

Effect on weaner pig performance and diet microbiology of feeding a liquid diet acidified to pH 4 with either lactic acid or through fermentation with *Pediococcus acidilactici*

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Abstract: The effect of feeding newly weaned pigs acidified liquid diets was investigated. The control diet was acidified to about pH 4 with lactic acid (LA). A second diet of the same formulation was acidified to about pH 4 by fermentation with *Pediococcus acidilactici* (PA). Forty-eight weaner pigs (weight 7 kg \pm 1 kg, age 24 \pm 4 days) were allocated to the two dietary treatments according to a randomised block design and fed *ad libitum* for 28 days. Food intake, daily gain and water intake were recorded, and a microbial assessment of the liquid diet was conducted. Reducing pH < 4.0 in either of the liquid diets was effective in eliminating coliform bacteria. There were no significant differences in any of the performance parameters measured. The average daily liveweight gain overall was 474 and 496 \pm 17.8 g d⁻¹ for PA and LA, respectively, with a feed conversion ratio overall of 1.15 and 1.11 \pm 0.025 for PA and LA, respectively. Fermentation of liquid diets for newly weaned piglets could provide a more cost effective means of acidifying diets than the use of organic acids. Reducing the pH of the liquid diet to 4.00 by fermentation with *Pediococcus acidilactici* was a cost effective method of eliminating enteropathogens and spoilage organisms from the diet.

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Keywords: pigs; weaners; feeding systems; liquid feeds; feed microbiology; fermentation; lactic acid; *Pediococcus acidilactici*

INTRODUCTION

Previous studies have shown that weaner pigs grow better on liquid feed than the same diet fed dry and that liquid diets with a wide range of dry matter concentrations are well accepted.^{1,2} Microbiological examination of the diets used in these studies revealed that a natural, lactic acid fermentation had occurred with a consequent reduction in pH of the liquid feed to about pH 3.5–4.0 within five days.

Gastric secretion of hydrochloric acid is very limited for the first few weeks of life.³ The suckling pig is able to overcome the disadvantages of having low HCl production by microbial fermentation of the lactose in sow's milk to lactic acid.^{4,5} This acid production inhibits the growth of pathogenic bacteria whilst allowing the commensal bacteria to flourish. *Lactobacilli*, for example *Lactobacillus fermentum* and *L. acidophilus*, are present on the teats of the sow (Campbell and Santos, 1996, unpublished data) and will be ingested during suckling. Recent studies⁶ have found that pigs fed a fermented liquid feed (FLF) have a lower stomach pH than pigs fed unfer-

mented feed. Formic acid supplementation of weaner diets has been reported to have a similar effect, but other organic acids did not reduce stomach pH.⁷

The problem of 3 and 4 day scouring, and post-weaning growth check, may occur because the piglets have been removed from the sow and hence from the supply of lactose and lactic acid bacteria. There exists a critical point immediately after weaning when numbers of *Lactobacilli* decline and pathogenic bacteria increase.⁸

Manufacturers of compound feeds add acids to dry feeds to compensate for the acid binding capacity of feed ingredients, to increase proteolysis and to reduce the incidence of scour.^{9,10} The addition of lactic, fumaric, formic and citric acid to the feed have all been shown to improve growth rate and feed efficiency in pigs.^{7,11} In pigs, colibacillosis can also be prevented by the addition of lactic acid to the drinking water.^{12–14}

With *ad libitum* liquid feeding for weaners, food is continuously present in both the feed delivery system and the troughs. Consequently, contami-

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nation with spoilage bacteria is possible and the growth of micro-organisms present in the feed and/or ingressing from the environment is inevitable. To date studies at this centre (Brooks and Verkamp, unpublished data) have failed to identify a sterilant which will prevent natural fermentation without radically changing either the nutrient value or the palatability of the diet. Therefore, the pragmatic response of pig producers has been to acidify liquid diets; a practice which has proved effective in preventing pathogen development in calf milk replacers.^{15,16}

In recent years there has been considerable interest in using probiotics instead of antibiotics in piglet diets.^{17,18} The probiotic virtues of lactic acid bacteria have been acknowledged for a long time.^{19–21} *Lactobacilli*, *Enterococci* and *Bifidobacteria* are commonly used as probiotics for pigs.^{18,22} In previous studies at this centre,^{1,2} weaner pigs were fed naturally fermented liquid diets *ad libitum*. The high levels of lactic acid bacteria present in the diets could have exerted a probiotic effect and may be an important determinant of success in the liquid feeding of weaners. These fermentations, however, were uncontrolled and relied upon naturally occurring organisms.

Strains of *Lactobacillus plantarum*, *L. brevis*, *L. fermentum*, *L. caesei* and *Pediococcus acidilactici* have all been used as inoculants to control fermentation in human foods such as soda crackers, fermented milks and sausages.^{19,21,23,24} In theory, such inoculants could also be used to control fermentation in a liquid feed system for pigs.

If the fermentation pattern of liquid feed for pigs could be successfully controlled by the use of such bacterial inoculants, then the risk of coliform scours developing post weaning could be reduced. This in turn would improve the growth rate and feed efficiency of weaned piglets. The use of bacterial inoculants which would lower the pH of the diet could replace expensive organic acids and/or antibiotics in piglet diets which are destined for use in liquid feed systems. The main objectives of the experiments reported here were:

- (1) to compare the growth performance, feed efficiency and health of newly weaned piglets fed liquid diets in which pH was reduced through acidification by lactic acid bacteria with that of pigs fed a conventional acidified diet;
- (2) to examine changes in the composition of acidified and inoculated diets over time.

MATERIALS AND METHODS

Forty-eight Large White × (Large White × Landrace) weaner pigs (Camborough hybrids, Pig Improvement Company, Fyfield Wick), average weight 7 ± 1 kg and age 24 ± 4 days, were allocated according to a randomised block design to compare the effects of feeding weaner pigs a control

diet acidified with lactic acid (LA) or the same diet acidified by fermentation with *Pediococcus acidilactici* (PA). The two dietary treatments were:

LA: Control piglets were fed *ad libitum* on an early weaner diet (Diet 1), supplied as a meal and mixed with water to provide a dry matter concentration of 255 g kg^{-1} (water feed ratio of 2.5:1) with the addition of DL-lactic acid (Ellis and Everard, Exeter), adjusted to a pH of 4.0. Lactic acid was added with each new batch of feed, at the rate of 440 ml per 10 kg of dry matter to maintain the desired pH. The acid was substituted for an equal volume of water in order to maintain the same dry matter (DM) concentration on both diets. The pigs received Diet 1 for the first 14 days post-weaning. The diet was changed to Diet 2 for the remainder of the trial. Residual feed in the tank ensured that the change from Diet 1 to Diet 2 was a gradual process. Feed was dispensed to the pigs automatically using an *ad libitum* feed delivery system.

PA: Piglets were fed as described for LA with the exception that lactic acid was omitted and *Pediococcus acidilactici* ('Bactocell', which comprises a single live strain (MA18/5M) of *Pediococcus acidilactici*, Encore (UK) Ltd, Preston, UK) was added at the rate of 1 g of *Pediococcus acidilactici* per 20 kg of dry matter. The inoculant, which was in freeze dried form, was introduced with each new batch of feed during mixing.

The treatments were replicated four times. A replicate consisted of two pen groups (12 pigs); each pen group consisted of three female and three male piglets. The dietary treatments were introduced at weaning and continued for 28 days.

The housing and feeding system and the management procedures used in this study have been described previously.¹

The experimental diets used were manufactured by A-One Feed Supplements Ltd (Thirsk, North Yorkshire). Diets 1 and 2 contained, respectively, (g kg^{-1}): crude protein 220 and 225; lysine 17 and 16; oil 65 and 80; fibre 23 and 25. The diets contained, respectively, 16.6 and 16.2 MJ DE kg^{-1} . Both diets contained 175 mg kg^{-1} copper (as CuSO_4) and 40 mg kg^{-1} Avilamycin (as Maxus, Elanco Products Ltd, Basingstoke, Hampshire) for growth promotion.

Diet 1 contained 45% cooked cereals in the form of oats and maize, in a ratio of approximately 1:1. Milk products (primarily skim milk and whey powder) contributed 15% lactose in the dry matter. In addition glucose was added to provide 2.5% of the dry matter. Protein and fat were supplied as steam dried fish meal (68–70% protein, 10–12% oil) and full fat soya bean oil. In Diet 2 the cereal component contained 49.5% of cooked cereals in the form of

Table 1. Performance of pigs fed liquid feed acidified with either lactic acid (control) or as a result of fermentation of the diet with *Pediococcus acidilactici*

Parameter	Period	Treatment		SE _D
		Control lactic acid	<i>Pediococcus acidilactici</i>	
Dry matter feed intake (g d ⁻¹)	week 1	277	296	44
	week 2	490	548	58
	week 3	630	645	97
	week 4	747	762	103
	Overall	536	563	71
Daily gain (g d ⁻¹)	week 1	299	366	42
	week 2	467	506	37
	week 3	559	568	43
	week 4	571	546	36
	Overall	474	496	25
Dry matter feed conversion ratio	week 1	0.99	0.77	0.15
	week 2	1.06	1.07	0.07
	week 3	1.13	1.14	0.06
	week 4	1.34	1.37	0.12
	Overall	1.15	1.11	0.09
Total water intake (ml pig d ⁻¹)	week 1	1090	1129	183
	week 2	1714	2237	258
	week 3	2484	2721	221
	week 4	3026	3047	391
	Overall	2078	2283	252
Average water intake from drinkers (ml pig d ⁻¹)	week 1	299	283	79
	week 2	312	670	132
	week 3	620	811	191
	week 4	814	790	221
	Overall	511	638	133
Average effluent production (ml pig d ⁻¹)	week 1	417	564	251
	week 2	911	1259	318
	week 3	1305	1680	244
	week 4	1837	1903	230
	Overall	1118	1359	185

maize and wheat, in a ratio of approximately 1 : 1. The main milk product was skim milk powder with some whey powder; together these contributed 10% lactose in the dry matter. Glucose was added to provide 2.5% of the dry matter. Protein and fat were supplied as steam dried fish meal (68–70% protein, 10–12% oil) and full fat soya bean oil. Both diets were supplemented with minerals, trace elements and vitamins.

A microbiological assessment was made of the liquid feed mixture for each treatment. The feeding system was cleaned prior to the introduction of new treatments to the facility. Each morning prior to replenishment of the feed system, samples (300 ml) of the liquid feed were removed from the mixing tank via the outfall pipe, after two minutes of mixing, and using aseptic procedures. The samples were plated within two hours of collection. The viable indigenous micro-organisms present in the dry diets (Diets 1 and 2) were assessed on days 1, 7, 14 and 21

of the experiment. Each sample was serially diluted in 0.25 strength Ringers solution (Unipath Ltd, Basingstoke, Hampshire) and appropriate dilutions were plated and incubated as follows. Total bacteria were assessed using Plate Count Agar (Unipath Ltd) and incubated aerobically at 30°C for three days. Coliforms were assessed on MacConkey's agar (Unipath Ltd) using the surface plate count method.²⁵ They were incubated, aerobically, at 37°C for 24 h. Lactic acid bacteria were assessed using the pour plate method of Banwart²⁵ on de Mann, Rogossa and Sharpe agar (Unipath Ltd) incubated anaerobically using the gas pak system (Unipath Ltd) at 37°C for three days. Yeasts and moulds were assessed using the surface plate method of Banwart²⁵ on Rose Bengal Chloramphenicol agar (Unipath Ltd) incubated, aerobically, at 22°C for five days. In addition, the pH of each sample was measured using a pH meter (Kent EIL 7015) and samples were assessed for alcohol by the distillation method for wines²⁶

Table 2. Indigenous populations of micro-organisms (\log_{10}) present in the initial dry feed components of Diets 1 and 2

Diet	Day	Coliforms	Yeasts	<i>lactic acid bacteria</i>
1	1	0.00	3.30	3.63
1	7	1.00	3.32	3.21
2	14	2.84	3.69	4.17
2	21	2.84	3.32	4.05

and changes in dietary composition by standard methods.²⁷

Piglets were weighed at weekly intervals throughout the experimental period. The health of the animals was monitored regularly and any medication and veterinary intervention was recorded. Feed intake, weight gain, water intake and effluent production records were maintained throughout the trial.

Feed intake was calculated on a dry matter food

intake (DMFI) basis. Dry matter feed conversion ratio (DMFCR) was the appropriate multiple of DMFI divided by the weight gain of the pigs. Performance data were subjected to two-way analysis of variance. Daily gain was also analysed using covariance analysis (using weaning age as the covariate). All statistical analyses were undertaken using Minitab v9.2 (Minitab Inc., State College, USA, 1993).

RESULTS

Animal health

There were no major health problems with the piglets and only an occasional sign of scouring in the control (LA) treatment which did not require veterinary intervention. Two pigs on PA who developed joint ill and one pig on LA which received a physical ear injury were given 1 ml injections of long acting Duphamox (Solvay Duphar Veterinary, Southampton). One LA pig had an aural haematoma

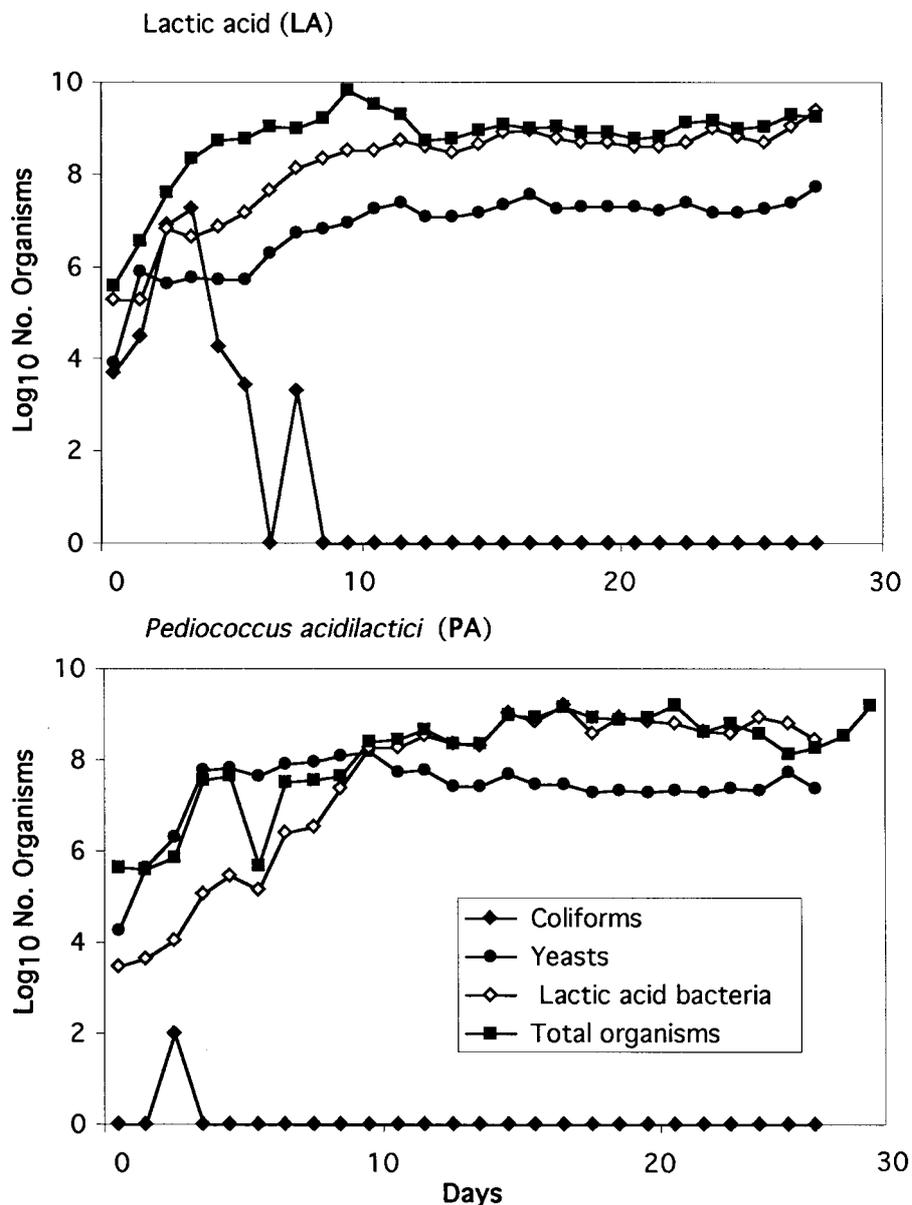


Figure 1. Populations of lactic acid bacteria, yeasts and coliforms in liquid feed, over time, when acidified with lactic acid (LA) or through fermentation with *Pediococcus acidilactici* (PA).

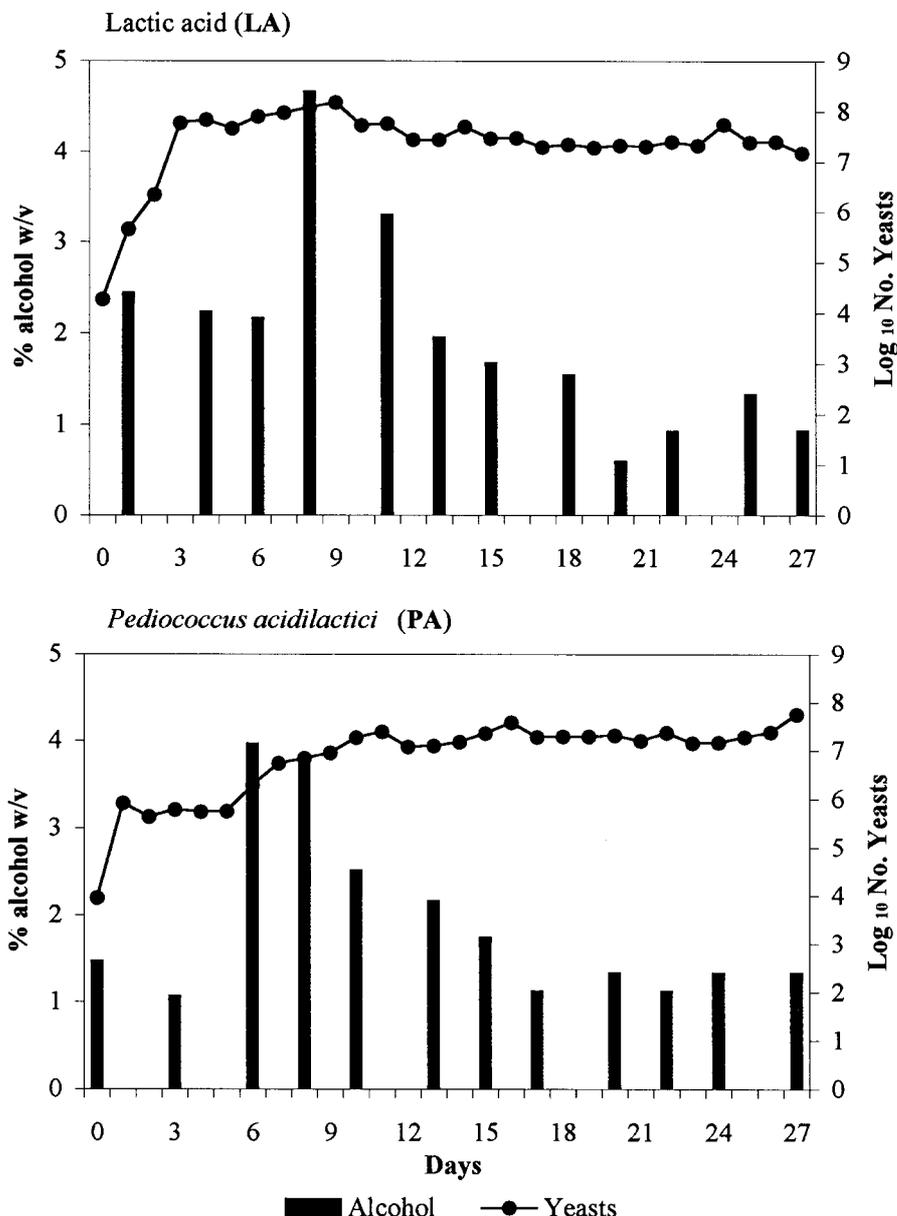


Figure 2. The relationship between the number of yeasts and alcohol production in the liquid feed, over time, when acidified either with lactic acid (LA) or through fermentation with *Pediococcus acidilactici* (PA).

Treatment	Week	Crude protein	Crude fat	Crude fibre	Ash	Nitrogen-free extractive	Digestible energy ^c
PA ^a	1	225	50	17	49	659	16.1
PA ^a	2	262	29	24	58	627	15.4
PA ^a	3	265	56	24	61	594	15.8
PA ^a	4	265	65	25	64	581	15.9
LA ^b	1	202	67	15	41	675	16.6
LA ^b	2	258	40	20	58	624	15.7
LA ^b	3	285	53	23	59	580	15.8
LA ^b	4	278	46	28	57	571	15.6

^a Treatment *Pediococcus acidilactici*

^b Treatment lactic acid

^c Megajoules (MJ) of digestible energy (DE) kg⁻¹ of dry matter (DM)

Calculated using the equation ME (MJ kg⁻¹ DM) = 0.016NFE + 0.032OIL + 0.018CP - 0.015CF. The ME was assumed to be 0.96% of DE, where ME is metabolisable energy, NFE is nitrogen-free extractive, OIL is crude fat, CP is crude protein, CF is crude fibre. Equation No. 8.22.²⁸

Table 3. Proximate analysis of the dietary components and calculated energy values of the liquid diets (g kg⁻¹ DM)

and on three consecutive days was given a 1 ml injection of Duphaphen (active ingredient Procaine Penicillin/Dihydrostreptomycin sulphate, Solvay Duphar Veterinary, Southampton).

Growth and feed utilisation

The biological performance and effluent output of the pigs is summarised in Table 1. Taken over the whole 28 days, treatment had no significant overall effect on any of the parameters of performance measured. The overall daily gain was 474 and $496 \pm 17.8 \text{ g d}^{-1}$ for PA and LA, respectively, and the overall dry matter feed conversion ratio was 1.15 and 1.11 ± 0.025 for the LA and PA treatments, respectively. There were no significant treatment effects on water usage or effluent production.

Microbiology of the liquid feed system

A microbiological assessment of the dry diets as supplied indicated that Diet 1 contained an indigenous population which comprised very small numbers of coliform bacteria but had a small population of yeasts and lactic acid bacteria. Diet 2 contained larger numbers of indigenous coliform bacteria, similar numbers of yeasts and a larger number of lactic acid bacteria than Diet 1 (Table 2).

The microbiology of the liquid feed system was studied for both treatments. The changes in \log_{10} numbers of coliforms, lactic acid bacteria and yeasts with time are presented in Fig 1. For both treatments the numbers of yeasts increased rapidly over the first four days and thereafter remained relatively stable.

In treatment PA, the numbers of lactic acid bacteria increased from day 1 to day 10, thereafter remaining relatively stable. A coliform bloom was observed in the liquid feed in the first four days of the PA treatment and coliforms were not eliminated until day 10 of the experiment when the pH had been reduced to 4.5. In the control treatment (LA), a natural colonisation by lactic acid bacteria developed slowly in the liquid feed system (observed to be gram-positive rods) reaching a peak on day 10 of the experiment and thereafter remaining relatively stable. In the control treatment (LA) acidification with lactic acid to pH 4.0 resulted in the elimination of coliforms from the diet after day 2.

As a result of yeast activity in both liquid feed systems, alcohol was produced (Fig 2). As the yeast populations increased rapidly in the first 9 days of the experiment, the alcohol content of both the liquid feeds increased from 24.4 and 14.7 on day 1 to 46.6 and 38.1 g l^{-1} on day 9 for treatments LA and PA, respectively. After day 9 the level of alcohol in both liquid feeds gradually decreased and stabilised at approximately 12.7 g l^{-1} (LA) and 14.4 g l^{-1} (PA) for the remaining 14 days of the experiments. The alcohol content did not appear to reduce the acceptability of the diet to the piglets.

Proximate analysis of the liquid feeds

The results of a proximate analysis, conducted at weekly intervals for both treatments, is presented in Table 3. The components measured differed little between treatments or with time. The difference in nitrogen-free extractives over time corresponded to the changing proportions of cereals and lactose in Diets 1 and 2. Using the equation of Whittemore,²⁸ the average energy value of the dry matter of the liquid diets for both treatments was calculated as $15.8 \text{ MJ DE kg}^{-1} \text{ DM}$ for PA and $15.9 \text{ MJ DE kg}^{-1} \text{ DM}$ for LA throughout the feeding period.

DISCUSSION AND CONCLUSIONS

In this study, pigs fed a liquid feed acidified by fermentation with *Pediococcus acidilactici* had a growth rate of 496 g d^{-1} , respectively, in the four weeks following weaning. This was not significantly different from the growth rate of pigs fed the conventional diet acidified with lactic acid (474 g d^{-1}). These growth rates exceeded the best growth rate of pigs fed dry pelleted feed (397 g d^{-1}) or naturally fermented feed (454 g d^{-1}) in our previous studies.^{1,2} It is known that newly weaned piglets have insufficient gastric secretions to optimise protein digestion³ and that supplementation with organic acids can help to overcome this problem. Dietary supplementation of weaning pig diets with organic acids has been used to improve growth performance.^{7,16,29-31} Roth et al³² supplemented the diet of weaner pigs of 7 to 28 kg liveweight with different levels of lactic acid and demonstrated that lactic acid improved the daily gain, feed intake and feed conversion ratio. They concluded that the most effective dosage of lactic acid added to a dry diet was 1.6%. In the current study the lactic acid added to the liquid diet was 1.25%. There is no published information on the effects of adding lactic acid to liquid feed for weaner pigs. Furthermore, in the absence of a sterilant which would prevent natural fermentation occurring in liquid feed offered *ad libitum*, it is not possible to make such an assessment.

It has been hypothesised that supplementation of the diet with organic acids reduces gastrointestinal pH and coliform number.^{12,33} Lactic acid has also been used as an effective means of controlling pathogenic organisms by administration in the drinking water of weaned piglets.^{12,14,33,34} They demonstrated that the numbers of haemolytic *Escherichia coli* were reduced in the gastrointestinal tract of weaned piglets which had been given lactic acid in their drinking water compared with the control pigs. In the current study it was found that, although the addition of lactic acid was effective in controlling coliform bacteria in the liquid feed system, it was not an effective means of controlling natural fermentation, since a natural lactic acid fermentation still developed.

While previous studies have indicated that organic acids can be used to overcome some of the challenges facing the weaner pig, the current study indicates that fermentation of the liquid feed could be regarded as an alternative approach. In this experiment *Pediococcus acidilactici* was introduced to promote lactic acid fermentation. As there was no significant difference in performance of the pigs on the two treatments, fermentation with PA would provide advantages over lactic acid as it is a cheaper option. The cost of inoculating the diet with *Pediococcus acidilactici* was less than £1.00 t⁻¹ of dry matter whereas the cost of achieving the same pH reduction using lactic acid was around £150.00 t⁻¹ dry matter. Providing that the appropriate inoculants can be identified for use in liquid feeding systems, the potential for very large savings per tonne of feed exists compared with using organic acids.

There are very few studies which have examined the effects of lactic acid fermented diets on pigs. *Lactobacillus* fermentation products, however, have been shown to stimulate growth and improve feed efficiency in pigs³⁵⁻³⁷ and feeding live *Lactobacilli* cells has been shown to reduce scouring in pigs.³⁸ The very limited published data available on the effects of lactic acid fermented diets on pigs is mainly concerned with its use in ensiled poultry or fish offal.³⁹⁻⁴¹ Recently, Mikkelsen and Jensen⁶ have reported that pigs fed fermented liquid feed ate 22% more than pigs fed non-fermented liquid feed in the first week post weaning. These authors also reported that pigs fed FLF had a significantly ($P < 0.02$) lower stomach pH and significantly reduced numbers of total cultivable bacteria, lactic acid bacteria and coliform bacteria in the gastrointestinal tract.

The *P. acidilactici* organism used in this study did not reduce the pH of the diet to as low a value or as fast as the naturally occurring lactic acid bacteria spp had done in previous experiments in this series,^{1,2} nor did PA become the dominant organism in the liquid feed system. This may have been because either the temperature or the pH in the liquid feed system was not at the optimum for the growth of *P. acidilactici* or because a more aggressive strain of unidentified lactic acid bacteria was able to out-compete it.

In the current study a population of unidentified yeasts, which produced alcohol, developed in both the liquid feed systems. The quantity of alcohol produced did not appear to have any detrimental effect on the piglets. Indeed, the alcohol content of the diet may have been responsible for beneficial changes in the behavior of the pigs, as the attendants noted that the pigs in this study appeared more restful than pigs in previous studies.^{1,2}

In this system it might have been expected that a small proportion of dry matter and DE would have been lost as a result of fermentation. When the energy contents of both of the liquid diets were cal-

culated (Table 3) there appeared to be a loss of energy, especially between the first and second week of fermentation (0.7 and 0.9 MJ DE kg⁻¹ DM for PA and LA, respectively). It should be noted, however, that when liquid diets are subjected to a proximate analysis, alcohol and volatile fatty acids present would be driven off and would not be accounted for in the energy value using the equation given by Whittemore.²⁸ It was known that alcohol was present in both of the liquid diets in this study, therefore the apparent decrease in energy value may be attributed almost entirely to the volatile fraction which was not accounted for by the prediction equation used.

In this study, acidification of the diet either with lactic acid or by fermentation with *Pediococcus acidilactici* resulted in similar pig performance. The latter, however, was a much less expensive means of acidifying the feed. Although both treatments were effective in restricting the growth of enteropathogens in the liquid diet, lactic acid was effective from day 1 while fermentation did not achieve this objective for several days. An important finding in this study was that even when the diet was acidified using lactic acid, a lactic acid bacteria flora still developed. Therefore, it was not possible from this study to determine whether it is the acidification of liquid diets or the presence of lactic acid bacteria which contribute most to their success. Clearly, it is important to provide a diet which has a pH which will prevent the proliferation of pathogenic bacteria and thereby improve the biosecurity of the feed. The question which still awaits resolution is whether the presence of large populations of live lactic acid bacteria and the presence in the diet of any antimicrobial products they produce confers additional benefits on the health and performance of the pig.

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