

The use of prebiotics and probiotics in pigs

A review

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1. The probiotic and prebiotic concept

The concept of probiotics evolved at the beginning of the 20th century from a hypothesis first proposed by the Nobel Prize winning Russian scientist Elie Metchnikoff. He suggested that the long and healthy life span of Bulgarian peasants was due to the consumption of fermented milk products (Metchnikoff, 1908). During the last few decades, research on probiotics has expanded beyond bacteria isolated from fermented dairy products to normal microbiota of the intestinal tract (Sanders and Huis in't Veld, 1999).

Vanbelle *et al.* (1990) defined probiotics as natural intestinal bacteria that, after oral administration in effective doses, are able to colonize the animal digestive tract, thus keeping or increasing the natural flora, preventing colonization of pathogenic organisms and securing optimal utility of the feed. Prebiotics are defined as non-digestible food ingredients that affect the host beneficially by selectively stimulating the growth and/or activity of bacteria in the colon (Gibson and Roberfroid, 1995). This definition was recently amended to 'A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health.' In practice, the beneficial bacteria that serve as targets for prebiotics are mostly lactobacilli and bifidobacteria (Gibson *et al.*, 1999; Bouhnik *et al.*, 2004). Unlike probiotics where allochthonous microorganisms are introduced in the gut, and have to compete against established colonic communities, an advantage of using prebiotics to modify gut function is that the target bacteria are already commensal to the large intestine (Macfarlane *et al.*, 2008). However, if for any reason like disease, ageing, antibiotic or drug therapy, the appropriate health-promoting bacteria are not present in the bowel, prebiotics are not likely to be effective. Combinations of prebiotics and probiotics are referred to as synbiotics.

Commercial probiotic products often do not meet expected standards in that the composition and viability of the strains may differ from information on the label (Hamilton-Miller *et al.*, 1999; Hamilton-Miller and Shah *et al.*, 2002; Weese, 2002; Fasoli *et al.*, 2003). Another major issue in relation to the application of probiotics is the poor evidence for efficacy based on clinical trials (Klaenhammer and Kullen, 1999). Three issues interfere with the identification of specific health effects of probiotics (Klaenhammer and Kullen, 1999). Firstly, the complexity and variability of the gastro-intestinal environment in relation to

gastro-intestinal diseases make it difficult to determine the effect probiotics have on health and disease. Secondly, confusion as to the identity, viability and properties of probiotics lead to strains being incorrectly identified. Lastly, single probiotic strains induce a multitude of effects among different hosts in a test population. A mono-strain probiotic is defined as containing one strain of a certain species whereas multi-strain probiotics contain more than one strain of the same species or genus. The term multi-species probiotics is used for preparations containing strains that belong to one or preferably more genera (Timmerman *et al.*, 2004). Multi-species preparations have an advantage when compared to mono- and multi-strain probiotics (Timmerman *et al.*, 2004). Multi-species probiotics benefit from a certain amount of synergism due to the combination of characteristics from different species.

The concept of probiotics plays an important role in animal health. Pig rearing has become an intensive commercial industry. Economic losses due to decreased health and performance brought about by intensive farming practices focused on increased production and low costs, are very important. Major efforts have been made to find different ways to improve the rearing of pigs. Antibiotics have been used successfully for more than 50 years to enhance growth performance and control the spread of disease (Gustafson and Bowen, 1997). Antibiotic resistance is as ancient as antibiotics, protecting antibiotic producing organisms from their own products (Phillips *et al.*, 2004). Antibiotic resistant variants and species that are inherently resistant can dominate and populate host animals.

Increased concern exists about the potential of antibiotics in animal feed and their contribution to the growing list of antibiotic-resistant human pathogens (Corpet, 1996; Williams and Heymann, 1998). Although the use of antibiotics for growth promotion is still allowed in certain countries, including the United States, Australia and South Africa, several European countries have implemented strict legislation to prevent the incorporation of antibiotics in animal feed (Ratcliff, 2000). In 1986 Sweden was one of the first countries to ban the incorporation of low-dose antibiotics into animal feed.

The question remains, does the use of antibiotics in production animals pose a risk to human health? In a recent review, it was stated that the actual danger appears small and the low dosages used for growth promotion (generally below 0.2% per ton feed) could not be regarded as a hazard (Phillips *et al.*, 2004). Antibiotics are used in animals and humans, and most of the resistance problem in humans arises from medicinal use. Resistance may develop in bacterial populations present in production animals, and resistant bacteria can contaminate

animal-derived food, but adequate cooking destroys most bacteria. Growth-promoting antibiotics predominantly active against Gram-positive bacteria have very little or no effect on the antibiotic resistance of salmonellae and consequently on infections caused by salmonellae. In some parts of the world, antibiotics used to treat animals and added to feed as growth promoters may have adverse effects when associated resistance is taken into account. The same antibiotics are often used to treat humans (Phillips et al., 2004). In contrast, Piddock (2002) could not find clear evidence that antibiotic-resistant bacteria isolated from animals, cause infections in humans, for example quinolone-resistant strains of *Salmonella* serovar Typhimurium DT104 are not transmitted through production animals. The flouroquinolones used therapeutically in animals appear to pose little threat to human health. Flouroquinolone resistance was recorded in bacteria isolated from humans, in countries where the use of this growth promoter is banned such as Sweden, Finland and Canada (Rautelin et al., 1993; Sjögren et al., 1993; Gaudreau and Gilbert, 1998). Faecal flora isolated from a healthy person may contain antibiotic resistant enterococci, but most enterococci isolated from animals do not colonize the human intestine (Dupont and Steele, 1987; SCAN, 1996, 1998; Bezoen et al., 1999; Butaye et al., 1999; Acar et al., 2000). *E. coli* resistance is more likely to be driven by antibiotic use in humans, although an animal origin for at least some clinical isolates cannot be excluded (Gulliver et al., 1999). The banning of antibiotic usage in animal feed remains a controversial issue especially in the way that it affects farming with production animals.

Many natural substances have been investigated as alternatives to conventional chemotherapeutic agents (Turner et al., 2002). Probiotics are one approach used to improve piglet health and deal with intestinal problems encountered during rearing (Vanbelle et al., 1990). Other approaches include acidification of feed or water (Chapman, 1988), altering dietary formulations for small piglets, the development of feeds with lower protein content (Lawrence, 1983), and vaccination with attenuated pathogens or with strains genetically modified (Greenwood and Tzipori, 1987; Trevallyn-Jones, 1987). The administration of growth hormones, somatostatin immunization and enzyme supplementation were also considered as alternatives to antibiotic treatment (Thacker, 1988). Treatment with psychopharmacological drugs (Björk et al., 1987), utilization of the lacto-peroxidase system (Reiter, 1985) and stimulation of hormone-like proteins (anti-secretory factors) capable of reversing intestinal hyper secretion to reduce symptoms of diarrhoea (Lönnroth et al., 1988) were proposed. Some esoteric substances such as zeolite have reduced diarrhoea in piglets

and increased feed efficiency (Mumpton and Fishman, 1977). Natural substances that enhance growth performance and immune function in pigs include plant products such as seaweed, saponins extracted from certain desert plants, spices and herbs (Turner et al., 2002). Probiotic preparations may be incorporated in prophylactic agents and it is important to know the mode of action to anticipate the dosage levels (Jonsson and Conway, 1992). The use of probiotics should not exclude other alternatives and a combination of treatments may be complimentary and more effective.

2. Aspects relevant to the use of probiotics in pigs

To understand the effect probiotics have on piglets, a thorough understanding of aspects affecting the rearing of pigs and their digestive tract is needed.

2.1 Rearing of pigs

One of the major problems in the rearing of pigs is the high mortality rate (*ca.* 20%) up to weaning age (Bäckström, 1973). In piggeries pre-weaning mortality is caused by diarrhoea, overlay, splay leg, anaemia, bacterial septicaemia, necrotic enteritis, cold exposure and/or congenital defects (Fahy et al., 1987). Piglets in a piggery are often born immature, which renders them more vulnerable to infections. Neonatal diarrhoea often manifests 48 h after birth and is largely attributed to the enterotoxigenic *E. coli* strains K88 (most frequent), K99, 987P or F41 (De Graaf and Mooi, 1986). *Salmonella* spp., *Campylobacter* spp., *Cryptosporidium*, transmissible gastroenteritis virus, rotavirus, porcine adenovirus and coronavirus may also cause diarrhoea (Tzipori, 1985; Fahy et al., 1987). The disease manifests by hypersecretion of fluids across the gut wall and into the lumen, triggering the host's immune system through the various toxins produced. Piglets are particularly susceptible to diarrhoea during the first three weeks after birth and at weaning age (21- to 28-days-old). During the first days, the piglet is protected by maternal immunoglobulins in the colostrum (Porter, 1969).

Post-weaning diarrhoea occurs approximately 4 to 10 days after weaning. Enteropathogenic *E. coli* is the major pathogen (Fahy et al., 1987). Many theories have been proposed as to why disease occurs at weaning. One hypothesis is the sudden deprivation of maternal antibodies and other protective factors in the sow's milk. Another possibility is

sudden changes in diet and/or a compromised metabolism (Fahy et al., 1987) that may lead to particles being metabolized by pathogens, which results in an increase of cell numbers. Changes in temperature, humidity and other environmental conditions may also affect the animal's immune system (Carghill, 1982), leading to diarrhoea (Björk et al., 1984). Traditionally, pigs have been weaned after 7 to 10 weeks, but piglets are now weaned after 3 to 4 weeks. At this young age the intestinal tract is not able to digest the diets developed for older pigs (Cranwell and Moughan, 1989). The correct feed formulation is thus of critical importance. Feed should contain easily digestible components. During the fattening stage (six months and older) swine dysentery is a problem and feed should be adapted to achieve desirable performances.

2.2 The porcine digestive tract

The length of the gastro-intestinal tract (GIT) in the newborn pig is only two meters compared to 20 meters in a mature animal (Slade, 2004). Probiotics need to resist low pH and proteolytic enzymes in the digestive tract. The retention time, mixing of the ingested material with gastric juices and previous digesta, influences the survival of the administered strains. In the anterior part of the small intestine, the most important defense is the fast flow rate that prevents microbial overgrowth, provided the microorganisms do not attach to the epithelium. The presence of bile in this region also represses survival and activity of the microorganisms. In the caecum and large intestine probiotics have to compete with a stable indigenous microflora in the healthy host animal, but the passage rate is slower and the microorganisms establish easier (Jonsson and Conway, 1992).

2.2.1 Stomach

The entrance of the stomach has the same type of keratinized squamous non-secreting epithelium as the esophagus (Noakes, 1971). In this region epithelial cells are released continuously and are covered with intestinal bacterial cells including lactobacilli (Lipkin, 1987). Released squamous cells colonized by these bacteria may help to regulate the composition of the digestive microflora by ensuring dominance of the lactic acid bacteria (Fuller et al., 1978; Barrow et al., 1980). In the stomach, gastric juices containing mucus, HCl, proteolytic enzymes and low pH are factors influenced by the age of the animal. The stomach pH may be as low as 2.0 in an adult pig, but as high as 5.0 in milk-fed piglets (Slade,

2004). The intestinal pH of pigs at different ages is listed in Table 1. The degree of mixing and the rate at which contents pass through the stomach influence the effectiveness of the digestion process. Mixing of the digesta depends on dry matter content and particle size. Liquid feed and finely ground feed are mixed more easily than drier or coarsely ground cereal diets (Maxwell et al., 1970).

Table 1 pH Values in the digestive tract of pigs

Age	Stomach	Small intestine		Caecum	Colon
		Anterior	Posterior		
Neonatal	4.0 - 5.9	6.4 – 6.8	6.3 – 6.7	6.7 – 7.7	6.6 – 7.2
Pre-weaned	3.0 – 4.4	6.0 – 6.9	6.0 – 6.8	6.8 – 7.5	6.5 – 7.4
Weaned	2.6 – 4.9	4.7 – 7.3	6.3 – 7.9	6.1 – 7.7	6.6 – 7.7
Adult	2.3 – 4.5	3.5 – 6.5	6.0 – 6.7	5.8 – 6.4	5.8 – 6.8

Compiled from Smith and Jones (1963), Smith (1965), Boucourt and Ly (1975), Clemens et al. (1975), Braude et al. (1976), Cranwell et al. (1976), Barrow et al. (1977), Schulze (1977), Schulze and Bathke (1977).

2.2.2 Small intestine

The acidified portions of digesta entering the duodenum are mixed with bile, pancreatic juice, enzymes and other substances. The pH increases in the small intestine, but variations are less than encountered in the stomach. The difference between piglets and adult pigs is less pronounced (Kidder and Manners, 1978). Variations are large in the duodenum (pH 2.0 to 6.0) and progressively smaller towards the ileum (pH 7.0 to 7.5). The activity of microflora in the distal part of the small intestine lowers the pH in this region (Friend et al., 1963). It normally takes 2.5 h for a food particle to pass through the small intestine (Kidder and Manners, 1978). At this flow rate, it is difficult for bacteria to multiply fast enough to prevent being washed out and probiotics should be administered in sufficient dosages. Attachment to epithelial cells is a prerequisite for bacteria to colonize the small intestine. Volumes measured for the small intestine can be as much as 0.1, 0.6 and 20 L for very young, weaned and adult pigs, respectively (Vodovar et al., 1964).

2.2.3 Large intestine

The large intestine consists of the caecum, spiral colon and the distal colon. The rate of passage is slower compared to the small intestine, leading to the establishment of a dense and complex anaerobic microflora. The first part of a meal reaches the anus after 10 to 24 h, but the mean retention time is much more variable and can be two to four days (Kidder and Manners, 1978). The large intestine can hold volumes up to 0.04, 1.0 and 25.0 L for very young, weaned and adult pigs, respectively (Kidder and Manners, 1978). The pH of the large intestine remains at approximately 6.0 (Kidder and Manners, 1978).

2.3 Lactic acid bacteria indigenous to pigs

The pig is a monogastric animal in which the foregut (stomach and small intestine) is colonized by a relatively large variety of microflora. Bacteria in the small intestine survive low pH conditions better and bacterial numbers are generally high (10^7 to 10^9 cfu/ml) in this section of the GIT (Conway, 1989). Lactic acid bacteria (LAB), mostly *Lactobacillus* and *Streptococcus* spp. dominate the small intestine (Fuller et al., 1978). LAB in the foregut helps the young pig to decrease the stomach pH by the production of lactic acid and other organic acids, mainly from lactose (Cranwell et al., 1976; Barrow et al., 1977). LAB may regulate the microflora of the small intestine by migrating with the digesta passing down the GIT (Fuller et al., 1978). Gram-negative bacteria dominate the caecum (Robinson et al., 1981) and Gram-positive species the colon (Salinatro et al., 1977). Species often found in the porcine digestive tract are *Lactobacillus acidophilus*, *Lactobacillus delbreuckii*, *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Lactobacillus salivarius*, *Enterococcus bovis*, *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus intestinalis*, *Streptococcus porcinus*, *Streptococcus salivarius*, *Bifidobacterium adolescentis* and *Bifidobacterium suis* (Raibaud et al., 1961; Zani et al., 1974; Barrow et al., 1977; Fuller et al., 1978; Collins et al., 1984; Robinson et al., 1984; Robinson et al., 1988). The selection and establishment of the indigenous LAB in the neonatal pig develops progressively from birth (Sinkovics and Juhasz, 1974; Schulze, 1977). A succession of *Lactobacillus* spp. occurs in the small intestine (Tannock et al., 1990) *L. reuteri* colonize animals on the first day of birth, with the *L. acidophilus* group appearing one week after birth (Naito et al., 1995). Lysozyme in sow's milk has a significant effect on bacterial colonization of the pre-weaned piglet (Schulze and Müller, 1980). Colostrum from the sow's milk provides a protective effect against pathogen-

induced diarrhoea (Ducluzeau, 1985). Adverse conditions may lead to changes in the intestinal flora. Markedly lower numbers of lactobacilli and bifidobacteria were detected in the foregut of piglets deprived of water and food for 72 h, while numbers of *E. coli* increased (Morishita and Ogata, 1970).

2.4 Detection and identification of lactic acid bacteria in the porcine gastro-intestinal tract

Understanding of the complex natural bacterial communities that colonize the GIT of monogastric mammals such as pigs and humans is far from complete. The identification of faecal flora by time-consuming methods where intestinal bacteria had to be isolated and cultured, revealed considerable species diversity (Moore and Holdeman, 1974; Salinatro et al., 1977; Moore et al., 1987). Over the past decade, molecular methods have been developed that may be used to study the diversity of the gut microflora (Wilson and Blitchington, 1996). Molecular biology plays an important role in the field of probiotics, where it is used as a taxonomic tool. Current techniques like genetic fingerprinting, gene sequencing, oligonucleotide probing and specific primer selection, discriminate closely related bacteria with varying degrees of success (McCartney, 2002). Additional methods that are used include DGGE, temperature gradient gel electrophoresis (TGGE) and fluorescent-*in-situ*-hybridization (FISH). FISH can be used to great effect in the identification of intestinal microorganisms. By applying fluorescently labeled oligonucleotides, individual whole fixed cells can be identified *in situ* (DeLong et al., 1989; Amann et al., 1990). FISH can be implemented in the detection of probiotic bacteria since rDNA targeted specific oligonucleotide probes can be designed for the probiotic strains administered, that would enable detection of the cells in the mucus. The addition of fermentable carbohydrates supports the growth of lactobacilli in the ileum and colon of weaning piglets. Future molecular biology studies on probiotics and gut flora will lead to a better understanding of the activity and function of microflora (McCartney, 2002). The quest will be to demonstrate the role of probiotic bacteria *in vivo*.

2.5 Immunology

In the healthy adult pig, immunoglobulins are released into the digestive tract and contribute to the host's defense against infection. This immune defense starts to function soon

after birth and continues up to about 3 weeks of age (Jonsson and Conway, 1992). After 3 weeks, IgA is secreted and provides immune protection (Porter, 1969). Sow milk immunoglobulins inhibit the growth of *E. coli* (Wilson and Svendsen, 1971), adhesion to enterocytes (Nagy et al., 1979) and neutralizes toxins (Brandenburg and Wilson, 1973). At weaning, the piglet is suddenly deprived of milk antibodies and some non-immunological factors such as lactoferrin, transferrin, vitamin B₁₂-binding protein and the bifidus factor (Cranwell and Moughan, 1989). Although the immune system of piglets is fully functional at the time of weaning, it may need to be stimulated to prevent diarrhoea. Probiotics may stimulate the immune system (Perdigón et al., 1987; Shahani et al., 1989). Hypersensitivity responses in the early-weaned piglet may be induced by dietary components. The intake of small amounts of certain proteins before weaning, particularly soy, sensitizes the immune system (Newby et al., 1984). Mild diarrhoea and some intestinal disturbances may result, leading to increased susceptibility to pathogenic infections.

3. Use of probiotics and prebiotics

The interest in probiotics increased during the 1940s, followed by a decline. However, interest is escalating again as can be seen from the number of recent publications. Emphasis has shifted from using milk fermented with microbes to selecting for indigenous bacteria. The species used in probiotic products for pigs include *L. acidophilus*, *Lactococcus lactis*, *L. reuteri*, combinations of *Lactobacillus* spp., *E. faecalis*, *E. faecium*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus subtilis* var. *toyoi*, *Bifidobacterium bifidum*, *Bifidobacterium pseudolongum*, *Bifidobacterium thermophilus*, *Clostridium butyricum*, *Saccharomyces* spp. and other yeasts. Mixed combinations of organisms used, include *Pediococcus acidilactici*, *Lactobacillus plantarum*, *Lactobacillus casei*, *L. fermentum*, *Lactobacillus brevis*, *Lactobacillus delbreuckii* subsp. *bulgaricus*, *L. casei*, *Streptococcus salivarius* subsp. *thermophilus*, *L. plantarum*, *L. acidophilus* and *E. faecium* (Jonsson and Conway, 1992).

Lactobacilli are strong acid producers and seldom pathogenic (Sharpe et al., 1973; Sharpe, 1981). Certain strains of *E. faecalis* and *E. faecium* are pathogenic (Hardie, 1986; Mundt, 1986). However, some non-pathogenic enterococci are incorporated in probiotic products (Strompfová et al., 2004). Many enterococci produce antimicrobial substances (enterocins) and have an effect on spoilage organisms (Cintas et al., 1997; Sabia et al., 2002). Enterococci can be used as probiotic organisms because of high growth rate, adhesion ability

and production of enterocins (Maia et al., 2001). Non-pathogenic strains of certain *E. coli* can be administered to prevent subsequent colonization of other pathogenic bacteria in the GIT (Duval-Iflah et al., 1983). One of the best examples of probiotic *E. coli* is strain Nissle 1917 (EcN), serotype O6:K5:H1 (Blum et al., 1995). This strain lacks typical virulence genes and prevents the invasion of *Yersinia enterocolitica*, *Shigella flexneri*, *Legionella pneumophila* and *Listeria monocytogenes* (Altenhoefer et al., 2004).

Probiotic preparations should be administered soon after birth, when disease is anticipated (preventive or curative). Administration could be orally (although this could be very stressful to the animals), or dispensed in water or feed (pelleted or ground). Probiotic bacteria can be given as viable organisms in wet, frozen or freeze-dried preparations or pastes (Tournut, 1989), or as fermented products (Pollman et al., 1984). Pelleting involves high temperatures and pressures that may be lethal to microorganisms (Jonsson and Conway, 1992). Some streptococci and *Bacillus* spp. are less affected by heat and may survive, but lactobacilli are more sensitive. Growth conditions of the bacteria, harvesting methods and exposure conditions prior to freeze-drying also influence survival of the cells.

Normal indigenous microflora of a healthy pig may not establish in the GIT when piglets are moved directly after birth into a scrupulously clean environment, or after antibiotic treatment. Preparations of LAB can be administered at these times to initiate the natural sequential colonization of the digestive tract (Cranwell et al., 1976). With normal pig rearing the piglets stay in close contact with the sow for the first weeks, and will be colonized by LAB. On farms with a high incidence of diarrhoeal disease, it may be appropriate to introduce a probiotic strain as early as possible to colonize the digestive tract with probiotic strains that inhibit pathogens. The characteristics and mechanisms of action of the specific strains used will determine whether a single or continuous dosage is preferable.

Therapeutic doses are 10^9 to 10^{12} viable organisms per animal per day or 10^6 to 10^7 added to feed (Vanbelle et al. 1990). The number of organisms given should be sufficient to elicit a beneficial response in the host, but should not induce digestive disorders (Jonsson and Conway, 1992). The issue whether administered probiotic microorganisms are transient or adhere in the GIT, influences the dosage required. Transient strains need to be administered at higher levels than strains adhering to and multiplying in the GIT (Conway, 1989).

Prebiotics are often administered in conjunction with probiotics. The dominant prebiotics used are fructo-oligosaccharides (FOS), oligofructose and inulin trans-galacto-oligosaccharides (TOS), gluco-oligosaccharides, glyco-oligosaccharides, lactulose, lactitol, malto-oligosaccharides, xylo-oligosaccharides, stachyose and raffinose (Monsan and Paul, 1995; Orban et al., 1997; Patterson et al., 1997; Collins and Gibson, 1999; Patterson and Burkholder, 2003). Although mannan oligosaccharides (MOS) have been used as prebiotics, they do not enrich probiotic bacterial populations, but act by binding and removing pathogens from the intestinal tract and by stimulating the immune system (Spring et al., 2000). The oligomers, galacto-oligosaccharides (GOS), soybean oligosaccharides, lactosucrose, isomalto-oligosaccharides and palatinose revealed prebiotic potential (Manning et al., 2004). The vast majority of studies on prebiotics focused on inulin, FOS, GOS and TOS. The latter group of carbohydrates has a history of safe commercial use (Macfarlane et al., 2008).

4. Efficacy and mode of action of probiotics and prebiotics

Administration of probiotic products often gives inconclusive or conflicting results in host animals and determination of the mode of action becomes more difficult (Jonsson, 1985; Tuschy, 1986; Conway, 1989). One important factor to consider is that host susceptibility varies from one animal to the other. Evaluations of probiotic use should include the effect on microflora in the digestive tract. Performance and health can be evaluated by growth rates, feed utilization, number of deaths and occurrence of diarrhoea (Jonsson and Conway, 1992).

The clinical conditions in which efficiency of probiotics have been reported range from infectious, allergic and inflammatory to neoplastic, suggesting that a single mechanism of action is unlikely (Marteau and Shanahan, 2003). Various hypotheses exist to explain the mode of action of probiotics, but these remain speculative (Vanbelle et al., 1990).

Health promoting advantages of probiotic preparations include production of antimicrobial substances, organic acids, and prevention of adhesion of pathogenic bacteria in the digestive tract. Other possible modes of action include production of metabolites able to neutralize bacterial toxins *in situ* or inhibition of their production. An increase in feed conversion by secretion of enzymes from the microflora, stimulation of the immune system, and proliferation in the GIT were also suggested as possible modes of action. Sakata et al. (2003) suggested that probiotics modify the metabolism in the microbial ecosystem of the large intestine by increasing the production of short chain fatty acids (SCFA). This leads to

an increase in sodium and water absorption and a decrease in colonic activity. The SCFA act as modulators for required functions to ensure a healthy GIT. One study assessed the body weight, weekly feed intake and fecal consistency after probiotic supplementation. One advantage recorded for probiotic supplementation in this study included reduction of weaning diarrhea in piglets (Taras *et al.*, 2007). The exact mechanism of action of probiotics remains largely unknown. Probiotics may contribute to host defense by reinforcing non-immunological defenses and stimulating both specific and non-specific host immune responses (Gill, 2003). Little is known about the relative importance of the probiotic-stimulated mechanisms in host protection.

Prebiotics have been shown to possess some immunomodulatory properties. To assess the effects of prebiotics such as FOS and GOS on the immune system, a large number of immunological parameters/markers needed to be assessed (Macfarlane *et al.*, 2008). Measurements of these markers had to take into account the fact that they can be affected by gender and age, and that they might vary because of external factors such as stress, smoking and alcohol intake, which necessitates careful selection of control subjects. The gut contains lymphoid tissue that forms a major part of the body's immune system. Experimental data obtained suggest that immunomodulation can occur through the use of functional foods such as prebiotics (Macfarlane *et al.*, 2008). Prebiotics like raffinose have been shown to reduce allergic reactions in children (Nagura *et al.*, 2002) and results obtained to date with prebiotics in relation to osteoporosis offer some promise (Macfarlane *et al.*, 2008) but studies are limited. Lactulose, FOS and GOS have laxative effects with lactulose well established in the treatment of constipation (De Schryver *et al.*, 2005). Latest studies indicate that there might be potential use of prebiotics on their own, or in combination with probiotics, to immunomodulate the diseases like rheumatoid arthritis and cancer (Macfarlane *et al.*, 2008) but these studies are however in a very early stage.

Research data demonstrating the efficacy of prebiotic application in pigs are scarce compared to human studies (Mountzouris *et al.*, 2006). Most prebiotic oligosaccharides incorporated into swine diets at levels ranging from 5 to 40 g/kg have resulted in mixed but generally not significant effects regarding beneficial modulation of microbial populations determined in various intestinal segments and faeces of pigs (Flickinger *et al.*, 2003; Mikkelsen *et al.*, 2003). Prebiotic inclusion levels in feeds at higher levels have resulted in significantly increased levels of bifidobacteria and lactobacilli in the porcine gut, but at the

higher cost of nutrient digestibility (Smiricky-Tjardes et al., 2003). However, depression of nutrient digestibility is linked to animal performance and health, therefore careful assessment is required. It was concluded that in terms of microflora metabolic activity, the substantially higher numerical trends seen in TOS and FOS treatments regarding total volatile fatty acid, acetate concentrations and glycolytic activities, it could be postulated that TOS and FOS promoted saccharolytic activities in the pig colon (Mountzouris et al., 2006). Overall, effects of prebiotics on porcine gut health have often been variable and inconsistent.

5. Selection of potential probiotic strains

Probiotic strains are selected based on resistance to lytic enzymes in saliva (lysozyme) and digestive enzymes, growth at low pH and bile salts, and their ability to prevent colonization of pathogenic bacteria. Stimulation of the immune system by the probiotic strains is required to increase cell-mediated immune response. Technological resistance and stability at high temperatures during pelleting, spraying etc. will ensure viability of the probiotic strains after dosage. Cell adhesion is one of the selection criteria that remain controversial. This aspect was derived from the concept of virulence factors in pathogenic bacteria. Adherence promotes certain virulence activities like production of toxins (Edwards and Puente, 1998; Klemm and Schembri, 2000). Similar interactions could be beneficial for probiotic organisms such as lactobacilli. Lactobacilli adhere to mucosal surfaces and thereby limit the adherence of pathogenic bacteria (Kotarski et al., 1997; Kirjavainen et al., 1998). Some lactobacilli lack the ability to bind mucus *in vitro* (Jonsson et al., 2001). Since many of these non-binders were isolated from mucosal surfaces it may be assumed that the growth environment affects the adhesion properties of bacteria (Jonsson et al., 2001). The adhesion property of probiotic LAB is species-specific (Barrow et al., 1980). Host specificity is a desirable property for probiotic bacteria and is one of the selection criteria (Salminen et al., 1988; Saarela et al., 2000). Adhesion of LAB in relation to host specificity in human, canine, possum, bird and fish mucus were investigated *in vitro* (Rinkinen et al., 2003). Results indicated that the adhesion trait was not host specific but rather characteristic of the species. This suggests that animal models in probiotic adhesion assays may be more applicable to other host species than earlier thought and highlights the fact that the selection criteria for a probiotic may vary according to the application of the probiotic (Rinkinen et al., 2003).

Numerous papers have been published on the isolation and selection of potential probiotic strains (Nemcova et al., 1997; Chang et al., 2001; Gusils et al., 2002). Results obtained with *in vivo* feeding trials were variable because of the complexity of the intestine and variation between individual animals (Simon et al., 2003). Competitive exclusion products containing undefined cultures were effective in pigs (Fedorka-Cray et al., 1999; Genovese et al., 2000), but the possibility that these products may contain pathogens remains (Gillian et al., 2004). Individual probiotic strains need to be identified before inclusion in a probiotic product (Gillian et al., 2004).

Selection characteristics for prebiotics differ from those proposed for probiotics. Prebiotics should not be hydrolyzed by digestive enzymes or absorbed by mammalian tissues. Substances used as prebiotics must selectively enrich beneficial bacteria (Simmering and Blaut, 2001).

5.1 The use of gastro-intestinal models to screen cultures for probiotic properties

To select suitable probiotics, the strains have to be studied in the environment where they function. The intestinal tract of humans and animals is not readily available for research purposes. This led to the development of various models simulating the gastro-intestinal tract (Miller and Wolin, 1981; Veilleux and Rowland, 1981; Edwards et al., 1985; Gibson et al., 1988; MacFarlane et al., 1989; Molly et al., 1993; Veenstra et al., 1993). A unique GIT model was developed at the TNO Nutrition and Food Research Organization, based in the Netherlands. It was the first *in vitro* model that included features like peristaltic movements, physiological transit characteristics, nutrient absorption and water retention (Veenstra et al., 1993). These unique features made the TNO model very expensive to develop and operate. Potential applications included research on the digestibility of carbohydrates and other food ingredients, interactions of fats and proteins, stability of fat and sugar replacers, availability of minerals and survival of bacteria used in fermented foods and probiotics (Veenstra et al., 1993). The TNO model could be used in both animal nutrition and pharmaceutical research. Latest research included studies of the absorption of mycotoxins in the GIT of pigs (Avantaggiato et al., 2004) and mechanistic studies on the intragastric formation of nitrosamines, resulting in valuable information being obtained regarding the human cancer risk from the combined intake of codfish and nitrate-containing vegetables (Krul et al., 2004). These *in vitro* models proved a popular tool for research concerning bacterial populations in

the GIT and probiotic bacteria administered to animals and humans. Advantages in the use of *in vitro* models compared to *in vivo* animal trials and experiments include cost-effectiveness, rapid results, reproducibility and most importantly, no ethical constraints (Veenstra et al., 1993).

5.2 The safety of probiotic bacteria

Theoretically, probiotic bacteria may be responsible for side effects such as systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals and gene transfer (Marteau, 2001). However, only a few cases of side effects in humans have been reported (Marteau, 2001). Limited information is available on the adverse effects of probiotics in animals, especially pigs. Future studies may focus on the degradation of the intestinal mucus layer by probiotics. No mucus degradation was observed in experiments with gnotobiotic rats (Ruseler-van Embden *et al.*, 1995). Antibiotic resistance genes, especially those encoded by plasmids, can be transferred between organisms (Marteau, 2001). This raises the question whether resistance genes can be transferred by probiotics to endogenous flora or to pathogenic microorganisms. Risk of gene transfer depends on the genetic material transferred, nature of the donor and recipient strains and on selective pressure. Probiotics currently used have been assessed as safe in fermented foods, but safety evaluation in microbial food supplements remains controversial since legislation differs between countries (Isolauri *et al.*, 2004). The ability of specific probiotic strains to survive gastric conditions and adhere to intestinal mucosa following oral administration may entail the risk of bacterial translocation, bacteraemia and sepsis (Table 2). It was proved that probiotics improve the microflora in the gut and thus the overall health status of the host animal and that probiotics have “GRAS” (generally regarded as safe) status (Anadon *et al.*, 2006).

6. Situation in South Africa

Although the use of antibiotics as a growth stimulant in pig rearing has not been banned thus far in South Africa, the legislation might be implemented in the future. Therefore the Livestock Business division of the Agricultural Research Council (ARC) already has a research programme in place called “Alternatives to antibiotics”. At the Animal Feed Manufacturers Association (AFMA) forum in 1998, an overview of the mechanisms of and role of probiotics

and probiotics in animal feeds was presented by the ARC. During the AFMA Forum in March 2007, it was reported that legislation banning the use of antimicrobial growth promoters (AGP's) was implemented on the 1st of January 2006 in the European Union (EU). The question remained, will the same legislation on AGP's await South Africa in the future?

It was reported at the AFMA forum by Maritz (2006) that varying results were obtained in countries where the use AGP's were banned. In Denmark, the use of antibiotics as therapeutic treatment increased after the banning of AGP's, reflecting increasing problems with diarrhoea (Maritz, 2006). The same problem was experienced in Sweden and was addressed by changes in farm management, hygiene, sectioning, zinc supplementation of piglet feed and the use of medicated feed in some herds (Wegener, 2005). The cost of production in the pig industry also increased after the banning of AGP's (Maritz, 2006).

The ARC-Irene is highly committed to the task of finding alternatives to antibiotics, and therefore frequently calls on the livestock, feed and pharmaceutical industries to assist us with research efforts in this regard. It is clear that an outright ban of AGP's in South Africa will not be feasible (also emphasized by above findings from countries in the EU), thereby supporting the approach of the United States. Specific AGP's linked directly to human medicinal use will have to be phased out. We hypothesize that a holistic approach will be required as a single alternative is unlikely to be the answer, rather a combination of alternatives might provide products and strategies that will enable AGP's to be phased out. Human health is of prime importance, especially with the high incidence of HIV/AIDS and other eroding conditions in the population, and the threat of antibiotic resistant bacteria to these individuals as well as to the population as a whole.

7. General discussion

The large population of LAB present in the digestive tract of a healthy animal makes piglets ideal candidates for probiotic dosage. The genetic background, physiological health status and diet of the animal may influence the effectiveness of probiotic preparations (Jonsson and Conway, 1992). It appears difficult to establish a probiotic permanently in the digestive tract of the host animal. Most studies indicate that the indigenous microflora are very efficient in preventing new organisms from establishing permanently (Jonsson and Conway, 1992). Other general disadvantages of probiotics include their high price and the

fact that a high dosage of administration is required (Guerra *et al.*, 2007). More basic knowledge of the digestive ecosystem is needed to obtain consistent effects from probiotics. With the introduction of molecular based techniques such as FISH, this might be achieved in the future.

Table 2 Potential clinical targets of probiotic intervention

Effect	Potential mechanism	Potential risks
Nutritional management of acute diarrhoea	Reduction in duration of rotavirus shedding, normalization of gut permeability and microbiota	Risk related to host and strain characteristics
Nutritional management of allergic disease and inflammatory bowel disease	Degradation/ structural modification of enternal antigens, normalization of properties of indigenous microbiota and gut barrier functions, local and systemic inflammatory response, increase in expression of mucins	Strains with pro-inflammatory effects, adverse effects on innate immunity, translocation, infection
Reducing the risk of infectious disease	Increase in IgA-secreting cells against rotavirus, the expression of mucins	Risk related to host and strain characteristics
Reducing the risk of allergic/inflammatory disease	Promotion of gut barrier functions, anti-inflammatory potential, regulation of the secretion of inflammatory mediators, promotion of the development of the immune system	Directing the microbiota towards other adverse outcomes, directing the immune responder type to other adverse outcomes

Modified from Isolauri *et al.* (2004)

Some of the problems that remain to be solved include the mode of action of probiotics, dose-response relationship, better knowledge of the importance of adhesion, the chemical nature of the receptor sites of different probiotic strains and retaining viability. Specific conditions where probiotics can be incorporated, as alternatives to antibiotics need to be determined and production costs kept low for these products to become more attractive to the farmer.

7. References

- Acar, J., Casewell, M.W., Freeman, J., Friis, C., Goossens, H., 2000. Avoparcin and virginamycin as animal growth promoters: A plea for science in decision-making. *Clin. Microbiol. Infect.* 6, 1-7.
- Altenhoefer, A., Oswald, S., Sonnenborn, U., Enders, C., Schulze, J., Hacker, J., Oelschlaeger, T.A., 2004. The probiotic *Escherichia coli* strain Nissle 1017 interferes with invasion of human intestinal epithelial cells by different enteroinvasive bacterial pathogens. *FEMS Immunol. Med. Microbiol.* 40, 223-229.
- Amann, R.L., Krumholz, L., Stahl, D.A., 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic and environmental studies in microbiology. *J. Bacteriol.* 172, 762-770.
- Anadon, A., Martinez-Larranaga, A., Martinez, M.A., 2006. Probiotics for animal nutrition in the European Union. Regulation and safety assessment. *Regulatory Toxicology and Pharmacology.* 45, 91-95.
- Avantaggiato, G., Havenaar, R., Visconti, A., 2004. Evaluation of the intestinal absorption of deoxynivalenol and nivalenol by an *in vitro* gastro-intestinal model, and the binding efficacy of activated carbon and other absorbent materials. *Food Chem. Toxicol.* 42, 817-824.
- Bäckström, L., 1973. Environment and health in piglet production. A field study of incidences and correlations. *Acta Vet. Scand.* 41, 1-240.
- Barrow, P.A., Brooker, B.E., Fuller, R., Newport, M.J., 1980. The attachment of bacteria to the gastric epithelium of the pig and its importance in the micro ecology of the intestine. *J. Appl. Bacteriol.* 48, 147-154.
- Barrow, P.A., Fuller, R., Newport, M.J., 1977. Changes in the microflora and physiology of the anterior intestinal tract of pigs weaned at 2 days, with special reference to the pathogenesis of diarrhoea. *Infect. Immun.* 18, 586-595.

- Bezoen, A., Hare, W., Hanekamp, J.C., 1999. Emergence of a debate: AGPs and public health. Human health and growth promoters (AGPs) reassessing the risk. Heidelberg Appeal Foundation, Amsterdam, The Netherlands.
- Björk, A.K.K., Christensson, E., Olsson, N.-G., Martinsson, K.B., 1984. The clinical effect of amperozide in pig production. Effects of amperozide on aggression and performance in weaners. Proc. Int. Vet. Soc. 8th Congress, Ghent, Belgium, 27-31 August, 1984, XV, p. 335.
- Björk, A.K.K., Olsson, N.-G.E., Martinsson, K.B., Göransson, L.A.T., 1987. A note on the role of behaviour in pig production and the effect of amperozide in growth performance. Anim. Prod. 45, 523-526.
- Blum, G., Marre, R., Hacker, J., 1995. Properties of *Escherichia coli* strains of serotype O6. Infection 23, 234-236.
- Boucourt, R., Ly, J., 1975. Microflora and fermentation in the gastro-intestinal tract of the young pig. Cuban J. Agric. 9, 163 -167.
- Bouhnik, Y., Raskine, L., Simoneau, G., Vicaut, E., Neaut, C., Flourie, B., Brouns, F., Bornet, F.R., 2004. The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. Am. J. Clin. Nutr. 80, 1658-1664.
- Brandenburg, A.C., Wilson, W.R., 1973. Immunity to *Escherichia coli* in pigs; IgG immunoglobulin in passive immunity to *E. coli* enteritis. Immunol. 24, 119-127.
- Braude, R., Fulford, R.J., Low, A.G., 1976. Studies on digestion and absorption in the intestines of growing pigs. Measurements of the flow of digesta and pH. Brit. J. Nutr. 36, 497-510.
- Butaye, P., Devriese, L.A., Haesebrouck, F., 1999. Glycopeptide resistance in *Enterococcus faecium* strains from animals and humans. Rev. Med. Microbiol. 10, 235-43.
- Carghill, C.F., 1982. Control of *E. coli* infections in pigs. Australian Vet. Sci. 8, 206-207.
- Chang, Y.H., Kim, J.K., Kim, H.J., Kim, W.Y., Kim, Y.B., Park, Y.H., 2001. Selection of a potential probiotic *Lactobacillus* strain and subsequent *in vivo* studies. Ant. van Leeuwenh. 80, 193-199.
- Chapman, J.D., 1988. Probiotics, acidifiers and yeast culture: A place for natural feed additives in pig and poultry production. In: Lyons, T.P. (Eds.), Biotechnology in the Feed Industry, Proc. Alltech's 4th Ann. Symp. pp. 219-233.

- Cintas, L.M., Casaus, P., Holo, H., Hernandez, P.E., Nes, I.F., Havarstein, L.S., 1997. Enterocins L50A and L50B, two novel bacteriocins from *Enterococcus faecium* L50 are related to staphylococcal hemolysins. *J. Bacteriol.* 180, 1988-1994.
- Clemens, E.T., Stevens, C.E., Southworth, M., 1975. Sites of organic acid production and pattern of digesta movement in the gastro-intestinal tract of swine. *J. Nutr.* 105, 759-768.
- Collins, M.D., Farrow, J.A.E., Katic, V., Kandler, O., 1984. Taxonomic studies on streptococci of serological groups E, P, U and V: Description of *Streptococcus porcinus* sp. nov. *System. Appl. Microbiol.* 5, 402-413.
- Collins, M.D., Gibson, G.R., 1999. Probiotics, prebiotics, and synbiotics: Approaches for modulating the microbial ecology of the gut. *Am. J. Clin. Nutr.* 69, 1042S-1057S.
- Conway, P.L., 1989. Lactobacilli: Fact and fiction. In: Grubb, R. (Ed.), *The regulatory and protective role of the normal microflora*. Macmillan Press, Basingstoke, pp. 263-281.
- Corpet, D.E., 1996. Microbiological hazards for humans of antimicrobial growth promoter use in animal production. *Rev. Med. Vet.* 147, 851- 862.
- Cranwell, P.D., Moughan, P.J., 1989. Biological limitations imposed by the digestive system to the growth performance of weaned pigs. In: Barnett, J.L., Hennesy, D.P. (Eds.), *Manipulating Pig Production. II*, Australian Pig Science association. Werribee, Victoria, Australia, pp. 140-159.
- Cranwell, P.D., Noakes, D.E., Hill, K.J., 1976. Gastric secretion and fermentation in the suckling pig. *Brit. J. Nutr.* 36, 71-86.
- De Graaf, F.K., Mooi, F.R., 1986. The fimbrial adhesions of *Escherichia coli*. *Adv. Microb. Physiol.* 28, 65-143.
- Delong, E.F., Wickham, G.S., Pace, N.R., 1989. Phylogenetic strains: Ribosomal RNA-based probes for the identification of single microbial cells. *Science* 43, 1360-1363.
- De Schryver, A.M., Keulemans, Y.C., Peters, H.P., Akkermans, I.M., Smout, A.J., De Vries, W.R., Van de Berge-Hene-gouwen, G.P., 2005. Effects of regular physical activity on defecation patterns in middle-aged patients complaining of chronic constipation. *Scand. J. Gastroenterol.* 40, 422-429.
- Ducluzeau, R., 1985. Implantation and development of the gut flora in the newborn piglet. *Pig News Inform.* 6, 415-418.
- Dupont, H.L., Steele, J.H., 1987. Use of antimicrobial agents in animal feeds: Implications for human health. *Rev. Infect. Dis.* 9, 447-460.

- Duval-Iflah, Y., Chappuis, J.P., Ducluzeau, R., Raibaud, P., 1983. Intraspecific interactions between *Escherichia coli* strains in human newborns and in gnotobiotic mice and piglets. *Prog. Food Nutr. Sci.* 7, 107-116.
- Edwards, C.A., Duerden, B.I., Read, N.W., 1985. Metabolism of mixed human colonic bacteria in a continuous culture mimicking the human faecal contents. *Gastroenterology* 88, 1903-1909.
- Edwards, R.A., Puente, J.L., 1998. Fimbrial expression in enteric bacteria: A critical step in intestinal pathogenesis. *Trends Microbiol.* 6, 282-287.
- Fahy, V.A., Connaughton, D., Driesen, S.J., Spicer, E.M., 1987. Prewaning colibacillosis. In: APSA Committee (Eds.), *Manipulating Pig Production*, Australian Pig Science association. Werribee, Victoria, Australia, pp. 176-188.
- Fasoli, S., Marzotto, M., Rizotti, L., Rossi, F., Dellaglio, F., Torriani, S., 2003. Bacterial composition of commercial probiotic products as evaluated by PCR-DGGE analysis. *Int. J. Food. Microbiol.* 82, 59-70.
- Fedorka-Cray, P.J., Bailey, J.S., Stern, N.J., Cox, N.A., Ladely, S.R., Musgrove, M., 1999. Mucosal competitive exclusion to reduce *Salmonella* in swine. *J. Food Prot.* 62, 1376-1380.
- Flickinger, E.A., Van Loo, J., Fahey, G.C., 2003. Nutritional responses to the presence of inulin and oligofructose in the diets of domesticated animals: A Review. *Crit. Rev. Food Sci. Nutr.* 43, 19-60.
- Fuller, R., Barrow, P.A., Brooker, B.E., 1978. Bacteria associated with the gastric epithelium of neonatal pigs. *Appl. Environ. Microbiol.* 35, 582-591.
- Gaudreau, C., Gilbert, H., 1998. Antimicrobial resistance of clinical strains of *Campylobacter jejuni* subsp. *jejuni* isolated from 1985 to 1997 in Quebec, Canada. *Antim. Agents. Chemother.* 42, 2106-2108.
- Genovese, K.J., Anderson, R.C., Harvey, R.B., Nisbet, D.J., 2000. Competitive exclusion treatment reduces the mortality and faecal shedding associated with enterotoxigenic *Escherichia coli* infection in nursery-raised neonatal pigs. *Can. J. Vet. Res.* 64, 204-207.
- Gibson, G.R., Cummings, J.H., MacFarlane, G.T., 1988. Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulphate reduction and methanogenesis by mixed populations of human gut bacteria. *Appl. Environ. Microbiol.* 31, 359-375.
- Gibson, G.R., Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 125, 1401-1412.

- Gibson, G.R., Rastall, R.A., Roberfroid, M.B., 1999. Prebiotics. In *Colonic Microbiota, Nutrition and Health* ed. Gibson, G.R. and Roberfroid, M.B. pp. 101-124. Dordrecht: Kluwer Academic Press.
- Gill, H.S., 2003. Probiotics to enhance anti-infective defences in the gastro-intestinal tract. *Best Pract. Res. Cl. Ga.* 17, 755-773.
- Gillian, E., Gardiner, G.E., Casey, P.G., Casey, G., Lynch, P.B., Lawlor, P.G., Hill, C., Fitzgerald, G.F., Stanton, C., Ross, R.P., 2004. Relative ability of orally administered *Lactobacillus murinus* to predominate and persist in the porcine gastro-intestinal tract. *Appl. Environ. Microbiol.* 70, 1895-1906.
- Greenwood, P.E., Tzipori, S., 1987. Protection of suckling piglets from diarrhoea caused by enterogenic *Escherichia coli* by vaccination of the pregnant sow with recombinant DNA derived pilus antigens. In: APSA Committee (Eds.), *Manipulating Pig Production*, Australian Pig Science Association. Werribee, Victoria, Australia, p.230.
- Guerra, N.P., Bernardez, P.F., Mendez, J., Cachaldora, P., Castro, L.P., 2007. Production of potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. *Anim. Feed Sci. Technol.* 134, 89-107.
- Gulliver, M.A., Bennett, M., Begon, M., 1999. Enterobacteria: Antibiotic resistance found in wild rodents. *Nature* 401, 233.
- Gusils, C., Bujazha, M., Gonzales, S., 2002. Preliminary studies to design a probiotic for use in swine feed. *Interciencia* 27, 409-413.
- Gustafson, R.H., Bowen, R.E., 1997. Antibiotic use in animal agriculture. *J. Appl. Microbiol.* 83, 531-541.
- Hamilton-Miller, J.M., Shah, S., Winkler, J.T., 1999. Public health issues arising from microbiological and labeling quality of foods and supplements containing probiotic Microorganisms. *Public Health Nutr.* 2, 223-229.
- Hamilton-Miller, J.M., Shah, S., 2002. Deficiencies in microbiological quality and labeling of probiotic supplements. *Int. J. Food Microbiol.* 72, 175-176.
- Hardie, J.M., 1986. Genus *Streptococcus*. In: P.H.A. Sneath, P.H.A. (Ed.), *Bergey's Manual of Systematic Bacteriology*, vol. 2. Williams & Wilkins, London, pp. 1043-1047.
- Isolauri, E., Salminen, S., Ouwehand, A.C., 2004. Probiotics. *Best Pract. Res. Cl. Ga.* 18, 299-313.
- Jonsson, E., 1985. *Lactobacilli* as probiotics to pigs and calves. Dissertation, Dept. Animal Nutrition Management, Swed. Univ. Agric. Sci. 148, pp. 1-65.

- Jonsson, E., Conway, P., 1992. Probiotics for pigs. In: Fuller, R. (Ed.), Probiotics: The scientific basis. Chapman & Hall, London, UK. pp. 260-316.
- Jonsson, H., Ström, E., Roos, S., 2001. Addition of mucin to the growth medium triggers mucus-binding activity in different strains of *Lactobacillus reuteri in vitro*. FEMS Microbiol. Lett. 204, 19-22.
- Kidder, D.E., Manners M.J., 1978. Digestion in the Pig, Sciencetechnica, Bristol.
- Kirjavainen, P.V., Ouwehand, A.C., Isolauri, E., Salminen, S.J., 1998. The ability of probiotic bacteria to bind to human intestinal mucus. FEMS Microbiol. Lett. 167, 185-189.
- Klaenhammer, T.R., Kullen, M.J., 1999. Selection and design of probiotics. Int. J. Food Microbiol. 50, 45-57.
- Klemm, P., Schembri, M.A., 2000. Bacterial adhesions: Function and structure. Int. J. Microbiol. 290, 27-35.
- Kotarski, S.F., Savage, D.C., 1997. Models for study of the specificity by which indigenous lactobacilli adhere to murine gastric epithelia. Infect. Immun. 63, 1698-1702.
- Krul, C.A.M., Zeilmaker, M.J., Schothorst, R.C., Havenaar, R., 2004. Intragastic formation and modulation of N-nitrosodimethylamine in a dynamic *in vitro* gastro-intestinal model under human physiological conditions. Food Chem. Toxicol. 42, 51-63.
- Lawrence, T.L.J., 1983. Dietary manipulation of the environment within the gastro-intestinal tract of the growing pig and its possible influence on disease control: Some thoughts. Pig Vet. Soc. Proc., Cambridge, 10, 40-49.
- Lipkin, M., 1987, Proliferation and differentiation of normal and diseased gastro-intestinal cells. In: Johnson, L.R. (Ed.), Physiology of the Gastro-intestinal Tract, 2nd ed. Raven Press, New York, pp. 255-284.
- Lloyd-Jones, G., Lau, P.C.K., 1998. A molecular view of microbial diversity in a dynamic landfill in Québec. FEMS Microbiol. Lett. 172, 219-226.
- Lönnroth, I., Martinsson, K., Lange, S., 1988. Evidence of protection against diarrhoea in suckling piglets by a hormone-like protein in sow-milk. J. Vet. Med. 356, 628-635.
- MacFarlane, G.T., Hay, S., Gibson, G.R., 1989. Influence of mucin on glycosidase, protease and arylamidase activities of human gut bacteria grown in a 3-stage continuous culture system. J. Appl. Bacteriol. 66, 407-417.
- Macfarlane, G.T., Steed, H., Macfarlane, S., 2008. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. J. Appl. Microbiol. 104, 305-344.

- Maia, O.B., Duarte, R., Silva, A.M., Cara, D.C., Nicoli, J.R., 2001. Evaluation of the components of a commercial probiotic in gnotobiotic mice experimentally challenged with *Salmonella enterica* subsp. Enterica ser. Typhimurium. *Vet. Microbiol.* 19, 183-189.
- Manning, T.S., Gibson, G.R., 2004. Prebiotics. *Best Pract. Res. Cl. Ga.* 18, 287-298.
- Maritz, J., 2006. Antimicrobial growth promoters in animal production: Possible implications of the removal from animal feed. *AFMA Matrix*, September, 4 – 8.
- Marteau, P., Shanahan, F., 2003. Basic aspects and pharmacology of probiotics: An overview of pharmacokinetics, mechanisms of action and side effects. *Best Pract. Res. Cl. Ga.* 17, 725-740.
- Marteau, P., 2001. Safety aspects of probiotic products. *Scand. J. Nutr.* 45, 22-24.
- Maxwell, C.V., Reimann, E.M., Hoekstra, W.G., Kowalczyk, T., Benevenga, N.J., Grummer, R.H., 1970. Effect of dietary particle size on lesion development and on the contents of various regions of the swine stomach. *J. Anim. Sci.* 30, 911-922.
- McCartney, A.L., 2002. Application of molecular biological methods for studying probiotics and the gut flora. *Br. J. Nutr.* 88, S29-S37.
- Metchnikoff, E., 1908. *Prolongation of Life*. G. Putnum's Sons. New York.
- Mikkelsen, L.L., Jakobsen, M., Jensen, B.B., 2003. Effects of dietary oligosaccharides on microbial diversity and fructo-oligosaccharide degrading bacteria in faeces of piglets in postweaning. *Anim. Feed Sci. Technol.* 109, 133-150.
- Miller, T.L., Wolin, M.J., 1981. Fermentation by the human large intestine microbial community in an *in vitro* semi-continuous culture system. *Appl. Environ Microbiol* 42, 400-407.
- Molly, K., Van de Woestyne, M., Verstraete, W., 1993. Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. *Appl. Microbiol. Biotechnol.* 39, 254-258.
- Monsan, P., Paul, F., 1995. Oligosaccharide feed additives. In: R.J. Wallace, R.J. Chesson, A. (Eds.), *VHC Biotechnology in Animal Feeds and Animal Feeding*, New York. pp. 233-245.
- Moore, W.E., Holdeman, L.V., 1974. Human faecal flora: The normal flora of 20 Japanese-Hawaiians. *Appl. Environ. Microbiol.* 27, 961-979.
- Moore, W.E., Moore, L.V.H., Cato, E.P., Wilkins, T.D., Kornegay, E.T., 1987. Effect of high-fibre and high-oil diets on the faecal flora of swine. *Appl. Environ. Microbiol.* 53, 1638-1644.

- Morishita, Y., Ogata, M., 1970. Studies on the alimentary flora of pigs. V. Influence of starvation on the microbial flora. *Japan. J. Vet. Sci.* 32, 19-24.
- Mountzouris, K.C., Balakas, C., Fava, F., Tuohy, K.M., Gibson, G.R., Fegeros, K., 2006. Profiling of composition and metabolic activities of the colonic microflora of growing pigs fed diets supplemented with prebiotic oligosaccharides. *Anaerobe.* 12, 178-185.
- Mumpton, F.A., Fishman, P.H., 1977. The application of natural zeolites in animal science and aquaculture. *J. Anim. Sci.* 45, 1118-1203.
- Mundt, J.O., 1986. Enterococci. In: Sneath, P.H.A. (Ed.), *Bergey's Manual of Systematic Bacteriology*, vol. 2. Williams & Wilkins, London, pp. 1063-1065.
- Nagura, T., Hachimura, S., Hashiguchi, M., Ueda, Y., Kanno, T., Kikuchi, H., Sayama, K., Kaminogawa, S., 2002. Suppressive effect of dietary raffinose on T-helper 2 cell-mediated activity. *Br. J. Nutr.* 88, 421-427.
- Nagy, L.K., Bhugal, B.S., McKenzie, T., 1979. Duration of anti-adhesive and bactericidal activities of milk from vaccinated sows on *Escherichia coli* O149 in the digestive tract of piglets during the nursing period. *Res. Vet. Sci.* 27, 289-296.
- Naito, S., Hayashidani, H., Kaneko, K., Ogawa, M., Benno, Y., 1995. Development of intestinal lactobacilli in normal piglets. *J. Appl. Bacteriol.* 79, 230-236.
- Nemcova, R., Laukova, A., Gancarcikova, S., Kastel R., 1997. *In vitro* studies of porcine lactobacilli for possible probiotic use. *Berl. Munch. Tierarztl. Wochenschr.* 110, 413-417.
- Newby, T.J., Miller, B., Stokes, C.R., 1984. Local hypersensitivity response to dietary antigens in early-weaned pigs, In: Haresign, W., Cole, J.A. (Eds.), *Recent Advances in Animal Nutrition*. Butterworth, London, pp. 49-59.
- Noakes, D.E., 1971. Gastric function in the young pig. Ph.D. Thesis, University of London. (Cited from Cranwell and Moughan, 1989).
- Orban, J.I., Patterson, J.A., Sutton, A.L., Richards, G.N., 1997. Effect of sucrose thermal oligosaccharide caramel, dietary vitamin-mineral level, and brooding temperature on growth and intestinal bacterial populations in broiler chickens. *Poult. Sci.* 76, 482-490.
- Patterson, J.A., Burkholder, K.M., 2003. Application of Prebiotics and Probiotics in poultry production. *Poult. Sci.* 82, 627-631.
- Patterson, J.A., Orban, J.I., Sutton, A.L., Richards, G.N., 1997. Selective enrichment of bifidobacteria in the intestinal tract of broilers by thermally produced kestoses and effect on broiler performance. *Poult. Sci.* 76, 497-500.

- Perdigón, G., Nader de Macais, M.E., Alvarez, S., Oliver, G., Pesce de Ruiz Holgado, A.A., 1987. Enhancement of immune response in mice fed with *Streptococcus thermophilus* and *Lactobacillus acidophilus*. J. Dairy. Sci. 70, 919-926.
- Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., Nightingale, C., Preston, R., Waddell, J., 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemother. 53, 28-52.
- Piddock, L.J.V., 2002. Fluoroquinolone resistance in *Salmonella* serovars isolated from humans and food animals. FEMS Microbiol. Rev. 26, 3-16.
- Pollmann, D.S., Kennedy, G.A., Koch, B.A., Allee, G.L., 1984. Influence of nonviable *Lactobacillus* fermentation product in artificially reared pigs. Nutr. Rep. Int. 29, 977-982.
- Porter, P., 1969. Transfer of immunoglobulins IgG, IgA and IgM to lacteal secretions in the sow and their absorption by the neonatal piglet. Biochem. Biophys. Acta. 18, 381-392.
- Raibaud, P., Caulet, M., Galpin, J.V., Mocquot, G., 1961. Studies on the bacterial flora of the alimentary tract of pigs. II. Streptococci: selective enumeration and differentiation of the dominant group. J. Appl. Bacteriol. 24, 285-306.
- Ratcliff, J., 2000. Antibiotic Bans – A European Perspective. Proceedings of the AFMA Symposium on: Improving animal performance through nutrition. Pretoria, South Africa.
- Rautelin, H., Renkonen, O.V., Kosunen, T.U., 1993. Azithro-mycin resistance in *Campylobacter jejuni* and *Campylobacter coli*. Eur. J. Clin. Microbiol. Infect. Dis. 12, 864-865.
- Reiter, B., 1985. Protective proteins in milk, biological significance and exploitation. Int. Dairy Fed. Bull. 191,1-35.
- Rinkinen, M., Westermarck, E., Salminen, S., Ouwehand, A.C., 2003. Absence of host specificity for *in vitro* adhesion of probiotic lactic acid bacteria to intestinal mucus. Vet. Microbiol. 97, 55-61.
- Robinson, I.M., Allison, M.J., Bucklin, J.A., 1981. Characterization of the cecal bacteria of normal pigs. Appl. Environ. Microbiol. 41, 950-955.
- Robinson, I.M., Stromley, J.M., Varel, V.H., Cato, E.P., 1988. *Streptococcus intestinalis*, a new species from the colons and faeces of pigs. Int. J. Syst. Bacteriol. 38, 245-248.
- Robinson, I.M., Whipp, S.C., Bucklin, J.A., Allison, M.J., 1984. Characterization of predominant bacteria from the colons of normal and dysenteric pigs. Appl. Environ. Microbiol. 48, 964-969.
- Ruseler-van Embden, J.G.H., Van Lieshout, L.M.C., Gosselink, M.J., Marteau, P., 1995. Inability of *Lactobacillus casei* strain GG, *L. acidophilus*, and *Bifidobacterium bifidum* to

- degrade intestinal mucus glycoproteins: Clearing the way for mucosa-protective therapy. *Scand. J. Gastroenterol.* 89, 1916-1917.
- Saarela, M., Mogensen, G., Fondén, R., Mättö, J., Mattila-Sandholm, T., 2000. Probiotic bacteria: Safety, functional and technological properties. *J. Biotechnol.* 84, 197-215.
- Sabia, C., Manicardi, G., Messi, P., de Niederhausern, S., Bondi, M., 2002. Enterocin 416KI, an antilisterial bacteriocin produced by *Enterococcus casseliflavus* IM 416KI isolated from Italian sausages. *Int. J. Food Microbiol.* 75, 163-170.
- Sakata, T., Kojima, T., Fujieda, M., Takahashi, M., Michibata, T., 2003. Influences of probiotic bacteria on organic acid production by pig caecal bacteria *in vitro*. *Proc. Nutr. Soc.* 62, 73-80.
- Salinatro, J.P., Blake, I.G., Muirhead, P.A., 1977. Isolation and identification of faecal bacteria from adult swine. *Appl. Environ. Microbiol.* 33, 79-84.
- Salminen, S., Deighton, M.A., Benno, Y., Gorbach, S.L., 1988. Lactic acid bacteria in health and disease. In: Salminen, S., von Wright, A. (Eds.), *Lactic Acid Bacteria. Microbiology and Functional aspects*, 2nd ed. Marcel Dekker, New York, pp. 211-253.
- Sanders, M.E., Huis in't Veld, J., 1999. Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labeling issues. *Ant. van Leeuwenh. Int. J. Gen. Mol. Microbiol.* 76, 293-315.
- SCAN, 1996. Report of the Scientific Committee for Animal Nutrition (SCAN) on the possible risk for humans of the use of avoparcin as a feed additive. Opinion expressed 21 May 1996. Office for EC Publications, Luxembourg.
- SCAN, 1998. Opinion of the Scientific Committee for Animal Nutrition (SCAN) on the immediate and longer-term risk to the value of streptogramins in human medicine posed by the use of virginiamycin as an animal growth promoter, 10 July 1998. Office for EC Publications, Luxembourg.
- Schulze, F., 1977. Quantitiven Magen-Darm-Flora-Analysen beim Ferkel vor und nach dem Absetzen unter Berücksichtigung der Pathogenese der Kolienterotoxämie. *Arch. Exp. Vet. Med., Leipzig*, 31, 299-316.
- Schulze, F., Bathke, W., 1977. Zur quantativen Zusammensetzung der Magen-Darm-Flora beim Läufer Schwein. *Arch. Exp. Vet. Med., Leipzig*, 31, 161-185.
- Schulze, F., Müller, G., 1980. Lysozym in der Sauenmilch und seine Bedeutung für die bakterielle Besiedlung des Magen-Darm-Kanals beim Saugferkel. *Arch. Exp. Vet. Med., Leipzig*, 34, 317-423.

- Shahani, K., Fernandes, H., Amer, V., 1989. Immunologic and therapeutic modulation of gastro-intestinal micro ecology by lactobacilli. *Microecol. Ther.* 18, 103-104.
- Sharpe, M.E., 1981. The genus *Lactobacillus*, In: Starr, M.P. (Ed.), *The Prokaryotes, a Handbook on Habitats, Isolation and Identification of Bacteria*, vol. 2. Springer-Verlag, Berlin, pp. 1653-1679.
- Sharpe, M.E., Hill, L.R., Lapage, S.P., 1973. Pathogenic lactobacilli. *J. Med. Microbiol.* 6, 281-286.
- Simmering, R., Blaut, M., 2001. Pro- and prebiotics – the tasty guardian angels? *Appl. Microbiol. Biotechnol.* 55,19-28.
- Simon, O., Vahjen, W., Scharek, L., 2003. Microorganisms as feed additives – probiotics. In: R. O. Ball (Ed.), *Proceedings of the 9th International Symposium on Digestive Physiology in Pigs*, vol. 1, University of Alberta, Edmonton, Alberta, Canada. pp. 295-318.
- Sinkovics, G., Juhasz, B., 1974. Development of the intestinal flora in suckling pigs. *Acta. Vet. Acad. Scient. Hungar.* 24, 375-381.
- Sjögren, E.E., Lindblom, G.B., Kaijser, B., 1993. Rapid development of resistance to quinolones in *Campylobacter* in Sweden. *Acta. Gastro-Enterologica Belgica* 46, Suppl. 10.
- Slade, R., 2004. Food tube. *Pig Int.* 34, 22-24.
- Smiricky-Tjardes, M.R., Grieshop, C.M., Flickinger, E.A., Bauer, L.L., Fahey, G.C., 2003. Dietary galactooligosaccharides affect ileal and total-tract nutrient digestibility, ileal and fecal bacterial concentrations, and ileal fermentative characteristics of growing pigs. *J. Anim. Sci.* 81, 2535-2545.
- Smith, H.W., 1965. The development of the flora of the alimentary tract in young animals. *J. Pathol. Bacteriol.* 90, 495-513.
- Smith, H.W., Jones, J.E.T., 1963. Observation on the alimentary tract and its bacterial flora in healthy and diseased pigs. *J. Pathol. Bacteriol.* 86, 387-412.
- Spring, P., Wenk, C., Dawson, K.A., Newman, K.E., 2000. The effect of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella*-challenged broiler chicks. *Poult. Sci.* 79, 205-211.
- Strompfová, V., Laukova, A., Ouwehand, A.C., 2004. Selection of enterococci for potential canine probiotic additives. *Vet. Microbiol.* 100, 107-114.
- Tannock, G.W., 1990. The micro ecology of lactobacilli inhabiting the gastro-intestinal tract, In: Marshall, K.C. (Ed.), *Advances in Microbial Ecology*, vol. 11. Plenum Press, New York, pp. 147-171.

- Taras, D., Vahjen, W., Simon, O., 2007. Probiotics in pigs – modulation of their intestinal distribution and on their impact on health and performance. *Livestock*. 108, 229-231.
- Thacker, P.A., 1988. Novel approaches to growth promotion in the pig, In: Haresign W., Cole, D.J.A. (Eds.), *Recent Advances in Animal Nutrition*. Butterworth, London, pp. 73-84.
- Timmerman, H.M., Koning, C.J.M., Mulder, L., Rombouts, F.M., Beynen, A.C., 2004. Monostrain, multistain and multispecies probiotics – A comparison of functionality and efficacy. *Int. J. Food Microbiol.* 96, 219-233.
- Tournut, J., 1989. Applications of probiotics to animal husbandry. *Rev. Sci. Tech. Off. Int. Epiz.*, 8, 551-566.
- Trevallyn-Jones, J., 1987. Field trials with recombinant DNA scours vaccine, In: APSA Committee (Eds.), *Manipulating Pig Production Australian Pig Science*. Weribee, Victoria, Australia, p. 231.
- Turner, J.L., Pas, S., Dritz, S., Minton, J.E., 2002. Review: Alternatives to conventional Antimicrobials in Swine Diets. *Prof. Anim. Sci.* 17, 217-226.
- Tuschy, D., 1986. Verwendung von ‘Probiotika’ als Leistungsförderer in der Tierernährung. *Übers. Tierernährg.*, 14, 157-178.
- Tzipori, S., 1985. The relative importance of enteric pathogens affecting neonates of domestic animals. *Adv. Vet. Sci. Comp. Med.* 29, 103-206.
- Vanbelle, M., Teller, E., Focant, M., 1990. Probiotics in animal nutrition: A review. *Arch. Anim. Nutr. Berlin* 7, 543-567.
- Veenstra, J., Minekus, M., Marteau, P., Havenaar, R., 1993. New Type Gastro-intestinal, A Tool to Test Physiological Aspects of Foods. *Int. Food Ingredients*. 3, 2-8.
- Veilleux, B., Rowland, I.R., 1981. Simulation of the rat intestinal ecosystem using a two-stage continuous culture system. *J. Gen. Microbiol.* 123, 103-115.
- Vodovar, N., Flanzky, J., François, A.C., 1964. Intestin grele du porc. I. – Dimensions en fonction de l’age et du poids, etude de la jonction du canal cholédoque et du canal pancreatic a celui-ci. *Ann. Biol. Anim. Biochem. Biophys.* 4, 27-34.
- Weese, J.S., 2002. Microbiological evaluation of commercial probiotics. *J. Am. Vet. Med. Assoc.* 220, 794-797.
- Wegener, H.C., 2005. Use of antimicrobial growth promoters in food animals: the risks outweigh the benefits. In: *Proceedings of the International Debate Conference on Antimicrobial Growth Promoters: Worldwide Ban on the Horizon?* Noordwijk on Zee, the Netherlands.

- Williams, R.J., Heymann, D.L., 1998. Containment of antibiotic resistance. *Science*, 279, 1153-1154.
- Wilson, K.H., Blichington, R.B., 1996. Human colonic biota studied by ribosomal DNA sequence analysis. *Appl. Environ. Microbiol.* 62, 2273-2278.
- Wilson, W.R., Svendsen, J., 1971. Immunity to *Escherichia coli* in pigs. The role of milk in protective immunity to *E. coli* enteritis. *Can. J. Comp. Med.* 35, 230-243.
- Zani, G., Biavati, B., Crociani, F., Matteuzzi, D., 1974. Bifidobacteria from the faeces of piglets. *J. Appl. Bacteriol.* 37, 537-547.