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Fermented Liquid Feed: The potential for eliminating enteropathogens from feed

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Summary: The aim of this study was to determine the effect of temperature on the survival of *Salmonella* and *E. coli* in fermented liquid pig feed (FLF). Liquid feed, fermented with *Lactobacillus plantarum*, was challenged with *Salmonella* or *E. coli* (six serovars of each) at 20, 30 or 37 °C. Temperature significantly affected the survival of *Salmonella* and *E. coli*. In FLF containing *ca* 230 mmol L⁻¹ lactic acid the mean decimal reduction time (D) for *Salmonella* was reduced from 157 (±10) min at 20 °C to 12 (±1.5) min at 30 °C and < 5 min at 37 °C. Likewise, the mean D for *E. coli* was significantly reduced from >180 min at 20 °C to 30 (±12.7) min at 30 °C and 21 (±6.2) min at 37 °C. These studies suggest that successful elimination of potential pathogens from liquid feed can be achieved through fermentation with appropriate lactic acid bacteria and temperature control.

Keywords: *Salmonella*, *E. coli*, pigs, lactic acid, *Lactobacillus*

Introduction: Liquid feed (LF) is often fed to grower and finisher pigs in UK and Europe. However, unless steps are taken to prevent it, liquid feed has the potential to be a vector for pathogenic microorganisms. The risk of proliferation of both pathogenic and spoilage organisms in LF can be reduced by fermenting LF with lactic acid bacteria (Brooks et al.,2001). A typical fermented liquid feed (FLF) has a pH of 3.8 – 4 and contains 150 – 250 mmol L⁻¹ lactic acid, which enables it to withstand contamination by other microorganisms including pathogens such as salmonellae. However, extrinsic environmental factors such as temperature may affect both the ability of lactic acid bacteria to grow and produce lactic acid and the survival of enteropathogens in FLF (Beal et al.,2002). The aims of this study were to determine the affect of temperature and fermentation time on the generation of lactic acid and the survival of *Salmonella* and *E.coli* in FLF.

Materials and Methods: A commercial piglet diet was sterilized (25 kGy g irradiation), mixed with sterile distilled water (2.5 water:1 feed), inoculated with *ca* 10⁶ cfu ml⁻¹ *Lactobacillus plantarum* and incubated at 20, 30 or 37 °C for 48, 72 or 96 h. Samples of the resultant FLF's were taken for lactic and acetic acid analysis by high performance liquid chromatography. FLF's were inoculated (in triplicate) with *ca* 10⁷ cfu g⁻¹ of the *Salmonella* serovars: Typhimurium, DT104B(342A), DT104B(342B), DT193(20), Derby(16), Goldcoast(245) and Anatum(41A) and *E.coli* serovars: K88(99), K88(100), K88(101), K99(185), K99(230) and O157:H7. FLF's were maintained at 20, 30 or 37 °C and samples taken at appropriate time intervals for the enumeration of *Salmonella* and *E. coli* using standard plate count techniques.

The decimal reduction time (D) was calculated for each strain.

Results: Different fermentation time/temperature regimes generated different quantities of lactic and acetic acids in FLF. However, there were no statistically significant differences ($p > 0.05$) in lactic acid concentration in feeds fermented at 30 °C for 72 or 96 h and feeds fermented at 37 °C for 48, 72 or 96 h (Table 1). There were significant ($p < 0.05$) differences in D_{30} between strains of *E. coli* and *Salmonella*.

Table 1. Lactic and acetic acid concentration (mmol L^{-1}) and decimal reduction time (D) of *E. coli* and *Salmonella* serovars at 20, 30 and 37 AC in FLF fermented over 48, 72 or 96 h.

Temperature °C	20			30			37			s.e.d
Ferm Time (h)	48	72	96	48	72	96	48	72	96	
Lactic acid	132 ^a	192 ^b	229 ^{bc}	168 ^a	224 ^{bc}	232 ^c	254 ^c	251 ^c	256 ^c	8.6
Acetic acid	9	11	22 ^a	nd	12	22 ^a	nd	25 ^a	29 ^a	3.4
D (min)										
<i>E. coli</i> K88(99)	> 180	> 180	> 180	46.6 ^{a1}	42.9 ^{a1}	35.4 ¹	25.2 ^{b1}	23.7 ^{b1}	22.9 ^{b1}	1.84
<i>E. coli</i> K88(100)	> 180	> 180	> 180	60.0 ^a	56.1 ^{ab}	50.7 ^b	26.1 ^{c1}	23.6 ^{c1}	17.4 ^{c12}	1.84
<i>E. coli</i> K88 (101)	> 180	> 180	> 180	44.2 ¹	36.9 ^{a1}	30.2 ^{a1}	22.5 ^{b1}	24.3 ^{b1}	24.3 ^{b1}	1.84
<i>E. coli</i> K99 (185)	> 180	> 180	> 180	35.9	21.9 ^{a2}	19.0 ^{a2}	22.0 ^{a1}	16.6 ^{a2}	15.9 ^{a2}	1.84
<i>E. coli</i> K99 (230)	> 180	> 180	> 180	43.7 ¹	38.1 ¹	28.8 ^{a1}	22.2 ^{a1}	14.6 ^{b2}	14.1 ^{b2}	1.84
<i>E. coli</i> O157:H7	> 180	> 180	> 180	20.6 ^a	18.7 ^{ab2}	15.0 ^{abc2}	12.2 ^{bc}	9.3 ^{c2}	10.3 ^{c2}	1.84
<i>Salmonella</i> :-										
DT104B(342A)*	> 1080	201.6	171.6	34.7	13.8 ¹	< 5.0	< 5.0	< 5.0	< 5.0	1.49
DT104B(342B)*	> 1080	190.8 ¹	162.0 ²	21.7 ¹	12.7 ¹	< 5.0	< 5.0	< 5.0	< 5.0	1.84
DT193 (20)*	> 1080	238.8	147.6 ¹	15.4 ^{a2}	11.6 ^{a1}	< 5.0	< 5.0	< 5.0	< 5.0	1.84
Derby (16)	> 1080	184.8 ¹²	150.0 ¹	25.8 ¹	11.3 ¹	< 5.0	< 5.0	< 5.0	< 5.0	1.84
Goldcoast (245)	> 1080	163.2	147.6 ¹	16.4 ^{a2}	14.2 ^{a1}	< 5.0	< 5.0	< 5.0	< 5.0	1.84
Anatum (41A)	> 1080	193.2 ²	164.4 ²	15.1 ^{a2}	10.2 ^{a1}	< 5.0	< 5.0	< 5.0	< 5.0	1.84

* *Salmonella* Typhimurium serovars < 5.0 = no organisms were detected after 5 min challenge

^{a,b,c} means with the same superscript in the same row are not significantly different ($p < 0.05$)

^{1,2} means with the same superscript in the same column are not significantly different ($p < 0.05$)

Of the *E. coli* strains, O157:H7 appeared to be the most sensitive to the conditions prevailing in FLF. Temperature had a significant ($p < 0.001$) affect on the death rate of both organisms. This was most apparent in FLF's fermented at 20 °C and 30 °C for 96 h; both contained comparable concentrations of lactic and acetic acid (230 and 22 mmol L^{-1} respectively). The 10 °C increase in temperature resulted in a 5–6 fold and > 35 fold increase in the death rate of *E. coli* and *Salmonella* respectively. Increasing the temperature to 37 °C resulted in a further increase in death rate.

Discussion: On most farms temperature of liquid feed is not controlled. However, this study demonstrates that temperature control is important in reducing the risk of enteropathogen transmission via fermented liquid feed. At 20 °C there was little reduction in numbers of *Salmonella* or *E. coli* within the first three hours of inoculation. This is in agreement with previous studies in which FLF was challenged with *Salmonella* species (Beal et al.,2002, van Winsen et al.,2001). In this study, a large inoculum size was used in order to generate D values and it is unlikely that such high levels of contamination would occur on farms. However, even with more realistic numbers of 10 – 100 organisms g^{-1} feed it could take

3 - 18 h to eliminate *Salmonella* or *E. coli* from feed at 20 °C compared with 30 min – 2 h at 30 °C and less than 30 min at 37 °C. Lactic acid concentration also plays an important role the elimination of enteropathogens from FLF and in this study high concentrations of lactic acid were achieved via inoculation with *Lb plantarum*. With proper management, i.e. temperature control and inoculation with lactic acid bacteria to ensure adequate lactic acid production, FLF systems have the potential to play an important role in the reduction of enteropathogens in the food chain.

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Population of a farrowing unit by *Salmonella* negative animals

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Summary: In order to obtain a better control of *Salmonella* in swine herd, it is important to ensure introduction of negative replacement animals. The objective of this project was to introduce negative sows in a new farrowing unit by use of a protocol based on a combination of bacteriology and serology to select replacement gilts in the finishing unit of origin. Animals were selected from a finishing unit known to be moderately contaminated by *Salmonella* based on previous bacteriological and serological analysis. Based on the results, animals were separated in 2 groups. The first group (group A) consisted of seronegative gilts from *Salmonella* negative pens, designed NP/NS. Group B was composed of seronegative gilts from positive pens, was designed PP/NS and gilts were treated with neomycin to reduce *Salmonella* shedding. All animals were also washed at their arrival in the farrowing unit. Results demonstrated that it is possible to populate new herds by negative animals (NP/NS) coming from a positive herd by selecting animal using bacteriology and serology, and by application of biosecurity and prophylactic measures.

Keywords: biosecurity, prophylactic, serology, excretion, salmonella-free

Introduction: Pork products have been associated with many cases of salmonellosis in human (Beran et al., 1995). Since *Salmonella* is a facultative intracellular pathogen, following the infection, many animals will remain healthy carrier up to the end of the fattening period (Letellier et al., 1999). When stressed (eg transport to slaughter), many healthy carriers will shed the bacteria and contaminate other