Microflora Management in the Gastrointestinal Tract of Piglets

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ABSTRACT: The stressful physiological and environmental conditions around weaning often promote the proliferation of pathogens in the digestive tract of piglets resulting in diarrhoea and reduced daily weight gain. Typical dietary practices to maintain growth performance and health have led to an increased use of antimicrobial growth promoters. Due to the advanced ban of antibiotics in pig production, new concepts have been developed to secure animal health and growth performance, feed efficiency, and product quality as well. Several naturally occurring compounds seem to beneficially affect the composition and activity of the microflora in the gastrointestinal tract (GIT) of pigs. These are, among others, organic acids, probiotics, prebiotics, and enzymes. Organic acids are already widely used, especially in pigs, due to their positive effects on GIT health and growth performance. Probiotics have been shown to be effective against diarrhoea though effects may be dependent on diet composition and environmental conditions. Prebiotics may influence composition and activity of the intestinal microflora. Additionally, pre- and probiotics may exert positive influences on immune response, whereas enzymes may enhance feed digestibility by breaking down anti-nutritional factors. In the following, the focus will be directed to the role of organic acids, probiotics, prebiotics, and feeding enzymes as potential modulators of GIT health. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 9 : 1353-1362)***

Keywords: Microflora, In-feed Antibiotics, Organic Acids, Probiotics, Prebiotics, Enzymes, Pigs, GIT

INTRODUCTION

Many feed additives are designed to secure animals' health status and to optimize growth performance. Among others, in-feed antibiotics are frequently used in intensive animal production systems. In pig production, the supplementation of in-feed antibiotics is widely practiced around weaning - the most critical period for a pig due to its immature gastrointestinal tract (GIT). However, because of growing public concern about the potential risk of antibiotic cross-resistance from animal products to humans an increasing number of antimicrobial growth promoters has been banned in Europe, the US, and Canada (Piva, 1998; Hillman, 2001). As a result of this ban, markedly increased incidences of subclinical diseases and a decrease in animal performance, such as a reduced average daily weight gain and increased feed conversion efficiency, have been reported (e.g. Brufau de Barberà, 2000). This is especially the case when animals are housed under poor management conditions. Consequently, there is major interest in developing suitable alternatives which support the indigenous microflora of the GIT in their approach to control pathogenic bacteria.

Besides, animal production systems without in-feed antibiotics require high hygiene standards in terms of animal housing conditions, feed processing and storage, an advanced production management (age of weaning, age-mates in clan facilities: all-in-all-out), as well as adequate nutrient supply (Simon et al., 2003). It is unlikely that alternative feed-based growth promoters will be able to overcome serious management problems that have been managed in the past through therapeutic antibiotic use (Cromwell, 2001). However, it should be considered that the potential of antibiotics to enhance performance is also directly affected by husbandry conditions, with benefits of antimicrobials being more pronounced when suboptimal conditions occur. Thus, the first step in reducing the use of growth promoting antibiotics should be to optimize the management of the pig facilities, particularly during critical growth periods and times of stress such as weaning.

Ban of antibiotic growth promoters in Europe

During the last four decades, the increase of antibiotic resistant bacteria has led to intensified discussions about the use of antimicrobial substances. It is generally accepted that there exists a relation between the use of antibiotics and the development of resistance in human beings and animals. Therefore, attempts were made to monitor use and resistance of antimicrobial substances in humans and food-producing animals.

In 1969, the Swann Committee of the United Kingdom recommended that antibiotics used in human chemotherapy should not be used as in-feed antibiotics (e.g. tetracyclines). Sweden was the leading country in banning antimicrobial growth promoters in Europe; the use of antibiotics as feed additives was banned in 1986. Since that time, antibiotics were only permitted as therapeutics to cure or prevent

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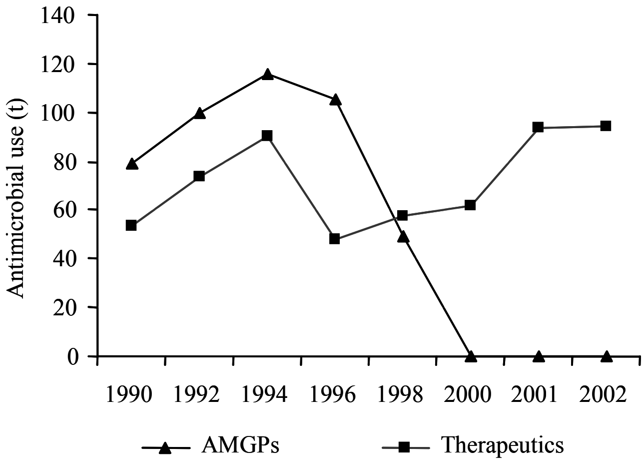

Figure 1. Antimicrobial growth promoter (AMGP) and therapeutic antimicrobial use in food-producing animals in Denmark (adapted from DANMAP 2002).

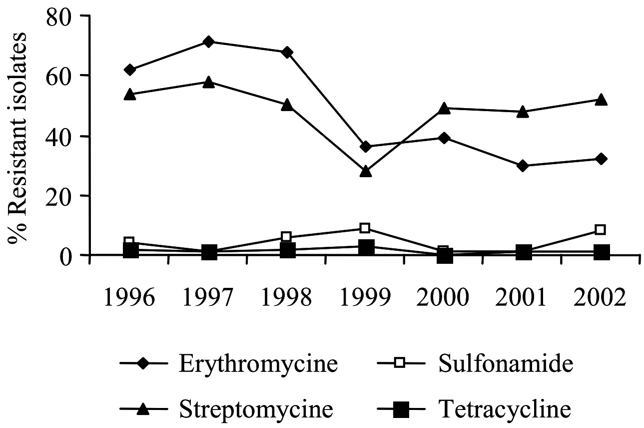

Figure 2. Trends in resistance to selected antibiotics among *Campylobacter coli* from pigs in Denmark (DANMAP 2002).

disease provided that a veterinary prescription had been issued. Sweden's experience from animal production without the use of in-feed antibiotics shows that under professional production conditions, excellent production results can be achieved without the use of in-feed antibiotics. Since 1987, the post-weaning mortality has decreased by 0.9% and the average body weight of 25 kg was reached by 1-2 days earlier. However, during the first years after the ban, the therapeutic use of antibiotics increased, especially in piglet production due to severe health problems. Therefore, major efforts were undertaken to improve management conditions and the hygienic standards. Since 1993, a gradual decrease in the use of antibiotics could be observed. In 1998, only 15% of piglets received either antibiotics or zinc-oxide during rearing (Wierup, 1998).

Denmark followed the example of Sweden and banned the use of feed-grade antibiotics in pork production at the finishing stage in 1998, and at the weaning stage in 2000. Since 2000, the use of antimicrobials as in-feed antibiotics in Denmark has almost been terminated (Figure 1). However, the ban of feed antibiotics at the weaning stage has also led to severe health problems, increased costs, and above all, it has augmented the therapeutic use of antibiotics.

The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) has implemented since 1997 a major effort to track antibiotic resistance in animal and human bacteria. Since 2000, the prescription of antibiotics additionally is monitored by means of a program called VetStat, based on type, farm, and veterinarian (Hayes and Jensen, 2003). The data of these monitoring programs suggest that the decreasing use of in-feed antibiotics has already reduced the incidence of resistance of different microbial strains (e.g. *Enterococcus faecium*, *Campylobacter spp.*, *E. coli*) to certain antibiotics, such as Avoparcin, Virginiamycin, and Macrolides (DANMAP 2002, Figure 2).

In 1999, the EU Scientific Steering Committee has reviewed the medical and non-medical use of antimicrobial substances in the EU (European Commission, Health & Consumer Protection Directorate, 2003). As a consequence, the use of in-feed antibiotics in the EU was restricted in 1999 as a precautionary measure to minimize the risk of the development of resistant bacteria and to preserve the efficacy of certain antibiotics used in human medicine. This ban included Bacitracin, Tylosin, Spiramycin, Virginiamycin, Olaquindox, and Carbadox. Avoparcin had already been banned since 1997. Nowadays, only four antimicrobial growth promoters are still approved as feed additives in the EU including lavophospholipol, salinomycin sodium, avilamycin and monensin sodium. However, these products are scheduled to be banned by 2006.

Mode of action of antimicrobial growth promoters versus alternative feed additives

In general, alternatives to in-feed antibiotics should reduce the colonisation of the GIT with pathogens, prevent enteric diseases, maximize daily gain and feed intake, improve the feed conversion efficiency and minimize pollution. However, the growth promoting mechanisms of antibiotics and those of alternative feed additives may differ considerably. Consequently, this likely will affect the efficacy of the alternatives compared to in-feed antibiotics. For example, antibiotics supplied at subtherapeutic levels act in part by decreasing the microbial load in the gut, resulting in a reduction in intestinal cell turn over, and thereby reducing the energy needs of the intestinal tissues (Mathew, 2002). This additional energy becomes available for the animal, thus contributing to growth performance. Furthermore, the feeding of antimicrobials suppresses the hostile microbes which allows the host animal to perform

Table 1. Mechanisms of organic acids and their salts (adapted from Kirchgessner and Roth, 1988)

Feed		pH decrease Antimicrobial effect (bacteria, yeast, fungi) Reduced buffering capacity
GIT	Proton	pH decrease in the stomach Increase in efficiency of pepsin (pH optimum 2.5 and 3.5) Antimicrobial effect
	Anion	Antimicrobial effect Complexing agent (Ca^{2+}, Mg^{2+}, Fe^{2+} etc.)
Intermediary metabolism		Energy source

more closely to its maximum genetic potential (Cromwell, 2001). By contrast, most alternatives to in-feed antibiotics have not been proven to reduce overall microbial loads in the GIT which means that they likely will not act in the same way as antimicrobial growth promoters. Instead, alternative compounds may for example alter the proportions of specific GIT bacteria through promoting the colonization of more favourable species, thereby suppressing harmful bacteria. Therefore, beneficial effects of alternative growth promoters on growth performance and health may be more subtle than those of antibiotics (Mathew, 2002).

GIT microflora

The GIT of pigs harbours about 10^{14} microorganisms whereby >90% of the microbes are gram-positive, strict anaerobic bacteria, such as streptococci, lactobacilli, eubacteria and peptostreptococci (Robinson et al., 1981; Moore et al., 1987; Leser et al., 2002). Most of the microorganisms inhabiting the GIT are harmless and do not cause intestinal diseases. However, due to the complexity of the intestinal microbial community, the majority of the bacteria in the GIT of pigs have not been characterized (Leser et al., 2000). Compared to the total number of species of bacteria in the GIT, only a few of them (e.g. *E. coli, Salmonella spp.*) are able to disturb the microbial balance. In the healthy pig, this balance is based on the constant competition between bacteria for adhesion sites and nutrients within the lumen of the digestive tract (Mosenthin, 2003).

The composition of the GIT microflora influences growth performance and the health status of the animal, whereby the composition of feed influences directly or indirectly the composition of the microbes (Diekenhorst, 2002). However, there exist many potential stressors that may interfere with the microbial balance between harmless and pathogenic bacteria including parasites, moulds and mycotoxins, poor water and feed quality, humidity, NH_3, H_2S, dust, temperature variation, social stress, and an immature digestive system. These stress factors may favour the proliferation of pathogenic bacteria so that they dominate their harmless counterparts in different sections of the GIT. As far as profitability of pig production is concerned, the most devastating effect of a disturbed microbial balance is the increased incidence of diarrhoea. This occurs mainly at the time around weaning or in the periods of sudden change in feed composition (Ewing and Cole, 1994; Mosenthin, 2003).

Organic acids

Organic acids have been used successfully in animal feeding for years. Their efficiency is well documented and extensively described in the literature. The antimicrobial and growth-promoting effects of organic acids and their salts render them a realistic alternative to antibiotic growth promoters in starter diets for early weaned pigs (Mosenthin, 2003). Mechanisms and sites of activity include preservative effects in animal feeds and the GIT following ingestion (Roth et al., 1993). More recently, it has been shown that short-chain fatty acids (SCFA), particularly butyric acid, may play an important role in controlling cell proliferation and differentiation, and induction or repression of gene expression (Davie, 2003).

The mechanisms of organic acids and their salts are summarized in Table 1. The influence of organic acids in the GIT can be split into two parts, the effect of the proton (acidification) and the effect of the anion. The proton acts primarily in the stomach whereas the major site of action of the anion is the small intestine. The addition of organic acids supports the acidification in the stomach, thereby increasing proteolytic enzyme activity which subsequently may improve the digestibilities of crude protein and amino acids (Gabert and Sauer, 1994). Associated with the enhanced amino acid digestibility, microbial fermentation of undigested dietary protein is reduced. This may be indicated by a reduced production of toxic polyamines (cadaverine and putrescine) as well as ammonia in the ileum and caecum (Blank et al., 1999, 2001). Likewise, the production of SCFA (e.g. acetic, propionic and butyric acids) in the GIT is reduced indicating decreased microbial growth. The antimicrobial effect of organic acids seems to be related to a decrease in pH and a specific effect of the anion affecting amino acid metabolism, DNA synthesis, energy metabolism and cell membrane permeability of

Table 2. Effect of organic acids and their salts on growth performance in weanling pigs (adapted from Mroz (2003) and Partanen (2001))

	Formic acid	Fumaric acid	Citric acid	Potassium diformate
Experiments	6	18	9	3
Observations	10	27	19	13
Acid levels (g/kg)	3-8	5-25	5-25	4-24
Feed intake (g/d)				
Control	667	613	534	764
Experimental	710	614	528	823
p<	0.01	0.42	0.14	0.001
Weight gain (g/d)				
Control	387	358	382	479
Experimental	428	374	396	536
p<	0.001	0.01	0.01	0.02
Feed:gain				
Control	1.64	1.59	1.67	1.60
Experimental	1.60	1.55	1.60	1.54
p<	0.02	0.01	0.01	0.02

Table 3. Species mainly used in probiotic preparations (adapted from Durst et al., 1998; Lee et al., 1999)

Species used as probiotics
Bacteria
Lactobacillus bulgaricus
L. acidophilus
L. paracasei
Streptococcus thermophilus
Enterococcus faecium
E. faecalis
Bifidobacterium pseudolongum
B. thermophilum
B. breve
B. bifidum
Bacillus cereus
B. toyoi
B. subtilis
Yeast
Saccharomyces cerevisiae
S. boulardi

specific bacteria (Adams, 1999).

Various acids seem to affect the population of different microorganisms differently. As pointed out by Canibe et al. (2001), potassium diformate and formic acid reduce the growth of yeasts in the GIT, while lactic acid may promote growth of yeast. It has been postulated that yeast can protect against invading pathogens by binding their toxins and stimulating the immune system (Mul and Perry, 1994). By contrast, yeast can also cause diseases in man and animals (Hurley et al., 1989).

Knarreborg et al. (2002) investigated *in vitro* the effects of several organic acids on changes in the populations of coliforms and lactic acid bacteria in the gastric digesta and in small intestine contents of pigs, at different pH values. This study demonstrated a clear selective removal of target species, i.e. coliform bacteria from the gastric digesta. The bactericidal effect towards coliform bacteria could be established according to the following order: propionic< formic<butyric<lactic<fumaric<benzoic acid. The survival rate of bacteria was strongly influenced by pH, irrespective of the GIT segment. Benzoic acid showed the strongest bactericidal effect on coliforms as well as on lactic acid bacteria. Furthermore, addition of potassium diformate resulted in a reduced growth of coliform and lactic acid bacteria. In a recent study, Franco et al. (2005), investigated the effect of combinations of organic acids given to 19-21 days old weaned piglets on composition of intestinal microflora. In this study, a combination of formic and lactic acid led to a decrease in small intestinal coliforms as compared to a control group, receiving no organic acids.

As summarized in Table 2, formic acid as well as potassium-diformate evoke the most pronounced responses of growth performance in piglets. According to a review by FB Agrarwirtschaft Soest (1998), sorbic acid is the most efficient organic acid in improving daily gain and feed conversion efficiency by 20 and 10%, respectively, compared to the control. However, it has to be mentioned, that sorbic acid is considerably more expensive than formic acid, and has not been frequently used in practical pig diets.

Probiotics

According to a widely accepted definition by Fuller (1989), probiotics can be characterized as "live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance". The probiotic effects of lactic acid-producing bacteria have received most attention, probably due to their predominance within the GIT microflora, the historical perception of health-links and, additionally, the observation that they are rarely pathogenic (Kelly, 1998). The species currently being used in probiotic preparations are summarized in Table 3.

The mode of action of probiotics with respect to beneficial effects on GIT health, growth performance and immune functions of pigs remains speculative (Cromwell, 2001). The most favoured hypothetical mode of action is the concept of competitive exclusion (Kelly, 1998). From the data available, it can be postulated that probiotics may exert influence on GIT microflora, epithelial lining, gut-associated lymphoid tissue (GALT), and the neuro-endocrine system. However, probiotics do not act like essential nutrients in terms of a clear dose-response relationship (Simon et al., 2003).

Since probiotics are discussed as alternatives to antimicrobial growth promoters in pig husbandry, their impact on performance is of major interest. However, the effect of different types of probiotics on growth performance in piglets varies considerably. In a review considering 44 studies (FB Agrarwirtschaft Soest, 1998), in which mainly lactic acid-producing bacteria or *Bacillus spp.* were used as supplements, daily gain and feed to gain ratio

Table 4. Incidence of diarrhoea in piglets fed probiotic supplemented feed (in comparison to non-treated animals) (adapted from Simon et al., 2003)

Probiotic strain	Age	Incidence of diarrhoea
B. cereus	8 weeks	Reduced*
B. cereus	Day 1-85	Reduced*
B. cereus	Day 7-21	Reduced*
B. cereus	Day 24-66	No effect
B. cereus	25 kg live weight	No effect
B. cereus	2 weeks post weaning	Reduced*
E. faecium	Day 1-70	Reduced*
E. faecium	8 days before/after weaning	Reduced*
Peptostreptococcus acidilactici	Day 5-28	Reduced*
P. acidilactici	Day 5-28	Reduced*
S. cerevisiae	Day 5-28	Reduced*

* $p<0.05$.

were significantly ($p<0.05$) improved in five studies only. There were no significant ($p>0.05$) growth promoting effects of probiotic supplementation to diets for grower-finisher pigs, and the results obtained for sows were equivocal. Among others main reasons for the variability of the results include the viability of microbial culture, strain differences, dose level and frequency of feeding of the culture, drug interactions and a lack of systematic research.

The effect of probiotics in relation to incidence of diarrhoea is well investigated, since diarrhoea is one of the main problems in piglet husbandry during the first weeks after weaning. From a recently published review, it seems that most of the studies, though not all, could show a significant reduction ($p>0.05$) in the incidence of diarrhoea (Table 4, Simon et al., 2003). More recently, Alexopoulos et al. (2004a) showed that administration of a probiotic product containing spores of *Bacillus licheniformis* and *Bacillus subtilis* to sows and their litters resulted in a significant decrease in the diarrhoea score of the piglets, as compared to untreated controls. In this study, the probiotic was administered starting two weeks prior to expected farrowing until the weaning day. Furthermore, administration of the same probiotic strains during weaning, growing and finishing stages resulted in a lower mortality as well as improved weight gain and feed conversion ratio, in comparison to untreated controls (Alexopoulos et al., 2004b). Taras et al. (2005) supplemented diets for pregnant sows and their litters with the probiotic *Bacillus cereus* var. toyoi. They found a significant reduction in the incidence of diarrhoea in weaned piglets. By use of the probiotic strain *Enterococcus faecium* NCIMB 10415, Schierack et al. (2004) could show up to 50% reduction of enterpathogenic *E. coli* serogroup O141. Similar results were observed for total β-haemolytic *E. coli*, but not for total coliform bacterial counts, suggesting that there is no general exclusion effect against *E. coli* strains caused by the probiotic *Enterococcus faecium*. However, Huang et al. (2004) observed significantly reduced *E. coli* and aerobe counts ($p<0.01$) and increased lactobacilli and anaerobe counts ($p<0.01$) in digesta and most sections of the GIT as well as a 66 and 69% decrease in diarrhoea index and diarrhoea incidence. In this study, weanling piglets were orally administered a probiotic preparation containing *L. gasseri*, *L. reuteri*, *L. acidophilus* and L. *fermentum*, which were isolated from the GIT of weanling piglets. On day 8 they were challenged by *E. coli* (serovars K99, K88 and 897P at the ratio 1:1:1). In conclusion, the effects of different probiotic strains on the reduction of diarrhoea may vary, however, these feed additives seem to be a suitable approach in terms of preventing diarrhoea.

Prebiotics

Another approach to protect the host against infections with pathogens may be to enhance the beneficial activity of the microflora by addition of specific ingredients to the diet. This idea has led to the introduction of the term “prebiotics”. Prebiotics are defined as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health” (Gibson and Roberfroid, 1995).

Currently, the most promising candidates for acting as prebiotics are the non-digestible-oligosaccharides (NDO). Physiologically, functional oligosaccharides are natural constituents of plants such as legume seeds (Bach Knudsen, 1997) and cereals (Henry and Saini, 1989) consisting of 2-10 sugar units. Oligosaccharides may contain similar or different sugars, different linkage structures and may be linear or branched. In addition, NDO such as fructo-oligosaccharides (FOS) and transgalacto-oligosaccharides (TOS) can be manufactured under commercial conditions to be used as functional ingredients in feed or food. In contrast, mannan-oligosaccharides (MOS) are derived from yeast cell material.

The physical and chemical properties of NDO depend on their chemical composition. In general, prebiotic oligosaccharides are resistant to mammalian enzymes, yet are susceptible to fermentation by certain microbial

Table 5. Response to NDO in pig feeds

Source	Parameters	Response	Authors
MOS	Performance, microflora	No	White et al., 2002
	Immune system	Yes	
MOS	Performance	Yes	Davis et al., 2002
	Immune system	No	
FOS	Performance	Yes	Xu et al., 2002
FOS, TOS	pH, performance	No	Houdijk, 1998
FOS, TOS	pH, SCFA, microflora	Yes	Houdijk et al., 1997
	Digestibility	No	
TOS, soy solubles[1]	Microflora	Yes	Smiricky-Tjardes et al., 2003
Soybean galactooligosaccharides[2]	Nutrient and energy digestibility	No	Zhang et al., 2001
Galactosyl lactose	Performance, digestibilities		Mathew et al., 1997
	SCFA, microflora	No	
STOC[3]	Performance	No	Orban et al., 1996
Lactulose, inulin	Microflora, digestibilities	No	Branner et al., 2004

[1] Containing raffinose, stachyose and sucrose; [2] Containing raffinose and stachyose; [3] STOC: Sucrose thermal oligosaccharide caramel.

populations (Patterson and Burkholder, 2003). For example, certain NDO specifically promote the proliferation of bifidobacteria (Hidaka et al., 1986), and are therefore also referred to as "bifido growth factor". According to various studies in different species including humans and pigs (e.g. Sghir et al., 1998; Nemcová et al., 1999) the site and extent of fermentation in the GIT is dependent on various factors including chemical properties of the NDO such as sugar composition, types of linkages, degree of polymerisation, and the physical structure. Many NDO may be exclusively fermented by saccharolytic bacteria, such as bifidobacteria. For example, FOS and TOS will primarily stimulate the production of acetate and lactate in the small intestine and, particularly, in the large intestine (Wang and Gibson, 1993). Due to the low pK_a of these acids, these NDO may increase the barrier effect against infections by enteric pathogens, such as clostridia, *Salmonella sp.* and *E. coli.* However, there is growing evidence that NDO are not fermented by saccharolytic bacteria only. Studies by Hartemink and Rombouts (1997) revealed that a significant proportion of different sources of NDO including FOS and TOS were fermented by other species than bifidobacteria including species such as clostridia, enterobacteria and *E. coli*. Thus, the bifidogenic effect of NDO, such as FOS and TOS will be diminished by these bacteria. However, the mode of action of MOS differs from the other NDO. MOS have a high affinity for specific glycoproteins (lectins) on pathogenic bacteria which prevent their attachment to the intestinal mucosa (Ewing and Cole, 1994). The attachment of pathogenic bacteria to the epithelium of the GIT is an essential step in the development of intestinal infections. As a result, beneficial bacteria are given the opportunity to attach and to colonize, therefore delivering beneficial effects to the host (Cromwell, 2001).

Results of the effects of prebiotics on parameters such as growth performance, SCFA production or microbial composition are equivocal (Table 5). There may be several factors involved that partly explain the lack of response to supplementation of NDO. This includes the superior housing conditions in research stations which often do not correspond to the conditions in practice (Mul and Perry, 1994), and the supplementation level because at higher dietary levels NDO may act as antinutritional factors (Benno et al., 1987; Fishbein et al., 1987).

However, Konstantinov et al. (2003, 2004) analysed the composition of the GIT microbial community in piglets fed combinations of various fermentable carbohydrates by use of molecular methods. In one study, weaning piglets were fed either sugar beet pulp and FOS (Konstantinov et al., 2003), or a control diet without any fermentable carbohydrates. In another study (Konstantinov et al., 2004), weaning piglets received a diet containing inulin, lactose, wheat starch and sugar beet pulp, as compared to a control diet without any fermentable carbohydrates. The authors found a greater diversity and more rapid stabilisation of the GIT community due to the introduction of fermentable carbohydrates to piglet's diets. It seems that, in addition to NDO, various fermentable carbohydrates might display prebiotic properties. In terms of health functionality, larger and more slowly fermentable polysaccharides might provide an advantage over the rapidly fermented NDO, in that they provide a carbohydrate source for SCFA production and suppression of protein metabolism more distally in the digestive tract. According to Rastall and Gibson (2002), long-chain inulin may exert a prebiotic effect in more distal colonic regions than the lower molecular weight FOS. Indeed, there is increasing evidence that some NDO are completely fermented either by the end of the terminal ileum (FOS) or within the proximal large intestine (TOS), and are therefore unavailable for microorganisms in the distal colon (Houdijk, 1998). Since plant-derived inulin is intrinsically limited in its degree of polymerisation, Rastall and Gibson (2002) suggest that polysaccharides such as the microbial fructan laevan may be fermented slower, thereby increasing chances of persistence until the distal colon.

Recently, the concept of "synbiotics", a mixture of probiotic strains and NDO has been proposed to characterize health-enhancing foods and supplements used as functional food ingredients in humans (Gibson and Roberfroid, 1995). Pairing NDO and probiotic strains that have the metabolic potential of fermenting the supplied NDO at a competitive rate compared to the indigenous microflora, is likely to be a successful strategy in controlling the intestinal ecosystem. The expected benefits are an improved survival rate during the passage of the probiotic bacteria through the upper GIT and a more efficient implantation in the colonic microflora together with a stimulating effect of the NDO on the growth and/or activity of both the exogenous (probiotic) and the indigenous bacteria (Roberfroid, 1998). It has been recognized that pre- and probiotics are most effective in young animals when the immature digestive system is still under development. Studies with piglets have revealed synergistic effects of combinations of different probiotics and prebiotics in terms of improved growth performance (Kumprecht and Zobac, 1998), decreased mortality rate (Nousianen and Setälä, 1993) and increased counts of total anaerobes, lactobacilli and bifidobacteria in fecal samples of young pigs (Nemacová et al., 1999). However, there is a need for refocusing on the fundamental principles of microbial ecosystems and host/bacterial interactions to elucidate the synbiotic mechanisms in more detail.

Enzymes

One reason for the addition of exogenous hydrolytic enzymes to weaning diets is to compensate potential deficiencies in endogenous enzyme secretion in weaning piglets. Exogenous enzymes may also enhance feed digestibility by breaking down anti-nutritive factors present in feedstuffs, including non-starch polysaccharides (NSP), mostly β-glucans and xylans, phytic acids and protease inhibitors present in certain cereals and legumes (Li et al., 2003). Enzyme preparations commercially used mainly consist of NSP-degrading enzymes as well as of microbial phytases (Mosenthin and Diebold, 2000).

The efficacy of NSP-degrading enzymes in the GIT has been attributed to several possible modes of action (Haberer, 1997). For example, they may lead to a decreased viscosity of digesta, associated with an enhanced absorption of nutrients. Furthermore, they may contribute to the removal of the so called "cage effect". This mainly affects nutrients (protein, starch, lipids) located within the cell. Non-starch polysaccharides protect these nutrients against enzymatic hydrolysis. Through breakdown of NSP by means of exogenous enzymes, the enclosed nutrients become available for the animal.

There seems also to be an influence on the composition of the microflora due to changes in transit time and morphology of the GIT, a shift in hydrolysis products available for fermentation, as well as interferences between bacteria (Simon, 2000). Relatively little is known about the effect of enzyme supplementation on the gastrointestinal microflora and possible implications for the host animal. One of the first studies investigating the effects of enzymes on the microflora of the GIT was performed in broilers (Vahjen et al., 1998). In this study, xylanase (ZY 68) supplementation to a diet with wheat as the sole cereal component caused shifts in the spectrum of microbial species in the GIT. Xylanase supplementation led to significantly lower colony forming units (CFU) per gram of wet weight for enterobacteria and gram-positive cocci in luminal and tissue samples during the first 3 weeks of life as well as to increased numbers of tissue-associated *Lactobacillus spp.* from week 2 of age. With respect to enterobacteria and gram-positive cocci these observations were confirmed in subsequent studies, but not those for *Lactobacillus spp.* (Hübener et al., 2002). In weaning piglets, the addition of xylanase to a diet with wheat and rye as sole cereal components caused an increased metabolic activity of *Lactobacillus spp.*, including *Lactobacillus acidophilus, L. amylophilus* and *L reuteri*, in the mid and terminal jejunum and in the ileum, determined by means of molecular methods (16S rRNA hybrdization) (Simon et al., 2002). Increasing concentrations of lactate in ileal digesta should reflect an increased population and activity of lactobacilli and streptococci. Since ileal lactate concentrations did not differ among treatments, Diebold et al. (2004) suggested that there was no effect of dietary supplementation of phospholipase, xylanase or both enzymes on streptococci and lactobacilli in early-weaned piglets fed a wheat-based diet. In the same study, the combination of phospholipase and xylanase led to the highest increase in ileal SCFA production, whereas phospholipase and xylanase alone showed no effect or tended to increase ileal SCFA concentrations, respectively. However, large variations in the results reveal that microbial communities and their metabolic activities in the small intestine of individual pigs may differ considerably in their response to enzyme supplementation (Simon et al., 2002).

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