1. GENERAL INTRODUCTION

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Weaning in piglets is characterized by nutritional, environmental and social stress, often associated with reduced body weight gain, nutrient malabsorption and increased incidence of diarrhea (Boudry et al., 2004; Hedemann and Jensen, 2004). To minimize post-weaning associated disorders in piglets, certain feed additives may be used to modulate intestinal microbial fermentation pathways, thereby improving gut health conditions (Awati et al., 2006a). Hence, manipulating the composition and metabolic activity of the gut microbiota through diet composition has gained increased interest in the post-antibiotic era. Since intestinal bacteria vary in their genetic potential for substrate utilization, there is great potential to beneficially manipulate microbial ecology in the gastrointestinal tract (GIT) by modulating diet composition (Pieper et al., 2009). Different strategies including the use of prebiotic carbohydrates ("non-digestible food ingredients that stimulate the growth and/or activity of selected bacteria strains", Gibson and Roberfroid, 1995) and other feed additives have been described to support the intestinal ecosystem (Pettigrew, 2006; Stein and Kil, 2006). In contrast, bacterial proteolysis limits amino acid availability to the host and results in the production of branched-chain fatty acids and several putrefactive compounds, including ammonia and amines (Swanson et al., 2002). These compounds can have toxic effects on pigs and microbes, and may influence metabolic function and diversity of the gut microbiota (Gaskins, 2003). Jeaurond et al. (2008) hypothesized that feeding fermentable substances such as carbohydrates seems to be suitable for reducing the negative impact of proteolytic fermentation in the GIT. The inclusion of carbohydrates with prebiotic properties, e.g. inulin, pectin, fibers, in pigs` diets to stimulate establishment of beneficial enteric microorganisms may inhibit the proliferation of enteric pathogens and decreases formation of toxic fermentation products (Williams et al., 2001). On the other hand, the environmental conditions in the GIT play also an important role in the development and metabolic function of the intestinal microbiota. Among others, the osmolarity of the environmental medium is one of the most variable parameters bacteria of the intestine have to cope with. This explains that much attention has been paid in recent years to understand the mechanisms of bacterial adaptation to increased osmolarity (Picherau et al., 1999). Recent results suggest that osmolytes, such as betaine (Eklund et al., 2006a,b), may have the potential to modify as feed additives the microbial community and activity in the porcine GIT, due to their

osmoprotective character which, in turn, may beneficially affect composition and metabolic activity of the intestinal microbiota.

1.2. THE INTESTINAL MICROBIOTA

1.2.1. Development of piglet`s intestinal microbiota

The GIT of piglets is sterile at birth, and is then colonized by microbes originating from feces and skin of the mother (Conway, 1997) as well as the ingested milk and the environment. The intestinal microbial community has also been shown to be age-dependant, as its development after birth is a complex process involving different colonization phases. There is sufficient evidence that colonization of the GIT starts with lactic acid bacteria, enterobacteria and streptococci (Lallès et al., 2007). In the immediate post-weaning period, the balance between the development of so-called healthy commensal microbiota and the establishment of bacterial intestinal disease can be easily deflected towards disease expression (Hopwood and Hampson, 2003). As it is shown in Table 1, the microbiota harbouring the GIT is unstable during the first week after weaning (Jensen, 1998), and is characterized by a marked decrease in biodiversity (Wallgren and Melin, 2001). The number of total bacteria as well as lactic acid bacteria in the small intestine, for example, declines within the first day after weaning, but increases until day 11 post weaning. On the other hand, there is a considerable decrease in the mean cell counts of bifidobacteria to numbers below the detection limit on day 11 after weaning.

After piglets` transformation from liquid to solid feed, obligate anaerobes increase in number and diversity until an adult-type pattern is achieved (Inoue et al., 2005; Konstantinov et al., 2006). Within a short period of time, the intestinal microbiota needs to change ultimately from a simple and unstable community into a complex and stable community, thereby generating a tight colonization resistance. This is described as mechanisms whereby the intestinal microbiota protects itself together with the host against incursion by new and often harmful microorganisms, e.g. through more successful competition for the same ecological niche or production of microbicidal substances (Lallès et al., 2007).

Table 1:Cell counts of bacterial groups (in log cells / g digesta) in the small intestinal digesta of piglets during the post-weaning period (adapted from Pieper et al., 2008)

	Days post-weaning		
	0	1	11
Total bacteria	8.96	8.44	9.35
Lactobacilli / enterococci	8.82	6.72	8.78
Bifidobacteria	6.46	5.70	n.d.
Eubacterium rectal / *Clostridium coccoides*	5.27	6.67	6.23
Bacteroides / *Prevotella*	5.68	6.23	6.41
Enterobacteria	7.35	7.19	6.50
Escherichia coli	7.24	7.05	n.d.

n.d.: Not detectable; below detection level of 4×10^4

According to Zoetendal et al. (1998), each individual harbours a specific and unique bacterial community in the GIT. In addition, there is general agreement that the density and composition of intestinal bacteria may vary considerably among the different segments of the porcine GIT (Jensen and Jørgensen, 1994; Leser et al., 2002), as it is shown in Figure 1. Proximal segments of the GIT are colonized by relatively few (Jensen and Jørgensen, 1994) but complex indigenous bacterial populations (Jensen, 2001), while the cecum and colon represent the major sites of bacterial activity in the pig gut (Bach Knudsen et al., 1991). In the acidic environment of the stomach, lactic acid bacteria, mainly lactobacilli and streptococci (Jensen, 2001), are the most important bacterial groups besides other groups including enterobacteria, clostridia, and bifidobacteria (Conway, 1994). For the distal small intestine, with its slightly altering conditions (higher pH and slower passage rate), lactobacilli, streptococci, clostridia, enterobacteria, *Bacillus* and *Bacteroides* spp. have been described as the predominant culturable bacteria (Conway, 1994; Jensen, 2001). The main metabolically active microbiota in the cecum and colon consists of Gram-positive anaerobe cocci (such as streptococci and peptococci), besides lactobacilli and clostridia (Russel, 1979; Robinson et al., 1984). In the colon, Gram-negative bacteria are present in relatively low percentages amounting to 7 – 29 % (Russel, 1979; Robinson, 1984; Leser et al., 2002), and the largest fraction is suggested to belong to the groups of *Bacteroides* and *Prevotella* (Leser et al., 2002). During the past decade, due to the development and use of molecular

techniques (e.g. denaturing gradient gel electrophoresis, real-time polymerase chain reaction; PCR), the composition of the bacterial community harbouring the GIT has been more specified. However, the majority of intestinal bacteria still remains uncharacterized (Leser et al., 2002).

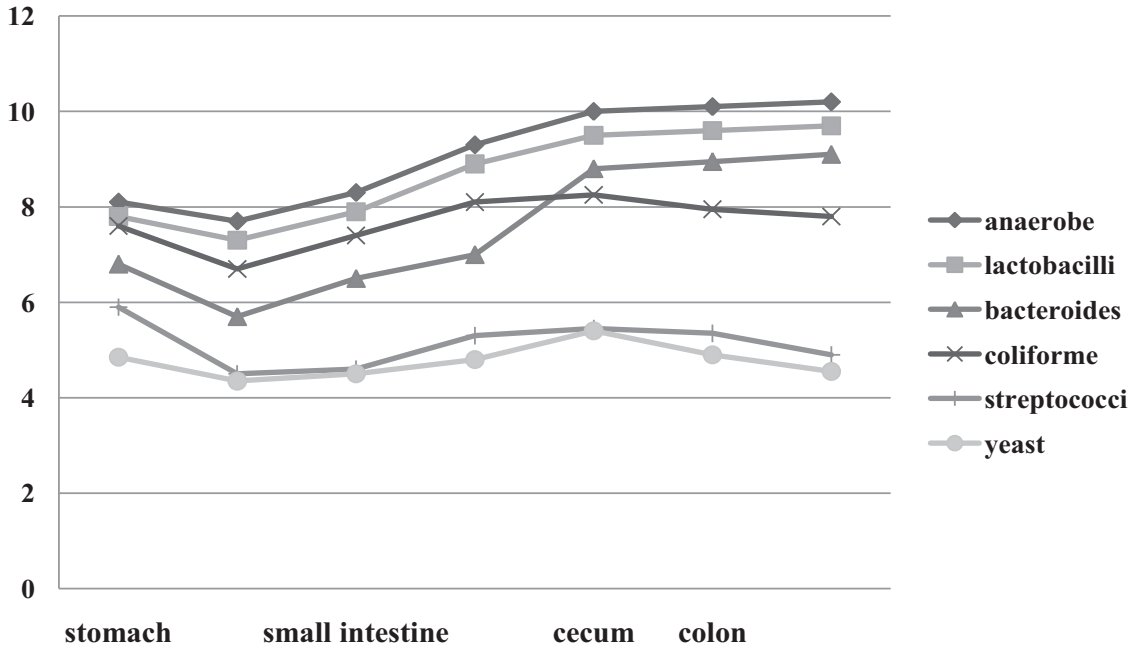

Figure 1: Cell counts of bacterial groups (in log CFU / g gut content) in the GIT of pigs (adapted from Jensen, 1993)

1.2.2. Interaction between intestinal microbiota and the host

The microbial colonization of the gut has been shown to exert several positive effects to the host (Berg et al., 1996), but these effects are also strongly related to intestinal disorders or diseases. The benefits of intestinal bacteria have been described in detail by different authors (e.g. Berg et al., 1996), and include among others, the inhibition or competitive exclusion of pathogens as well as the stimulation of the intestinal development and function.

Lactic acid bacteria are present throughout the entire GIT and are considered to be favorable to the host. They produce organic acids such as acetic or lactic acid due to the fermentation of lactose in the stomach and small intestine as well as fiber degradation in the large intestine (Jensen, 2001). The lactic acid producing bacteria mainly consist of bifidobacteria and

lactobacilli, but streptococci and enterococci are also capable of producing lactic acid (Duncan et al., 2004). In the upper parts of the GIT, organic acid production supports the maintenance of a lower pH, thereby inhibiting the proliferation of potential disease-causing microbes (Hodgson and Barton, 2009). In the large intestine, considerable amounts of lactic acid are utilized by other bacterial species, and mostly converted into acetate and propionate, but also into butyrate (Hashizume et al., 2003).

The genus *Enterococcus* consists of Gram-positive, facultative anaerobic bacteria which are present both as single cocci and in chains (Fisher and Philips, 2009). Large numbers of *Enterococcus* species also belong to the group of organisms known as lactic acid bacteria, which have proven their beneficial effects as probiotics in several studies in livestock (e.g. Jin et al., 2000). Their main benefit is described as strong inhibitory effect on the adhesion or proliferation of intestinal pathogens. The high antagonistic activity of *Enterococcus* strains (e.g. *Enterococcus faecalis*, *Enterococcus mundii*, *Enterococcus faecium*) against a pathogenic *Escherichia coli* (*E. coli)* strain was found *in vitro* by García-Galaz et al. (2004), where the proliferation of pathogenic *E. coli* was reduced by at least about 80 %. In contrast, Teixeira et al. (2001) could recover two new *Enterococcus* species (*Enterococcus porcinus* subsp. *nov*. and *Enterococcus ratti* subsp. *nov.)* from pigs and rats with enteric disorders, which are suspected to be new diarrheagenic pathogens for animals.

Clostridiaceae family contains speciess which show cellulolytic, pectinolytic and hemicellulolytic activities (Metzler and Mosenthin, 2008), therefore being particularly important in fiber digestion (Ziemer and Kerr, 2009). Since clostridia are among the main producers of the favored butyric acid, they play a vital role in energetic supplementation of the colonocytes (Gottschalk, 1986; Sanz et al., 2005). However, pathogenicity of some clostridia strains could be shown in several studies as well (Yaeger et al., 2002; García, 2009).

As *E. coli* is economically one of the most important pathogenic bacteria in pig production (Nagy and Fekete, 2005), it is considered to be an undesired inhabitant of the porcine GIT. Diarrheal diseases in livestock are often due to infection by one of various pathotypes of *E. coli* (Nagy and Fekete, 2005). In particular, enterotoxigenic *E. coli* (ETEC) is a major cause of watery diarrhea in suckling and weaning piglets (Nagy and Fekete, 2005). However, there are also non-pathogenic *E. coli*, which mainly occur in the lower gut (Arbuckle, 1970).

The ratio between lactobacilli and enterobacteria has been used as a simple index for health-promoting microbiota in the GIT, and an increase in this ratio implied a higher resistance to intestinal disorders (Muralidhara et al., 1977; Reid and Hillmann, 1999). Particularly in weaning pigs, the supplementation of dietary lactobacilli as probiotic ("Live microorganisms which when administered in adequate amounts confer a health benefit to the host", FAO/WHO, 2001) could have an important role in controlling colibacillosis, which is one of the most common intestinal disorders during the first month of life (Tortuero et al., 1995; Nemcova et al., 1999). Konstantinov (2008) found that probiotic supplementation containing *Lactobacillus sobrius* significantly reduced ileal levels of ETEC when fed directly to piglets after weaning. Zhang et al. (2010) could confirm these findings after supplementation of a probiotic *Lactobacillus* strain in a study with weaned barrows.

1.3. MICROBIAL FERMENTATION AND DIETARY MANIPULATION OF GASTROINTESTINAL MICROBIOTA

Since intestinal bacteria vary in their genetic potential for substrate utilization, there is also a considerable potential to beneficially manipulate microbial ecology in the GIT by diet composition (Pieper et al., 2009). The amount and composition of substances reaching the large intestine can be readily modified by diet composition. As main fraction, beside others, are carbohydrates most important in terms of providing bacterial substrates (Snel et al., 2002). Especially the fiber fraction mainly consisting of non-starch polysaccharides (NSP), resistant starch and non-digestible oligosaccharides (Bauer et al., 2006), is the main energy source for intestinal bacteria (Bach Knudsen et al., 1991). Their fermentation results in the production of short-chain fatty acids (SCFA), mainly acetic, propionic and butyric acid, as well as in different gases (hydrogen, carbon dioxide, methane) (Bach Knudsen et al., 1991). According to Williams et al. (2001), about 68 % of the energy value of fermented carbohydrates can be metabolized into SCFA. The SCFA can be rapidly absorbed by the intestinal epithelia (Argenzio and Meuten, 1991), and are known to play important roles, e.g. in affecting colonic mucosal blood flow, cecal mucin secretion, or mucosal cell proliferation among others (Scheppach, 1994). Especially butyrate is known to have implications on colon health, and to be the preferred energy source for colonocytes (Ritzhaupt et al., 1998; Scheppach et al., 1998). According to some studies (e.g. Varel and Pond, 1985; Moore et al., 1987), diets high in fiber promote the presence of cellulolytic bacteria without changing the total number of microorganisms in the GIT. Högberg and Lindberg

(2004) reported for weaned piglets, that an increase in dietary content of NSP influenced the distribution of organic acids in the ileum, indicating a shift in dominating bacteria.

However, there are differences in the fermentability between the carbohydrates due to differences in their chemical composition and structure (e.g. Smiricky-Tjardes et al., 2003). For example, while soluble NSP, such as pectin and inulin, tend to be highly fermentable (Bach Knudsen, 2001), insoluble NSP, such as cellulose, provide a substrate that is more slowly fermented by the intestinal microbiota (Freire et al., 2000). For example, in previous *in vitro* studies (e.g. Salminen et al., 1998), highest acetic acid production was obtained for the carbohydrate pectin, while starches produced the highest amount of butyric acid. In addition, the inclusion of carbohydrates with prebiotic properties, e.g. inulin and pectin, in pigs` diets to stimulate establishment of beneficial enteric microorganisms may limit the proliferation of enteric pathogens and decreases formation of toxic fermentation products (Williams et al., 2001). In a continuous *in vitro* fermentation system simulating pigs` GIT fermentation, a higher density of bifidobacteria was reported upon supplementation with inulin (Tzortzis et al., 2005). Such an increased number of *Bifidobacterium* spp. has been associated with production of beneficial SCFA *in vitro* (Tzortzis et al., 2005), together with a reduced growth of pathogenic bacteria in the intestine *in vivo* (Zhang et al., 2010), thereby promoting piglets` health conditions. Interestingly, in a study of Loh et al. (2006) the addition of inulin to different basal diets increased the proportion of piglets with counts of bifidobacteria above the detection level, while lactobacilli counts remained unaffected. In contrast, in a study of Tako et al. (2008) in pigs, an increase of lactobacilli and bifidobacteria upon inulin supplementation was determined. Additionally, in a study with weaned pigs, a reduction in the count of coliforms was observed due to inulin supplementation (Wellock et al., 2008). In grower pigs, ileal infusion of pectin largely stimulated the pectinolytic activity determined at the fecal level, likely reflecting changes in the composition of the microbiota (Metzler et al., 2008). Generally, based on *in vitro* studies, pectin was found to be degraded by different intestinal bacterial groups including *Bacteroides* spp., clostridia and bifidobacteria (Dongowski et al., 2000; Olano-Martin et al., 2002), suggesting that pectin represents a preferential substrate for these strains.

In addition to fermentable NSP, dietary starch may also be used as a major substrate for colonic fermentation (Morita et al., 1998). Starch that enters the large intestine is believed to be the most important fuel for colonic bacteria (Cummings and Macfarlane, 1991). This so-called resistant starch is known to selectively stimulate butyric acid producing bacteria, e.g.