soluble fraction compared to wheat (Bach Knudsen 2014; Rodehutscord et al. 2016; McGhee and Stein 2018; McGhee and Stein 2020). Content of CP is lower in rye, but it contains a higher proportion of lysine within CP (Rodehutscord et al. 2016; Stein et al. 2016; McGhee and Stein 2018). RSM and SBM differ mainly regarding the higher concentration of CP and lysine content in SBM, as well as the higher content of fibre, especially insoluble fibre fractions in RSM (Bach Knudsen 2014; Mejicanos et al. 2016; Stein et al. 2016; Landero et al. 2018; Mejicanos et al. 2020). Moreover, ME is higher in SBM than in RSM (14.5 vs. 11.6 MJ/kg as-fed) (Stein et al. 2016). Compared to SBM, RSM contains more sulphuric AA methionine and cysteine (Mejicanos et al. 2016).

**Table 1. Concentration of dietary fibre in rye, wheat, RSM, and SBM (as-fed basis)**

Item, % <sup>1</sup>	Rye	Wheat	RSM	SBM	Reference
TDF	15.2-16.6	11.1	-	-	McGhee and Stein (2018)
	15.2-18.1	11.3	-	-	McGhee and Stein (2020)
	-	-	31.7	21.8	Mejicanos et al. (2016)
	11.7	9.8	29.7	18.3	Stein et al. (2016)
	-	-	37.56	22.17	Hong et al. (2021a)
	-	-	33.01	-	Mejicanos et al. (2020)
SDF, % TDF	10.9-21.1	4.5	-	-	McGhee and Stein (2018)
	11.2-14.9	7.1	-	-	McGhee and Stein (2020)
	-	-	9.1	8.2	Hong et al. (2021a)
NSP	14.7	11.3	22.0	21.0	Bach Knudsen (2014)
	13.9	9.8	-	-	Rodehutscord et al. (2016)
	-	-	18.0	17.8	Mejicanos et al. (2016)
	-	-	20.54	-	Mejicanos et al. (2020)
Soluble NSP, % NSP	25.6	21.7	15.5	27.6	Bach Knudsen (2014)
AX	9.5	7.3	-	-	Bach Knudsen (2014)
	8.54	6.37	-	-	Rodehutscord et al. (2016)
Soluble AX, % AX	33.7	24.7	-	-	Bach Knudsen (2014)
	36.2	21.8	-	-	Rodehutscord et al. (2016)
β-glucans	1.7	1	-	-	Bach Knudsen (2014)
	2.01	0.61	-	-	Rodehutscord et al. (2016)
	2-2.1	0.8	-	-	McGhee and Stein (2018)
NDF	14.6	12.0	-	-	Rodehutscord et al. (2016)
	13.99-15.05	11.21	-	-	McGhee and Stein (2018)
	17.38-17.94	10.74	-	-	McGhee and Stein (2020)
	-	-	26.0	12.0	Mejicanos et al. (2016)
	12.3	14.9	27.1	8.9	Stein et al. (2016)
	-	-	28.44	14.94	Hong et al. (2021a)
ADF	-	-	26.20	10.95	Mejicanos et al. (2020)
	2.96	3.14	-	-	Rodehutscord et al. (2016)

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	2.46-2.84	2.84	-	-	McGhee and Stein (2018)
	2.58-3.4-	2.79	-	-	McGhee and Stein (2020)
	-	-	18.2	7.5	Mejicanos et al. (2016)
	2.5	3.6	18.4	5.8	Stein et al. (2016)
	-	-	21.71	11.51	Hong et al. (2021a)
	-	-	20.21	6.86	Mejicanos et al. (2020)
ADL	0.858	0.783	-	-	Rodehutscord et al. (2016)
	0.61-0.76	0.82	-	-	McGhee and Stein (2018)
	0.81-1.05	0.74	-	-	McGhee and Stein (2020)
Klason lignin	2.1	1.8	13.3	1.8	Bach Knudsen (2014)

<sup>1</sup> only Bach Knudsen (2014) and Rodehutscord et al. (2016) DM basis

**Table 2. Concentration of crude protein and AA of rye, wheat, RSM, and SBM (as-fed basis)**

Item, %	Rye	Wheat	RSM	SBM	Reference
<b>Crude protein</b>	10.2	14.0	37.3	48.1	Stein et al. (2016)
	8.65-9.08	11.4	-	-	McGhee and Stein (2018)
	-	-	38.6	46	Landero et al. (2018)
	-	-	40.2	48.1	Kaewtapee et al. (2018)
<b>Indispensable AA</b>					
Arginine	0.58	0.66	2.21	3.54	Stein et al. (2016)
	0.44-0.45	0.53	-	-	McGhee and Stein (2018)
	-	-	2.24	3.30	Landero et al. (2018)
Histidine	-	-	2.45	3.52	Kaewtapee et al. (2018)
	0.24	0.35	1.04	1.31	Stein et al. (2016)
	0.21	0.26	-	-	McGhee and Stein (2018)
Isoleucine	-	-	0.96	1.35	Landero et al. (2018)
	-	-	1.04	1.23	Kaewtapee et al. (2018)
	0.35	0.49	1.45	2.21	Stein et al. (2016)
Leucine	0.32	0.41	-	-	McGhee and Stein (2018)
	-	-	1.29	2.09	Landero et al. (2018)
	-	-	1.56	2.13	Kaewtapee et al. (2018)
Lysine	0.65	0.97	2.51	3.75	Stein et al. (2016)
	0.56-0.57	0.73	-	-	McGhee and Stein (2018)
	-	-	2.59	3.55	Landero et al. (2018)
Methionine	-	-	2.79	3.59	Kaewtapee et al. (2018)
	0.40	0.41	2.07	3.01	Stein et al. (2016)
	0.36	0.37	-	-	McGhee and Stein (2018)
Phenylalanine	-	-	2.00	2.95	Landero et al. (2018)
	-	-	2.18	2.93	Kaewtapee et al. (2018)
	0.16	0.24	0.71	0.98	Stein et al. (2016)
Threonine	0.15	0.18	-	-	McGhee and Stein (2018)
	-	-	0.76	0.60	Landero et al. (2018)
	-	-	0.81	0.65	Kaewtapee et al. (2018)
Tryptophan	0.48	0.68	1.48	2.45	Stein et al. (2016)
	0.42-0.44	0.50	-	-	McGhee and Stein (2018)
	-	-	1.45	2.27	Landero et al. (2018)
Valine	-	-	1.65	2.40	Kaewtapee et al. (2018)
	0.34	0.42	1.55	1.84	Stein et al. (2016)
	0.29-0.30	0.33	-	-	McGhee and Stein (2018)



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	-	-	1.61	1.76	Landero et al. (2018)
	-	-	1.80	1.88	Kaewtapee et al. (2018)
Tryptophane	0.10	0.17	0.43	0.68	Stein et al. (2016)
	0.08-0.10	0.11	-	-	McGhee and Stein (2018)
	-	-	0.56	0.66	Kaewtapee et al. (2018)
	-	-	0.43	0.62	Landero et al. (2018)
Valine	0.49	0.62	1.82	2.28	Stein et al. (2016)
	0.43-0.44	0.51	-	-	McGhee and Stein (2018)
	-	-	1.71	2.18	Landero et al. (2018)
<b>Dispensable AA</b>					
Alanine	0.43	0.51	1.60	2.15	Stein et al. (2016)
	0.38-0.39	0.43	-	-	McGhee and Stein (2018)
	-	-	1.71	1.99	Kaewtapee et al. (2018)
Aspartic acid	0.74	0.76	2.59	5.48	Stein et al. (2016)
	0.63	0.63	-	-	McGhee and Stein (2018)
	-	-	3.00	5.43	Kaewtapee et al. (2018)
Cysteine	0.20	0.34	0.85	0.71	Stein et al. (2016)
	0.20-0.21	0.27	-	-	McGhee and Stein (2018)
	-	-	0.91	0.68	Kaewtapee et al. (2018)
Glutamic acid	2.47	4.23	6.22	8.58	Stein et al. (2016)
	1.99-2.12	2.95	-	-	McGhee and Stein (2018)
	-	-	6.83	8.50	Kaewtapee et al. (2018)
Glycine	0.45	0.59	1.80	2.03	Stein et al. (2016)
	0.39-0.40	0.48	-	-	McGhee and Stein (2018)
	-	-	1.96	1.99	Kaewtapee et al. (2018)
Proline	1.16	1.48	2.18	2.50	Stein et al. (2016)
	0.88-0.94	1.00	-	-	McGhee and Stein (2018)
	-	-	2.31	2.24	Kaewtapee et al. (2018)
Serine	0.42	0.66	1.41	2.34	Stein et al. (2016)
	0.36-0.37	0.45	-	-	McGhee and Stein (2018)
	-	-	1.74	2.41	Kaewtapee et al. (2018)
Tyrosine	0.24	0.37	1.06	1.70	Stein et al. (2016)
	0.19-0.20	0.26	-	-	McGhee and Stein (2018)

### 2.4.4. Anti-nutritional factors of rye and rapeseed

The feeding of rye was limited in the past mainly due to the idea of low palatability and the high content of NSP and trypsin inhibitors (Schwarz et al. 2015). Scientific evidence on rye reducing growth performance and feed intake is scarce but was attributed to the presence of alkylresorcinols which are concentrated higher in rye than in other cereals (Schwarz et al. 2015). The high content of NSP was considered detrimental primarily due their ability to reduce pre-caecal nutrient digestibility and feed intake by creating a false sensation of satiety (Misir and Marquardt 1978; Schwarz et al. 2015). However, the positive effects of a high hindgut fermentability of NSP described above were not considered. Cereal trypsin inhibitors can reduce activity of digestive enzymes and are present in rye as well as in wheat (Franco et al. 2002). Moreover, oral uptake of alpha-amylase trypsin inhibitors from wheat was associated with allergic and inflammatory immune responses in humans but has not been studied in pigs

or in comparison to trypsin inhibitors from rye yet (Zevallos et al. 2017; Bellinghausen et al. 2019).

The primary components of RSM considered as detrimental to the nutritional value are fibre, glucosinolates, phytic acid, tannins, and sinapine (Mejicanos et al. 2016). The high content of fibre, especially compared to SBM is considered to lower the levels of ME and to decrease nutrient digestibility. Therefore, some studies investigated the effects of dehulled RSM which considerably lowers the content of IDF and increases CP and AA content (Danielsen et al. 1994; Kracht et al. 2004; Mejicanos and Nyachoti 2018). Dehulling resulted in varying effects on ileal digestibility of AA being either slightly increased or not changed (Kracht et al. 2004; Mejicanos and Nyachoti 2018). However, as discussed above, the feeding of DF may also hold benefits for animal health and dehulling may also increase the concentration of other ANF such as glucosinolates and sinapine (Kracht et al. 2004). The content of glucosinolates is below 30 µg/g in double-zero-varieties of rapeseed. This level should not be exceeded due to the toxicity of glucosinolate degradation products such as isothiocyanates, thiocyanates and nitriles (Lee et al. 2020). Isothiocyanates and thiocyanates can impair function of the thyroid gland by hindering iodine uptake which leads to a lower production of thyroid hormones and hypothyroidism (Lee et al. 2020). Nitriles may be detrimental to liver function (Lee et al. 2020). Previous suggestions of a decreasing effect of glucosinolates on ME value of RSM were not confirmed in a recent study with growing pigs (Tripathi and Mishra 2007; Zhang et al. 2020). 36-70 % of phosphorus in RSM is bound to phytic acid (Mejicanos et al. 2016). Phytic acid binds to cations such as Zn, Ca, and Fe as well as to digestive enzymes and reduces their bioavailability and activity, respectively (Woyengo and Nyachoti 2013; Mejicanos et al. 2016). The addition of phytase to pigs' diet may reduce the negative effects of phytate (Woyengo and Nyachoti 2013). Sinapine is a phenolic compound contributing to the bitter taste of RSM which may lower voluntary feed intake (Mejicanos et al. 2016). Plant breeding efforts may reduce levels of sinapine in rapeseed grains by 71 % (Emrani et al. 2015). In comparison to SBM, RSM does not contain trypsin inhibitors (Chmielewska et al. 2021). Rapeseed storage proteins napin and cruciferin have been associated with cross-reactivity to other *Brassica*-proteins in humans but not with an immunomodulatory or allergenic potential comparable to glycinin and β-conglycinin from SBM (He et al. 2015; Radcliffe et al. 2019; Chmielewska et al. 2021). However, effects of napin and cruciferin have not been studied in pigs, whilst soy proteins may hold immunomodulatory capacities in pigs (Radcliffe et al. 2019).

### 2.4.5. Recommended inclusion levels

Due to the idea of low palatability, the perception of NSP as anti-nutritive components and the risk of ergot contamination, the recommended inclusion level of rye was limited to 10 % for piglets up to 15 kg BW, 20 % for piglets with minimum 15 kg BW and 30-50 % for growers from 28 kg BW (LfL-Bayern 2019). In contrast, the recommendations for wheat are up to 50 % for weaner and grower pigs (LfL-Bayern 2019). Taking into account anti-nutritional glucosinolates dietary inclusion of RSM is recommended to be maximum 10 % for weaner pigs and 15 % for growers (LfL-Bayern 2019). SBM inclusion is recommended to be up to 20 % in weaner and grower diets (LfL-Bayern 2019).

**3. Part I: Effects of cereal and protein source on performance, apparent ileal protein digestibility and intestinal characteristics in weaner piglets**

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## Article

# Effects of Dietary Cereal and Protein Source on Fiber Digestibility, Composition, and Metabolic Activity of the Intestinal Microbiota in Weaner Piglets

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**Simple Summary:** Rye and rapeseed meal can be alternative feed components for weaner piglets instead of wheat and soybean meal. Both components can help to meet current challenges in pig nutrition, such as increasingly dry weather conditions and the high amount of imported soybean. Since they contain more and differently composed fiber, effects on digestive physiology and intestinal microbiota might help to maintain gut health and prevent post-weaning diarrhea. This study shows that despite a similar composition of the large intestinal microbiota, the higher amount and solubility of complex carbohydrates from rye lead to a higher fermentative activity compared to wheat, which is considered a beneficial effect. The high amount of insoluble dietary fiber in rapeseed-based diets lowered bacterial metabolic activity and caused a shift toward insoluble fiber degrading bacteria.

**Abstract:** This study aimed to investigate the effect of fiber-rich rye and rapeseed meal (RSM) compared to wheat and soybean meal (SBM) on fiber digestibility and the composition and metabolic activity of intestinal microbiota. At weaning, 88 piglets were allocated to four feeding groups: wheat/SBM, wheat/RSM, rye/SBM, and rye/RSM. Dietary inclusion level was 48% for rye and wheat, 25% for SBM, and 30% for RSM. Piglets were euthanized after 33 days for collection of digesta and feces. Samples were analyzed for dry matter and non-starch-polysaccharide (NSP) digestibility, bacterial metabolites, and relative abundance of microbiota. Rye-based diets had higher concentrations of soluble NSP than wheat-based diets. RSM-diets were higher in insoluble NSP compared to SBM. Rye-fed piglets showed a higher colonic and fecal digestibility of NSP ( $p < 0.001$ ,  $p = 0.001$ , respectively). RSM-fed piglets showed a lower colonic and fecal digestibility of NSP than SBM-fed piglets ( $p < 0.001$ ). Rye increased jejunal and colonic concentration of short-chain fatty acids (SCFA) compared to wheat ( $p < 0.001$ ,  $p = 0.016$ , respectively). RSM-fed pigs showed a lower jejunal concentration of SCFA ( $p = 0.001$ ) than SBM-fed pigs. Relative abundance of *Firmicutes* was higher ( $p = 0.039$ ) and of *Proteobacteria* lower ( $p = 0.002$ ) in rye-fed pigs compared to wheat. RSM reduced *Firmicutes* and increased *Actinobacteria* (jejunum, colon, feces:  $p < 0.050$ ), jejunal *Proteobacteria* ( $p = 0.019$ ) and colonic *Bacteroidetes* ( $p = 0.014$ ). Despite a similar composition of the colonic microbiota, the higher amount and solubility of NSP from rye resulted in an increased fermentative activity compared to wheat. The high amount of insoluble dietary fiber in RSM-based diets reduced bacterial metabolic activity and caused a shift toward insoluble fiber degrading bacteria. Further research should focus on host–microbiota interaction to improve feeding concepts with a targeted use of dietary fiber.

**Keywords:** cereal; dietary fiber; microbiome; nutrient digestibility; pigs; protein source; rapeseed; rye; soybean; wheat



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## 1. Introduction

An important approach to stabilize gut health of weaner pigs is the optimization of intestinal microbial colonization in the sense of intestinal eubiosis and beneficial bacterial

metabolic activity [1]. Here, the inclusion of dietary fiber is a promising strategy. Dietary fiber influences digestion and fermentation processes and many sources of dietary fiber were shown to increase the potentially beneficial microbiota, to reduce pathogens, and to improve intestinal barrier function [2–5]. Non-starch-polysaccharides (NSP) are the major component of dietary fiber. They are not digestible in the small intestine but are fermented in the upper and lower intestinal tract by the resident microbiota [6]. Fermentation products of intestinal bacteria can have different impact on the microbial community and on the host. Proteolytic bacterial activities produce potentially harmful metabolites such as ammonia and other degradation products [7]. Beneficial metabolites are short-chain fatty acids (SCFA) and lactic acid. They are products of substrate fermentation and may hamper the growth of pathogens and provide energy for beneficial bacteria [8]. Acetic, propionic, and butyric acid are the predominant forms of SCFA. After absorption, they serve as an energy substrate for the pig [9]. Moreover, butyric acid is the main energy source of the colonic epithelial cells [10]. Most intestinal bacteria preferably ferment carbohydrates including NSP, which implies that diets containing high amounts of complex structured NSP might improve gut health by promoting growth of fermentative bacteria and providing beneficial metabolites to the host. Dietary fiber may also influence the integrity of the epithelial mucus layer and on mucus production [11]. *N*-acetylneuraminic acid is a sialic acid present in mucin molecules and can be considered a marker for mucus [12], but data on its intestinal concentration in pigs fed different fiber sources are scarce.

Rye and rapeseed meal (RSM) are fiber-rich feed components and interesting alternatives to wheat and soybean meal (SBM). Rye is not used much in pig nutrition due to the idea of low palatability, its high content of fiber and its susceptibility to the infection with *Claviceps purpurea* [13]. However, recent studies investigating the feeding of rye instead of wheat at dietary inclusion up to 69% showed no reduction of feed intake and growth performance [14,15]. This might be related to the use SCFA from large intestinal fiber fermentation as an energy substrate [16]. Moreover, increasing pollen fertility and selection of resistance genes lowered the risk of ergot infections in rye [17]. Generally, wheat and rye have a similar concentration of carbohydrates, but rye has a higher concentration of total dietary fiber (TDF) and fermentable fractions [16]. The content of total NSP, soluble NSP (sNSP), arabinoxylans (AX) and  $\beta$ -glucans is higher in rye compared to wheat [18,19]. Thus, rye increases intestinal butyrate production compared to wheat [20,21] and helps to prevent *Salmonella* infection in weaner pigs [15]. Moreover, rye might be effective against enterotoxigenic *E. coli* (ETEC) colonization [5] and promote the growth of *Bifidobacterium* and *Prevotella* spp., both important lactic acid-producing bacteria. Lactic acid is considered to inhibit growth of pathogens [22]. A high abundance of proteolytic *Clostridium* can impair gut health and might be reduced by increasing dietary AX and  $\beta$ -glucans [23]. Consequently, replacing wheat by rye might induce a favourable shift in the large intestinal microbiome by providing more readily fermentable substrate and thereby promoting the production of useful metabolites.

Next to SBM, RSM is the second most important protein meal in pig nutrition in Europe. RSM and SBM contain similar amounts of NSP, but RSM has less soluble NSP and more lignin, total, and soluble AX [18]. Insoluble fiber helps to maintain normal gut function but might decrease feed intake and nutrient digestibility [9]. Very little research has been done on the fermentability of RSM carbohydrates. However, some studies showed that RSM might increase relative abundance of SCFA producing genera in the hindgut and decrease major proteolytic bacteria compared to SBM [24,25].

Providing non-digestible fiber substrates using rye and RSM in piglet diets could support gut health. Knowledge of the effects of feeding rye in combination with RSM is scarce. For the present study, we hypothesized that rye and RSM would increase microbial fiber fermentation and SCFA production in the large intestine compared to wheat and SBM and cause a shift in the microbial community toward potentially beneficial bacteria. The aim of this study was to investigate the effect of diets containing either rye or wheat as a cereal combined with either RSM or SBM as a protein meal on fiber digestibility, on the

mucus marker *N*-acetylneuraminic acid, as well as on composition and metabolic activity of intestinal microbiota.

## 2. Materials and Methods

### 2.1. Animals and Diets

The animal study was described in detail previously [14]. In brief, 88 piglets (German Landrace, bred at the Institute of Animal Nutrition, Freie Universität Berlin,  $8.3 \pm 1.1$  kg body weight) were weaned at day 28 of life and randomly allocated to four feeding groups in a  $2 \times 2$  factorial design with wheat/SBM (W-SBM), wheat/RSM (W-RSM), rye/SBM (R-SBM), and rye/RSM (R-RSM). Piglets were housed with two animals per pen and 11 pens per treatment. The pen was used as statistical unit ( $n = 11$ ). The health status was monitored daily by controlling general condition, feed intake, and fecal consistency of the piglets. Water and feed were supplied ad libitum during the experimental period of 33 days. The diets were formulated to meet or exceed the recommendations for piglet nutrition [26] and calculated to be isonitrogenous. Wheat (*Triticum aestivum*) or hybrid rye (*Secale cereale*) were included as cereals at 48 and 25% of SBM or 30% of RSM as protein rich ingredient. The average concentration of crude protein in the complete diet was 220 g/kg. Crude fiber varied among the groups with 22 g/kg in W-SBM, 52 g/kg in W-RSM, 18 g/kg in R-SBM, and 48 g/kg in R-RSM. The exact feed formulation, results of the nutrient analysis, and digestibility determinations are available elsewhere [14].

### 2.2. Sampling

One piglet per pen was dissected after 33 days of trial duration. Pigs were chosen to achieve balanced numbers of males and females in each treatment group. After anaesthesia by 20 mg of ketamine hydrochloride (Ursotamin, 10%; Serumwerk Bernburg AG, Bernburg, Germany) and 2 mg of xylazine (Xylazin, 2%, Serumwerk Bernburg AG, Bernburg, Germany) per kg body weight, pigs were euthanized with an intracardial injection of tetracaine hydrochloride, mebezonium iodide, and embutramide (T61; Intervet, Unterschleißheim, Germany). The gastrointestinal tract was removed and digesta samples were collected from jejunum, ileum, colon ascendens, and rectum. From each intestinal segment, one subset of digesta samples was stored at  $-20$  °C for analysis of dry matter (DM), fiber and *N*-acetylneuraminic acid. Another subset was snap frozen with liquid nitrogen and stored at  $-80$  °C for analysis of bacterial metabolites and 16S rRNA sequencing.

### 2.3. Determination of Digesta Content and Apparent Digestibility of DM and NSP

Digesta samples from jejunum, colon ascendens, and feces were dried in an oven at 103 °C overnight to determine the DM content. Colonic and fecal samples were lyophilized and total and insoluble NSP content was measured as described for NSP in the diets [14]. To determine apparent digestibility of DM and NSP, titanium dioxide content was determined as described before [27] in lyophilized and grinded (0.5 mm particle size) digesta, feces, and in the diets. Apparent digestibility of DM and NSP was calculated as follows [28]:

$$\text{Digestibility (\%)} = 100 - (\text{TiO}_2 \text{ in feed (\%)} / (\text{TiO}_2 \text{ in digesta/feces (\%)})) \times (\text{Nutrient in digesta/feces (\%)} / (\text{Nutrient in feed (\%)})) \times 100$$

Apparent digestibility determined in feces was further considered as apparent total tract digestibility (ATTD).

### 2.4. Analysis of *N*-Acetylneuraminic Acid as Marker of Intestinal Mucus Production

Lyophilized and grinded samples of ileal digesta were hydrolyzed with acetic acid (2 mol/L). After centrifugation, the supernatant was analyzed for *N*-acetylneuraminic acid via HPIC with an amperometrically pulsed detector cell (Thermo Fisher Scientific, Waltham, MA, USA) using a commercial standard of *N*-acetylneuraminic acid as reference substance (Sigma–Aldrich, Taufkirchen, Germany).



### 2.5. Analysis of Bacterial Metabolites

In jejunal and colonic digesta samples and feces, concentration of SCFA, D- and L-lactate, and ammonia was analyzed as described before [29]. Briefly, SCFA were determined via gas chromatography (Agilent Technologies 6890N, autosampler G2614A, and injection tower G2613A; Network GC Systems, Böblingen, Germany) using caproic acid as an internal standard. D- and L-lactate were measured by HPLC (Agilent 1100; Agilent Technologies, Böblingen, Germany). Ammonia was analyzed colorimetrically using the Berthelot reaction in microtitration plates at 620 nm in a Tecan Sunrise microplate reader (Tecan Austria GmbH, Grödig, Austria).

### 2.6. DNA Extraction and 16S rRNA Sequencing

To extract total DNA from 0.25 g of jejunal and colonic digesta and feces, a commercial extraction kit (QIAamp PowerFaecal Pro DNA Kit, Qiagen, Hilden, Germany) was used in accordance with the manufacturer's instructions with an additional lysis step at 65 °C. Homogenization was carried out using FastPrep-24™ 5G (M.P. Biomedicals LLC, Santa Ana, CA, USA) at 6 m/s for 10 min (4 times 5 × 30 s and 15 s pause time). DNA extracts were stored at −30 °C until further analysis. Extracts were subjected to amplicon sequencing using an Illumina NextSeq500 sequencer (LGC, Berlin, Germany) with two 150–base pair reads. After demultiplexing, BBMerge tool [30] was used for combining paired reads. Resulting 16S rDNA sequences were analyzed using QIIME2 pipeline [31] and the SILVA SSU database [32]. Quality control and determination of sequence counts were performed using the DADA2 [33]. Further details were described previously [34]. Indices of bacterial diversity (Richness, Shannon index, and Evenness) were calculated from ASV level data. Principal component analysis of 16S rRNA data was carried out using the online software ClustVis [35].

### 2.7. Statistical Analysis

Statistical analyses were carried out in SPSS 26.0 (IBM, Chicago, IL, USA). The distribution of data was tested by Kolmogorov–Smirnov test. Normally distributed data were analyzed by 2-factorial ANOVA with cereal (CER) and protein meal (PM) as fixed factors. Group differences were assessed by post hoc Tukey test. *p*-values below 0.05 were considered significant. Pearson's correlation was analyzed between colonic *Bacteroidetes* and average daily gain (ADG) and between jejunal *Proteobacteria* and apparent ileal digestibility (AID) of crude protein and total amino acids.

## 3. Results

Results of growth performance and fecal score were described in detail previously [14]. In brief, average daily gain, average daily feed intake, feed conversion ratio and final body weight were not influenced by the feeding of rye compared to wheat. RSM in comparison to SBM reduced average daily gain and average daily feed intake in the overall trial period. Fecal score was within the physiological range throughout the trial.

### 3.1. NSP Concentration in Diets

The four experimental diets contained more iNSP (insoluble non-starch-polysaccharides, 76.9–93.0 g/kg) than sNSP (12.8–34.6 g/kg) with glucose (27.0–48.2 g/kg), xylose (24.2–30.6 g/kg), and arabinose (18.2–30.5 g/kg) being the predominant sugars in the NSP fractions (Table 1). The two rye-based diets had higher concentrations of soluble dietary fiber (SDF) and sNSP than the wheat-based diets (47 and 118% more, respectively). RSM-diets were higher in iNSP and the A/X-ratio was higher compared to SBM-diets (15 and 35% more, respectively).

**Table 1.** Analyzed content of DM and dietary fiber of the experimental diets.

Item, g/kg (as-Fed Basis)	Diet			
	W-SBM	W-RSM	R-SBM	R-RSM
DM	931	937	931	928
NDF	154	153	163	141
ADF	26.3	74.1	23.0	70.5
ADL	2.2	28.2	4.3	24.9
NSP				
Total	89.6 (12.8) <sup>1</sup>	102 (14.2)	104 (24.2)	128 (34.6)
Fucose	0.86 (0.03)	0.82 (0.02)	0.99 (0.01)	0.91 (0.08)
Rhamnose	0.79 (0.07)	0.81 (0.09)	0.76 (0.11)	1.00 (0.13)
Arabinose	18.2 (2.02)	25.1 (2.82)	21.3 (4.46)	30.5 (7.67)
Galactose	13.1 (2.13)	8.20 (1.82)	14.1 (2.38)	7.72 (0.90)
Glucose	27.0 (0.86)	38.0 (2.50)	30.7 (5.35)	48.2 (11.8)
Mannose	3.43 (2.17)	2.83 (1.90)	4.81 (4.03)	5.21 (3.22)
Xylose	24.2 (5.18)	23.9 (4.92)	27.5 (7.13)	30.6 (10.8)
Galacturonic acid	1.90 (0.26)	1.71 (0.08)	4.00 (0.65)	3.39 (0.08)
Glucuronic acid	0.18 (0.06)	0.18 (0.05)	0.20 (0.05)	0.14 (0.03)
Total AX <sup>2</sup>	42.4 (7.2)	49.0 (7.7)	48.9 (11.6)	61.1 (18.5)
Ratio A/X <sup>3</sup>	0.75	1.05	0.77	1.00
TDF	135	164	152	188
SDF <sup>4</sup>	27.6	27.1	37.1	42.4
IDF	108	137	115	146

W-SBM, wheat/soybean meal; W-RSM, wheat/rapeseed meal; R-SBM, rye/soybean meal; R-RSM, rye/rapeseed meal; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; NSP, non-starch-polysaccharides; AX, arabinoxylans; TDF: total dietary fiber; SDF, soluble dietary fiber; IDF, insoluble dietary fiber. <sup>1</sup> Values in parentheses are concentration of soluble fraction: total concentration of each component–concentration of the respective insoluble fraction. <sup>2</sup> Arabinose + xylose. <sup>3</sup> A/X: Arabinose/Xylose. <sup>4</sup> SDF, soluble dietary fiber: TDF–IDF.

### 3.2. Apparent Digestibility of DM and NSP

Fecal DM digestibility was lower in rye-fed piglets than pigs receiving wheat-based diets ( $p = 0.035$ , Table 2). DM digestibility was lower in RSM-pigs than in SBM-pigs in the jejunum, colon and feces (each  $p < 0.001$ ).

Colonic NSP digestibility (70.7–88.2%) was higher than fecal NSP digestibility (59.5–74.8%) across the four feeding groups. Both sites showed a higher digestibility of soluble than of insoluble NSP and AX. Rye-fed piglets showed a higher colonic and fecal digestibility of NSP, sNSP, glucose and soluble AX (colon: each  $p < 0.001$ ; feces:  $p = 0.001$ ,  $p < 0.001$ ,  $p < 0.001$ ,  $p = 0.005$ , respectively). Compared to SBM, RSM-fed piglets showed a lower colonic and fecal digestibility of NSP and iNSP (each  $p < 0.001$ ). Fecal digestibility of iNSP was highest in R-SBM-fed pigs and lowest in R-RSM-fed pigs ( $p < 0.001$ ). Fecal digestibility of insoluble AX was highest in W-RSM and lowest in R-RSM-fed piglets ( $p = 0.009$ ).

### 3.3. Concentration of N-Acetylneuraminic Acid in the Ileum Digesta

Ileal digesta concentration of N-acetylneuraminic acid and ratio of N-acetylneuraminic acid to titanium dioxide were measured to estimate mucus production in the small intestine. Nor concentrations neither the ratio were affected by the dietary treatments ( $p > 0.05$ , Table 3).

### 3.4. Bacterial Metabolites

Concentration of SCFA was highest in colonic digesta, followed by feces and jejunal digesta (Table 4). Acetic acid was the predominant fraction of SCFA followed by butyric acid in jejunal digesta and by propionic acid in colonic digesta and feces. In comparison to wheat, rye increased concentration of SCFA ( $p < 0.001$ ), acetic acid ( $p < 0.001$ ), and propionic acid ( $p = 0.024$ ) in the jejunum and of SCFA ( $p = 0.016$ ), acetic acid ( $p = 0.014$ ), propionic acid ( $p = 0.034$ ), and i-butyric acid ( $p = 0.035$ ) in the colon. RSM-fed pigs showed a lower concentration of SCFA ( $p = 0.001$ ), acetic acid ( $p = 0.002$ ), and n-butyric acid ( $p = 0.010$ ).

in the jejunum and a lower colonic concentration of acetic acid ( $p = 0.049$ ), i-butyric acid ( $p = 0.001$ ), n-butyric acid ( $p = 0.041$ ), and i-valeric acid ( $p = 0.002$ ) than SBM-pigs.

**Table 2.** Effects of the experimental diets on apparent digestibility of DM and NSP in weaned piglets <sup>1</sup>.

Digestibility, %	Diet				SEM	CER	p-Value	
	W-SBM	W-RSM	R-SBM	R-RSM			PM	CER * PM
Jejunum								
DM	73.3	53.1	70.6	57.4	1.9	0.787	<0.001	0.246
Colon ascendens								
DM	86.7	80.0	85.4	78.4	0.8	0.224	<0.001	0.925
NSP								
Total	70.4	64.5	77.4	71.1	1.0	<0.001	<0.001	0.903
Fucose	81.8	67.8	79.0	64.4	1.7	0.241	<0.001	0.890
Rhamnose	55.0	26.5	53.9	37.8	4.7	0.573	0.018	0.495
Arabinose	69.5	72.9	74.2	75.3	0.7	0.011	0.097	0.375
Galactose	92.0	70.1	89.5	64.1	2.1	0.043	<0.001	0.394
Glucose	62.0	61.3	75.3	72.1	1.2	<0.001	0.222	0.448
Mannose	74.8 <sup>a</sup>	48.8 <sup>b</sup>	78.7 <sup>a</sup>	67.6 <sup>a</sup>	2.3	0.001	<0.001	0.020
Xylose	70.0	71.1	76.9	76.7	0.9	<0.001	0.757	0.639
Galacturonic acid	83.1 <sup>a</sup>	−29.7 <sup>c</sup>	91.7 <sup>a</sup>	31.3 <sup>b</sup>	7.6	<0.001	<0.001	<0.001
Glucuronic acid	47.3 <sup>b</sup>	40.6 <sup>b</sup>	72.3 <sup>a</sup>	42.0 <sup>b</sup>	2.6	0.001	<0.001	0.002
Soluble NSP <sup>2</sup>	70.7	71.4	86.3	88.2	1.8	<0.001	0.624	0.821
Insoluble NSP	70.4	63.4	74.8	64.7	1.1	0.116	<0.001	0.405
Total AX	69.8	72.1	75.7	76.0	0.8	0.001	0.370	0.467
Soluble AX <sup>3</sup>	81.9	88.3	94.5	96.2	1.5	<0.001	0.130	0.362
Insoluble AX	67.3	69.0	69.9	67.2	0.8	0.807	0.783	0.189
Ratio A/X <sup>4</sup>	0.82	1.05	0.94	1.14	0.02	<0.001	<0.001	0.640
Feces <sup>5</sup>								
DM	87.7	80.7	87.1	77.8	0.7	0.035	<0.001	0.160
NSP								
Total	65.3	59.5	74.8	62.5	1.2	0.001	<0.001	0.073
Fucose	80.9	64.0	74.0	56.7	1.9	0.011	<0.001	0.948
Rhamnose	41.5	1.0	25.4	0.5	3.7	0.129	<0.001	0.155
Arabinose	60.5 <sup>b</sup>	68.1 <sup>a</sup>	67.7 <sup>a</sup>	67.1 <sup>a,b</sup>	1.0	0.096	0.058	0.027
Galactose	90.6 <sup>a</sup>	67.6 <sup>b</sup>	87.4 <sup>a</sup>	51.3 <sup>c</sup>	2.5	<0.001	<0.001	<0.001
Glucose	51.1 <sup>b</sup>	51.2 <sup>b</sup>	71.4 <sup>a</sup>	59.4 <sup>b</sup>	1.7	<0.001	0.018	0.017
Mannose	83.7 <sup>a</sup>	57.4 <sup>c</sup>	87.1 <sup>a</sup>	74.3 <sup>b</sup>	2.0	<0.001	<0.001	0.002
Xylose	66.7	69.2	73.4	70.3	1.0	0.040	0.865	0.131
Galacturonic acid	85.5 <sup>a</sup>	−45.2 <sup>c</sup>	93.4 <sup>a</sup>	11.7 <sup>b</sup>	9.1	<0.001	<0.001	<0.001
Glucuronic acid	30.8 <sup>b</sup>	29.9 <sup>b</sup>	66.3 <sup>a</sup>	28.5 <sup>b</sup>	2.9	<0.001	<0.001	<0.001
Soluble	71.4	54.9	85.8	79.7	2.5	<0.001	0.004	0.162
Insoluble	64.1 <sup>b</sup>	59.9 <sup>bc</sup>	71.2 <sup>a</sup>	55.7 <sup>c</sup>	1.2	0.413	<0.001	0.003
Total AX	64.0	68.7	70.9	68.7	0.9	0.060	0.508	0.061
Soluble AX	81.5	81.9	90.9	93.2	1.8	0.005	0.686	0.779
Insoluble AX	60.4 <sup>a,b</sup>	66.2 <sup>a</sup>	64.7 <sup>a,b</sup>	58.0 <sup>b</sup>	1.0	0.282	0.788	0.001
Ratio A/X	0.95	1.18	1.01	1.19	0.02	0.143	<0.001	0.367

W-SBM, wheat/soybean meal; W-RSM, wheat/rapeseed meal; R-SBM, rye/soybean meal; R-RSM, rye/rapeseed meal; SEM, standard error of the mean; CER, cereal; PM, protein meal; DM, dry matter; NSP, non-starch-polysaccharides; AX, arabinoxylans. <sup>1</sup> Data are presented as means ( $n = 11$ ); p-values indicate effects of the factors cereal (CER), protein meal (PM) and their interaction (CER \* PM). <sup>2</sup> Soluble NSP: total NSP−insoluble NSP. <sup>3</sup> Soluble AX, total AX−insoluble AX. <sup>4</sup> A/X, ratio of concentration of arabinose and xylose in digesta or feces. <sup>5</sup> R-SBM:  $n = 10$  (lack of collectable feces). <sup>a, b, c</sup> Values within a row with different superscripts differ significantly at  $p \leq 0.05$  (Tukey test).

**Table 3.** Effects of the experimental diets on *N*-acetylneuraminic acid concentration in ileal digesta <sup>1</sup>.

Item	Diet					<i>p</i> -Value		
	W-SBM	W-RSM	R-SBM	R-RSM	SEM	CER	PM	CER * PM
Neu5Ac, g/kg DM	1.15	1.41	1.25	1.47	0.11	0.702	0.294	0.932
Ratio Neu5Ac/TiO2	11.7	21.3	14.1	21.6	2.3	0.761	0.070	0.813

W-SBM, wheat/soybean meal; W-RSM, wheat/rapeseed meal; R-SBM, rye/soybean meal; R-RSM, rye/rapeseed meal; SEM, standard error of the mean; CER, cereal; PM, protein meal; Neu5Ac, *N*-acetylneuraminic acid; DM, dry matter. <sup>1</sup> Data are presented as means (W-SBM, W-RSM, R-SBM: *n* = 7; R-RSM: *n* = 6, lack of collectable digesta); *p*-values indicate effects of the factors cereal (CER), protein meal (PM) and their interaction (CER \* PM).

**Table 4.** Effects of the experimental diets on bacterial metabolites in digesta and feces of weaned piglets <sup>1</sup>.

Concentration, $\mu$ mol/g DM	Diet					<i>p</i> -Value		
	W-SBM	W-RSM	R-SBM	R-RSM	SEM	CER	PM	CER * PM
Jejunum								
SCFA	89.6	30.8	192	104	13.6	<0.001	0.001	0.511
Acetic acid	79.0	27.8	172	96.1	12.1	<0.001	0.002	0.517
Propionic acid	0.47	0.68	3.60	1.26	0.43	0.024	0.188	0.115
<i>i</i> -butyric acid	1.33	0.94	1.42	1.33	0.11	0.288	0.286	0.509
<i>n</i> -butyric acid	8.43	1.12	14.0	5.51	1.58	0.097	0.010	0.845
<i>i</i> -valeric acid	0.08	0.00	0.27	0.03	0.05	0.195	0.072	0.353
<i>n</i> -valeric acid	0.24	0.28	0.36	0.28	0.03	0.351	0.709	0.342
Ammonia	37.9	29.7	53.7	37.8	3.4	0.073	0.070	0.551
L-lactate	177	213	303	133	32	0.717	0.295	0.112
D-lactate	5.64	9.83	8.92	7.15	2.04	0.944	0.775	0.483
Colon ascendens								
SCFA	990	922	1316	1040	48	0.016	0.057	0.244
Acetic acid	536	489	743	566	30	0.014	0.049	0.243
Propionic acid	251	236	314	280	12	0.034	0.323	0.706
<i>i</i> -butyric acid	11.0	9.56	14.2	9.78	0.48	0.036	0.001	0.064
<i>n</i> -butyric acid	153	141	191	143	7	0.172	0.041	0.197
<i>i</i> -valeric acid	10.9	8.58	13.9	7.61	0.74	0.430	0.002	0.133
<i>n</i> -valeric acid	28.9	37.0	39.9	33.7	2.3	0.402	0.836	0.124
Ammonia	51.5	52.9	65.5	41.5	3.6	0.849	0.116	0.078
L-lactate	22.4	34.1	40.8	28.4	3.9	0.425	0.965	0.133
D-lactate	7.69	11.0	9.17	7.80	1.31	0.754	0.723	0.390
Feces <sup>2</sup>								
SCFA	592	529	688	571	34	0.308	0.187	0.694
Acetic acid	283	257	358	291	17	0.112	0.171	0.537
Propionic acid	154	133	163	140	9	0.642	0.236	0.972
<i>i</i> -butyric acid	15.1	15.9	21.6	15.6	1.0	0.106	0.168	0.079
<i>n</i> -butyric acid	97.1	80.8	93.2	81.3	6.5	0.898	0.296	0.870
<i>i</i> -valeric acid	21.0	21.5	31.0	21.5	1.4	0.065	0.094	0.063
<i>n</i> -valeric acid	22.7	20.8	21.9	22.0	1.3	0.943	0.755	0.711
Ammonia	78.4	84.5	86.0	78.1	5.6	0.958	0.939	0.552
L-lactate	12.0	19.2	12.9	15.5	2.0	0.714	0.223	0.562
D-lactate	6.42	11.7	6.45	10.0	1.45	0.778	0.135	0.770

DM, dry matter; W-SBM, wheat/soybean meal; W-RSM, wheat/rapeseed meal; R-SBM, rye/soybean meal; R-RSM, rye/rapeseed meal; SEM, standard error of the mean; CER, cereal; PM, protein meal; SCFA, short-chain fatty acids; <sup>1</sup> Data are presented as means (*n* = 11); *p*-values indicate effects of the factors cereal (CER), protein meal (PM), and their interaction (CER \* PM). <sup>2</sup> R-SBM: *n* = 10 (lack of collectable feces).

In caudal direction, ammonia increased, and L-lactate decreased. Ammonia, D-, and L-lactate were not affected by the dietary treatments (*p* > 0.05).

### 3.5. Microbial Diversity Indices

Microbial richness was reduced in jejunal digesta of piglets fed rye compared to wheat ( $p = 0.047$ ; Table 5). Shannon index and evenness were not affected ( $p > 0.05$ ).

**Table 5.** Effects of the experimental diets on ecological indices of the intestinal microbiota of weaned piglets <sup>1</sup>.

	Diet				SEM	CER	p-Value	
	W-SBM	W-RSM	R-SBM	R-RSM			PM	CER * PM
Jejunum <sup>2</sup>								
Richness	42.3	72.8	37.2	38.9	5.1	0.047	0.098	0.138
Shannon Index	1.53	1.94	1.61	1.65	0.08	0.522	0.165	0.247
Evenness	0.420	0.475	0.448	0.462	0.011	0.781	0.223	0.475
Colon ascendens								
Richness	186	202	203	190	6	0.811	0.899	0.246
Shannon Index	3.46	3.65	3.69	3.88	0.06	0.055	0.112	0.957
Evenness	0.666	0.688	0.699	0.743	0.010	0.060	0.149	0.633
Feces								
Richness	159	177	192	182	6	0.163	0.748	0.286
Shannon Index	3.20	3.38	3.35	3.74	0.09	0.176	0.128	0.558
Evenness	0.633	0.652	0.639	0.720	0.023	0.213	0.096	0.300

W-SBM, wheat/soybean meal; W-RSM, wheat/rapeseed meal; R-SBM, rye/soybean meal; R-RSM, rye/rapeseed meal; SEM, standard error of the mean; CER, cereal; PM, protein meal. <sup>1</sup> Data are presented as means ( $n = 11$ ); p-values indicate effects of the factors cereal (CER), protein meal (PM), and their interaction (CER \* PM). <sup>2</sup> W-RSM:  $n = 10$  (DNA-extract not amplifiable).

### 3.6. Relative Abundance of Bacterial Phyla, Order, and Genera

Most abundant phyla in jejunum, colon, and feces were *Firmicutes* and *Bacteroidetes* (Table 6). In rye-fed pigs, jejunal *Firmicutes* were higher and *Proteobacteria* lower than in wheat-fed pigs ( $p = 0.039$ ,  $p = 0.002$ , respectively). Compared to SBM, RSM reduced *Firmicutes*, and increased *Actinobacteria* at the three sampling sites ( $p < 0.050$ ). RSM increased *Proteobacteria* in the jejunum and *Bacteroidetes* in the colon ( $p = 0.019$ ,  $p = 0.014$ , respectively). Correlation ( $r$ ) between the relative abundance of jejunal *Proteobacteria* and AID of crude protein was 0.119 ( $p = 0.484$ ) and with AID of total amino acids it was 0.097 ( $p = 0.605$ ). Correlation ( $r$ ) between colonic *Bacteroidetes* and ADG of days 0–33 was  $-0.106$  ( $p = 0.498$ ) and of days 28–33  $-0.302$  ( $p = 0.049$ ). In the jejunum, W-RSM-fed pigs showed a lower relative abundance of *Firmicutes* compared to the other three groups ( $p = 0.002$ ).

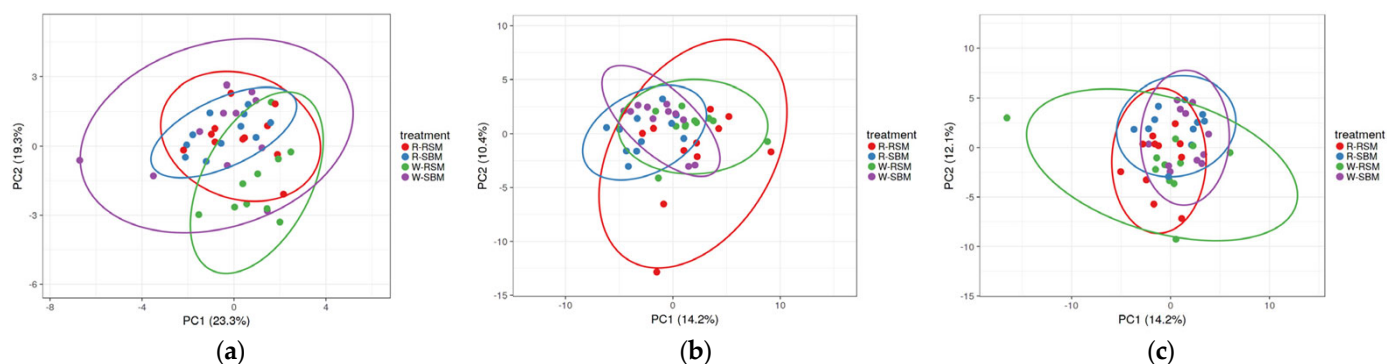
At the genus level, rye increased relative abundance of *Clostridium sensu stricto 1* in the jejunum ( $p = 0.005$ ) and *Terrisporobacter* in the jejunum and feces ( $p = 0.018$ ,  $p = 0.004$ , respectively). Compared to SBM, RSM increased *Bifidobacterium* spp. in the jejunum ( $p = 0.046$ ) and the genera *Prevotella 9*, *Blautia*, and *Syntrophococcus* in feces ( $p = 0.022$ ,  $p = 0.039$ ,  $p = 0.024$ , respectively). RSM decreased *Clostridium sensu stricto 1* in the three sampling sites ( $p < 0.05$ ), *Terrisporobacter* in jejunum and feces ( $p = 0.033$ ,  $p = 0.004$ , respectively), and unknown *Ruminococcaceae* and *Christensenellaceae R-7* group in feces ( $p = 0.042$ ,  $p = 0.031$ , respectively). Results at the order level are displayed in the Supplementary Materials.

Principal component analysis showed higher variabilities of the relative abundance of bacterial genera in jejunal digesta ( $\leq 23.3\%$ ) than in colonic digesta and feces ( $\leq 14.2\%$ ; Figure 1). Clusters of the experimental diets were overlapping to a high extent.

**Table 6.** Effects of the experimental diets on the relative abundance (%) of bacterial phyla <sup>1</sup>.

	Diet				SEM	CER	<i>p</i> -Value	
	W-SBM	W-RSM	R-SBM	R-RSM			PM	CER * PM
<b>Jejunum <sup>2</sup></b>								
Actinobacteria	0.550	5.40	1.36	2.72	0.673	0.467	0.019	0.177
Bacteroidetes	0.064	3.11	0.089	0.174	0.686	0.292	0.258	0.284
Firmicutes	98.4 <sup>a</sup>	88.7 <sup>b</sup>	98.2 <sup>a</sup>	96.7 <sup>a</sup>	1.1	0.039	0.004	0.032
Proteobacteria	0.749	2.60	0.131	0.407	0.255	0.002	0.019	0.077
<b>Colon ascendens</b>								
Actinobacteria	0.344	2.61	1.44	3.00	0.435	0.380	0.029	0.678
Bacteroidetes	17.5	23.4	18.2	26.1	1.4	0.540	0.014	0.713
Firmicutes	80.0	72.3	79.1	68.2	1.7	0.423	0.005	0.607
Proteobacteria	1.82	1.00	0.702	2.10	0.358	0.989	0.688	0.131
Spirochaetes	0.202	0.082	0.350	0.133	0.044	0.245	0.053	0.569
<b>Feces</b>								
Actinobacteria	0.749	3.61	2.08	4.86	0.516	0.182	0.005	0.965
Bacteroidetes	10.6	12.3	11.0	16.3	1.1	0.322	0.123	0.415
Firmicutes	86.7	82.4	85.6	76.9	1.3	0.184	0.012	0.377
Proteobacteria	0.961	1.19	0.247	1.35	0.221	0.533	0.137	0.323
Spirochaetes	0.671	0.098	0.387	0.179	0.145	0.730	0.189	0.537
Tenericutes	0.061	0.056	0.049	0.058	0.012	0.844	0.956	0.783

W-SBM, wheat/soybean meal; W-RSM, wheat/rapeseed meal; R-SBM, rye/soybean meal; R-RSM, rye/rapeseed meal; SEM, standard error of the mean; CER, cereal; PM, protein meal. <sup>1</sup> Data are presented as means ( $n = 11$ ); *p*-values indicate effects of the factors cereal (CER), protein meal (PM) and their interaction (CER \* PM). <sup>2</sup> W-RSM:  $n = 10$  (DNA-extract not amplifiable). <sup>a, b</sup> Values within a row with different superscripts differ significantly at  $p \leq 0.05$  (Tukey test).



**Figure 1.** PCA showing the effect of the experimental diets on the relative abundance (%) of bacterial genera in digesta of jejunum (a), colon ascendens (b), and feces (c) in weaned piglets; R-SBM, rye/soybean meal; R-RSM, rye/rapeseed meal; W-SBM, wheat/soybean meal; W-RSM, wheat/rapeseed meal. Principal component analysis was performed with ClustVis.

#### 4. Discussion

##### 1. Fiber Composition of the Experimental Diets

The use of fiber-rich feed components might help to stabilize the intestinal milieu during the post-weaning period. As rye contains more SDF and sNSP [18,36] the rye-based diets used in the current study were characterized by higher concentrations of soluble fiber fractions than the wheat-based diets. Similarly, the high concentrations of iNSP in RSM [18] resulted in a higher concentration of iNSP in the RSM-based diets compared to the SBM-based ones.

AX are the predominant fraction of cereal NSP and consist of a xylan backbone substituted by arabinose to varying degrees [18]. The degree of substitution can be described by the A/X-ratio, which is similar in rye and wheat [18,20], but higher in RSM compared to SBM [18]. Readily fermentable and more soluble AX are usually more substituted and

characterized by a higher A/X-ratio [37]. However, solubility and fermentability of AX are reduced with increasing cross-linkages to lignin, ferulic acid or other polysaccharides and by a higher molecular weight of AX [18,38]. As discussed below, intestinal microbiota was shaped differently by the RSM-based diets in the present study compared to SBM. This might be related to the higher dietary and intestinal A/X-ratio of RSM. However, the high content of insoluble dietary fiber (IDF) and acid detergent lignin (ADL) in the RSM-based diets indicating a high degree of lignification might have reduced fermentability, as indicated by the reduced concentration of SCFA in RSM-fed pigs. Therefore, A/X-ratio could not be used as an indicator of degradability of AX in the current study.

#### 4.2. Apparent Digestibility of NSP

The apparent colonic and total tract digestibility of total and soluble NSP in rye-fed pigs was higher compared to pigs receiving wheat-based diets. A higher ATTD of TDF and arabinose in rye-fed pigs was associated with an increased microbial fermentation in the large intestine [16]. In rye compared to wheat a higher fermentative activity might be related to the higher solubility of rye AX, the higher content of  $\beta$ -glucans and the higher total amount of dietary fiber [16,37]. Therefore, the digestibility of NSP in the current study might have been caused by a higher uptake of fermentable fiber with the rye-based diets, including more sNSP and soluble AX. Moreover, wheat AX might be less degradable, because of cross-linkages to other polysaccharides such as cellulose [37]. Cellulose is a major plant cell wall component, which is highly insoluble, poorly fermentable, and has a higher concentration in wheat than in rye [18]. The increased concentrations of digesta SCFA and the higher relative abundance of major plant cell wall-degrading bacterial species in the jejunum indicate an enhanced bacterial fiber degradation and can further explain the higher digestibility of NSP in rye-fed pigs of the present study.

With respect to NSP digestibility in RSM-fed pigs, values for colonic and total tract digestibility of total and iNSP was lower than in SBM-fed pigs. Similarly, ATTD of neutral detergent fiber (NDF), acid detergent fiber (ADF), carbohydrates, and dietary fiber was lower in other studies investigating the feeding of RSM instead of SBM to pigs [39,40]. However, one of these studies did not show a different ATTD of NSP and cellulose between RSM and SBM [39]. Microbial fiber fermentation and metabolic activity might be reduced as RSM is more lignified and insoluble than SBM [18]. This is reflected in the greater amount of IDF and iNSP in RSM-based diets of the current study and the lower SCFA concentration in jejunum, colon, and feces of RSM-fed pigs compared to SBM.

Surprisingly, the present values for colonic digestibility of NSP were higher than ATTD of NSP. In contrast, other studies show an increasing digestibility of NSP and AX in caudal direction [16,38]. However, the values of NSP digestibility were numerically in the same range, and furthermore, the determination of digestibility in feces is susceptible to interference. Bacteria may act as “non-dietary interfering substance” in NSP analytical procedures resulting in overestimated values of NSP concentration that are higher than the true diet derived NSP concentration [41]. Considering the higher number of bacteria in feces compared to colonic digesta and that in pigs and humans bacterial mass represents 40–50% of fecal DM [42,43], the interfering effect would be more pronounced with respect to ATTD of NSP compared to colonic digestibility. Additionally, some bacteria produce exopolysaccharides such as colonic acid (*Enterobacteria*) or dextran (*Lactobacillales*), which might have contributed to increased fecal concentration of NSP [44].

#### 4.3. Bacterial Metabolites and Composition of the Microbiome

In the current study, compared to wheat, rye-fed pigs had a higher concentration of SCFA in the jejunum and colon. As shown in a study comparing the feeding of an AX-rich diet (65% rye-flakes) with a diet based on wheat flour, complex cereal polysaccharides such as AX are not degraded enzymatically in the small intestine and might therefore promote the growth of SCFA-producing bacteria in the distal parts of the intestine [38]. Another study focused on the feeding of wheat and rye breads to pigs, demonstrating

that increased SCFA-production in the small intestine might be related to the more soluble fraction of dietary fiber, which is readily fermentable, whilst insoluble fractions would be degraded more distally [37]. Moreover, rye might cause a higher bacterial production of butyrate due to the structure of AX and the lower content of cellulose [45]. Therefore, the high concentration of TDF and SDF in the rye-based diets of this study may have provided more substrate for growth of SCFA-producing bacteria. Positive effects of SCFA derived from fermentation of dietary fiber on gut and animal health have been widely reviewed, including the use of acetate as an energy source and butyrate as the main fuel for colonocytes and its efficiency against possible pathogens [7,46]. In the present study, only data on digesta concentration of SCFA are available, but SCFA are absorbed rapidly via the gut wall [47]. Rye-derived SCFA might be of systemic use as peripheral blood concentration of SCFA was increased by an AX-rich diet based on rye-flakes compared to a wheat flour-based diet [48]. Despite the increased colonic concentration of SCFA and especially butyrate in the current study, no changes of the microbial community were determined in colonic digesta. This might be related to cross-feeding of AX-degrading bacteria, e.g., between *Bifidobacteria* and butyrate-producing bacteria, and to an increased abundance of the phosphotransferase system (kl02060) regulating carbohydrate uptake into bacterial cells [22]. Nevertheless, it is likely that rye compared to wheat enhanced the production of bacterial metabolites by providing a higher amount of easier fermentable substrate without a shift in the microbial community.

Compared to SBM-fed pigs, concentration of SCFA was lower in RSM-fed pigs in the jejunum and tendentially in the colon which indicates a lower metabolic activity of the resident microbiota. In contrast, previous studies showed an equal level of SCFA between RSM and SBM [24,25,28]. However, compared to these studies, the inclusion level of RSM and consequently the content of lignin and IDF was higher in the present study. A high degree of lignification may hamper enzymatic as well as microbial fiber degradation in RSM-fed pigs [39]. In combination with the lower digestibility of NSP and DM, this could explain the lower SCFA compared to SBM-fed pigs. Additionally, SCFA might have been absorbed rapidly or used by other microbiota in the sense of cross-feeding [49].

In accordance with a recent meta-analysis that identified *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* as the most abundant phyla of the core microbiome in pigs [50], *Firmicutes* and *Bacteroidetes* were the predominant phyla of the pigs in the current study.

In rye-fed piglets of this study, composition of the microbial community was only different to wheat-fed pigs in the jejunum. This was unexpected, because according to other studies, the major impact of rye on the microbial community is expected in the proximal parts of the large intestine [16,23,38]. A higher relative abundance of *Firmicutes* in the small intestine might be explained by the higher content of sNSP in the rye-based diets, since more insoluble substrate would be degraded further distally [7]. The phylum *Firmicutes* contains many plant cell wall-degrading species including *Clostridium sensu stricto 1* and *Terrisporobacter*. These two genera are known to ferment complex indigestible plant polysaccharides such as hemicellulose and cellulose [51]. Therefore, the fiber provided by the rye-based diets might have served as a substrate and stimulated *Firmicutes'* proliferation. *Firmicutes* contains many butyrate-producers [52], therefore an increased abundance could be considered as a positive effect of the feeding of rye. However, SCFA are absorbed primarily in the caecum and proximal colon, and might not be utilized by the host in the same extent in the jejunum [47].

*Proteobacteria* had a lower jejunal relative abundance in rye-fed pigs of the current study. Many putative pathogens such as *E. coli* and *Salmonella* belong to *Proteobacteria* and a lower presence of *Proteobacteria* was associated with an increased intestinal barrier function and a higher anti-inflammatory capacity of the local immune system [53].

In contrast to an increased concentration of SCFA in jejunal digesta, the analysis of microbial diversity in rye-fed pigs showed a lower richness compared to wheat. It is possible that the increased production of metabolites might be driven by only a few selected genera of *Firmicutes* which were more abundant in the rye-fed pigs.



With respect to effects of RSM on the relative abundance of microbiota compared to SBM, the present study resulted in a reduced abundance of *Firmicutes* at the three sampling sites. As mentioned above, *Firmicutes* contains many fiber-degrading species and is known to produce SCFA. The lower NSP and DM digestibility together in RSM-fed pigs compared to SBM indicate that the fiber provided by the RSM-based diets was not used as a suitable substrate for fermentation by microbiota, especially fiber-degrading *Firmicutes*. Consequently, the metabolic activity and growth of microbiota was lower as illustrated by the lower concentration of SCFA and the lower relative abundance of *Firmicutes*. In line with this, compared to alfalfa meal, wheat bran and pure cellulose containing a higher amount of IDF also reduced the relative abundance of *Firmicutes* in large intestinal mucosa of suckling pigs [54]. However, another study investigating the feeding of RSM instead of SBM showed an increase of *Firmicutes*, although the lower inclusion level and lower content of IDF might have prevented an inverse result [55]. A higher ratio of *Firmicutes*:*Bacteroidetes* was associated with a reduction of the incidence of diarrhea and infections [55]. Nonetheless, previously published results of the present study showed that the fecal score of the pigs was in a physiological range throughout the trial [14]. Within *Firmicutes*, the predominant genera *Clostridium sensu stricto 1* and *Terrisporobacter* were also decreased by RSM in the current study. This might explain the reduced concentration of SCFA in RSM-fed pigs because another study showed a strong correlation between the relative abundance of these genera with the production of large amounts of metabolites from plant fiber [51].

In line with another study investigating the feeding of RSM [25], RSM increased the relative abundance of *Actinobacteria* along the gastro-intestinal tract in the current study. In contrast, another study did not show differing values of abundance of *Actinobacteria* in RSM- or SBM-fed pigs [55]. *Actinobacteria* efficiently use hemicellulose and cellulose [52], both mainly insoluble fiber fractions. Despite RSM and SBM containing similar amounts of cellulose [18] the higher inclusion of RSM compared to SBM and the higher content of IDF in RSM-diets might have promoted growth of *Actinobacteria* in RSM-fed pigs. In the jejunum, *Bifidobacterium*, a genus belonging to *Actinobacteria*, was also increased by the feeding of RSM. *Bifidobacterium* is considered to improve gut health [7,56] and was increased by the feeding of cellulose in another study [57].

As mentioned above, an increased relative abundance of *Proteobacteria* may be a risk for animal health. Nevertheless, the pigs of both RSM and SBM groups were in a good condition throughout the trial. *Proteobacteria* contains many proteolytic genera [58]. RSM may lead to a lower AID of protein and amino acids than SBM [59] which also was demonstrated in this study [14]. However, there was no correlation detected between AID of crude protein and total amino acids and the jejunal relative abundance of *Proteobacteria*. It is likely that the protein was not available as a substrate for growth of *Proteobacteria*, because IDF in RSM caused an encapsulation in the rigid cell wall and an increased digesta passage rate [39].

In RSM-fed pigs, the increased relative abundance of *Bacteroidetes* is most likely related to the higher content of IDF than in SBM-based diets. *Bacteroidetes* as the second most abundant phylum of the gut microbiota in pigs [50] was also increased by resistant starch in humans [60] and by corn bran in pigs [61], both sources of IDF. In contrast, a study investigating the feeding of RSM instead of SBM to pigs showed a lower abundance of *Bacteroidetes* which was related to the high pectin content [25]. An increased abundance of *Bacteroidetes* is associated with weight loss in humans, mice [62], and pigs [63]. The negative correlation between abundance of *Bacteroidetes* and ADG in the last six days of the trial might suggest that the shift towards more colonic *Bacteroidetes* was connected to the reduced weight gain in RSM-fed pigs.

## 5. Conclusions

In conclusion, compared to wheat, the higher amount and solubility of NSP from rye resulted in an increased degradation and fermentation of NSP and in a higher metabolic activity of intestinal microbiota. However, relative abundance of large intestinal microbiota

was not different between pigs fed rye and wheat. RSM caused a lower bacterial metabolic activity than SBM. The higher fiber content of RSM-based diets was expected to increase fermentation, but 30% inclusion of RSM might have provided an excess of IDF. Still, RSM lead to a lower abundance of common fiber degraders of the predominant phylum *Firmicutes* and an increased abundance of IDF degrading *Actinobacteria* and *Bacteroidetes*. Further research is needed to better understand host–microbiota interaction and to improve feeding concepts with a targeted use of dietary fiber.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/ani12010109/s1>, Table S1: Effects of the experimental diets on the relative abundance (%) of bacterial orders, Table S2: Effects of the experimental diets on the relative abundance (%) of bacterial genera.

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**Data Availability Statement:** Data supporting reported results is contained within the article and the Supplementary Materials.

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## **5. General discussion and conclusion**

Rye and RSM are fibre-rich alternative feed components to wheat and SBM in pig nutrition. This study aimed to investigate the effects of rye and RSM compared to wheat and SBM on growth performance and digestive physiology of weaned piglets. A trial with weaner pigs fed one of four different experimental diets containing either rye or wheat combined with either RSM or SBM was conducted to monitor growth performance and faecal consistency. At the end of the trial, a dissection served to analyse physico-chemical properties of digesta, nutrient digestibility, intestinal morphology, and the intestinal microbiome.

### **5.1. Key findings of this study**

The fibre composition of rye and RSM was reflected in the experimental diets. The inclusion of rye increased soluble fibre fractions, RSM raised the concentration of insoluble fibre. Even though these differences could be associated to many parameters investigated in this study, growth performance was hardly or not affected at all. Additionally, the piglets were in a healthy condition throughout the trial. In line, recent research on the feeding of rye and RSM to pigs in comparison to wheat and SBM showed similar growth performance between treatments (Chuppava et al. 2020; Hansen et al. 2020). The slightly reduced performance of RSM-fed piglets in the current study might mainly be related to the high inclusion level of RSM and therefore relatively high concentration of IDF in the RSM-based diets hampering protein digestibility. However, within a physiological range the feeding of SBM instead of RSM led to softer faeces which might also be related to a lower content of ADF in SBM. Similarly, the feeding of a high-fibre diet led to a decreased incidence of diarrhoea compared to a diet containing less ADF (Chen et al. 2013).

The effects of the diets on digestibility of CP, AA and NSP could also be related to the differences of their fibre profile. The decreasing effect of high-fibre diets, especially of IDF-rich diets on CP and AA digestibility is well known. This effect may be explained by several factors such as an entrapment of nutrients in the fibre matrix, an increased passage rate of digesta, and an increase of digesta viscosity (Agyekum and Nyachoti 2017). With respect to rye, it is not clear, if rye generally decreases AID of CP and AA, since studies on this topic are scarce and results inconsistent (Cervantes-Pahm et al. 2014; McGhee and Stein 2018). The high concentration of TDF might have caused the lower AID of CP and most AA in this study. Small intestinal digesta viscosity was increased most likely due to the high concentration of SDF in the rye-based diets and might also explain the reduced AID of CP. However, one study of the few other studies investigating these parameters showed a higher digesta viscosity in rye-fed

pigs without affecting AID of CP (Cervantes-Pahm et al. 2014). Since growth performance was not affected in rye-fed piglets, the effect of rye of AID of CP and AA might therefore be of minor relevance in practice. In this context, the digestibility of NSP is interesting as rye-fed piglets showed higher values for total and soluble NSP in the colon and faeces compared to wheat. This indicates a higher fibre degradation in the large intestine in rye-fed pigs and may be explained by increased microbial fermentation of fibre, especially readily fermentable SDF. In line, concentration of SCFA as microbial metabolites were increased in colonic digesta of rye-fed pigs. An increase of large intestinal SCFA concentration was also demonstrated in other studies investigating the feeding of rye instead of wheat (Le Gall et al. 2009; Nielsen et al. 2014). Results of other studies imply that the high content of fibre, including more soluble AX and  $\beta$ -glucans, makes rye a more fermentable substrate than wheat, which additionally is richer in less fermentable cellulose (Le Gall et al. 2009; McGhee and Stein 2020). Consequently, the increased large intestinal concentration of SCFA might indeed serve as an energy source for the pig and this might potentially amend effects of a reduced pre-caecal digestibility of CP and AA on growth performance.

In RSM-fed pigs compared to SBM, a reduction of AID of CP and AA was described in several other studies and was mainly attributed to the higher content of fibre in RSM (Mejicanos et al. 2017; Perez de Nanclares et al. 2017; Mejicanos et al. 2020). Especially IDF including lignin were associated with an increased passage rate, poorly accessible protein within the plant cell wall fibre matrix and increased endogenous nitrogen-losses due to abrasive effects on the mucosal surface of the intestine (Montagne et al. 2004; Wilfart et al. 2007). The high concentration of IDF and ADL of the RSM-based diets compared to SBM used in this study may therefore likely be responsible for the reduction of AID of CP and AA. Moreover, unlike soluble NSP from rye, insoluble NSP from RSM resulted to be less digestible in the large intestine compared to SBM and concentrations of SCFA were even lower in RSM-fed pigs. A lower digestibility of DF from RSM in comparison to SBM was also shown in other recent studies (Huang et al. 2018; Perez de Nanclares et al. 2019). This suggests that IDF could not be used by large intestinal microbiota to produce useful SCFA and losses of protein digestibility could not be compensated via this mechanism which may be related to the lower growth performance in RSM-fed pigs.

With respect to the intestinal microbiome, in this study *Firmicutes* and *Bacteroidetes* were the predominant phyla which are considered part of the core microbiota of the pig (Cox 2017; Holman et al. 2017). Rye caused neither a shift of bacterial phyla nor an increased microbial diversity in the colon or faeces despite the higher intestinal concentration of SCFA compared to wheat. This is surprising, since the higher content of fermentable substrate in rye, especially

soluble fibre fractions, were expected to increase the growth of carbohydrate fermenters and alpha-diversity indices in the large intestine. Other studies suggest rye DF might be fermented mainly in the caecum, but also in the proximal part of the colon (Nielsen et al. 2014; Gorham et al. 2017; McGhee and Stein 2020). It is therefore likely, that rye DF was fermented accompanied with microbial shifts in the caecum and that microbiota exhibited an enhanced fermentative activity without shifting their composition in the proximal colon and faeces. Among SCFA, butyrate is considered relevant for multiple beneficial effects on gut and animal health (Guilloteau et al. 2010). Rye was shown to increase absorption of SCFA, especially butyrate, in pigs compared to wheat (Ingerslev et al. 2014). Moreover, the reduced shedding of pathogenic *Salmonella* was suggested to be related to an increased butyrate production in the caecum of rye-fed piglets compared to wheat (Chuppava et al. 2020). In this context, the results of this study might imply an advantage of rye over wheat since in the jejunum the relative abundance of *Firmicutes*, a phylum containing many butyrate-producing genera (Long et al. 2020), was increased, and since in the colon concentration of butyrate was higher in rye-fed pigs.

The RSM-based diets provided a higher concentration of DF, especially IDF, than SBM, which was reflected in an increased relative abundance of *Actinobacteria* and *Bacteroidetes* along the intestinal tract. Both phyla were demonstrated to degrade IDF in pigs, *Actinobacteria* from RSM, cellulose and hemicelluloses (Umu et al. 2018; Long et al. 2020) and *Bacteroidetes* from corn-bran (Zhao et al. 2019). In contrast, RSM compared to SBM did not increase the relative abundance of *Actinobacteria* in another study (Hong et al. 2020). This contrast might be explained by the higher inclusion level of RSM compared SBM in the present study. Nonetheless, the higher abundance of *Actinobacteria* and *Bacteroidetes* was accompanied by a decrease of SCFA and of the digestibility of insoluble NSP in RSM-fed piglets. This might imply that insoluble fibre from RSM was poorly degradable. Moreover, the lower SCFA concentration in RSM-fed piglets was possibly related to the lower relative abundance of carbohydrate fermenting, SCFA producing *Firmicutes* compared to SBM.

### **5.2. Critical evaluation of the study design and implications for future research**

The current study showed promising results for the replacement of wheat and SBM by rye and RSM in pig nutrition. Several results were related with the differing effects of soluble and insoluble fibre fractions which demonstrated that the DF profile of feed components should always be considered when calculating diets in practice. The investigation of *N*-acetylneuraminic acid as a marker of intestinal mucus is rather new in pig nutrition research



and reference data are scarce. Thus, despite not showing different values across the treatments, data were provided for referring studies in the future.

However, some aspects regarding the presented data of this study might be perceived with care or indicate future research activities. This includes the interpretation of the concentration of SCFA in digesta. As mentioned before, SCFA are absorbed fast from the intestinal lumen and luminal concentrations may not automatically be equalled with neither microbial production rate nor peripheral blood levels. This is illustrated by a study showing decreased digesta SCFA and concurrent increased SCFA in plasma from the jugular vein after the uptake of a high-DF diet (Xu et al. 2020). Another difficulty with respect to SCFA analysis is the dependency of blood levels on the time after feeding (Bach Knudsen et al. 2005). However, other studies showed that a higher amount of AX increased SCFA flow in the portal and mesenteric vein compared to RS, low-AX or cellulose which suggests that an increased intestinal fermentation of DF might indeed result in a higher blood concentration of SCFA (Ingerslev et al. 2014; Bach Knudsen et al. 2016a).

Since host-microbiota-interactions are still not fully understood, it might have been indicated to validate the data of the relative abundance of microbiota findings by quantifying important bacterial species by qPCR to get a better picture of the microbial community influenced by the different treatments. Moreover, possible effects of the feeding of rye instead of wheat to prevent the colonisation of pathogens were investigated in few previous studies (Chuppava et al. 2020), but not subject of this thesis. Nonetheless, despite revealing a similar composition of the microbiome it was shown that rye was more fermentable than wheat indicating an advantage of rye in this context. Additionally, the investigation of protective effects of DF from rye or RSM against intestinal infections with pathogens and PWD would have required a challenge trial which might be a future research project. With respect to fibre digestibility and microbial parameters, digesta samples from the caecum might have completed findings of this study. Apparently, degradation of rye DF started in the jejunum and caused a shift of microbiota and an increase of SCFA concentration in this intestinal section. In contrast, the colonic and faecal samples did not show any differences of the relative abundance of bacteria. AX however, especially soluble AX, are fermented primarily in the caecum (Bach Knudsen and Laerke 2010; Nielsen et al. 2014; Xu et al. 2021). Therefore, it is likely, that especially SDF from rye was fermented to a larger extent than wheat DF in the caecum and that expected shifts of the microbial community could have been observed there.

Regarding effects of the feeding of rye and RSM on the environment, especially on soil quality, the analysis of the ATTD of nitrogen or CP might have been interesting in addition to the data

on AID. Input of nitrogen into the soil by pig manure should be limited and therefore, a high total tract digestibility and consequently low excretion of these nutrients are required (Millet et al. 2018). Rye and RSM used in this study showed a lower CP content than wheat and SBM which might be of advantage in this context (Wang et al. 2018c). In contrast, the experimental diets were balanced for CP and lysine, and AID of CP and AA was comparably lower to wheat and SBM which might increase nitrogen flow in rye and RSM-fed pigs. Nonetheless, it is likely that large intestinal CP fermentation was similar across the treatments, since  $\text{NH}_3$  concentration did not differ indicating similar proteolytic activity of resident microbiota. In other studies, RSM decreased nitrogen excretion and  $\text{NH}_3$  emissions in growing-finishing pigs compared to SBM, and rye did not change nitrogen excretion of growing pigs compared to wheat (McDonnell et al. 2010; Swiech et al. 2012). Thus, it remains unclear if nitrogen excretion was increased by lower AID of CP and if large intestinal digestive processes attenuated this effect in the current study.

Finally, to better understand the interaction between different fractions of DF or rye and RSM on intestinal microbiota and the host, future research ought to focus on effects on histomorphological alterations of the gut wall, reactions of the intestinal and systemic immune system, activity of digestive enzymes, as well as intestinal barrier function. Moreover, large scale field trials are indicated to validate the explanatory power of the outcome of this study.

### **5.3. Linking the intestinal microbiome to growth performance, protein digestibility and digesta pH**

The possible impact of the interaction between the intestinal microbiota and the host on gut and animal health was described above. Consequently, results of the feeding of rye and RSM on the relative abundance and diversity of intestinal microbiota might be related to the results of growth performance, nutrient digestibility, and physico-chemical characteristics of digesta. With respect to growth performance, a negative correlation was found between colonic *Bacteroidetes* and the ADG for the whole trial period. Reduced ADG in RSM-pigs might therefore not only be due to a lower palatability or nutrient digestibility, but also to a different composition of intestinal microbiota, such as an increased growth of IDF degraders like *Bacteroidetes* (Martínez et al. 2010; Zhao et al. 2019). In line, RSM replacing SBM resulted in a reduced growth performance of growing-finishing pigs as well as in an increased abundance of fibre degraders such as *Prevotella* (Umu et al. 2020). However, another study resulted in an increase of ADG accompanied with a decrease of faecal *Bacteroidetes* when replacing SBM by up to 20 % RSM (Hong et al. 2020). Therefore, the relation of bacterial shifts with growth parameters in RSM-fed pigs compared to SBM is still not clear and requires further investigation.

AID of CP and total AA was not correlated to jejunal *Proteobacteria* in the present study, a phylum containing many proteolytic genera (Pieper et al. 2016). This may be explained by the different fibre composition of the diet ingredients. In rye-fed pigs, AID of CP and AA was most likely reduced due to the high content of fibre in general or other factors known to decrease protein digestibility, rather than either SDF or IDF, since jejunal digestibility of total NSP was higher than in wheat-fed pigs and *Proteobacteria* decreased in the jejunum indicating they did not use CP as a substrate for growth. In contrast, in RSM-fed pigs it was more likely the high content of IDF that impaired protein digestibility hampering the access of proteolytic bacteria without shifting *Proteobacteria* either. The lower jejunal digestibility of insoluble NSP and insoluble AX in RSM-pigs compared to SBM supports this explanation. Consequently, the reduction of AID of CP could not be linked to proteolytic bacterial activity in this study.

A higher microbial diversity is generally considered beneficial for the host. In contrast, the lower jejunal richness, one of the alpha-diversity indices, in rye-fed pigs was not accompanied with an impaired growth performance. However, the colonic and faecal diversity indices were not affected in the current study and the lack of impact on pigs' growth might be explained by overall lower bacterial numbers in the small intestine and because alterations of microbial diversity in the large intestine might have more impact in this context. This is also reflected in other studies investigating sources of DF showing no effects regarding microbial diversity but varying outcome of growth performance. A study investigating RSM instead of SBM resulted in higher ADG of RSM-fed pigs and in unaltered values of faecal microbial diversity (Hong et al. 2020). Moreover, the comparison of SDF-rich sugar beet pulp to IDF-rich soybean hulls did not result in differences of neither growth performance nor colonic alpha-diversity (Hong et al. 2021b).

Intestinal microbiota may shift due to changes in the digesta pH. In fact, pH was shown to decrease in the colon in high-DF compared to low-DF diets and was negatively correlated to the relative abundance of colonic *Prevotella* (Xu et al. 2020). Moreover, a study comparing readily fermentable sugar beet pulp to less fermentable soybean hulls suggested a relation between an increased relative abundance of *Proteobacteria* with lower digesta pH (Hong et al. 2021b) which is also reflected by the results of the comparison of rye and wheat from the current study. In line, diets enriched with soluble AX and  $\beta$ -glucans compared to control resulted in a higher SCFA concentration and lower digesta pH values in the caecum as well as in increased abundance of *Prevotella* spp. and *Veillonellaceae* (Gorham et al. 2017). In contrast, caecal digesta pH was not correlated to the abundance of any phylotype in pigs fed either a rye bran or a cellulose-rich wheat-based diet and concentration of caecal SCFA was lower in rye bran-fed pigs (Xu et al. 2021). It is still likely, that the jejunal and ileal digesta pH

in rye-fed pigs decreased with the increased fermentation of DF and the resulting presence of more acidic metabolites such as SCFA. Thus, jejunal pH and relative abundance of *Proteobacteria* both seem associated with the higher amount and fermentability of DF of rye compared to wheat.

In total, the extent and mechanisms of the interaction between microbiota and physiological functions need more clarification. Recently, several studies tried to predict the metabolic function of the intestinal microbiome of pigs by assessing 16S rRNA marker gene sequences (Long and Venema 2020; Umu et al. 2020; Miragoli et al. 2021). The abundance of marker genes were used as indicators of the functional capacity of the microbiome regarding the metabolism of carbohydrates and AA, genetic information processing, e.g., DNA replication, or cellular processes like bacterial chemotaxis and might implicate the effects of the composition of the microbiome on its host (Langille et al. 2013; Umu et al. 2020). However, interpretation of these data should be undertaken with precaution since marker gene profiles are based on research on humans (Langille et al. 2013).

### **5.4. Transfer to practice**

#### 5.4.1. The dietary inclusion level of rye and rapeseed meal

Since the feed components investigated in this study can be used as basic ingredients for diets in pig farming, the outcome of the study may have direct implications for the formulation of diets in practice. The recommendation for a limited inclusion of rye and RSM in diets for weaner pigs might be revised. The inclusion of a high percentage of rye did not lead to any reduction of ADFI, ADG, FCR and final BW which is in line with other recent studies investigating up to 69 % of rye (Chuppava et al. 2020; Wilke 2020). Previous prejudices of SDF-rich rye being less palatable and leading to detrimental effects on feed intake, growth performance and animal health are not supported by neither those studies nor by the current study. The inclusion of RSM reduced ADG and ADFI, so it is likely that 30 % dietary inclusion is too high for weaner pigs. However, the reduction was not substantial and other studies with weaner pigs investigating the inclusion of up to 40 % showed no decreasing effects on weight gain (Hansen et al. 2020). This indicates that the recommended level of 10 % (LfL-Bayern 2019) may be very low. In any case, the four ingredients might be used in combination in practice. The inclusion of RSM in addition to SBM might be a good solution to attenuate the negative effects of RSM while limiting the use of SBM. Many studies investigated the replacement of SBM with increasing levels of RSM and showed equal performance parameters between treatments (Pedersen et al. 2016; Mejicanos et al. 2017).

Considering the comparable results of growth performance and health of weaner piglets in the present study, the advantages regarding challenges accompanying climate change and antibiotic resistance may outweigh the slightly reduced performance of RSM-pigs and emphasize the suitability of rye as a feed component in weaner diets. However, to support the aspect of a more sustainable production chain when feeding of rye and RSM, life cycle assessment and life cycle costing analyses of slaughter pigs from farms feeding rye and RSM are warranted (Baum and Bienkowski 2020).

### 5.4.2. The relation between dietary fibre and the nutritional value of rye

The high fermentability of rye DF seems to require a more distinct consideration when evaluating its nutritional value as an energy source. The current formula for the estimation of ME of feed components considers the concentration of starch, CP and crude fibre but not the fermentability of DF (GfE 2008). However, cereal DF can increase available energy substantially by being metabolised to SCFA by the resident microbiota. Rye has the lowest ME value compared to other cereals including wheat (Cervantes-Pahm et al. 2014; McGhee and Stein 2020). Additionally, AID and ATTD of GE was lower but higher for TDF in rye than in wheat and corn (McGhee and Stein 2020). This difference was mainly attributed to its low concentration and digestibility of pre-caecal digestible starch and the use of DF as fermentable substrate in the large intestine (McGhee and Stein 2020). In line with this, rye-fed pigs of the present study showed an increased colonic and faecal digestibility of NSP and concentration of SCFA, but also a similar growth performance compared to wheat. This indicates that with respect to the expected growth performance an isolated focus on ME of a cereal, without considering fermentability of DF, might mislead to an underestimation of DF-rich cereals with a lower pre-caecal availability of starch and CP such as rye.

## 5.5. Conclusion

This study showed that rye and RSM can be used as alternatives to wheat and SBM in compound feed for weaner piglets without or with only a minor reduction of growth performance and protein digestibility and without any negative effect on animal health. In fact, the inclusion level of rye and RSM may be higher than previously recommended. Effects on digestive physiology and intestinal microbiota were mainly associated with the different composition of dietary fibre of the investigated feed components. Future research to investigate effects of the feeding of rye and RSM on structural changes of the gut wall, immune function and host-microbiota interaction is warranted to elucidate further possible beneficial features of rye and RSM.

### 6. Summary

Summary of the doctoral thesis:

#### **Effects of dietary rye and rapeseed on growth performance, nutrient digestibility, digesta characteristics and the intestinal microbiome of weaner piglets**

Rye and rapeseed meal (RSM) are interesting alternatives to wheat and soybean meal (SBM) for the nutrition of weaner piglets, especially regarding current challenges of pig production with respect to climate change and globalized trading of feed components. Rye tolerates more extreme temperatures and lower soil quality than wheat, rapeseed is adapted better to local climate conditions than soybean. The level of inclusion of rye and RSM into piglets' diets was limited in the past due to the practical evidence of a lower palatability and anti-nutritional factors. Nonetheless, the content of dietary fibre increases with the inclusion of rye and RSM instead of wheat and SBM which might have beneficial effects on digestive physiology and resident microbiota. Moreover, recent studies showed that the feeding of high levels of rye and RSM did not reduce growth performance and feed intake of young pigs. However, research on the combined feeding of rye and RSM in piglets is scarce.

Therefore, the aim of this study was to investigate the effects of rye and RSM included at a high dietary inclusion level in comparison to wheat and SBM on growth performance, protein, amino acid, and non-starch-polysaccharide (NSP) digestibility, physical characteristics of the digesta, intestinal morphology, and the composition and metabolic activity of intestinal microbiota in weaner pigs.

A feeding trial was conducted with 88 piglets. At weaning at the age of 28 days, the piglets were randomly allocated to four groups and housed two piglets per pen. They were fed one of four isonitrogenous, pelleted diets *ad libitum* ( $n = 11$ ): wheat/SBM, wheat/RSM, rye/SBM, and rye/RSM. Dietary inclusion level was 48 % for the cereals, 25 % for SBM, and 30 % for RSM. The diets were analysed for nutrient composition including total dietary fibre and NSP. Body weight gain and feed intake were recorded weekly and faecal consistency was scored daily. After 33 days, one piglet per pen was euthanized for the assessment and collection of digesta and the measurement of intestinal morphology. Digesta samples were analysed for pH value, viscosity, dry matter and nutrient digestibility, bacterial metabolites, and relative abundance of microbiota.

The rye-based diets contained more soluble NSP than wheat-based diets. The RSM-based diets were higher in insoluble NSP compared to SBM. Growth performance was not affected

## Summary

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by rye, but RSM reduced weight gain ( $p = 0.024$ ) and feed intake ( $p = 0.037$ ) compared to SBM. Rye and RSM decreased apparent ileal digestibility of crude protein and amino acids ( $p < 0.05$ ). Colonic and faecal digestibility of NSP was higher in rye-fed piglets ( $p < 0.001$ ,  $p = 0.001$ , respectively), lower in RSM-fed piglets ( $p < 0.001$ ). Rye-fed pigs showed a more acidic and viscous small intestinal digesta ( $p = 0.045$ ,  $p = 0.013$ , respectively), and a higher relative weight of the colon tissue ( $p < 0.001$ ). Concentration of short-chain fatty acids was higher in rye-fed piglets in jejunum and colon ( $p < 0.001$ ,  $p = 0.016$ , respectively). RSM lowered jejunal concentration of short-chain fatty acids ( $p = 0.001$ ). Rye increased the relative abundance of *Firmicutes* and decreased *Proteobacteria* in the jejunum ( $p = 0.039$ ,  $p = 0.002$ , respectively). *Firmicutes* were decreased and *Actinobacteria* (jejunum, colon, faeces:  $p < 0.050$ ), jejunal *Proteobacteria* ( $p = 0.019$ ) and colonic *Bacteroidetes* ( $p = 0.014$ ) were increased in RSM-fed pigs compared to SBM.

In conclusion, this study showed that rye and RSM can be used as alternatives to wheat and SBM in compound feed for weaner piglets without or with only a minor reduction of growth performance and protein digestibility and without any negative effect on animal health. In fact, the inclusion level of rye and RSM may be higher than previously recommended. Effects on digestive physiology and intestinal microbiota were mainly associated with the different composition of dietary fibre of the investigated feed components. The high content of soluble dietary fibre in rye was more degradable than wheat fibre, caused an increased microbial fermentative activity but did not induce a shift of the colonic microbiota. RSM was most likely too high in insoluble dietary fibre which led to the reduction of protein digestibility and bacterial metabolic activity as well an increased relative abundance of *Actinobacteria* and *Bacteroidetes* which are known to degrade insoluble fibre.

Future research is warranted to elucidate further possible beneficial features of rye and RSM, to evaluate the optimum dietary inclusion level of these feed components, and to improve the understanding of the mechanisms, including host-microbiota interaction, that facilitate targeted practical use of rye and RSM instead of wheat and SBM in pig nutrition.

### 7. Zusammenfassung

Zusammenfassung der Doktorarbeit:

#### **Auswirkungen der Fütterung von Roggen und Raps auf Wachstumsleistung, Nährstoffverdaulichkeit, Charakteristika der Digesta und auf das intestinale Mikrobiom von Absetzferkeln**

Roggen und Rapsextraktionsschrot (RSM) sind interessante Alternativen zu Weizen und Sojaextraktionsschrot (SBM) für die Fütterung von Absetzferkeln, vor allem im Kontext aktueller mit dem Klimawandel und dem globalisierten Handel von Futterkomponenten einhergehenden Herausforderungen für die Schweineproduktion. Roggen toleriert extremere Temperaturen und schlechtere Bodenqualität als Weizen, Raps ist an das lokale Klima besser angepasst als Soja. Die Höhe der Einsatzempfehlungen für Roggen und RSM in Ferkelfutter war in der Vergangenheit aufgrund bestehender Vorbehalte bezüglich niedriger Schmackhaftigkeit und aufgrund antinutritiver Faktoren limitiert. Allerdings steigt der Anteil der Faserstoffe, sogenannter „dietary fibre“, mit der Verwendung von Roggen und RSM statt Weizen und SBM. Dies könnte eine positive Wirkung auf die Verdauungsphysiologie und die intestinale Mikrobiota haben. Neue Studien zeigten außerdem, dass die Fütterung hoher Anteile von Roggen und RSM Wachstum und Futteraufnahme junger Schweine nicht reduzierte. Es gibt jedoch nur wenige Studien zur kombinierten Fütterung von Roggen und RSM. Daher war es Ziel dieser Studie die Wirkung hoher Anteile von Roggen und RSM im Vergleich zu Weizen und SBM auf Wachstum, Protein-, Aminosäuren- und Nicht-Stärke-Polysaccharid (NSP)-Verdaulichkeit, physikalische Eigenschaften der Digesta, intestinale Morphologie und die Zusammensetzung und metabolische Aktivität der intestinalen Mikrobiota bei Absetzferkeln zu untersuchen.

Es wurde ein Fütterungsversuch mit 88 Ferkeln durchgeführt. Beim Absetzen im Alter von 28 Tagen wurden die Ferkel randomisiert auf vier Gruppen aufgeteilt und zu zweit in Buchten eingestallt. Sie wurden jeweils mit einer von vier isonitrogenen, pelletierten Rationen *ad libitum* gefüttert ( $n = 11$ ): Weizen/SBM, Weizen/RSM, Roggen/SBM und Roggen/RSM. Die beiden Getreide wurden zu je 48 % in die Ration eingemischt, SBM zu 25 % und RSM zu 30 %. In den Rationen wurde die Nährstoffzusammensetzung einschließlich „total dietary fibre“ und NSP analysiert. Körpermassezunahme und Futteraufnahme wurden wöchentlich erfasst, die Kotkonsistenz täglich gescort. Nach 33 Tagen wurde ein Ferkel pro Bucht euthanasiert, um Digesta-Proben zu sammeln und zu analysieren und um Maße der Darmmorphologie zu



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erfassen. Die Digesta wurde hinsichtlich pH-Wert, Viskosität, Trockenmasse- und Nährstoff-Verdaulichkeit, bakterieller Metabolite und der relativen Häufigkeit der Mikrobiota untersucht.

Die Roggen-Rationen enthielten mehr lösliche NSP als die Weizen-basierten. Die RSM-Rationen hatten mehr unlösliche NSP im Vergleich zu SBM. Das Wachstum wurde von Roggen nicht beeinflusst, aber RSM reduzierte die Körpermasseszunahme ( $p = 0,024$ ) und die Futteraufnahme ( $p = 0,037$ ) im Vergleich zu SBM. Roggen und RSM senkten die scheinbare ileale Verdaulichkeit von Protein und Aminosäuren ( $p < 0,05$ ). Die Verdaulichkeit der NSP in Colon und Faeces war höher in den Roggen gefütterten Ferkeln ( $p < 0,001$  bzw.  $p = 0,001$ ) und niedriger in RSM gefütterten Ferkeln ( $p < 0,001$ ). Die Roggen gefütterten Ferkel hatten saurere und visköser Digesta im Dünndarm ( $p = 0,045$  bzw.  $p = 0,013$ ) und schwereres Colon-Gewebe ( $p < 0,001$ ). Die Konzentration der kurzkettigen Fettsäuren in Jejunum und Colon war höher in den Roggen gefütterten Ferkeln ( $p < 0,001$  bzw.  $p = 0,016$ ). RSM minderte die Konzentration der kurzkettigen Fettsäuren im Jejunum ( $p = 0,001$ ). Roggen erhöhte im Jejunum die relative Häufigkeit der *Firmicutes* und senkte die der *Proteobacteria* ( $p = 0,039$  bzw.  $p = 0,002$ ). *Firmicutes* waren in RSM gefütterten Ferkeln im Vergleich zu SBM niedriger, *Actinobacteria* höher (Jejunum, Colon, Faeces:  $p < 0,050$ ) und *Proteobacteria* im Jejunum ( $p = 0,019$ ) und *Bacteroidetes* im Colon ( $p = 0,014$ ) höher.

Zusammenfassend zeigt diese Studie, dass Roggen und RSM als Alternativen zu Weizen und SBM in Mischfutter für Absetzferkel eingesetzt werden können ohne bzw. nur mit geringgradigen Einbußen hinsichtlich des Wachstums und der Proteinverdaulichkeit und ohne eine Beeinträchtigung der Tiergesundheit. Tatsächlich könnte dabei die Einsatzmenge in der Ration höher sein als bisher empfohlen. Die Effekte auf Verdauungsphysiologie und intestinale Mikrobiota standen vor allem mit der unterschiedlichen Zusammensetzung der „dietary fibre“ der untersuchten Futterkomponenten in Zusammenhang. Der hohe Gehalt löslicher Faserstoffe in Roggen war zu einem höheren Grad abbaubar als Weizenfaserstoffe, verursachte eine erhöhte mikrobielle Fermentationsaktivität, aber führte nicht zu einer Verschiebung der Mikrobiota im Colon. RSM enthielt vermutlich einen zu hohen Anteil unlöslicher Faserstoffe, was zur verminderten Proteinverdaulichkeit und bakterieller metabolischer Aktivität führte und zu einer Erhöhung der relativen Häufigkeit von *Actinobacteria* und *Bacteroidetes*, die bekanntermaßen unlösliche Faserstoffe verwerten.

Dies gibt Anlass für künftige Studien, um weitere mögliche positive Eigenschaften von Roggen und RSM zu erforschen, sowie die optimale Einsatzmenge zu ermitteln und die Mechanismen, einschließlich der Interaktion zwischen Wirt und Mikrobiota, besser zu verstehen, welche die

## Zusammenfassung

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gezielte praktische Nutzung von Roggen und RSM statt Weizen und SBM in der Schweinefütterung ermöglichen.

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Ellner C, Wessels AG, Zentek J. Effects of Dietary Cereal and Protein Source on Fiber Digestibility, Composition, and Metabolic Activity of the Intestinal Microbiota in Weaner Piglets. *Animals* **2022**, 12, 109. doi: 10.3390/ani12010109.

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### Congress contributions

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Ellner C, Zentek J (**2022**). Effects of dietary rye and rapeseed meal on digestibility and transport physiology of crude protein and lysine in the small intestine of weaner piglets. The 7th EAAP International Symposium on Energy and Protein Metabolism and Nutrition (ISEP), 12.-15.09.2022, Granada, Spain. Animal Science Proceedings, Proceedings of the 7th EAAP International Symposium on Energy and Protein Metabolism and Nutrition, Elsevier, Volume 13, Issue 3, ISSN: 2772-283X

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The author declares no conflict of interest.



## **Selbstständigkeitserklärung**

Hiermit erkläre ich an Eides statt, die vorliegende Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet zu haben. Die Arbeit ist in dieser Form noch keiner anderen Prüfungsbehörde vorgelegt worden.

Carola Ellner

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