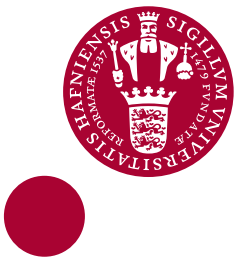

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A review on early gut maturation and colonization in pigs, including biological and dietary factors affecting gut homeostasis.

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Abstract (400 words max)

During the prenatal, neonatal and post-weaning periods, the mammalian gastrointestinal tract undergoes various morphological and physiological changes alongside with an expansion of the immune system and microbial ecosystem. This review focuses on the time period before weaning and summarizes the current knowledge regarding i) structural and functional aspects ii) the development of the immune system, and iii) the establishment of the gut ecosystem of the porcine intestine. Structural and functional maturation of the gastrointestinal tract gradually progress with age. In the neonatal period colostrum induces gut closure, leads to an increase in intestinal weight, absorptive area and brush border enzyme activities. During the first weeks of life, an increased secretion of stomach and pancreatic enzymes and an increased uptake of monosaccharides and amino acids are observed. The development in digestive function coincides with development in both the adaptive and innate immune system. This secures a balanced immune response to the ingested milk-derived macromolecules, and colonizing bacteria. Husbandry and dietary interventions in early life appear to affect the development of multiple components of the mucosal immune system. Furthermore, the composition of the intestinal microbial communities seems to be affected by the early postnatal environment, which might also contribute to gut maturation, metabolic and immune development. Understanding the interplay between morphological, functional and immunological maturation, as influenced by early microbial colonization and ingestion of dietary factors, is of utmost importance to identify management and feeding strategies to optimize intestinal health. We discuss some possible implications related to intrauterine growth restriction, and preterm delivery as these both dramatically increase the risk of mortality and morbidity. In addition, some nutritional interventions during the perinatal period in both sows and piglets will be discussed in the light of possible health consequences early in life and later on.

Abbreviations:

GD: days of gestation

GIT: gastrointestinal tract

HP: high protein

IUGR: intrauterine growth restriction

MMC: migrating myoelectric complex

NEC: necrotizing enterocolitis

PND: postnatal days

PP: Peyer's patches

SCFA: short chain fatty acids

scFOS: short-chain fructooligosaccharides

SWE: seaweed extract

Introduction

The development of the mammalian intestine is driven by intrinsic (e.g. ontogenetically programmed) and extrinsic (e.g. nutrients, microbiota) factors during the prenatal, the neonatal and the post-weaning period (Buddington and Sangild, 2011). In pigs, it is well established that weaning causes substantial changes in the intestinal structure, microbial composition and intestinal functional properties (e.g. Pluske et al., 1997; Montagne et al., 2007). In current pig production, an abrupt weaning process usually is imposed between 3 to 5 weeks of age, leading to increased susceptibility to intestinal dysfunction from which putative pathogens such as *Escherichia coli, Clostridium perfringens* can take advantage and cause intestinal disease (Ewing and Tucker, 2008). Understanding the factors that disturb development of the gastrointestinal tract (GIT) in the pre-weaning period, may be important to better understand physiological and immunological reactions and the susceptibility to gut disorders around weaning and later in life. This review specifically summarizes the current knowledge regarding i) structural and functional aspects ii) the development of the immune system, iii) the establishment of the gut ecosystem in early life. Regarding the development of the intrinsic neuro-endocrine system and its role in modulating the structural and functional maturation of the gastrointestinal tract, we refer to other reviews (Van Ginneken, 2012; Thymann, 2016). An overview of important developmental changes after birth is provided in Table 1. In addition, some factors disturbing or modulating gut colonization and maturation (i.e. preterm birth, intrauterine growth restriction, nutritional interventions) will be discussed in the light of possible health consequences early in life and later on.

1. Structural and functional aspects of early life gut maturation

The GIT, i.e. the stomach, small intestine and large intestine, originates from the primitive gut during embryonic development and is composed of 4 distinct layers, i.e. the tunica mucosa, tela submucosa, tunica muscularis and tunica serosa. Although all layers are present in the stomach,

small and large intestine, distinct spatiotemporal differences in morphology occur in these 3 parts of the GIT during development (Van Ginneken et al., 2002), which is also reflected in their functional capacity (Henning et al., 1994). In pigs enterocytes with a high endocytotic activity (fetal-type) are gradually replaced during the first 3 weeks of life by new adult-type cells with markedly reduced endocytotic activity (Smith and Peacock, 1980, Klein, 1989). Additionally, this shift in enterocyte types occurs concomitantly with the marked shift in expression of the enterocyte brush border disaccharidases with decreased lactase and increased sucrase and maltase activities with age (Le Huërou-Luron, 2002). These maturational changes gradually progress with age, but become most evident at weaning, when the gut digestive and absorptive capacity rapidly adapts to effectively utilize the weaning diet instead of the easily digestible milk diet. In addition, the stomach acid secretion amplifies and besides an increased production of gut enzymes an elevated pancreatic function is realised (Cranwell, 1995).

1.1. Development of the gastrointestinal tract's structure

1.1.1. Stomach

The gastric corpus and mucosal layer of the cardiac and fundic region show the most pronounced alterations during development (Xu et al. 1992). The fundus region of the stomach of the pig shows 3 periods of intensive growth, i.e. around the 3rd month of gestation, around birth and between postnatal days (PND) 10 and 20. At about 45 days of gestation (GD), gastric pits develop and around the 3rd month of gestation fundic glands are formed. The parietal cells will be the first ones to differentiate at approximately GD60, whereas the mucous neck cells and chief cells take up to the 3rd month of gestation for differentiation although mucous secretion can already be observed around GD45-50 (Georgieva and Gerov, 1975a). As such, these differences have consequences for the onset of the digestive enzymes and hormones secretion (see section 1.2.1). At birth, hypertrophy and hyperplasia realize the massive growth of the

stomach, whereas later - during the first weeks after birth - hyperplasia is the key driver for growth (Lindemann et al., 1986).

1.1.2. Small intestine

From GD40 onwards, villi are observed in the largest part of the small intestine of the developing piglet, the jejunum. The crypts and muscularis mucosae layer are formed around the 3rd month of gestation. By then epithelial cells lining the lumen also differentiate into enterocytes, goblet cells and enteroendocrine cells (Georgieva and Gerov, 1975b; Van Ginneken et al., 2001, Willemen et al., 2012; Willemen et al., 2013). The presence of Paneth cells in pigs is still under debate (Burkey et al., 2009). However, they might only be found around birth (Georgieva and Gerov, 1975b). Towards the end of gestation, the small intestine will grow more rapidly than the body itself, resulting in an increase of its relative weight by 70-80% during the last 3 weeks of gestation (McPherson et al., 2004). After birth, enteral nutrition and bioactive substances in the colostrum and milk stimulate intestinal mucosal proliferation and facilitate the gut closure (Takeda et al., 2004) (see also 2.). This results in an increase of the relative volume of the tunica mucosa after birth, whereas the relative volume of the tela submucosa and tunica muscularis decreases (Van Ginneken et al., 2002, Van Ginneken and Weyns, 2004). The intestinal epithelium is unique because cell proliferation, cell differentiation and apoptosis occur in an ordered manner along the crypt-villus axis. Cell proliferation occurs mainly in the crypts, whereas the villus houses the differentiating and differentiated cells. Subsequently cells die by apoptosis towards the villus tip in adult animals (Hall et al., 1994, Yen and Wright, 2006), These mechanisms allow the small intestine to maintain a relatively constant cell number (Hall et al., 1994, Potten, 1997). However, another pattern is observed in neonatal piglets. In these young animals, apoptotic cells are present along the entire length of the villi and cells seem to die in groups (Biernat et al., 2003, Godlewski et al., 2005). Furthermore, an enhanced mitosis and a significant decline in apoptosis rate is present during the first few days after birth (Wolinski et al., 2003, Godlewski et al., 2007), which is reflected in the postnatal enlargement of the intestinal

mucosa and increase in villus length. Nevertheless, this intestinal epithelial cell turnover is a dynamic process that is markedly affected by nutritional status and specific nutrients in the diet (see further) (Carver and Barness, 1996; Ziegler et al., 1999; Ziegler et al., 2003).

During development regional differences in morphology - which reflect slightly different functions - appear along the small intestine. In a 'mature' small intestine, the length of the villi increases from the duodenum to the mid jejunum but decreases again towards the ileum (Skrzypek et al., 2005; Van Ginneken et al., 2002; Wiyaporn et al., 2013); villi in the duodenum and jejunum have a more regular shape in comparison to in the ileum (Skrzypek et al., 2005), and crypts are usually deeper in the proximal part compared to in the middle and distal parts of the small intestine (Oste et al., 2010). Furthermore, villus height and shape change with ageing. At birth the small intestinal mucosa is lined with finger-like villi (Skrzypek et al., 2010). During the first days after birth, the length of the intestinal villi quickly increases from approximately 200 to 300 μm at birth up to >500 μm at 3 days of age (Skrzypek et al., 2010). After 3 days of age the length of the villi decreases and at the same time the villus diameter increases, resulting in leaf-like shaped villi (Cera et al., 1988). In neonates, intestinal crypt depth is lower than in 3-day old piglets, whereas crypt depth again decreases when the piglet becomes older (Skrzypek et al., 2005). In these morphological changes colostrum (see 4.3.1) plays an essential role. Feeding colostrum leads to an increase in intestinal weight, absorptive area and brush border enzyme activities (see section 1.2.2) (Wang et al., 1996; Zhang et al., 1997). Moreover the presence of milk-based nutrients continue to affect gut maturation since Huygelen et al. showed it resulted in crypt deepening and cell proliferation (Huygelen et al., 2014).

1.1.3. Large intestine

The colonic epithelium is still immature at birth (Montedonico et al., 2006). At PND1, the weight of the large intestine increases by approximately 30% and by PND3 its weight has doubled by a contribution of all layers (Bach and Carey, 1994). In this respect, the proximal colon of the neonate looks similar to the small intestine, i.e. villus-like structures, with a not yet defined

function, are transiently observed in the proximal large intestine the first days after birth (Cremaschi et al., 1979; Xu et al., 1992a). They were presumably also present during late gestation. This morphological similarity with the small intestine is also accompanied with functional similarities as these colon enterocytes can transport amino acids until 2 weeks after birth (Xu et al., 1992). Most probably these neonatal colon enterocytes are gradually replaced by newly synthesized colon enterocytes that lack this ability (Sepulveda and Smith 1979). The decline in this transport function is independent of age at birth but seems to be affected by the neurohormonal status of the animal and food passage (Wooding et al., 1978).

1.2. Development of the gastrointestinal tract's basic functions

1.2.1. Stomach enzymes and secretion

Gastric acid secretion is low at birth but increases rapidly during the first week of life as the parietal cells increase in size and number (Xu and Cranwell, 1990, Sangild et al., 1992) and maximal acid secretion is reached from 5-6 weeks of age (Cranwell, 1995). The gastric proteases show a characteristic shift in their expression during development linked to the nutrition of the young pig (Sangild et al., 1991): chymosin, having primary milk clotting function and low proteolytic activity. After birth, the chymosin concentration declines steadily up to 3-4 weeks of age and after 2 months of age chymosin activity is undetectable. Instead, pepsinogen A, only found in traces at birth, gradually increases, take over and becomes the main protease (together with gastricsin) in the fundic mucosa from 4-5 weeks of age (Sangild et al. 1991). In addition to piglet age, creep feeding before weaning and weaning to solid feed increase the gastric acid and the protease secretion capacity of the stomach (Cranwell, 1985).

1.2.2. Intestinal brush border enzymes

When looking at the activities of the brush border peptidases during the suckling period, the overall trend is that the activities are relatively high at birth and then decrease with age in

suckling pigs (Le Huerou-Luron, 2002). The brush border carbohydrases in the small intestinal enterocytes do not follow the same pattern as the peptidases and moreover develop differently after birth (Le Huerou-Luron 2002). Lactase activity, which cleaves milk lactose, undergoes a marked decrease during the first 2-5 weeks of life. In contrast, from one week of age, the activity of both maltase and sucrase markedly increase. These changes of brush border disaccharidase activity seem to be substrate-independent and ontogenetically programmed. For example, pigs fed a formula with lactose as sole carbohydrate source (50% of diet dry matter) already showed reduced lactase activity and increasing maltase activity at 2 weeks of life (Pieper et al., 2016a).

1.2.3. Intestinal absorption and intestinal closure

Uptake of macromolecules to the enterocytes by endocytosis is a feature of the foetal and/or pre-weaning periods of mammals (Brambell, 1970; Baintner, 1986). In the enterocytes, internalised macromolecules are broken down in large digestive vacuoles, formed after coalescence with lysosomes, and are used in the metabolism or further transferred in undegraded form into the general circulation. Although the transfer of passive immunity (immunoglobulin G) is most important, other macromolecules, like hormones and growth factors, are transferred from mother to offspring during the pre-closure period (Sanderson and Walker, 1993). Additionally this mechanism could aid in, the surveillance of dietary and microbial macromolecules (antigens) in the gut after their absorption and exposure to the immune system may be also important, especially for tolerance induction. This non-selective absorption is further facilitated by a low degradative capability in the intestinal lumen, due to a low secretion of pancreatic enzymes (Pierzynowski et al., 1995), and the presence of proteinase inhibitors from colostrum and piglet blood plasma (Weström et al., 1985), thus enhancing the absorption to as much as 50-80 % of the amount fed. The high transfer of colostrum-derived macromolecules into blood, however, ceases abruptly 18-36 h after birth during intestinal closure. This results in an exclusion of molecules with a molecular weight greater than a few

kilo-daltons, while smaller molecules are absorbed independently of the closure process (Weström et al., 1984). In case of deprivation of colostrum intake or starvation, intestinal closure can be delayed and uptake of proteins is prolonged (Payne et al., 1962; Lecce et al., 1973).

The small intestinal transport mechanisms for the end products of the digestion, i.e., monosaccharides, amino acids or peptides (di- and tri-peptides) and fat digestion products (e.g., fatty acids and monoglycerides) and their development have been described earlier (Buddington and Malo, 1996, Buddington et al., 2001).

After closure, the uptake of macromolecules into the enterocytes will continue for some time, but there is no further transmission to the blood and the macromolecules will remain in the enterocytes to be degraded or to finally disappear from the mucosa due to cell shedding at the villus tip. These fetal-type enterocytes will be gradually replaced by new adult-type cells having low endocytotic activity (Smith and Peacock, 1980). This cell replacement proceeds in a proximal-distal direction along the intestine, being completed in the distal part by the time the pigs are 3-4 weeks of age. Although the mechanism involved in the induction of closure remains obscure, components present in colostrum and humoral factors released in response to feeding have been implicated (Ekström et al., 1988). Nevertheless, these adult type cells have a more efficient enzymatic brush border membrane machinery resulting in an increase in rate of uptake of monosaccharides and amino acids during the suckling period (see also 1.2.2.). There is limited information available about whether these adult type enterocytes can absorb macromolecules in the pig. The studies performed show that the transfer is low, but not insignificant, and could be enhanced by decreasing intestinal degradation (Svendsen et al., 1990). Results similar to pigs have been obtained in other species and it has become obvious that the absorption might be increased during injuries and inflammatory conditions in the intestines, opening up a paracellular pathway between the enterocytes for leakage of macromolecules (Sanderson and Walker, 1993).

1.2.4. Gastrointestinal transit

Complex interactions between myogenic, neural and hormonal mechanisms determine the rate of gastric emptying and are associated with meal volume and content (Low, 1990; Olsson and Holmgren, 2011). In piglets, there is a rapid emptying of liquid nutrients (complete after 15 min) immediately after suckling. As the milk clots, a period of inhibition is established. This is then followed by a slow phase of emptying, representing the phase of clot hydrolysis, and liquefaction occurs (Decuypere et al., 1986). When the piglets reach an age of 4 to 6 weeks, 50 - 70% of the ingested milk empties within 1 h (Wangsness and Soroka, 1978; Moughan et al., 1991) and a total volume of 80 to 90% is expelled within 3 h (Moughan et al., 1991), although others reported that gastric emptying is already completed within 2 h (Kidder and Manners, 1968; Braude et al., 1970). Neonatal piglets, 2 to 6 days after parturition, show a similar pattern of gastric emptying (Wright et al., 1998). However, when using non-disintegrating radio-opaque pellets faster gastric emptying was observed in suckling piglets (PND21) compared to recently weaned piglets (Snoeck et al., 2004).

Nutrients and hormones also control intestinal motility, and thus transit, but the autonomic nervous system that includes extrinsic and intrinsic (enteric) pathways plays the most important role (Hansen, 2003; Olsson and Holmgren, 2011)(for review see Van Ginneken, 2012). In suckling piglets, no alterations in intestinal passage of barium sulphate were detected between PND7 and PND21 (Kidder and Manners, 1968). Similar observations were made in a study with Evans blue in PND0, PND3 and PND10 piglets (Huygelen et al., 2015). However, at PND28 the geometric center, a marker for intestinal transit (Miller et al., 1981), was higher than in the younger age groups, implying a faster small intestinal transit in weanling piglets (Huygelen et al., 2015).

2. Early-life development of the gut immune system

In the ungulate species, including the pig, no macromolecular passage between mother and offspring can take place during the fetal period, due to the epitheliochorial placenta consisting of four epithelial layers between the fetal and maternal blood circulations (Baintner, 1986). Consequently, the newborn piglet and neonates of the ungulate species are born hypogammaglobulinaemic and must acquire passive immunity (IgG) via mammary secretions for their survival (see also 1.2.3). Secondly, because the placenta of the pig is essentially impermeable to macromolecules in the absence of infection, most piglets are born essentially antigen-naïve and, as a consequence, the immune system is extremely poorly developed. Thus a quick maturation of the immune system is essential (Bailey et al., 2005).

2.1. Organised lymphoid tissues

In adult animals, Peyer's patches are clearly visible in the wall of the small intestine. In the pig, multiple discrete Peyer's patches occur throughout the jejunum, while a single, large patch extends from the ileocaecocolic junction for perhaps 1 meter through the ileum into the jejunum (Rothkotter and Pabst, 1989; Barman et al., 1997). These Peyer's patches are present at birth but are very difficult to identify without microscopic examination: at this stage they contain very small, primordial follicles and almost no T-cells (Makala et al., 2000). Within the first two weeks of life, expansion of the follicles occurs and the T-cell zones begin to be populated (Barman et al., 1997; Makala et al., 2000). However, while the size of B-cell and T-cell compartments expands, function remains limited for several weeks. In the neonate, immunoglobulin heavy chain gene rearrangements are much more restricted than in adults, and development to use the full range of adult V-segment genes does not occur until around six weeks (Sun et al., 1998; Wilson et al., 2007). Similarly, B-cells use primarily the mu, or IgM heavy chain for the first 6 weeks or so, and IgA positive follicles do not appear until about 6 weeks (Wilson et al., 2005). Thus, while piglets under 6 weeks can make antigen-specific responses, the quality of the response may be limited compared to older animals.

294 Studies in sheep have suggested that the ileal Peyer's patch may have a specific role in
295 expanding the antibody repertoire in young animals, as the Bursa of Fabricius does in birds
296 (Yasuda et al., 2006). In pigs, there are several features of the ileal patch which have suggested
297 that the same may be true: one specific segment within the patch contains follicles but no T-cell
298 zones and does not recruit lymphocytes from blood, similar to the bursa in chickens (Pabst et al.,
299 1991). However, detailed analysis has found no evidence for repertoire diversification in early
300 B-cells within this patch in the pig (Sinkora et al., 2011), and it has also been proposed that the
301 ileal Peyer's patch may simply be the primary source of undiversified IgA antibodies (Butler et
302 al., 2016).

303

304 2.2. Diffuse lymphoid tissues – the intestinal mucosa

305 In adult pigs, the intestinal mucosa is heavily infiltrated with multiple types of lymphocytes,
306 apparently engaged in surveillance and maintenance of homeostasis rather than in expression of
307 active immune responses. Although the mucosa is not considered an organized lymphoid
308 structure, lymphocyte subsets clearly occupy distinct spatially environments and, presumably,
309 co-operate within them. Within the epithelial layer, CD8alpha-positive T-cells predominate:
310 most of these are CD8alpha/beta positive, true cytotoxic T-cells, but many are CD8alpha/alpha
311 positive and include both unconventional T-cells and subsets which appear not to express T-cell
312 receptors at all. Deep to the basement membrane of the villi are conventional CD4 T-helper cells,
313 and these are mixed with antigen-presenting cells bearing the T-cell restriction molecule, MHC
314 class II (Vega-Lopez et al., 1993). In the pig, the antigen-presenting cell population includes both
315 conventional dendritic cells and capillary endothelium, and both appear to interact with CD4 T-
316 cells (Wilson et al., 1996; Inman et al., 2010a). Beneath the villi, around the crypts, plasma cells
317 secreting IgA and IgM are present, together with significant numbers of eosinophils.

318 Even less of this diffuse architecture is present in the newborn piglet than in the organized
319 Peyer's patches. However, the cell types appear after birth in a well-ordered sequence rather

than all at once. Initially, antigen-presenting cells appear in the intestinal mucosa during the first two weeks of life. CD4 T-cells appear in the mucosa during weeks three and four, and CD8 T-cells in the epithelium start to appear from four to 6 weeks old, such that the normal architecture apparent in adult animals is not properly developed until 6 weeks after birth (Bianchi et al., 1992; Vega-Lopez et al., 1995). Even where the architecture appears normal, the interactions between cell types may be unusual in young animals: mucosal CD4 T-cells appear to interact both with resident dendritic cells and with capillary endothelial cells, whereas interactions in adults appear to be exclusively with dendritic cells (Inman et al., 2010a).

2.3. The influence of microbiota

Most of this expansion appears to be driven by microbial colonization. True antigen-naïve pigs are very hard to generate, since food contains intact molecules and may contain microbial products even when autoclaved or irradiated. Such animals have been reared but their immune systems have not been well characterised. However, several groups have reared germ-free piglets and compared them either with conventional animals or with defined-colonized animals. While some development of the mucosal immune system may occur in germ-free piglets, it is very limited compared to conventionals. Colonization with a defined, limited microbiome can recapitulate most of the development of antigen-presenting cell, B-cell and T-cell compartments which is apparent in conventional piglets (Sun et al., 1998; Inman et al., 2012), Thus, there is the potential for altered nutrition to change the rate at which the immune system develops, or to affect the types of cell which appear sequentially, or their interactions. Early, pre-weaning interventions in husbandry and diet have been shown to affect the establishment of intestinal microbiome and the development of the immune system, although the causal link between the two is difficult to establish directly. Piglets removed from the sow and reared in high containment units on bovine-based milk formula develop different microbiomes from their littermates on the sow and marked differences in the mucosal immune system in all three

compartments: antigen-presenting cells (more rapid recruitment), T-cells (fewer regulatory T-cells); and B-cells (increased antibody responses to weaning diet) (Inman et al., 2010b; Lewis et al., 2012). Similarly, piglets reared on indoor and outdoor farms develop different microbiomes and differences in expression of genes associated with MHC-dependent antigen presentation in the intestinal mucosa (Mulder et al., 2009; Schmidt et al., 2011; Mulder et al., 2011). Thus, husbandry and dietary interventions in early life do appear to affect the rate of development of multiple components of the mucosal immune system and provide potential targets for optimizing enteric health.

3. Early-life gut colonization and establishment of the gut ecosystem

The growing (weaned) and adult pig intestinal tract is colonized by highly diverse microbial consortia. Similar as in other mammalian species, factors such as host phylogenetic background, the early environment, and diet have likely been the major driving forces for the co-evolution of close microbe – host relationship (Ley et al., 2006). The adult pig gastrointestinal tract harbours likely more than 1,000 different bacterial species, of which most belong to the phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria, Actinbacteria* and *Spirochaetes* (Kim et al., 2011; Ramayo-Caldas et al., 2016). Recent advances in metagenomic deep sequencing or comparative analysis of whole genome sequences have also increased our understanding about the metabolic potential of the porcine gut microbiome (Lamendella et al., 2011; Looft et al., 2014; Xiao et al., 2016). In addition, a high similarity between functional bacterial pathways between the human and the porcine gut microbiome was recently observed, supporting the potential use of pigs as model for humans (Xiao et al., 2016). The developing pig microbiome has recently also been linked with growth traits, thus opening translational potential for the pig industry (Ramayo-Caldas et al., 2016).

Although the establishment of the intestinal microbiome of pigs has yet not been studied in such detail and over longer periods as in humans, it is likely that similar patterns occur in dependence of certain life events such as environmental or dietary changes, or medical intervention. The

establishment of microbial communities in the human neonatal GIT undergoes several successional events from birth until adulthood – usually characterized by an overall increasing microbial diversity and activity (Koenig et al., 2011; Yatsunenko et al., 2012). In the developing intestine, microbial succession continues until a point called 'climax' community is reached, where bacterial populations remain relatively stable over time and even return to their initial composition after perturbations (Detlefsen et al., 2007). Whether this concept can be transferred to pigs with a relatively short life span is unclear and data rather point towards an ongoing change in bacterial composition in growing pigs between the ages of 10 to 22 weeks (Kim et al., 2011). In pigs, only few studies exist regarding the colonization patterns very early (<14 days of age) in life. Apparently, the very early colonizers between birth and 2 days of age are mainly members of the genera *Escherichia*, *Clostridium*, *Fusobacterium, Streptococcus* and *Enterococcus*, whereas *Lactobacillus, Bacteroides, Prevotella* and *Ruminococcus* increase in abundance afterwards during undisturbed colonization processes (Bian et al., 2016; Kubasova et al., 2017). Similarly, *Clostridium difficile*, a putative pathogen associated with an increased risk for pre-weaning mortality in pigs, can be found in high numbers in the GIT of neonatal piglets but disappears at the age of approximately 14 days (Grzeskowiak et al., 2016). Although the early colonizing communities in pigs cannot be clearly linked to the intestinal microbiota of the sow (Bian et al., 2016; Kubasova et al., 2017), there are indications that the early postnatal environment has strong influence on the composition of the intestinal microbial communities later in life (Thompson et al., 2008; Schmidt et al., 2011; Starke et al., 2013). This early life "microbial programming" might be essential for gut maturation, metabolic and immune development later in life (Merrifield et al., 2015)(see also 2.3). Changing this early life environment (e.g. by moving piglets from their mother into artificial rearing units) leads to a different development of the intestinal microbiota (Schmidt et al., 2011). Feeding formula-based diets (e.g. in breeding lines with large litters of >14 piglets) may amplify these conditions and increase the risk for enteric disease. For example, a high level of lactose in the formula (by replacing maltodextrin) reduced the incidence of necrotizing enterocolitis, increased the

abundance of certain lactic acid bacteria in the small intestine, reduced the concentration of short chain fatty acids (SCFA) in the stomach and increased the concentration of lactate and SCFA in the large intestine (Thymann et al., 2009). With respect to the proliferation of potential harmful bacteria, some studies reported increased *C. perfringens*-like and *Streptococcus*-like phylotypes in the distal small intestine of formula-fed preterm pigs, and higher abundance of clostridia and coliforms in the colon (Siggers et al., 2008; Thymann et al., 2009). However, systematic studies about microbial succession in term-born and formula fed piglets are still scarce. A recent study showed that high levels of lactose (>40%) in formula for neonatal piglets exceeded the small intestinal digestive capacity and increased large intestinal concentration of fermentation metabolites almost 3-fold, and the abundance of enterobacteria and members of clostridial cluster I as compared to suckling piglets (Pieper et al., 2016b). A further understanding of the dynamics of early life intestinal microbial colonization in relation to dietary interventions may help to identify risk factors for intestinal disease and the possible need for medical interventions later in life.

4. Lessons learned from suboptimal gut maturation and nutritional interventions

As the digestive tract of piglets undergoes structural, functional, immunological maturation and as the colonization interplays during these processes, several factors might impact these developmental changes. Dietary interventions can modulate all the piglets of one litter, but yet, not every piglet reacts on a certain event to the same extent. This can be partially explained by inter-individual differences in gut maturity. Hence, intra-uterine growth restriction and prematurity will be discussed first as factors affecting gut colonization and maturation, followed by an overview of dietary interventions.

4.1. Intrauterine growth restriction

4.1.1. Introduction

In comparison with other livestock animals, pigs exhibit the highest number of naturally occurring low birth weight pigs (Cooper, 1975). This low birth weight is mainly the result of intrauterine growth restriction (IUGR) most often caused by placental insufficiency (Ashworth et al., 2001). The prevalence of IUGR piglets increases in highly prolific sows due to uterine crowding and in gilts due to competition with the maternal resources for growth (De Vos et al., 2014). This high prevalence of IUGR pigs affects the profitability of pork production since these pigs exhibit higher mortality and morbidity rates, poorer growth rates and poorer carcass quality when compared with their normal littermates (Tuchscherer et al., 2000, Gondret et al., 2002, Quiniou and Gaudré, 2002, Bee, 2007, Beaulieu et al., 2010, Paredes et al., 2012). This poor profitability and high loss of piglets has driven research to explore the link between intrauterine impaired growth and gut maturation and colonization. Moreover, given the concept of 'metabolic programming', the long-term effects of intrauterine growth restriction gain importance.

4.1.2. IUGR gut maturation and colonization

Many authors have reported differences in intestinal architecture between IUGR and normal birth weight pigs during the neonatal period. In these first days of life, the intestinal absorptive surface was smaller in IUGR as indicated by reduced intestinal villus height and crypt depth (Xu et al., 1994, Che et al., 2010, D'Inca et al., 2010, D'Inca et al. 2011, Mickiewicz et al., 2012, Ferenc et al., 2014). According to D'Inca et al. (2010) this reduction in surface area results from a disturbed proliferation-apoptosis homeostasis possibly in association with an altered gene expression pattern of growth-related proteins (Wang et al., 2005). The smaller intestinal surface area in IUGR piglets is reflected in diminished activities of brush border enzymes, especially lactase (Xu et al. 1994, Che et al. 2010, D'Inca et al. 2010, D'Inca et al. 2011, Ferenc et al. 2014), and affects the gut barrier function. In IUGR piglets, transcellular and paracellular permeability is transiently increased (Wang et al., 2015). The higher transcellular permeability is probably related to a delayed 'gut closure' (Sangild et al., 1999, Jensen et al., 2001) since IUGR piglets

ingest less colostrum (Amdi et al., 2013). Nonetheless, the increased paracellular permeability indicates a compromised barrier, and can explain the higher translocation of antigens and microorganisms in neonatal IUGR piglets as shown by D'Inca et al. (2011).

These structural and functional differences appear not to be present in IUGR piglets that have survived the first critical days after birth (Boudry et al., 2011, Huygelen et al., 2014, Mickiewicz et al., 2012, Wang et al., 2015), although proteomic analysis suggests a continuous impairment (Wang et al., 2010). Proteome analysis revealed that proteins involved in key biological processes, such as absorption, digestion, transport, apoptosis, metabolism and redox homeostasis are affected in IUGR piglets throughout the suckling period (D'Inca et al., 2010, Wang et al., 2010). The interplay between gut maturation and colonization is evident (see 3). Therefore it would not be unexpected to see differences in gut colonization in IUGR. In IUGR pigs the adherent bacterial flora contained a higher number of colony-forming units but only in the neonatal period (D'Inca et al., 2010). Thus, similar as the structural and functional characteristics of the intestinal epithelium, differences between IUGR and normal birth weight pigs with regard to gut colonization and composition are not present in IUGR piglets older than 1 week (D'Inca et al., 2010, De Vos et al., 2014, Prims et al., 2016). Nevertheless, newborn IUGR pigs that face difficulties in receiving sufficient nutrition are at higher risk of dying in the immediate postnatal period due to a compromised digestion, a failing barrier function, and an altered colonization. These factors can be held responsible for the higher morbidity and mortality rates in the neonatal period (Quiniou and Gaudré, 2002). IUGR piglets that receive sufficient support can catch up with the normal maturation pattern.

4.2. Prematurity

4.2.1. Introduction

Following conception the pig fetus develops and matures to reach full term. The number of days to reach full term can vary substantially between litters but will in most cases range between

114-117 days. In a retrospective study of more than 60.000 sow records, Vanderhaeghe and coworkers found a prevalence of 10% early deliveries (defined as <114 days) and that early birth coincided with a higher litter size and higher number of stillborn (Vanderhaeghe et al., 2011). As in humans, it is plausible that early delivery in pigs *per se* is a risk factor for mortality and morbidity. Likewise, it may be possible that piglets within a litter will display variation in degree of maturity at the time of birth. Provided that newborn pigs show varying degrees of maturity at the time of birth, this may account for some of the morbidity and mortality seen in pigs compared with any other mammal species. From this notion, there is a rationale to understand the pathophysiological mechanisms of prematurity in pigs, to identify ways to improve survival in the first days after birth.

To achieve a better understanding of the influence of prematurity, several studies were conducted with cesarean-derived newborn pigs. Strategies to improve survival have included respiratory support, controlled gut colonization, immunological support and nutritional interventions. To maximize the sensitivity toward these interventions, newborn piglets with a much higher degree of prematurity (i.e. born 10-12 days before expected term) than seen under normal circumstances were studied. In brief, pregnant sows were subjected to cesarean section on day 106-108 (Sangild et al., 2013). At this stage of pregnancy pigs require respiratory support, very controlled environmental conditions and careful nutrition, as neither the pulmonary-, circulatory-, thermoregulatory-, immunological-, or digestive system is fully developed. Under experimental conditions, these systems can be sufficiently supported to achieve a similar mortality rate as in pigs born at full term.

4.2.2. Influence of prematurity on gut function and immunity

During fetal life, and particularly in the last trimester, the pig fetuses display swallowing movements, and ingest amniotic fluid. Amniotic fluid contains some of the same components as found in maternal colostrum and milk (e.g. IL-10, TGF-β, EGF, IGF-1) (Siggers et al., 2013) and during the last trimester the fetuses swallow substantial amounts of amniotic fluid with marked

504 trophic effects on gut growth (Sangild et al., 2002; Trahair and Sangild, 2000). If fetal life is
505 disrupted by premature birth, the gut trophic and maturational effects of amniotic fluid are not
506 fully achieved and the intestine displays clear characteristics of prematurity. Postnatal feeding of
507 preterm pigs with collected amniotic fluid has shown gut maturational effects (Siggers et al.,
508 2013) albeit with varying degree of protection from gut disease (Ostergaard et al., 2014; Siggers
509 et al., 2013). This may illustrate how the clinical effects of amniotic fluid depend on whether the
510 intestine is sterile (during fetal life) or colonized with microbes after birth. From this notion,
511 colostrum and milk may be more tailored to secure gut health with the concomitant influence of
512 gut microbial colonization.
513 The prematurity characteristics of the gut include increased permeability (Hansen et al., 2016),
514 reduced digestive and absorptive capacity (Buddington et al., 2008), increased sensitivity to
515 necrotic and hemorrhagic changes, particularly in the colon and distal small intestine (Sangild et
516 al., 2006). These pathological changes are collectively referred to as necrotizing enterocolitis
517 (NEC). Although both etiology and pathogenesis remain enigmatic, there are three wellknown
518 risk factors, i.e. prematurity, gut colonization and enteral feeding. The prematurity *per se* results
519 in insufficient pulmonary surfactant production with poor expansion of the alveoli as a result.
520 Together with a compromised circulatory function, this can result in poor saturation and poor
521 tissue perfusion. The gut is sensitive to poor perfusion and it is plausible that ischemia and
522 hypoxia are key etiologic factors for NEC. In this review we put more emphasis on the the other
523 risk factor, i.e. gut colonization and its relation with enteral feeding (third risk factor).
524

525 4.2.3. Prematurity and gut colonization

526 Following birth the gut is rapidly colonized. While the early colonizers and the succession of
527 microbes depend on environmental bacteria, it is also partly dictated by the immaturity of the
528 host (Cilieborg et al., 2011a). In human preterm neonates it is also influenced by extensive use of
529 antibiotics, resulting in a dysbiotic and unstable gut microbiota. Although the advantages of
530 antibiotics use outweigh the negative effects in preterm human infants, there is a need to

identify ways to stabilize the gut microbiota after antibiotics use. Similarly, antibiotics are commonly used for neonatal pigs under farming conditions to prevent or treat against pathogens. Using a combination of ampicillin, gentamicin and metronidazole, Jensen et al. (2014) showed that suppression of the early colonizers, allows the preterm gut to better adapt to postnatal life and that the protective effect was more pronounced if antibiotics were administered enterally relative to parenteral administration (Birck et al., 2016; Nguyen et al., 2016). Considering the dysbiosis seen in preterm infants, and considering frequent antibiotics use as a premise, it becomes relevant to use probiotics, prebiotics and synbiotics to support gut homeostasis after preterm birth (Johnson-Henry et al., 2016; Sawh et al., 2016). Use of probiotics in preterm human infants has been studied to some extent, yet the evidence for a positive and reproducible outcome is still weak. Whereas most studies do find positive effects of probiotics use against NEC and/or sepsis (Sawh et al., 2016), the effect can also be entirely neutral as indicated in the largest preterm infant probiotics experiment to date (Costeloe et al., 2016). Likewise, in preterm piglets both positive effects (Siggers et al., 2008) and negative effects (Cilieborg et al., 2011c) have been observed, indicating that details regarding choice of strain, timing, dosing and route of administration still need to be optimized to achieve a reproducible and positive clinical outcome.

The influence of gut dysbiosis after preterm birth is exacerbated if enteral nutrition is suboptimal. Following preterm birth, it is well known that mother's milk is more protective against NEC and sepsis than artificial formulas (Gupta and Paria, 2016). For pigs this is even more important as there is no transplacental transfer of immunoglobulins before birth, and the passive immunization therefore has to take place via colostrum ingestion immediately after birth (see also 2). Colostrum provides not only immunoglobulins, but also a range of other compounds including antibacterial, anti-inflammatory, immunoregulatory and growth stimulating factors, that support the gut in the transition to postnatal life outside the uterus. In preterm pigs, provision of colostrum is the most effective way to prevent NEC (Sangild et al., 2006) and the effect appears to be present also when using bovine colostrum (Bjornvad et al.,

558 2008). Although there are species-specific compounds in colostrum (e.g. porcine IgG), there also
559 appears to be a protective effect of factors not specific to pigs. The gut protective effects of
560 bovine colostrum in preterm pigs were studied in a number of studies (Andersen et al., 2016;
561 Cilieborg et al., 2011b; Hansen et al., 2016; Sangild et al., 2006; Sty et al., 2016), and shown how
562 it in most cases can prevent NEC-like lesions. Next, this creates an incentive for formulation of
563 milk replacers that can mimic the effects of colostrum. Factors with immunomodulatory effects,
564 e.g. osteopontin, gangliosides, sialic acid (Moller et al., 2011) or gut trophic effects e.g. GLP-2 and
565 EGF (Benight et al., 2013; Sangild et al., 2006), have been tested in preterm pigs but in general
566 the responses have been minor when tested using milk replacer as base diet. Among the
567 nutritional interventions tested, replacing maltodextrin with lactose has shown most
568 pronounced and reproducible effects (Buddington et al., 2008; Thymann et al., 2009a)(see also
569 1). It remains unclear whether this should be interpreted as protective effects of lactose or
570 detrimental effects of maltodextrin. In pigs, the lactase enzyme is the most dominating
571 carbohydrase after both preterm and term birth, which may in part explain the positive
572 response to lactose relative to maltodextrin. In addition, lactose may serve other important
573 functions directly in the mucosa. Blood samples collected directly from the portal vein under
574 controlled jejunal infusions of lactose, showed that the derivatives of lactose, i.e. glucose and
575 galactose, where not recaptured in a 1:1 ratio in blood although this is how they exist in the
576 lactose molecule (Thymann et al., 2009b). Only half of the infused galactose was recaptured in
577 the portal vein, indicating that it may be used for mucin synthesis or it may be a preferred
578 substrate for mucosal or microbial metabolism.
579
580 4.3. Nutritional modulation
581 4.3.1. Sow's colostrum and milk

582 The intake of colostrum and milk is of crucial importance for the development of the GIT and
583 immune system and therefore affects the survival and growth of the neonatal piglets (see
584 sections above). Colostrum and milk are composed of macronutrients, but contain as well

585 immunoglobulins and immune cells, bioactive molecules such as hormones (e.g. insulin,
586 neurotensin, bombesin, progesterone) and growth factors (e.g. IGF-I, EGF, TGF-β) and prebiotic
587 and antimicrobial compounds. The latter two also playing a role on the establishment of the gut
588 microbiota (Devillers and Lessard, 2007). The concentrations of the macronutrients undergo
589 large changes during the lactation period. A fast drop in the protein concentration, mainly due to
590 a decrease in immunoglobulins, is observed, while the concentration of the energetic molecules,
591 i.e. lipids and lactose increase (Devillers and Lessard, 2007). Concerning porcine milk
592 oligosaccharides, about 30 molecules have been identified, and changes in their composition
593 also occur throughout lactation (Tao et al., 2010; Salcedo et al., 2016). For an extensive
594 overview on the composition and importance of colostrum and milk, we refer to the paper of
595 Devillers and Lessard (2007). In the following sections, we describe interventions through the
596 maternal diet or by direct interventions to piglets in the pre-weaning period.

597

598 4.3.2. Indirect intervention through maternal effects

599 Several studies have been investigating the effect of the supplementation of biological active
600 compounds, such as prebiotics, or probiotics to sows and their effects on performance, immune
601 development and the gut microbiome of their progeny. While very often effects on intestinal
602 health or microbiota are observed, the performance of piglets until weaning remains sometimes
603 unaffected (Leonard et al., 2012; Le Bourgot et al., 2014).

604 The programming and modulation of maternal dietary changes will be discussed in the following
605 sections, but the proof-of-principle has been nicely shown by a study on maternal antibiotic
606 treatment modulating the progeny-pigs and its microbiota on a short- and long-term (Arnal et
607 al., 2014). Indeed, maternal antibiotic treatment from 10 days before the estimated farrowing
608 date until 21 days after farrowing, transiently modified both mother fecal and offspring ileal
609 microbiota during the first weeks of life, without effects on offspring's microbiota on a long-term
610 (169 days of age). The maternal antibiotic treatment transiently induced diverse temporal and

611 regional patterns of selective modifications in crypt depth (reduction), intestinal alkaline
612 phosphatase activity and HSP70 protein production that suggest a lower or delayed response to
613 bacteria especially in the ileum. In addition, site- and sometimes diet-specific long-term effects
614 on key components of intestinal homeostasis (intestinal alkaline phosphatase, DPP-IV) were
615 observed, without alterations on growth performance (Arnal et al., 2014).

616 4.3.2.1. Effects on the microbiota

617 A nutritional modulation of the intestinal microbiota of sows affects bacterial community in the
618 gastrointestinal tract of their suckling piglets, as contact with sow's faeces contributes to the
619 microbial colonization in their offspring. This modulation has been shown to occur for probiotics
620 (Macha et al., 2004; Baker et al., 2013; Starke et al., 2013) and for prebiotics (Paßlack et al.,
621 2015). Modifications of microbial profiles were observed in suckling piglets although the altered
622 profile did not completely mirror the quantitative composition in the sow suggesting
623 modifications took place within the intestine of suckling piglets (Vahjen et al., 2007; Starke et al.
624 2013). Moreover, the influence diminished after weaning probably due to the natural bacterial
625 development as piglets age (see also section 3), superseding the priming effect of a modified
626 maternal microbiota (Starke et al., 2013). In their study, the authors observed that some sows
627 reacted differently to the probiotic supplementation (*E. faecium* NCIMB), which led to the
628 responder hypothesis that proposes an individual response to probiotic supplementation
629 depending on the actual microbiota composition (Starke et al., 2013).

630 Dietary inulin supplemented to sows during the gestation and lactation period, increased the cell
631 numbers of enterococci in sows' feces and in caecal digesta of the suckling piglets, showing the
632 connection between the composition of the intestinal microbiota of mothers and their offspring
633 (Bian et al. 2016). In line with this, Thompson et al. (2008) showed that cohoused piglets, raised
634 by a sow milk replacer, developed very similar communities, indicating the direct environment
635 and contact with other animals as important external factors affecting the development of
636 bacterial communities (see also section 3).

4.3.2.2. Effects on the immune system of the progeny

Supplementing sows with oligosaccharides such as short-chain fructooligosaccharides (scFOS), mannan-oligosaccharides (MOS) or a seaweed extract containing laminarin, a ß- (1-3)/(1-6)glucan, might induce a nonspecific immune response responsible to increase colostral immunity (IgA, IgG or TGFß) (Czech et al., 2010; Leonard et al., 2010; 2012; Le Bourgot et al., 2014). The altered synthesis of key cytokines into mammary secretions might then influence the maturation and development of immune cells in offspring (Donnet-Hughes et al., 2000; Nguyen et al., 2007). This was observed in the study of Leonard et al. (2010) where gestating and lactating sows were supplemented with a seaweed extract (SWE) and piglets had a greater percentage of *E. coli* phagocytizing leukocytes and a decreased percentage of *E. coli* phagocytizing lymphocytes at weaning. Moreover the results after an *ex vivo* LPS challenge on ileal biopsies indicated that piglets from SWE supplemented sows had an enhanced immune function (Leonard et al., 2012). A later study on the supplementation of laminarin and/or fucoidan to sows showed that laminarin induced positive effects on intestinal architecture and health on day 8 postweaning and improved growth performance during the grower-finisher period (Heim et al., 2015). The authors proposed that the down-regulation of IL-6 gene expression may be attributed to the increased Lactobacillus spp. gene numbers in the colon of piglets and that the down-regulation of the pro-inflammatory cytokines may provide more nutrients available for growth. Alternatively, an increased secretion of pro-inflammatory cytokines (IFNγ) by Peyer's Patches (PP) and mesenteric lymph nodes cells, a higher proportion of activated T cells in ileal PP, and increased secretion of sIgA by PP cells were observed in suckling offspring of scFOS supplemented sows (Le Bourgot et al., 2014). The authors hypothesized that the colostral immunoglobin and cytokine concentration induced by scFOS may modify the early process of commensal microbiota establishment in the intestine and of sIgA responses needed to maintain gut homeostasis.

Besides prebiotics, also conjugated linoleic acid or oils rich in omega-3 fatty acids provided to the sows during gestation and/or lactation have been shown to increase colostral immunoglobulins (Bontempo et al., 2004; Mitre et al., 2005; Corino et al., 2009), improve piglet body weight (Mitre et al., 2005; Corino et al., 2009), increase immune components (Fritsche et al., 1993; Bontempo et al., 2004; Mitre et al., 2005; Patterson et al., 2008; Corino et al., 2009) and reduce intestinal inflammation (Patterson et al., 2008).

4.3.3. Direct interventions in piglets during the pre-weaning period

4.3.3.1. Formula versus maternal milk

Current pig breeding uses hybrid sows with a high prolificacy (De Vos et al 2014), leading to the need of supplementing piglets with formula milk. As sow's milk contains bioactive compounds such as cytokines, hormones, immunoglobulins, stimulating and regulating the growth and maturation of the intestine (Devillers and Lessard, 2007), the composition of formula milk might also be enriched with several compounds to enhance gut development and modulate microbiota composition. The comparison between sow-reared and formula-fed piglets showed a comparable daily weight gain on day 28 of age, a greater absorptive area, deeper crypts, and higher maltase and sucrase activities in the small intestine compared to suckling piglets, after a delay in the functional maturation, reflected by a decreased lactase, dipeptidylpeptidase IV and sucrase activities on day 10 (De Vos et al., 2014). In the cecal contents of 21-d old piglets, *Prevotella* was the dominant genus within mother-fed samples and *Bacteroides* was most abundant within formula-fed samples (Poroyko et al., 2010). A metatranscriptomic analysis revealed increased transcripts for utilization of L-arabinose, a sugar known to accumulate in the distal intestine of formula-fed pigs due to the hydrolysis of plant-based non-starch polysaccharides additives (Schutte et al., 1992), and for utilization of the sugar alcohol mannitol in formula fed samples. Several differences were observed for transcripts encoding amino acid metabolism, and sow-fed samples had enriched sequences assigned to oxidative stress, which could be consistent with antioxidant properties of maternal milk (Poroyko et al., 2010). Other

688 studies support differential microbiota composition and metabolic activity between sow-reared
689 and formula-fed piglets (Li et al., 2012; Wang et al., 2013).

690 4.3.3.2. Milk composition

691 Prebiotics can be added to artificial milk to mimic the bioactive compounds present in maternal
692 milk. The addition of scFOS and polydextrose to formula milk modulated microbial colonization
693 and produced short-chain fatty acids pattern closer to that of sow-reared piglets, as seen by
694 similar propionate and butyrate concentrations on d14 (Wang et al., 2013). In agreement with
695 this modulation, Howard et al. (1995) observed that FOS was able to stimulate the bifidobacteria
696 population in piglet's feces and Alizadeh et al. (2015) observed an increase in fecal lactobacilli
697 and bifidobacteria in formula-fed piglets supplemented with galacto-oligosaccharides.
698 Moreover, an increased cecal butyrate concentration was observed, which is known to prevent
699 the colonization of various pathogens such as *E. coli*, to inhibit inflammation and to improve gut
700 barrier function (Hinnebusch et al., 2003; Peng et al., 2009). The latter seemed to be stimulated
701 by dietary GOS, given for 26 d (Alizadeh et al., 2015). Prebiotics in formula milk can furthermore
702 improve intestinal architecture (Alizadeh et al., 2015) and enhance epithelial cell proliferation
703 (Howard et al., 1995). In addition, prefeeding of inulin reversed the inhibitory effects of a later
704 supplemented laxative dose of lactulose on cecal cell proliferation and diarrhea (Kien et al.,
705 2004).

706 Besides prebiotics, high protein (HP) concentration in formula milk has been shown to affect
707 microbiota proliferation on d7 (Chatelais et al., 2011), without changing the microbiota
708 composition on d28, increase intestinal transcellular permeability (Chatelais et al., 2011; Boudry
709 et al., 2013) and modify immune development (Chatelais et al., 2011; Boudry et al., 2013). Later
710 in life, HP formula diet increased colonic permeability (Boudry et al., 2013) and affected the ileal
711 and colonic response to inflammatory mediators in a gender specific way (Chatelais et al., 2011;
712 Boudry et al., 2013), probably through microbiotal and hormonal factors (Boudry et al., 2013).

713

Conclusion

In conclusion, the GIT of neonatal piglets undergoes important maturation processes and establishes its ecosystem, both being far from complete by the time of weaning. Even more, these processes will be disturbed during the weaning period, and strategies should focus to minimize the impact of this abrupt weaning on the GIT and its microbial community. Furthermore, preterm birth in pigs, even a few days before the expected term date, and reflected by a low birth weight is a major risk factor for gut dysbiosis and mucosal dysfunction. Dietary strategies such as the use of pre- and probiotics applied during the perinatal period should try to stabilize the gut microbiota and stimulate the innate immunity. It becomes more and more evident that long-term effects are provoked by early-life interventions on microbiota composition and gut homeostasis. Thus, while most of the studies on nutritional modulation report mainly on a short-term, it is expected that future research will also reveal strategies to improve gut homeostasis at the physiological, immunological and microbial level on the long-term.

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Table 1. Overview of developmental changes after birth in pigs

Developmental changes after birth	**References**
Structural aspects	
Massive growth of the stomach by hypertrophy and mainly hyperplasia	Lindemann et al. 1986
Shift in enterocyte types	Smith and Peacock, 1980, Klein, 1989
Postnatal enlargement of the intestinal mucosa, changes in villus length and crypt depth, accompanied by changes in the shape of the villi	Wolinski et al., 2003; Godlewski et al., 2007; Skrzypek et al, 2005; Skrzypek et al, 2010
Uptake of macromolecules to the enterocytes by endocytosis which ceases at a certain moment due to intestinal closure	Brambell, 1970; Baintner, 1986
Bioactive substances in the colostrum and milk stimulate intestinal mucosal proliferation and facilitate the closure of the small intestine	Takeda et al., 2004
Morphological changes of colonocytes, with disappearance of villi-like structures	Cremaschi et al. 1979; Xu et al. 1992a
Functional aspects	
Gastric acid secretion is low at birth but increases rapidly during the first week of life	Xu and Cranwell 1990 , Sangild et al. 1991; Sangild et al. 1992
Activities of the brush border peptidases are relatively high at birth and then decrease with age in suckling pigs	Le Huerou-Luron, 2002
Shift in expression of the enterocyte brush border disaccharidases	Le Huërou-Luron 2002
Lactase activity decreases with age, while maltase and sucrase activities increase	Le Huerou-Luron 2002
As the milk clots in the stomach, a period of inhibition of motility is established, followed by a slow phase of emptying	Decuypere et al., 1986
During aging, a faster small intestinal transit is observed	Huygelen et al., 2015
Gut immune system	
The newborn piglet are born hypogammaglobulinaemic and must acquire passive immunity (IgG) via mammary secretions for their survival	Bailey et al, 2005
Peyer's patches are present at birth and contain very small, primordial follicles and almost no T-cells	Makala et al, 2000
While the size of B-cell and T-cell compartments expands, function remains limited for several weeks	Barman et al, 1997; Makala et al, 2000

While piglets under 6 weeks can make antigen-specific responses, the quality of the response may be limited compared to older animals	Wilson et al, 2005
Antigen-presenting cells appear in the intestinal mucosa during the first two weeks of life	Bianchi et al, 1992; Vega-Lopez et al, 1995
CD4 T-cells appear in the mucosa during weeks three and four, and CD8 T-cells in the epithelium start to appear from four to 6 weeks old	Bianchi et al, 1992; Vega-Lopez et al, 1995
Gut colonization	
The establishment of the intestinal microbiome of pigs occurs early in life and is dependent on events such as environmental or dietary changes, or medical intervention	Koenig et al., 2011; Yatsunenko et al., 2012; Thompson et al., 2008; Schmidt et al., 2011; Starke et al., 2013
The very early colonizers between birth and 2 days of age are mainly members of the genera *Escherichia*, *Clostridium*, *Fusobacterium, Streptococcus* and *Enterococcus*, whereas *Lactobacillus, Bacteroides, Prevotella* and *Ruminococcus* increase in abundance afterwards during undisturbed colonization processes	Bian et al., 2016; Kubasova et al., 2017
Pre-weaning interventions in husbandry and diet have been shown to affect the establishment of intestinal microbiome and the development of the immune system	Inman et al, 2010b; Lewis et al, 2012
Colostrum and milk	
A fast drop in the protein concentration of the milk, mainly due to a decrease in immunoglobulins, is observed, while the concentration of the energetic molecules, i.e. lipids and lactose increase	Devillers and Lessard, 2007
About 30 porcine milk oligosaccharides have been identified, and changes in their composition also occur throughout lactation	Tao et al., 2010; Salcedo et al., 2016