Chapter Eleven

Probiotics for pigs

EVA JONSSON AND PATRICIA CONWAY

11.1. INTRODUCTION

Although the use of the term 'probiotic' to describe a feed supplement is recent (Parker, 1974), there are a few earlier reports presenting the concept of using living microbes and substances to improve piglet health. For example, in 1946 Møllgaard proposed that the phytic acid found in ungerminated seeds interfered with absorption of calcium and phosphorus, an effect which they showed could be inhibited by compounds such as lactic acid. In order to increase levels of lactic acid within the digestive tract, a lactic acid bacillus originating from the piglet intestine was introduced and subsequent improved health and skeletal formation was observed (Møllgaard, 1947).

As pig raising has become more industrialized with intensive or semi-intensive commercial units, the risk for major economic losses due to decreased performance and health has become of paramount importance and massive efforts have been made to find different ways to improve production. Intestinal disturbances, e.g. diarrhoea, contribute significantly to the piglet health and reported decreased performance. Medical products such as antibiotics and chemotherapeutics have been used very successfully against the reported decreased performance. The use of low dose antibiotics in the pig raising seems to have facilitated large-scale animal production by allowing good growth rates under sometimes suboptimal conditions. For reviews of modes of growth promotion by antibiotics see Visek (1978) and Walton (1980).

There is an increasing concern from both consumers and authorities about the health risks involved in consuming meat containing residues from feed additives as well as the potential hazards from spreading of resistance factors by indiscriminate use of antibiotics. This problem is even worse if no distinction is made between those compounds used for curing of diseases and those used as feed additives. Furthermore, there is an increase in awareness of the ethical aspects of animal production. The general use of low-dose antibiotics in animal feed has hence been

R. Fuller, *Probiotics*

prohibited in Sweden since 1986. The awareness of problems connected with antibiotic treatment of enteric conditions increased the interest in the use of prophylactic *Escherichia* coli vaccines in the early 1970s. Today the vaccination of sows in Sweden before farrowing is common and has contributed to the effective reduction in outbreaks of *E. coli* K88 induced neonatal diarrhoea during the first week of life (Söderlind *et al.*, 1988).

Other approaches that have been used to deal with intestinal problems include: acidification of feed or water (Chapman, 1988), altering dietary formulations, e.g. release of new feeds for small piglets and feeds of lowered protein concentration (Lawrence, 1983); vaccination with attenuated strains of the pathogens or with strains produced by recombinant gene technology (Greenwood and Tzipori, 1987; Trevallyn-Jones, 1987); administration of growth hormone, somatostatin immunization, repartitioning agents and enzyme supplementation (Thacker, 1988); utilization of the lactoperoxidase system (Reiter, 1985): treatment with psychopharmacological drugs (Björk *et al.*, 1987) and the stimulation of levels of hormone-like protein (antisecretory factors) that is capable of reversing intestinal hypersecretion and thus reducing the symptoms of diarrhoea (Lönnroth *et al.*, 1988). More esoteric substances such as the natural mineral zeolite which had been shown to reduce diarrhoea in piglets and increase feed efficiency by as much as 25% (Mumpton and Fishman, 1977) should also be cited.

Parker's original definition of probiotics encompassed 'organisms and substances'. Subsequent references to probiotics seem to be limited to microorganisms and/or their metabolites. To be strictly in accordance with Parker, compounds such as enzymes, growth hormones, etc., could also be referred to as probiotics. We shall limit our discussion, however, to probiotic preparations which contain viable microorganisms.

Today, treatment of diarrhoea consists largely of the use of electrolyte solutions, vaccination and administration of antibiotics but there is a need for new treatments. It appears that the concept of low protein feeds supplemented with essential amino acids is now receiving considerable interest with the release of some commercial preparations in Sweden. The lower protein levels should result in less undigested protein which can be a source of nutrients by pathogens in the small intestine. Because pathogenic *E. coli* K88 grows well in the mucus secreted by the small intestinal mucosa (Conway *et al.*, 1990), the reduction of dietary proteins does not automatically protect the host from intestinal colonization by such strains. Probiotics may have the potential to become part of the treatment and be given as prophylactic agents. However, we must understand their modes of action and when and to what extent they can be anticipated to have beneficial effects, for such preparations to be used routinely. Because the causes of digestive

disturbances are multifactorial, it cannot be expected that, a single agent or treatment will be effective against all causative agents. The use of probiotics is not mutually exclusive to other alternatives and in fact combined approaches are more likely to be complementary and the most effective. For example, probiotic preparations with antibacterial activity could be used together with special dietary formulations, or with compounds which suppress symptoms of diarrhoea without directly influencing the pathogenic microbes.

11.2. SPECIAL FEATURES OF PIGS RELEVANT TO THE USE OF PROBIOTICS

An understanding of factors affecting piglet growth is required for a discussion of the action of probiotics and the scientific basis of probiotic functions. At present, probiotics are only being evaluated for oral administration to piglets. Such preparations have therefore directly to exert their effects inside the digestive tract on the activities connected with digestion, microbial competition and immunological defence. However, it is feasible that *Lactobacillus* metabolites produced in the digestive tract could be absorbed and reach many sites and organs in the body. Consequently, some aspects of the raising of pigs and their special features with reference to the state of health of the animals, the digestive tract and factors influencing piglet weight gain and survival will be discussed.

11.2.1. Rearing of pigs

In the production of meat from pigs, one of the major problems is the high mortality of around 20% especially up to weaning (Bäckström, 1973). Other countries report up to 47% and 22% incidence of diarrhoea and death, respectively (Fahy *et al.*, 1987a, b). An example of the causes of pre-weaning mortality in a piggery with a moderate diarrhoea problem is presented in Table 11.1. It is not possible to convert the entire mortality rate into profit regardless of how effective a probiotic or antibiotic may be. The mortality rate cannot only be judged from the human point of economics but the biological reasons have to be taken into account as well. The sow can bear large litters of up to 12–15 piglets. The biological cost is low for bearing an extra piglet which could survive in favourable circumstances, because the newborn piglet corresponds to 0.5–1% of the weight of the sow, as compared with about 6% for humans. The piglets are born quite immature, however, and this makes them sensitive to infections. One must emphasize the importance of the sow and her individual experience and ability to take

care of and feed her young. In many cases, the raising system limits her possibilities to function optimally.

Table 11.1 Causes of pre-weaning mortality in a piggery with a moderate diarrhoea problem (compiled from Fahy *et al.*, 1987a).

Cause of death	Percentage of total
Diarrhoea	41
Overlay	17
Splayleg	9
Anaemia	6
Bacterial septicaemia	5
Necrotic enteritis	3
Cold exposure	3
Congenital defects	11
Unknown	5

It is probable that only a part of the high morbidity rate can be targeted by probiotics, namely that part induced by diarrhoea. Piglet diarrhoea can be induced by a number of agents (Table 11.2), and manifests itself by hypersecretion of fluids across the gut wall and into the lumen. This host response can be triggered by, for example, the toxins produced by enteropathogenic *E. coli* strains and functions as the host's defence. Such a rapid fluid flow into the intestine facilitates flushing the pathogen from the site. Samples submitted to a typical diagnostic laboratory in USA (Hoefling, 1989) showed that enterotoxic *E. coli* (EEC) was the primary cause of diarrhoea in 26% of cases. Other cases that were analysed showed that transmissible gastroenteritis (26%), clostridial enteritis (18%), coccidiosis (14%) and rotavirus (8%) were the causative agents, while in 8% no already characterized agent was found. Fahy and co-workers (1987a, b) reported that EEC was responsible for diarrhoea on average in 25% of the cases and even up to 80% after weaning.

The piglets are particularly susceptible to diarrhoea during three periods, i.e. during the first week, at 2 to 3 weeks of age and at weaning. During the early stages of life, the piglet is protected by maternal immunoglobulins in the colostrum (Porter, 1969). A loop-hole for infection of the piglets is the period when the piglet nuzzles the skin of the sow trying to locate the teats. If the environment is heavily contaminated as in many rearing systems, the piglets are exposed to an extensive contamination of microbes. This time period can be up to 60 minutes (Spicer *et al.*, 1986). Neonatal diarrhoea often occurs within 48 hours after birth. It can be largely attributed to enterotoxic *E. coli* strains carrying K88, K99 or 987P fimbriae (reviewed by De Graaf and

Mooi, 1986) and often producing ST enterotoxin. The disease is firstly seen in one or two pigs before spreading to the entire litter. The cause of the enhanced susceptibility to diarrhoea at about 2 to 3 weeks of age is multifactorial, including infections by the protozoan *Isospora suis* and rotavirus. One suggestion for this sensitivity to infection has been that there is a marked decline in antibodies in the sow's milk. Post-weaning diarrhoea occurs approximately 4 and 10 days after weaning and is also multifactorial with enteropathogenic *E. coli* strains as the major infection agent (Fahy *et al.*, 1987b). Recently *Treponema hyodysenteriae* has been more often isolated from younger pigs (O. Söderlind pers. comm.).

Table 11.2 Causative agents of diarrhoea in pre-weaning piglets (prepared from Tzipori, 1985; Fahy *et al.*, 1987a).

Agent	*Percentage of piglets* Canada	USA	Australia
Enterotoxigenic *Escherichia coli*, e.g. K88 (most frequent), K99, 987P and F41	22	31	82
Salmonella spp. *Campylobacter* spp.			
Clostridium perfringens type A and C	ND	ND	ND
Cryptosporidium	15	18	1
Transmissible gastroenteritis virus	52	16	0
Rotavirus	9	14	4
Porcine adenovirus, coronavirus	ND	ND	ND

ND = No data.

Piglets prior to weaning and newly weaned pigs may carry enteropathogenic *E. coli* strains but remain healthy. Many theories have been proposed as to why disease occurs at weaning and these include: (1) sudden deprivation of maternal antibodies and other protective factors in the sow's milk; (2) the altered diet could result in undigested material being available for growth of the pathogen until the host digestive enzymes are induced; (3) extremes of temperature and humidity can be detrimental to lymphocytes which secrete protective antibodies into the mucus layer of the intestines (Carghill, 1982); (4) dietary stress because piglets often do not eat for 24–48 hours (Fahy *et al.*, 1987b); (5) social stress increases the frequency of diarrhoea (Björk *et al.*, 1984). Any of these factors may enhance sensitivity to infection and if they are combined, the risk for infection is amplified.

The process of weaning piglets exposes them to a multitude of physiological shocks which make the piglets more sensitive to infections. These shocks are sometimes referred to collectively as stress.

Traditionally, weaning was done at 7–10 weeks of age; however, today piglets are often weaned earlier. In Sweden weaning generally is done at 5–6 weeks with some incidences at 3–4 weeks or even earlier, as as is more common in other countries. The weaned pig requires a relatively larger digestive system than a sucking pig if it is to satisfactorily digest and absorb the less digestible post-weaning diets (Cranwell and Moughan, 1989). At 3 weeks the digestive and immunological systems are not fully developed and it takes some time for the piglets to adapt their digestive system to the necessary size and enzyme production when changing from sow milk to pig feed (for review see Cranwell and Moughan, 1989). Also, abnormal behaviour might occur with inferior health as a result (Algers, 1980). Some of the problems in connection with early weaning can be overcome by using suitable food, good hygiene and careful management (English, 1984; Campbell, 1989; Partridge, 1989).

During the fattening stage, swine dysentery is a considerable problem. To achieve desirable performance at this stage, it is necessary for the pigs to be in good health, and for digestion to function optimally. During this period, probiotics are most likely to be used as a means of obtaining the desired high growth rate rather than to prevent infections, as is the case for younger pigs. During this stage diseases unrelated to the digestive tract and its microflora cannot be expected to be influenced by the use of probiotics.

Many factors will add to the stress of the animals. If pigs are raised in a suboptimal environment with a poor atmosphere (manure gases, particles in the air), overcrowding, lack of stimulation and unsuitable temperatures, the stress on the animals will increase and as a result they become more susceptible to infections. Stress, including dietary stress, has been shown to affect the microbial flora in the digestive tract of mice, humans and piglets (Tannock and Savage, 1974; Pollman *et al.*, 1980c; Lencner *et al.*, 1984; Tannock, 1984).

11.2.2 Digestive tract

If probiotic preparations are to survive and be active in the digestive tract, they have to be suitable for that environment and resist the host's protective mechanisms which are inhibitory to microbes. For example, there are powerful stomach defences such as low pH and proteolytic enzymes. The retention time as well as the degree of mixing of the ingested material with the gastric juices and previous digesta also influences survival of administered strains. In the anterior small intestine, the most important defence is the very fast flow rate which prevents microbial overgrowth unless the microorganisms can be attached to the epithelium in this site. Among other factors, the presence

of bile in this region also negatively influences survival and activity of the microbes. A relatively rapid transit time in the posterior small intestine also protects the host unless invading microbes can adhere to the epithelial mucosa. In the caecum and large intestine the passage rate is lower and the microbes can establish, however, they must compete with a stable indigenous microflora in the healthy host. The extent of survival in the stomach, together with the volume of the digest found in the different parts of the digestive tract, influence the numbers of the probiotic organisms required for dosage. For reviews of digestion in the pig see Kidder and Manners (1978) and Rerat *et al.* (1976).

(a) Stomach

At the entrance to the stomach there is an area, *pars oesophagea*, which has the same type of keratinized squamous non-secreting epithelium as the oesophagus and constitutes approximately 5% of the surface area of the stomach (Noakes, 1971). (See Figure 11.1.) The size of the entire *pars oesophagea* area varies from 1–2 cm^2 in the neonatal pig to about 10–15 cm^2 in the adult pig. The squamous cells in this region undergo continuous desquamation, thereby releasing cells covered with lactobacilli and exposing new epithelial cells which are very rapidly colonized by lactobacilli. The rate of this is not known for pigs, but as a comparison it can be mentioned that in rodents the oesophageal cells undergo a proliferative cycle of 54 h with a life span of the cells of 80 h (Lipkin, 1987). It is believed that the released squamous cells that are colonized by lactobacilli may help to regulate the composition of the digestive microflora by ensuring a dominance of the lactic acid bacteria (Fuller *et al.*, 1978; Barrow *et al.*, 1980).

In the stomach, gastric juice which contains mucus, HCl and proteolytic enzymes are secreted. The composition of the gastric juice is affected by many factors, for example, the type of diet. For a review of this topic see Cranwell and Moughan (1989). Gastric pH is influenced by the age of the pig, the degree of mixing of gastric juice with the stomach contents and the rate of passage of the contents through the stomach. The mixing of the digesta depends, among other things, on its dry matter content and particle size. Liquid feed and finely ground feed can mix more easily than drier or coarsely ground cereal diets (Kvasnitskii, 1951; Maxwell *et al.*, 1970). In suckled piglets, the pH of the stomach digesta ought to be homogeneous as the curd of sows milk is soft (White *et al.*, 1969).

The pH in the stomach varies according to the site. The lowest values are generally found in the pyloric antrum near the exit to the small intestine. The contents near the *pars oesophagea*, as well as the region itself, have the highest pH. This depends on the slow mixing in the

proximal part of stomach and the fact that gastric juice is only secreted in the distal part of the stomach. In neonatal piglets, the pH is relatively high in the pyloric antrum, pH 5.2–5.9 (Smith, 1965) but at 4–10 days of age it reaches 4.1–4.4 (Barrow *et al.*, 1977) and drops successively with age. Lawrence (1970) showed in cannulated pigs that the *in vivo* gastric pH varied according to type of diet and time after feeding from a maximum pH of 3.8–4.8 at 30 min after feeding through a gradual decrease down to about pH 2 after 7.5 h. He also showed that *ad lib* feeding gave higher pH values. In the very young piglet, the pH remains close to that of the diet even in the outer layer of the digesta, whereas in the adult, pH falls rapidly to approximately pH 2 in the outer layer (Manners, 1976). See Table 11.3.

Stomach emptying is affected by contractions passing down the pyloric region into the duodenum. Nervous and hormonal feedback mechanisms mainly in the duodenum regulate the passage. Factors triggering the passage include volume of gastric contents and its composition. Parameters of the duodenal digesta which decrease the stomach emptying are low pH, high contents of fat and fatty acids and amino acids and high osmolality (Kidder and Manners, 1978). These factors are generally the same in the pig as for other animal species. Stomach emptying in the pig seems, however, to be somewhat different in two aspects. Firstly, the pH of the emptied gastric contents only plays a minor role for the passage out from the stomach. Secondly, it appears that not only influences from the duodenum but from a larger area in the small intestine affects the gastric emptying (Auffray, 1965).

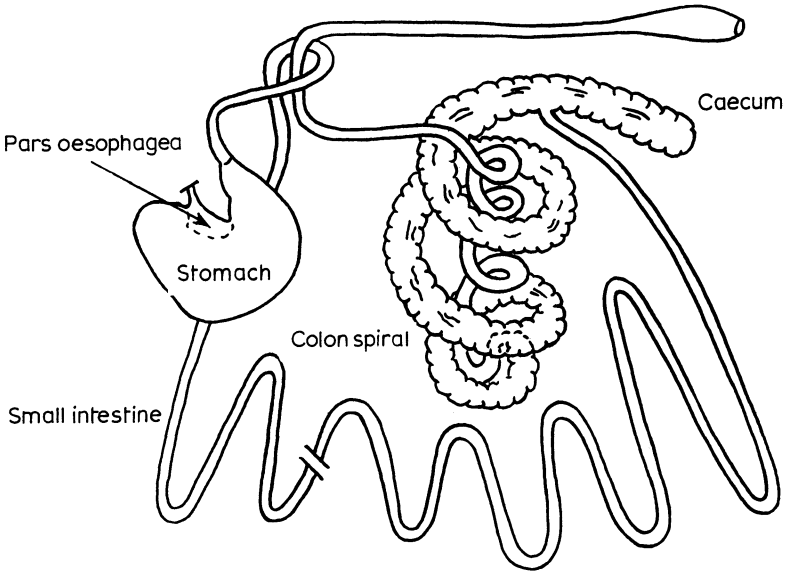

Figure 11.1 The gastro-intestinal tract of pigs, from stomach to rectum.

After a small feed or initially after a large feed, the stomach emptying is approximately exponential. The stomach does not, however, empty completely between meals (Braude *et al.*, 1976; Kidder and Manners, 1978). Food is likely to remain longer in the stomach if the pig is fed at infrequent intervals (Kidder and Manners, 1978). In piglets who suckle frequently (Jensen and Recén, 1989), the stomach empties almost completely between meals (Kvasnitskii, 1951). Reiter (1985) reported that undiluted cow's milk fed to artificially reared piglets may accumulate in the stomach and cause death, while this is not the case for diluted cow colostrum. However, in our experience UHT-treated cow milk with 7% fat has proved satisfactory for the raising of gnotobiotic piglets. After a meal, the liquid part of the feed will rapidly start passing out from the stomach and reach the colon about 12h after feeding (Clemens *et al.*, 1975). In very young piglets, scours was always preceded by gastric stasis (White *et al.*, 1969). These authors considered that gastric stasis with the following lack of nutrients for the body gave general unthriftiness which predisposed the piglets to bacterial infections. Although not discussed by these workers, gastric stasis may result in decreased transit time in the small intestine. In turn, this may reduce the hosts' defence against pathogen colonization of the epithelia, because the pathogens will not be removed as rapidly and therefore could proliferate.

Table 11.3 pH values in the digestive tract of pigs.

Age	Stomach	Small intestine		Caecum	Colon
		Anterior part	Posterior part		
Neonatal	4.0–5.9	6.4–6.8	6.3–6.7	6.7–7.7	6.6–7.2
Unweaned	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: Barrow *et al.* (1977); Boucourt and Ly, (1975); Braude *et al.* (1976); Clemens *et al.* (1975); Cranwell *et al.* (1976); Schulze (1978); Schulze (1987); Schulze and Bathke (1977); Smith and Jones (1963); Smith (1965).

Volumes measured for different parts of the digestive tract can be found in the literature. Some of these values have to be regarded as unrealistic judging from the volumes available within the animal. Also, it should be remembered that not all organs are filled with contents at all times. In very young piglets, the contents in the stomach might be up to 50 ml, while in weaned pigs it may be up to 1l. In adult pigs the stomach may contain 3–7 kg of digesta (Smith and Jones, 1963; Kidder and Manners, 1978).

(b) Small intestine

The acidified small portions of digesta let out from the stomach into duodenum will be mixed with bile and pancreatic juice containing enzymes and buffer substances. The pH will increase along the passage through the small intestine. The variations in pH values in the small intestine is less than for the stomach and also the difference between piglets and adults is less pronounced (Kidder and Manners, 1978). The variations are large in the duodenum (pH 2–6) and progressively smaller down to the ileum (pH 7.0–7.5). There is also a considerable influence of the type of diet on the lumenal pH (Braude *et al.*, 1976). The activity of the microflora in the distal part of the small intestine (Friend *et al.*, 1963) will lower the pH in this region.

The passage rate through the small intestine is very fast in the anterior part but slows down successively as the digesta progresses towards the anus. Normally, it takes about 2.5 h for a digesta particle to pass through the small intestine (Kidder and Manners, 1978). At this flow rate, it would be difficult for bacteria to multiply sufficiently fast to avoid being washed out. Attachment to the epithelial cells is therefore virtually a prerequisite for microorganisms to colonize this region. Because the epithelium is continuously regenerating and sloughing off cells and overlying mucus, bacteria can colonize this region only if their generation time is faster than the sloughing rate. For conventional mice, cells in the small intestine migrate from the crypts to the top of the villi in about 2 days (Abrams *et al.*, 1963). This is much slower than the anticipated generation time for many bacteria. In addition to bacteria adhering to epithelial cells *via* specific adhesins, it has recently been proposed that bacteria may also colonize the mucus overlaying the epithelial cells (Conway *et al.*, 1990, 1991). This mucus supports rapid growth of an *E. coli* strain with an *in vitro* generation time of 26 min.

Volumes measured for the small intestine can be up to 0.1, 0.6 and 20 litres for very young piglets, weaned and adult pigs respectively (Vodovar *et al.*, 1964). In the newborn piglets the length of the small intestine is 2–3 m (review Cranwell and Moughan, 1989) and the surface (not taking the enlargement by microvilli into account) can be estimated to be *c.* 1 m^2 at birth and by day 10 to be 2 m^2 (Smith and Jarvis, 1978). In a 25 kg pig the length of the small intestine is approximately 16 m (Pettersson, 1990), which approaches that of the adult pig (Nickel *et al.*, 1967). Based on the assumptions given above and our experiences, the area in the 25 kg pig could be estimated to be 15–20 m^2.

(c) Large intestine

The large intestine consists of the caecum, the spiral colon and the distal colon. The caecum and centripetal part of the spiral are wide and the

rate of passage of digesta is low, thus allowing activity of a dense and complex anaerobic microflora. The passage rate of a meal is not very easily determined as particles from different meals become randomly distributed. Generally, the first part of a meal will reach the anus after 10–24 hours. The mean retention time is much more variable and can be 2–4 days (Kidder and Manners, 1978).

Volumes measured for the large intestine can be up to about 0.04, 1 and 25 litres for very young piglets, weaned and adult pigs respectively (Kvasnitskii, 1951). The pH in the large intestine (around pH 6.0) does not fluctuate as much as in the foregut as the contents are more homogeneous and microbial fermentation is dominant (Kidder and Manners, 1978).

11.2.3 Indigenous lactic acid bacteria

The pig is a monogastric animal in which the foregut (stomach and small intestine) is colonized by a relatively rich microflora. The flora is not as rich as in ruminants which have a specialized foregut fermentation system, but stomach contents still contains about 110^7g 10^8 bacteria per gram of digesta. As the killing by low pH is not so great bacterial numbers found in the small intestine are generally also high, 10^7–10^9. See Table 11.4. The microflora of the pig foregut is dominated by lactic acid bacteria (LAB), mostly *Lactobacillus* and *Streptococcus* spp. They are found both in the digesta and attached to the epithelia. The non-secreting *pars oesophagea* area in the stomach is densely colonized with layers of LAB (Fuller *et al.*, 1978), as illustrated in Figure 11.2. Attachment to the epithelial cells in the small intestine has also been demonstrated (McAllister *et al.*, 1979). There is a difference in the composition of the microflora in the caecum and colon with Gram-negative organisms dominating the caecum (Robinson *et al.*, 1981) and Gram-positive bacteria dominating the colon (Salanitro *et al.*, 1977).

Several functions within the pig digestive tract enable the LAB microflora to be dominant. These include the fact that lactic fermentation in the stomach is facilitated by the relatively high stomach pH. Furthermore, food entering the stomach is inoculated with the indigenous LAB as the ingesta is mixed with gastric contents and continuous inoculation of LAB is ensured by the sloughing of *pars oesophagea* cells with attached LAB. The relatively high pH in the greater part of the stomach will also mean that the killing of LAB in the gastric content is not so great, hence they will become a major component of the microflora in the small intestine.

The importance of LAB microflora in the foregut relates to physiological, microbiological and digestive functions. It helps the young pig to

lower the pH in the stomach by the production of lactic acid and other organic acids formed mainly from lactose in the sow's milk (Cranwell *et al.*, 1976; Barrow *et al.*, 1977). Both the organic acids and the low pH value in the pyloric antrum are important in decreasing the numbers of bacteria passing into the small intestine (Smith, 1965; Schulze, 1978). LAB may regulate the microflora of the small intestine by inoculating

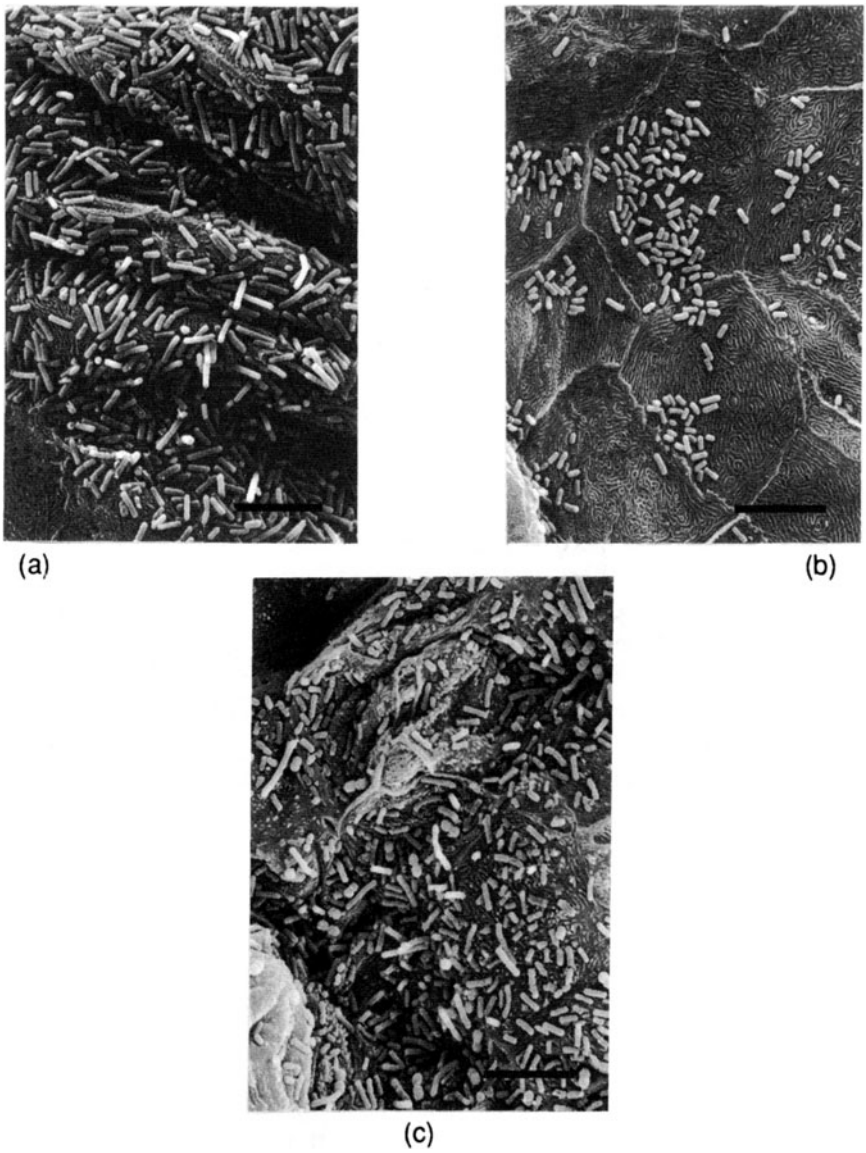

Figure 11.2 Scanning electron micrographs of the squamous stomach epithelium from piglets aged (a) 5 days, (b) 26 days and (c) 47 days. Bar markers = 10 μm. (Blomberg and Conway, 1989; reprinted by permission of John Wiley & Sons, Ltd.)

the digesta passing down the digestive tract (Fuller *et al.*, 1978). In addition to the pH effect, LAB can compete with other bacteria and in this way reduce the contamination in the lower digestive tract (Barrow *et al.*, 1980). It has also been shown that LAB can improve the digestion of mixed-linked (1→3, 1→4) β-D-glucans in fibres (Graham *et al.*, 1986). For reviews of the pig microflora see Cranwell (1968), Schulze (1987) and for its activities see Kenworthy (1973) and Ratcliffe (1985). For a review of the ecology of the lactobacilli of the digestive tract see Tannock (1990).

Species often found are *Lactobacillus acidophilus, L. delbrueckii, L. fermentum, L. reuteri, L. salivarius* (Fewins *et al.*, 1957; Fuller *et al.*, 1978; Mäyrä-Mäkinen *et al.*, 1983; Axelsson and Lindgren, 1987) and *Enterococcus bovis, Ent. durans, Ent. faecalis, Ent. faecium, Streptococcus intestinalis, S. porcinus* and *S. salivarius* (Raibaud *et al.*, 1961; Barrow *et al.*, 1977; Fuller *et al.*, 1978; Collins *et al.*, 1984; Robinson *et al.*, 1988). Bifidobacteria detected from the digesta are *Bifidobacterium adolescentis* and *Bif. suis* (Zani *et al.*, 1974; Robinson *et al.*, 1984).

Table 11.4 Occurrence of bacteria in the digestive tract of healthy pigs older than 2 weeks. Numbers given as colony-forming units per gram digesta or per square centimetre of epithelium (wet weight).

	Digesta						Epithelia	
Bacteria	1	2	3	4	5	6	3	5
Total count	5.5–9.3	5.5–8.4	5.5–9.5	8.4–9.7	7.8–9.8	5.5–5.9		9.4–10.0*
Lactobacilli	5.2–8.9	5.4–8.5	6.1–9.6	7.8–9.3	7.6–9.6	5.1–7.9	6.9	7.3– 7.6*
Streptococci	4.0–6.8	4.0–6.7	5.1–7.4	7.6	5.9–8.0	5.0	4.3–5.8	*6.8– 7.4*
Bifidobacteria	4.3–6.7	3.9–5.3	5.6–7.3	5.1–7.9	5.5–8.7			

1, stomach; 2, anterior small intestine; 3, posterior small intestine; 4, caecum; 5, colon; 6, non-secreting epithelium in the stomach.

*Per gram of mucosa.

Compiled from: Conway (1989); Horvath *et al.* (1958); Kenworthy and Crabb (1963); Kovacs *et al.* (1972); McAllister *et al.* (1979); McGillivery *et al.* (1984); Ogata *et al.* (1968); Russel (1979); Schulze (1977); Smith (1961); Smith and Crabb (1961); Tannock and Smith (1970).

Although both McGillivery (1984) and Cain (1989) reported that lactobacilli were the dominant group of bacteria associated with the duodenal mucosa, and McAllister and co-workers (1979) reported that lactobacilli and *Bacteroides* and *Clostridium* were predominant throughout the small intestine, no characterization of the various species has been reported for the small or large intestinal mucosa. However, Fuller and co-workers (1978) found *L. fermentum* to be the most common species (93%) of isolates from 2–10 day old piglet *pars*

oesophagea. Subsequently, it has been shown that there is a succession of *Lactobacillus* spp. in this region in neonatal pigs (Tannock *et al.*, 1990) and that these populations vary between 5, 26 and 47 day old piglets as illustrated in Figure 11.2 (Blomberg and Conway, 1989).

Piglets should be born in a conventional environment to allow the development of a normal adult microflora. If they are moved at a later stage to an environment devoid of indigenous microorganisms, the microflora will continue to develop normally (Gouet *et al.*, 1984). The selection and establishment of the indigenous LAB microflora in the neonate occurs progressively from birth (Sinkovics and Juhasz, 1974; Schulze, 1978). Ducluzeau (1985) concludes that several investigations point to the fact that the effect of sow milk on the digestive microflora is not so important because no major change in composition of the digestive microflora occurs at weaning, while sow colostrum does indisputably have a protective effect against diarrhoea caused by pathogenic *E. coli*. This conclusion of Ducluzeau may only apply to the indigenous microflora at weaning, because lysozyme in sow's milk has a significant effect on bacterial colonization of the unweaned piglet (Schulze and Müller, 1980) and factors such as lactoferrin, transferrin and vitamin B_{12} binding protein will also play a role (Cranwell and Moughan, 1989). For reviews of the development of the digestive microflora in the milkfed pig and milk-fed human infant, see Ducluzeau (1985) and Cranwell (1990).

Type and amount of diet have been shown to affect the lactobacilli in the digestive tract. Brockett and Tannock (1981) concluded that the relative amounts of palmitic and oleic acids in the food can influence the number of tissue-associated lactobacilli in the mouse stomach. Jonsson and Henningsson (1991) showed a correlation between the diet of the piglet and the occurrence of faecal lactobacilli with ability to degrade mixed-linked (1→3, 1→4) β-D-glucans. These bacteria that were commonly found in the neonatal pig probably originated from the faeces of the sow. During the sucking period, they could not be detected but they reappeared at 7 weeks when the piglets had consumed creep feed containing such fibre for some time.

Lactobacilli in the digestive tract appear to be more affected than, for example, *E. coli*, by lack of nutrients. In rats fed restrictively, Brownlee and Moss (1961) found decreased numbers of lactobacilli adhering to the non-secreting part of the stomach. In mice deprived of nutrients for up to 4 days, the surface associated lactobacilli of the stomach were lost; however, colon lactobacilli levels remained unchanged and coliform numbers increased (Conway *et al.*, 1986). Ogata and Morishita (1969) and Morishita and Ogata (1970) showed markedly decreased numbers of lactobacilli and bifidobacteria in the foregut when pigs were starved for 24 h, and in the ileum when they were deprived

of food and water for 72 h, while numbers of *E. coli* and *Bacteroides* increased in the ileum. Ducluzeau and co-workers (1986) used pigs with ileo-rectal anastomosis. They found that in the contents of the large intestine 14–54 days after the operation, the strictly anaerobic bacteria still remained at the same level, while the numbers of lactobacilli decreased and clostridia could no longer be detected. Morishita and Ogata (1970) suggested that *E. coli* and *Bacteroides* rely more on endogenous nutrients than do the lactobacilli, which have complex nutrient requirements (Sharpe, 1981). Another reason could also be that these other groups of bacteria can grow more quickly and hence are able to compete more efficiently for limiting nutrients (Freter, 1983). Survival during nutrient deprivation has been extensively studied *in vitro* for many species of *Vibrio* and *Pseudomonas* as well as strains of *E. coli*. A very elaborate survival mechanism involving the synthesis of new proteins has been elucidated (reviewed in Kjelleberg *et al.*, 1990). Preliminary studies of the starvation survival capacity of *Lactobacillus* strains of digestive origin have shown excellent survival in the total absence of nutrients; however, in the presence of low levels of a single amino acid, the cells started to grow and then died (Conway, unpublished data). It was hypothesized that the starvation survival mechanisms of *Lactobacillus* have a very low threshold for nutrients, above which growth is initiated. We are not aware of *in vitro* starvation survival studies of any *Bacteroides* spp.

11.2.4 Immunology

In the healthy adult pig, immunoglobulins are released into the digestive tract and thereby contribute to the host's defence against infection. This immune defence does not begin to function in the newborn piglet until about 3 weeks of age. During the first weeks of life, the piglet is protected by maternal antibodies and non-immunological defence factors (Porter, 1969). After 3 weeks of age, IgA is secreted. Sow milk immunoglobulins have been shown to inhibit *E. coli* growth (Wilson and Svendsen, 1971), adhesion to enterocytes (Nagy *et al.*, 1979) and to neutralize the toxins (Brandenburg and Wilson, 1973). The milk immunoglobulins are produced by plasma cells which are derived from lymphocytes sensitized in the gut against intestinal pathogens (Hartmann *et al.*, 1984). Activation of the gut–mammary gland link by feeding sows with either live cultures of *E. coli* (Stevens and Blackburn, 1967) or killed *E. coli* cells (Porter and Linggood, 1983), induced IgM-mediated protection against neonatal scours. A further example of the phenomenon is the fact that vaccinating the sow against *E. coli* K88 has led to protection of the neonatal piglets from K88-induced diarrhoea (Söderlind *et al.*, 1982, 1988). Following the introduction

of such a vaccine, the main pre-weaning diarrhoeal period has been transposed from the neonatal period to the 14–21 days period.

The success of protection *via* ingested maternal lactogenic antibodies has led to applications such as concentrating porcine serum from abattoir blood and adding it to the diet of early weaned pigs (Drew and Owen, 1988). Several non-immunological host defence factors, e.g. lactoferrin, transferrin, vitamin B_{12} binding protein and the bifidus factor, have been identified in sow milk (reviewed by Cranwell and Moughan, 1989). At weaning, the piglet is suddenly deprived of both the milk antibodies as well as these non-immunological factors. Although the immune system of the piglets is generally functioning at the time of weaning, it may need to be stimulated to overcome the cessation of the protective factors in the sow milk. Perdigón and co-workers (1987) report stimulation of the immune response in mice fed with LAB. Similarly, Shahani and co-workers (1989) also proposed that one of the functions of an administered probiotic at this time could be to stimulate the immune system.

The piglet immune system could also contribute to digestive disturbances should dietary components induce hypersensitivity responses in the early weaned piglet (Newby *et al.*, 1984). These workers suggested that the intake of small amounts of certain proteins, particularly soya, before weaning sensitizes the immune system and consequently when the post-weaning piglet consumes larger quantities of the same protein, a hypersensitivity reaction is induced. Mild diarrhoea and intestinal damage could result and thereby increase susceptibility to infections by pathogens. As the risk of hypersensitivity is a difficult parameter to assess, there appears to be considerable confusion about the significance of nominal antigenic ratings of some dietary ingredients (Campbell, 1989).

Various types of stress have been shown to exert negative effects on the immune system. For example, extremes of temperature and humidity have a detrimental effect on lymphocytes which secrete protective antibodies into the gut (Carghill, 1982). Furthermore, an association between a depressed immune response and stress-induced elevated corticosteroid concentration in the blood has been shown for sows (Barnett *et al.*, 1987).

11.3. CURRENT USE OF PROBIOTICS

The interest in probiotics has fluctuated during the twentieth century, with a peak in the 1940s. Thereafter the interest waned, but it is increasing again as can be seen from the amount of published literature

containing more positive interpretations and predictions. Emphasis has now shifted from using milk fermented with microbes selected for their capacity to yield organoleptically desirable fermented dairy products, to selecting indigenous microbes. The pioneering work in this area was carried out in chickens (as presented in the chapter by Barrow). For reviews see Kinsey (1980), Wolter and Henry (1982), Søgaard (1987), Fuller (1986, 1989), Sissons (1988, 1989), Conway (1989) and Vanbelle *et al.* (1989).

Currently, probiotics are being sold as solitary microbial additives or mixed with different substances. Reported dosages range from 10^9 to 10^{12} microbes per animal per day for administration at different times and in different ways. One common problem for LAB preparations used earlier was that the numbers found in some of the preparations was considerably lower than claimed.

Table 11.5 Microorganisms used as probiotics for pigs.

Organism	Form	Reference(s)
Lactobacillus acidophilus	Cultured milk	11, 16, 17, 38, 40
	Frozen culture	33, 34, 36
	Freeze-dried	29
	Dried	10
	FP	35
L. lactis	Frozen	26
L. reuteri	Cultured milk	37
Lactobacillus spp.	Cultured milk	12, 24, 25
	Dried	32
	FP	2, 8
Enterococcus faecalis	Spray-dried	31
Ent. faecium	Freeze-dried	3,4,6,7,15,17,18,22,23,27,39,42
Bacillus licheniformis	Spores	19
B. subtilis	Spores	30
B. subtilis var. *toyoi*	Spores + FP	13, 28, 40, 41
Bifidobacterium bifidum	Dried	5
Bif. pseudolongum	Dried	14
Bif. thermophilus	Dried	14
Clostridium butyricum	Dried	9
Saccharomyces spp.	Dried	1
Yeasts	Dried	17
Mixed organisms:		
Pediococcus acidilactici	Dried	19
L. plantarum		
L. casei		
L. fermentum		
L. brevis		

L. delbrueckii subsp. *bulgaricus* *L. casei* *Streptococcus salivarius* subsp. *thermophilus*	FP	21
L. plantarum *L. acidophilus* *Ent. faecium*	Dried	32
Yoghurt organisms	Yoghurt	37
Lactic culture	Freeze-dried ± FP	28

FP = Fermentation product.

References: 1 Burnett and Neil (1977); 2 Cowman *et al.* (1978); 3 Danek (1986); 4 Deprez *et al.* (1989); 5 Ervolder *et al.* (1984); 6 Gualtieri and Betti (1984); 7 Gudding and Larssen (1985); 8 Hale and Newton (1979); 9 Han *et al.* (1984); 10 Ingram *et al.* (1973); 11 Jensen (1974); 12 Jonsson (1986); 13 Jørgensen (1988); 14 Kimura *et al.* (1983); 15 Kluber *et al.* (1985); 16 Kornegay (1985/86); 17 Kornegay and Thomas (1973); 18 Krarup (1987); 19 Landsudvalget (1988); 20 Landsudvalget (1989); 21 Lessard and Brisson (1987); 22 Maeng *et al.* (1989); 23 Moen (1982); 24 Møllgaard (1946); 25 Møllgaard (1947); 26 Muralidhara *et al.* (1977); 27 Neupert (1988); 28 Ogle and Inborr (1987); 29 Olsson (1966); 30 Ozawa *et al.* (1981); 31 Ozawa *et al.* (1983); 32 Pollman *et al.* (1980a); 33 Pollman *et al.* (1980b); 34 Pollman *et al.* (1980c); 35 Pollman *et al.* (1984); 36 Premi and Bottazzi (1974); 37 Ratliffe *et al.* (1986); 38 Redmond and Moore (1965); 39 Roth and Kirchgessner (1986); 40 Roth and Kirchgessner (1988); 41 Spriet *et al.* (1987); 42. Underdahl *et al* (1983).

11.3.1 Organisms used

Several organisms are used as probiotics for pigs while others are under investigation as potential probiotics (Table 11.5). For a review, see also Tuschy (1986), who gives details about different commercial brands, and Tournut (1989). Lactobacilli are strong acid producers and they are seldom pathogenic (Sharpe *et al.*, 1973; Sharpe 1981). Some strains of *Ent. faecalis* and *Ent. faecium* have been found to be pathogenic (Hardie, 1986; Mundt, 1986), however, streptococci are more tolerant to harsh conditions, and therefore would yield more stable preparations than those containing lactobacilli. *Bifidobacteria* are more oxygen sensitive than most lactobacilli, and some strains are strictly anaerobic. Consequently, their survival in air will be more of a problem than for the other LAB used.

The bacteria can be largely divided into two main types: those which originate from the indigenous digestive microflora and those which do not. Species normally found in the pig indigenous microflora are *L. acidophilus, L. fermentum, L. reuteri, Ent. faecium* and *Ent faecalis*. In

the preservation of food or feed, *L. plantarum*, *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* have been employed as starter cultures.

The tradition to use bifidobacteria has developed in Japan, where three preparations of bifidobacteria, namely *Bif. bifidum*, *Bif. pseudolongum* and *Bif. thermophilum* have been used for animals since 1968 (Kurman, 1983). Other bacterial genera used as probiotics include *Bacillus* and *Clostridium*, for example, *Bacillus licheniformis* and *B. subtilis* and the related *B. toyoi* and *Clostridium butyricum* (Han *et al.*, 1984; Tournut, 1989). *Saccharomyces* sp. and unspecified yeast preparations were used by Kornegay and Thomas (1973) and by Burnett and Neil (1977).

Bacillus spp. are mostly soil organisms. Some species are used for the production of antibiotic substances. It is not known if *Bacillus* spp. constitute a component of the indigenous microflora or whether they are transient in the digestive tract (Savage, 1977). It has been claimed (Ozawa *et al.*, 1981) that the spores would germinate in the anterior part of the digestive tract and compete with enterotoxigenic *E. coli*. They may increase the *Lactobacillus* flora (Roth and Kirchgessner, 1988) or stimulate the immune system against *E. coli* (Pollman, 1986). It is feasible that their action could be mediated by preformed antibiotic substances especially if they are given as spores dried in their propagation medium. It has been proposed that *B. licheniformis* will produce antibiotic substances in the upper digestive tract of mice (Ducluzeau *et al.*, 1978a) and that a mixture of *B. subtilis* and *B. licheniformis* may be effective because of enzymatic activity and/or by suppressing pathogens and production of compounds such as NH_3 and amines (Landsudvalget, 1989).

Another approach is the use of competitive colonization where non-pathogenic strains are given in order to prevent subsequent colonization by pathogenic strains. For example, Davidson and Hirsch (1976) and Duval-Iflah *et al.* (1983) administered non-pathogenic *E. coli* to pigs and Borriello and Barclay (1985) non-toxic strains of *C. difficile* to hamsters. As with vaccinations, this approach will only protect the host from very closely related strains to that administered since its action is solely to compete for the same site. A related approach was that of Nakamura and co-workers (1983) who showed that drug-sensitive *E. coli* strains fed to young animals prevented establishment of resistant strains. Another way to try to improve animal health is that tested by Smith and Huggins (1983) using phages which attach specifically to K-antigens. This method also has the disadvantage of being too specific, however. Fuller (1986) proposed that phage probiotics may have a potential if the phage could attach to receptors to which

the pathogens attach. Alternatively, this phage could attach to the adhesive determinants of the pathogens, e.g, K88, K99 fimbriae (Smith and Huggins, 1983).

11.3.2 Usage

Probiotic preparations can be given soon after birth, at times when the farmer expects diseases (preventive or curative), or mixed into the food for continuous supply. The microorganisms can be injected orally or distributed in the water or in the feed, which can be pelleted or ground. They can be given as viable organisms in wet, frozen or freeze-dried preparations or as pastes (Tournut, 1989), or as fermentation products with or without dead organisms (Pollman *et al.*, 1984).

The form of presentation of the microorganisms as probiotics is largely dependent on convenience for distribution and administration, provided essential characteristics are maintained. For example, if the microbes must be metabolically active for effective action, the preparation must contain viable microbes. Although pelleted food is often preferred, the pelleting process involves high temperatures and high pressures which can be lethal to many microorganisms. Some streptococci and the spore-forming bacteria such as *Bacillus* ssp. are less affected and survive quite well. Lactobacilli are more sensitive, but Lyons (1988) reported a process of microencapsulation which gives better survival ability, with about 40% of the microencapsulated bacteria remaining viable after pelleting. Growth conditions of the microorganism, methods used for harvesting and protection prior to freeze-drying will also influence survival of the bacteria. Storage stability of viable *Lactobacillus* preparations is influenced by the storage environment, length of storage and type of medication in the feed. Storage at refrigeration or ambient temperatures is to be preferred to storage in nurseries at higher temperature (32°C) (Pollman and Bandyk, 1984). *L. acidophilus* cells are quite sensitive to both freeze-drying and air-drying, and the drying medium and the residual moisture content influences their survival. If too much of the bound water is lost, damage to the cell wall and the cytoplasmic membrane appears to occur with increased sensitivity of *L. acidophilus* cells to bile and lysozyme (De Valdez *et al.*, 1985; Brennan *et al.*, 1986). Gilliland and Rich (1990) found that two strains of *L. acidophilus* could be stored at 5°C for 3 weeks if cultured and maintained in skim milk at pH 5. At higher pH the viability of these strains declined for the same storage conditions. Freezing and frozen storage for 4 weeks at −196°C had no significant effect on the viability of the two strains.

(a) Time of administration

The probiotics may be given at different ages of the pig depending on their proposed mechanism of function. If there is reason to believe that the normal indigenous microflora of a healthy pig will not be established, preparations containing solely LAB are probably most desirable because the natural sequential colonization of the digestive tract can be initiated. Such situations might occur when the piglets are moved directly after birth into a scrupulously clean environment, as described by Cranwell *et al.*, (1976), or for example after antibiotic treatment. For normal pig rearing, the piglets stay in close contact with the sow for the first weeks of life and will therefore be colonized by LAB. It should be stated, however, that some LAB strains have a greater capacity than other LAB strains to inhibit the establishment of pathogens, for example, by the production of antagonistic metabolites (see section 11.5.2(a)). Consequently, in farms with a high incidence of diarrhoeal disease, it may be appropriate to introduce a probiotic strain as early as possible and thereby colonize the digestive tract with the probiotic strains that have capacity to inhibit pathogens.

If the reason for using probiotic preparations is to counteract a high level of potentially pathogenic organisms such as *E. coli*, the preparations should be given prior to and during periods of risk for disease unless colonization can be ensured with limited dosages. The question is often raised as to whether single doses or continuous dosage should be used. The characteristics and mechanisms of action of the strains to be used as probiotics will influence this point. For example, if the desired strain functions by producing desirable metabolites in the digestive tract during passage through the system, but lacks the capacity to colonize the tract, then continuous or daily dosage would be required. If the probiotic strain has characteristics which facilitate colonization of the tract, e.g. adhesins, then single dosages may suffice until such time as the pig is exposed to some form of stress or treatment that could result in loss of the probiotic strain.

(b) Dosage levels

The numbers of organisms given as therapeutic doses are often 10^9–10^{12} per animal per day or 10^6–10^7 g^{-1} feed. These levels are mostly arbitrary as no dosage studies for different strains have been reported. The numbers given should be sufficient to elicit a beneficial response in the host and therefore be given in significant numbers in relation to the indigenous flora or achieve such numbers by growth within the digestive tract. Conversely, levels should not be so high that they induce digestive problems. It has been reported that overdosing humans with LAB could have a laxative effect (Gordon *et al.*, 1957). These workers

report cases of diarrhoea in patients dosed with milk fermented with *L. acidophilus* such that individuals received 10^9 cells per day. For pigs, a dose of 10^9 per day corresponds to the LAB already present in only 10–100 g of digesta. This is similar to the numbers of lactobacilli found in the stomach of sucking piglets. A comparable dose in weaned pigs would have to be $10^{9.7}$–$10^{10.5}$. To our knowledge, no reports have shown that too high doses of LAB given to pigs would bring about diarrhoea. Conway and co-workers (manuscript) observed no detrimental effects of dosing 4 weeks old piglets with 10^9–10^{10} *L. murinus* cells twice daily for 10 days as compared to treatment for only 1 day.

When discussing dosage levels required to achieve a certain level of bacteria in the anterior small intestine, one has to take into account killing in the stomach and dilution with digestive secretions to 3–6 times the volume. Studies by Jonsson and co-workers (1985) in pigs with cannulas in the anterior and posterior part of the small intestine show that the numbers of lactobacilli per gram of digesta are between 4 and 15 times higher in the ileum compared to the numbers found in the anterior part of the small intestine. For the same sites, a *Lactobacillus* strain with good survival ability fed once to these pigs increased in numbers 3–5 times. Passage time between these sites in the intestine is only a few hours, hence multiplication of the organisms would not be extensive. The origin of *Lactobacillus* strains and their bile tolerance were reported to affect survival in the digestive tract of pigs (Jonsson *et al.*, 1985). Consequently the capacity of the potential probiotic strain to survive *in vivo* ought to be tested for individual strains. This aspect is further discussed in section 11.5.

The issue of whether administered probiotic microorganisms will be transient or whether they will colonize the digestive tract also influences the dosage required. Transient strains will probably need to be administered in higher levels than those strains that will multiply within the tract if similar numbers are required to achieve the desired function. For bacteria to colonize, the strains will probably need to associate with the epithelial mucosa (discussed in Conway, 1989). Required dosage levels of strains with colonizing potential have not been reported to date. As cited previously, the *pars oesophagea* of the healthy stomach is colonized by indigenous lactic acid bacteria. Approximately 10^8 bacteria cm^{-2} would be needed to cover the mucosal surface and thus one could anticipate that the entire area would thus have about 10^8 LAB in the neonatal pig and about 10^9 LAB in the 25 kg pig. If a LAB strain could multiply and colonize within the stomach it would require administration levels considerably less than 10^8 and 10^9 per neonatal pig and 25 kg pig, respectively.

If the small intestine of a newborn pig is viewed as a straight tube, it should take *c.* 10^{11} LAB to cover the surface area totally. In the 25

kg pig corresponding values would be up to 100 times larger (10^{13}). Although these values are much higher than for the stomach, dosage levels may not need to be very great for colonization to occur because those released from the stomach due to multiplication at this site could serve as an inoculum for the intestine. While such calculations yield bacterial numbers consistent with the *in vivo* reports for the *pars oesophagea* region, it is more difficult to confirm values for the small intestine. Fossum and Liven (1974) pointed to the fact that the small intestine contained parts which were almost sterile with little contents other than intestinal mucus and other dilated regions which were full of digesta, bacteria and enzymatic activity. However, the mucosa was not studied, and hence no conclusions can be drawn regarding LAB colonizing the epithelium.

11.4. EFFICACY

Use of probiotics often gives inconclusive or conflicting results (reviewed by Jonsson 1985; Tuschy 1986; Conway 1989), and there is no direct evidence to explain these differences. Strains used as probiotics can vary in their characteristics and thus their actions; for example, not all *L. acidophilus* strains have exactly the same characteristics. However, one can hypothesize that the host susceptibility may vary from one study to the next. Altered *in vivo* conditions could also result in altered activities of the probiotic cells. The viability or survival potential of the probiotic cells would also be important facts.

If the host's body is already functioning optimally, animals will most likely be growing at their maximum capacity and therefore it may not be possible to achieve any improvement by administering beneficial bacteria. It is interesting to note, however, that in SPF pigs kept under strictly hygienic conditions, advantages in health were seen by administering of antibiotics (Jucker *et al.*, 1973). This may mean that non-pathogenic microbes may exert some stress on the animal. A lot of research and trials using probiotics are being done, both by commercial companies, experimental stations and universities. As there are strong commercial interests, some of the work in this area is not accessible. In addition, the practice of not publishing inconclusive or negative results contributes to the difficulty of obtaining the overall picture.

Evaluations of the use of probiotics *in vivo* include studies of

- influence on the digestive microflora, including pathogenic bacteria
- influence on the digestive tract, its function and morphology
- performance and health of animals

- various effects where animals are used as models for humans, for example, anticholesterolaemic properties, stimulation of the immune system and anticarcinogenic activity.

For further details see review of the nutritional and therapeutic role of dietary LAB (Fernandes *et al.*, 1987, Bourlioux and Pochart, 1988)

11.4.1 Influence on the digestive microflora

To exert any beneficial effects, the bacteria or possibly the active substances have to reach the site of function. In order to do so, first of all they have to be consumed. This means that the preparations have to be appetizing or at least not repulsive to the pig. Pigs are sensitive to the long-term effects as well as the flavour and taste of their feed (Houpt *et al.*, 1979). This fact is also stressed by Premi and Bottazzi (1974). The next important step is that the preparation should contain viable and active organisms or substances in sufficiently large amounts. In the following discussion, emphasis will be put on viable organisms.

One of the reasons for using lactic acid bacteria as probiotics is that they are said to stabilize the digestive microflora and compete with pathogenic microbes. These statements probably arise because LAB are strong acid producers; they contribute to the lowered pH and decrease in numbers of bacteria entering the small intestine in neonatal pigs (Cranwell *et al.*, 1976, Barrow *et at.*, 1977). In fact, half of the lactose in sow's milk is metabolized to yield organic acids in the stomach (Cranwell *et al.*, 1976). Once the complex indigenous microflora in the digestive tract develops, the system is relatively stable. Addition of further microbes to the stable indigenous microflora should not give any change in numbers if the other conditions remain the same (Hungate, 1984). However, although the microflora is a stable system, it is also dynamic. Strains will replace each other and the metabolism will adapt itself to the substrates available. In his discussion about the large intestine, Freter (1983) states that competition for limiting nutrients is one of the determining factors that has received the most scientific support. If this is the case, the addition of small numbers of bacteria or their metabolites could have substantial influence on the microflora. Of course, the composition of the diet would also be of major importance.

The composition of the microflora in the digestive tract, except for faeces, is difficult to study in the living animal. Unfortunately, the faecal flora cannot be used as an indicator of microbial populations in the anterior digestive tract as these populations vary enormously (Pollman *et al.*, 1980b).

Several reports of administering different *Lactobacillus* strains to neonatal or weaned piglets state that the numbers of *E. coli* and

lactobacilli are influenced. When feeding *L. acidophilus* to piglets, Pollman and co-workers (1980b) found no influence on the succession of the microflora in the digestive tract, except an increase in the numbers of lactobacilli and coliform bacteria in the non-secreting part of the stomach (cardia region). Higher ratios of lactobacilli to coliforms in the colon microflora were obtained after administration of *L. murinus*, but not *L. fermentum*, to piglets up to 4 weeks old (Conway *et al.*, manuscript) and in faeces of 3 weeks old piglets fed *L. acidophilus* (Premi and Bottazzi, 1974). Olsson (1966) showed decreased levels of *E. coli* and haemolytic *E. coli* in the faeces of weaned pigs dosed with *L. acidophilus*. A lactobacillus fermentation product fed to artificially raised piglets challenged with *E. coli*, suppressed the counts of *E. coli* but not the lactobacilli in the stomach (Pollman *et al.*, 1984). Ratcliffe and co-workers (1986) found that both yoghurt and milk fermented with *L. reuteri* given to 2-day-old piglets increased the numbers of lactobacilli and decreased numbers of *E. coli* throughout the gut. When milk was acidified by lactic acid to the same pH both numbers of coliform bacteria and lactobacilli decreased. *L. delbrueckii* subsp. *bulgaricus* could be detected in the stomach and duodenum but not in the colon.

For streptococci, *Ent. faecium* Cernelle 68 fed to piglets in herds, with or without *E. coli* enterotoxaemia, decreased the faecal excretion of coliforms and haemolytic *E. coli*. However, no prevention of disease occurred (Deprez *et al.*, 1989). Using a preparation containing *Ent. faecium*, Danek (1986) influenced the faecal microflora by decreasing numbers of coliform bacteria, and increasing lactic fermentation and streptococci counts. Ozawa *et al.* (1983) fed *Ent. faecalis* to weaning pigs and reported that the microflora (bifidobacteria, streptococci and lactobacilli) was stabilized and that *Ent. faecalis* was antagonistic to *Salmonella* and yeasts.

Kimura *et al.* (1983) observed that animals with diarrhoea had disturbed microflora with decreased numbers of bifidobacteria and lactobacilli and increased numbers of enterobacteria. Oral administration of *Bif. thermophilum* and *Bif. pseudolongum* reinforced the normal intestinal flora and alleviated clinical symptoms in scouring animals and had a prophylactic effect on diarrhoea in suckling piglets.

Bacillus spp. given to slaughter pigs did not influence the intestinal microflora (Spriet *et al.*, 1987). However, Ozawa *et al.* (1981) showed significantly increased numbers of streptococci and bifidobacteria in the proximal small intestine and decreased levels and detection frequencies of *Bacteroides*, by administration of *B. subtilis* strain BN.

A marked change in microbial flora after 3 weeks of administration of *C. butyricum* ID was observed with increased numbers of *C. butyricum* and lactobacilli and reduced counts of staphylococci and coliforms (Han *et al.*, 1984).

To summarize, it seems that administering some probiotics might, in many instances, influence the microbial flora in the digestive tract, especially when the indigenous microflora has been disturbed. This so-called balancing of the flora is difficult to analyse and may not always be clearly connected with improvements in health or performance. Should this stabilizing effect not occur, it is possible that detrimental effects on health and performance could well be demonstrable.

11.4.2 Influence on the digestive tract, its function and morphology

(a) Digestion

Møllgaard (1946, 1947) observed improved skeleton formation in pigs given skim milk cultures of intestinal lactobacilli. He proposed that this was due to improved absorption of calcium by increased lactate production and lower pH in the small intestine. Administration of *Bacillus* spp to cannulated pigs did not affect the pH, the concentration of bacterial metabolites (lactic acid, ammonia, volatile fatty acids) or the apparent digestibility of protein or organic matter (Spriet *et al.*, 1987). Hale and Newton (1979) fed a *Lactobacillus* fermentation product to weaned piglets. Although scouring was reduced, the average daily gain was not significantly improved and the only improvement in digestibility was noted for crude fibre. The authors proposed that this was may be attributable to a decreased transit time.

(b) Morphology

Germ-free animals are frequently used to study the effect of microorganisms on the host. The presence of a microflora causes many changes in the physiology and morphology of the animal. The digestive tract in germ-free animals has more slender and finger-like villi, thinner small intestines with reduced *lamina propria*, longer cell renewal times and higher activities of digestive enzymes (for review see Coates, 1973). Savage *et al.* (1981) found that monoassociation of germ-free mice with a *Lactobacillus* strain did not affect the renewal time of cells in the small intestine. The authors, however, expressed a caution that more microbial species may be needed to cause the changes seen in conventional animals. It has recently been shown using germ-free rodents that the presence of LAB does not stimulate any of the host characteristics referred to by these workers as microflora-associated characteristics (Norin *et al.*, 1991).

Pollman *et al.* (1984) found that artificially reared pigs fed a *Lactobacillus* fermentation product and challenged with *E. coli* had no difference in morphology of the small intestine. By contrast, notable

differences in the structure of the small intestine in conventional piglets were shown by Jonsson and Henningsson (1991) using two different herds. In this study, the villi of the control piglets were more or less damaged whereas the pigs receiving *L. reuteri* had more normal villi about 1 week after weaning. Adding *L. acidophilus* to the feed of pigs to be slaughtered was suggested by Premi and Bottazzi (1974) to yield intestines of a better quality for use in the sausage industry.

11.4.3 Performance

A major aim with using probiotics is to improve the performance and health of the animals. Growth rates, feed utilization, number of deaths and days with diarrhoea, sometimes irrespective of cause, are most commonly measured. These studies do not, however, yield information about the mode of action. Depending on the experimental design and the types of controls, it can be difficult to specify with certainty which factors have contributed to the reported changes. It should not be overlooked that the probiotic preparations may have a nutritive effect in addition to other reported functions. It is very laborious to carry out studies in such a way that there should be as little doubt as possible over the results. Many factors influence the performance and health of the animals. In order to conduct experiments in such a way that the addition of the probiotics is the only factor being varied between the groups requires control over a multitude of factors, for example, the environment, handling of the animals, genetic background of the animals, different stress factors and chances for cross-contamination. Variations in these factors could lead to inconclusive or contradictory results. It may be difficult to judge from published results if controlled and comparable conditions were used unless specifically stated. To reduce interference of the factors which are difficult to control and to obtain statistically meaningful results a large number of animals need to be tested. It should also be acknowledged that results can seem more favourable than in reality by incorrect usage or understanding of the statistical analyses. Ideally, a standardized method in which conditions could be exactly specified is clearly needed for probiotic testing. However, because of the different types of microbes and preparations available as probiotics, one standardized method may be too general to be really valuable.

It has not been possible to obtain the experimental details from all reports of probiotic testing, hence the experimental design cannot be extensively judged. Many reports deal with young piglets and it seems that streptococci have the best effect on piglets exposed to suboptimal conditions. For growing pigs, the reports are fewer and the effects are more variable.

(a) Lactobacilli

L. acidophilus has also been reported in many trials to improve performance and health. Sucking piglets with chronic diarrhoea problems showed improvements after administration of *L. acidophilus* strains (Redmond and Moore, 1965; Jensen, 1974; Premi and Bottazzi, 1974). Similarly, Olsson (1966) improved the health of weaning pigs with an *L. acidophilus* strain. However, Kornegay (1985/86) did not find any improvement in growth rate of nursing pigs. This points to the fact that there might be situations when the pig is sensitive to digestive tract disturbances and therefore benefits from enrichment by lactobacilli.

In some trials, starter pigs given *L. acidophilus* have shown improved average daily gain and feed conversion; however, growing-finishing pigs have not been influenced (Kornegay and Thomas, 1973; Pollman *et al.*, 1980a, b, c). Dietary carbohydrates were found to be of importance and the addition of lactose improved the observed beneficial effects. In other trials, no consistent beneficial effects were found (Ingram *et al.*, 1973). It should be emphasized that not all *L. acidophilus* strains are identical and hence strain variation will give rise to conflicting results. A significantly improved weight gain and decreased incidence of diarrhoea was noted for piglets receiving an *L. fermentum* strain (Conway *et al.*, manuscript). Ratcliffe *et al.* (1986) used the closely related *L. reuteri* and found significantly decreased weight gain for very young piglets and tendencies to lower feed conversion.

(b) Streptococci

Almost all reported studies using streptoccocci have utilized various strains of *Ent. faecium*. The greater part of the experiments reported for *Ent. faecium* M 74 show positive effects on health and growth of neonatal piglets (Moen, 1982; Gualtieri and Betti, 1984; Gudding and Larssen, 1985; Danek, 1986; Roth and Kirchgessner, 1986 and Maeng *et al.*, 1989). In contrast, Kluber *et al.* (1985) failed to show these effects and in fact reported higher mortality figures for the groups receiving the strain M 74. For *Ent. faecium* C68 the effects depended on the conditions under which the animals were reared (Jørgensen, 1988). Under suboptimal conditions, but not for pigs raised under good hygienic conditions, improvements of performance and health were shown. Increased weight gain, feed consumption and feed conversion as well as reduced scouring were found by Maeng *et al.* (1989) when dosing piglets up to 4 months with *Ent. faecium* C68. Decreased incidence of diarrhoea was found by Krarup (1987) for neonatal piglets dosed with this strain. Pollman *et al.* (1980a) showed an additive effect of the strain C68 and an antibiotic for piglets with an increased average daily weight gain and feed conversion while for older growing and

fattening pigs the effects of the probiotic were not significant. For starter pigs, the C68 strain was ineffective in promoting enhanced performance (Kornegay and Thomas, 1973; Pollman *et al.*, 1980a), whereas Neupert (1988) showed better feed conversion and shorter fattening times and a tendency towards more lean meat for growing pigs by administration of the C68 strain.

(c) Bacillus spp.

The use of *Bacillus* spp. as probiotics is more recent. Jørgensen (1988) showed that sows treated with *B. toyoi* had lower frequencies and milder symptoms of the mastitis-metritis-agalactiae syndrome. For piglets, Roth and Kirchgessner (1988) and Ogle and Inborr (1987) reported tendencies to increased daily gain, feed consumption and feed conversion ratio as well as fewer deaths and fewer treatments with antibiotics when administering *B. toyoi*. Pollman (1986) summarized some studies in which *B. subtilis* was given to pigs. When sows were dosed with *B. subtilis* before farrowing, the piglet survival rate was improved. When the sows had previously received an *E. coli* vaccine there was no effect on performance of either the sow or the piglet. For starter pigs no consistent benefit from *B. subtilis* addition to diets containing carbadox was noted when no major post-weaning diarrhoea was evident. He concluded that administration of *Bacillus* spp. to pigs would be most promising for sows which have not received an *E. coli* vaccine, and that this treatment had little effect on starter pigs.

(d) Various organisms

A mixture of *L. plantarum, L. casei, L. brevis. L. fermentum* and *Pediococcus acidilactici* (Landsudvalget, 1988) was tested in herds with problems with weaning diarrhoea. A non-significant effect towards decreased diarrhoea was seen. However, the numbers of viable bacteria in the preparation was only 0.1% of the desired value. One can speculate that the effect could have been more pronounced if the mixture had contained higher counts. Ogle and Inborr (1987) found significant effects on daily gain when including LAB in the feed of starter pigs.

When yoghurt was administered to 2–12 days old piglets Ratcliffe *et al.* (1986) found decreased feed to weight gain ratio. Using a human *Bif. bifidum* strain Ervolder *et al.* (1984) reported less incidence of diseases in suckling and weaned piglets after dosage. The dosage of *C. butyricum* ID to growing pigs showed a tendency towards better daily weight gain and greater feed consumption as well as significantly improved feed conversion. By varying the dosage, the digestibility of the diet and nitrogen retention rate could be slightly increased (Han *et al.*, 1984). Yeast preparations have also been used but neither Kornegay and

Thomas (1973) nor Burnett and Neil (1977) detected any improvement in performance.

(e) Fermentation products

Fermentation products of lactic acid bacteria (LFP) have been administered separately or in combination with freeze-dried cultures. The organisms involved are not always stated. Generally these investigation show some positive results for weaned pigs, but it must be emphasized that the success of LFP is dependent on the bacterial strain(s) being cultured. Cowman *et al.* (1978) reported briefly that in an extensive investigation they obtained positive responses to different levels of LFP on average daily gain and feed consumption in post-weaned pigs. In contrast, Hale and Newton (1979) found reduced scouring but no significant difference in average daily gain. Pollman and his group (1984) used artificially raised piglets which were housed individually. These authors found increased weight gain and decreased numbers of *E. coli*. They related the weight gain to the reduction of *E. coli* in the stomach without giving any plausible explanation as to how this connection could occur. Lessard and Brisson (1987) used a LFP made from *L.*$^{\text{delbrueckü}}$ *bulgaricus*, *L. casei* and *S. salivarius* subsp. *thermophilus* and demonstrated stimulated growth and feed intake. When feeding a fermentation product together with the mixed LAB culture, Ogle and Inborr (1987) showed a tendency to increased daily gain up to 9 weeks of age.

In summary, it seems as if there might be situations where the total microflora of the piglets is not optimal and the animals therefore benefit from the use of probiotics. Many of the reports deal with young piglets and it seems generally as if the most positive effects are reported for the young animals held under suboptimal conditions. Lactobacilli seem to be the most efficient in influencing the microflora in the digestive tract while streptococci seem to have the best effect on piglet performance. For pigs after weaning the reports are fewer and the effects are more variable.

11.4.4 Immunology

Kluber *et al.* (1985) administered Ent. *faecium* M74 to artificially reared pigs. The *in vivo* cell-mediated immunity was not affected. It is noteworthy that the mortality was greater for pigs treated with the LAB. These pigs also had an increase in mature and immature neutrophils with suppressed lymphocyte counts. Pollman and his group (1980b) report stable levels of *L. acidophilus* 9 days after dosage to gnotobiotic

pigs. The animals had elevated counts of white blood cells but similar levels of serum metabolites as compared with gnotobiotic piglets, which however were shown also to be colonized by some other lactobacilli. In a later investigation, this group found an increased white blood cell count in artificially raised piglets challenged with *E. coli* after dosage with LAB metabolites (Pollman *et al.*, 1984). Lessard and Brisson (1987) fed metabolites from dairy LAB (see above) to weaned pigs and found slightly increased serum levels of IgG.

11.5. FUNCTIONAL CHARACTERISTICS OF POTENTIAL PROBIOTIC STRAINS

Lists of criteria to be fulfilled for probiotic organisms have been compiled and new items have been added as the knowledge about the interrelationships between the host and the microbes increased (see, for example, Tannock, 1984). Procedures for selection of new strains are treated elsewhere in this book and hence are only discussed briefly here in relation to the experiences obtained in isolating and selecting *Lactobacillus* strains from the indigenous microflora of pigs (cf. also Jonsson, 1985). Factors to consider when developing dietary adjuncts containing LAB and bifidobacteria have also been described by Klaenhammer (1982), Kurman (1983) and Gilliland and Walker (1990).

11.5.1 Isolation techniques

The common practice of using standard selective media (e.g. Rogosa and MRS agar) for isolating LABs from the indigenous gut microflora yields the most dominant species. Consequently, to reduce the isolates needing screening as described below, care must be taken in the selection of the source of the inoculum. In addition, the target for the intended use of the probiotic strain must be clearly defined. For example, if post-weaning bacterial-induced diarrhoea is the target, it would be reasonable to select the digestive tract microflora of an extremely healthy post-weaning pig as the inoculum source. While it is feasible to propose that the isolation medium could be specially designed to enrich for LABs with specific characteristics, in practice this could be difficult to implement. Most of the reported desirable characteristics of LABs for porcine use cannot be selected for by compositional changes of the medium, e.g. capacity to adhere to epithelial mucosa or to produce metabolites inhibitory to pathogen

colonization. It can be noted, however, that Jonsson and co-workers (unpublished data) prepared media from mucosal homogenates from various regions of the gut and showed a tendency for these media to yield a greater number of isolates which were adhesive to the mucosal region used in the medium on which they had been isolated.

It is often specified that anaerobic conditions are required for culturing isolates from the digestive tract. The redox-potential of the digestive tract of the piglet immediately after birth is high, *c.*+400 mV in the stomach, +150 to −150 mV in the small intestine and +100 to −200 mV in the large intestine. After 5 days the Eh decreases to +50 mV, 0 to −250 mV and −150 to −250 for the respective sites. There is no great change in Eh during weaning, despite the changed feeding and rearing systems. For growing pigs, the Eh found was −100 to −250 mV, with the highest values found in the stomach and anterior small intestine (Schulze and Jacob, 1981). Consistent with other reports, the authors observed that these values were reflected in the values obtained for the total counts of bacteria. Lactobacilli grow best *in vitro* in microaerophilic and anaerobic conditions. Although lacking a cytochrome system and therefore not able to utilize oxygen for aerobic growth, lactobacilli can grow in the presence of oxygen using fermentative metabolism. This taxonomic description may not be entirely correct for lactobacilli of digestive origin because *L. acidophilus* Po13 isolated from the *pars oesophagea* area of a pig only grows in anaerobic conditions (Jonsson *et al.*, 1985).

Lactobacillus strains can often have distinct colony morphology and Klaenhammer and Kleeman (1981) raised the issue that colony morphology was important because isolates producing smooth colonies were more resistant to bile acid and freeze damage than isolates producing rough colonies. In contrast, recent studies have shown that the rough variant of an *L. fermentum* strain of porcine origin was generally more resistant to bile acids and low pH than a smooth colony variant of the same strain (Szewzyk *et al.*, manuscript).

To date, the *Lactobacillus* flora from the digestive tract has been poorly studied taxonomically and many new species from this habitat are now being described (reviewed by Tannock, 1990). The morphology and physiology of the isolated strains might be different to what has previously been considered normal for lactobacilli. The morphology of these wild types may sometimes cause some confusion as coccoid rods are often found and the morphology also might change as the strain is propagated, (see Kandler and Weiss, 1986). On isolation, such strains may also have an increased sensitivity to oxygen.

11.5.2 Evaluation of strains

Probiotic preparations have been said to have several beneficial roles in pigs. Although their modes of action are not known, it is assumed that strains intended to be used as probiotics should be isolated from the indigenous microflora and have characteristics such as colonization capacity, and capacity to inhibit the activity as well as colonization ability of pathogenic microbes. Initially, such screening relies on *in vitro* test systems, the result of which can be confirmed *in vivo*. Subsequently, extensive field testing is needed to confirm that the strain(s) do in fact have a beneficial influence on performance and health of the pig.

The success of a probiotic preparation will depend not only on its biological effect under controlled experimental conditions but also relies on its dependability when produced on a larger scale. It has to be possible to propagate the strain or strains on a commercial scale such that essential strain characteristics are maintained without excessive production costs. The commercial preparation should also withstand storage under conditions likely to be encountered in the distribution and farm situations without great loss of viability or strain characteristics, (see Pollman, 1986).

To exert beneficial effects, the bacteria or possibly the active substances have to reach the site of function. In order to do so, first of all they have to be consumed. This means that the preparations have to be appetizing or at least not repulsive to the pig. Pigs are sensitive to the long-term effects as well as the flavour and taste of their feed (Houpt *et al.*, 1979). This fact is also stressed by Premi and Bottazzi (1974). The next important step is that the preparation should contain viable and active organisms or substances in sufficiently large amounts.

(a) In vitro *versus* in vivo *tests*

Ideally, strain selection should be performed under *in vivo* conditions but logistics require the development of *in vitro* assays. Consequently, the probiotic characteristics which mediate the beneficial effects on health and performance of the pig need to be defined such that specific parameters can be measured *in vitro*, e.g. colonization capacity is measured as the *in vitro* adhesive capacity. Designing *in vitro* and *in vivo* studies to evaluate the potential of a probiotic strain raises more questions: for example, should conventional or gnotobiotic pigs be used? These *in vitro* and *in vivo* issues are discussed below in terms of survival, colonization and inhibition of pathogen colonization.

Many *in vitro* tests for studying strain characteristics of probiotic

organisms have been reported, however, not all studies have included comparisons of the results with results obtained under *in vivo* conditions. As discussed below for the various characteristics, this comparison of the *in vivo* and *in vitro* results is feasible for factors such as survival and colonization of the strain(s), because these can be measured directly *in vivo*. Other factors such as the production of antagonistic substances can only be measured indirectly unless the active substances are characterized and probes for the component are produced. Unfortunately, measuring survival and colonization *in vivo* is also limited because of difficulties in detecting administered strains in the complex indigenous microflora, especially if the strain originates from the digestive tract. This aspect is discussed in detail in section (b) below on gnotobiotic and conventional animals.

Survival

Survival of administered bacteria in the anterior digestive tract is mainly discussed and studied *in vitro* in terms of bile acid and low pH tolerance. The effects of proteolytic enzymes in the stomach and small intestine on survival of bacteria have been seldom investigated but it cannot be excluded that these enzymes also may have some influence on the survival of probiotic strains. Lactobacilli of pig stomach origin have been shown to survive passage of the pig stomach and small intestine (Jonsson *et al.*, 1985). In addition, these workers showed that strains isolated from the *pars oesophagea* area varied in their bile tolerance *in vitro* and a similar variation could be observed *in vivo* in the small intestinal contents of cannulated pigs. Even a *L. delbrueckii* subsp. *bulgaricus* strain from yoghurt was found to survive in the small intestine of pigs after ingestion (Ratcliffe *et al.*, 1986). Using lactobacilli of human origin and low pH buffer or human gastric juice, survival in gastric juice *in vitro* and *in vivo* were almost comparable, with *in vivo* studies showing slightly better survival than those using low pH buffer (Conway *et al.*, 1987).

In an attempt to develop an *in vitro* survival assay which simulated *in vivo* stomach conditions, sterile pig feed was slowly mixed with artificial saliva, bacterial culture and acid mixture and incubated anaerobically at 40°C for 24 h (Jonsson, unpublished). The results obtained with this test did not correspond very well to what was found *in situ* in the pig. One explanation could be that the influence of the gastric LAB microflora was missing; this is very relevant for the conditions in the pig. In other experiments where the pH of MRS-broth was decreased stepwise by 0.5 units by using HCl to pH 2.5, the results of bacterial growth showed better correspondence with the *in vivo*

survival through the stomach. The strains with the best *in vivo* survival grew well at pH 3.5.

Colonization

It has been proposed that LAB as probiotics could colonize the piglet digestive tract and thereby continually exert their influence. For example, LAB may protect the host from infections by colonizing sites and thereby exclude invading pathogens. This latter concept is often referred to as competitive exclusion and has been extensively studied for complex probiotic type preparations for chickens since the first experiments reported by Nurmi and Rantala (1973). This was also studied in piglets for example by Corpet and Nicolas (1979). By colonization, one means that the LAB can remain and multiply within the digestive tract and are not eliminated from the system. This could be achieved either by attachment to epithelia and/or growth in the digesta. In the small intestine with its rapid flow of digesta, attachment to the epithelium is most likely a prerequisite for colonization, whereas in the stomach and large intestine the flow rate is lower and the LAB may survive solely by multiplication within the lumenal contents. In the stomach the squamous cells sloughed off from the *pars oesophagea* area with their attached LAB will also continuously inoculate the ingesta. The fact that the stomach seldom empties completely between meals and that the conditions will not become too acid also facilitates colonization in this site.

Colonization can be confirmed *in vivo* by detecting the strain in the tract beyond the time accepted as the lowest possible for transit of particles through the tract. Generally, detection of a strain for more than 7 days after dosage would be strong evidence of colonization. The detection can be made in faecal samples, although to obtain information as to what habitats are colonized within the digestive tract, samples from the various regions of the gut must be analysed for presence of the strain.

The potential for *in vivo* colonization is presently assessed *in vitro* by studying the capacity of the strains to attach to the epithelia of the digestive tract. When discussing adhesion to epithelial mucosa, it is important to distinguish between the various epithelial surfaces to which bacteria can attach. The *pars oesophagea* region consists of keratinized squamous non-secreting cells while the remainder of the epithelia in the digestive tract consists of columnar cells which are covered by a mucus layer. Barrow *et al.* (1980) found considerable differences in the capacity of LAB to adhere to squamous cells and also reported that strains with such ability did not associate with columnar cells. Subsequently it has been shown that stomach and colon are

colonized by different *Lactobacillus* strains (Tannock *et al.*, 1990). It is most probable that different adhesion mechanisms mediate attachment to squamous and columnar secreting cells. Another site for colonization may be the mucus layer overlying the epithelia. This has recently been shown *in vitro* for pathogenic *E. coli* on piglet ileal mucosa (Conway *et al.*, 1990) and it is reasonable to propose that LAB may also colonize this niche.

Bacteria can adhere specifically or non-specifically to surfaces as defined by Rutter *et al.* (1984). The former mechanism involves adhesin–receptor interactions while the latter is mediated by physico-chemical factors such as, hydrogen bonding, surface charge and the degree of surface hydration. Specific adhesion occurs when an adhesin on the bacterial cell binds to a receptor on the epithelial cell, in what is often defined as a lock and key function. To date, no adhesin–receptor complexes have been characterized for adhesion of lactobacilli to porcine epithelia. It has been proposed that adhesion of lactobacilli to squamous cells was mediated by an extracellular polysaccharide (Barrow *et al.*, 1980). More recently, proteinaceous components have been proposed (Henriksson *et al.*, 1991) as demonstrated for adhesion of lactobacilli to rodent squamous cells (Conway and Kjelleberg, 1989). Subsequent studies suggest that the mechanism of adhesion may involve other components as well (Henriksson and Conway, manuscript. unpublished). Wadström *et al.* (1987) studied the adhesion of lactobacilli to cells from pig small intestinal epithelium *in vitro* and showed a correlation between hydrophobic bacterial cell surface and adhesion.

Non-specific adhesion, which is demonstrable *in vitro* in adhesion assays, may not have any significance in the colonization of epithelia *in vivo* but may possibly be important in the colonization of luminal contents. For example, non-specific adhesion may enhance substrate uptake and thus utilization for growth. Defined adhesin–receptor interactions have been extensively studied for pathogens and evidence is now appearing that some of these interactions may have no *in vivo* significance either. For example, the P-fimbriae on uropathogenic *E. coli* bind specifically to a gal-gal receptor but from recent studies it is apparent that this interaction is not important for *in vivo* colonization (C.Svanborg-Edén, pers. comm.). In this connection, it might also be emphasized that competitive colonization does not imply that the lactobacilli and the pathogenic bacteria will bind to the same receptors on the epithelial cells, but rather that the lactobacilli may sterically hinder the adhesion of the pathogens as demonstrated *in vitro* (Conway, 1985). It should also be pointed out that *in vivo* colonization of a site involves more aspects than simply adhesion to the epithelium. LAB growing at the site of colonization may utilize nutrients otherwise

available for the pathogens. Furthermore, the growth of LAB may also result in production of metabolites inhibitory to the pathogens.

When testing the adhesion capacity of LAB *in vitro*, epithelial cell preparations or mucosal pieces washed free of the mucus layer have most often been used. After the bacteria and the epithelial cells are incubated together, adhesion is sometimes assessed by direct microscopy. This technique of not washing the epithelial cells prior to microscopy means that loosely bound bacteria with low affinity for the epithelial cells are not removed, thus some weakly adhesive strains may be considered adhesive. A standardized washing procedure is required after the incubation to remove loosely bound bacteria.

To check if the adhesion is specific or non-specific it can be tested if the bacteria have a higher affinity for epithelial cells than control surfaces such as protein-coated plastic as reported for *L. fermentum* adhesion to squamous cells (Henriksson *et al.*, 1991). If the affinity for the control surface is comparable or higher compared to the epithelial cells, non-specific adhesion might be involved. Unfortunately, these types of controls are often not reported.

Many *in vitro* investigations have been done to test the adhesive capacity of LAB and to show that this adhesion is host species-specific, thus confirming the rationale for selecting probiotic strains from the piglet microflora rather than from another animal host. These tests have not been strictly discriminating for species-specificity as some LAB strains from other host species can attach to piglet cells (Barrow *et al.*, 1980; Conway *et al.* 1987). In the latter investigation even some yoghurt strains adhered to human and pig ileal cells. Species-specificity of LAB in pigs was also demonstrated *in vivo* in gnotobiotic pigs by Tannock *et al.* (1982). Pedersen and Tannock (1989) tested eight strains of lactobacilli isolated from the digestive tract of pigs for their ability to adhere *in vitro* to cells collected from the stratified squamous epithelium of newborn piglets. *In vivo* colonization capacity of these strains was studied in piglets dosed with the individual strains. The results showed that the *in vitro* tests did not predict whether a *Lactobacillus* strain would associate with stratified epithelium *in vivo*. None of these strains was dominant in the digestive tract 7 days after the inoculation of the piglets with a single dose of the bacteria confirming the results obtained by Jonsson (1986). A heterofermentative *Lactobacillus* strain could be found by Pedersen and Tannock (1989) during the first 7 days in piglet faeces but not after 19 days. No permanent establishment on the *pars oesophagea* region was noted in spite of the *in vitro* adhesive properties of the strain to the *pars oesophagea* cells. So far, only on one occasion has long-term establishment been reported. The *L. murinus* used in this study was of porcine gastric origin and was extremely adhesive in an *in vitro*

adhesion assay (Henriksson *et al.*, 1991). Piglets were dosed at either 2 days, 2 weeks or 4 weeks of age and harboured the strain continually for the duration of the 9-week study (Conway *et al.*, manuscript).

In summary, inconsistencies between *in vitro* adhesion and *in vivo* colonization can depend on factors such as the survival capacity and *in vivo* growth potential. In addition, it is also quite plausible that the design and interpretation of results of the *in vitro* adhesion assay should be closely examined. It is difficult to extrapolate from *in vitro* adhesion results stating how many bacteria bind per epithelial cell, because one cannot predict the threshold value below which it can be stated that the strain under investigation is not adhesive or rather will not be adhesive *in vivo*. It should also be noted that *in vitro* adhesive capacity *per se* does not necessarily confirm that the strain is equipped with colonization capacity. *In vitro* adhesive capacity can be combined with other parameters, such as survival capacity and growth in the gastrointestinal milieu, and the total picture of these *in vitro* analyses may yield a prediction of the colonization capacity which correlates better with the *in vivo* results.

Microbial interactions

Interations between the probiotic strain and other microbes can be discussed in terms of effects of the probiotic microbe on the indigenous microflora and its effects on potentially pathogenic microorganisms.

In vitro, microbial interactions are largely measured as effects on pathogenic bacteria by low and high molecular weight metabolites of the LAB (reviewed by Fernandes *et al.*, 1987; Lindgren and Dobrogosz, 1990). The effects on the indigenous microflora can also be assessed by monitoring changes in the metabolism and profile of indigenous population. This aspect has been studied *in vitro* by Aimutis *et al.* (1985). They isolated bacteria from the piglet small intestine and propagated them in different selective media and found that apart from the selective effect of the media the bacteria growing in the different substrates also showed antagonistic effects for *E. coli*. For example, *Bifidobacterium*, *Clostridium*, *Lactobacillus* and *Leptotrichia* spp. serially transferred in sow colostrum broth or piglet feed infusion broth inhibited the growth of *E. coli*.

In vivo, microbial interactions can be assessed in terms of the effects of administration of the probiotic strain on the levels of groups of microorganisms in any region of the digestive tract. The studies can be done as challenge studies or administration of probiotics to animals on farms with a known history of diseases. For the latter case, the results are more difficult to evaluate as there are many factors which cannot be controlled. For example, when enumerating the number of *E. coli*

in the digestive tract, there are no convenient assays for pathogenic *E. coli* or for distinguishing them from the indigenous ones. Because the indigenous *E. coli* levels are not stable, if total *E. coli* levels are decreased as a result of administration of a probiotic microbes or its metabolites, one cannot state conclusively that the effect is on the pathogenic *E. coli*. Some *in vivo* studies have reported changes in levels of lactobacilli and coliforms after dosage of the probiotic strain, see section. 11.4.2. The effect of lactobacilli on the metabolism of the microflora has been studied in gnotobiotic mice colonized with human digestive tract microflora (Norin *et al.*, 1991). No changes in microflora associated biochemical characteristics were observed by these workers. Gnotobiotic piglets and gnotobiotic adult mice were associated with microflora from conventional piglets and adult pigs in a study by Ducluzeau *et al.* (1978b). They found that the ex-gnotobiotic mouse was a better model for studies of association of the flora from piglets than ex-gnotobiotic mice associated with the flora of adult pigs. Provided that the inoculation was made anaerobically, the gnotobiotic mouse was an adequate model to preserve the bacterial strains, but the equilibrium between the strains was markedly affected by the host. This equilibrium changed with time but upon transfer into a new piglet or mouse, the equilibrium was restored. It is therefore feasible to propose testing probiotic preparations on ex-gnotobiotic mice associated with piglet digestive tract microflora.

Probiotic strains producing well-characterised low and high molecular weight antagonistic metabolites may offer the only possibility of yielding *in vitro* and *in vivo* studies which can be directly compared. This will be possible if a probe can be prepared which is specific for the antagonistic metabolite. Such a probe would allow *in vivo* confirmation of the production of the active component. To our knowledge, no such probes are presently available for testing in pigs. Consequently, *in vitro* antagonistic activities can only be compared with indirect observations *in vivo* such as decreases in coliforms levels, decreased incidence of diarrhoea and death as well as improved weight gain. For example, an *L. fermentum* strain which produces low and high molecular weight metabolites antagonistic to growth and adhesion of *E. coli* K88 *in vitro*, was used to dose piglets. Improved weight gain and a decreased incidence of diarrhoea was noted in these piglets, which suggest that the strain expressed its antagonistic metabolites *in vivo* (Conway *et al.*, manuscripts). It should be emphasized that direct comparisons of *in vitro* data to *in vivo* results is presently only based on extrapolations. Another example is the study of Ducluzeau and Raibaud (1974) who found significant differences between *in vitro* and *in vivo* interactions; *E. coli* could suppress the *Shigella* population *in vitro* whereas they could coexist *in vivo*. Freter (1974) claimed that he could cultivate any

two organisms in different ways to make either one show antagonism towards the other. This comment highlights the erroneous conclusions which can be drawn from *in vitro* studies if culturing conditions are not controlled appropriately.

The above comments on *in vivo* studies apply specifically to conventional animals. Gnotobiotic animals may be useful for eliminating some of the problems with these indirect measurements. This aspect is discussed further in the following section.

(b) Gnotobiotic versus conventional animals

Gnotobiotic animals have been very important for understanding of the interrelationships between host and microbes and between microbes *in vivo*. Results from such animals are not necessarily transferable to conventional animals, because of the absence of the contributions to the system from the indigenous microflora (cf. Coates and Gustafsson, 1984). Gnotobiotic animals may be valuable for testing probiotic strains, in particular, for testing the colonization capacity, survival and microbial interactions in the absence of the indigenous microflora. For the latter two points, the difficulties of detection as discussed below are eliminated. Gnotobiotic animals can also be valuable for evaluating the effects of the probiotic strain on host physiological factors, e.g the immune system. Luckey (1987) has raised the issue that such studies should pay close attention to the diet of control animals. He proposes that stimulation of the immune system may occur simply because of a deficiency in general antigens of the diet rather than a specific effect of the administered microorganism. The gnotobiotic animal can be a valuable first step for testing survival and colonization potential of a probiotic strain. That is, if the strain fails to establish in either the gnotobiotic animal or the ex-gnotobiotic animal colonized with a complex flora, one can conclude the strain lacks colonizing capacity. This type of a study has been performed using caesarian derived colostrum-deprived piglets by Underdahl *et al.* (1983) and Ushe and Nagy (1985) who studied *Ent. faecium* C68 and M74, respectively. *Ent. faecium* C68 established in the piglets while M74 did not. It should be added that non-indigenous LAB or even strains originated from dairy products have been reported to establish in the digestive tract of germ-free animals (Morishita *et al.*, 1971, Bianchi-Salvadori *et al.*, 1984). When studying colonization in the digestive tract of germ-free animals, it has to be kept in mind that the animals may be continuously reinoculated by contaminated equipment, faeces and left-over food. If the administered strain has a good survival capacity in the digestive tract, the levels found, especially in the digesta, might reflect the continuous addition of transient bacteria. The numbers

found associated with the epithelia could be less influenced by such reinoculation or recycling of the probiotic strain.

Jonsson and Björck (submitted) tested a strain of *L. reuteri* in a series of consecutively harsher *in vivo* steps beginning with establishment in germ-free piglets and finishing with conventional pigs. The strain established in the digestive tract (mucosa and digesta) of the germ-free piglets but when challenged after a week with a faecal SPF-flora, the strain persisted but in decreased numbers. When the strain was administered at the same time as the faecal SPF-flora, it only established in the digesta but not on the epithelia. When fed once to conventional cannulated pigs, the strain decreased successively in numbers in the small intestinal digesta and after 3 days the numbers had declined to below the detection level. This series showed that the harsher the pressure on the ex-gnotobiotic animal, the lower the establishment of this strain. It may be concluded from this study that this strain will probably not colonize conventional pigs. This type of experiment, in which colonization is tested in a range of progressively more rigorous conditions, could be useful as a standard test system.

To obtain comparable results when using conventional animals, considerably larger numbers of animals need to be investigated because of variable factors such as genetic background and environmental stresses. For experimental purposes, cannulated pigs could be used (e.g. Jonsson, 1985), which enable more controlled studies that can be repeated in the same individual animal, hence minimizing the influence of the above-mentioned factors.

The difficulties of directly demonstrating *in vivo* antagonistic activities of probiotic strains on specific pathogens are eliminated when using gnotobiotic animals. For such studies, the animals can be monoassociated with the probiotic and then challenged with the pathogen, and *vice versa*. Ushe and Nagy (1985) showed decreased ileal colonization by enterotoxigenic *E. coli* in caesarian derived colostrum-deprived piglets dosed with *Ent. faecium* M74. As another alternative to gnotobiotic animals, piglets have been treated with antibiotic at birth and then artificially reared (Muralidhara *et al.*, 1977). These workers showed the successful competition of an *L. lactic* strain of human origin over pathogenic *E. coli*.

A promising alternative to gnotobiotic or conventional animals has been developed in mice by Tannock and co-workers. Specially derived mice which have a gastrointestinal microflora devoid of lactobacilli have been established and used to confirm species-specificity of lactobacilli (Tannock and Archibald, 1984). This animal model is closer to conventional animals and holds promise as a tool for evaluating directly the role of lactobacilli in the mouse.

While these types of studies provide controlled conditions, it must

be emphasized that from such results it cannot be assumed that we can predict exactly what would occur in conventional animals. Ultimately, probiotics which appear potentially valuable must be evaluated in conventional animals in the open environment to make sure of their commercial potential.

When studying survival of administered LAB in the digestive tract, the administered strain has to be redetected. In gnotobiotic animals and ex-gnotobiotic animals with a known microflora this is easier than within the rich LAB microflora of conventional pigs. Most investigators have not attempted to identify the administered bacteria within the microflora of the digestive tract but have only analysed the total count of the species used. Different methods have been used for detection of lactobacilli of intestinal origin and these include biochemical and serological tests (Muralidhara *et al.*, 1977; Jonsson *et al.*, 1985; Conway *et al.*, manuscript), genetic probes (Betzl *et al.*, 1990, Tannock *et al.*, 1990) and antibiotic resistance (Jonsson and Björck, submitted). Yoghurt lactobacilli have been detected using selective inhibitory media (Ratcliffe *et al.*, 1986). While serology is extremely valuable in confirming detection of an administered strain, caution should be taken to ensure the antibody is treated against cells growing under conditions comparable to those used for preparation of the antibodies as described by Conway and co-workers (manuscript). Bacteria can express different surface antigens depending on growth conditions (P.S. Cohen, pers. comm.). Genetic markers may make it possible to redetect strains conveniently and with certainty in conventional animals in the future. It may be difficult, however, to find naturally occurring traits in the administered strain that are not common to a relatively large part of the present indigenous microflora. Introduction of such markers into a strain may also decrease its competitive ability within the digestive microflora (Wells *et al.*, 1979).

11.6 GENERAL DISCUSSION

Piglets are ideal candidates for probiotic administration, especially preparations containing lactic acid bacteria, because of the large population of these microorganisms in the digestive tract of the healthy animal. Unfortunately, beneficial effects of probiotic administration on growth and health of pigs are not always achieved. Many factors could influence the effectiveness of such preparations, for example the genetic, physiological or health status of the animal, as well as the fact that the diet or the environment and its microbial load could vary between herds. In addition, the probiotic preparation could vary

with regard to the type(s) of microbe used, its (their) viability and physiological state as well as the form, time and levels of dosage.

Although pronounced beneficial effects on growth and health have not always been demonstrable, there are often trends towards improvement especially in neonatal and pre- and post-weaning piglets. For example, administering lactobacilli has eliminated long-term diarrhoea in some herds and administration of streptococci in some cases has prevented diarrhoea in piglets. These facts support the belief that the digestive microflora of young pigs could be favourably influenced by administering LAB. For these animals, the digestive ecosystem is less stable than in the adults and is, hence, more easily invaded by opportunistic pathogens.

The probiotic preparation and the time for administering would have great influence on the induced effects. The preparations can be either based on preformed antagonistic substances or on viable microorganisms. For the former type, the activity of the preparations is related to the type(s) and concentration of the compound(s). This concept may be the easier to monitor and to control. For the latter type, many more factors have to be considered, for example, the viability and genetic stability of the organisms in the preparation as well as their effect on the digestive ecosystem. Presently, it seems as if it is very difficult in most situations to implant permanent probiotic organisms in the digestive tract. Although one report points in this direction, most studies indicate that the indigenous microflora are very efficient in preventing new organisms from establishing permanently.

To obtain more consistent effects of both types of probiotics, more basic knowledge of the digestive ecosystem is needed. For example, the interactions between animal, diet, microbes, digestion and the immune response need to be better understood (see Fig 11.3). We postulate that probiotics would have greater influences when pigs are exposed to negative stress of some kind. This stress may be imposed from the outside of, or from within, the animal, and directly predispose the animal to infections or indirectly influence the health. Much research has been put into optimizing the rearing systems, however, there may still be further improvements to be obtained, especially when the genetic material of the stock is changing due to the breeding programs. In addition, the expanding knowledge of animal behaviour and its influence on the physiology of the animal will be valuable in finding ways to improve conditions for raising of the pigs and their well-being and performance.

To understand better the digestive ecosystem and hence the modes of action of probiotics, improved methods for studying all the complex interactions *in situ* are required. One possibility could be to concentrate on the microenvironments in the digestive tract and thus understand

the conditions for the microbes in these sites. Increased knowledge about hormonal reactions and the immune responses are probably also relevant for this kind of study. Benefit can probably also be gained by connecting knowledge from the spheres of digestive microbiology with that of physiology. One example of this can be seen from the early studies of germ-free rodents which have enlarged caeca. The reason for this enlargement was not obvious until it was apparent that the digestive tract microflora have mucinolytic activity and that there is a retrograde transportation of digesta in the hindgut of these animals. A better understanding of the complex interactions within the digestive tract will allow predictions as to which situations could be improved by probiotic treatments.

In summary, the current awareness of the risks involved with excessive antibiotic usage and the recognition that in the past, many of the probiotic preparations were largely ineffective, herald a new generation of probiotic strains. The signature of these new probiotics is that the functional characteristics are defined. In addition, the target and associated causative agents must be identified in order to establish which conditions can be improved by which probiotic preparations. Conversely, it is also important to define conditions of health which

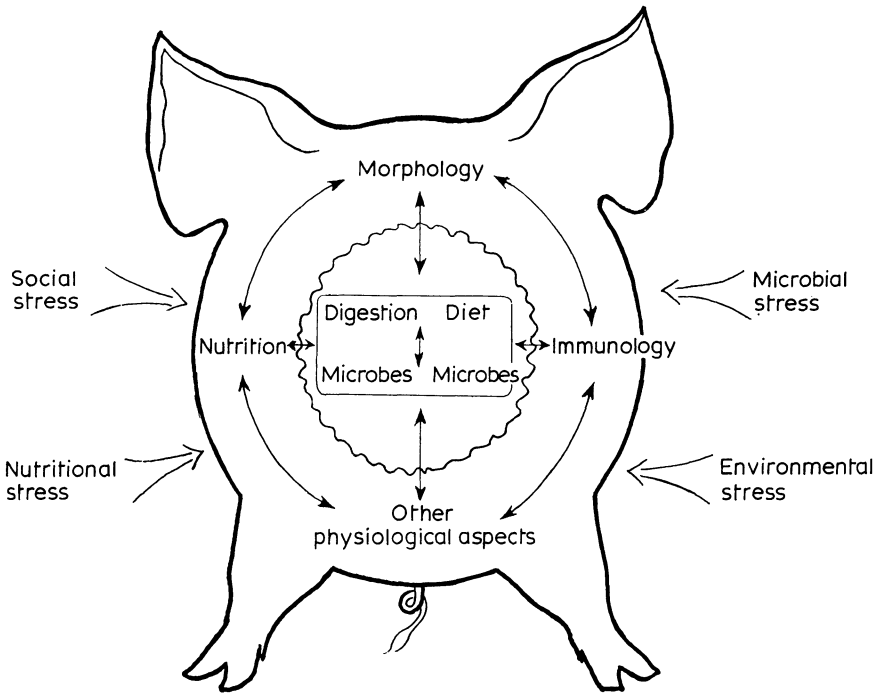

Figure 11.3 To further understand how probiotics exert their effects, studies on the total ecosystem of the pig in its specific environment will be needed.

are unlikely to be improved by known probiotic activities. Situations most certainly exist whereby probiotic strains function cooperatively with other developing concepts in the treatment and care of piglets, thus providing the opportunity to have multifaceted prophylactic piglet care.

From studies to date, it is feasible to postulate that probiotic preparations could contain microbes with the capacity to improve piglet health by their direct inhibitory effects on enteropathogenic bacteria. The *in vitro* and *in vivo* demonstration and characterization of inhibitory components will ensure functional preparations. Identification of adhesins which mediate *in vivo* colonization will allow better predictions if probiotic preparations have the capacity to colonize the digestive tract. Probiotic strains with demonstrable direct effects may also function indirectly by stabilizing the digestive microflora at times when the ecosystem is stressed. This latter vital role is essentially restricted until the complex interactions within the digestive tract are better understood.

REFERENCES

Abrams, G.D., Bauer, H. and Sprinz, H. (1963) Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. *Lab. Invest.*, **12**, 355–64.

Aimutis, W.R., Kornegay, E.T. and Eigel, W.N. (1985) *In vitro* antagonism of *Escherichia coli* by piglet small intestinal bacteria isolated by selective enrichment in media containing sow colostrum, piglet feed or brain heart infusion. *J. Gen. Microbiol.*, **31**, 135–46.

Algers, B. (1980) Djurhälsa och beteende vid burhållning av avvanda smågrisar (Animal health and behaviour in flatdeck rearing of weaned piglets). Dept animal hygiene with farrier's school, Swed. Univ. Agric. Sci., Skara, Sweden, Report, No. 4, pp. 1–82.

Axelsson, L. and Lindgren, S (1987). Characterization and DNA homology of *Lactobacillus* strains isolated from pig intestine. *J Appl. Bacteriol.*, 62, 433–40.

Auffray, P. (1965) Cited in Rerat *et al.* (1976).

Bäckström, L. (1973) Environment and health in piglet production. A field study of incidences and correlations. *Acta Vet. Scand. Suppl.*, **41**, 1–240.

Barnett, J.L., Hemsworth, P.H., Winfield, C.G. and Fahy, V.A. (1987) The effects of pregnancy and litter number on behaviour and physiological responses related to welfare status of individual and group housed pigs. *Appl. Anim. Behaviour Sci.*, **17**, 229–43.

Barrow, P.A., Fuller, R. and 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 diarrhea. *Infect. Immun.*, **18**, 586–95.

Barrow, P.A., Brooker, B.E., Fuller, R. and Newport, M.J. (1980) The attachment of bacteria to the gastric epithelium of the pig and its importance in the microecology of the intestine. *J. Appl. Bacteriol.*, **48**, 147–54.

Betzl, D., Ludwig, W. and Schleifer, K.N. (1990) Identification of lactococci and enterococci by colony hybridization with 23S rRNA-targeted oligonucleotide probes. *Appl. Environ. Microbiol.*, **56**, 2927–9.

Bianchi-Salvadori, B., Camaschella, P. and Bazzigaluppi, E. (1984) Distribution and adherence of *Lactobacillus bulgaricus* in the gastroenteric tract of germ-free animals. *Milchwissenschaft*, **39**, 387–91.

Björk, A.K.K., Christensson, E., Olsson, N.-G. and Martinsson, K.B. (1984) The clinical effect of amperozide in pig production. I.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. and 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**, 523–6.

Blomberg, L. and Conway, P.L. (1989) An *in vitro* study of ileal colonisation resistance to *Escherichia coli* strain Bd 1107/7508 (K88) in relation to indigenous squamous gastric colonisation in piglets of varying ages. *Microbial Ecol. Health Dis.*, **2**, 285–91.

Borriello, S.P. and Barclay, F.E. (1985) Protection of hamsters against *Clostridium difficile* ileocaecitis by prior colonisation with non-pathogenic strains. *J. Med. Microbiol.*, **19**, 339–50.

Boucourt, R. and Ly, J. (1975) Microflora and fermentation in the gastrointestinal tract of the young pig. 2. Bacterial population. *Cuban J. Agric.*, **9**, 163.

Bourlioux, P. and Pochart, P. (1988) Nutritional and health properties of yoghurt. *Wld Rev. Nutr. Diet.*, **56**, 217–58.

Brandenburg, A.C and Wilson, W.R. (1973) Immunity to *Escherichia coli* in pigs; IgG immunoglobulin in passive immunity to *E. coli* enteritis. *Immunology*, **24**, 119–27.

Braude, R., Fulford, R.J. and 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.

Brennan, M., Wanismail, B., Johnson, M.C. and Ray, B. (1986) Cellular damage in dried *Lactobacillus acidophilus*. *J. Food Protect.*, **49**, 47–53.

Brockett, M. and Tannock, G.W. (1981) Dietary components influence tissue-associated lactobacilli in the mouse stomach. *Can. J. Microbiol.*, **27**, 452–5.

Brownlee, A. and Moss, W. (1961) The influence of diet on lactobacilli in the stomach of the rat. *J. Pathol. Bacteriol.*, **82**, 513–16.

Burnett, G.S. and Neil, E.L. (1977) A note on the effect of probioticum feed additive on the live-weight gain, feed conversion and carcass quality of bacon pigs. *Anim. Prod.*, **25**, 95–8.

Cain, C.J. (1989) Observations on indigenous and non-indigenous lactic acid

bacteria as potential probiotic organisms in the pig. M.Agr.Sc. Thesis, La Trobe University, Australia.

Campbell, R.G. (1989) The nutritional management of weaner pigs, in *Manipulating Pig Production*, II (eds J.L. Barnett. and D.P. Hennesy.), Australasian Pig Science Association, Werribee, Victoria, Australia, pp. 170–5.

Carghill, C.F. (1982) Control of *E. coli* infections in pigs. *Austral. Adv. Vet. Sci.*, pp. 206–7.

Chapman, J.D. (1988) Probiotics, acidifiers and yeast culture: a place for natural additives in pig and poultry production, in *Biotechnology in the Feed Industry, Proc. Alltech's 4th Ann. Symp.* (ed. T.P. Lyons), pp. 219–33.

Clemens, E.T., Stevens, C.E. and Southworth, M. (1975) Sites of organic acid production and pattern of digesta movement in the gastrointestinal tract of swine. *J. Nutr.*, **105**, 759–68.

Coates, M.E. (1973) Gnotobiotic animals in nutrition research. *Proc. Nutr. Soc.*, **32**, 53–8.

Coates, M.E. and Gustafsson, B.E. (eds) (1984) *The Germfree Animal in Biomedical Research*, Laboratory Animals, London.

Collins, M.D., Farrow, J.A.E., Katic, V. and 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**, 402–13.

Conway, P.L. (1985) *Lactobacillus* spp. of the intestinal tract and mechanisms of attachment. *SBF-Nytt*, **31**, 32–6.

Conway, P.L. (1989) Lactobacilli: Fact and fiction, in *The Regulatory and Protective Role of the Normal Microflora*, (eds R. Grubb, *et al.*), Macmillan Press, Basingstoke, pp. 263–81.

Conway, P.L. and Kjelleberg, S. (1989) Protein-mediated adhesion of *Lactobacillus fermentum* strain 737 to mouse stomach squamous epithelium. *J. Gen. Microbiol.*, **135**, 1175–86.

Conway, P.L., Maki, J., Mitchell, R. and Kjelleberg, S. (1986) Starvation of marine flounder, squid and laboratory mice and its effect on the intestinal microbiota. *FEMS Microbiol. Ecol.*, **38**, 187–95.

Conway, P.L., Gorbach, S.L. and Goldin, B.R. (1987) Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *J. Dairy Sci.*, **70**, 1–12.

Conway, P.L., Welin, A. and Cohen, P.S. (1990) Presence of K88 specific receptors in porcine ileal mucus is age dependent. *Infect. Immun.*, **58**, 3178–82.

Conway, P.L., Blomberg, L., Welin, A. and Cohen, P.S. (1991) The role of piglet intestinal mucus in the pathogenicity of *Escherichia coli* K88, in *Molecular Pathogenesis of Gastrointestinal Infections*, (eds T. Wadström, et al.), Plenum Press, New York. pp 335–8

Corpet, D. and Nicolas, J.-L. (1979) Antagonistic effect of intestinal bacteria from the microflora of holoxenic (conventional) piglets, against *Clostridium perfringens* in the digestive tract of gnotoxenic mice and gnotoxenic piglets, in *Clinical and Experimental Gnotobiotics, Zbl. Bakteriol. Suppl* 7, Gustav Fischer, Stuttgart, pp. 167–74.

Cowman, G.L., Davis, L.W., Duncan, M.S. and Trammell, J.H. (1978) Dose-response relationship in post-weaned pigs fed a non-viable *Lactobacillus* fermentation product. *J. Anim. Sci.*, **47**, (Suppl. 1), 298

Cranwell, P.D. (1968) Microbial fermentation in the alimentary tract of the pig. *Nutr. Abstr. Rev.*, **38**, 721–30

Cranwell, P.D. (1990) Development of the gastrointestinal microflora in the milk-fed pig and milk-fed human infant. La Trobe University, School of Agriculture, Melbourne, Bundoora 3083, Australia, Occasional Paper 21, 1–40.

Cranwell, P.D. and Moughan, P.J. (1989) Biological limitations imposed by the digestive system to the growth performance of weaned pigs, in *Manipulating Pig Production* II, (eds J.L. Barnett, and D.P. Hennesy), Australasian Pig Science Association, Werribee, Victoria, Australia, pp. 140–59.

Cranwell, P.D., Noakes, D.E. and Hill, K.J. (1976) Gastric secretion and fermentation in the suckling pig. *Brit. J. Nutr.*, **36**, 71–86.

Danek, P. (1986) Zmeny fekalni mikroflory selat behem aplikace *Streptococcus faecium* M-74 (Lactiferm) v krmne smesi. *Zivocisna Vyroba*, **31**, 1019–27.

Davidson, J.N. and Hirsch, D.C. (1976) Bacterial competition as a means of preventing neonatal diarrhea in pigs. *Infect. Immun.*, **13**, 1773–4.

De Graaf, F.K. and Mooi, F.R. (1986) The fimbrial adhesins of *Escherichia coli*. *Adv. Microb. Physiol.*, **28**, 65–143.

Deprez, P., van den Branden, J., de Geest, J. and Muylle, E. (1989) De invloed van *Streptococcus faecium* toediening op de excretie van *Escherichia coli* en het coorkomen van slingerziekte bij gespeende biggen. *Vlaams Diergeneeskde Tijdschr.*, **58**, 113–17.

De Valdez,G.F., de Giori, G.S., Ruiz Holgardo, A.P. and Oliver, G. (1985) Effect of drying medium on residual moisture content and viability of freeze-dried lactic acid bacteria. *Appl. Environ. Microbiol.*, **49**, 413–15.

Drew, M.D. & Owen, B.D. (1988) The provision of passive immunity to colostrum-deprived piglets by bovine or porcine serum immunoglobulins. *Can. J. Anim. Sci.*, **68**, 1277.

Ducluzeau, R. (1985) Implantation and development of the gut flora in the newborn piglet. *Pig News Inform.*, **6**, 415–18.

Ducluzeau, R. and Raibaud, P. (1974) Interaction between *Escherichia coli* and *Shigella flexneri* in the digestive tract of 'gnotobiotic' mice. *Infect. Immun.*, **9**, 730–3.

Ducluzeau, R., Dubos, F., Raibaud, P. and Abrams, G.D. (1978a) Production of an antibiotic substance by *Bacillus licheniformis* within the digestive tract of gnotobiotic mice. *Antimicrob. Agents Chemother.*, **13**, 97–103

Ducluzeau, R., Rapine, P., Courvalin, C. and Raibaud, P. (1978b) Transfert de la flore microbienne fécale de porcelets et de porcs adultes holoxéniques à des souris adultes et des porcelets axéniques: Effet de l'animal hôte et du régime alimentaire sur le faciès microbien du tube digestif des divers animaux. *Ann. Microbiol (Inst. Pasteur).*, **129B**, 597–612.

Ducluzeau, R., Ladire, M. and Laplace, J.P. (1986) Évolution de la flore microbienne caecale après isolement chirurgical du gros intestin chez le porc. *Ann. Microbiol (Inst. Pasteur).*, **137B**, 123–8.

Duval-Iflah, Y., Chappuis, J.P., Ducluzeau, R. and Raibaud, P. (1983) Intraspecific interactions between *Escherichia coli* strains in human newborns and in gnotobiotic mice and piglets. *Prog. Food Nutr. Sci.*, **7**, 107–16

English, P.R. (1984) Establishing the early weaned pig. *Pig Vet. Soc. Proc.*, **7**, 29–37.

Ervolder, T.M., Gudkov, A.V., Gudkov, S.A. *et al.* (1984) Use of bifidobacteria in control of gastrointestinal disease in piglets and broiler chicks. *Molochnaya Promyshlennost*, **8**, 18–20. (*in Dairy Sci. Abstr.*, 1985, **47**, 714).

Fahy, V.A., Connaughton, D., Driesen, S.J. and Spicer, E.M. (1987a) Preweaning colibacillosis, in *Manipulating Pig Production*. (eds APSA Comittee), Australian Pig Science Association, Werribee, Victoria, Australia, pp. 176–88.

Fahy, V.A., Connaughton, D., Driesen, S.J., and Spicer, E.M. (1987b) Post-weaning colibacillosis, in *Manipulating Pig Production*, (eds APSA Committee), Australian Pig Science Association, Werribee, Victoria, Australia, pp. 189–201.

Fernandes, C.F., Shahani, K.M. and Amer, M.A. (1987) Therapeutic role of dietary lactobacilli and lactobacillus fermented dairy products. *FEMS Microbiol. Rev.*, **46**, 343–56.

Fewins, B.G., Newland, L.G.M. and Briggs, C.A.E. (1957) The normal intestinal flora of the pig. III. Qualitative studies of lactobacilli and streptococci. *J. Appl. Bacteriol.*, **20**, 234–42.

Fossum, K. and Liven, E. (1974) The distribution of enzymes and bacteria in the small intestines of slaughter pigs. *Acta Path. Microbiol. Scand.*, **382**, 644–52.

Freter, R. (1974) Interactions between mechanisms controlling the intestinal microflora. *Amer. J. Clin. Nutr.*, **27**, 1409–16.

Freter, R. (1983) Mechanisms that control the microflora in the large intestine, in *Human Flora in Health and Disease*. (ed. D. Hentges), Academic Press, New York, pp. 33–54.

Friend, D.W., Cunningham, H.M. and Nicholson, J.W.G. (1963) The production of organic acids in the pig. II. The effect of diet on the levels of volatile fatty acids and lactic acid in sections of the alimentary tract. *Can. J. Anim. Sci.*, **43**, 156–68.

Fuller, R. (1986) Probiotics. *J. Appl. Bacteriol. Suppl.*, 1S-7S.

Fuller, R. (1989) Probiotics in man and animals. *J. Appl. Bacteriol.*, **66**, 365–78.

Fuller, R., Barrow, P.A. and Brooker, B.E. (1978) Bacteria associated with the gastric epithelium of neonatal pigs. *Appl. Environ. Microbiol.*, **35**, 582–91.

Gilliland, S.E. and Rich, C.N. (1990) Stability during frozen and subsequent refrigerated storage of *Lactobacillus acidophilus* used as a dietary adjunct to produce a hypocholesterolemic effect in humans. *J. Dairy Sci.*, **73**, 1187–92.

Gilliland, S.E. and Walker, D.K. (1990) Factors to consider when selecting a culture of *Lactobacillus acidophilus* grown at different pH. *J. Dairy Sci.*, **73**, 905–11.

Gordon, D., Macrae, J. and Wheater, D. (1957) A *Lactobacillus* preparation for use with antibiotics. *Lancet*, May 4, 899–901.

Gouet, P., Fonty, G., Jouany, J.P. and Nebout, J.M. (1984) Cellulolytic bacteria establishment and rumen digestion in lambs isolated after birth. *Can. J. Anim. Sci. Suppl.* **64**, 163–4.

Graham, H., Hesselman, K., Jonsson, E. and Åman, P. (1986) Influence of

β-D-glucanase supplementation on digestibility of a barley-based diet in the pig gastrointestinal tract. *Nutr. Rep. Intern.*, **34**, 1089–96.

Greenwood, P.E. and Tzipori, S. (1987) Protection of suckling piglets from diarrhoea caused by enterotoxigenic *Escherichia coli* by vaccination of the pregnant sow with recombinant DNA derived pilus antigens, in *Manipulating Pig Production*, (eds APSA Comittee), Australian Pig Science Association, Werribee, Victoria, Australia, p. 230.

Gualtieri, M. and Betti, S. (1984) Effects of the administration of *Streptococcus faecium* to suckling piglets. *Riv. Suinicoltura*, **25**, 39–43.

Gudding, R. and Larssen, R.B. (1985) Some effects of *Streptococcus faecium* M 74 in piglets. *Nord. Vet. Med.*, **37**, 48–9.

Hale, O.M. and Newton, G.L. (1979) Effects of a nonviable *Lactobacillus* species fermentation product on performance of pigs. *J. Anim. Sci.*, **48**, 770–5.

Han, I.K., Kim, J.D., Lee, J.H., *et al.* (1984) Studies on the growth promoting effects of probiotics. III. The effects of *Clostridium butyricum* ID on the performance and the changes in the microbial flora of the feces of growing pigs. *Korean J. Anim. Sci.*, **26**, 166–71.

Hardie, J.M. (1986) Genus Streptococcus, in *Bergey's Manual of Systematic Bacteriology*, vol. 2. (ed. P.H.A. Sneath), Williams & Wilkins, London, pp. 1043–7.

Hartmann, P.E., McCauley, I., Gooneratne, A.D. and Whitely, J.L. (1984) Inadequacies of sow lactation: survival of the fittest, in *Physiological Strategies in Lactation* (eds M. Peaker *et al.*), Academic Press, London, pp. 301–26.

Henriksson, A., Szewzyk, R. and Conway, P.L. (1991) Characteristics of the adhesive determinants of *Lactobacillus fermentum* 104, *Appl. Environ. Microbiol.* **57**, 499–502.

Hoefling, D. (1989) Tracking the culprits behind diarrhea in neonatal pigs. *Vet. Med.*, April, 427.

Horvath, D.J., Seeley, H.W., Warner, R.G. and Loosli, J.K. (1958) Microflora of intestinal contents and feces of pigs fed different diets including pigs showing parakeratosis. *J. Anim. Sci.*, **17**, 714–22.

Houpt, K.A., Zahorik, D.M., Anika, S.M. and Houpt, T.R. (1979) Taste aversion learning in suckling and weanling pigs. *Vet. Sci. Comm.*, **3**, 165–9.

Hungate, R.E. (1984) Microbes of nutritional importance in the alimentary tract. *Proc. Nutr. Soc.*, **43**, 1–11.

Ingram, S.H., Lennon, A.M., Albin, R.C. *et al.* (1973) *Lactobacillus acidophilus* for pigs at weaning. *J. Anim. Sci.*, **36**, 207

Jensen, H. (1974) Biological effects of feeding pigs with *Lactobacillus acidophilus*. *Proc. XIX Intern. Dairy Congr., 1974, India.* 1E, p. 579.

Jensen, P. and Recén, B. (1989) When to wean – observations from free-ranging domestic pigs. *Appl. Anim. Behaviour Sci.*, **23**, 49–60.

Jonsson, E. (1985) Lactobacilli as probiotics to pigs and calves. Dissertation, Dept. Animal Nutrition Management, Swed. Univ. Agric. Sci., No. 148, pp. 1–65.

Jonsson, E. (1986) Persistence of a *Lactobacillus* strain in the gut of sucking piglets and its influence on performance and health. *Swed. J. Agric. Res.*, **16**, 43–7.

Jonsson, E. and Henningsson, S. (1991) Establishment in the piglet gut of lactobacilli capable of degrading mixed-linked β-D-glucans. *J. Appl. Bacteriol.* **70**, 512–6.

Jonsson, E., Björck, L. and Claesson, C.O. (1985) Survival of orally administered *Lactobacillus* strain in the gut of cannulated pigs. *Livest. Prod. Sci.*, **12**, 279–85.

Jørgensen, J.H. (1988) Probiotika till svin. En litteraturoversigt og en afprøvning at 2 probiotikas effekt under forskellige produktionsformer. *Dansk Vet. Tidskr.*, **71**, 1211–21.

Jucker, H., Pfirter, H.P., Wenk, C. and Scgürch, A. (1973) Untersuchungen über die Wirkung antimikrobiell wirksamer Zulagen zum Futter von Ferkeln und Mastschweinen. *Wien Tierärtzl. Monatschr.*, **60**, 100–3.

Kandler, O. and Weiss, N. (1986) Regular, non-sporing gram-positive rods in *Bergey's Manual of Systematic Bacteriology*, vol. 2. (ed. P.H.A. Sneath.), Williams & Wilkins, London, pp. 1208–34.

Kenworthy, R. (1973) Intestinal microflora of the pig. *Adv. Appl. Microbiol.*, **16**, 31–54.

Kenworthy, R. and Crabb, W.E. (1963) The intestinal flora of young pigs, with reference to early weaning, *Escherichia coli* and scours. *J. Comp. Pathol.*, **73**, 215–28.

Kidder, D.E. and Manners, M.J. (1978) *Digestion in the Pig*, Scientechnica, Bristol.

Kimura, N., Yoshikane, M., Kabayashi, A. and Mitsuoka, T. (1983) An application of dried bifidobacteria preparation to scouring animals. *Bifidobacteria Microflora*, **2**, 41–55.

Kinsey, C.M. (1980) Use of microbial additives in feed. A literature review. *Proc. 40th Semiannual Meeting, San Antonio, Texas, USA, Nov. 12–14, 1980*, pp. 25–30.

Kjelleberg, S., Nyström, T., Albertson, N. and Flärdh, K. (eds) (1990). Symposium 'Nutrient limitation: Global responses and prokaryotic development'. *FEMS Microbiol. Ecol.*, **74**, 91–241.

Klaenhammer, T.R. (1982) Microbial considerations in selection and proparation of *Lactobacillus* strains for use as dietary adjuncts. *J. Dairy Sci.*, **65**, 1339–49.

Klaenhammer, T.R. and Kleeman, E.G. (1981) Growth characteristics, bile sensitivity, and freeze damage in colonial variants of *Lactobacillus acidophilus*. *Appl. Environ. Microbiol.*, **41**, 1461–7.

Kluber, E.T., Pollman, D.S. and Blecha, F. (1985) Effect of feeding *Streptococcus faecium* to artificially reared pigs on growth, haematology, and cell-mediated immunity. *Nutr. Rep. Intern.*, **32**, 57–66.

Kornegay, E.T. (1985/86) Effect of dosing or feeding *Lactobacillus acidophilus* on blood cholesterol levels and growth rate of weanling and growing pigs. Virginia Polytechnic Institute and State University, Animal Science Research Report, No. 5, Blacksburg, Virginia, USA, pp. 14–19.

Kornegay, E.T. and Thomas, H.R. (1973) Bacterial and yeast preparations for starter and grower rations. Virginia Polytechnic Institute and State University, Research Division Report, No. 151, Blacksburg, Virginia, USA.

Kovacs, F., Nagy, B. and Sinkovics, G. (1972) The gut bacterial flora of

healthy early weaned piglets, with special regard to factors influencing its composition. *Acta Vet. Acad. Sci. Hung.*, **22**, 327–38.

Krarup, L.T. (1987) Forebyggelse af diarré hos spædgrise. *Dansk Vet. Tidskr.*, **70**, 324–8.

Kurman, J.A. (1983) The development and significance of new cultures with bifidobacteria as an example. *North Eur. Dairy J.*, **3**, 65–74.

Kvasnitskii, A.V. (1951), *Voprosy fiziologii pishchevareniya u svinei*. Sel'khozgir, Moscow. (Cited from Kidder and Manners, 1978.)

Landsutvalget for svin (1988) Probilact H til smågrise. Den rullende Afprøfning af stald- og produktionssystemer, Copenhagen, Meddelse, No. 144, pp. 1–6.

Landsutvalget for svin (1989) Bioplus 2B till smågrise. Den rullende Afprøfning af stald- og produktionssystemer, Copenhagen, Meddelse, p. 162.

Lawrence, T.L.J. (1970) Some effects of including differently processed barley in the diet of the growing pig. 2. *In vivo* gastric pH changes. *Anim. Prod.*, **12**, 151–63.

Lawrence, T.L.J. (1983) Dietary manipulation of the environment within the gastrointestinal tract of the growing pig and its possible influence on disease control: some thoughts. *Pig Vet. Soc. Proc., Cambridge*, **10**, 40–9.

Lencner, A.A., Lencner, C.P., Mikelsaar, M.E. *et al.* (1984) Die quantitative Zusammensetzung der Lactoflora des Verdauungstrakts vor und nach kosmischen Flügen unterschiedlicher Dauer. *Die Nahrung*, **28**, 607–13.

Lessard, M. and Brisson, G.J. (1987) Effect of a *Lactobacillus* fermentation product on growth, immune response and fecal enzyme activity in weaned pigs. *Can. J. Anim. Sci.*, **67**, 509–16.

Lindgren, S. and Dobrogosz, W.J. (1990) Antagonistic activities of lactic acid bacteria in food and feed fermentations. *FEMS Microbiol. Rev.*, **87**, 149–64.

Lipkin, M. (1987) Proliferation and differentiation of normal and diseased gastrointestinal cells, in *Physiology of the Gastrointestinal Tract*, 2nd edn (ed. L.R. Johnson), Raven Press, New York, pp. 255–84.

Lönnroth, I., Martinsson, K. and Lange, S. (1988) Evidence of protection against diarrhoea in suckling piglets by a hormone-like protein in sow milk. *J. Vet. Med.*, **356**, 628–35.

Luckey, T.D. (1987) Misconceptions in gnotobiology. *Microecol. Ther.*, **17**, 19–20.

Lyons, T.P. (1988) Probiotics: An alternative to antibiotics. *The Bovine Practitioner*, **23**, 64–9.

Maeng, W.J., Kim, C.W. and Shin, H.T. (1989) Effects of feeding lactic acid bacteria concentrate (LBC, *Streptococcus faecium* Cernelle 68) on the growth rate and prevention of scouring in piglets. *Korea J. Anim. Sci.*, **31**, 318–23.

Manners, M.J. (1976) The development of digestive function in the pig. *Proc. Nutr. Soc.*, **35**, 49–55.

Mäyrä-Mäkinen, A., Manninen, M. and Gyllenberg, H. (1983) The adherence of lactic acid bacteria to the columnar epithelial cells of pigs and calves. *J. Appl. Bacteriol.*, **55**, 241–5.

Maxwell, C.V., Reimann, E.M., Hoekstra, W.G. *et al.* (1970) Effect of dietary particle size on lesion development and on the contents of various regions of the swine stomach. *J. Anim. Sci.*, **30**, 911–22.

McAllister, J.S., Kurtz, H.J. and Short, E.C. (1979) Changes in the intestinal flora of young pigs with postweaning diarrhea or edema disease. *J. Anim. Sci.*, **49**, 868–79.

McGillivery, D.J., Cranwell, P.D., Cain, C.J. and Chandler, K.D. (1984) The microbiology of the *pars oesophagea* of sucking and weaned pigs. *Int. Pig Vet. Soc. Proc.*, **8**, 235.

Moen, J.H. (1982) *Streptococcus faecium* mot spedgrisdiaré. *Norsk Vet. Tidskr.*, **94**, 629–31.

Møllgaard, H. (1946) On Phytic acid, its importance in metabolism and its enzymic cleavage in bread supplemented with calcium *Biochem. J.*, **40**, 589–603.

Møllgaard, H. (1947) Resorptionen af kalcium og fosforsyre. *Beretning fra forsøgslab.*, **228**, 1–55.

Morishita, Y. and Ogata, M. (1970) Studies on the alimentary flora of pig. V. Influence of starvation on the microbial flora. *Japan. J. Vet. Sci.*, **32**, 19–24.

Morishita, Y., Mitsuoka, T., Kaneuchi, C. *et al.* (1971) Specific establishment of lactobacilli in the digestive tract of germ-free chickens. *Japan. J. Microbiol.*, **15**, 531–8.

Mumpton, F.A. and 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 *Bergey's Manual of Systematic Bacteriology*, vol. 2, (ed P.H.A. Sneath), Williams & Wilkins, London, pp. 1063–5.

Muralidhara, K.S., Sheggeby, G.G., Elliker, P.R. *et al.* (1977) Effect of feeding lactobacilli on the coliform and *Lactobacillus* flora of intestinal tissue and feces from piglets. *J. Food Protect.*, **40**, 288–95.

Nagy, L.K., Bhugal, B.S. and 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**, 289–96.

Nakamura, M., Yoshimura, H., Koeda, T. and Sato, S. (1983) Prevention of drug-resistant *Escherichia coli* colonization in chickens by treatment with a faecal fluid. *J. Appl. Bacteriol.*, **55**, 225–31.

Neupert,B. (1988) Milchsäurebakterien CYCLACTIN* in Verbindung mit AVOTAN* erhöhen den Deckungsbeitrag in der Schweinemast. *Kraftfutter*, **4**, 126.

Newby,T.J., Miller,B., Stokes,C.R. *et al.* (1984) Local hypersensitivity response to dietary antigens in early weaned pigs, in *Recent Advances in Animal Nutrition* (eds W. Haresign. and D.J.A. Cole), Butterworth, London, pp. 49–59.

Nickel, R., Schummer, A. and Seiferle, E. (1967) *Lehrbuch der Anatomie der Haustiere. Band 2. Eingeweide*, 2nd edn., Paul Parey, Berlin, p. 138.

Noakes, D.E. (1971) Gastric function in the young pig. Ph.D. Thesis, University of London. (Cited from Cranwell and Moughan, 1989.)

Norin, K.E., Persson, A-K., Saxerholt, N. and Midtvedt, T. (1991) Establishment of *Lactobacillus* and *Bifidobacterium* species in germ-free mice and their influence on some microflora associated characteristics. *Appl. Environ. Microbiol.* **57**, 1850–2.

Nurmi, I.E. and Rantala, M. (1973) New aspects of *Salmonella* infection in broiler production. *Nature*, **241**, 210–11.

Ogata, M. and Morishita, Y. (1969) Studies on the alimentary flora of pigs. IV. The alimentary flora of pigs infected with hog cholera. *Japan. J. Vet. Sci.*, **31**, 71–82.

Ogata, M., Morishita, Y., Tanaka, Y. and Shinjo, T. (1968) Studies on the alimentary flora of pig. III. Influence of fermented chlorella-diet on the fecal flora. *Japan. J. Vet. Sci.*, **30**, 281–7.

Ogle, R.B. and Inborr, J. (1987) Alternatives to low dose antibiotics in piglet feeds in Sweden. *38th Meeting of the European Association for Animal Production, Portugal, Commission on Pig Production*, Session 4, pp. 1–6.

Olsson, T. (1966) Tarmrubbningar på svin – profylax och terapi med *Lactobacillus acidophilus*. *Svensk Vet. Tidn.*, **18**, 355–63.

Ozawa, K., Yokota, H., Kimura, M. and Mitsuoka, T. (1981) Effects of administration of *Bacillus subtilis* strain BN on intestinal flora of weanling piglets. *Japan. J. Vet. Sci.*, **43**, 771–5.

Ozawa, K., Yabu-Ochi, K., Yamanaka, K., *et al.*, (1983) Effect of *Streptococcus faecalis* BIO-4R on intestinal flora of weanling piglets and calves. *Appl. Environ. Microbiol.*, **45**, 1513–18.

Parker, R.B. (1974) The other half of the antibiotics story. *Anim. Nutr. Health*, **29**, 4–8.

Partridge, I.G. (1989) Alternative feeding strategies for weaner pigs, in *Manipulating Pig Production* II, (eds J.L. Barnett and D.P. Hennesy), Australasian Pig Science Association, Werribee, Victoria, Australia, pp. 140–59.

Pedersen, K. and Tannock, G.W. (1989) Colonization of the porcine gastrointestinal tract by lactobacilli. *Appl. Environ. Microbiol.*, **55**, 279–83.

Perdigón, G., Nader de Macias, M.E., Alvarez, S. *et al.* (1987) Enhancement of immune response in mice fed with *Streptococcus thermophilus* and *Lactobacillus acidophilus*. *J. Dairy. Sci.*, **70**, 919–26.

Pettersson, H. (1990) *Genotype x nutrition interactions in the performance testing of pigs*. Dissertation, Dept Animal Breeding and Genetics, Swed. Univ. Agric. Sci., No. 93, p. 157.

Pollman, D.S. (1986) Probiotics in pig diets, in *Recent Advances in Animal Nutrition* (eds W. Haresign and D.J.A. Cole), Butterworth, London, pp. 193–205.

Pollman, D.S. and Bandyk, C.A. (1984) Stability of *Lactobacillus* products. *Anim. Feed Sci. Technol.*, **11**, 261–7.

Pollman, D.S., Danielson, D.M. and Peo, E.R. Jr (1980a) Effect of microbial feed additives on performance of starter and growing-finishing pigs. *J. Anim. Sci.*, **51**, 577–81.

Pollman, D.S., Danielson, D.M., Wren, W.B. *et al.* (1980b) Influence of *Lactobacillus acidophilus* inoculum on gnotobiotic and conventional pigs. *J. Anim. Sci.*, **51**, 629–37.

Pollman, D.S., Danielson, D.M. and Peo, E.R. Jr (1980c) Effect of *Lactobacillus acidophilus* on starter pigs fed a diet supplemented with lactose. *J. Anim. Sci.*, 51, 638–44.

Pollman, D.S., Kennedy, G.A., Koch, B.A. and Allee, G.L. (1984) Influence

of nonviable *Lactobacillus* fermentation product in artificially reared pigs. *Nutr. Rep. Int.*, **29**, 977–82.

Porter, P. (1969) Transfer of immunoglobulins IgG, IgA and IgM to lacteal secretions in the paturient sow and their absorption by the neonatal piglet. *Biochem. Biophys. Acta*, **18**, 381–92.

Porter, P. and Linggood, M. (1983) Development of oral vaccine for prevention of scouring caused by enteropathogenic *Escherichia coli*. *J. Infect.*, **6**, 111–21.

Premi, L. and Bottazzi, V. (1974) Use of lactobacilli in the control of intestinal disturbances of pigs. *Proc. XIX Int. Dairy Congr., 1974 India*. 1E, p. 89.

Raibaud, P., Caulet, M., Galpin, J.V. and 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.

Ratcliffe, B. (1985) The influence of the gut microflora on the digestive processes. *Beretning fra Statens Husdyrbrugsforsøg*, **580**, 245–67.

Ratcliffe, B., Cole, C.B., Fuller, R. and Newport, M.J. (1986) The effect of yoghurt and milk fermented with a porcine intestinal strain of *Lactobacillus reuteri* on the performance and gastrointestinal flora of pigs weaned at two days of age. *Food Microbiol.*, **3**, 203–11.

Redmond, H.E. and Moore, R.W. (1965) Biologic effect of introducing *Lactobacillus acidophilus* into a large swine herd experiencing enteritis. *Southw. Vet.*, **18**, 287–8.

Reiter, B. (1985) Protective proteins in milk, biological significance and exploitation. *Int. Dairy Fed. Bull.*, **191**, 1–35.

Rerat, A., Corring, T. and Laplace, J.P. (1976) Protein digestion and absorption, in *Protein Metabolism and Nutrition* (eds D.J.A., Cole *et al.*), Butterworth, London, pp. 97–138.

Robinson, I.M., Allison, M.J. and Bucklin, J.A. (1981), Characterization of the cecal bacteria of normal pigs. *Appl. Environ. Microbiol.*, **41**, 950–5.

Robinson, I.M., Whipp S.C., Bucklin, J.A. and Allison, M.J. (1984) Characterization of predominant bacteria from the colons of normal and dysenteric pigs. *Appl. Environ. Microbiol.*, **48**, 964–9.

Robinson, I.M., Stromley, J.M., Varel, V.H. and Cato, E.P. (1988) *Streptococcus intestinalis*, a new species from the colons and feces of pigs. *Int. J. Syst. Bacteriol.*, **38**, 245–8.

Roth,F.X. and Kirchgessner,M. (1986) Zur nutritiver Wirksamkeit von *Streptococcus faecium* (Stamm M74) in der Ferkelaufzucht. *Landwirtsch. Forsch.*, **39**, 198–205.

Roth, F.X. and Kirchgessner, M. (1988) Nutritive Wirksamkeit von Toyocerin. 1. Mitteilung: Ferkelaufzucht. *Landwirtsch. Forsch.*, **41**, 58–62.

Russel, E.G. (1979) Types and distribution of anaerobic bacteria in the large intestine of pigs. *Appl. Environ. Microbiol.*, **37**, 187–93.

Rutter, P.R., Dazzo, F.B., Freter, R., Gingell, D. *et al.* (1984) Mechanisms of adhesion, in *Microbial Adhesion and Aggregation*, (ed. K.C. Marshall), Springer-Verlag, Berlin, pp. 5–19.

Salanitro, J.P., Blake, I.G. and Muirhead, P.A. (1977) Isolation and identification of fecal bacteria from adult swine. *Appl. Environ. Microbiol.*, **33**, 79–84.

Savage, D.C. (1977) Microbial ecology of the gastrointestinal tract. *Ann. Rev. Microbiol.*, **31**, 107–33.

Savage, D.C., Siegel, J.E., Snellen, J.E. and Whitt, D.D. (1981) Transit time of epithelial cells in the small intestines of germfree mice and ex-germfree mice associated with indigenous microorganisms. *Appl. Environ. Microbiol.*, **42**, 996–1001.

Schulze, F. (1977) Quantitative 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. (1978) Zur quantitativen Zusammensetzung der Magen-Darm-Flora bei Ferkeln in den ersten zwei Lebenswochen. 1. Mitteilung: Zur Entwicklung der Magen-Darm-Flora bei natürlich aufgezogenen Ferkeln. *Arch. Exp. Vet. Med., Leipzig*, **32**, 155–70.

Schulze, F. (1987) Die Gastrointestinalflora des Schweines und ihre Regulationsmechanismen. *Fortschrittsber. Landw. Nahrungsgüterwirtsch.*, **25**, 1–72.

Schulze, F. and Bathke, W. (1977) Zur quantitativen Zusammensetzung der Magen-Darm-Flora beim Läuferschwein. *Arch. Exp. Vet. Med., Leipzig*, **31**, 161–85.

Schulze, F. and Jacob, H.-E. (1981) Das Redoxpotential im Magen-Darm-Kanal von Schweinen unterschiedlichen Alters. *Arch. Exp. Vet. Med., Leipzig*, **35**, 349–57.

Schulze, F. and 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**, 317–24.

Shahani, K., Fernandes, H. and Amer, V. (1989) Immunologic and therapeutic modulation of gastrointestinal microecology by lactobacilli. *Microecol. Ther.*, **18**, 103–4.

Sharpe, M.E. (1981) The genus *Lactobacillus*, in *The Procaryotes, a Handbook on Habitats, Isolation and Identification of Bacteria* (eds M.P. Starr *et al.*), vol. 2, Springer-Verlag, Berlin, pp. 1653–79.

Sharpe, M.E., Hill, L.R. and Lapage, S.P. (1973) Pathogenic lactobacilli. *J. Med. Microbiol.*, **6**, 281–6.

Sinkovics, G. and Juhasz, B. (1974) Development of the intestinal flora in suckling pigs. *Acta Vet. Acad. Scient. Hungar.*, **24**, 375–81.

Sissons, J.W. (1988) The potential of probiotic organisms to prevent diarrhoea and promote digestion. *Proc. 4th Int. Sem. on Digestive Physiology in the Pig, Jablonna, Poland, 1988*, pp. 357–70.

Sissons, J.W. (1989) Potential of probiotic organisms to prevent diarrhoea and promote digestion in farm animals – a review. *J.Sci. Food Agric.*, **49**, 1–13.

Smith, H.W. (1961) The development of the bacterial flora of the faeces of animals and man: The changes that occur during ageing. *J. Appl. Bacteriol.*, **24**, 235–41.

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. and Crabb, W.E. (1961) The faecal bacterial flora of animals and man: its development in the young. *J. Pathol. Bacteriol.*, **82**, 53–66.

Smith, H.W. and Huggins, M.B. (1983) Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. *J. Gen. Microbiol.*, **129**, 2659–75.

Smith, H.W. and Jones, J.E.T. (1963) Observations on the alimentary tract and

its bacterial flora in healthy and diseased pigs. *J. Pathol. Bacteriol.*, **86**, 387–412.

Smith, M.W. and Jarvis, L.G. (1978) Growth and cell replacement in the new-born pig intestine. *Proc. R. Soc. London, B.*, **203**, 69–89.

Söderlind, O., Olson, E., Smyth, C.J. and Möllby, R. (1982) Effect of parenteral vaccination of dams and intestinal *Escherichia coli* in piglets with diarrhea. *Infect. Immun.*, **36**, 900–6.

Söderlind, O., Thafvelin, B. and Möllby, R. (1988) Virulence factors in *Escherichia coli* strains isolated from Swedish piglets with diarrhea. *J. Clin. Microbiol.*, **26**, 879–84.

Søgaard, H. (1987) Det teoretiske grundlag for probiotikakonceptet. *Dansk Vet. Tidskr.*, **70**, 310–20.

Spicer, E.M., Driesen, S.J., Fahy, V.A., *et al.* (1986) Causes of preweaning mortality on a large intensive piggery. *Austral. Vet. J.*, **63**, 71–5.

Spriet, S.M., Decuypere, J.A. and Henderickx, H.K. (1987) Effect of *Bacillus toyoi* (Toyocerin) on the gastrointestinal microflora, concentration of some bacterial metabolites, digestibility of the nutrients and the small intestinal mean retention time in pigs. *Med. Fac. Landbouww. Rijksuniv. Gent*, **52**, 1673–83.

Stevens, A.J. and Blackburn, P.W. (1967) An indirect approach to piglet scours *Vet. Record*, **80**, 637–8.

Tannock, G.W. (1984) Control of gastrointestinal pathogens by normal flora, in *Current Perspectives in Microbial Ecology* (eds M.J. Klug and C.A. Reddy), Proc. 3rd Int. Symp. Microbial Ecology, Michigan State Univ., 7–12 Aug 1983. American Society for Microbiologists, Washington, DC, pp. 374–82.

Tannock, G.W. (1990) The microecology of lactobacilli inhabiting the gastrointestinal tract, in *Advances in Microbial Ecology*, vol. 11 (ed. K.C. Marshall), Plenum Press, New York, pp. 147–71.

Tannock, G.W. and Archibald, R.D. (1984) Derivation and use of mice which do not harbour lactobacilli in the gastrointestinal tract. *Can. J. Microbiol.*, **30**, 849–53.

Tannock, G.W. and Savage, D.C. (1974) Influences of dietary and environmental stress on microbial populations in the murine gastrointestinal tract. *Infect. Immun.*, **9**, 591–8.

Tannock, G.W. and Smith, J.M.B. (1970) The microflora of the pig stomach and its possible relationship to ulceration of the *pars oesophagea*. *J. Comp. Pathol.*, **80**, 359–67.

Tannock, G.W., Szylit, O., Duval, Y. and Raibaud, P. (1982) Colonization of tissue surfaces in the gastrointestinal tract of gnotobiotic animals by *Lactobacillus* strains. *Can. J. Microbiol.*, **28**, 1196–8.

Tannock, G.W., Fuller, R. and Pedersen, K. (1990) *Lactobacillus* succession in the piglet digestive tract demonstrated by plasmid profiling. *Appl. Environ. Microbiol.*, **56**, 1310–16.

Thacker, P.A. (1988) Novel approaches to growth promotion in the pig, in *Recent Advances in Animal Nutrition* (eds W. Haresign and D.J.A. Cole), Butterworth, London, pp. 73–84.

Tournut, J. (1989) Applications of probiotics to animal husbandry. *Rev. Sci. Tech. Off. Int. Epiz.*, **8**, 551–66.

Trevallyn-Jones, J. (1987) Field trials with a recombinant DNA scours vaccine,

in *Manipulating Pig Production* (eds APSA Comittee), Australian Pig Science Association, Werribee, Victoria, Australia, p. 231.

Tuschy, D. (1986) Verwendung von 'Probiotika' als Leistungsförderer in der Tierernährung. *Übers. Tierernährg*, **14**, 157–78.

Tzipori, S. (1985) The relative importance of enteric pathogens affecting neonates of domestic animals. *Adv. Vet. Sci. Comp. Med.*, **29**, 103–206.

Underdahl, N.R. Torres-Medina, A. and Doster, A.R. (1983) Effect of *Streptococcus faecium* C-68 in control of *Escherichia coli*-induced diarrhea in gnotobiotic pigs. *Amer. J. Vet. Res.*, **43**, 2227–32.

Ushe, T.C. and Nagy, B. (1985) Inhibition of small intestinal colonization of enterotoxigenic *Escherichia coli* by *Streptococcus faecium* M74 in pigs. *Zbl. Bakteriol. Hyg., I Abt. Orig. B*, **181**, 374–82.

Vanbelle, M., Teller, E. and Focant, M. (1989) Probiotics in animal nutrition; a review. *Lab. Biochim. Nutr., Belgium*, **55**, 1–59.

Visek, W.J. (1978) The mode of growth promotion by antibiotics. *J. Anim. Sci.*, **46**, 1447–69.

Vodovar, N., Flanzy, J. and François, A.C. (1964) Intestin grele du porc. I. – Dimensions en fonction de l'age et du poids, étude de la jonction du canal cholédoque et du canal pancréatique a celui-ci. *Ann. Biol. Anim. Biochem. Biophys.*, **4**, 27–34.

Wadström, T., Andersson, K., Sydow, M. *et al.* (1987) Surface properties of lactobacilli isolated from the small intestine of pigs. *J. Appl. Bacteriol.*, **62**, 513–20.

Walton, J.R. (1980) Modes of action of growth promoting agents. *Fortschr. Vet. Med.*, **33**, 77–82.

Wells, C.L., Johnson, W.J., Kan, C.M. and Balish, E. (1979) Inability of a genetically engineered strain of *Escherichia coli* (c 1776) to colonize germfree animals, in *Clinical and Experimental Gnotobiotics* (eds Fliedner *et al.*), *Zbl. Bakteriol. Suppl.*, **7**, 197–200.

White, F. Wenham, G., Sharman, G.A.M. *et al.* (1969) Stomach function in relation to a scour syndrome in the piglet. *Brit. J. Nutr.*, **23**, 847–58.

Wilson, W.R. and 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**, 230–43.

Wolter, R. and Henry, N. (1982) Les 'probiotiques' en alimentation animale. *Rec. Méd. Vét.*, **158**, 283–90.

Zani, G., Biavati, B., Crociani, F. and Matteuzzi, D. (1974) Bifidobacteria from the faeces of piglets. *J. Appl. Bacteriol.*, **37**, 537–47.