

LIQUID FEEDING AS A MEANS TO PROMOTE PIG HEALTH

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ABSTRACT

Surveillance studies have shown that liquid feeding of pigs reduces the incidence of salmonella. This has been particularly associated with the use of acidic residues derived from the food industry. More recently, and particularly because producers wish to feed liquid diets *ad libitum*, there has been much interest in the concept of feeding fermented liquid feed (FLF) to pigs. Natural, uncontrolled fermentation has produced very mixed results on commercial units. However, when selected lactic acid bacteria inoculants are used and fermentation conditions are carefully controlled, an acidic diet is produced that rapidly and effectively excludes enteropathogens.

When diets are produced by controlled fermentation they are readily accepted by pigs. Such feed has been shown to enhance post-weaning growth and reduce coliform levels in the lower gut. Because of this, FLF may be a useful alternative to antibiotic growth promoters and avoid the problems associated with the development of antibiotic resistance. Although results in experimental units are impressive, more research is needed before we can provide Standard Operating Procedures relevant to different conditions. These are needed to enable the transfer of this exciting technology to commercial pig units.

INTRODUCTION

It is important to define liquid feeding and differentiate it from other feeding systems. Liquid feeding involves the use of a diet prepared either from a mixture of liquid food industry co-products and conventional dry components, or from dry raw materials mixed with water. Generally, the diets are mixed at a central point on the pig unit. If the pigs are ration-fed, a single pipeline can be used to transfer the mixed feed direct to target pigs, wherever on the unit they may be. If the pigs are to be fed *ad libitum* the feed is often moved to 'satellite' tanks, from which specific groups of pigs are fed. Thus in a modern system, one feed preparation area can produce a range of diets to match the nutrient requirements of pigs of different ages and stages of production.

A liquid diet will typically contain 200-300 g dry matter (or dry ingredients) per kg. This type of feeding system should not be confused with wet/dry feeder systems where water and feed are kept separate up to the point of delivery to the pig. A key difference between these two feeding systems is the length of time that the dry matter fraction of the diet is in a liquid medium before it is consumed by the pig. This has important implications for the microbiology of, and nutrient availability from, the feed.

POTENTIAL ADVANTAGES OF LIQUID FEEDING

Traditionally, producers have perceived a number of advantages in using liquid feeding. These include:

- Reduction of food loss, as dust, during handling and feeding.
- Improvement in the pigs' environment and health due to the reduction of dust in the atmosphere.
- Improved pig performance and feed conversion ratio (FCR).
- Flexibility in raw material use (opportunity to utilize more economic food sources and reduce cost per kg gain).
- Improved materials handling (system can act as both a feed mixing and distribution system).
- Increased accuracy of rationing (computer control brings a degree of accuracy to the system that it is difficult to emulate with dry feeding systems).
- Improved dry matter intake in problem groups (e.g. weaners and lactating sows).
- Improved intakes at high ambient temperatures.

Jensen and Mikkelsen (1998) reviewed nine recent studies in which the performance of pigs fed dry or liquid diets were compared (Table 1). Grow-finish pigs fed liquid diets generally had improved daily live-weight gain and feed conversion ratio.

Table 1. Improvement (%) in growth rate and feed conversion ratio in nine experiments conducted to compare liquid and dry feeding for grow-finish pigs (Jensen et al. 1998).

Improved daily weight gain		Improved feed conversion ratio	
Mean \pm SD	Range	Mean \pm SD	Range
4.4 \pm 5.4	-2.6 - 15.0	6.9 \pm 3.5	1.9 - 12.7

To this improved performance must now be added other benefits that can accrue to the environment through:

- The utilization of co-products from the human food industry which would otherwise incur a cost for environmentally acceptable disposal
- Reduction in nitrogen loading through the easy adoption of 'step' and 'phase feeding'
- Reduction in phosphate loading through activation of endogenous phytase in cereal grains and / or the addition of exogenous enzymes to diets.

The latter are outside the scope of this paper and will not be discussed further.

UTILISATION OF LIQUID HUMAN FOOD INDUSTRY CO-PRODUCTS

In discussing the potential health benefits of liquid feed it is appropriate to consider the use of food industry liquid co-products, as these play an important role in the maintenance of animal health on many units. This aspect will be discussed in the next section.

Liquid co-products of many sorts are used for pig feeding around the world. However, we should look to The Netherlands for the best example of co-product utilization. In The Netherlands, it has been estimated that about 6.5 million tonnes of co-products are used annually on farms (de Haas 1998). The quantity of material has increased dramatically in recent years and it is predicted that it will continue to increase. Currently, demand exceeds supply and consequently, co-products are being shipped into The Netherlands from France, Poland and even the UK. Of the 6.5 million tonne total, approximately 35% (2.3 million tonnes) is fed to pigs (Table 2). Of this, 70% consists of carbohydrate rich materials (Scholten *et al.* 1999). The importance of these co-products to pig production is put into perspective when it is remembered that the net production of pork in The Netherlands in 1996 was 1.62 million tonnes (Meat and Livestock Commission 1998). Such detailed data are not available from other countries, but using information from trade sources we estimate that approximately 30% of pigs in Northern and Western Europe are fed liquid diets and a majority of these incorporate at least some food industry co-products.

Table 2. Amount of liquid co-products (tonnes) from the food industry delivered directly to pig farms in the Netherlands (Scholten *et al.* 1999).

Product	1993	1996
Wheat starch industry	650,000	885,000
Potato processing industry	350,000	525,000
Dairy industry	300,000	300,000
Fermentation industry	80,000	120,000
Beer industry	80,000	100,000
Sugar industry	25,000	50,000
Other	170,000	360,000
Total amount (tonnes)	1,655,000	2,340,000

A major problem for the nutritionist and the pig producer wishing to use co-products is the variability in their composition (Table 3). This means that if they are going to be used efficiently diets have to be continually reformulated to compensate for the changes in composition that can occur from one load to the next.

Despite the variability of liquid products, they can be used efficiently and without detriment to pig performance if diets are formulated accurately. For example, Scholten *et al.* (1997) used combinations of liquid wheat starch, liquid potato steam peelings and cheese whey to replace 35% of the dry matter in growing pig diets and 55% in finishing diets at a water to feed ratio of 2.6:1. The results of their study (Table 4) show that, when the diet is properly balanced, the inclusion of co-products does not adversely affect pig performance.

In a recent study ‘wheat bottom stills’ (Greenwich Gold™), a residue left after the production of ethanol, was substituted in diets for grow-finish pigs (Table 5). The basal diet was a conventional UK diet based on wheat and barley with extracted soyabean and rapeseed as the principle protein sources and substitutions were made using best-cost formulation. The growth rate and carcass quality of pigs was not affected by substituting conventional raw materials with up to 30% of this co-product (Brooks *et al.* 2001).

Table 3. Variability in composition of some feed components (Brooks et al. 1995).

		Dry matter (g kg ⁻¹)	Crude protein (g kg ⁻¹ DM)	DE (MJ kg ⁻¹ DM)
Liquid Products	Yoghurt	22 – 191	139 - 389	17.3 – 21.8
	Whey	20 – 58	115 - 234	13.4 – 15.9
	Delactosed whey	210 – 406	206 - 293	6.8 – 15.1
	Milk	126 - 193	211 - 396	14.6 – 24.3
	Wheat bottom stills (a)	155 – 193	207 - 258	12.6 – 17.2
	Wheat bottom stills (b)	76 – 160	192 - 367	13.9 – 16.3
	‘C’ starch	133 – 159	68 - 106	15.6 – 16.2
	Poultry processing residue	84 – 239	211 – 364	16.2 – 23.5
Dry Products	Biscuit meal	87 – 95	76 – 126	15.4 – 17.9
	Wheatfeed	87 – 89	152 – 187	12.4 – 13.1
	Maize gluten	87 – 90	203 – 227	12.7 – 13.1
	Hi-pro Soya bean meal	87 – 90	421- 514	12.9 – 16.2

Table 4. Performance of growing-finishing pigs (25-112kg) fed a liquid diet with or without liquid co-products (Scholten et al. 1997).

	Control diet (meal + water)	Co-product diet	SE _M
Daily gain (g day ⁻¹)	740 ^a	768 ^b	4.7
Feed intake (kg day ⁻¹)	1.99 ^a	1.98 ^b	0.01
Feed conversion ratio	2.69 ^a	2.58 ^b	0.02
Lean meat percentage	55.3 ^c	54.8 ^d	0.16

Data in a row with a different superscript differ significantly (^{a,b} P<0.001; ^{c,d} P<0.05)

Table 5. Effect of inclusion level of wheat bottom stills (Greenwich Gold™) in the diet on the performance of growing-finisher pigs (2-90 kg) (Brooks et al. 2001).

	Greenwich Gold % [†]				SE _D
	0	15	22.5	30	
Daily gain (g)	779	759	746	793	15.9
Daily DM intake (g)	1608 ^a	1511	1523	1504 ^a	33.0
DM FCR	2.08 ^a	2.00	2.05 ^b	1.91 ^{ab}	0.04
					1
Carcass weight (kg)	63.57	61.83	62.22	62.42	0.97
Backfat P2 (mm)	11.00	10.73	11.20	10.16	0.68
Killing-out %	70.38	70.85	71.70	69.70	0.93

^{a,b} Means with the same superscript differ at P<0.05 or greater

[†]Greenwich Gold contained (g kg⁻¹ fresh material) DM 192; crude protein 6.25; NDF 1.6; ash 1.28 and DE 3.3 MJ kg⁻¹. The diets were formulated to provide (at a nominal 87% DM) 13.4 MJ DE kg⁻¹ and 12 and 9.5 g lysine kg⁻¹ in the grower and finisher diets respectively.

The use of liquid food industry products can help solve an environmental problem for the food industry. However, it is important that while solving an environmental problem for the human food industry the livestock industry does not transfer the problem to the farm. Some environmentalists have been concerned that liquid feeding may increase environmental loading. By their nature, liquid diets tend to increase effluent volume. Liquid diets may also increase water consumption. Many co-products have high mineral content and it is essential that pigs be allowed access to a separate supply of water in order that they can maintain their homeostatic balance (Brooks *et al.* 1990). This is a requirement of the UK Welfare Codes (DEFRA 2003) and should be mandatory whenever liquid diets are used. In addition to its need to maintain homeostasis, the pig appears to have a behavioural need for water separate from any that it consumes with its food. Producers should not see this as a disadvantage, for our studies have shown that in grow-finish pigs performance is improved if they consume more water with their feed, and as a result have greater total water intake (Table 6). Subsequently, Barber *et al.* (1991) demonstrated that increasing the water content of liquid diets fed to growing pigs increased dry matter digestibility (Table 7).

Table 6. Voluntary water use and performance of grow-finish pigs offered liquid diets at different water to meal ratios (Gill *et al.* 1987).

	Water to dry ingredients ratio			
	2:1	2.5:1	3:1	3.5:1
Meal intake (kg d ⁻¹)	1.48	1.49	1.46	1.47
Voluntary water use (kg d ⁻¹)	1.26 ^a	0.78 ^b	0.44 ^c	0.24 ^d
Total water use (kg d ⁻¹)	4.23 ^a	4.51 ^b	4.86 ^c	5.60 ^d
Daily gain (kg d ⁻¹)	0.73 ^a	0.74 ^a	0.75 ^{a,b}	0.77 ^b
Dry Matter Feed Conversion Ratio	2.01	2.00	1.95	1.90
Water to feed ration (w/w)	2.97	3.12	3.36	3.68

^{a, b, c} Means with the same superscript do not differ significantly at $P < 0.05$.

Table 7. Effect of water to feed ratio on diet digestibility (Barber *et al.* 1991).

	Water to dry ingredient ratio			
	2:1	2.67:1	3.33:1	4:1
Dry matter digestibility (%)	79.1 ^a	77.8 ^a	80.3 ^{a, b}	82.9 ^b
Estimated DE (MJ kg ⁻¹ DM)	15.1	15.0	15.4	15.8

^{a, b} Means with the same superscript do not differ significantly at $P < 0.05$.

If this finding is taken together with the improved FCR found when pigs are fed liquid diets (see data reviewed by Jensen and Mikkelsen (1998) summarised in Table 1) we can conclude that pigs are able to extract more nutrients from liquid diets than from dry ones. It follows that the use of liquid diets, with or without co-products, may at the same time increase effluent volume and reduce nutrient load per litre. Therefore, it is very important when making comparisons of environmental loading produced by different feeding systems that these are expressed in terms of nutrients voided per kg growth made by the pig, or better still nutrients voided per kg meat produced.

Scholten *et al.* (1997) considered the outputs of pigs fed diets based on meal and water, or on co-products. They found that ammonia emissions were similar for pigs fed conventional liquid diets and those fed diets that included co-products (Table 8); and that manure production of pigs fed co-products was 2.4% higher when based on manure produced per kg growth. However, they did not measure the nutrient content of the manure. In studies with young pigs (7-25 kg), it was found that effluent volume increased when pigs were fed on liquid diets rather than dry pelleted diets (Table 9).

Table 8. Environmental impact of growing-finishing pigs fed a liquid diet with or without liquid co-products (Scholten *et al.* 1997).

	Control diet (meal + water)	Co-product diet
Ammonia emission (kg place ⁻¹ year ⁻¹)	1.9	2.0
Manure production (l place ⁻¹ year ⁻¹)	1,092	1,156
Manure production (l kg growth ⁻¹)	4.1	4.2
Dry matter content of the manure (%)	8.3	6.8
pH of the manure	7.3	7.5

Table 9. Production of effluent by weaner pigs fed dry and liquid diets (Russell *et al.* 1996).

	Trial 1			Trial 2		
	Dry	Liquid	SE _D	Dry	Liquid	SE _D
Daily gain (g d ⁻¹)	343	428	21***	397	454	14***
Total water use (ml pig ⁻¹ d ⁻¹)	1306	2298	64***	1499	2028	84**
Effluent production (ml pig ⁻¹ d ⁻¹)	754	1058	46**	982	1189	31*
Effluent production (l kg gain) ^a	2.20	2.47	+12.3%	2.47	2.62	+6.1%

^a Note that in Trial 2 trough design for the liquid fed pigs was improved and resulted in a considerable reduction in effluent production per kg gain.

Some of the co-products that are used in liquid form could be dried and incorporated into conventional dry compounded feed. However, feeding them in liquid form removes the cost of drying and reduces dependence on non-renewable energy sources. On the debit side, the use of liquid co-products increases transportation costs, as more water is shipped with the dry matter. Consequently, there is an increased demand for non-renewable energy for transportation. As a result, disposing of some products by processing them through a pig may only be efficient if pig production units are situated close to the source of supply. However, in Europe many products are transported considerable distances as 'back loads' in tankers that would otherwise travel empty. Thus in real terms, there is only a marginal increase in fuel cost to set against the material (i.e. the difference between running the tanker empty and full).

Drying and subsequent incorporation into dry diets would not be a viable economic option for co-products with very low dry matter content. These materials would still have to be disposed of in an environmentally acceptable manner. The alternative routes for disposal of these materials would be through a sewerage system (either public or privately owned), land

application or through addition to landfill sites. In developed economies both the economic and the environmental cost of such disposal continues to increase.

Therefore, when deciding whether to utilize co-products as feed stocks or make them environmentally non-damaging through waste treatment it is important to audit the alternative systems in their entirety to ensure that they are ultimately beneficial to the environment.

HEALTH BENEFITS OF LIQUID FEEDING SYSTEMS

There is considerable concern about the incidence of zoonoses in animal feeds and in particular the transmission of *Salmonella* through the food chain. 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. Two hypotheses can be advanced to explain this. First, changes to the non-starch polysaccharide fraction of the diet resulting from heat treatment may produce a gastrointestinal environment that is more favourable to the colonisation of *Salmonella*. Secondly, non-pathogenic *Salmonella* may exclude pathogenic strains. Elimination of non-pathogenic *Salmonella* from feed may create ecological niches that are subsequently colonised by pathogenic strains.

A study of *Salmonella* incidence on German farms (von Altrock *et al.* 2000) identified the use of pelleted feed as a common risk factor for *Salmonella*. Bush (cited by United States Animal Health Association 1999) found that operations feeding a pelleted finisher diet had a 26 times greater risk of being *Salmonella* positive than those that fed a meal diet. They suggested that pelleted diets either influenced the gut environment such that pigs are more susceptible to *Salmonella* or, that pigs shed *Salmonella* that were already present. The same review (United States Animal Health Association 1999) also cited a study by Wong *et al.* who looked at the herd-level risk factors for the introduction and spread of *Salmonella* in Danish, German, Greek, Swedish and Dutch pig herds. They found that the incidence of *Salmonella* was 8.2% in herds feeding pelleted dry feed, 4.2% in herds feeding non-pelleted and dry feed but only 1% in herds feeding non-pelleted and wet feed. They also observed that the odds of a herd using whey being seropositive was 1% compared with 5.6% in herds not using whey. Danish studies have also shown a reduction in *Salmonella* when pigs are fed meal rather than pellets and fewer *Salmonella* positive pigs when using coarse ground rather than finely ground meal. (Jørgensen *et al.* 1999; Sloth *et al.* 1998).

It is clear from surveillance data that liquid feeding has a positive effect on gut health and reduces the incidence of *Salmonella*. In a survey of 320 farms in Holland, the incidence of sub-clinical *Salmonella* infection was found to be ten times lower on farms with liquid feeding than on farms feeding dry compound diets. The incidence was particularly low on farms that fed acidified cheese whey (Tielen *et al.* 1997). A more recent study (van der Wolf *et al.* 1999), found that automated liquid feeding of food industry co-products was associated with a decreased risk of infection.

It is important to note that most of the studies in which reductions in *Salmonella* incidence have been associated with liquid feeding have come from Denmark and The Netherlands. In both these countries, there is a tradition of using food industry co-products. As Scholten *et al.* (1999) have pointed out, the majority of these 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 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. This has led workers in Europe to look more closely at the microbiology of liquid feed and to develop controlled fermentation of feed.

MICROBIAL ACTIVITY IN LIQUID FEED

Liquid feeding alters both the physico-chemical properties of the diet and its microbiology. Both of these factors are important in terms of pig health and performance. As noted above, there are benefits from including fermented co-products, with high levels of lactic acid, in diets for pigs. However, not all producers have access to liquid co-products. Nevertheless, a similar benefit can be obtained even when traditional dry diets are fed in liquid form. Twenty-five years ago, Smith (1976) showed that *Lactobacillus* species, which occur naturally on cereal grains, proliferate in a wet feed and reduce the pH. In his study, adding water to the meal at feeding time produced a pH of 5.8. Soaking the mixture for 24 h resulted in a massive proliferation of *Lactobacilli*, which produced lactic acid and reduced the pH to 4.1.

Virtually any combination of feed ingredients will ferment if left to steep in water. Almost all raw materials have a natural flora (mainly lactic acid bacteria and yeasts). Many may also have an undesirable microflora (coliforms, salmonellas and moulds). Generally, the dominant microflora that develops in liquid feed is the lactic acid bacteria (LAB). However, at low operating temperatures and particularly with some feed ingredients (e.g. by-products from brewing and ethanol production), yeasts will dominate. LAB fermentation is beneficial as it produces organic acids, primarily lactic acid. When incorporated into dry diets, lactic acid has a beneficial effect on feed intake, daily gain and FCR of piglets (Table 10). It seems likely that it is also having similar effects in liquid feeding systems. Importantly, recent research indicates that lactic acid is utilised as well as cornstarch (Everts *et al.* 2000), so the lactic acid makes a valuable contribution to the pig's energy supply.

Table 10. Effect of lactic acid percentage in diets on the performance of pigs (% increase over negative control) (Roth *et al.* 1993).

Lactic acid %	Daily gain	Feed Intake	FCR
0.8	+4.7	+6.1	+1.2
1.6	+8.1	+6.1	-1.8
2.4	+7.3	+5.4	-1.8

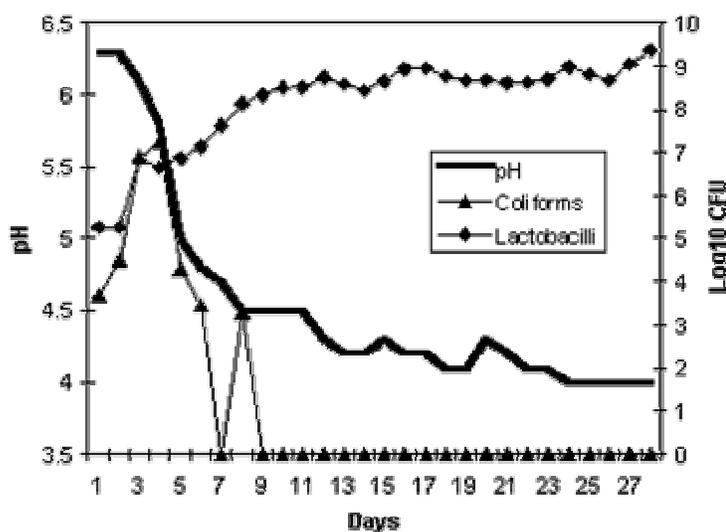
Yeast fermentation is not desirable. Starch is turned into alcohol and carbon dioxide. The alcohol can become too prevalent and the carbon dioxide indicates a significant loss of energy

value. More importantly, just as in beer and wine making, fermentation by inappropriate yeasts can produce taints, which render the food unpalatable.

Because pipeline wet feeding systems are not sterilised between feeds it is inevitable that they are microbiologically active. Hansen and Mortenson (1989) conducted a large survey in Denmark and found that it took 3-5 days for the lactobacillus levels to elevate and stabilise in pipeline feeding systems. In their studies, they found that it was detrimental to sterilise pipeline-feeding systems as this removed the lactobacilli and increased the feed pH by 1.5-2.0 units. This in turn allowed coliform bacteria to proliferate for 1-5 days until the lactobacilli re-established themselves and lowered the pH. They found that sterilisation of pipeline feeding systems was actually disadvantageous, as outbreaks of diarrhoea often resulted from the coliform 'bloom' which followed the sterilisation of pipeline systems.

Studies at the University of Plymouth (Geary 1997; Geary *et al.* 1999; Geary *et al.* 1996; Russell *et al.* 1996) have shown that a lactobacillus population develops in *ad libitum* liquid feeding systems for weaner pigs and that this is accompanied by a reduction of pH and *E. Coli* population (Figure 1).

Figure 1. Change in pH and microbial population of liquid feed over time (Geary *et al.* 1999).



Jensen *et al.* (1998) have reviewed the effects on performance of presenting weaner pigs with dry, freshly prepared liquid, or fermented liquid feed (Table 11). Compared with feeding dry diets weight gain was improved by an average of 12.3% when feed was presented in liquid form and by a further 13.4% if the liquid feed was fermented. However, FCR was generally worse on liquid feed (LF) and FLF than on dry feed. This is in contrast to the results obtained with finishing pigs (Table 1) and probably reflects differences in feeding behaviour between older and newly weaned pigs.

Table 11. Improvement (%) in growth rate and food conversion ratio in experiments in which the performance of pigs fed dry feeding (DF), liquid feed (LF) or fermented liquid feed (FLF) was compared (After Jensen et al. 1998).

	Number of trials	Improved daily weight gain		Improved food conversion ratio	
		Mean \pm SD	Range	Mean \pm SD	Range
LF v. DF	10	12.3 \pm 9.4	-7.5 - 34.2	-4.1 \pm 11.8	-32.6 - 10.1
FLF v. DF	4	22.3 \pm 13.2	9.2 - 43.8	-10.9 \pm 19.7	-44.3 - 5.8
FLF v. LF	3	13.4 \pm 7.1	5.7 - 22.9	-1.4 \pm 2.4	-4.8 - 0.6

FERMENTATION REDUCES THE INCIDENCE OF ENTEROPATHOGENS IN LIQUID FEED

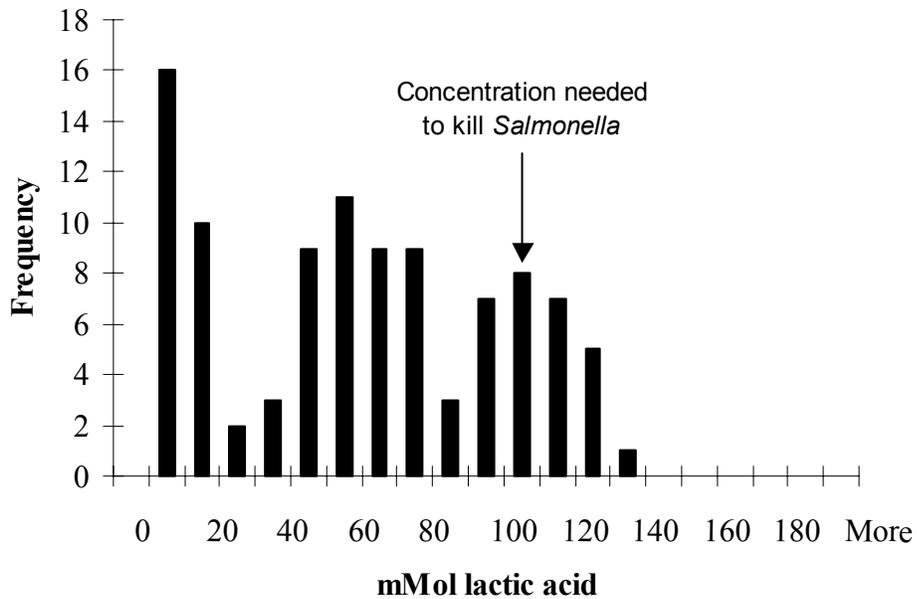
As noted earlier, the animal feed industry makes strenuous efforts to reduce the incidence of enteropathogens (particularly *Salmonella* spp.) from dry diets. However, no matter how effective this process is, there remains the possibility that the feed can become re-contaminated between leaving the mill 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.

A lactic acid concentration in feed of 70 mMol was found to be bacteriostatic to *Salmonella*, but higher levels (>100 mMol) are needed in order to be bactericidal (Beal, Niven and Brooks unpublished data). Unfortunately, natural fermentations cannot be relied upon to produce these concentrations of acid. For example, in samples of wheat from across the UK, fermented for 24h at 30°C, the lactic acid level varied from 0 to 50 (8.7 \pm 12.2) mMol. After 72h the range was from 0.14 to 135 (48 \pm 38) mMol lactic acid (Figure 2).

Only circa 10% of natural fermentations achieved the threshold level of 100 mMol lactic acid needed to eliminate *Salmonella*.

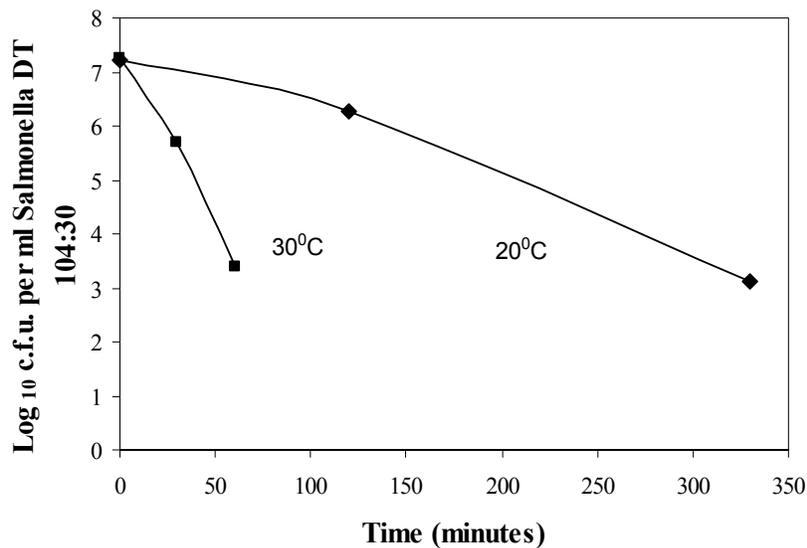
This problem can be overcome by inoculating liquid feed with LAB that produce lactic acid rapidly, and to a high concentration. We have identified a number of LAB species that are capable of producing in excess of 100 mMol lactic acid with less than 30 mMol acetic acid in 24 h. However, in the EU only microorganisms that have been registered as zootechnical additives can be used as inoculants. This is extremely restrictive and most organisms that have been registered are probiotics, that have been developed for other purposes, and may not have particularly good lactic acid producing properties. The reason that the EU requires registration of LAB is concern about the possible transfer of antibiotic resistant genes. In a number of other countries around the world LAB have 'Generally Recognised as Safe' (GRAS) status, so a wider range of organisms could be used as inoculants.

Figure 2. Lactic acid produced by the natural fermentation of wheat at 30°C for 72h (Beal, Niven and Brooks unpublished data).



In studies at Plymouth, *Salmonella typhimurium* was rapidly excluded when it was introduced into feed that has been fermented for 48, 72 or 96 h with *Pediococcus pentosaceus* (Beal *et al.* 2002). However, the death rate of *Salmonella typhimurium* is very temperature dependent and was much faster at 30°C than at 20°C (Figure 3).

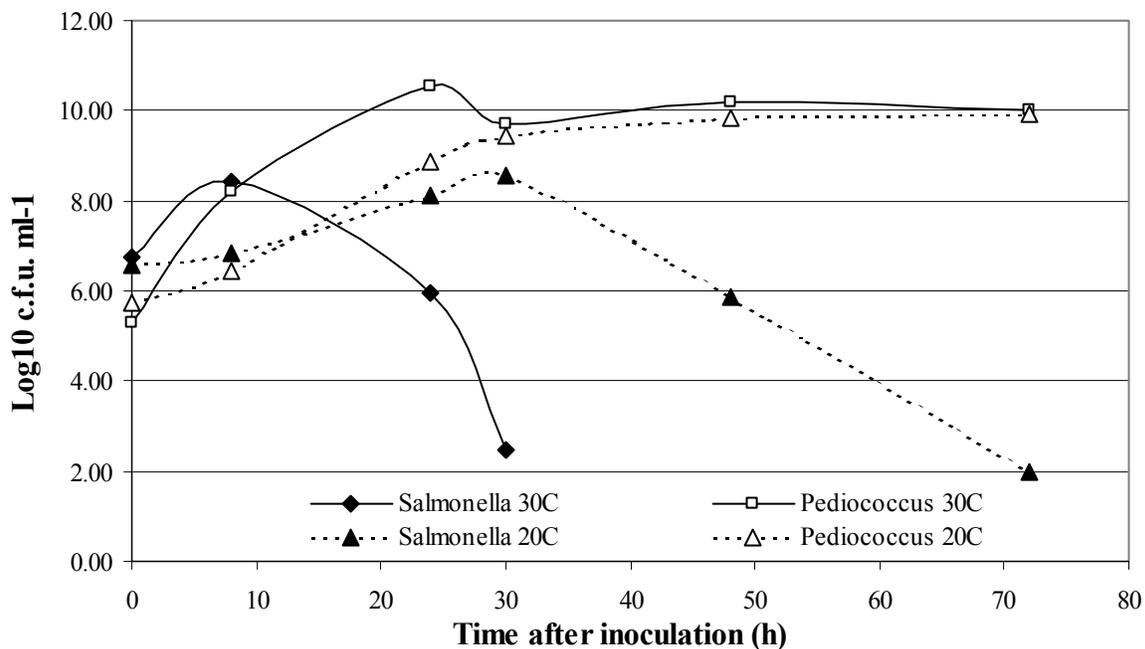
Figure 3. Disappearance of Salmonella after inoculation into fermented feed maintained at 20°C or 30° C (Beal *et al.* 2002).



In another study (van Winsen *et al.* 1997), it was found that pig feed fermented with *Lact. plantarum* had a bacteriostatic effect on *Salmonella* during the first two hours following inoculation and a bactericidal effect thereafter. Six hours after inoculation *Salmonella typhimurium* could not be detected in the FLF. In contrast, *Salmonella typhimurium* added to non-fermented feed survived and multiplied (van Winsen *et al.* 1997). More recently, it was been shown that it is the lactic acid concentration of the fermented feed that is responsible for this effect (van Winsen *et al.* 2000).

Studies in our laboratory have shown that when *Salmonella typhimurium* DT104:30 and *Pediococcus pentosaceus* were co-inoculated into liquid feed, the *Pediococcus pentosaceus* rapidly dominated the fermentation and reduced *Salmonella typhimurium* to undetectable levels (Beal *et al.* 2002). However, this effect was also temperature dependent (Figure 4). The decimal reduction time (D_{value}) was significantly better at 30° C (D_{value} 34 – 45 min) than at 20° C (D_{value} 137 – 250 min).

Figure 4. Disappearance of Salmonella after co-inoculation with *Pediococcus pentosaceus* at 20° C or 30° C (Beal *et al.* 2002).



FLF has also been shown to be effective in reducing the incidence of pathogenic *E.coli* (Beal *et al.* 2001) (Table 12). However, LAB species differ markedly in their effects on the survival of enterotoxigenic *E. coli* (Hillman *et al.* 1994a; Hillman *et al.* 1994b; Hillman *et al.* 1995).

Jensen and Mikkelsen (1998) demonstrated the importance of temperature in controlling fermentation and lowering the pH of the feed. They used a 0.5 residue, and eight-hourly replenishment of the tank. They found that pH reached a steady state in 50 h when the tank was maintained at 25°C, but that it took around 100 h when the tank was maintained at 15°C.

We now favour even higher operating temperatures (circa 30° C). At this temperature, and using the right inoculant it is possible to achieve the desired lactic acid concentration (i.e. >100 mMol) in 24h.

Table 12. Decimal reduction time (min) of selected micro-organisms added to FLF that had been fermented with *Lact. plantarum* for 24 h, 48 h, 72 h and 96 h (Beal et al. 2001).

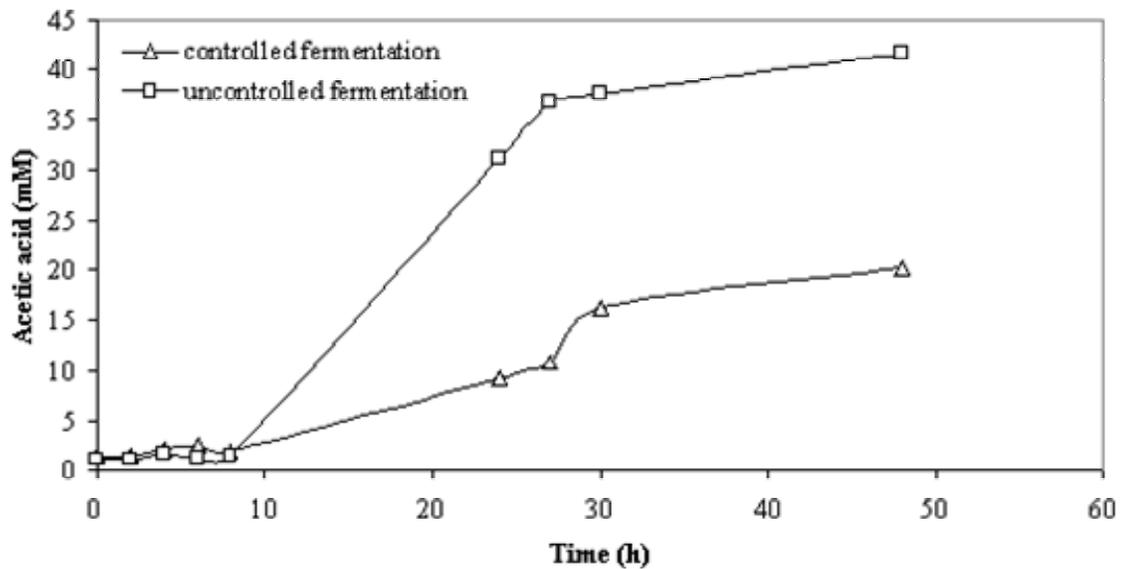
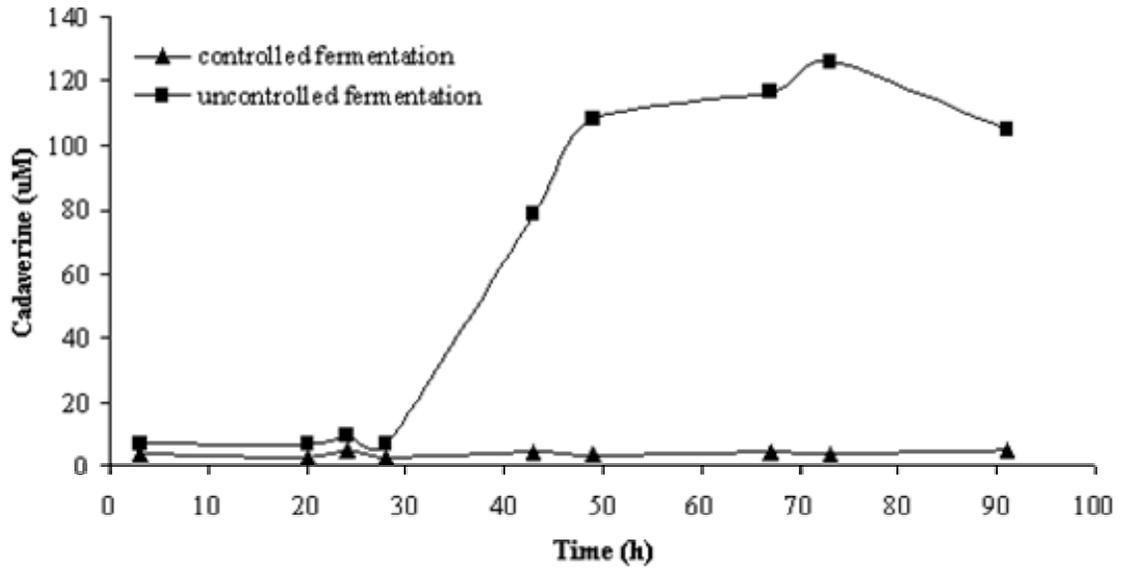
Serotype	Decimal reduction time (min)			
	24 h	48 h	72 h	96 h
E.coli K88 (99)	nt	25.2 ^a	23.7 ^a	22.9 ^a
E.coli K88 (100)	nt	26.1 ^a	23.6 ^{ab}	17.4 ^b
E.coli K88 (101)	nt	22.3 ^a	24.2 ^a	24.3 ^a
E.coli K99 (185)	nt	22.0 ^a	16.5 ^b	15.8 ^b
E.coli K99 (230)	nt	22.2 ^a	14.6 ^b	14.0 ^b
E.coli 0157:H7	nt	12.2 ^a	9.3 ^b	10.3 ^b
Salm. typhimurium DT104B (342A)	46.5 ^a	31.6 ^b	13.8 ^c	nt
Salm. typhimurium DT104B (342B)	35.3 ^a	20.9 ^b	nt	nt
Salm. typhimurium DT193 (20)	32.0 ^a	15.7 ^b	nt	nt
Salm. derby (16)	38.2 ^a	25.3 ^b	nt	nt
Salm. goldcoast (245)	38.8 ^a	15.6 ^b	nt	nt
Salm. anatum (41A)	26.4 ^a	14.4 ^b	11.9 ^c	nt

^{a, b, c} Means in the same row with the same superscript do not differ at $P < 0.05$

Palatability is also an important issue, particularly when feeding FLF to young pigs. Our studies have shown that pigs are tolerant of dietary lactic acid concentrations up to 200 mMol, but low levels of other short chain fatty acids like acetic acid can adversely affect intake. The presence of biogenic amines in the diet may also have adverse effects on intake. The data in Figure 5 shows the comparative development of acetic acid and a biogenic amine (cadaverine) in fermented feed produced by either a controlled fermentation (feed inoculated with a selected LAB) or an uncontrolled fermentation (fermented by the native flora). This data reinforces the importance of using selected LAB inoculants to control fermentation and helps explain some of the palatability problems that we have observed on commercial pig units.

Our initial studies of FLF used continuous fermentation. However, this created many problems on commercial units. Producers were unable to deal with the complexity of the system or with the number of diets that had to be prepared. Therefore, current work is centring on the use of batch fermentation. This is more easily controlled and if problems occur, such as the development of a mal-fermentation, they can be recovered from more easily. Much current work in Europe is based on fermentation of just the carbohydrate fraction of the diet. This creates a product that can then be incorporated into a range of different diets. In addition, the fermentation process is simpler, more predictable and more reliable.

Figure 5. Development of acetic acid and cadaverine in controlled and uncontrolled fermentations of liquid feed (Niven, Beal and Brooks unpublished data).



BENEFICIAL EFFECTS OF FERMENTED LIQUID FEED

As noted earlier fermented liquid feed may reduce the incidence of Salmonella in slaughter pigs. In addition, there is growing evidence that FLF may confer specific benefits to newly weaned pigs. FLF may benefit the weaner pig by:

- Improving feed intake. Well fermented feed may maintain the growth of the gut epithelium
- Providing an acid diet. This may help control pathogens both in the feed and in the pig's gut. It also helps with protein digestion.
- Supplying lactic acid bacteria. The presence of large populations of LAB in the diet may have a beneficial effect on the lower gut microflora.

The fastest growing tissue in the pig's body is the epithelial lining of the small intestine. Many of the nutrients needed for growth are directly absorbed from the gut lumen. Even transient starvation will result in a rapid reduction in villus height and thus reduce the absorptive capacity of the gut (Pluske *et al.* 1996a; Pluske *et al.* 1996b). Conversely, a diet that is palatable and well accepted by the newly weaned pig will ensure an adequate supply of nutrients to the brush border.

The weaned pig has an insufficiency of stomach acid, which is the first line of defence against bacterial invasion (Cranwell *et al.* 1976; Smith *et al.* 1963). Manipulation of stomach acidity, through lactic acid supplementation of the diet (Thomlinson *et al.* 1981) feeding fermented milk (Dunshea *et al.* 2000; Ratcliffe *et al.* 1985; Ratcliffe *et al.* 1986) or through lactic supplementation of water (Cole *et al.* 1968) all reduced gastric pH and the number of coliforms in the stomach. Similarly, Mikkelsen and Jensen (1997) found that fermented liquid feed results in a significant increase in lactic acid content in the stomach and some small but significant changes in other sections of the gut.

Recent studies have shown that feed form can have an effect on the pH of the GIT (Table 13) with the lowest pH being found in pigs that continued to suckle their dams (Moran 2001).

Table 13. Effect of dietary treatment on the pH of the intestinal contents of piglets 14 days post-weaning (Moran 2001).

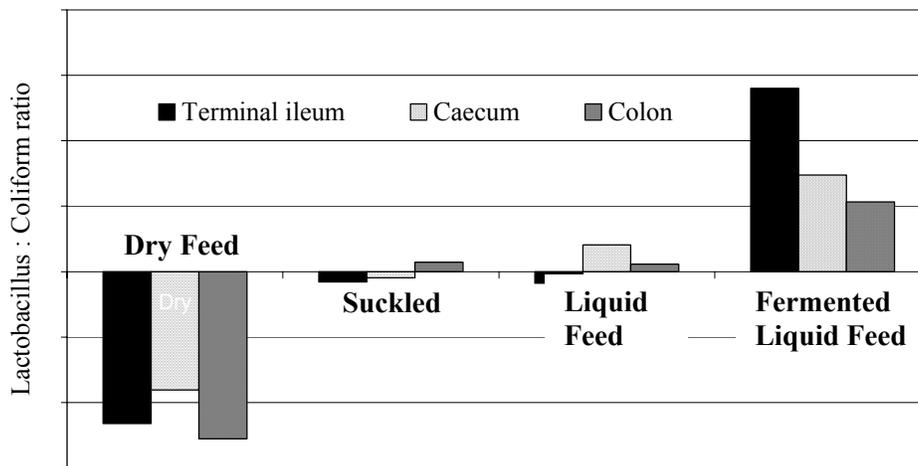
	Dietary treatment			
	Suckled	Dry pelleted feed	Liquid feed	FLF
Stomach	2.4 ^b	3.9 ^{ab}	4.8 ^a	3.9 ^{ab}
Terminal ileum	5.9 ^{bc}	6.3 ^{abc}	6.4 ^{abc}	6.1 ^{abc}
Caecum	6.1 ^{ab}	5.8 ^b	6.0 ^{ab}	6.0 ^{ab}
Colon	6.6 ^a	5.9 ^b	6.0 ^{ab}	6.2 ^{ab}

^{a, b, c} Means in the same row with the same superscript do not differ at $P < 0.05$

Feeding fermented liquid feed does not appear to produce any significant effect on the number of lactic acid bacteria throughout the gut but it does dramatically reduce the number of coliforms in the lower small intestine, caecum and colon (Jensen *et al.* 1998; Moran 2001; Muralidhara *et al.*

1977; van Winsen *et al.* 2001). The ratio of lactic acid bacteria to coliforms in the lower gut of pigs weaned onto liquid diets was very similar to that of pigs that continued to suckle the sow (Moran 2001). However, when the pigs were weaned onto dry diets there was a significant shift in the ratio towards the coliform bacteria. Conversely, when they were weaned onto FLF the number of coliforms was reduced and the ratio shifted in favour of the lactic acid bacteria (Figure 6). This is similar to the response that occurs when some antibiotic growth promoters are fed (Jensen 1998), and suggests that FLF might have a valuable role as part of a strategy for the management of pigs in the absence of antibiotic growth promoters.

Figure 6. Ratio of Coliforms to Lactic Acid Bacteria in the gut of post-weaned pigs fed dry pellets, non-fermented liquid feed (NFLF) or fermented liquid feed (FLF) compared with pigs that continued to suckle their dam without creep feeding (Moran 2001).

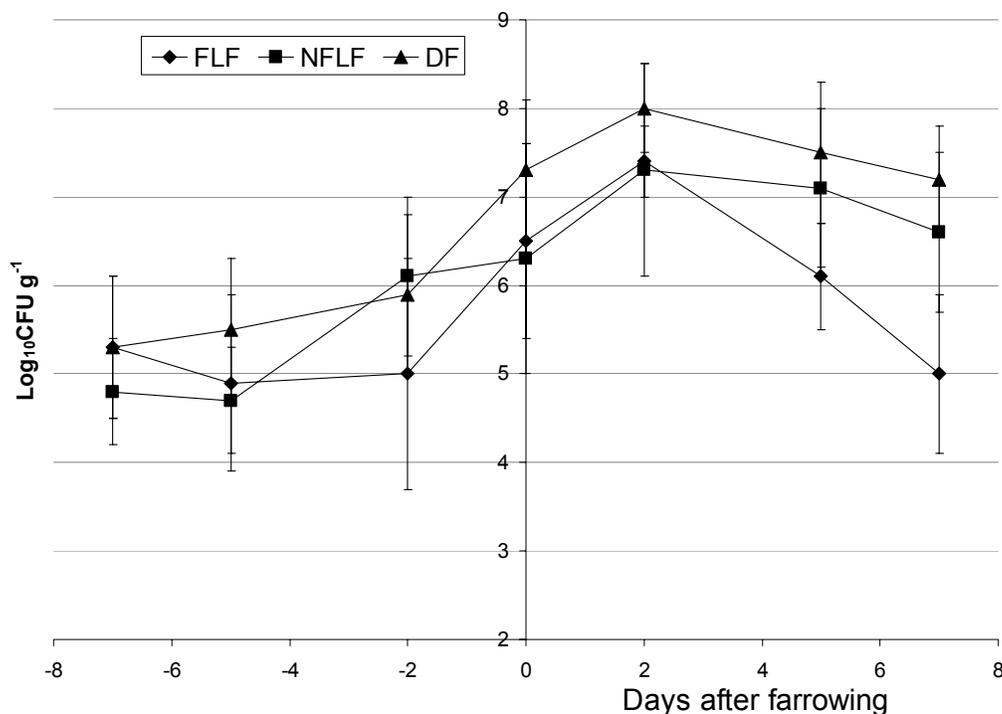


FEEDING LACTIC ACID BACTERIA TO THE SOW IS A WAY OF INFLUENCING GUT COLONISATION IN HER PIGLETS

At birth the pig usually has a sterile gut and acquires its characteristic flora during and following birth 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 were fed diets fermented with aggregating *Lab. salivarius* derived from healthy sows and were compared with sows fed dry diets or non-fermented liquid diets prepared immediately before feeding (Demečková *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 (Figure 7). 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 quality of the sow's colostrum was also affected by diet. Colostrum from sows fed FLF had significantly greater mitogenic activity on both intestinal cells and blood lymphocytes compared with colostrum from sows fed a dry diet. It can be anticipated that an increase in the proliferation of intestinal epithelium will result in an overall increase in the epithelial cell population and a corresponding increase in villus height. Thus, colostrum with a higher mitogenic activity has the potential to both accelerate the maturation of the newborn's GI tract and provide the piglet with better protection by maintaining the integrity of the intestinal mucosa. The observed increase in the mitogenic activity of lymphocytes is very important. It is clear that the immunostimulatory effects depend on both the organism used and the dose (Donnet-Hughes *et al.* 1999; Gill *et al.* 2001), and generally requires continued ingestion of LAB. Dose levels required to produce an immunostimulatory effect appear to be of the order 10^9 CFU. This level is consistent with the daily dose of LAB provided by FLF.

Figure 7. Faecal counts of coliforms and LAB in the sows fed different diet for the period of 1 week before farrowing till 1 week after parturition (Demečková *et al.* 2002).



CONCLUSIONS

In some counties, the adoption of liquid feeding, has significantly reduced the incidence of *Salmonella*, particularly when it is allied to the use of acidic food industry co-products or fermented feed. Fermentation, is one of the oldest, safest and most natural methods of feed preservation and it is pleasing to see that it is being rediscovered and put to good use in the pig

industry. The use of acidic components and the controlled fermentation of liquid feed provide a simple mechanism whereby the bio-safety of feed can be increased. The ability of fermented feed to exclude pathogens such as *Salmonella* can make an important contribution to food safety. This capability will increase in importance as legislators press the pig industry to remove antibiotic growth promoters from their diets. However, legislators will also need to be sympathetic to the needs of the industry and ensure that legislation governing the use of LAB inoculants ensures food safety but does not become a deterrent to the development of this exciting and beneficial technology.

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