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2 Metabolism and population dynamics of the intestinal microflora in the growing pig

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The intestinal flora of pigs contains several hundred microbial species, mostly strict anaerobes. A great amount of these bacteria reside in the large intestine, which in adult pigs consists of mainly Gram-positive bacteria such as cocci, lactobacilli, eubacteria and clostridia. The composition of the intestinal microflora is a result of the interaction between the microorganisms that colonize the gut and the intestinal physiology of the pigs. The initial inoculum is usually derived from the sow at the time of birth and the climax flora is developed through a gradual process in which there is a shift in relative abundance of various microorganisms, especially throughout the first month of the pig’s life. While a part of this microflora is constantly present in the gut (resident flora), some microorganisms have a short residence and dynamically change the composition of the microflora. The turnover of this flora (also known as the transient flora) in the gut depends on both the composition of the resident flora and the degree of contamination of ingested food and other sources which in traditional indoor farming include the sow’s skin and the pen’s environment. The stability and diversity of this flora has a tremendous role in maintaining the health status of the pigs, especially during the suckling and post-weaning period. Most investigations of the intestinal flora in pigs focus on classical and/or molecular methods, aiming to isolate, enumerate and/or qualitatively identify different bacterial groups. Other recent studies that measure the metabolic capability and functional status of the intestinal microflora in pigs have added knowledge about the composition and dynamics of the gut flora, especially in pre- and post-weaning pigs.

1. INTRODUCTION

The intestinal microflora of pigs comprises hundreds of bacterial species most of which are residing in the lower part of the gastrointestinal tract. This flora develops
through a process of ecological succession and plays a tremendous role in the state of health and disease of pigs, especially during suckling and post-weaning periods. Among the important factors in this process is the influence of the interaction between the microorganisms that contaminate the animal, diet regime and food composition, immunological status of the pig and environmental factors on the intestinal physiology. Several studies have tried to identify the types of bacteria colonizing the intestine of growing pigs. Most of these studies utilize selective media, which lead to enumeration of few particular bacterial groups. The problems associated with culture-based techniques are yet exacerbated in anaerobic habitats. Using conventional techniques of culturing and identification, only about 30–40 separate species have been generally recovered from any individual animal. Recent development of more refined molecular techniques has opened new windows of opportunity to study unculturable bacterial components of the alimentary tract or of members of the intestinal microflora that cannot tolerate exposure to oxygen.

In addition to this new and promising approach, recent in vitro methods focus on measuring the metabolic activities of the major intestinal flora. These methods, alone or in combination, have been extensively used to investigate the population structure and the functional status of the intestinal flora in growing pigs.

In this chapter we discuss the available information on population dynamics of the intestinal flora in growing pigs, and address factors involved in changes of this flora during different stages of the animal’s life and in health and disease.

2. PIG HUSBANDRY

Despite the fact that adult pigs may weigh over 300 kg, they only weigh between 1 and 2 kg at birth. A sow normally gives birth to a litter of around 10 piglets and, in accordance with modern agricultural systems, piglets are allowed to suckle their dam for a comparably short period. Many countries practise weaning when piglets are around 3 weeks old. However, the length of the suckling period varies somewhat between countries and rearing systems. Thus, it could be summarized that piglets in modern systems have access to their dam and her milk for a period ranging from 2 to 7 weeks. From the time of weaning until the weight of approximately 25 kg, piglets are referred to as weaners or weaning pigs. From then, and until slaughter, pigs are denoted fatteners or finishing pigs. Market weight varies over the world but is commonly around 100 kg live weight. The age at slaughter varies with health status, breed, feed intensity and rearing system, but fatteners generally are around half a year when slaughtered.

The breeding stock is often primarily selected soon after birth in terms of prospective gilts and boars. After a secondary selection at puberty, the selected gilts are mated at approximately 7 months of age. After a pregnancy period of 116 days they deliver their first litter at the age of 11 months. After that time they may deliver slightly more than two litters annually. In modern husbandry, sows could give birth to up to 10 litters, but they are generally replaced far earlier.
3. PHYSIOLOGY AND ANATOMY OF THE GASTROINTESTINAL TRACT OF PIGS

The digestive tract of the newborn piglet is specialized on a diet comprising milk. Therefore, a high lactase activity is present in the small intestine (Hampson and Kidder, 1986), at least for as long as milk comprises the dominant feed source. Other organs important for the digestive system, such as the pancreas (Kelly et al., 1991a), are not quite active during intensive suckling. Owing to the abrupt change from milk to cereal consumption at weaning, the lactase activity rapidly declines during the post-weaning period (Hampson and Kidder, 1986). Instead, α-amylase is increasingly produced by saliva, stimulated by chewing. Further, the pancreas becomes active at weaning, and starts to excrete pancreatic juice (Kelly et al., 1991a,b).

The sow’s milk constitutes a compact food, and the intestine (especially the large intestine) is comparably small during the suckling period (Kelly et al., 1991a). At weaning, the domesticated piglets are offered cereals instead of milk. As a consequence, the stomach and the intestine rapidly increase in size (Kelly et al., 1991a). Despite this, the ability to absorb nutrients might decrease due to a reduction in the height of the intestinal villi during the post-weaning period (Hampson, 1986), resulting in a decreased total area of the surface of the intestinal lumen. The diet might also influence the size of the intestine during the subsequent rearing of pigs. Fibre-rich feed sources are correlated to an enlargement of both stomach and large intestine (Anugwa et al., 1989). The latter is rather expected, because fermentation as well as absorption of electrolytes and fluids takes place in the large intestine. However, a lower capacity to absorb water during the first 2 weeks following weaning makes recently weaned piglets vulnerable to loss of fluid from the intestine (van Beers-Schreurs et al., 1998), and may possibly contribute to outbreaks of post-weaning diarrhoea (see below).

4. DIET REGIMES AND ALTERATIONS OF FOOD COMPOSITIONS DURING THE GROWTH OF PIGS

It is of decisive importance that the newborn piglet consumes colostrum, not only to get energy, but also to obtain immunoglobulins, since the porcine placenta does not allow transfer of passive immunity from the sow. Therefore, the intestine of the piglet allows digestion of macromolecules during the first 24–36 h of life. At farrowing, the colostrum comprises around 160 g (16%) protein per litre, which rapidly declines. Twenty-four hours later the protein content of the milk is around 6%. During the first day post-farrowing the lactose content increases from 3 to 5%. In contrast, the fat content is rather stable around 5.5–6.5% (Klobasa et al., 1987).

The young piglet is continuously dependent on milk until weaning. The composition of the milk varies somewhat over the suckling period, but generally comprises 5.0–6.5% protein, 5.5–6.5% lactose and 5.5–6.5% fat (Klobasa et al., 1987). The milk also contains IgA, which may protect the piglet from enteric diseases by acting locally in the gut.
The sow eagerly offers her milk to the litter during their first week of life. However, sucking milk is generally initiated by the constantly hungry offspring from the second week of life onwards (Algers, 1993). To protect herself from catabolism, the wild sow copes with this situation by avoiding contact with her litter during a great part of the day. Thereby, wild piglets are weaned in a gradual process. The access to milk will continuously be reduced in comparison to the energy required, and the piglets are forced to successively search for alternative energy sources. The final weaning takes place at around 16 weeks of age (Jensen and Recen, 1985), and at that age the piglets are well adapted to other foodstuffs than milk. Further, they are well developed with respect to immune functions at that age (Joling et al., 1994).

A domesticated sow shares pen with her offspring during the suckling period. She is thereby denied the ability to shun the litter and, as piglets prefer milk as a source for energy, she will be intensively suckled. In order to protect domesticated sows from their hungry brood, piglets in modern agricultural systems are weaned between 2 and 7 weeks of age. However, the early weaning system is chiefly employed to improve production, i.e. to increase the number of piglets produced per sow per year. Generally, this weaning is effected by removal of the dam from the offspring. As a consequence, the domesticated piglet will experience weaning at an unexpected point of time. There is a potential risk to develop disease at weaning due to:
1. an abrupt change of the feed composition where the diet is switched from milk-based to solid-based feed, mainly cereals. This change also includes a sudden withdrawal of the protective IgA that is also present in the milk;
2. a poorly developed immune system. In this context it is relevant to point out that piglets aged 5–6 weeks are not fully developed with respect to immune functions, and that piglets aged 2–3 weeks are even more immature (Wallgren et al., 1998);
3. the social alterations at weaning, which contribute to a long-lasting unpleasant situation for the piglet at weaning.

To prevent disturbances at weaning (and at other occasions), so-called growth promoters have generally been added to the feed of growing pigs for decades. The term “growth promoter” in this context refers to low dose administrations of antimicrobials (i.e. antibiotics or chemotherapeutics). Recently such a routine administration of antimicrobials to animal feed has been questioned, both from ethical and from ecological and medical perspectives. For instance, a ban for routine in-feed medication was effected in Sweden during 1986 (Swedish statute-book; SFS 1985: 295, Stockholm, Sweden). According to that act, antimicrobials may only be incorporated in animal feed for the purpose of preventing, alleviating or curing disease, i.e. not for growth or yield promoting purposes. The European Communities (EC) have followed this example regarding 8 out of 12 permitted substances during 1999 (Council directive 70/524/EEC on Feed additives, EC, Brussels, Belgium), and the remaining substances are to be discussed (COM 2002, 153: final, EC, Brussels, Belgium).
In this chapter it is assumed that antibiotics are not added to the food for growth promotion.

The pigs will never again experience such a dramatic alteration of feeding habits as they do at weaning. From that time they are offered feed based on cereals. The cereal-based feed may be supplemented with protein-rich sources, such as fishmeal and soybeans. Also bone meal and meat meal have been used as a protein source. However, the present discussion concerning transmissible spongiforme encephalitis (TSE) makes the future of the abattoir waste as protein source for meat producing animals less clear. High protein levels in the feedstuff are known to stimulate growth. However, protein may also provoke the enteric flora, which might lead to diarrhoea (Newport, 1980; Shone et al., 1988; van der Peet-Schwering and van der Binnendijk, 2000). Predigestion of proteins, for instance casein, is proven to decrease the risk of developing diarrhoea (Miller et al., 1984). Aiming to reduce feed provocation without reducing the growth, feed proteins can therefore, to some extent, be substituted with pure amino acids (Inborr and Suomi, 1988). In spite of this, proteins will always be an important source of nitrogen because purified amino acids are expensive.

Historically the pigs’ feed has been served as a dry feed, and this is still the most prevalent feed for weaners. Liquid feeds on the other hand, are becoming more popular. They were initially introduced aiming to get rid of whey at cheese production. However, as the access to whey is limited, liquid feeds based on water are being used extensively. To avoid uncontrolled growth of bacteria in liquid feeds, their pH should be below 4.

5. INDIGENOUS INTESTINAL MICROFLORA OF PIGS AND ITS IMPORTANCE

The gastrointestinal (GI) tract of pigs is a dynamic ecosystem consisting of microbes that colonize the gut and become established in the intestine (indigenous or autochthonous) and those that are simply passing through (transient or allochthonous). The normal microflora (also known as normal microbiota) develops as a result of the influence of the intestinal ecophysiology, and the interaction between the microorganisms that colonize the gut (Drasar and Barrow, 1985). It is believed that the initial inoculum is usually derived from the sow at the time of birth (Drasar and Hill, 1974; Savage, 1977). The climax flora is different in different animal species and alters as the host ages.

The predominant microorganisms are anaerobes, which require special cultivation techniques, involving rigorous exclusion of oxygen. In this habitat, the anaerobic bacteria outnumber the aerobes by a factor of at least 3 to 5 $\log_{10}$. The obligate and facultative anaerobic bacteria are of diverse genera and range over a wide spectrum of taxonomic species. Implantation of bacteria in the GI-tract occurs by an elaborate process of ecological succession in which the composition of microflora constantly changes (table 1). Organisms which dominate the intestine early in this process, are
suppressed by other groups of microorganisms, which are in turn suppressed by new
groups and so forth until a balanced ecosystem is established dominated by anaero-
bic species (Lee and Gemmell, 1972; Savage, 1977; Varel and Pond, 1985). This
orderly ecological succession makes the pig’s intestine a complex milieu of mixed
bacterial populations, which is suggested to contain between 400 and 500 species of
bacteria (Moore and Holdeman, 1974; Drasar and Barrow, 1985). The population
size of each bacterial species is regulated by the whole ecosystem. Various micro-
organisms are completely eliminated from the digestive tract as a result of this effect.

5.1. Interference and protection

The indigenous intestinal microflora is known to be of substantial benefit to the host
(Hentages et al., 1985; Wilson and Freter, 1986; Wilson et al., 1988). It is generally
agreed that this flora serves as one of the major defence mechanisms that protects the
host’s body against colonization by invading bacteria, an effect which is referred to
as “colonization resistance” (van der Waaij, 1979; Finegold et al., 1983; Tancrede,
1992; Rolfe, 1997). This effect is postulated to be due to the competition for attach-
ment sites, nutrients and production of antimicrobial substances such as bacteriocins,
defensins and volatile fatty acids. This function of the flora, although of great impor-
tance to the host, has yet to be fully exploited in veterinary practice.

The pathogenic bacteria may colonize the host either by expressing specific
bacterial virulence factors which may overcome the colonization resistance, or by
taking advantage of an already reduced colonization resistance, such as that induced
by antibiotic treatment. For instance, it has been shown that the normal flora is
suppressed during antibiotic treatment and that this suppression is often correlated
to simultaneous colonization and overgrowth of potentially pathogenic bacteria
(Gorbach et al., 1987; Tannock, 1995). Both bacterial interactions and host defence
mechanisms are important weapons against colonization by pathogenic bacteria.

Data from experimental models reinforce conclusions about the efficacy of using
even some members of normal flora as biotherapeutic agents (probiotics) (Axelsson

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Table 1. The density* (log10/g fresh weight contents) of microorganisms in various sections of the
gastrointestinal tract of pigs

|                     | Stomach | Duodenum | Ileum | Caecum | Rectum |
|---------------------|---------|----------|-------|--------|--------|
| Lactobacilli        | 7–8     | 6–7      | 7–8   | 8–9    | 6–9    |
| Coliforms           | 5–6     | 4–5      | 6–7   | 7–8    | 6–8    |
| Enterococci         | 0–7     | 0–6      | 3–8   | 4–8    | 5–8    |
| Cl. perfringens     | Nil     | Nil      | 0–7   | 5–6    | 0–6    |
| Bacteroides         | Nil     | Nil      | 0–7   | 5–8    | 5–10   |
| Total anaerobes     | 5–6     | 5–8      | 7–9   | 9–11   | 9–10   |
| Yeast               | 0–7     | 0–7      | 0–7   | 5–7    | 5–7    |

*The density of bacteria may alter with age. For details, see the references.
et al., 1989; Gorbach, 1990; Fuller, 1992). For instance, some members of lactobacilli have been successfully used as a preventive measure against colonization of pathogens (Gorbach et al., 1987; Lidbeck and Nord, 1993; Salminen and Arvilommi, 2001). It should be noted, however, that not all members of the intestinal microflora are beneficial to the host.

6. METHODS OF ANALYSING INTESTINAL MICROFLORA

Under normal conditions, with an intact immune system and normal ecology of the gut, a high diversity of bacterial species is observed in the intestine. Most of these bacteria are permanent inhabitants of the GI-tract. They are mostly strict anaerobes and difficult to cultivate. Traditional cultivation techniques separate about 30−40 species from any individual (Moore and Holdeman, 1974; Drasar and Barrow, 1985). Yet, owing to the complexity of this flora, not all of them can be fully investigated. In this section we will briefly evaluate some of the available quantitative and qualitative methods for sampling in order to understand the present limitations for analysing the intestinal microfloras in animals.

6.1. Quantitative analyses

Of the various techniques used to study the intestinal microflora, most deal with the investigation of physiological capabilities of specific species of bacteria (Lee and Gemmell, 1972; Moore and Holdeman, 1974; Daniel et al., 1987). These techniques require initial isolation steps, and do not represent the entire intestinal bacterial flora. The total microscopic count of faecal samples together with viable counts of the numerically most important groups of microorganisms, may suffice for some samples of the intestinal contents. Such procedures, apart from problems of diluting faecal samples under a reduced condition (Meynell and Meynell, 1970), require a number of selective media. A wide range of selective media has been used to estimate the number of easily recognized groups of intestinal microflora such as coliforms, staphylococci, streptococci, yeast and lactobacilli. For strict anaerobes such as Bacteroides, Fusobacterium, Clostridium, Eubacterium, etc., highly enriched media containing antibiotics such as neomycin, kanamycin and/or vancomycin to prevent growth of Gram-negative facultative anaerobes are used. It should be noticed, however, that these media are not always highly selective and lactobacilli frequently grow well on them, especially when strict anaerobes are present in small numbers (Drasar and Barrow, 1985). Some of these anaerobes are extremely sensitive to oxygen, dying within 10 min after exposure to air, which adds to the technical problems in culturing the intestinal microflora. In addition, the pure culture condition is not a natural state of bacteria in a community, and characterization of bacteria chosen for study under these circumstances may not be of great ecological importance.
6.2. Qualitative analyses

Qualitative analysis of the intestinal flora has also been used to estimate microbial activity of the GI-tract. For instance, *in vitro* systems have been used to assess the fermentation capacity of colonic microflora by measuring the ability of this complex ecosystem to metabolize specific carbohydrate(s) (Ehle et al., 1982; Edwards et al., 1985; McBurney et al., 1985; Wyatt and Horn, 1988), as well as the metabolites evolved by the sugar fermentation including the gas (Clarke, 1977; Smith and Bryant, 1979; Cummings, 1984; Ross and Shaffer, 1989). Since studying all microbial types present in the GI-tract is virtually impossible, a convenient way would be to study the metabolic activities of all or selected groups of bacteria by assessing, *in vitro*, their capacity to metabolize a number of substrates (Clarke, 1977). Again, owing to the complexity of the intestinal flora, study of the metabolic activities would be facilitated if rapid and multiple assay methods were used (Katouli et al., 1997a).

Still, one complementary way to study a complex flora is to investigate what the microbes have done during their presence in the gut. Over the years, long series of biochemical and microbial transformation processes have been studied in materials from germ-free animals and their conventional counterparts (Midtvedt, 1999; Norin and Midtvedt, 2000). As a result, a complementary way to study the metabolic capacity of the intestinal microflora has been established to evaluate what the microbes can do and/or what the microbes have done. With a slight travesty of the terms initially used by Claude Bernhard, the mammalian organisms, or the host side of the ecosystem, can be defined as Milieu interior (MI), and the non-host side as the Milieu exterior (ME). MI plus ME together are referred to as Milieu total (MT) (Midtvedt, 1985). A simple equation of MT minus MI gives ME or “what the microbes have done”. The approach for such studies is investigating mammals without any normal microflora, i.e. germ-free animals, thereby establishing the functions of the microorganisms per se. When various microorganisms are associated with these animals, their influence on host-derived structures and functions can easily be studied. These findings have been described as germ-free animal characteristics (GAC) and microflora-associated characteristics (MAC) (Norin and Midtvedt, 2000). MAC is defined as the recording of any anatomical structure, or physiological or biochemical function in an animal that has been influenced by the microflora. When the microbes that actually influence the parameter under study are absent, as in germ-free animals, this particular recording is defined as GAC.

6.3. New methods to analyse the intestinal microflora

6.3.1. Application of nucleotide probes

The use of molecular probes to characterize the intestinal microflora has recently been the centre of attention by many investigators. Using the most refined molecular methods together with the cultural-based methods to describe the natural
communities of the gut, have clearly shown the extent of the unknown microbial diversity of the gut (Raskin et al., 1995). These methods are mainly based on the use of oligonucleotide probes complementary to conserved tracts of the 16S rRNA of phylogenetically defined groups of bacteria.

Using 11 DNA oligonucleotide probes targeting the small sub-unit rRNA of major microbial groups, Lin and co-workers (Lin et al., 1997) have successfully quantified several phylogenetically defined groups of methanogens and sulphate-reducing bacteria of the GI-tracts of various domestic animals. This technique has also been used to assess and analyse fibre-digesting bacteria of the gut (Stahl et al., 1988; Lin et al., 1994; Lin and Stahl, 1995).

Apart from the high specificity and accuracy, another advantage of these methods for detecting defined groups of bacteria is that the faecal samples can be frozen on dry ice and stored at −80°C immediately after sampling until they are processed. One should, however, realize that not all laboratories have equipment to utilize rRNA techniques since the probes should be synthesized, purified by high-performance liquid chromatography (HPLC) and labelled. Besides, high numbers of probes are required to fully quantify different microbial groups of the gut.

6.3.2. Metabolic fingerprinting

Competition for nutrients in a mixed bacterial population depends, to a great extent, on the population size and degree of affinities of each bacterial species to the available substrates. Two similar bacterial populations normally yield similar patterns of metabolic activities upon utilization of similar substrates. Any changes in the population size or type of bacteria in a sample would be reflected in the overall metabolic fingerprint of that population. Therefore, measuring the metabolic activities of a bacterial population will not only yield the metabolic potential of that flora, but will also help to identify changes in functional status of that flora. Characterization of certain microbial populations of the gut on the basis of their metabolic activities has also been used to define the effect of environmental factors or nutritional status on the natural structure of microbial populations (Rowe et al., 1979; Edwards et al., 1985; McBurney et al., 1985; MacFarlane et al., 1992). Changes in the pattern of substrate utilization have then been correlated to the environmental parameters that regulate microbial populations/communities.

Katouli et al. (1997a) evaluated a microplate-based fingerprinting system (PhPlate system) for characterizing and measuring the metabolic capacity of mixed bacterial populations. This system is based on interval measurements of the colour changes generated by an indicator caused by bacterial utilization of different sole carbon sources and production or consumption of acids in microtitre plates (Möllby et al., 1993). The bacterial strains chosen for this evaluation and their concentration in the synthesized mixtures represented those commonly found in the colon of man and animals (Katouli et al., 1997a). This simple approach successfully yielded metabolic
fingerprints that varied among samples. The results, however, should be interpreted with care since these workers found that exclusion or addition of different bacterial types did not cause a change in the resultant function of a microbial community on some occasions. They also examined the suitability of the PhPlates system to detect changes in the composition and function of the intestinal microflora in pigs (Katouli et al., 1997b). The system proved to be efficient in detecting changes in the composition and metabolic function of the intestinal flora of the animals during different nutritional and pathophysiological statuses. Among the useful information that they obtained from such biochemical fingerprints, was the capacity of a given flora to ferment different carbohydrates, an ability that they referred to as fermentative capacity (FC) since most tests used in their system were carbohydrates. These workers also concluded that several factors might contribute to the FC-value of a given microflora. A flora with numerous but similar bacterial strains normally yields higher FC-values than a flora with fewer strains. On the other hand, a flora with a few but metabolically more active strains, capable of fermenting a vast number of carbon sources, also yields a high FC-value. The differences in types and numbers of the utilized carbohydrates can also be used to compare different microflora (Katouli et al., 1992).

7. **IN VIVO MODELS AND SAMPLE COLLECTION STRATEGIES**

Most of our present knowledge about the composition of the intestinal flora has come from animal studies. Faeces comprise the final phase of the intestinal flora. As the consistency of the faeces reflects the status of the intestine, faecal samples are assumed to represent the intestinal flora. Indeed, a major problem when studying the intestinal microflora, is obtaining samples which truly represent parts of the GI-tract that are normally inaccessible. For instance, samples from gastric, small intestinal and colonic contents can only be obtained through a peroral or nasal tube, abdominal surgery, excised appendices, or the use of open-ended tubes. Withdrawal of the contents at various levels by a magnetically guided tube has also been used (Wilson, 1974), but this method only affords a sample of the organisms that are free in the lumen. Therefore, microorganisms that are attached to the villi or other parts of the surface, which often are present in large numbers may be left out.

Samples from different parts of the intestinal content can be obtained by removing the relative portion of the alimentary tract while the animal is anaesthetized or in abattoirs and immediately after the animal is slaughtered. The latter has been used extensively for analysis of the caecum and colon contents of the rumen (Stewart and Bryant, 1988; Lin et al., 1997). A way to scrutinize the enteric bacterial populations in vivo would be to surgically insert cannulas at strategic spots of the intestine, and to collect samples via these fistulas. Surgical insertions of cannulas have previously been used in pigs, mainly to study the utilization of feed (Sauer et al., 1983; Rainbard et al., 1984; Johansen and Bach Knudsen, 1994). One location often used has been the ileo-caecal ostium, representing the transition from the small to the large intestine (van Leeuwen et al., 1991). An advantage of using this method is that
a fistula can remain in place for a long period, and courses of events can be followed *in vivo* at correct spots of the intestine. The surgical insertion of such a fistula at the ileo-caecal ostium has recently been shown not to affect the intestinal coliform flora by itself, not even close to the surgery (Högberg et al., 2001).

For obvious ethical reasons associated with these routes of sampling described above, many investigators prefer to analyse bacterial flora found in faecal samples or to collect rectal samples. Although the rectal bacterial flora differ from those located in anterior parts of the intestine (Zoric et al., 2001), they share certain properties. For instance, the diversity among coliform populations collected from different sites of the intestinal tract in healthy pigs, has been shown to be equally high (Zoric et al., 2001). In the pig, the ampulla of the rectum generally contains ingesta that can easily be collected by swabs or specula. However, in newborn piglets, collection of rectal samples may be obstructed because the anus is small and the ampulla may be empty for long periods during the first week of life.

8. **IN VITRO MODELS**

Development of multistage reactors to simulate the gastrointestinal microbial ecosystems has opened a new window to investigate the fermentation fluxes and products (e.g. volatile fatty acids, enzymatic activities and head space gases) of this complex system. These reactors are normally designed to simulate both the small and large intestine. For instance, Molly and co-workers (1994) have developed a reactor, in which the small intestine is simulated by a two-step “fill and draw” chamber and the large intestine by a three-step reactor. These workers have used this system to compare the composition and activity of microbial flora grown under various concentrations and combinations of carbon sources such as arabinogalacton, xylan, pectin, dextrin and starch with those described in the literature. The supply of different media or enzymes at each stage of the reactor, to support microbial communities resembling those of the GI-tract, is an additional advantage of such a system.

Construction of such bioreactors to simulate the GI-tract may be of high value for monitoring microbial community structures during biological processes. These *in vitro* models may be used for comparisons of microbial population changes over time, and for assessing the diversity of microbial communities under certain conditions. However, the input of host-derived substances and osmotic conditions and redox-potential differences are very difficult to mimic within these systems.

9. **MICROFLORA OF DIFFERENT REGIONS OF THE ALIMENTARY TRACT OF PIGS**

Development of the intestinal flora in pigs takes place through an ecological process. During this process of succession, organisms which are dominant at the early stage of life, are suppressed by other groups of microorganisms, which are in turn also suppressed and so forth. This process will continue until a stable and
complex flora dominated by anaerobic species is established in the gut. Unfortunately, due to the complexity of this flora, it is impossible to measure both quantitatively and qualitatively all types of microorganisms present and this imposes a great restriction in assessing changes in the composition of the intestinal flora of the animal at any given time.

Since the beginning of the 20th century, an increasing number of investigators have been engaged in studying the intestinal microorganisms in pigs. Many of these studies comprise extensive quantitative and qualitative analyses of the intestinal flora of conventional pigs at varying ages. The normal flora most studied include *Escherichia coli* and other coliform bacteria, streptococci, and lactobacilli (Komarew, 1940; Quinn et al., 1953a,b; Briggs et al., 1954) and, in some cases, the total number of aerobic and anaerobic bacteria and yeasts (Willingale and Briggs, 1955; Horvath, 1957; Wilbur, 1959). Some later studies also report findings of *Bacteroides* and *Veillonella* (Smith and Crabb, 1961; Smith and Jones, 1963; Smith, 1965a) and *Clostridium perfringens* (Månsson and Olsson, 1961; Van der Heyde and Henderickx, 1964). Some of these workers even compared the bacterial flora in faeces with that in the caecum (Briggs et al., 1954) or observed variation in the total faecal counts between individual pigs and between days for one animal. In the case of caecal samples, it appeared that the variations in the total count were small and of the same order as in faeces. Using more selective media and rigorous techniques to exclude oxygen, Kovacs et al. (1972) investigated variation in microflora of different gut segments of pigs. These workers found that the bacteriological status of the stomach, and small and large intestines, is strongly contrasted, as would be expected from the anatomical and physiological differences of these functional units. Among the four segments of the small intestine studied, the duodenum contained reduced numbers of all bacterial flora studied (except coliforms) compared to the stomach. These workers suggested that the inhibitory factors operative in the duodenum affect coliforms the least, compared to other groups such as streptococci, lactobacilli and clostridia. On the basis of these and many other detailed studies it has been concluded that certain groups of bacteria such as *E. coli*, streptococci and clostridia are among the early groups of microorganisms that colonize the stomach within few hours after birth; all obtained from the dam and the immediate surroundings (Savage, 1977; Ducluzeau, 1983; Drasar and Barrow, 1985). Using a biochemical fingerprinting method to measure the stability and diversity of coliforms and enterococcal flora of rearing pigs, Kühn et al. (1995) established a population similarity model and used this to measure similarity among the coliform and enterococcal populations of piglets in a litter and their dam. These workers found that all studied piglets acquired a diverse coliform and enterococci bacterial flora during the first day of life, which, although common among the piglets, was different from that of their sow. Both the enterococcal and coliform floras from different piglets were more similar to each

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1 The term “coliforms” is used to avoid incorrect citation of the literature (see box).
other during the suckling period than after weaning. These workers also found that the enterococcal flora in the piglets was more persistent than the coliforms.

Pigs are continuously exposed to microorganisms from their surrounding environment. Microorganisms that pass through the GI-tract (transient microflora) may be found in the luminal contents or in faeces (Savage, 1977). However, it is highly likely that most of them are eradicated by host factors such as acids in the stomach, or bile in the upper small intestine. In contrast, resident microflora represent microorganisms that colonize different regions of the GI-tract. The type and number of these organisms in these regions are highly variable. For instance, Lactobacillus was shown to represent 67% of the bacterial population of the non-secreting stomach region in healthy unweaned pigs (McGillivery and Cranwell, 1992). Their level ranges between $10^7$ and $10^8$ CFU per gram caecal content in suckling piglets (Jonsson, 1986). The Lactobacillus species that are most frequently isolated from the stomach, intestine and faeces of healthy piglets are L. fermentum (Fuller et al., 1978), L. acidophilus and L. delbrueckii (Mäyrä-Mäkinen et al., 1983). The majority of these isolates can attach to epithelial cells of the small intestine. In addition to lactobacilli, other bacterial groups have been isolated from the non-secreting part of the stomach. These include Streptococcus (Fuller et al., 1978), Eubacterium, E. coli, Bifidobacterium, Staphylococcus, Clostridium and Bacteroides (McGillivery and Cranwell, 1992). However, their population size in the stomach is much smaller than in the large intestine (McAllister et al., 1979). The viable cells of lactobacilli are continuously being released from the non-secreting region, together with desquamated epithelial cells and thereby inoculate the stomach and intestinal luminal content (Fuller et al., 1978; Jonsson and Conway, 1992). Using a combination of protein profile analysis and colony morphology, Henriksson et al. (1995) characterized the lactobacilli colonizing various regions of the porcine GI-tract and detected several different groups of lactobacillus. Specific groups of lactobacilli were associated with, and often unique for the stomach, jejunal, caecal, and colonic regions of the GI-tract. These workers also found that there were major differences between population densities of the gastric mucosal Lactobacillus population of individual pigs.

The term coliforms has been traditionally used to refer to Escherichia coli (E. coli)-like bacteria (coli-form) since these bacteria could be readily isolated from faecal materials of warm-blooded animals. During the early 1900s, the technology was not available to easily distinguish E. coli from other coliforms and therefore most of the coliforms recovered from human and animal faeces were assumed to reflect the presence of E. coli. As a result, the term “coliforms” was considered to be equivalent to E. coli. It is now known that coliform bacteria comprise of at least four genera of the family Enterobacteriaceae that can all ferment lactose. These genera are Escherichia, Klebsiella, Enterobacter and Citrobacter and collectively they represent only 1% of the total bacterial populations in human and animal faeces. Among coliforms, however, E. coli represents the majority of the population (90–95%). During the early 1950s, although more specific tests were developed to easily identify E. coli from the rest of coliforms, the use of “faecal coliforms” was so commonplace that the term was not dropped in favour of E. coli.
Digesta transferred from the stomach to the duodenum are subjected to a dramatic environmental change, mainly due to the introduction of host factors such as bile, enzymes and bicarbonate (Drasar and Barrow, 1985). Compared to the large intestine, this region is less densely populated by microorganisms, partly due to the high flow rate of the luminal content through the small intestine. Lactobacilli are the dominant species in the piglet’s small intestine, while *E. coli*, *Clostridium*, *Bacteroides* and oxygen tolerant anaerobes are also present in large numbers. In addition low levels (100–1000-folds) of *Streptococcus*, *Enterococcus* and *Staphylococcus* have been detected on the mucosa of the small intestine (McAllister et al., 1979). The densities of the small intestinal microflora tend to increase in the distal part. In piglets, this is a major site for colonization by certain diarrhoegenic *E. coli* strains. The large intestine, on the other hand, is the major site of microbial activities in the digestive tract of the healthy pig, and the slowly moving digesta contains the most dense microbial population of the entire GI-tract. The large intestine includes the caecum and the colon. The pig is a monogastric herbivore with a relatively large caecum and colon. Consequently, the transient time through this region is considerable, allowing large populations of bacteria to be accumulated in the large intestine. Obligate anaerobes dominate the microflora of this region and increase in number from the ileum to the spiral colon (Cranwell, 1990). It is generally acknowledged that between 10¹¹ and 10¹² CFU bacteria are present per gram dry weight of the colon contents (Onderdonk, 1999; Borriello, 2002). This figure for facultative anaerobes such as *Bacteroides* and clostridia can be as high as 10⁹ CFU per gram (Smith, 1965b; Drasar, 1974; Salanitro et al., 1977; McAllister et al., 1979).

10. DYNAMICS OF THE INTESTINAL MICROFLORA IN HEALTHY CONVENTIONAL PIGS

Detailed identification of different bacterial species in the pig’s intestinal tract is an extremely laborious and lengthy process. For this reason, studies investigating the dynamics of the intestinal flora focus on methods that can yield information on the functional status of the intestinal flora. These methods include measuring the fermentative capacity (FC) of the microflora, which evaluates the amount of sugar metabolized by the faecal microflora and the metabolites evolved (McBurney et al., 1985), and testing the reaction of the whole or part of the gut flora against a relatively high number of substrates (Clarke, 1977). Using a combination of 48 substrates, Katouli and co-workers examined the pattern of metabolic response of the intestinal flora of healthy pigs and compared that with diseased pigs (Katouli et al., 1997b). They found that the response of the animal’s intestinal microflora to different carbohydrates varied among individual piglets at different sampling occasions. Similar results have also been reported by others (Edwards et al., 1985). Despite these individual differences, the overall FC-values in most stages of animal life were similar among piglets (Katouli et al., 1997b). These workers also showed that piglets receive
a high proportion of their intestinal microflora from their dams during the first few days of their life. However, despite the close contact with their dams, they develop intestinal floras that are very different from the sow’s flora. This suggested that the milk-based diet in piglets would yield a flora that is different from the initial “birth flora” derived from sows. This finding is supported by the fact that microfloras of piglets during the suckling period show more similarity to each other than during the post-weaning and fattening period.

The post-weaning period in piglets is associated with a dietary shift from milk to solid food, which will be replaced with a high-energy fattening diet when piglets are allocated into fattening stables. This dietary shift will result in substitution and/or establishment of new microflora in the pig intestine. It has been shown that the intestinal flora of pigs during the fattening period is more diverse than that of the suckling period. This might be due to the fact that in fattening stables, pigs from different pens may be mixed together and a direct contact between adjacent pens is established. As a result, pigs are exposed to more diverse bacterial species (Katouli et al., 1997b). The fermentative capacity of the intestinal flora, which is normally high during the suckling period, decreases during post-weaning and fattening periods, indicating that organisms dominating the pigs’ intestine very early in life are able to utilize more diverse carbon sources than those dominating the animal during post-weaning and fattening periods (fig. 1).

### 10.1. Intestinal microflora of sows

Several studies have attempted to determine the type of bacteria that are present in the intestinal tract of sows. Such efforts, using selective plate media, have led to the enumeration of only bacterial groups such as lactobacilli, streptococci, *Bacteroides*, *E. coli* and *C. perfringens* (Rall et al., 1970; Terada et al., 1976; Salnitro et al., 1977). These studies have clearly shown that Gram-negative anaerobic species of *Bacteroides,*
Veillonella, Fusobacterium and Peptostreptococcus can be isolated from different segments of the intestinal tract (Aalbaek, 1972; Mitsuoka et al., 1974). Other groups using strict anaerobic methods have isolated streptococci, Eubacterium species, Clostridium species and Propionibacterium acnes as the predominant flora in adult pigs (Salnitro et al., 1977). Kühn and co-workers measured phenotypic diversity and stability of the intestinal coliforms (Kühn et al., 1993) and enterococcal floras (Kühn et al., 1995) in piglets during their first 3 and 5 months of age, respectively, and compared the results with those of their sows. They found that the diversity of bacterial flora of the sows was higher than in most of the piglets during the first week of the pig’s life. In a more comprehensive study, Katouli et al. (1997b) investigated similarities between biochemical fingerprints of the whole intestinal microflora of sows and their offspring. They found that the sow’s flora had a considerably lower FC-value than those of the piglets at the time of birth, which remained so over the entire suckling period. The fact that the bacterial floras of sows had lower FC-values (even lower than those of pigs at the end of the fattening period) suggests that the loss of fermentative capacity will continue as the animal ages (see fig. 1).

10.2. Intestinal microflora of piglets during the suckling period

As mentioned before, the piglet intestine is sterile at birth (Kenworthy and Crabb, 1963). Piglets receive their initial microflora from the sow’s teats and skin as well as maternal faeces (Arbuckle, 1968; Berschinger et al., 1988). In fact, it has been reported that piglets eat considerable amounts of their sow’s faeces during the suckling period (Sansom and Gleed, 1981). Studies carried out during the early 1960s by Smith and Crabb (1961) and Kenworthy and Crabb (1963), and more recently by Melin et al. (1997) and Katouli et al. (1995), have shown that despite differences in genetics and feeding strategies, healthy piglets reared in different environments develop a very comparable intestinal flora. During the early life when diet consists of mainly milk and the species-specific differentiation of the intestinal tract is low, several bacterial groups increase and decrease in a similar way. For instance, Melin et al. (1997) have shown that the number of faecal coliforms, *E. coli*, enterococci and *C. perfringens* decrease over the first 9 weeks of the piglet’s life. *C. perfringens* even reaches undetectable levels after 3 weeks.

During the first week of life the coliforms and enterococci in the piglets’ intestines may differ considerably from that of the dam, suggesting that these floras are coming from sources other than sows (Katouli et al., 1995; Kühn et al., 1995). However, this may contribute very little to the overall similarity between the gut microflora of the sows and their offspring. The diversity of these floras in piglets is very high already from the first week and remains so during the suckling period. The fact that piglets housed together develop highly similar floras during the first week and onwards, also confirms the environmental nature of these floras among litter and pen-mates (Katouli et al., 1999). The early differences among coliform and enterococci floras between
piglets and sows will gradually decrease during the suckling period so that before weaning these specific floras of littermates are fairly similar to each other and to that of their dams (Melin et al., 1997; Katouli et al., 1999).

The intestinal colonization of *E. coli* in piglets comprises successive waves of different strains. Most of these strains are transient bacteria, the tenure of which varies between a few days to 2 weeks. On the other hand, most resident *E. coli* strains colonize the intestine of young piglets during the suckling period. Katouli et al. (1995) have shown that during this period each piglet may carry more than one type of resident strain in their gut. In a herd or stable, several piglets may be colonized by the same resident strains, which indicates that both strain and host specificity, especially during the suckling period, are important for colonization and persistence of *E. coli* in piglets.

Comparison of metabolic fingerprints of the faecal samples from piglets and their dams has shown that despite the difference in the *E. coli* floras, most members of the intestinal flora in pigs are similar to those of their dams, suggesting that sows are the initial source of most microflora for piglets. However, it seems that despite the close contact of piglets with their dams during the suckling period, they will eventually develop floras that might not be very similar to that of their sow (Katouli et al., 1997b).

10.3. **Intestinal microflora of piglets following weaning including effects of regroupings and movements**

In modern agricultural systems, weaning is generally achieved by abruptly removing the sow. However, these circumstances expose the piglets to a considerable amount of stress that affects the immune system negatively (Blecha et al., 1985; Bailey et al., 1992; Hessing et al., 1995; Wattrang et al., 1998). The stress and the sudden alteration of diet also contribute to a disturbed enteric flora of the piglets during the post-weaning period (Kühn et al., 1993, 1995; Katouli et al., 1995, 1997b; Melin et al., 1997, 2000a). The diversity of the intestinal flora may decrease dramatically during the first 3 days post-weaning among apparently healthy piglets. Since a high microbial diversity of the gut is believed to protect the animals not only from intrinsic microbes but also from microorganisms of external origin (Pielou, 1975; Kühn et al., 1993), this points to a situation of potential danger due to a decreased colonization resistance. It has been shown that the population size of bacteria is not altered during this period (Melin et al., 1997), indicating that the decreased microbial diversity must be achieved by proliferation of some strains. Melin et al. (1997) also showed that the similarity of the intestinal flora among pen-mates decreases during this period. This in turn points to the fact that while some strains proliferate in one pig, other strains proliferate in other pigs. Thus, if a pig develops diarrhoea due to proliferation of a pathogenic clone, there is an increased risk that also pen-mates will be diseased as their colonization resistance is decreased due to the low diversity of the intestinal flora at the actual time. In apparently healthy pigs the enteric microflora will again stabilize.
between 2 to 3 weeks after weaning. However, if invaded by pathogenic strains of *E. coli*, the intestinal flora may be disturbed for an even longer period (Melin et al., 2000a).

Some pig herds practise mixing and moving of piglets at weaning. This will potentially increase the risk of developing diarrhoea not only due to an increased level of stress imposed on the piglets, but also because a larger number of pathogenic strains (from more than one pen) have the chance of proliferating and invading the vulnerable piglets (Katouli et al., 1999). It should be noted, however, that the increased number of strains might also increase the piglets’ colonization resistance. Therefore, the mixing practice may not always be detrimental to pigs especially if it is done under proper hygienic management. Under high hygienic and management standards, the disturbed normal flora will be restored in around 2–3 weeks and pigs regain a high microbial diversity and a high similarity between microbial populations within groups (Katouli et al., 1999). Analysis of the intestinal microflora of pigs after weaning has shown that the post-weaning coliform populations differ from those of the suckling period (Katouli et al., 1997b). Despite this, the overall similarity between intestinal populations of pen-mates may remain high (Katouli et al., 1997b).

### 10.4. Intestinal microflora of pigs during the fattening period

Pigs are generally transferred from weaning facilities to the fattening enterprises at the weight of approximately 25 kg, corresponding to an age of 10–14 weeks. This transfer may provoke the pigs in a similar way as weaning (Wallgren et al., 1993), and the provocations increase if the animals are transported and regrouped (Lund et al., 1998). However, pigs are more immunologically (Wallgren et al., 1998) and physiologically mature during this transfer than at weaning. Consequently, the effect on the enteric flora because of this transfer is much less evident than at weaning (Katouli et al., 1995). If healthy, the fattening pigs show a high diversity of the enteric flora throughout the fattening period (Kühn et al., 1995). However, transient microbes are continuously present, and the similarity of the intestinal flora between pigs at this stage may be considerably lower than at the suckling or post-weaning periods (Katouli et al., 1995; Kühn et al., 1995).

As mentioned before, an alteration of the composition of the intestinal populations takes place when pigs are allocated into fattening units and mixed with other pigs. The intestinal populations may differ considerably between pigs, mainly due to the fact that pigs are exposed to the diverse bacterial species, a situation which is normally expected in stables of mixed pigs. The overall fermentative capacity of the flora of the animals during this period is far less than during the suckling period. This loss of fermentative capacity is a gradual process but will be accelerated during the late post-weaning period (Katouli et al., 1997b). Changes in the composition and fermentative capacity of the intestinal flora of pigs after weaning and after allocation of pigs into the fattening stables, coincide with the dietary shift from milk to solid food and further to a high-energy fattening diet (see fig. 1).
11. INTESTINAL MICROFLORA OF SPECIFIC PATHOGEN FREE PIGS

Specific pathogen free (SPF) pigs are declared free from a defined number of microorganisms pathogenic to pigs. However, it should be noticed that these pigs are not reared under germ-free conditions. Diseases such as salmonellosis and swine dysentery (induced by *Brachyspira hyodysenteriae*) are not present, but microorganisms such as *E. coli* are in reality impossible to avoid. As feed and straw are often of similar sources as those offered to conventional pigs, the intestinal microflora is virtually the same for SPF pigs as for conventional pigs. Indeed, when comparing intestinal microflora obtained from SPF pigs with those obtained from conventional pigs they basically share a similar composition throughout their life. On the other hand, owing to the absence of certain pathogenic microbes and precautions undertaken to avoid introduction of infections, development of clinical diarrhoea is rarely seen in SPF herds.

We have recently studied the biochemical fingerprints and fermentative capacity of the whole and/or selected intestinal microflora of SPF pigs during weaning, post-weaning and the fattening period (Katouli et al., unpublished data). We found that, as in conventional pigs, the fermentative capacity of the SPF pigs also decreased as the pigs grew older and that there was a decrease in the fermentative capacity values of the intestinal flora immediately after weaning and after the pigs were transferred to the fattening stable (fig. 2). However, we also found that both SPF piglets and their sows had much higher FC-values than their conventional counterparts during the first week of life. These values, however, dropped to a level close to what we have normally obtained from conventional pigs during this period (see fig. 2). Interestingly, the FC-values of sows reached the same level as those of piglets at the time of weaning. Similar patterns were basically observed among selected groups of normal flora in SPF piglets except for *Lactobacillus* flora. The fermentative capacity

![Fig. 2](image_url)

**Fig. 2.** FC - values of the whole intestinal microflora of specific pathogen free (SPF) pigs during suckling, post-weaning and fattening periods. FC - values are the mean of four pigs and their standard errors. W = Weaning, F = Pigs were transferred to the fattening stable.
of this flora, which was high during the first 4 weeks of weaning, showed a dramatic decrease just before weaning, reaching its minimum level at the time of weaning (fig. 3).

12. ALTERATIONS OF THE INTESTINAL MICROFLORA OWING TO STRESS AND DISEASE

Physiological stresses and disease especially during suckling and early post-weaning, are a major concern within piglet production, and disturbances in the composition of the intestinal microflora constitute the greatest problem (Cutler et al., 1999). It is believed that changes in the stability of the intestinal flora will result in the development of a low diversity of the flora making the animal susceptible to
gastrointestinal diseases. These factors and their effect on the health status of growing pigs are discussed below.

12.1. Intestinal coliforms during the suckling and post-weaning periods

As described earlier, piglets rapidly develop a highly diverse intestinal coliform flora. Unless diarrhoea develops, this flora will remain stable until weaning and the intestinal coliform populations of pen-mates are fairly similar, indicating a high colonization resistance of the piglets within the pen.

Introduction of weaning, more or less, leads to a collapse of the intestinal coliform population. At this time the piglets are highly vulnerable to disease and if they are exposed to a low pathogen load, because of good herd management, they may be able to resist developing diarrhoea before the disturbed flora is completely recovered. In healthy pigs, the coliform flora will remain stable throughout the weaning period. At transfer to the fattening enterprises, a situation similar to weaning may occur. However, as the pigs are growing older and their feed composition changes less dramatically, the intestinal coliform floras are restored faster following this allocation.

12.2. At-risk situations

The intestinal bacterial populations may be influenced by changes in the life of a pig. Consequently, all adjustments should be defined as situations that may threaten the stability of the enteric microflora. The younger the pig and the more dramatic the alteration(s), the larger the risk will be. The influence of alterations of the intestinal flora on the pig’s life has been thoroughly described earlier in this chapter. Examples of induced at-risk situations are weaning, regrouping, transportation and alterations in feed regiments. In addition, non-optimized management may provide some additional risk situations, such as high pathogen load, chill, draught and moisture.

12.3. Diarrhoea pre-weaning

Pre-weaned piglets are frequently infected with enteropathogens at two stages: as newborn and at the age of 2–3 weeks. In systems effectuating weaning at the age of 2–3 weeks, the latter stage coincides with the weaning and thereby could possibly be referred to as post-weaning diarrhoea. Factors such as immune defence, indigenous flora, pH, food composition and environmental errors may influence the defensive capacity of the animals at these occasions and therefore exposure to enteropathogens may be hazardous. A number of host mechanisms have evolved which protect the GI-tract from invading pathogens. E. coli, Salmonella and B. hyodysenteriae are among the globally most economically important causes of bacterial induced diarrhoea in piglets (Bergeland and Henry, 1982; Edfors-Lilja and Wallgren, 1999; Straw et al., 1999).
The two most important pathogenic microorganisms that affect newborn piglets are *E. coli* (summarized by Fairbrother, 1999) and *C. perfringens* (summarized by Taylor, 1999). Both these microorganisms may induce neonatal diarrhoea in large numbers of piglets and may become fatal. However, owing to the unifactorial cause of these diseases, vaccination of sows and the subsequent transfer of their protective immunity to the offspring via colostrum have effectively prevented outbreaks of diseases caused by these species in newborn pigs. Other microorganisms associated with diarrhoea in neonatal animals include *Bacteroides fragilis*, *Campylobacter* spp. and *Yersinia enterocolitica* (Holland, 1990) and a number of viruses (Straw et al., 1999).

It should be noted, however, that the maternal immunity declines with increasing age of the piglets (Saito et al., 1986; Fu et al., 1990; Wallgren et al., 1998). Therefore, diarrhoea induced by the species mentioned above may occur at the age of 2−3 weeks if the pathogen load of the environment is high enough. Furthermore, these species may be found in association with large numbers of other potentially pathogenic microbes (summarized by Straw et al., 1999). Virulent organisms such as the protozoan *Isospora suis* as well as rotavirus and coronavirus, have frequently been correlated to diarrhoea in suckling piglets (Glock, 1981). The number of microbes that could potentially contribute to development of gastrointestinal disturbances increases as the piglets grow. Consequently, diarrhoea among somewhat older piglets may well reflect mixed infections, and can certainly be influenced by environmental conditions and hygiene.

When diarrhoea is observed during the first days of life, the causative agent can often be re-isolated in pure culture from faecal samples, and under such conditions the correlation between infection and signs of disease is obvious. Somewhat older piglets will receive a rather diverse enteric flora prior to infection. Kühn and co-workers (1993) have shown that during an *E. coli* associated outbreak of diarrhoea, the diseased piglets had a lower diversity of the intestinal coliforms, indicating that the pathogenic strain had outgrown the others. While studying the diversity of coliform populations in a group of pigs, we also noticed that pigs that received antibiotic during an outbreak of diarrhoea showed a lower diversity of coliforms. This effect, however, was not seen among all piglets. We also noticed that the piglets affected with diarrhoea did not recover from the low coliform diversity until long after weaning (Katouli et al., unpublished data) (fig. 4).

### 12.4. Diarrhoea post-weaning

As described above, the enteric microflora is severely disturbed following weaning, thereby paving the way for potentially pathogenic microbes. Toxin-producing strains of *E. coli* (mainly serogroups O138, O139 and O141) associated with oedema disease, may act as the main sources of post-weaning diarrhoea, a disease that is often fatal for newly weaned piglets (Berschinger, 1999). On the other hand, it should be mentioned that experimental challenge of healthy, newly weaned piglets...
with any of the above pathogens, in most cases might not result in a state of diarrhoea. This may be due to the lack of environmental factors necessary for complete disturbance of the flora. For instance, Melin et al. (2000a) used a highly virulent strain of *E. coli* to challenge a set of weaned piglets. The challenge strain belonged to serogroup O149 and carried surface antigen K88, which confer adhesion to the F4 receptor on the epithelial cells of the pigs. Furthermore, the strain was a potent toxin producer (STa, STb and LT) and the challenged piglets all had receptors for F4 (Edfors-Lilja et al., 1995) and the pigs were proven truly infected. Still, post-weaning diarrhoea (PWD) was not achieved, indicating that although PWD is strongly associated with *E. coli*, it should be considered as a multifactorial syndrome rather than a specific infection. Indeed, these workers succeeded in inducing an experimental PWD by exposing piglets to a cascade of different pathogenic strains of *E. coli* (Melin et al., 2000b,c), possibly imitating conditions that could occur post-weaning in a herd (see above).

Microbes should not be regarded as the sole cause of PWD in practical pig production. The influence of pathogenic microorganisms can be amplified by environmental stress such as chill, draught, moisture, etc. Further, insufficient management may contribute to the development of PWD. The newly weaned pig is poorly developed with respect to immune functions (Blecha et al., 1985; Bailey et al., 1992; Wallgren et al., 1998; Wattrand et al., 1998) and therefore vulnerable to infections.

Pigs affected by PWD will express a decreased diversity of the intestinal flora during the course of the disease owing to the overgrowth of one or several bacterial strains (Melin et al., 2000c). Because of the influence of the strain(s) causing disease, the similarity between intestinal coliform populations of diseased pigs may be larger than between apparently healthy pigs. However, this type of similarity does

[Fig. 4. A representative figure showing the effect of sulfametoxasol/trimethoprim (administered during an outbreak of diarrhoea) on the diversity of coliforms in conventional pigs. Four pigs (P1 to P4) from four litters were studied. Diversity of coliforms was measured as Simpson’s index of diversity after testing randomly 40 coliforms from MacConkey plates.

W = Weaning. DO = Onset of the diarrhoeal outbreak in the herd. F = Pigs were transferred to a fattening stable.]
not indicate any colonization resistance, as seen among suckling pigs (Katouli et al., 1999). Instead, it is achieved by infection of several individuals by the same pathogenic strain(s).

The balance of the intestinal microflora may be severely affected for as long as 4 weeks following an infection with coliforms at weaning, regardless of whether clinical PWD has been developed or not (Melin et al., 2000a). Of course, this may facilitate infections with other pathogenic microorganisms, such as *Brachyspira* species or *Salmonella*.

Taken together, the weaning is a critical physiological period for the pig. It is often accompanied by an abrupt multiplication of some strains of *E. coli* in the digestive tract and may result in development of diarrhoea and/or oedema disease. To avoid PWD, good management should be applied.

13. PRECAUTIONS AIMING AT STABILIZING THE INTESTINAL MICROFLORA

The negative influence of environmental disturbances and infections on the intestinal flora could possibly be reduced. Different strategies are discussed briefly below.

13.1. Feed composition

The food itself can be a provoking factor in causing disease and/or disturbance of the gut flora of the pigs. As an optimized growth of pigs is of economical importance, feed consumption and feed utilization are of great importance in modern pig husbandry. Pig feed is often processed, i.e. pre-heated, and the digestive ability of the food is facilitated. However, this normally leads to a reduced chewing and a shorter residence period of food in the stomach. As a consequence, the digestive and bactericidal effects of saliva and hydrochloric acid will be reduced and disturbances in the digestive tract may be facilitated. By avoiding pre-heating of dry feed, the natural protective effect of intestinal flora to infection will increase. An increased amount of fibre in the diet will further stimulate the natural protection towards disease owing to a slower passage through the gut (Heidelberg et al., 1984; Hampson and Kidder, 1986).

In a situation of increased risk, the food composition may have a big impact on the clinical outcome with respect to enteric health in a herd. For instance, the provocation of the abrupt change of food at weaning may be minimized by adding lactose to the food, thereby resembling the milk from the sow to some extent. In this context it should be mentioned that commercially available milk substitutes generally emanate from either cow milk, that will include proteins from foreign species, or even from soya.

Protein, which is required to stimulate the growth of pigs, may also affect the composition of the enteric flora, leading to diarrhoea (Newport, 1980; Shone et al., 1988; van der Peet-Schwering and van der Binnendijk, 2000). In fact, some protein
sources, such as soya have actually been linked to outbreaks of diarrhoea (Jager et al., 1986; Nabuurs, 1986). Predigestion of proteins is proven to decrease the risk of developing diarrhoea (Miller et al., 1984) and feed proteins can therefore to some extent be substituted with pure amino acids (Innborr and Suomi, 1988). However, as purified amino acids are expensive, the proteins themselves form the most important source of nitrogen in pig feed.

13.2. Antibiotics and feed additives

Antibiotics have commonly been used to control the enteric health and improve the growth rate. This is certainly achieved by suppression of the intestinal bacterial activity. Animals given antibiotics in the feed generally perform better than those offered probiotics (Eidelsburger et al., 1992). However, it should be remembered that intestinal treatments with antibiotics may affect the normal flora severely (Barza et al., 1987; Thijm and van der Waaij, 1979; Hashimoto et al., 1996; Wilson et al., 1996). Further, a continuous use of antibiotics will increase the risk of development of bacterial resistance to antibiotics used (Linton et al., 1988; Aarestrup, 2000). Consequently, a permanent use of antimicrobial agents in feed ought to be avoided and replaced with proper management systems and well-designed feed that maintain and stabilize the intestinal flora. As a result, development of diseases would be minimized and the number of medical treatments would be reduced.

13.3. Zinc oxide supplementation of the feed

Zinc is an essential component of several enzyme systems and plays an important role in stabilizing membrane integrity. As epithelial cells are the first line of defence against microbial invasion, zinc has a special role in resistance to infections. Zinc in the form of zinc oxide (ZnO) has been successfully used to prevent outbreaks of PWD (Holm, 1988; Holmgren, 1994). By adding a high concentration of the ZnO to the feed, it has been possible to preserve the integrity of the coliform population in weaned pigs (Melin et al., 1996; Katouli et al., 1999). This may partly explain the protective effect of the ZnO against post-weaning diarrhoea as the colonization resistance of the gut flora is preserved. No similar effect can be achieved if an equal amount of zinc is given parenterally (Shell and Korneay, 1994). This calls for a local effect of the ZnO in the intestine and, since a high concentration of zinc is required, it is possibly toxic. Piglets given ZnO-supplemented feed may grow faster than non-treated piglets close to weaning. However, a continuous feeding of high amounts of ZnO in the food should be avoided, because pigs that were offered a feed with 2500 ppm ZnO for 4 weeks expressed signs of intoxication (Jensen-Waern et al., 1998). Further, as most of the zinc oxide will pass through the pig’s intestine, the environmental aspects must be considered. Katouli et al. (1999) found that loss of diversity and disruption of the integrity of coliform flora in weaned piglets supplemented
with ZnO in their feed could be restored within 14 days post-weaning. On the basis of this finding, these workers concluded that feeds supplemented with ZnO should be restricted to only 2 weeks post-weaning in veterinary practice.

13.4. Probiotics

Using probiotics to stimulate the intestinal flora has been tempting, mainly due to the facts that probiotics can be used to improve colonization resistance of the intestinal flora and thereby potentially reduce the dependence on antibiotics in order to prevent and/or treat bacterial infection of the gut in animals (Kyriakis, 1989; Kyriakis et al., 1999). Studies on the suitability of the members of the intestinal flora have suggested potential candidates such as *Lactobacillus* species (Toit et al., 1998), and *Bacillus cereus* (Kirchgessner et al., 1993) as probiotics of microbial origin. Acidifiers have also been suggested and used as probiotics. These include fumaric acid, hydrochloric acid and sodium formate (Eidelsburger et al., 1992).

The existing reports on the use of probiotics in pigs range from positive effects on enteric health and weight gain (Eidelsburger et al., 1992) to no effects at all (McLeese et al., 1992). Also increased weight gains have been reported without any visible positive effects with respect to intestinal health (Eidelsburger et al., 1992; Kirchgessner et al., 1993). Presently, much work is focused on probiotics. However, the variations in the results obtained may indicate an influence of the management and environmental conditions. Therefore, great efforts should be made to scrutinize the effects of probiotics under unbiased conditions. The effect of probiotics on the health and well being of animals is discussed elsewhere in this book.

14. FUTURE PERSPECTIVES

The control of diarrhoeal diseases still presents a challenge in pig husbandry. Recent developments in management and production facilities, as well as availability of potential vaccines, has reduced mortality associated with diarrhoea in piglets. Changes in the composition and stability of the intestinal flora of piglets have been shown to play an important role in the development of diarrhoea during the suckling and early post-weaning periods. Factors such as stress, especially at weaning and early post-weaning periods, are among the main causes of disruption to the integrity of the intestinal flora. Approaches to challenge enteric diseases should include establishing diverse intestinal floras in piglets during the suckling period and maintaining the stability and diversity of this flora after weaning. While several methods, including molecular-based techniques, are available to detect and identify unculturable bacterial flora of the gut, there is a need for more advanced techniques to measure the functional status of the normal flora in response to dietary feed and environmental stress. The recent practice of withdrawing growth promoters in pigs in some countries should be monitored with respect to the composition of the gut
flora and the development of enteric disease. Furthermore, there is a growing interest in ecological agricultural practice and the farming of pigs outdoors. This may have a significant impact on the composition of the intestinal flora, which may differ from that of the traditional indoor microflora. Application of new methods alone or in combination with classical methods can be used to identify the stability and the impact of the outdoor flora on the general health of pigs.

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