

# THE EFFECT ON BIOLOGICAL PERFORMANCE AND FAECAL MICROBIOLOGY OF FEEDING FINISHING PIGS ON LIQUID DIETS FERMENTED WITH LACTIC ACID BACTERIA.

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**Abstract** Thirty-two Hermitage-Seaborough, Hybrid pigs, were allocated at 16 weeks of age to a randomized block design with four treatments and four replicates. Pigs were fed to scale (*ca.* 0.85 *ad libitum*) one of the four dietary treatments, namely; non-fermented liquid feed (NFLF) or liquid feed fermented for 24h at 30°C with either *Lactobacillus salivarius* (FLF-SAL), *Pediococcus acidilactici* (Bactocell™) (FLF-BAC), or a mixture of *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactococcus lactis*, and *Lactobacillus plantarum* (Stabisil™) (FLF-STAB). Treatment did not significantly affect average daily live weight gain or food conversion ratio. The coliform population in faecal samples from pigs fed FLF-SAL was significantly lower ( $1.44 \pm 0.422 \log^{10}$  CFU ml<sup>-1</sup>;  $P < 0.05$ ) than in pigs fed NFLF. Compared with pigs fed NFLF, the LAB:coliform ratio in the faeces of pigs fed FLF-SAL and FLF-STAB was significantly ( $P < 0.05$ ) reduced by  $1.47 \pm 0.46$  and  $1.91 \pm 0.46$ , respectively.

**Introduction** Liquid feed (LF) and particularly fermented liquid feed (FLF) has been shown to reduce the incidence of *Salmonella* in pigs (Tielen *et al.*, 1997; van der Wolf *et al.*, 1999; Lo Fo Wong *et al.*, 2002). A lactic acid concentration of 70mMol was found to be bacteriostatic to *Salmonella* spp., but higher concentrations (>100mMol) were needed to be bactericidal (Beal *et al.*, 2002; Brooks, 2005). Unfortunately, natural fermentations have produced unpredictable results on commercial units. In a recent study (Beal *et al.*, 2005), only 3% (9 of 300) of fermentations of wheat and barley produced more than 75 mmol lactic acid kg<sup>-1</sup> after 24 h fermentation. Thus natural fermentations, which rely on the indigenous flora present on grains, cannot be relied upon to produce bactericidal levels of lactic acid.

More predictable fermentation can be achieved by inoculating liquid feed with lactic acid bacteria (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 Beal, Niven and Brooks (unpublished data) have identified a number of LAB species capable of producing 180-230 mmol kg<sup>-1</sup> lactic acid in 24 hours with <30 mmol kg<sup>-1</sup> 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).

The objectives of the experiment reported here were to examine the effect on biological performance and faecal microbiology of pigs fed liquid diets in which pH was reduced through inoculating diets with specific lactic acid bacteria.

**Materials and Methods** Protocols for the study were approved by the University's Animal Ethics Committee and followed the Code of Recommendations for the Welfare of Livestock (DEFRA, 2003).

The experiment was conducted according to a randomized block design with four treatments and four replicates. A replicate consisted of eight pigs (four males and four females) each fed one of the four dietary treatments. The 32 pigs used were Hermitage-Seaborough Hybrids, which were 16 weeks old at the start of the experiment. The pigs were penned in single-sex groups of four pigs of similar weight. The four dietary treatments were: -

NFLF: Control. Freshly prepared non-fermented liquid feed

FLF-SAL: Liquid feed fermented with *Lactobacillus salivarius* [NC IMB 41229]).

FLF-BAC: Liquid feed fermented with Bactocell™, (*Pediococcus acidilactici* [CNCM No. MA 18/5 M]. Lallemand SA, Toulouse, France).

FLF-STAB: Liquid feed fermented with Stabisil™ (*Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactococcus lactis*, and *Lactobacillus plantarum*. Medipharm, SE-260 23, Kågeröd, Sweden).

The basal diet comprised (kg per tonne) wheat 393; barley 310; Hipro soya 196; rapeseed meal 75; mineral vitamin premix 25. Antibiotics were excluded, but copper (cupric sulphate) was included.

ed at 165 mg kg<sup>-1</sup>. The diet supplied 13.3 MJ DE kg<sup>-1</sup>; 196 g kg<sup>-1</sup> crude protein and 10.5 g kg<sup>-1</sup> lysine (of which 0.7g kg<sup>-1</sup> was crystalline lysine).

The non-fermented liquid feed (NFLF) was prepared daily by mixing dry feed with water in a ratio of 2.5:1 water to feed. No attempt was made to eliminate the natural microflora in NFLF. All the fermented liquid feeds (FLF) were prepared by inoculating NFLF with a starter culture and fermenting for 24 hours at 30°C, to give a final concentration of between 6 and 7 log<sub>10</sub> CFU ml<sup>-1</sup> liquid feed and a pH of *ca.* 4.0. In the case of (FLF-SAL) the inoculant was an overnight culture of *Lactobacillus salivarius* in MRS broth at 35°C. To prepare (FLF-BAC) and (FLF-STAB) a freeze-dried culture of respectively Bactocell™ or Stabasil™ was inoculated at 1 g per 10 g liquid feed.

The pigs were housed for 7 weeks in pens of four. The mean air temperature throughout the trial was 21±3.3°C. Each pen comprised a kennelled area and a slatted dunging area and four individual feeding stalls. The treatments were allocated to give one pig per diet per pen. The pigs weighed 54.3±6.3 kg at the beginning of the trial, and were slaughtered on reaching *ca.* 95 kg.

Pigs were fed in a trough, in individual feed stalls, twice daily, at 8:30 and 16:00 hours, according to a feeding scale that allowed *ca.* 0.85 *ad libitum* feed intake (to avoid weigh-backs). Pigs were allowed 30 minutes to feed after which any residual feed was removed and weighed. Feed allowances were adjusted weekly on the basis of live weight. Fresh water was available *ad libitum* from nipple drinkers.

Fresh faecal samples (approximately 100 g) were collected from the rectum of each pig at the end of the experimental period and microbiological analyses were performed immediately. To enumerate lactic acid bacteria and coliforms, gut contents were homogenized for 4 minutes in a stomacher (Stomacher 400, Seward, London, U.K.) and 10-fold dilutions were made in Maximum Recovery Diluent (Oxoid Ltd., Basingstoke, Hampshire, U.K.). Appropriate dilutions were plated onto selective media. Coliforms were enumerated on MacConkey agar (MCA) using the pour-plate technique and incubated aerobically at 37°C for 24 hours. Lactic acid bacteria were enumerated on modified deMan Rogosa Sharpe agar (MRS agar plus cystein HCl and Aniline blue) and incubated aerobically at 37°C for 48 hours. After incubation, plates containing 30-300 colonies were counted and the number of colony forming units (CFU) per gram of digesta calculated for each type of organism.

Data were statistically analysed using a General Linear Model with Minitab v 13.31 (Minitab Inc., Pennsylvania, USA, 2000). The bacterial count data was log transformed before statistical analysis. Significant differences between treatments means were compared by Tukey's HSD test.

**Results** The health of the pigs was good. After 24-h fermentation at 30°C, the pH of the feed dropped to 3.9 and remained around this value for the rest of the experiment for each dietary treatment. The pigs were fed to scale and over the 53 day trial period there were no significant treatment effects on average daily live weight gain. Daily gain was 899, 942, 937 and 917 (s.e.m. 18.8) g d<sup>-1</sup> respectively, for the pigs fed the NFLF, FLF-SAL, FLF-BAC and FLF-STAB diets. There were no significant treatment differences in FCR.

Fresh faecal samples were collected at the end of the finishing period. Numbers of faecal LAB, coliforms and the LAB:coliform ratio are presented in Table 1. There were no statistically significant differences in the concentration of LAB in the faeces of pigs from the different dietary treatments. The faecal coliform concentration was significantly ( $P < 0.05$ ) lower in pigs fed FLF-STAB than in pigs fed NFLF. The faecal LAB:Coliform ratio of pigs fed FLF-SAL and FLF-STAB was significantly higher ( $P = 0.023$ ) than for pigs fed NFLF.

**Discussion** The pigs were fed to scale and there was no significant difference in daily gain or FCR between treatments. These data indicate that fermenting the liquid feed did not reduce its nutritional value. There have been suggestions that fermentation can result in a loss of energy (Jensen and Mikkelsen, 1998) or synthetic lysine (Pedersen, 2001) from the diet. However, recent studies at the University of Plymouth have demonstrated that synthetic lysine is not lost from the diet when fermentation is controlled by the use of inoculants and the pH of the feed is reduced rapidly to *ca.* pH4 (Niven, Beal and Brooks in press).

An important feature of this study was that the water used to prepare the feeds (NFLF and FLFs) was at 30°C. After 24-h fermentation the pH of the feed dropped to 3.9 and remained close to this value for the rest of the experiment. It is very important that water used to prepare the

feeds (NFLF and FLFs) be at the correct temperature. Water added direct from the tap (around 5°C) will 'cold shock' the system and can adversely affect the growth of the LAB and hence the rate at which pH is lowered. Additionally, it can allow the yeast to become dominant. This can result in loss of nutritional value and reduce pathogen exclusion. Finally, the 'cold shock' can induce the production of 'cold shock' proteins in enteropathogens. These 'cold shock' proteins protect the pathogens and allow them to persist in the feed (Brooks *et al.*, 2001).

Beal *et al.* (2002) demonstrated that any *Salmonella* contaminating FLF during the initial mixing and fermentation process have the potential to remain viable for up to 72 h when fermentation is carried out at 20°C; whereas, if the temperature is raised to 30°C, the risk period is reduced to 48 h (Beal *et al.*, 2002).

A number of factors may account for the beneficial effect of FLF, and these may act independently or synergistically. The low pH (<4.0) of the diet, the high numbers of LAB (6-7 log<sub>10</sub> CFU ml<sup>-1</sup> liquid feed) and high concentration of lactic acid represent the most important characteristics of FLF in terms of its protective effect (Demeckova, 2003).

Despite feeding very large numbers of LAB (6 and 7 log<sub>10</sub> CFU ml<sup>-1</sup> liquid feed), the faecal LAB counts did not increase significantly. The main effect of feeding fermented diets is to reduce the concentration of coliform bacteria in the lower small intestine, caecum and colon (Muralidhara *et al.*, 1977; Jensen and Mikkelsen, 1998; Lawlor *et al.*, 2002). This is similar to the response that occurs when some antibiotic growth promoters are fed 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 (Jensen, 1998). However, the results of the current study indicate that the choice of LAB used to ferment the diet can influence the extent of this reduction in coliforms.

**Conclusion** This study demonstrates that feed fermented with selected LAB inoculants can have a greater effect on gut microbiology than naturally fermented feed. Selected inoculants used to ferment feed may provide a mechanism for controlling the incidence of *Salmonella* both in liquid diets and in the pig.

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	Non-fermented liquid feed NFLF	Liquid feed fermented with:			SE <sub>D</sub>
		L. salivarius FLF-SAL	P. acidilactici (Bactocell™) FLF-BAC	P. acidilactici, P. pentosaceus, L. lactis, and L. plantarum (Stabisil™) FLF-STAB	
Coliforms	6.40a	5.20a	5.78a	4.96b	0.422
Lactic acid bacteria (LAB)	9.28a	9.36a	9.38a	9.48a	0.083
LAB:coliform ratio	3.08a	4.16b	3.60a	4.50b	0.462

Table 1. Effect on the faecal microflora ( $\log_{10}$  CFU ml<sup>-1</sup>) of finishing pigs of feeding non-fermented liquid feed (NFLF) or liquid feed fermented with different lactic acid bacteria. <sup>a, b</sup> Means with the same superscript do not differ at P<0.05