FEMS Microbiology Letters, 366, 2019, fnz029

doi: 10.1093/femsle/fnz029
Advance Access Publication Date: 15 February 2019
Minireviews

MINIREVIEWS – Food Microbiology

The potential of pectin to impact pig nutrition and health: feeding the animal and its microbiome

Maria Wiese[1,2,*,†]

[1]Department of Food Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg, Denmark and [2]CP Kelco ApS, Ved Banen 16, 4623 Lille Skensved, Denmark

[*]**Corresponding author:** Department of Food Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg, Denmark. CP Kelco ApS, Ved Banen 16, 4623 Lille Skensved, Denmark. E-mail: marwie@dtu.dk
Maria Wiese, http://orcid.org/http://orcid.org/0000-0002-1010-1927

Editor: Egon Bech Hansen

[†]Maria Wiese, http://orcid.org/0000-0002-1010-1927

ABSTRACT

The increasing efforts to substitute antibiotics and improve animal health combined with the acknowledgement of the role of gut microbiota in health have led to an elevated interest in the understanding on how fibre with prebiotic potential, such as pectin, can improve animal growth and health via direct or gut microbiota mediated effects. Various reports exist on the antiviral and antibacterial effects of pectin, as well as its potency as a modulator of the immune response and gut microbial community. Comprehensive insights into the potential of pectin to improve animal growth and health are currently still hampered by heterogeneity in the design of studies. Studies differ with regard to the dosage, molecular structure and source of the pectin implemented, as well as concerning the set of investigations of its effects on the host. Harmonisation of the study design including an in-depth analysis of the gut microbial community and its metabolome will aid to extract information on how pectin can impact growth and overall animal health. Studies with an increased focus on pectin structure such as on pectin-derived rhamnogalacturonan I (RG-I) are just starting to unravel pectin-structure-related effects on mammalian health.

Keywords: Gut Microbiome; Pig; Pectin; Short Chain Fatty Acids; Feed; Health

PECTIN IN ANIMAL NUTRITION AND HEALTH

Plant food has been consumed in the animal kingdom for millions of years and the mammalian gastrointestinal tract (GIT) co-evolved and adapted to pectin exposure. Pectin is a major building block of the cell wall of all land plants and displays a plethora of molecular structures. The three major pectic polysaccharides are homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) (Willats *et al.* 2001). The structure to function related mode of action of pectin in the GIT remains poorly understood. Addition of pectin to modern animal feed formulations has been studied for various animals such as fish, (Volpe *et al.* 2015; Irvin *et al.* 2016; Goulart *et al.* 2017) horses (reviewed in (Silva, de Rezende and da Silva Inácio 2016)), pigs (reviewed in (Drochner, Kerler and Zacharias 2004)), broilers (Wang *et al.* 2016), cows (Huhtanen 1988; Gressley and Armentano 2005), as well as dogs (Biagi *et al.* 2010) and cats (Barry *et al.* 2010). This review focuses on study examples of the impact of pectin on pig nutrition and health, performed *in vivo*, *ex vivo* and *in vitro*. Within this context, the review explores the potential of pectin to improve growth and health via direct and indirect mechanisms related to the modulation of the gut microbiome, the immune system and the elimination of pathogens and viruses.

Existing studies on pectin in animal feed display a vast heterogeneity in study design, which hampers the evaluation of its efficacy, as a feed ingredient for improved growth and health. Study variability relates to the specific pectin implemented, its

Received: 26 August 2018; **Accepted:** 13 February 2019

source, molecular characteristics such as the degree of esterification (DE), the degree of acetylation, fine branching of sugar structures and molecular weight. Further variability exists concerning the feed matrix and, its protein, fat and fibre ratios. The level of information provided on these aspects also differs between studies, ranging from none to relatively detailed information on a few of the substrates (see Table 1 for an overview). The inclusion level of pectin, the adaptation and study period, the experimental hygienic conditions and general farm practices are additional factors that differ between studies (Larsen 1980; Lagreca and Marotta 1985; Baekey *et al.* 1988; Potkins, Lawrence and Thomlinson 1991; Langhout *et al.* 1999; Laerke *et al.* 2001; Dongowski, Lorenz and Proll 2002; Zhu, Rademacher and De Lange 2005; Buraczewska *et al.* 2007; Zhu, Rademacher and de Lange 2007; Strube *et al.* 2015a; Tarasenko and Rud 2016; Wu *et al.* 2016; Tian *et al.* 2017).

Various parameters have been studied for the evaluation of pectin in feed as growth and health modulating ingredient, (see Table 1). Parameters include gut development (Hedemann *et al.* 2006; Buraczewska *et al.* 2007; Święch *et al.* 2012), more specifically muscle layer width in the intestine and motility of the duodenum and mid-jejunum (Buraczewska *et al.* 2007), plasma glucose, mucosal protein synthesis in the intestine (Libao-Mercado *et al.* 2007), intestinal morphology and enzymatic activity (Hedemann *et al.* 2006; Wu *et al.* 2016; Pluschke *et al.* 2018). Studies on the direct interactions of pectin with the mucosa exist mostly *ex vivo* (Schmidgall and Hensel 2002; Liu *et al.* 2005; Thirawong *et al.* 2007).

Investigations of the impact of pectin on the ion exchange and buffering capacity, digestibility of organic matter (Metzler-Zebeli *et al.* 2010; Tian *et al.* 2017), dry matter content in feces, amino acid digestibility (Buraczewska *et al.* 2007; Święch *et al.* 2012), digesta viscosity (Laerke *et al.* 2001; Buraczewska *et al.* 2007; Wu *et al.* 2016) and mineral absorption (Metzler-Zebeli *et al.* 2010), as well as effects of pectin on the profile and transport of intestinal bile acids (BA) (Fang *et al.* 2018) are valuable contributions to the understanding of the interactions of pectin with feed components and the animal's physiology (Table 1). Within this context, information on pectin source and its molecular structure is essential and is often missing even in recent studies. For instance, in the study by Fang *et al.* 2018, who presented a detailed analysis of the impact of pectin on the profiles and transport of bile acids in young pigs. They reported benefits of pectin supplementation for the cholesterol metabolism, as well as its impact on BA composition and upregulation of intestinal BA transporters and receptors. Unfortunately, the authors did not provide information on the molecular structure and source of the pectin substrate implemented. Furthermore, the analysis of the piglet GM would have been an interesting addition to the study. BAs are known to be metabolised by the GM (Sayin *et al.* 2013; Ridlon *et al.* 2014) and the correlation of the changes in the BA pool with potential pectin induced changes in the GM could provide information on such metabolism.

Characteristics of pectin such as the DE impact its viscous properties, that can affect daily feed intake (DFI) and nutrient absorption. Bruggeman *et al.* (2018) recently published an invention, that describes the energy redistribution in animals by pectin with a DE of less than 65%. The findings are explained by a change of the feed intake pattern, characterised by initial latency to feed intake followed by smaller but more frequent meals. The report indicates a role of pectin in the regulation of hunger and satiety (Bruggeman *et al.* 2018). When included in weaner diets, citrus pectin with a DE of 70% reduced daily feed intake and average daily gain (DG) as reported by Hedemann *et al.* 2006. Unfortunately, the study did not include investigations of the impact of pectin on the piglet GM and SCFA production (Hedemann *et al.* 2006). The DG, a measure of animal growth is valuable information.

Nevertheless, DG is not only affected by a varying DFI, and the absorption or retention of specific nutrients, it can also be influenced by the level of pectin fibre derived short chain-fatty acids (SCFAs). SCFAs are the end products of fermentation of dietary fibres by the GM and have been shown to exert multiple beneficial effects on mammalian energy metabolism, body weight and mammalian health (den Besten *et al.* 2013). It is hence essential to understand how different pectin substrates influence the endogenous microbial community, which is the source of SCFA production *in vivo*. Comprehensive studies with a detailed analysis of pectin associated modulation of the GM and associated metabolites, such as SCFAs in combination with data on DFI and DG for piglets and pigs are still missing.

Organic acids can also act as antimicrobials due to their pH lowering properties and are among the candidate replacements for antibiotics. They can increase pancreatic secretion and exhibit trophic effects on the gastrointestinal mucosa as reviewed in Dibner and Buttin 2002 (Dibner and Buttin 2002). These acids have been used in swine diets for decades (Partanen and Mroz 1999; Namkung *et al.* 2004) and several studies exist on the impact of pectin on SCFA levels (Lærke *et al.* 2007; Metzler *et al.* 2009; Metzler-Zebeli *et al.* 2010; Tian *et al.* 2017). Zacharias *et al.* have reported changes in SCFA levels in porcine ceacel and fecal matter after administration of apple pectin (Zacharias, Kerler and Drochner 2004). Metzler-Zebeli *et al.* found increased ileal d-lactate levels and an increase of the molar proportion of isobutyrate after ileal pectin infusion. In feces, pectin infusion tended to increase total SCFAs and acetate. The study did not include a comprehensive analysis of the whole microbial community, solely selected microbial populations were analysed by quantitative PCR in DNA extracts of ileal digesta, and a reduction in the ileal gene copy number of the *Clostridium leptum* cluster was reported (Metzler-Zebeli *et al.* 2010).

Recent advances in GM community analysis via 16S rRNA gene sequencing at a reduced cost allow for comprehensive investigations of GM community changes, as demonstrated by Tian *et al.* who investigated the changes of the whole GM community structure by the supplementation of weaner feed with low methoxyl pectin (LMP) and high methoxyl pectin (HMP). The inclusion of the citrus pectin led to a higher relative abundance of Bacteroidetes, whereas that of Firmicutes was lower in the colon and feces, but not in the ileum. An increased relative abundance of unclassified bacteria within the Lachnospiraceae family was found in the feces of both LMP- and HMP-fed piglets. The increase in relative abundance of unclassified microbes within the family Prevotellaceae also occurred in the colon of both the LMP- and HMP-fed piglets. Whereas, the increase in relative abundance of the *Dialister* genus in the colonic digesta was LMP specific. The SCFA patterns in the piglet feces were not significantly affected by the diet and intervention time (Tian *et al.* 2017). *In vivo* SCFA are absorbed by the host, it would be hence beneficial also to monitor the SCFA levels in the piglet plasma.

Understanding the microstructure related mode of action of pectin and its impact on the GM and its metabolites along the GIT is critical for the successful tailoring of pectin formulations for in feed applications. Fig. 1 depicts an overview of direct and indirect interactions of pectin with the GM as well as with the host- associated gut barrier and immune system.

Table 1. Pectin in pig feed *in vivo* study examples

Pectin in feed	Animal and or model	Observations, parameters under investigation	Reference
LMP, HMP citrus (Classic CU-L 020/13, Classic CU-L 021/13 Herbstreith & Fox, Neuenburg, Germany) at 3% w/w of basal diet, aSBM soy bean meal, control diet (CONT)	Piglet (Dutch Landrace × Large White) (mean 6.1 ± 0.02 kg) at weaning (age of 3 weeks) 4 piglets per diet for 28 days. Piglets were housed *ad libitum* in metabolic cages.	The LMP, HMP and aSBM, differently affected the digestion processes and shaped the colonic microbiota from a *Lactobacillus*-dominating flora to a *Prevotella*-dominated community. Pectin supplemented diets led to a higher relative abundance of Bacteroidetes, and reduced abundance of Firmicutes in the colon and feces, but not in the ileum. A LMP related increase in the relative abundance of the genus *Dialister* was detected in the colonic digesta. LMP was more efficiently fermented in the ileum than HMP, which was mainly fermented in the proximal colon. The protein digestibility was lower for LMP and HMP fed pigs than for CONT fed pigs throughout the ileum and the large intestine. In the large intestine, lowest digestibility of NSP was observed for aSBM-fed pigs, followed by those for LMP- and HMP-fed pigs. Overall, the SCFA patterns in the pig feces were not significantly affected by the diet and intervention time.	Effects of pectin on fermentation characteristics, carbohydrate utilisation and microbial community composition in the gastrointestinal tract of weaning pigs (Tian *et al.* 2017).
73, 104 or 145 g per/kg of DM (Genu pectin type B Rapid Set, CP Kelco) citrus pectin	50 pigs from 10 L weaned at 4 weeks of age (BW 8.6 ± 1.4 kg) and divided into 5 treatment groups. Pigs fed for 9 days, and were then euthanised, entire GIT was removed.	Pectin decreased ADFI and ADG, pectin decreased the area of mucin in the crypts of the small intestine. Shorter villi and crypts, unaltered villous height/crypt depth ratio, no dosage effect. Maybe indirect effect of ADFI, but could be a direct effect too. No effect on crypt depth in the colon. Increased luminal viscosity and water binding capacity (J. E. Lindberg, unpublished data), which may have slowed down digesta passage and increased the satiety of the pigs, leading to lower feed intakes. Citrus pectin included in the diets may have had a negative impact on taste, mouth feel of the diets or both.	Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fiber concentrations and fiber properties (Hedemann *et al.* 2006).
0.03% enzyme-to-substrate ratio, of the enzymes pectin lyase and polygalacturonase in combination with potato pulp	20 piglets (30 days of age) 2 days after weaning, the animals were of mixed breed and had been given a wheat-based creep feed since 7 days of age.	Using low doses, 0.03% enzyme-to-substrate ratio, of the enzymes pectin lyase and polygalacturonase in combination with potato pulp, a low-value industrial by-product, Strube *et al.* show that high molecular weight RG-I can be solubilised in the stomach of weaning piglets. The release of this fibre is in the order of 22%–38% of the theoretical amount, achieved within 20 min. The catalysis takes place mainly in the stomach of the animal and is then followed by distribution through the small intestines.	*In situ* prebiotics: enzymatic release of galacto-rhamnogalacturonan from potato pulp *in vivo* in the gastrointestinal tract of the weaning piglet (Strube *et al.* 2015a)
Cornstarch–SBM (Control) or Control with 12% pectin. Intravenously 1.5 mmol/kg BW of L-1-/react-text 13 react-text: 172 C valine (40 mol%)	12 barrows (21 kg mean BW)	Dietary pectin inclusion increased plasma levels of glucose, isoleucine and glutamine but had no effect on insulin or urea nitrogen. There were no differences in FSR (Fractional Synthesis Rate) and ASR (Absolute Synthesis Rate) of whole intestinal protein in jejunum and colon. The FSR of mucosal proteins in colon, not in jejunum, was increased with dietary pectin supplementation. Assuming mucosal protein mass is constant, these results imply that the higher protein synthesis in colon mucosa contributes to the reduced THR efficiency observed in pectin-supplemented diet.	Effect of feeding fermentable fiber on synthesis of total and mucosal protein in the intestine of the growing pig (Libao-Mercado *et al.* 2007).

Table 1. Continued

Pectin in feed	Animal and or model	Observations, parameters under investigation	Reference
Two experiments were carried out using a balanced feed mixture composed of wheat, corn, soyabean meals, casein, and crystalline amino acids (AA) and supplemented with 0, 4, or 8% of apple pectin.	Pigs (25–40 kg BW) with the post valve T-caecum (PVTC) cannula to determine AA digestibility and ileal digesta viscosity. Second experiment, the N-balance on pigs 20 and 28 kg BW six animals per diet and DFI and BW measured twice during the 28, 40 day	The added pectin decreased standardized ileal AA digestibility (on average up to 5% dig. units) and increased digesta viscosity from about 1 to 88 mPas. The higher pectin level did not affect motility of the duodenum and mid-jejunum, measured as responses to electrical field stimulation and to acetylcholine. However, the response of the duodenum to electrical field stimulation was after feeding the diet with 4% pectin increased compared with other diets. Pectin supplementation did not alter the weight and the length of the small intestine, but induced changes in the intestinal morphology. Muscle layer width increased significantly in the duodenum, the mid-jejunum, and the ileum while villi length increased in the duodenum and ileum. The addition of pectin decreased N retention and increased the F/G ratio but did not affect significantly the ADG of the pigs.	The effect of pectin on amino acid digestibility and digesta viscosity, motility and morphology of the small intestine, and on N-balance and performance of young pigs (Buraczewska *et al.* 2007).
Daily infusion of either 60 g of pectin dissolved in 1.8 L of water or 1.8 L of water as a control infusion	2 × 8 barrows growing pigs (BW 30.1 ± 1.3 kg) (German Landrace × Piétrain), the pigs were surgically fitted at the distal ileum with a simple T-cannula	In Pectin infusion reduced ($P = 0.005$) ileal gene copy number of the *C. leptum* cluster. Ileal bacterial populations and fermentation patterns are susceptible to changes in the intestinal availability of Ca and P as well as to the supply of pectin as a fermentable substrate.	Ileal microbiota of growing pigs fed different dietary calcium phosphate levels and phytase content and subjected to ileal pectin infusion (Metzler-Zebeli *et al.* 2010).
Cereal-based diets with 0, 40 or 80 g of Apple pectin (P) or 270 g of rye (R) per kg	Four groups (six pigs each), pigs of 15 kg BW	Digesta viscosity was increased more by 80 g than by 40 g P or by R. Ileal digestibility of AA and nitrogen retention were negatively affected by P, whereas growth performance was decreased by R. The effect of the supplements on intestinal morphology was variable, except for the increase in myenteron thickness by P and crypt depth by R. The number of goblet cells containing acidic mucins was decreased by 40 g P in crypts in the mid-jejunum and by 40 g P and R in villi in the ileum. Fasting and postprandial plasma levels of free threonine and of threonine dehydrogenase activity in the liver and pancreas were not affected. In conclusion, feeding P or R negatively affects ileal AA digestibility and provokes irregular changes of small intestinal morphology. These effects cannot be attributed to the increase of digesta viscosity as the main factor.	The effects of pectin and rye on amino acid ileal digestibility, threonine metabolism, nitrogen retention and morphology of the small intestine in young pigs (Święch *et al.* 2012).
Citrus Pectin (Genu pectin type B Rapid Set, CP Kelco)	150 castrated piglets, five diets with varying contents of citrus pectin (soluble fibre) and barley hulls (insoluble fibre)	There was no difference in SCFA caused by dietary treatment, but across treatments there was a correlation between concentration of SCFA and the proportion of butyrate. The correlation was lowest in the caecum and highest in the distal colon.	Association between butyrate and short-chain fatty acid concentrations in gut contents and faeces in weaning piglets (Lærke *et al.* 2007).
Mango pectin	30 large Whitemale pigs with an initial weight of ∼19 kg (10 pigs per group)	The rheology of ingredients does not necessarily reflect the rheological effect when ingested. Viscous property of pectin hinders amylase activity in pig stomach.	Rheological and microstructural properties of porcine gastric digesta and diets containing pectin or mango powder (Wu *et al.* 2016).
Pectin (0%–12% inclusion level) CP Kelco, Wilmington, DE	Two groups of eight Yorshire barrows, average BW of 14.3 ± 1.4 kg (Lysine study) and 17.2 ± 1.3 kg (Threonine study)	Utilisation of ileal digestible threonine, but not of ileal digestible lysin intake, for PD (protein deposition) decreased linearly with dietary pectin level, and was not influenced by diet cellulose level.	Increasing dietary pectin level reduces utilization of digestible threonine intake, but not lysine intake, for body protein deposition in growing pigs (Zhu *et al.* 2005).

Table 1. Continued

Pectin in feed	Animal and or model	Observations, parameters under investigation	Reference
0.5 and 10% pectin	Weaned piglets three groups of six castrated male crossbred pigs with an average BW of 8 kg were fitted with T-cannulas at the caecum fed with diets supplemented with 0%, 5% and 10% pectin. Faeces were collected over a period of 3 days.	Addition of 5% pectin to the diet lowered the content of dry matter and lactic acid in fecal matter. The pH and the digestibility of pectin, the concentration of total SCFA, acetate, propionate, butyrate, bicarbonate and chloride increased. Dietary pectin of 10% increased the content of total SCFA and acetate further. It was concluded that pectin might be beneficial for the development of fermentative processes in the large intestine.	The influence of 5% and 10% dietary apple pectin on parameters of fermentation in faeces and caecal digesta of weaning pigs (Zacharias *et al.* 2004).
4 Citrus pectins with DE (DM) 80, 60%, 46%, 5% Two citrus pectins classified as calcium sensitive (CAS) and calcium insensitive (CAN) and SBP were tested.	Piglets (eight per group, n = 32 for control animals) were weaned at 4 weeks of age. Killed 7 weeks of age.	Dissimilarity of viscosity between water and GI due to GI juice interaction with pectin structures. DM46 and DM 80 reduced digestibility of organic matter, no difference of digestibility was seen for DM 60, DM5 CAS and CAN compared to the control group. Concluding that the rheological characteristics of pectins in water differ in the GIT.	29 Isolated Pectins: vary in their Functional Properties in the Gut of Piglets (Laerke *et al.* 2001).
Grapefruit pectin	18 female miniature Swine of the Pitman–Moore strain	Grapefruit pectin supplementation inhibits hypercholesterolemia and appears to be proportionately protective against atherosclerosis.	Grapefruit pectin inhibits hypercholesterolemia and atherosclerosis in miniature swine (Baekey *et al.* 1988).
Control treatment: Corn-SBM basal diet + ZnO (phase 1: 0.05%; phase 2; 0.03%) and four different levels of sugar beet pulp were supplemented in Corn-SBM basal diet (3%, 6%, 9% or 12%). Two phase feeding programs (phase 1: 1–2 weeks; phase 2: 3–5 weeks) were used for 5 week of growth trial.	200 weaning pigs [(Yorkshire × Landrace) × Duroc], averaging 9.01 ± 1.389 kg. Each treatment was composed of four replicates with 10 pigs per pen.	No significant differences in growth performance and incidence of diarrhea among treatments. Linear response was observed in *Lactobacillus* counts as sugar beet pulp supplementation increased ($P < 0.05$). In addition, IGF-1, IgA and IgG were not affected by dietary treatments. However, the BUN concentration was decreased when pigs were fed the treatments of diets with SBP compared to that of control treatment ($P < 0.05$). In nutrient digestibility, crude fiber and NDF digestibilities were improved as the sugar beet pulp increased ($P < 0.05$). However, digestibilities of crude ash, crude fat, crude fiber and nitrogen retention were not affected by dietary sugar beet pulp levels.	Effect of Dietary sugar beet pulp supplementation on growth performance, nutrient digestibility, fecal microflora, blood profiles and diarrhea incidence in weaning pigs (Yan *et al.* 2017).
40 days on one of four diets differing in the level and type of polysaccharide. Control diet with or without 4 or 8% pectin or a diet with rye instead of part of the wheat.	24 male growing pigs with a high lean gain potential (synthetic line 990, Poland), divided into four groups (six pigs each) initial BW of about 20 kg	The influence of polysaccharidesonthe epithelial structure was evaluated in the duodenum, mid-jejunum and ileum. Increased crypt depth and mucosa thickness were found after feeding the diet with rye in all measured segments, whereas feeding the diets with 4% and 8% pectin caused an increase in myenteron thickness of the mid-jejunum and ileum, in comparison with other diets.	The structure of small intestinal tissue in pigs as influenced by indigestible polysaccharides (Tusnio *et al.* 2006).
Pectin was supplied at levels of 0 and 334 g dry matter day−1 together with a semi-synthetic basal diet to. In addition to Pectin treatments two different levels of thiamine, 0.66 and 2.57 mg day−1 were applied.	12 adult sows weighing 166 ± 17 kg	The pectin fermentation rate was determined by three different methods s to account for 94%–96% of pectin intake. The apparent digestibility of nitrogen was reduced by more than 20% at an almost constant true N digestibility. The increased fecal nitrogen excretion with pectin caused a reduction in protein retention to zero, and was composed of 82% bacterial nitrogen. The changes in the proteinaceous water-soluble fraction of fecal nitrogen indicated an increase in the excretion of endogenous proteins.	Effects of pectin supplementation on the digestion of different structural carbohydrate fractions and on bacterial nitrogen turnover in the hindgut of adult sows (Roth-Maier *et al.* 1993).

Table 1. Continued

Pectin in feed	Animal and or model	Observations, parameters under investigation	Reference
Sugar beet fibre	Two groups of pigs/ 36 female weanling piglets (14 ± 2 kg) of the Large White breed	After 4 week, total serum cholesterol and high-density-lipoprotein cholesterol concentrations were similar on both diets. By contrast, the fasting triacylglycerols were 21% lower ($P < 0.05$) and apparent feed-conversion efficiency was 47% higher ($P < 0.01$) on the SBF diet than on the control diet. Accordingly, the effect of SBF did not appear to be mediated by an impairing effect on dietary lipid absorption. The results suggest that the decreasing effect of SBF on triacylglycerols was due to a reduction in very-low-density-lipoprotein synthesis without changes in the size of particles. The low-density-lipoprotein receptor activity of a liver plasma membrane-enriched fraction was not influenced by the dietary treatment; however, a significant negative relationship between cholesterol concentrations and the receptor activity was observed irrespective of the diet.	Effects of sugar beet fiber feeding on serum lipids and binding of low-density lipoproteins to liver membranes in growing pigs (Fremont, Gozzelino and Bosseau 1993).
Each group of piglets was fed with corn–soybean meal diets containing 5% apple pectin (PEC, purchased from Yuzhong Biotech Corporation, Henan, China) or cornstarch (purchased from Yufeng Cornstarch and Sugar Company, Hebei, China) as the control (CON)	Male Duroc × Large White crossbred piglets with an average body weight of 11.05 ± 0.11 kg were randomly divided into 2 groups of 6 animals each and allowed to adapt for 1 week before the start of the 72-day experiment.	Growth performance was not affected by the inclusion of 5% pectin in the conventional corn–soybean meal diet over the 72-d trial period n pigs fed with pectin, total cholesterol and low-density lipoprotein cholesterol were lowered but high-density lipoprotein was increased ($P < 0.05$). Serum triglycerides tended to be lower in the pectin-fed animals ($P = 0.093$), whereas no change was noted in serum total bile acid. Bile acid pool: In pigs fed with 5% pectin, only cecal ursodeoxycholic acid ($P = 0.097$) and hyocholic acid ($P = 0.088$) showed a decreasing tendency. In the ileum, pectin increased in-and-out BA transport on the apical membrane, but it increased the overall BA transport in the cecum.	Effects of dietary pectin on the profile and transport of intetinal bile acids in young pigs (Fang *et al.* 2018).

Feeding the animal and its microbiome: gut microbiota related challenges in pig production

Neonatal piglet diarrhea caused by pathogens or viral infections is leading to considerable economic losses in the pig industry (Wittum *et al.* 1995; Wieler *et al.* 2001; Jacobson *et al.* 2003). Diarrhea impairs the welfare of the animals in the short term and may disrupt the normal bacterial succession in the GIT and hence affect animal health in the long run as well. There is increasing pressure to replace the antibiotics and zinc oxides, that are implemented to counteract diarrhea in pig production. This pressure is leading to increased efforts to explore other avenues to promote gut health, such as the implementation of pro- and prebiotics (Brüssow 2017; Li 2017), which are thought to strengthen and stabilise gut health via a balanced gut microbiome. Bacteria colonise the GIT from birth onwards (Kenworthy and Crabb 1963). This colonisation impacts the maturation of the GIT, the immune system and its capacity to defend against pathogens (Bezirtzoglou 1997; Ley *et al.* 2008). Mammalian milk plays a vital role in the nurturing of a healthy GM. The milk oligosaccharides (MOs) are a group of diverse and complex glycans (≈200 structures) and an abundant component of the mammalian milk. MOs evolved to nurture the mammalian GM, nursing beneficial microbes and inhibiting pathogens. These glycans thus play an essential role in the establishment of health during early life (Sela and Mills 2010). They are known to lower the risk for viral, bacterial, protozoan, parasite infections and necrotising enterocolitis (Jantscher-Krenn *et al.* 2011). Porcine milk oligosaccharides (PMOs) are the representatives of these oligosaccharides in pigs (Poroyko *et al.* 2010). In earlier farm practice piglets were weaned spontaneously after 10–12 weeks. In current days piglets are weaned at the age of 3–5 weeks, a time when the sows produce the maximum amount of milk. The sudden weaning results in various adverse reactions such as diarrhea (Nabuurs 1998). The absence of PMOs contributes to these effects, through the weakening of the endogenous GM and reduced pathogen defence.

It is, therefore, necessary to use prebiotic ingredients as components in milk replacers and weaner feed formulations. Examples of current alternatives for PMOs are galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS). Mannan oligosaccharides have also been shown to promote health in piglets (Castillo *et al.* 2008; Che *et al.* 2011). Whereas, Chen *et al.* demonstrated the improvement of growth performance and immunity after administration of dietary pectic oligosaccharides (POS) in weaned pigs infected by rotavirus (Chen *et al.* 2017). Unfortunately, the study did not include an analysis of the impact of POS on the whole piglet microbiome. The inclusion of specific POS in weaner formulations might aid to mimic PMOs and promote GIT health during animal production at an early age.

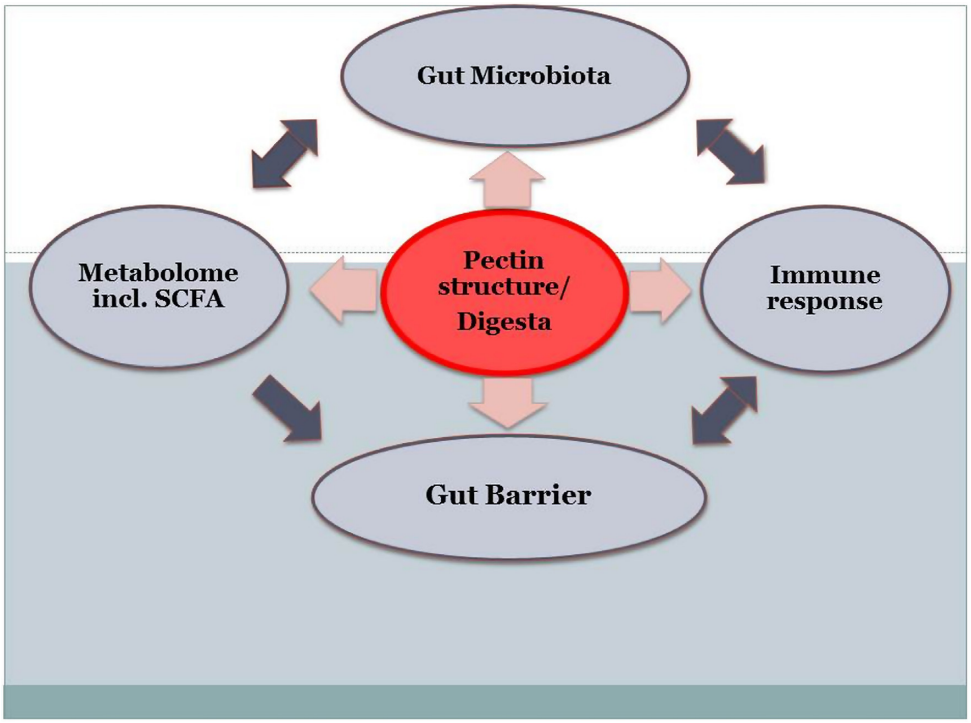

Figure 1. Overview of direct and indirect interactions of pectin within the gut. Interactions are indicated with arrows. The host and gut barrier are displayed as coloured background..

POS with varying degrees of polymerisation can be obtained via the hydrolysis of α and β-glucosidic links of the principal chains of HG, RG-I and RG-II and their side chains (Gullón *et al.* 2011). POS have been suggested as a new class of prebiotics (Hotchkiss Jr *et al.* 2003) and this subject has been recently reviewed in detail in 'Pectic oligosaccharides and other emerging prebiotics' by Míguez *et al.* (2016). Various *in vitro* studies have shown evidence for the prebiotic effects of POS when fermented by human fecal inoculum (Olano-Martin, Gibson and Rastall 2002; Suzuki *et al.* 2004; Al-Tamimi *et al.* 2006; Holck *et al.* 2011; Onumpai *et al.* 2011; Chen *et al.* 2013; Gómez *et al.* 2014; Moon *et al.* 2015; Gómez *et al.* 2016; Li *et al.* 2016). A recent study examined fermentability of POS from sugar beet pulp with human and pig fecal inoculum (from multiparous non-lactating sows, Dutch Landrace) *in vitro*. The study demonstrated lowered degradation of acetylated RG oligosaccharides by pig fecal microbiota (Leijdekkers *et al.* 2014). Few studies exist to date on POS in animal feed applications (Wang *et al.* 2016; Chen *et al.* 2017), also due to current limitations in the production of POS. These are hence just the beginnings of the exploration of POS for in feed applications. *In vitro* models such as the CoMiniGut model (Wiese *et al.* 2018) operating at a low fermentation volume, requiring only small amounts of substrate are ideal for the initial investigation of novel pectic substrates just available in small quantities.

Diet is a principal factor in shaping the GM composition and functionality (Scott *et al.* 2013). Depending on the age of the animal target group and the associated maturity and carbohydrate degradation capacity of the target group's GM, different pectin substrates can be matched for in feed applications. In a grown mammal the matured GM has an expanded capacity to degrade more complex carbohydrates and continues to have a profound influence on its nutrition, organ, tissue and immune system (O'Hara and Shanahan 2006). The GM provides a large set of metabolic functions for the host, that go beyond the production of SCFA, it also plays a role in the synthesis of K- and B-vitamins, the protection from pathogenic bacteria through competitive exclusion, stimulation of enterocyte turnover (Sekirov *et al.* 2010; LeBlanc *et al.* 2013) and assistance in development and maturation of the GIT and the mucosal system (Sommer and Bäckhed 2013). Emerging studies such as the study by Ramayo-Caldas *et al.* 2016 provide an integrated overview of the porcine GM and can identify microbial enterotypes associated with specific traits such as growth. An enterotype is a robust microbial cluster, driven by species composition including a functional understanding of the overall community (Arumugam *et al.* 2011). An enterotype-like group with a significant over-abundance of genera such as *Prevotella* and *Streptococcus* was reported to be correlated to increased body weight and average daily weight gain during the post-weaning period (Ramayo-Caldas *et al.* 2016). The study would have benefited from additional measurements and correlations of SCFA levels in colon content, feces and plasma with the identified enterotypes and body weights.

In vitro and *ex vivo* studies on the effects of pectin on the gut microenvironment

Several *ex vivo* studies investigated the mucoadhesive effect of pectin using porcine mucosa tissue or material, from different segments of the GIT, such as the buccal, stomach, small intestine and the large intestine. Mucoadhesion facilitates contact with the gut epithelium and may aid in the modulation of the immune response (Furness *et al.* 2013). It may also be used for drug delivery solutions (McConnell, Fadda and Basit 2008; Wong, Colombo and Sonvico 2011), as well as for the topical coating of the damaged intestinal wall in inflammatory diseases. The mucoadhesive properties of pectin have been shown to differ between different GIT sites and also vary based on molecular

properties such as the net charge, molecular weight and DE (Schmidgall and Hensel 2002; Liu *et al.* 2005; Thirawong *et al.* 2007). An overview of studies is displayed in Table 2.

The mucosal glycome can differ based on genetic factors as well as the developmental stage of the animal (Hesselager *et al.* 2016; Priori *et al.* 2016). In the future, knowledge should be also acquired on the mucoadhesive properties of pectin in the GIT of animals of varying age.

There is increasing acknowledgement and development of advanced *in vitro* screening tools for the investigation of digestive processes and gut microbial fermentation dynamics *in vitro* reviewed in Macfarlene & Macfarlane and Payne *et al.* (Macfarlane and Macfarlane 2007; Payne *et al.* 2012). Several studies have investigated the effect of pectin on pig associated gut microbial communities *in vitro* (Sunvold *et al.* 1995; Van Laere *et al.* 2000; Bauer *et al.* 2001; Jonathan *et al.* 2012; Leijdekkers *et al.* 2014; Taciak *et al.* 2015). Examples are given in Table 2. *In vitro* studies such as by Bauer *et al.* who tested the microbial activities of feces from unweaned and adult pigs, reported significant differences in the fermentation patterns of carbohydrates both due to the source of substrate, as well as the inoculum source and age (Bauer *et al.* 2001). There is hence a potential to implement *in vitro* tools for a pre-selection of pectin substrates based on animal target group specific GM activity. Initiatives, such as the one by the european infogest community, (www.cost-infogest.eu), to harmonise and validate *in vitro* simulation protocols (Egger *et al.* 2016), with *in vivo* data in pigs (Egger *et al.* 2017; Portmann *et al.* 2017), are currently setting the stage for an increased application of *in vitro* models in research on gut microbial processes, avoiding the high costs and ethical constraints of *in vivo* studies. The lack of ethical constraints, for instance, facilitates the possibility to study the potential efficacy of carbohydrates to reduce pathogen load (Martín-Peláez *et al.* 2010). The reduced cost of *in vitro* screening tools allows the investigation of the bioavailability of pectin within different feed matrices. The model-driven experimentation using a combination of *in vitro* gut fermentation and *in vitro* cell, tissue or mucoadhesion models (for examples see Table 2) can lead to an advanced strategy to identify structure-related modes of action of pectin on the gut microenvironment before *in vivo* studies. The novel *in vitro* methods should be implemented to expand knowledge on pectin compounds and their potential to improve animal growth and health via the modulation of the GM and its metabolome.

THE POTENTIAL OF PECTIN TO CONTROL AND ELIMINATE PATHOGENS

The gut microbiota plays a pivotal role in the inhibition of pathogen growth in the gut lumen right from the beginning of life. The phenomenon is referred to as colonization resistance and efficiently blocks infections by *Clostridium difficile*, *Salmonella* spp. and many other pathogenic bacteria. Addition of fermentable fibre such as pectin to pig diets may stimulate growth and metabolic activity of beneficial bacterial species in the GIT (Van der Waaij, Berghuis-de Vries and Lekkerkerk-Van der Wees 1971; Berends *et al.* 1996; Schley and Field 2002; Knudsen, Lærke and Hedemann 2008). Furthermore, various studies have demonstrated the antiadhesive properties of pectin. For example, it has been shown that POS purified from the root of *Panax ginseng* displays selective inhibitory effects against the adhesion of *Helicobacter pylori* to gastric epithelial cells (Lee *et al.* 2006). Rhoades *et al.* 2008 have reported orange peel derived POS – mediated inhibition of the adhesion of pathogenic *Escherichia coli* strains to gut epithelial cells *in vitro* (Rhoades *et al.* 2008). The use of antiadhesives can aid in pathogen control and represents an alternative to antibiotic therapy. Decoy oligosaccharides bind to the microbe's carbohydrate-binding proteins, and pathogens are cleared by the bulk fluid movement in the GIT (Zopf and Roth 1996; Sharon and Ofek 2000). Reports also exist on the capacity of pectin substrates to inhibit pathogen-derived toxins (Olano-Martin *et al.* 2003). The potential of pectin to limit bacterial infections via the stabilisation of a healthy microbiome as well as via decoy mechanisms should be further explored.

ANTIVIRAL PROPERTIES OF PECTIN

Rotavirus infection is one of the main causes of gastroenteritis and diarrhea in young animals (Chung *et al.* 2017; Yodmeeklin *et al.* 2017). A few studies have reported the antiviral properties of pectin. As early as 1947 Green *et al.* investigated a number of simple and complex carbohydrates containing large amounts of galacturonic acid for their ability to inhibit agglutination of chicken red blood cells by influenza A virus. Apple pectin was found (Green and Woolley 1947) to be able to inhibit virus hemagglutination and virus multiplication in embryonated eggs. Whereas, the hemagglutination-inhibiting citrus pectin, did not reduce the multiplication of the virus (Green and Woolley 1947; Woolley 1949). The application of pre and probiotics against rotaviral infections have been recently reviewed by Gonzalez-Ochoa *et al.* (Gonzalez-Ochoa *et al.* 2017). Fibres with prebiotic potential may improve the generalised antiviral response and may reduce the rotaviral infectivity leading to a shorter duration and severity of rotavirus-associated diarrhea (Gonzalez-Ochoa *et al.* 2017). Recent reports suggest antiviral properties of POS. Rigo-Adrover *et al.* 2017 have demonstrated, that a mixture of prebiotics scGOS, lcFOS, POS and heat-treated probiotics in fermented milk components, has led to reduced clinical signs in suckling rats with rotavirus (RV)-induced diarrhea (Rigo-Adrover *et al.* 2017). Decreased viral shedding may be attributed to the interaction of prebiotics with the viral particles, avoiding the entry into enterocytes and therefore reducing its replication. Other potential mechanisms are reviewed in Gonzalez-Ochoa *et al.* (Gonzalez-Ochoa *et al.* 2017). Chen *et al.* recently reported that the dietary POS supplementation could improve the growth performance and immunity in weaned pigs infected by porcine rotavirus (PRV), possibly because POS administration improved the immune function and the utilisation of nutrients in the PRV-infected piglets (Chen *et al.* 2013).

MODULATION OF THE IMMUNE RESPONSE

Complex interactions among diet, external pathogens and local immunological and non-immunological processes are constantly integrated in the GIT. The consumption of fibre can affect the immune system directly and via changes in the gut microbial community composition. The GM can mediate immune changes through the contact of bacteria with immune cells, the production of SCFA or by changes in the synthesis of mucin. Fermentable dietary fibre can modulate various properties of the immune system, including those of the gut-associated lymphoid tissues, secondary lymphoid tissues and peripheral circulation, effects of dietary fibre on the immune-system have been reviewed by Schley and Field (Schley and Field 2002). The structural features of the pectin macromolecule determine its effect on the immune system. Popov and Ovodov 2013 have reviewed

Table 2. *In vitro* (and *ex vivo*) studies investigating the effect of pectin on the gut microenvironment with focus on pigs.

In Vitro Model	Pectin/substrate	Observations	Reference
In vitro: pig colonic digesta (C) or faeces (F) Batch: 24 or 48 hr, no pH control	Apple pectin from ZPOW Pektow in Sp. z o.o. (Jasło, Poland) Potato protein and casein were fermented each with cellulose, pectin or raw potato (resistant) starch.	The total SCFA concentration was greater after C than F fermentation, regardless of the substrates. The total amines concentration after C and F fermentation of potato protein with all fibres was the same, while after C fermentation of casein it was affected by the type of fibre ($P = 0.001$ and $P = 0.000$ after 24 and 48 hr, respectively). It was very high with cellulose, lower with starch and the lowest with pectin. Concluding that *in vitro* bacterial proteolysis is greatly affected by the interaction of type of protein and fibre.	The effects of type of protein and fibre fermented *in vitro* with different pig inocula on short-chain fatty acids and amines concentrations (Taciak *et al.* 2015).
In vitro: pig and human fecal inocula: Pig Fecal Inoculum. Three multiparous non-lactating sows (Dutch Landrace, aged 4 years) Pooled fecal inoculum	Sugar beet pectic oligosaccharides SBPOS consisted mainly of partially acetylated RGOS and partially methyl-esterified/acetylated HGOS. Some SBPOS contained an unsaturated galacturonic acid residue at their non-reducing end.	SBPOS could be completely fermented by human and pig fecal microbiota, thereby producing butyrate yet mainly acetate and propionate as metabolites. The degradation of SBPOS by pig fecal microbiota was different and much slower compared to human fecal microbiota. In general, RGOS were degraded slower than HGOS. Acetylation of RGOS lowered the degradation rate by pig fecal microbiota but not by human fecal microbiota. No classic bifidogenic effect was shown for SBPOS using human fecal inoculum. However, several other potentially interesting modifications in the microbiota composition that can be associated with host health were observed.	*In Vitro* Fermentability of Sugar Beet Pulp Derived Oligosaccharides Using Human and Pig Fecal Inocula (Leijdekkers *et al.* 2014).
In vitro: single strain fermentations: 18 bacterial strains most strains were of human origin; two strains originated from porcine feces: *Lactobacillus acidophilus L. fermentum swine*	Soy arabinogalactan (AGPS), sugar beet arabinan (AOS), wheat flour arabinoxylan, polygalacturonan, rhamnogalacturonan from apple, FOS	*Lactobacillus acidophilus L. fermentum* (Porcine origin) degraded FOS and AGPS partially. Overall: AOS of DP 2–6 were fermented by *Bi. longum* and *C. clostridiiforme*. *E. coli* and *B. adolescentis* fermented only arabinotriose while *Clostridium beijerinckii* and *Clostridium sartagoformum* only degraded the arabinotriose and arabinotetraose to some extent. *Clostridium butyricum* degraded only arabinotetraose were unable to degrade the oligosaccharides. Rhamnogalacturonan-Enriched Polysaccharide Fraction (RGAPS) and (Rhamno)galacturonooligosaccharides ((R)GAOS).	Fermentation of plant cell-wall-derived polysaccharides and their corresponding oligosaccharides by intestinal bacteria (Van Laere *et al.* 2000).
In vitro: pig fecal inoculum from feces of three multiparous sows (Dutch Landrace). BW 264–368 kg. fed twice daily with a high-fat (18.3% (w/w)), low-fibre (7.1% (w/ w)) diet, the diet contained wheat (20% (w/w)) and barley (20% (w/w)) as fibre sources. Human inoculum faeces of three healthy donors	High methyl esterified (HM) citrus pectin (C74) from CP Kelco (Lille Skensved, Denmark) other fibres were: Guar gum, alginatye, tapioca starch, glucomannan. Maize starch, oat b-glucan, Inulin, FOS, soy pectin (Soyafibe-S-DA-100), xanthan gum.	Pig inoculum produced less SCFA than human inoculum for most of the fibres. Both inocula produced the highest amount of total SCFA from soy pectin (9.06–10.59 mmol/g). Fibres containing uronic acids induced the production of acetate, whereas fibres containing neutral sugars induced the production of propionate or butyrate. Except pectin and xanthan gum, the soluble fibres in this experiment were well fermented by both inocula with less than 5% (w/w) of the fibres remaining after fermentation. For xanthan gum, the analysis of sugar composition of the remaining soluble sugars showed that human inoculum did not ferment 13% of all glucose, 16% of all mannose and 14% of all glucuronic acid. For pig inoculum, it was shown that 30% of all glucose, 41% of all mannose and 54% of all glucuronic acid were not fermented.	*In vitro* fermentation of 12 dietary fibres by faecal inoculum from pigs and humans (Jonathan *et al.* 2012).

Table 2. Continued

In Vitro Model	Pectin/substrate	Observations	Reference
In vitro: incubated 6, 12, 24, 48 hr with ruminal fluid from cattle or feces from dogs, pigs, horses and human	Cellulose (Solka Floc), beet pulp (Michigan sugar), citrus pulp (Freeman Industries) and citrus pectin (HM rapid; TIC Gums Belcamp, MD)	When data were pooled across all species and substrate disappearance and SCFA production ranked from the least to greatest in the following order: cellulose< beet pulp< citrus pulp< citrus pectin. The fermentability of different fibrous substrates by fecal or ruminal microbiota from various species seem to be dependant not only on the fermentative activity of the microbial population but other factors as well, perhaps lag time and rate of digesta passage.	*In vitro* fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using faecal inoculum from cats, dogs, horses, humans, and pigs and ruminal fluid from cattle (Sunvold *et al.* 1995).
In vitro: faeces from the adult pigs from four castrated finisher pigs (Dutch Landrace × Great Yorkshire. The unweaned piglet faeces were collected from 19 unweaned piglets (male and female) which were 27 to 30 days old	Pectin	Comparison of *in vitro* microbial activity was made between inocula from faeces of adult and unweaned pigs, using a range of carbohydrate-rich substrates. The substrates tested were classified into groups (fibre-rich, grains, gums, pectin, saccharides, storage carbohydrates and miscellaneous). There were significant differences in the fermentation patterns both due to source of substrate and inoculum. It would appear that the metabolic activity of the microflora does differ significantly between adult and weanling pigs, though this varied for the different carbohydrates tested.	Microbial activities of faeces from unweaned and adult pigs, in relation to selected fermentable carbohydrates (Bauer *et al.* 2001).
In vitro: gut contents of piglet terminal ileum	Potato RGI	The fibers showed high fermentability, with lactate in particular being increased. A significant increase in the numbers of *Lactobacillus* and *Veillonella* organisms and an insignificant increase in the numbers of *Clostridium* organisms as well as a decrease in the numbers of *Streptococcus* organisms was observed. Multivariate analysis showed clustering of the treatment groups, with the group treated with purified potato fibre being clearly separated from the other groups, as the microbiota composition was 60% *Lactobacillus* and almost free of *Clostridium*.	*In situ* prebiotics for weaning piglets: *in vitro* production and fermentation of potato galactorhamnogalacturonan (Strube *et al.* 2015b).
Ex vivo: porcine GI mucosa, i.e. buccal, stomach, small intestine and large intestine, texture analyser with mucoadhesive platform. Buccal mucosa with simulated saliva fluid pH 6.75 (SSF), gastric mucosa with simulated gastric fluid USP without pepsin (SGF) or citric-phosphate buffer, pH 4.8 (representing the fasted or fed state, respectively), small intestinal mucosa (duodenum part) and large intestinal mucosa with simulated intestinal fluid pH 6.8 (SIF)	Four pectins with different DEs Herbstreith & Fox KG (Germany) HMP (CU201 70% De, MW 200 kDa, 0% DA), CU501 56% De 0% DA, Mw 180 kDa), LMP (CU701 (38% De, Mw 80 kDA, 0% DA CU020 29% De, 20% DA, Mw 150 kDA).	Two parameters derived from texture analysis, namely maximum detachment force (Fmax) and work of adhesion (Wad) were used as parameters for comparison of mucoadhesive performance. Pectins showed a stronger mucoadhesion on large intestinal mucosa than on small intestinal mucosa. The mucoadhesive properties of pectins on gastric mucosa depended on pH of the medium, a higher Fmax and W ad in a pH 4.8 medium than a pH 1.2 medium was revealed. The results also demonstrated that the mucoadhesive performance of pectins largely depended on their characteristics, i.e. higher degree of esterification and molecular weight gave a stronger mucoadhesion. The rank order of mucoadhesive performance of examined pectins on to the GI mucosa appeared to be similar to the rank order of their degree of esterification and molecular weight (i.e. CU201 > CU501 > CU020 > CU701).	Mucoadhesive properties of various pectins on gastrointestinal mucosa: An in vitro evaluation using texture analyzer (Thirawong *et al.* 2007).

Table 2. Continued

In Vitro Model	Pectin/substrate	Observations	Reference
Ex Vivo: In an ex vivo system based on porcine colonic tissue	Rhamnogalacturonan (RG) derived from pectin.(apple pectin, citrus fruit pectin and pectin acid from Roth (Karlsruhe, Germany) and sugar beet pectins Sûdzucker AG (Obrigheim, Germany).	Rhamnogalacturonans with a low degree of esterification and linear oligogalacturonids derived from pectin showed significant bioadhesion against colonic mucous membranes. In contrast, highly esterified pectins and neutral polysaccharides were ineffective. Within a structure activity relationship linear, strongly acidic homogalacturonides were shown to be most adhesive agents. Esterification, branching or non-linear backbone structures will reduce the adhesive properties. The bioadhesive effects were concentration-dependent.	Bioadhesive properties of polygalacturonides against colonic epithelial membranes (Schmidgall and Hensel, 2002).
The *ex vivo* studies on the mucosal adsorption of pectins and incorporated proteins were conducted on the surfaces of porcine colonic mucus membranes	Citrus pectins with the DE of 25% (P-25) or 94% (P-94), Pectin derivatives carrying side chain primary amine groups (P-N) were prepared from P-25.	Pectins with higher net charges were more mucoadhesive than the other pectins. Both the negatively charged pectin formulation, P-25, and the positively charged formulation, P-N, were able to synergise with the mucus to produce rheologically strengthened gels. The highly esterified pectin, P- 94, also synergised with the mucosal glycoproteins to form a gel structure via coil entanglements. When incubated with porcine intestinal mucus membrane, P-94 gels were found generally bound to the lumen area, P-25 gels were able to penetrate deeply near the wall area, P-N gels interacted with mucins via electrostatic bonding and dispersed into the whole area from the lumen to the wall. The mucin–gel complex formed between P-N or P-94 and mucus tissue constructs a surface barrier, which effectively prohibited the penetration of Triton X-100. P-25 gel did not inhibit Triton X-100 irritation of the mucus tissue as effectively as the gel formed by P-N/mucus complex	Interaction of various pectin formulations with porcine colonic tissues (Liu *et al.* 2005).
In vitro assay	Six pectin types from three manufacturers (Pectin classic, Genu Pectin, Grinsted Pectin) The factors which varied were; DM (three levels; high, low and medium), degree of amidation (DA) (two levels; ami-dated or nonamidated). Due to the manufacturing process, the molecular weight was correlated with the DM.	Mucoadhesive properties were measured using a texture analyzer. It was found that an intermediate degree of methoxylation (35% and 36%) improved the specific mucin interaction. Amidation did not increase mucin interaction. Samples from different manufacturers did not alter these conclusions. This study indicates that the general classification of pectin as a poor mucoadhesive, without differentiating between the amount and type of substituents, probably is an oversimplification.	*In Vitro* measurements of mucoadhesive properties of six types of pectin (Hagesaether and Sande 2007)

the poly-potency of pectin substrates as immunomodulatory agents, that can both stimulate and suppress the immune response (Popov and Ovodov 2013). Recent reports demonstrated the potential of citrus pectin to attenuate an endotoxin shock via the suppression of a toll-like receptor signalling in payer's patch myeloid cells (Ishisono, Yabe and Kitaguchi 2017).

Furthermore, Tang *et al.* 2017 reported a unique phenotype of macrophages treated with RG-I from apple pectin (Tang *et al.* 2017). Such publications illustrate the expansion of knowledge of the potential of pectin substrates to be implemented for the modulation of the mammalian immune response. These studies are providing additional guidance for the tailoring of pectin formulations for in feed applications.

STRUCTURE AND FUNCTION: EXAMPLE RG I

Aside from the above-mentioned studies by Tang *et al.* 2017, further studies exist on the direct effects of RG-I on the immune system via immunomodulation of the activity on Peyer's patches and macrophages (Grønhaug *et al.* 2011), stimulation of macrophages (Ho *et al.* 2015), inhibition of colon cancer

cell proliferation and cell cycle progression (Cheng *et al.* 2013), prevention of tumor cell migration and cell-to-cell and cell-to-substratum adhesion (Platt 2009). A recent study examined the digestion of RG-I from potato pulp simulating the upper piglet GIT *in vitro*. The potato RG-I was highly fermentable by terminal ileal piglet gut content *in vitro*, reflected by a dose-dependent decrease in pH and an increase in the organic acid content, in particular lactate. Analysis of the GM in fermentates revealed a significant increase in the numbers of *Lactobacillus*, *Veillonella* and *Clostridium,* as well as a decrease in the numbers of *Streptococcus* (Strube *et al.* 2015b). Strube *et al.* also demonstrated an enzymatic release of RG-I from potato pulp *in vivo*. Despite the demonstration of the enzymatic release of potato RG-I *in vivo*, the data presented by Strube *et al.* 2015a did not justify the claim of the production of an *in situ* prebiotic as stated in the title 'In situ prebiotics: enzymatic release of galacto-RG from potato pulp *in vivo* in the GIT of the weaning piglet', as the authors did not investigate potential prebiotic effects of the released RG-I in the different colon regions *in vivo* (Strube *et al.* 2015a).

Several studies have investigated the fermentability of RG-I by human fecal microbiota. Interestingly, Khodaei *et al.* 2016 produced galactose-rich oligosaccharides (oligo-RG-I) from potato RG-I and examined their fermentability with human fecal microbiota *in vitro.* They have found oligo-RG-I to be a more selective prebiotic and lead to higher absolute amounts of SCFAs than RG-I fermentations (Khodaei *et al.* 2016). The production of oligo-RG-I substrates might also be an interesting avenue to be explored for weaner feed or other in feed applications.

CONCLUDING REMARKS

Pectin should be further explored for a wide range of applications including prebiotics, milk replacers and as an antimicrobial and antiviral agent. The challenge lies in the unraveling of structure-to-function relationships of different pectin substrates in the GIT and the appropriate matching of specific pectin structures and formulations with the animal target group and application. The current state-of-art in molecular biology and *in vitro* tools can aid to unravel the modes of action and aid to develop a systematic approach for the design of *in vivo* studies.

ACKNOWLEDGEMENTS

I want to thank Todd Hutcherson for cross-checking the grammar.

Conflicts of interest. None declared.

FUNDING

This work was supported by the Innovation Foundation Denmark, Grant Number 5190-00025B.

REFERENCES

Al-Tamimi M, Palframan R, Cooper J *et al.* In vitro fermentation of sugar beet arabinan and arabino-oligosaccharides by the human gut microflora. *J Appl Microbiol* 2006;**100**:407–414.

Arumugam M, Raes J, Pelletier E *et al.* Enterotypes of the human gut microbiome. *Nature* 2011;**473**:174.

Baekey P, Cerda J, Burgin C *et al.* Grapefruit pectin inhibits hypercholesterolemia and atherosclerosis in miniature swine. *Clin Cardiol* 1988;**11**:595–600.

Barry K, Wojcicki B, Middelbos I *et al.* Dietary cellulose, fructooligosaccharides, and pectin modify fecal protein catabolites and microbial populations in adult cats. *J Anim Sci* 2010;**88**:2978–2987.

Bauer E, Williams B, Voigt C *et al.* Microbial activities of faeces from unweaned and adult pigs, in relation to selected fermentable carbohydrates. *Anim Sci* 2001;**73**:313–322.

Berends B, Urlings H, Snijders J *et al.* Identification and quantification of risk factors in animal management and transport regarding Salmonella spp. in pigs. *Int J Food Microbiol* 1996;**30**:37–53.

Bezirtzoglou E. The intestinal microflora during the first weeks of life. *Anaerobe* 1997;**3**:173–177.

Biagi G, Cipollini I, Grandi M *et al.* Influence of some potential prebiotics and fibre-rich foodstuffs on composition and activity of canine intestinal microbiota. *Anim Feed Sci Technol* 2010;**159**:50–58.

Bruggeman G, Bruininx E, Van Den Berg M *et al.* Pectins improving energy redistribution in animals. 2018 . Google Patents.

Brüssow H. Adjuncts and alternatives in the time of antibiotic resistance and in-feed antibiotic bans. *Microb Biotechnol* 2017;**10**:674–677.

Buraczewska L, Święch E, Tuśnio A *et al.* The effect of pectin on amino acid digestibility and digesta viscosity, motility and morphology of the small intestine, and on N-balance and performance of young pigs. *Livestock Sci* 2007;**109**:53–56.

Castillo M, Martin-Orue S, Taylor-Pickard J *et al.* Use of mannan-oligosaccharides and zinc chelate as growth promoters and diarrhea preventative in weaning pigs: effects on microbiota and gut function1. *J Anim Sci* 2008;**86**:94.

Cheng H, Zhang Z, Leng J *et al.* The inhibitory effects and mechanisms of rhamnogalacturonan I pectin from potato on HT-29 colon cancer cell proliferation and cell cycle progression. *Int J Food Sci Nutr* 2013;**64**:36–43.

Chen H, Hu H, Chen D *et al.* Dietary pectic oligosaccharide administration improves the growth performance and immunity in weaned pigs infected by rotavirus. *J Agric Food Chem* 2017;65:2923-9.

Chen J, Liang R-h, Liu W *et al.* Pectic-oligosaccharides prepared by dynamic high-pressure microfluidization and their in vitro fermentation properties. *Carbohydr Polym* 2013;**91**:175–182.

Che T, Johnson R, Kelley K *et al.* Mannan oligosaccharide improves immune responses and growth efficiency of nursery pigs experimentally infected with porcine reproductive and respiratory syndrome virus. *J Anim Sci* 2011;**89**:2592–2602.

Chung N, Wang S-M, Shen C-F *et al.*. Clinical and epidemiological characteristics in hosptialized young children with acute gastroenteritis in southern Taiwan: According to major pathogens. *J Microbiol Immunol Infect* 2017;**50**:915–22.

den Besten G, van Eunen K, Groen AK *et al.* The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013;**54**:2325–2340.

Dibner J, Buttin P. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. *J Appl Poult Res* 2002;**11**:453–463.

Dongowski G, Lorenz A, Proll J. The degree of methylation influences the degradation of pectin in the intestinal tract of rats and in vitro. *J Nutr* 2002;**132**:1935–1944.

Drochner W, Kerler A, Zacharias B. Pectin in pig nutrition, a comparative review. *J Anim Physiol Anim Nutr (Berl)* 2004;**88**:367–380.

Egger L, Ménard O, Baumann C *et al*. Digestion of proteins in milk: comparing different in vitro systems with in vivo data. 5th International Conference on Food Digestion 2017.

Egger L, Ménard O, Delgado-Andrade C *et al*. The harmonized INFOGEST in vitro digestion method: From knowledge to action. *Food Res Int* 2016;**88**:217–225.

Fang W, Zhang L, Meng Q *et al*. Effects of dietary pectin on the profile and transport of intestinal bile acids in young pigs. *J Anim Sci* 2018;**96**:4743–4754.

Fremont L, Gozzelino M-T, Bosseau AF. Effects of sugar beet fiber feeding on serum lipids and binding of low-density lipoproteins to liver membranes in growing pigs. *Am J Clin Nutr* 1993;**57**:524–32.

Furness JB, Rivera LR, Cho H-J *et al*. The gut as a sensory organ. *Nat Rev Gastroenterol Hepatol* 2013;**10**:729–740.

Gonzalez-Ochoa G, Flores-Mendoza LK, Icedo-Garcia R *et al*. Modulation of rotavirus severe gastroenteritis by the combination of probiotics and prebiotics. *Arch Microbiol* 2017;**199**:953–961.

Goulart F, Silva L, Loureiro B *et al*. Effects of dietary fibre concentrates on growth performance and digestive enzyme activities of jundiá (Rhamdia quelen). *Aquacult Nutr* 2017;**23**:358–366.

Green R, Woolley D. Inhibition by certain polysaccharides of hemagglutination and of multiplication of influenza virus. *J Exp Med* 1947;**86**:55–64.

Gressley T, Armentano L. Effect of abomasal pectin infusion on digestion and nitrogen balance in lactating dairy cows. *J Dairy Sci* 2005;**88**:4028–4044.

Grønhaug TE, Kiyohara H, Sveaass A *et al*. Beta-d-(1→ 4)-galactan-containing side chains in RG-I regions of pectic polysaccharides from Biophytum petersianum Klotzsch. contribute to expression of immunomodulating activity against intestinal Peyer's patch cells and macrophages. *Phytochemistry* 2011;**72**:2139–2147.

Gullón B, Martínez M, Sanz Y *et al*. Pectic-oligosacchar-ides from sugar beet pulp: membrane purification and preliminary evaluation of its prebiotic potential. 6th International CIGR Technical Symposium- Towards a sustainable Food Chain: Food Process, Bioprocessing and Food Quality Management. 2011

Gómez B, Gullón B, Yáñez R *et al*. Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: a comparative evaluation. *J Funct Foods* 2016;**20**:108–121.

Gómez Bn, Gullón B, Remoroza C *et al*. Purification, characterization, and prebiotic properties of pectic oligosaccharides from orange peel wastes. *J Agric Food Chem* 2014;**62**:9769–9782.

Hagesaether E, Sande SA. In vitro measurements of mucoadhesive properties of six types of pectin. *Drug Dev Ind Pharm* 2007;**33**:417–25.

Hedemann MS, Eskildsen M, Lærke HN *et al*. Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fiber concentrations and fiber properties. *J Anim Sci* 2006;**84**:1375–1386.

Hesselager MO, Everest-Dass AV, Thaysen-Andersen M *et al*. FUT1 genetic variants impact protein glycosylation of porcine intestinal mucosa. *Glycobiology* 2016;**26**:607–622.

Ho GTT, Ahmed A, Zou Y-F *et al*. Structure–activity relationship of immunomodulating pectins from elderberries. *Carbohydr Polym* 2015;**125**:314–322.

Holck J, Lorentzen A, Vigsnæs LK *et al*. Feruloylated and nonferuloylated arabino-oligosaccharides from sugar beet pectin selectively stimulate the growth of Bifidobacterium spp. in human fecal in vitro fermentations. *J Agric Food Chem* 2011;**59**:6511–6519.

Hotchkiss AT, Jr, Olano-Martin E, Grace WE *et al*. Pectic oligosaccharides as prebiotics. In: *ACS Symposium Series*. Washington: ACS Publications, 849, American Chemical Society, 2003, 54–62.

Huhtanen P. The effects of barley, unmolassed sugar-beet pulp and molasses supplements on organic matter, nitrogen and fibre digestion in the rumen of cattle given a silage diet. *Anim Feed Sci Technol* 1988;**20**:259–278.

Irvin S, Blyth D, Bourne N *et al*. A study of the discrete and interactive effects of different polysaccharides on the digestibility of diets fed to barramundi (Lates calcarifer). *Aquacult Nutr* 2016;**22**:1047–1054.

Ishisono K, Yabe T, Kitaguchi K. Citrus pectin attenuates endotoxin shock via suppression of Toll-like receptor signaling in Peyer's patch myeloid cells. *J Nutr Biochem* 2017;50:38-45.

Jacobson M, af Segerstad CH, Gunnarsson A *et al*. Diarrhoea in the growing pig–a comparison of clinical, morphological and microbial findings between animals from good and poor performance herds. *Res Vet Sci* 2003;**74**:163–169.

Jantscher-Krenn E, Zherebtsov M, Nissan C *et al*. The human milk oligosaccharide disialyllacto-N-tetraose prevents necrotising enterocolitis in neonatal rats. *Gut* 2011, doi: 10.1136/gutjnl-2011-301404.

Jonathan MC, van den Borne JJ, van Wiechen P *et al*. In vitro fermentation of 12 dietary fibres by faecal inoculum from pigs and humans. *Food Chem* 2012;**133**:889–897.

Kenworthy R, Crabb W. The intestinal flora of young pigs, with reference to early weaning, Escherichia coli and scours. *J Comp Pathol Ther* 1963;**73**:215–228.

Khodaei N, Fernandez B, Fliss I *et al*. Digestibility and prebiotic properties of potato rhamnogalacturonan I polysaccharide and its galactose-rich oligosaccharides/oligomers. *Carbohydr Polym* 2016;**136**:1074–1084.

Knudsen KB, Lærke HN, Hedemann MS. The role of fibre in piglet gut health. *Gut Efficiency* 2008;65–95.

Laerke HN, Hellwing AF, Knudsen KB *et al*. *29 Isolated Pectins Vary in their Functional Properties in the Gut of Piglets*. Uppsala, Sweden: CABI, 2001, 127.

Lagreca L, Marotta E. Nutritional effect of pectin in swine during growth and before slaughter. *Arch Latinoam Nutr* 1985;**35**:172–179.

Langhout D, Schutte J, Van Leeuwen P *et al*. Effect of dietary high- and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. *Br Poult Sci* 1999;**40**:340–347.

Larsen J. Effect of pectin on secretion in pig jejunal loops challenged to enteropathogenic *E. coli* or enterotoxin (LT). A preliminary report. *Nord Vet Med* 1980;**33**:218–223.

LeBlanc JG, Milani C, de Giori GS *et al*. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr Opin Biotechnol* 2013;**24**:160–168.

Lee J-H, Shim JS, Lee JS *et al*. Inhibition of pathogenic bacterial adhesion by acidic polysaccharide from green tea (Camellia sinensis). *J Agric Food Chem* 2006;**54**:8717–8723.

Leijdekkers AG, Aguirre M, Venema K *et al*. In vitro fermentability of sugar beet pulp derived oligosaccharides using human and pig fecal inocula. *J Agric Food Chem* 2014;**62**:1079–1087.

Ley RE, Hamady M, Lozupone C *et al*. Evolution of mammals and their gut microbes. *Science* 2008;**320**:1647–1651.

Libao-Mercado A, Zhu C, Fuller M *et al*.. Effect of feeding fermentable fiber on synthesis of total and mucosal protein in the intestine of the growing pig. *Livest Sci* 2007;**109**:125–8.

Li P-J, Xia J-l, Nie Z-Y *et al*. Pectic oligosaccharides hydrolyzed from orange peel by fungal multi-enzyme complexes and their prebiotic and antibacterial potentials. *LWT-Food Science and Technology* 2016;**69**:203–210.

Liu L, Fishman ML, Hicks KB *et al*. Interaction of various pectin formulations with porcine colonic tissues. *Biomaterials* 2005;**26**:5907–5916.

Li Y. Effects of prebiotics and probiotics on gut health, immunity, and growth of weanling pigs. 2017.

Lærke HN, Hedemann MS, Knudsen KB *et al*. Association between butyrate and short-chain fatty acid concentrations in gut contents and faeces in weaning piglets. *Livestock Science* 2007;**108**:163–166.

Macfarlane GT, Macfarlane S. Models for intestinal fermentation: association between food components, delivery systems, bioavailability and functional interactions in the gut. *Curr Opin Biotechnol* 2007;**18**:156–162.

Martín-Peláez S, Costabile A, Hoyles L *et al*. Evaluation of the inclusion of a mixture of organic acids or lactulose into the feed of pigs experimentally challenged with Salmonella Typhimurium. *Vet Microbiol* 2010;**142**:337–345.

McConnell EL, Fadda HM, Basit AW. Gut instincts: explorations in intestinal physiology and drug delivery. *Int J Pharm* 2008;**364**:213–226.

Metzler-Zebeli B, Vahjen W, Baumgärtel T *et al*. Ileal microbiota of growing pigs fed different dietary calcium phosphate levels and phytase content and subjected to ileal pectin infusion. *J Anim Sci* 2010;**88**:147–158.

Metzler BU, Mosenthin R, Baumgärtel T *et al*. Effects of fermentable carbohydrates and low dietary phosphorus supply on the chemical composition of faecal bacteria and microbial metabolites in the gastrointestinal tract of pigs. *J Anim Physiol Anim Nutr (Berl)* 2009;**93**:130–139.

Moon JS, Shin SY, Choi HS *et al*. In vitro digestion and fermentation properties of linear sugar-beet arabinan and its oligosaccharides. *Carbohydr Polym* 2015;**131**:50–56.

Míguez B, Gómez B, Gullón P *et al*. Pectic oligosaccharides and other emerging prebiotics. In: *Probiotics and Prebiotics in Human Nutrition and Health*. 2016; 301-30. www.intechopen.com.

Nabuurs M. Weaning piglets as a model for studying pathophysiology of diarrhea. *Vet Q* 1998;**20**:42–45.

Namkung H, Li J. Gong M, Yu H *et al*. Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. *Can J Anim Sci* 2004;**84**:697–704.

O'Hara AM, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep* 2006;**7**:688–693.

Olano-Martin E, Williams MR, Gibson GR *et al*. Pectins and pectic-oligosaccharides inhibit Escherichia coli O157: H7 Shiga toxin as directed towards the human colonic cell line HT29. *FEMS Microbiol Lett* 2003;**218**:101–105.

Olano-Martin E, Gibson GR, Rastall R. Comparison of the in vitro bifidogenic properties of pectins and pectic-oligosaccharides. *J Appl Microbiol* 2002;**93**:505–511.

Onumpai C, Kolida S, Bonnin E *et al*. Microbial utilization and selectivity of pectin fractions with varying structures. *Appl Environ Microbiol* 2011;**77**:5747–54, AEM. 00179-00111.

Partanen KH, Mroz Z. Organic acids for performance enhancement in pig diets. *Nutr Res Rev* 1999;**12**:117–145.

Payne AN, Zihler A, Chassard C *et al*. Advances and perspectives in in vitro human gut fermentation modeling. *Trends Biotechnol* 2012;**30**:17–25.

Platt D. Modified pectin. p. *p p*.2009. *P atent No* : *U S*7, 491, 708*B*1.

Pluschke AM, Williams BA, Zhang D *et al*. Dietary pectin and mango pulp effects on small intestinal enzyme activity levels and macronutrient digestion in grower pigs. *Food Funct* 2018;**9**:991–999.

Popov S, Ovodov YS. Polypotency of the immunomodulatory effect of pectins. *Biochemistry (Moscow)* 2013;**78**:823–835.

Poroyko V, White JR, Wang M *et al*. Gut microbial gene expression in mother-fed and formula-fed piglets. *PLoS One* 2010;**5**:e12459.

Portmann R, Ménard O, Baumann C *et al*. Digestion of Proteins in Milk: comparing different in vitro systems with in vivo data. 2017. 5 International Conference on Food Digestion.

Potkins Z, Lawrence T, Thomlinson J. Effects on ileal apparent digestibility in the growing pig of replacing barley with bran, oatmeal by-product, guar gum and pectin. *Anim Feed Sci Technol* 1991;**35**:171–179.

Priori D, Colombo M, Koopmans S-J *et al*. The A0 blood group genotype modifies the jejunal glycomic binding pattern profile of piglets early associated with a simple or complex microbiota. *J Anim Sci* 2016;**94**:592–601.

Ramayo-Caldas Y, Mach N, Lepage P *et al*. Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. *The ISME Journal* 2016;10:2973-7.

Rhoades J, Manderson K, Wells A *et al*. Oligosaccharide-mediated inhibition of the adhesion of pathogenic Escherichia coli strains to human gut epithelial cells in vitro. *J Food Prot* 2008;**71**:2272–2277.

Ridlon JM, Kang DJ, Hylemon PB *et al*. Bile acids and the gut microbiome. *Curr Opin Gastroenterol* 2014;**30**:332.

Rigo-Adrover M, Pérez-Berezo T, Ramos-Romero S *et al*. A fermented milk concentrate and a combination of short-chain galacto-oligosaccharides/long-chain fructo-oligosaccharides/pectin-derived acidic oligosaccharides protect suckling rats from rotavirus gastroenteritis. *Br J Nutr* 2017;**117**:209–217.

Roth-Maier DA, Machmüller A, Kreuzer M *et al*.. Effects of pectin supplementation on the digestion of different structural carbohydrate fractions and on bacterial nitrogen turnover in the hindgut of adult sows. *Anim Feed Sci Technol* 1993;**42**:177–91.

Sayin SI, Wahlström A, Felin J *et al*. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metabolism* 2013;**17**:225–235.

Schley P, Field C. The immune-enhancing effects of dietary fibres and prebiotics. *Br J Nutr* 2002;**87**:S221–S230.

Schmidgall J, Hensel A. Bioadhesive properties of polygalacturonides against colonic epithelial membranes. *Int J Biol Macromol* 2002;**30**:217–225.

Scott KP, Gratz SW, Sheridan PO *et al*. The influence of diet on the gut microbiota. *Pharmacol Res* 2013;**69**:52–60.

Sekirov I, Russell SL, Antunes LCM *et al*. Gut microbiota in health and disease. *Physiol Rev* 2010;**90**:859–904.

Sela DA, Mills DA. Nursing our microbiota: molecular linkages between bifidobacteria and milk oligosaccharides. *Trends Microbiol* 2010;**18**:298–307.

Sharon N, Ofek I. Safe as mother's milk: carbohydrates as future anti-adhesion drugs for bacterial diseases. *Glycoconj J* 2000;**17**:659–664.

Silva RHP, de Rezende ASC, da Silva Inácio DF. Pectin-rich by-products in feeding horses—A review. *Cogent Food & Agriculture* 2016;**2**:1193925.

Sommer F, Bäckhed F. The gut microbiota–masters of host development and physiology. *Nat Rev Microbiol* 2013;**11**:227.

Strube ML, Jensen TK, Meyer AS *et al*. In situ prebiotics: enzymatic release of galacto-rhamnogalacturonan from potato pulp in vivo in the gastrointestinal tract of the weaning piglet. *Amb Express* 2015a;**5**:66.

Strube ML, Ravn HC, Ingerslev H-C *et al*. In situ prebiotics for weaning piglets: in vitro production and fermentation of potato galacto-rhamnogalacturonan. *Appl Environ Microbiol* 2015b;**81**:1668–1678.

Sunvold G, Hussein H, Fahey G *et al*. In vitro fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculum from cats, dogs, horses, humans, and pigs and ruminal fluid from cattle. *J Anim Sci* 1995;**73**:3639–3648.

Suzuki Y, Tanaka K, Amano T *et al*. Utilization by intestinal bacteria and digestibility of arabino-oligosaccharides in vitro. *J Jpn Soc Hortic Sci* 2004;**73**:574–579.

Święch E, Tuśnio A, Taciak M *et al*. The effects of pectin and rye on amino acid ileal digestibility, threonine metabolism, nitrogen retention, and morphology of the small intestine in young pigs. *J Anim Feed Sci* 2012;**21**:89–106.

Taciak M, Barszcz M, Tuśnio A *et al*. The effects of type of protein and fibre fermented in vitro with different pig inocula on short-chain fatty acids and amines concentrations. *J Anim Feed Sci* 2015;**24**:235-43.

Tang Y, Govers C, Wichers HJ *et al*. Macrophages treated with non-digestible polysaccharides reveal a transcriptionally unique phenotype. *J Funct Foods* 2017;**36**:280–289.

Tarasenko LO, Rud VO, The effect of pectin usage as feed additive on pigs excretion metabolism and blood biochemical parameters. 2016;65: 237-42.

Thirawong N, Nunthanid J, Puttipipatkhachorn S *et al*. Mucoadhesive properties of various pectins on gastrointestinal mucosa: an in vitro evaluation using texture analyzer. *Eur J Pharm Biopharm* 2007;**67**:132–140.

Tian L, Bruggeman G, den Berg M *et al*. Effects of pectin on fermentation characteristics, carbohydrate utilization, and microbial community composition in the gastrointestinal tract of weaning pigs. *Mol Nutr Food Res* 2017;**61:160-86**.

Tusnio A, Swiech E, Taciak M *et al*.. The structure of small intestinal tissue in pigs as influenced by indigestible polysaccharides. *J Anim Feed Sci* 2006;**15**:89.

Van der Waaij D, Berghuis-de Vries J, Lekkerkerk-Van der Wees J. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. *Epidemiol Infect* 1971;**69**:405–411.

Van Laere KM, Hartemink R, Bosveld M *et al*. Fermentation of plant cell wall derived polysaccharides and their corresponding oligosaccharides by intestinal bacteria. *J Agric Food Chem* 2000;**48**:1644–1652.

Volpe M, Santagata G, Coccia E *et al*. Pectin-based pellets for crayfish aquaculture: structural and functional characteristics and effects on redclaw Cherax quadricarinatus performances. *Aquacult Nutr* 2015;**21**:814–823.

Wang Z, Yu H, Wu X *et al*. Effects of dietary zinc pectin oligosaccharides chelate supplementation on growth performance, nutrient digestibility and tissue zinc concentrations of broilers. *Biol Trace Elem Res* 2016;**173**:475–482.

Wieler L, Ilieff A, Herbst W *et al*. Prevalence of enteropathogens in suckling and weaned piglets with diarrhoea in southern Germany. *Zoonoses Public Hlth* 2001;**48**:151–159.

Wiese M, Khakimov B, Nielsen S *et al*. CoMiniGut—a small volume in vitro colon model for the screening of gut microbial fermentation processes. *PeerJ* 2018;**6**:e4268.

Willats WG, McCartney L, Mackie W *et al*. Pectin: cell biology and prospects for functional analysis. *Plant Mol Biol* 2001;**47**:9–27.

Wittum TE, Dewey CE, Hurd HS *et al*. Herd-and litter-level factors associated with the incidence of diarrhea morbidity and mortality in piglets 1 to 3 days of age. *Swine Health Prod* 1995;**3**:99–104.

Wong TW, Colombo G, Sonvico F. Pectin matrix as oral drug delivery vehicle for colon cancer treatment. *Aaps PharmSciTech* 2011;**12**:201–214.

Woolley D. Purification of an influenza virus substrate, and demonstration of its competitive antagonism to apple pectin. *J Exp Med* 1949;**89**:11–22.

Wu P, Dhital S, Williams BA *et al*. Rheological and microstructural properties of porcine gastric digesta and diets containing pectin or mango powder. *Carbohydr Polym* 2016;**148**:216–226.

Yan C, Kim H, Hong J *et al*.. Effect of Dietary sugar beet pulp supplementaiton on growth performance, nutrient digestibility, fecal Microflora, blood profiles and Diarrhea incidence in weaning pigs. *J Anim Sci Technol* 2017;**59**:18.

Yodmeeklin A, Khamrin P, Chuchaona W *et al*.. Analysis of complete genome sequences of G9P rotavirus strains from human and piglet with diarrhea provides evidence for whole-genome interspecies transmission of nonreassorted porcine rotavirus. *Infect Genet Evol* 2017;**47**:99–108

Zacharias B, Kerler A, Drochner W. The influence of 5% and 10% dietary apple pectin on parameters of fermentation in faeces and caecal digesta of weaning pigs. *Arch Anim Nutr* 2004;**58**:149–156.

Zhu C, Rademacher M, De Lange C. Increasing dietary pectin level reduces utilization of digestible threonine intake, but not lysine intake, for body protein deposition in growing pigs. *J Anim Sci* 2005;**83**:1044–1053.

Zhu C, Rademacher M, de Lange C. Intake of fermentable fibre and body protein deposition in pigs fed methionine or tryptophan limiting diets. *Publicat Eur Associat Anim Product* 2007;**124**:553.

Zopf D, Roth S. Oligosaccharide anti-infective agents. *Lancet North Am Ed* 1996;**347**:1017–1021.