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Probiotics – do they have a role in the pig industry?

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The delivery of certain living microorganisms in food has long been suggested as having positive health effects in humans. This practice has extended into food animal production, with a variety of microorganisms being used; lactic acid bacteria, various Bacillus *species and the yeast* Saccharomyces cerevisiae *have been particularly used in the pig industry. The increased interest in probiotics is essentially due to the problem of microbial resistance to antibiotics and following the ban of the use of antibiotics in animal production, probiotics being considered an alternative means to reduce pathogen infection and improve animal health especially around the time of weaning. However, there is still a need to clarify the probiotic effectiveness in pigs, and the underlying mechanisms. When assessing the efficacy of probiotics one must consider the particular strain of organism being used and the production stage of the pigs being treated. The reproducible delivery of probiotics in industrial pig production is problematic as maintenance of viability is key to their beneficial activity, but difficult to achieve with commonly used feed processing technologies. One specific context where probiotics organisms may be reliably delivered is in systems utilising fermented liquid feeds. Liquid feed may be fermented by the activity of wild lactic acid bacteria or may be stimulated using specific isolates as 'starters'; the latter system has advantages in terms of reproducibility and speed of fermentation. The farm context in which the organism is used is likely to be critical; the use of probiotics is more likely to result in measurable economic gains in animals living in sub-optimal conditions rather than in those reared in the highest welfare and husbandry conditions. The establishment of a beneficial lactic acid bacteria population at birth may lead to healthier animals, this may be most effectively achieved by treating sows, which provide an amplification step and flood the neonatal pigs' environment with desirable bacterial strains. In contrast, it may be sufficient to provide a supportive, protective microbiota around the time of weaning as this is a time of major crisis with instability and loss of certain bacterial populations.*

Keywords: pig, probiotic, perfomance, health

Implications

This review provides the scientific background to the use of probiotics in the pig industry to control bacterial gut infection. Given the European Union ban on the use of prophylactic antibiotics, this approach could have a significant positive effect upon the economic viability of pig producers.

Introduction

The concept of probiotics, defined as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001)', was first noted by Metchnikov in his book 'The Prolongation of Life' in 1908. He ascribed the noted longevity of certain Bulgarian peasants to their high consumption of milk products fermented with lactic acid bacteria (probably *Lactobacillus delbrueckii* subspecies *bulgaricus*). The mechanism by which this happened was supposed to be via modification of the community of bacteria present in the colon; Metchnikov postulated that many human ills were due to the overgrowth of undesirable colonic bacteria.

A large amount of work on the efficacy of probiotics in human disease has been carried out (for recent reviews see Marchesi and Shanahan, 2007; Doron *et al.*, 2008; Parkes *et al.*, 2009; Collado *et al.*, 2009; Lomax and Calder, 2009). Certain aspects of this work can be applied to the pig, particularly mechanistic studies looking at the interaction of probiotics with host mucosal surfaces or pathogenic bacteria (Madsen *et al.*, 2001; Roselli *et al.*, 2007). However, this 'human model' does not give many insights into the efficacy of probiotics in terms of production parameters in the pig industry.

In this review, we will describe key aspects of the biological interactions between various mammals and probiotics

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with particular emphasis on possible underlying mechanisms. We will then review the literature on the use of probiotics in pigs, including information with respect to the most effective time of application. We will also discuss the use of fermented liquid feed in the pig industry as a means of delivering probiotic organisms. The delivery of probiotics to pigs is problematic due to the harsh processes used in feed processing and the inherent fragility of bacteria; technological aspects of delivery that may address these problems will be discussed.

Gut bacteria and health

Most of the health benefits ascribed to the administration of probiotics are linked to modulation of either host or bacterial factors in the gastrointestinal tract. It is thus appropriate to spend some time here considering the significance of bacteria to the host's well-being. The gastrointestinal tracts of humans and pigs are colonised by a wide array of bacteria, yeasts and viruses (Sears, 2005). In humans, the number of bacterial cells outnumbers the cells composing the host's body by 10-fold. This bacterial component of the host, particularly the bacteria of the gut, may be seen as an extra, indispensable organ, which contributes an array of gene products not native to the host, such as a plethora of specific glycosidases (Kim *et al*., 2007; Klaenhammer *et al*., 2008). Animals raised in the absence of bacteria show profound retardation in the developmental adult gut morphology and immune function (Nanthakumar *et al*., 2003; Wagner, 2008).

The endogenous microbiota provides critical support to the host in areas such as vitamin and co-factor production, usage of otherwise indigestible feed ingredients, detoxification of food components, coating the gut with a benign microbiota to physically exclude pathogens, production of natural antibiotics and antifungals, maintainance of gut barrier function and promotion of anti-inflammatory response (Madsen *et al*., 2001; Hooper *et al*., 2002; Ouwehand *et al*., 2002; Roselli *et al*., 2007). A novel role in regulating fat storage has been recently ascribed to microbiota by recent studies, a promotion of monosaccharides absorption from the gut resulting in induction of *de novo* hepatic lipogenesis has been shown by comparing germ-free mice with conventionalised mice (Bäckhed *et al*., 2004). Furthermore, an increased capacity to harvest energy from the diet has been observed by comparing gut microbiota of obese and lean mice (Turnbaugh *et al*., 2006).

Gut microbiota plays a critical role in 'educating' the neonatal gut immune system to generate functional adult systems for recognising pathogens and dealing with novel food antigens (Calder *et al*., 2006; Williams *et al*., 2006; Boirivant *et al*., 2008).

Early-life experience of the environment is critical in programming, or 'imprinting' the range of microbial biotypes which will accompany the host for their subsequent life (Zoetendal *et al*., 2001; Favier *et al*., 2002). The similarity of bacterial microbiota's varies between individuals on the basis of genetic relatedness and environmental experience (Mueller *et al*., 2006). A complex microbiota (many different biotypes) may confer advantages to the hosts by allowing rapid adaptation to environmental changes (Marchesi and Shanahan, 2007). The importance of early-life exposure on subsequent development of a rich, diverse microbiota is shown in studies comparing the microbiota of children normally delivered and those delivered by caesarean section; the latter children had markedly less complex microbiota (Gronlund *et al*., 1999; Biasucci *et al*., 2008). Children born and raised in relatively clean environments have been shown to have higher rates of atopy in later life, possibly reflecting the importance of bacterial diversity in the development of a competent, efficient immune system, this connection between over-clean environments and subsequent immunological dysfunction is referred to as 'The Hygiene Hypothesis' (Strachan, 1989).

However, although most studies indicate an association of gut microbiota composition with atopic disease, the specific harmful or protective microbes have not yet been identified (Penders *et al*., 2007). Recent results have highlighted the need to enlarge the concept of the hygiene hypothesis, and these aspects are well discussed in a recent review by Isolauri *et al*. (2009). According to these authors, three aspects should be considered in the re-evaluation of the hygiene hypothesis: the importance of gut microbiota composition in consolidation of healthy immune responsiveness; the new knowledge of immunomodulatory and suppressive immune responses extending the original 'T helper 1/T helper 2' paradigm; the role of host-microbe interaction in the development of not only atopic disease but also of other inflammatory diseases, including obesity.

It is unlikely that newborn pigs would be suffering from a deficit of microbial complexity in their environment under natural conditions. It is important to note that their endogenous microbiota is largely established at this time (Konstantinov *et al*., 2006; Thanantong *et al*., 2006). However, it is possible that piglets born into regularly sterilised farrowing accommodation may acquire a substantially different microbiota from the substrate than they would in an outdoor farrowing situation. This has indeed been shown in recent studies in pigs raised in different high *v.* low hygiene environments, which showed that such differences significantly affect not only intestinal microbiota composition but also the mucosal innate immune function in neonates, as well as in adult animals (Mulder *et al*., 2009; Inman *et al*., 2010).

Increasingly, research is taking place to look at bacterial 'imprinting' in early life. In an ideal world, the piglet should pick up a protective gut microbiota at birth which would improve nutrient availability by providing vitamins, short chain fatty acids or aminoacids (Cheeson, 1994; Metges, 2000; Resta, 2009), while protecting against environmentally acquired pathogens by direct and indirect (stimulation of the host immune system) means (Ouwehand *et al*., 2002; Bailey, 2009). This may be the most important 'window' for establishing a potentially beneficial bacterial community, in order to set up life-long, stable associations between the host and microbe.

Do probiotics work? – an overview

Anecdotally, probiotics have been thought to be useful in the treatment of numerous gastrointestinal disturbances in

humans and farm animals (Calder *et al*., 2006; Lallès *et al*., 2007; Marchesi and Shanahan, 2007; Collado *et al*., 2009; Setia *et al*., 2009; Shanahan, 2009). Especially, earlier studies have often suffered from a lack of rigorous study design, characterisation of probiotic strains, sufficient duration of treatment or description of host microbiota. Where studies have been carefully carried out, the commercial claims for many probiotics are difficult to substantiate. However, in humans, a number of gastrointestinal tract conditions appear to be alleviated by treatment with various lactic acid bacteria (LAB); for example, various types of inflammatory bowel disease have been shown to respond well to treatment with LAB (Mimura *et al*., 2004; Bibiloni *et al*., 2005). Similarly, there appears to be some benefit in prophylactic and reactive treatment with probiotics in traveller's diarrhoea (usually caused by enterotoxigenic *E. coli*), rotaviral diarrhoea in infants using *L. rhamnosus* GG and dirrhoea related to antibiotic use (Sazawal *et al*., 2006; Henker *et al*., 2008).

Historically, the situation in pigs is complicated by the fact that until recently, the industry routinely used antibiotic compounds as growth promoters. Although generally used at sub-therapeutic, these compounds almost certainly reduced intestinal pathogen loads. The most vulnerable times in the pig's life are immediately after birth and the 2-week period post-weaning. The legislative withdrawal of in-feed antimicrobials was expected to lead to increased mortality or decreased hardiness in these life stages. In fact, the situation seems to be more complex than this intuitive picture. In Sweden, the pig industry was, indeed, hit by the reduction in antimicrobial cover. However, the institution of relatively inexpensive husbandry modifications has allowed the industry to return to levels of productivity similar to those seen before the ban (Wierup, 2001). In addition, numerous institution-based experiments (as opposed to real farm conditions) have shown that in-feed antimicrobials are at their most effective in animals being raised under sub-optimal conditions (Dritz *et al*., 2002). Where high welfare, high health status animals are used, as in agricultural research centres, the gains produced by in-feed antimicrobials are marginal, at best (Zeyner and Boldt, 2006). These findings may inform subsequent research into the efficacy of probiotics.

Although many studies looking at the role of probiotics in pigs have been published (Bomba *et al*., 2002; Konstantinov *et al*., 2008; Bird *et al*., 2009; Lessard *et al*., 2009; Martin *et al*., 2009; Pieper *et al*., 2009; Szabo *et al*., 2009; Wang *et al*., 2009), it is very difficult to perform any meaningful metanalysis as the organisms, strains, doses and duration of the experiments are so varied, not to mention the confusing effects of husbandry, environment and genotype on the immune system and microbiota of the animals being studied. Certainly, it would be desirable to compare different probiotics and to follow the piglets through the weaning phase in well-controlled large-scale experiments.

To conclude, it seems unequivocal that certain bacterial supplements, under the appropriate conditions, can have a positive effect on the physiology of recipients. The difficulty lies in exact determination of the appropriate organism, dose of viable organisms and the life cycle stage of the recipient animal.

How do probiotics work?

We will limit the discussion here to the interactions occurring in the intestine. Probiotics might be expected to act either directly on the host, strengthening anti-pathogen defences, or the observed benefits may reflect the ability of a particular probiotic organism to adversely affect the survival of deleterious bacteria. Probiotics may also influence the availability of feedstuffs, enhancing the supply of some nutrients. Most bacteria used as probiotics are common intestinal bacteria such as species of *Bacillus*, *Lactobacillus* and *Bifidobacterium*. The roles of such commensal bacteria have received much attention in recent years; in particular, the ability to mono-colonise gnotobiotic animals has allowed us to see how important the bacteria of the gut are to its development (McFall-Ngai, 2002).

Probiotic actions on host physiology

Carriage of a large number of bacteria in the gut of a pig (and all other vertebrates) obviously has a cost in terms of energy. This cost may either be assimilation of nutrients by bacteria, thus reducing the availability of dietary components to the animal, or in terms of mounting an immune response to the bacteria. The effects of subclinical infections with pathogens are likely to be important with respect to production parameters, as energy spent fighting non-beneficial bacteria is energy lost to the animal, and farmer, in terms of growth and efficient feed conversion. It is in these compromised, but not overtly ill, animals that probiotics or other interventions to reduce the load of damaging bacteria may be most useful. To this end, it is interesting to note that a recent study showed a positive effect of probiotic treatment of *E. coli* F4 infected weaned piglets with *L. sobrius* not only on pathogen levels, but also on average daily weight gain (Konstantinov *et al*., 2008).

Cheeson (1994) identified a number of factors that may be expected to change with alterations to the intestinal bacterial microbiota in pigs, including an increase in the proportion of the amino acid pool that is available to other tissues (e.g. skeletal muscle), a reduction of endogenous nitrogen losses and a corresponding increase in apparent nitrogen digestibility and absorption. In fact, metabolic requirement is met not only by the diet but also by amino acids provided by the gastrointestinal microbiota, and from 1% to 20% of plasma, urinary and body lysine of the host has been calculated to derive from intestinal microbial sources (Metges, 2000).

Probiotics may also affect the absorption/secretion activity of intestine in pigs A slightly higher L-glutamine transport and increased ion secretion was observed in *Bacillus cereus* or *Enterococcus faecium* treated pigs, at 28 days of age (Lodemann *et al*., 2006; Lodemann *et al*., 2008). In a study carried out in pigs to screen lactic acid bacteria producing active dietary enzymes, such as amylase, lipase, phytase and protease, *Lactobacillus* sp. PSC101 was selected as a strong probiotic candidate due to its resistance to both acid and bile and production of dietary enzymes promoting animal growth

(Kim *et al*., 2007). A previous study in germ-free mice using the organism *Bacteroides thetaiotamicron* has shown that introduction of the bacteria is critical for induction of critical glycolytic enzymes in the enterocytes (Bry *et al*., 1996). Considering all these data, it follows that in immature animals there is a scope for enhancing positive interactions between host and microorganisms in the gut. It has now also been generally accepted that gut microbiota has to be considered a pivotal factor in shaping the host's metabolism, where differences in microbiota composition have strong effects on overall energy yield from the diet, and thus body weight (for a recent review, see (Vrieze *et al*., 2010)). In the strict definition of the word 'probiotic', pre-emptive administration of bacterial strains capable of stimulating the widest possible range of food substrate degrading enzymes in the young pigs' gut would be desirable as a means of maximising the efficiency of food assimilation.

In *ex vivo* or *in vitro* models, it has been shown that incubation of intestinal cells with various *Lactobacilli* species protect against pathogen-induced disruption of membrane barrier. This appears to be a multi-factorial process involving both induction of mucus secretion from goblet cells (Mack *et al*., 1999; Caballero-Franco *et al*., 2007) and maintenance of the tight cell junctions between cells (Madsen *et al*., 2001; Roselli *et al*., 2007; Putaala *et al*., 2008). This function may be most important in counteracting the effects of pathogens, which often exert gastrointestinal effects by weakening the junctions between cells allowing for translocation of the pathogens and activation of inflammatory signals or establishment of local inflammatory lesions. Studies on protective activity of probiotics on membrane barrier of pigs are rare.

Other than the described mechanisms, probiotics may provide defence to the cells through induction of anti-inflammatory cytokines, and reduction of pro-inflammatory cytokines, from enterocytes and intestinal immune cells recruited to sites of inflammation by probiotics (O'Hara *et al*., 2006; Walsh *et al*., 2008; Wang *et al*., 2009). Cytokines are also involved in the maintainance of barrier integrity induced by probiotics (Roselli *et al*., 2007). However, the exact mechanisms of probiotic protection are still largely unknown.

One system that is an attractive target by which probiotics may exercise strong influence is the innate immune system. The intestine has a range of non-specific anti-bacterial weapons that are constitutively produced by enterocytes or specialist cell types. Of particular interest are the defensins, pore-forming antimicrobial peptides produced by Paneth cells and other cells included neutrophils and macrophages; these molecules act as antimicrobials by directly inhibiting pathogen growth, as well as potentiating branches of the innate, humoral and cell-mediated immune system (Linde *et al*., 2008). Defensin induction seems to be a common and important mechanism of probiotic treatment (Mondel *et al*., 2009). *In vitro* work has shown that a commonly used probiotics cocktail VSL#3, containing four *Lactobacillus* species, three *Bifidobacterium* species and one *Streptococcus* is a powerful inducer of β-defensin synthesis. The mechanism appears to be via nuclear factor (NF)-κB and activator protein-1 (AP1) intermediates, which is interesting as probiotics are intuitively regarded as being anti-inflammatory (Schlee *et al*., 2008). In a recent *in vivo* study, cells of *L. plantarum* WCFS1 were given to healthy volunteers, and their effect on duodenal gene expression was investigated, showing cellular pathways and mucosal gene expression patterns correlating with the establishment of immune tolerance in healthy adults (van Baarlen *et al*., 2009).

The toll-like receptors (TLR) are regarded as one of the gut's primary means of detecting and initiating responses to microbial molecular markers. Ligation of TLR initiates a signalling cascade that results in the activation of the transcription factor NF-κB and subsequent up-regulation of co-stimulatory molecules as well as inflammatory cytokines and chemokines (Kumar *et al*., 2009). Thirteen mammalian TLRs have been identified so far, and they are expressed in diverse cell types including gut epithelial cells, B cells, mast cells, dendritic cell, macrophages, neutrophils and T regulatory (Treg) cells (Sutmuller *et al*., 2006), the ubiquitous nature of TLR mRNA expression in pigs is also emerging (Shimosato *et al*., 2005; Tohno *et al*., 2005; Thomas *et al*., 2006). There is currently much research focussed on how these sensors are able to distinguish between commensal and pathogenic bacteria, which bear the same microbial patterns, in such a way that the appropriate 'danger' signals can be generated to pathogens but not inappropriately to benign organisms. Evidence that TLR signalling, especially TLR9, is implicated in the protective effects of probiotics on various models of colitis has been reported in recent studies (Rachmilewitz *et al*., 2004). *B. longum* and *L. plantarum* were shown to improve colitis by inhibiting inflammatory cytokine expression via TLR-4-linked NF-κB activation and by inhibiting intestinal bacterial glycosaminoglycan degradation (Lee *et al*., 2009). Studies on pigs reported that suplementation with *B. animalis* affected the expression of TLR-2 in the lymph nodes when fructo-oligosaccharides were added to the diet (Trevisi *et al*., 2008). In addition, tumour necrosis factor-α was positively correlated with TLR-2 and negatively correlated with bifidobacteria DNA. A recent study highlighted a diverse innate and adaptive immune responses induced by *L. acidophilus* and *L. reuteri v.* rotavirus infection in gnotobiotic pigs (Wen *et al*., 2009).

The ability of probiotics to influence the adaptive immune system of pigs has been described in several studies. There have been a number of studies looking at the effects of probiotics on serum and faecal immunoglobulin concentrations. A recent study has found that *E. faecium* treatment enhanced the course of infection in weaning piglets challenged with *Salmonella serovar Typhimurium*, however, the probiotic treatment resulted in greater production of specific antibodies against *Salmonella* (Szabo *et al*., 2009).

In a study, in which pregnant sows were given either *B. cereus* or *E. faecium* significant decreases were seen in the serum IgG levels of the piglets post-weaning, perhaps reflecting the increased stability of the gut wall, with a concomitant reduction in translocation of bacteria from the gut into the systemic circulation (Scharek *et al*., 2007).

Interestingly, increased levels of faecal IgA were seen in the group given *B. cereus* compared to the *E. faecium* group and the other controls.

Similar increases in faecal IgA, following probiotic treatment, has been observed in human infants (Fukushima *et al*., 1998; Rinne *et al*., 2005). These studies have been aimed at using probiotics to ameliorate the symptoms of food allergy. It is postulated that mucosal IgA may 'mop up' potentially harmful food antigens preventing them from causing inflammatory consequences leading to pathology. It is worth noting in all cases mentioned that the specificity of the IgA molecules has not been determined. It should be noted also that a role for probiotics in accelerating or amplifying the process of immunological tolerance to food antigens has been proposed (Savilahti *et al*., 2008).

The effect of probiotics on immune cells is less clear. The distribution of intestinal immune cells (granulocytes, mast cells, CD4+, CD8+, CD25+, IgA+ lymphocytes) and the mucosal expression of cytokines (IFN-γ, TNF-α, TGF-β, IL-10) of young pigs were not changed by *E. coli* Nissle administration (Duncker *et al*., 2006). On the other hand, *L. acidophilus* and *L. reuteri* were able to down-regulate the rotavirus induced activation/recruitment of monocytes/macrophages and CD14 expression in the intestine of neonatal gnotobiotic pigs, thereby limiting inflammation (Zhang *et al*., 2008). More intriguing was the response to *L. fermentum* in weaned pigs, that induced an increase in the pro-inflammatory cytokines IFN-γ and TNF-α, in the ileum, and an increase in the percentage of CD4+ lymphocyte subset in blood (Wang *et al*., 2009).

Action of probiotics on other bacteria

Bacteria form complex associations within the ecosystem of the gut. The different organisms modulate their environment in ways that facilitate the growth of certain microbes while inhibiting the growth of others. The aim of therapeutic probiotics is to facilitate the growth of one or more organisms which inhibit the growth of potentially deleterious organisms (Servin, 2004).

The most closely studied group of organisms in this respect is the *Lactobacillus* genus. The reduction in pH mentioned earlier, a consequence of their preferred fermentative metabolism, is recognised as important in reducing the growth rates of potential pathogens, particularly enterobacteria such as *Salmonella* and *E. coli*. It is worth noting that unionised lactic acid is an effective, non-specific permeabiliser of Gram-negative cell membranes (Alakomi *et al*., 2000). More specifically, lactobacilli in general elaborate a range of peptide-based molecules generically referred to as 'bacteriocins' (Cotter *et al*., 2005). Colicins are generally most effective against closely related, Gram-positive organisms. However, there have been numerous reports of lactobacilli and bifidobacteria inhibiting the growth of Gram-negative bacteria by a mechanism(s) that do not involve pH reduction or volatile fatty acid production (e.g. Coconnier-Polter *et al*., 2005; Fayol-Messaoudi *et al*., 2005).

In addition to actively inhibiting the growth of potential pathogens a general mode of action for probiotics is their ability to competitively exclude access of pathogens to the luminal surface of the gut epithelial cells. This may be due to direct competition for specific receptors or by steric hindrance where the bulk of the probiotic organisms on the cell surface prevent access of pathogens to their cognate receptors (e.g. Jin and Zhao, 2000; Roselli *et al*., 2007).

Fermented liquid feeds

Since lactobacilli have direct and indirect actions against spoilage and pathogenic bacteria, as well as potential health-promoting effects, they are attractive candidates as additives to feed stuffs. Fermentation of liquid pig feed by LABs occurs naturally on farms but the organisms responsible and the extent of the fermentation is uncontrolled. The literature on the efficacy of fermented liquid feeds (FLF) indicates that they are generally positive in terms of reducing pathogen load in feed and environment (van der Wolf *et al*., 2001; van Winsen *et al*., 2002), improving growth/production parameters (Kyriakis *et al*., 1999), and reducing carriage of pathogens in pigs fed on FLF compared to conventionally fed animals (Boesen *et al*., 2004). However, there is consistent production of research studies showing that the gains associated with feeding FLF are marginal, at best (e.g. Lawlor *et al*., 2002; Canibe and Jensen, 2003; Canibe *et al*., 2007). The lack of consistency between experimental designs make direct comparisons, and logical interpretation, of the many studies difficult (Plumed-Ferrer and von Wright, 2009). The primary difficulty is that the wide range of organisms used to cause fermentation obviously do not all have the same fermentation characteristics; add to this the different feed substrates (whole feed or just cereal components) and different starter concentration, duration of fermentation and temperature; it becomes evident that a clear picture would be a surprise rather than an expectation!

One of the repeated claims against the use of FLF is that there is a reduction in the available lysine (a growth limiting nutrient for pigs) in fermented compared to unfermented feed (P. Brookes, personal communication). It appears that early in the course of natural fermentations *Enterobacteriaceae* grow and utilise significant amounts of lysine before the LABs can produce sufficient lactate (with a subsequent drop in pH). Where LABs are inoculated in sterile feed there is only a very slight drop in available lysine supporting the contention that the enterobacterial bloom, rather than LAB growth, is responsible for the lysine depletion (Niven *et al*., 2006). This highlights the need for controlled, highly reproducible fermentations where relatively large quantities of highly active LAB cultures are used to initiate fermentations (Plumed-Ferrer and von Wright, 2009). In a well-controlled fermentations LAB numbers can reach 10^{10} cfu/ml. The criticism of many clinical trials of probiotics in humans is that insufficient numbers of viable organisms are delivered; this would not be the case in pigs eating exclusively fermented feed.

In field experiments the effect of an *L. plantarum* fermented diet on *Salmonella* carriage and shedding was equivocal, although the total *Enterobacteriaceae* population was reduced (van Winsen *et al*., 2002). Under more controlled laboratory conditions we have shown a clear reduction in *Salmonella* carriage in FLF-fed animals where a different strain of *L. plantarum* was employed and lactate levels of 200 to 250 mM and a pH of <4 were consistently maintained (Kenny *et al*., awaiting publication) with no difference in food conversion ratio between the FLF and control groups. On the farm fermentations, using a defined medium have shown that a stable, high lactate, low yeast fermentation can be achieved using an *L. plantarum* starter (Plumed-Ferrer *et al*., 2005).

In conclusion, the assessment of the efficacy of FLF is even more problematic than that for probiotics in general. The careful choice of fermentation organism, feed substrate and pig life-cycle stage, coupled with a relatively complex and expensive agrotechnical fermentation and delivery system are necessary for this method of probiotic delivery to achieve its potential.

Direct fed microbials

The challenge of delivering viable beneficial microbes to swine (and other target species) has exercised the pharmaceutical and agricultural feed industry for many years. The human probiotic field has been embarrassed on several occasions by exposés clearly demonstrating that the quantity, type and quality of organisms in commercial preparations was wildly different to that described on the packaging (Huys *et al*., 2006; Marcobal *et al*., 2008). For optimal use in a farm setting, any microbial feed additives should be cost-effective, stable to moisture (or portion packed) and temperature. These criteria are difficult to meet reliably for most bacteria, however, bifidobacteria in particular have short shelf lives if not maintained carefully. Nevertheless, a number of commercial preparations are available to pig farmers and have been tested relatively rigorously. The most critical periods in which the probiotics have been tested are the period around farrowing, the first week of life and the post-weaning period.

In biological terms, the easiest microbes to manipulate are those that produce spores; spores are extremely robust and stable yet non-replicating under normal storage conditions. In addition, many *Bacillus* species produce antibiotics called bacitracins, which are effective against many Gram-positive organisms. Several spore-forming species of the genus *Bacillus* (*B. subtilis*, *B. licheniformis* and *B. cereus* var toyoi) have been used in the pig industry. Interestingly, these organisms are not usually part of the indigenous porcine gut microbiota; they are however common soil bacteria, which are likely to be transient passengers through the guts of most outdoor reared pigs.

Treatment of sows and their litters with feed supplemented with *B. cereus* var Toyoi reduced carriage of pathogenic *E. coli* strains and resulted in altered absolute numbers and distributions of immune cells in the piglets (Scharek *et al*., 2007). Piglets from the group given the microbial supplement had a reduced incidence of diarrhoea and liquid faeces; they also had higher average daily gains and feed : gain ratios (Taras *et al*., 2005). Another study describes a large-scale study (nearly 22 000 piglets) comparing the production characteristics when sows were fed the same diet with either a proprietary mix of *B. licheniformis* and *B. subtilis* or a standard mixture of anti-microbial growth promoters (Kritas and Morrison, 2005). The cost of producing each kilogram of pork and all other production parameters were statistically the same showing that the probiotic supplementation was effective at replacing the non-specific chemical inhibition traditionally used in the pig industry.

It is becoming clear that the gut microbiota of animals, including humans, is critically determined at the very earliest stages after birth ('microbial imprinting'; Favier *et al*., 2002; Konstantinov *et al*., 2006). Organisms that are abundant in the piglet's environment at this time have a high chance of forming a permanent association with the piglet's intestinal mucosa (true 'colonisation'). It may transpire that this is the most efficient time to deliver probiotics to ensure the establishment of life-long health benefits and to produce a robust microbiota, resistant to adverse ecological shifts at times like weaning. The most efficient way to deliver probiotics to piglets may be to dose sows before and during farrowing so that she, and her environment, is saturated with desirable organisms in a form whereby the piglet can acquire them as part of its natural development.

Conclusion

The use of live bacterial cultures in the pig industry, whether to improve resistance to specific pathogens or to non-specifically enhance pig health, is likely to continue and expand as economic pressures to improve production parameters and public resistance to the use of 'chemicals' in meat production increase. The general public is familiar with the concept of probiotics ('friendly bacteria') and would welcome their use in sustainable animal production strategies.

There is an increasing body of well-designed *in vitro* and *in vivo* studies, which suggest that certain microbial supplements are useful in protecting particularly young pigs from intestinal infections around weaning. This period, and other stressful mixing events during their lives, is probably important as the point at which pigs pick up important zoonotic pathogens, such as *S. enterica*, but also *Streptococcus suis* (Su *et al*., 2008). It is likely that appropriate probiotic treatments: whether as direct fed microbials or fermented liquid feed will be useful in reducing the burden of pig pathogens.

The challenge before the feed additive industry is to identify organisms, which reliably enhance pig health, at a defined stage in the production process, and to formulate the viable organisms in a way that maintains their viability in the hostile farm environment. The use of single types of bacteria in the pig industry is likely to be superseded by logically constructed mixtures of different organisms. Mixtures of organisms are already available commercially, but detailed comparisons of these mixtures with other treatments are difficult to find.

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