For a successful recall, the product in question must be traced rapidly. Therefore an adequate system of traceability is an additional requirement in the production of safe food and feed. Since recalls may be very costly for the producer an early warning system to reduce the probability of a recall would be beneficial.

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Fermented Liquid Feed (FLF) can reduce the transfer and incidence of Salmonella in pigs.

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Summary. Surveillance studies have shown that feeding pigs liquid diets, and particularly fermented liquid diets reduces the incidence of Salmonella positive herds. Studies have shown that a concentration
of 70 mmol kg⁻¹ lactic acid is bacteriostatic to Salmonella and that concentrations >100 mmol kg⁻¹ are bactericidal. Uncontrolled natural fermentation results in lactic acid concentrations varying between 0 and 140 mmol kg⁻¹ so cannot be relied upon to produce bactericidal levels of lactic acid. However, if selected lactic acid bacteria are used as inoculants and the temperature of the fermentation is controlled (circa 30°C), acid conditions can be produced within 24 h that rapidly and effectively exclude enteropathogens from the diet.

FLF has beneficial effects on the gut architecture and significantly reduces coliform numbers in the lower gut. Sows fed FLF, fermented using pig-derived LAB, have reduced numbers of coliforms in their dung. Piglets suckling sows fed FLF have reduced numbers of faecal coliforms and the LAB species used for fermentation can be recovered from the piglets’ faeces. The colostrum from sows fed FLF has increased immunoglobulin concentration and increased mitogenic activity for both lymphocytes and epithelial cells. This suggests that feeding sows FLF could reduce vertical transmission of enteropathogens.

Taken together, these results indicate that FLF can reduce the potential for enteropathogen transfer via the food, can beneficially influence the ecophysiology of the gut and can stimulate the pig’s immune system.

Health benefits of liquid feeding systems.
In Europe, the animal feed industry has reduced the incidence of Salmonella in feed by stringent quality control and the use of high temperature treatments to kill any residual Salmonella in raw materials. Despite this, there is growing evidence that this approach has been unsuccessful in reducing the incidence of Salmonella in pigs on production units. The incidence of Salmonella is lower when pigs are fed liquid diets than when they are fed dry and particularly pelleted diets (Tielen et al., 1997; United States Animal Health Association, 1999; van der Wolf et al., 1999; von Altrock et al., 2000; van der Wolf et al., 2001; Wong et al., 2002). The incidence was particularly low on farms that fed acidified cheese whey (Tielen et al., 1997) or fermented food industry co-products (van der Wolf et al., 1999).

As Scholten et al. (1999) have pointed out the majority of food industry co-products have been fermented by lactic acid bacteria and as a result have a low pH and contain significant quantities of lactic acid. This high lactic acid concentration inhibits Salmonella in the feed (Geary et al., 1999; Beal et al., 2002) and hence eliminates it at the start of the food chain. Consequently, the inclusion of fermented co-products in liquid diets for pigs makes a significant contribution to food safety. Producers who do not have access to liquid co-products can gain similar benefits by using traditional dry diets if these are fed in liquid form and fermented with lactic acid bacteria (LAB).

Virtually any combination of feed ingredients will ferment if left to steep in water. Almost all raw materials have a natural flora, which includes potentially beneficial LAB and yeasts. Many may also have an undesirable microflora, which can include coliforms, salmonelllas and moulds. In the initial stages of fermentation there is a ‘blooming’ of coliforms (Hansen and Mortenson, 1989; Russell et al., 1996; Geary, 1997) followed by a proliferation of LAB, which normally dominate natural fermentations. However, at low operating temperatures and particularly in some feed ingredients (e.g. by-products from brewing and ethanol production) yeasts will dominate. As they can tolerate low pH yeasts will nearly always become a dominant feature of natural fermentations over time. Yeast fermentation is not desirable as starch is turned into alcohol and carbon dioxide reducing the energy value of the feed. In addition, fermentation by inappropriate yeasts can produce ‘off’ flavours and taints that can make the food unpalatable.

Fermentation can reduce the incidence of enteropathogens in liquid feed.
As noted earlier, the animal feed industry makes strenuous efforts to reduce the incidence of enteropathogens (particularly Salmonella spp.) from dry diets. However, no matter how effective this
process is, there remains the possibility that the feed can become re-contaminated between leaving the mill and being eaten by the pig. An advantage of properly fermented liquid feed is that the acid content of the feed significantly reduces the risk of re-contamination.

*Salm. typhimurium DT104:30* was rapidly excluded when it was introduced into feed that has been fermented for >48 h with *Pd. Pentosaceus* (Beal et al., 2002). However, the death rate was very temperature dependent. *Salm. typhimurium DT104:30* died four to five times faster in feed maintained at 30 °C (D\textit{value} 34-45 min) compared with feed maintained at 20 °C (D\textit{value} 137-250 min). It appears that this effect is due to the lactic acid concentration of the fermented feed rather than the presence of any bacteriocins produced by LAB (Geary et al., 1999; van Winsen et al., 2000).

An important practical consideration is the rate at which potential enteropathogens are excluded at the start of the fermentation process. As noted earlier, fermentations are characterised by an initial coliform bloom and this is only reversed when lactic acid levels rise. Studies in our laboratory have shown that when *Salm. typhimurium DT104:30* and *Pd. pentosaceus* are co-inoculated into liquid feed the *Pd. pentosaceus* rapidly dominate the fermentation and reduce *Salm. typhimurium* to undetectable levels (Beal et al., 2002). However, this effect is also temperature dependent. The decimal reduction time (D\textit{value}) of *Salmonella* was significantly better at 30 °C (D\textit{value} 34-45 min) than at 20 °C (D\textit{value} 137-250 min). It was found that a lactic acid concentration of 70 mmol kg\(^{-1}\) was bacteriostatic, but higher levels (>100 mmol kg\(^{-1}\)) were needed in order to be bactericidal.

Studies in our laboratory have demonstrated that FLF is also effective in excluding a wide range of potentially pathogenic coliform bacteria from FLF (see paper by Beal et al. at this conference).

Unfortunately, natural fermentations have produced unpredictable results on commercial units. Recent studies (Beal, Niven and Brooks in press) have provided an explanation for this. Samples of wheat and barley were obtained, at harvest, from across the UK. These samples were allowed to ferment and their acid production was assessed. There was a great deal of variation in the amount of lactic acid produced by the samples. After fermentation for 24 h at 30 °C lactic acid concentration ranged from 0.14-134.9 mmol kg\(^{-1}\) (mean 59.6±40.0). Only 3% (9 of 300) of fermentations conducted produced more than 75 mmol kg\(^{-1}\) after 24 h fermentation. Thus natural fermentations, which rely on the indigenous flora present on grains, cannot be relied upon to produce bacteriocidal levels of lactic acid.

More predictable fermentation can be achieved by the inoculating liquid feed with LAB that produce lactic acid rapidly and have a high terminal lactic acid concentration. Beal, Niven and Brooks (unpublished data) have identified a number of LAB species are capable of producing 180-230 mmol kg\(^{-1}\) lactic acid in 24 hours with <30 mmol kg\(^{-1}\) acetic acid (a low level of acetic acid is needed to maintain palatability). Regrettably, these organisms cannot be used as inoculants in the EU because of an anomaly in the current legislation (Brooks et al., 2003).

**Fermented feed or fermented feed components?**

A number of studies have been undertaken using continuous fermentation of complete diets (i.e. a proportion of the feed is retained each day to act as an inoculum for the next day’s feed) (Geary et al., 1996; Russell et al., 1996; Jensen and Mikkelsen, 1998; Geary et al., 1999; Pedersen, 2001; Pedersen et al., 2002a; Scholten et al., 2002). However, although some of these gave good results it is difficult to obtain a reliable and consistent fermentation. Concern has also been expressed that fermenting complete diets could reduce nutrient availability. Specifically, synthetic amino acids added to the diet have been degraded in the fermentation process (Pedersen, 2001; Pedersen et al., 2002b; Pedersen et al., 2002c). If the diet contains no synthetic amino acids fermentation does not appear to have any adverse effect on lysine levels (Pedersen, 2001). Studies in our laboratory indicate that LAB used as inoculants do not degrade lysine, and that the loss of synthetic lysine may result during
the coliform bloom when there could be an acid induced activation of the adaptive pathway involving lysine decarboxylase possessed by Salmonella and E.coli (Meng and Bennett, 1992; Park et al., 1996). This is supported by our observation that uncontrolled fermentations result in a significant increase in the production of biogenic amines (Niven unpublished data).

Fermenting only the cereal fraction has significant practical advantages compared with fermenting complete diets. However, if only the cereal component is fermented, a higher concentration of acid must be generated (in order to compensate for dilution and buffering effects when the complete diets is produced) and this can only be achieved using selected LAB as inoculants to stabilise the process.

**Effects of FLF on the gastrointestinal tract.**

Young pigs have an insufficiency of stomach acid, which is the first line of defence against bacterial invasion (Smith and Jones, 1963; Cranwell et al., 1976). Feeding FLF reduces gastric pH and the number of coliforms in the stomach (Mikkelsen and Jensen, 1997; Moran, 2001; van Winsen et al., 2001; Scholten et al., 2002; Canibe and Jensen, 2003). This is due not only to the concentration of acid but also to high concentration in an undissociated form (Russell and Diez-Gonzalez, 1998; van Winsen et al., 2001). Similarly, chickens fed FLF had reduced susceptibility to infection with Salmonella (Heres et al., 2003a) and this was attributed to the barrier function provided by increased acidity in the gizzard and proventriculus (Heres et al., 2003b).

Post weaning anorexia can have a significant effect on the villous architecture of the pig and this can be reduced by feeding liquid diets - see review by Brooks et al. (2001). Furthermore there has been evidence in some studies that feeding FLF has additional benefits in maintaining gut architecture (Scholten et al., 1999; Moran, 2001; Scholten et al., 2002). This may result from a combination of factors including, improved intake of the liquid diet, reduced viscosity of feed and digesta, alterations to the nutrient supply for the lower gut microbiota and the probiotic or immunostimulatory properties of the LAB present in the FLF.

It has been demonstrated that increasing digesta viscosity predisposes pigs to coliform proliferation (Hopwood et al., 2002). Liquid feed and particularly FLF, has reduced viscosity (Niven and Brooks unpublished data) and reduces the dry matter content of the digesta (Canibe and Jensen, 2003). These physico-chemical differences may contribute to the observed changes in the eco-physiology of the pig’s GIT.

Generally, feeding FLF does not produce any significant increase in the number of lactic acid bacteria present in the GIT (after the stomach) but it does dramatically reduce the number of coliforms in the lower small intestine, caecum and colon (Jensen and Mikkelsen, 1998; Hansen et al., 2000; Moran, 2001; van Winsen et al., 2001; Canibe and Jensen, 2003). The ratio of lactic acid bacteria to coliforms in the lower gut of pigs weaned onto freshly prepared (non-fermented) liquid diets was very similar to that of pigs that continued to suckle the sow. However, when the pigs were weaned onto dry diets there was a significant shift in the ratio towards the coliform bacteria. Conversely, when pigs were weaned onto FLF the number of coliforms was reduced and the ratio shifted in favour of the lactic acid bacteria (Moran, 2001).

Recently, van Winsen et al. (2002) conducted a longitudinal study to measure the effect of fermented feed, in particular of its components lactic acid and Lactobacillus plantarum, on gastrointestinal bacterial ecology. Their results showed that fermented feed reduced the Enterobacteriaceae population in the faeces of pigs, which is supported by studies in our laboratory.

At the University of Plymouth we have been using this effect with a different objective. The newborn pig usually has a sterile gut at birth and acquires its characteristic flora through contact with its mother and its surroundings. The most significant contributor of bacteria to the piglet’s surroundings
is the sow. Therefore, we reasoned that if the gut microflora of the sow could be manipulated this
would impact on the development of the piglet’s gut microflora. To this end sows have been fed
diets fermented with aggregating LAB derived from healthy sows and compared them with sows fed
dry diets or non-fermented liquid diets prepared immediately before feeding (Demecková et al.,
2002). The treatments had no effect on the number of LAB in sows’ faeces, but feeding FLF significantly
reduced the number of coliforms shed. The faeces of piglets suckled by sows fed FLF contained
significantly more lactic acid bacteria (7.7 vs. 7.3 log\(_{10}\) CFU g\(^{-1}\)) and significantly less coliforms (7.5 vs.
8.1 log\(_{10}\) CFU g\(^{-1}\)) than the faeces of piglets suckling sows fed dry feed. The colostrum of sows fed FLF
had increased immunoglobulin activity and increased the mitogenic activity of lymphocytes and
enterocytes (see paper by Demecková et al. in this publication).

A detailed discussion of the reported immunostimulatory effects of LAB is beyond the scope of this
paper and raises important issues about when LAB inoculants become probiotics. This inevitably raises
more legislative issues. However, it is clear that the immunostimulatory effects depend on the both the
organism used and the dose (Donnet-Hughes et al., 1999; Gill and Rutherfurd, 2001), and generally
require continued ingestion. Dose levels required to produce an immunostimulatory effect appear to
be of the order 10\(^7\) CFU. This level is consistent with daily dose of Lab. plantarum provided by FLF.

**Conclusions**

Liquid feeding appears intrinsically to reduce the incidence of *Salmonella* in pigs. The inclusion
of acidic components in diets and the controlled fermentation of liquid feed can provide a simple
mechanism whereby the bio-safety of feed can be increased. The ability of fermented feed to exclude
pathogens such as *Salmonella* could make an important contribution to food safety. Furthermore,
the immunostimulatory effects of lactic acid bacteria could be harnessed to improve gut health
following the reduction in the use of antibiotic growth promoters. This capability will increase in
importance as legislators press the pig industry to remove antibiotic growth promoters from their
diets. However, it is the legislators who will also determine whether the potential of LAB and fermented
feed will be realised. Current EU legislation is preventing the development of this technology and unless
this problem is addressed in a rational and constructive way an extremely useful technology may
fail to be implemented on commercial units.

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KEYNOTE LECTURES


Emerging Antimicrobial Resistance in Foodborne Pathogens

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Summary: Foodborne microbial illnesses are an important public health issue worldwide. Although these illnesses are usually mild to moderate self-limiting gastroenteritis, invasive diseases and complications may occur. Many foodborne bacteria (pathogenic and commensal varieties) colonize the gastrointestinal tracts of a wide range of wild and domestic animals, especially animals raised for human consumption. Food contamination with these pathogens can occur at multiple steps along the food chain, including production, processing, distribution, and preparation. An additional concern is the growing incidence of antimicrobial-resistant foodborne pathogens. This paper will focus on antimicrobial resistance among three of the most relevant foodborne bacterial pathogens, Salmonella, Campylobacter, and E. coli.

Keywords: Salmonella; Campylobacter; E. coli; Multiple antimicrobial resistance; food animals

Introduction: The fact that microbial ecosystems are interconnected must be underscored. Bacteria present today in a pig's intestine may a week later be in packaged pork products, and two months thereafter, in a community reservoir. Antimicrobial resistance in foodborne bacteria, therefore, should not necessarily be considered distinct from that in isolates from humans, food animals, or other niches. Yet because food consumption is an important pathway for bacteria to enter humans, the presence of antimicrobial-resistant bacteria in foods warrants particular attention.

Antimicrobial-resistant bacteria have been recovered from a wide variety of foods, and several thorough reviews have been written on the broad subject of antimicrobial-resistant bacteria in the food production