The effect of fermented liquid feeding on the faecal microbiology and colostrum quality of farrowing sows

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Abstract

This study investigated the effects of fermented liquid feed (FLF) on the lactic acid bacteria (LAB):Coliform (L:C) ratio in the faeces of farrowing sows and the quality of sow colostrum. Eighteen multiparous sows were randomly allocated to one of three dietary treatments for approximately 2 weeks prior to farrowing and for 3 weeks after parturition. The three dietary treatments were dry pelleted feed (DPF), nonfermented liquid feed (NFLF), and fermented liquid feed (FLF). A rifampicin-resistant mutant of *Lactobacillus plantarum* was used to ferment liquid feed. Coliforms and lactic acid bacteria (LAB) in the faeces of farrowing sows and piglets were estimated by standard microbiological techniques. Intestinal epithelial cells (IEC-6) and blood lymphocytes were used to evaluate the mitogenic activity of colostral samples taken at parturition. Results demonstrated that while the LAB population was not significantly affected by dietary treatment, significant differences in coliform population were observed in the sow faecal samples taken 7 days after parturition. Faeces excreted from sows fed FLF had significantly ($P < 0.001$) lower numbers of coliforms than sows fed NFLF or DF. Piglets from sows fed FLF excreted faeces that were higher in LAB (7.7 vs. 7.3 $\log_{10}$ CFU g^{-1}; $P < 0.01$) and lower in coliforms (7.5 vs. 8.1 $\log_{10}$ CFU g^{-1}; $P < 0.001$) than faeces from the piglets of DF-fed dams. Colostrum from sows fed FLF had a significantly greater ($P < 0.001$) mitogenic activity on both intestinal cells (IEC-6) (79326 ± 3069 CPM) and blood lymphocytes (1903 ± 204 CPM) compared with colostrum from dry feed fed sows (53433 ± 1568 and 1231 ± 61.4 CPM, respectively). The combined effects of enhanced maternal/passive immunity and the reduction in the level of environmental contamination with faecal pathogens, achieved by FLF, may be important in achieving improved health status for both sows and piglets.
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1. Introduction

Under normal circumstances, the newborn pig, which is sterile at birth, rapidly acquires its characteristic microflora by contact with its mother and its birth environment. The most significant factor affecting the microflora of the piglet's environment is the sow. It has been shown that sucking piglets can ingest large quantities of both sow's faeces and bedding material (Sansom and Gleed, 1981). In addition, the sow's teats can be heavily contaminated with microbes from faeces and the environment. Therefore, management interventions and nutritional regimes that influence, in a beneficial way, the microbiology of the sow's faeces

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are also likely to influence that of neonate. The relative proportion of different organisms in the sow's faeces and the extent to which the piglet comes into contact with the faeces may affect colonization of the gut (Conway, 1996). Colonization of the pig gut occurs very rapidly after birth. By 24 h, *Escherichia coli*, *Streptococci*, and *Clostridia* were found in high numbers (over 10^8 g^{-1}) in the faeces of piglets. After a further 24 h, these bacteria were accompanied by similarly high numbers of Lactobacilli (Smith and Crab, 1961). A potential problem for the newborn piglet is that this rapid bacterial colonization cannot be efficiently controlled as at this stage of development its immune system is very naïve and hence functionally immature (Klobasa et al., 1981). In addition, the gastrointestinal tract is also in the process of developing which means that pathogenic bacteria have potential, through this route, to access the peripheral body systems. An additional source of contamination is the environment. Therefore, if the microflora that the sow introduces into the environment can be modified in a beneficial way this may also exert a beneficial effect on colonization of the piglet's gut.

The most well studied external factor in influencing the establishment of the intestinal microbiota is the diet. Work at the University of Plymouth and at Foulum in Denmark has shown that feed may be fermented successfully with lactic acid bacteria (LAB) and that this process reduces the number of salmonellae and coliforms in the feed (Brooks et al., 1996; Geary et al., 1996; Mikkelsen and Jensen, 1997; Moran, 2001). Feeding such a diet to pigs does not appear to produce any significant effect on the number of LAB throughout the gut 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). In addition, the presence of high numbers of LAB in the FLF can also contribute to the health status of the pig. Recent studies strongly support the hypothesis that orally administered LAB stimulate the immune system, both at the local and systemic level (von der Weid et al., 2001; Madsen et al., 2001; Gill and Rutherfurd, 2001; Grangette et al., 2001). The stimulation of the mucosal immune system can result in potentially higher levels of colostral immune factors contributing to improved colostrum quality and immune protection of sucking piglets. Such combination of effective immunity and

reduced level of environmental contamination with faecal pathogens is the basis for maintenance of good health for both sow and piglet.

Therefore, the aims of this study were to investigate whether feeding the sows fermented liquid feed (i) modifies the faecal microflora at farrowing especially at parturition when a coliform bloom usually occurs (ii) whether any changes in faecal microflora affect bacterial colonization of the gut of the newborn piglet (iii) whether feeding FLF has a beneficial effect on the quality of colostrum produced by the sow.

2. Materials and methods

The experiment was conducted according to a randomised block design, with six replicates. A replicate consisted of three animals, each fed one of three dietary treatments.

2.1. Experimental animals

Eighteen multiparous (range 2nd to 9th parity) sows (Large White × Landrace) and their piglets were used in the experiment. They were selected on the basis of expected farrowing date. All piglets in the litter of each sow were tagged and weighed at birth, 1 and 3 weeks of age. Neither sows nor piglets received any medication before or during the experiment.

2.2. Dietary treatments

Sows were randomly allocated to one of three dietary treatments for 2 weeks before anticipated farrowing day and for 3 weeks after parturition. The three dietary treatments were dry pelleted feed (DPF); nonfermented liquid feed (NFLF); fermented liquid feed (FLF). A lactation diet in pelleted form was supplied by BOCM Pauls, and provided 14 MJ/kg digestible energy (Table 1). The sows were given 3.5 kg of DPF/day. NFLF was prepared daily by mixing 3.5 kg of DPF with water in a feed to water ratio of 1:2. In order to eliminate natural microflora present in the feed, the feed was sanitized with 300 ppm chlorine dioxide (ClO_2) (Sanitech 2%; Alltech, Kentucky) for 24 h. Active chlorine dioxide was prepared by mixing 125.4 ml of ClO_2 concentrate and 12.5 g of citric acid in a 25-l fermentation bin. The reaction was allowed to proceed for 5 min until the pH

Table 1
Declared composition of the experimental diet fed as either a liquid meal or in a dry pelleted form

Ingredient	Inclusion
Oil	65 g kg^{-1}
Protein	175 g kg^{-1}
Fibre	50 g kg^{-1}
Ash	65 g kg^{-1}
Lysine	10 g kg^{-1}
Moisture	138 g kg^{-1}
Copper	25 mg kg^{-1}
Sodium selenite–selenium	0.40 mg kg^{-1}
Vitamin A—retinol	10 000 iu
Vitamin D3—cholecalciferol	2 000 iu
Vitamin E α-tocopherol	100 iu
Digestible energy (DE)	14 MJ kg^{-1}

had dropped to approximately 2.6 and the activated solution turned yellow-green. FLF was prepared by inoculating the liquid diet, sanitized with 300 ppm ClO_2 (Sanitech; Alltech) with rifampicin-resistant *Lactobacillus plantarum* (PC-81-11-02, Alltech) to give a final concentration of between 6 and 7 $\log_{10}$ CFU/ml liquid feed. The feed was then allowed to ferment for 72 h at 30 °C. Sows were offered the diets in two equal meals per day. Piglets were not offered any supplementary creep feed during the trial.

2.3. Selection of rifampicin-resistant mutant of L. plantarum

Antibiotic resistant isolates of *L. plantarum* strain (PC-81-11-02, Alltech) were obtained by serial culturing on MRS medium (Oxoid, Basingstoke, England) containing increasing concentration of rifampicin (Sigma-Aldrich, Dorset, England) to a final concentration of 100 μg/ml. This strain had previously been shown to be an effective LAB for fermenting liquid pig feed (Moran, 2001) as it produces more than 150 mM lactic acid and less than 30 mM acetic acid in 24 h at 30 °C. Rifampicin ([3-(4-methylpiperazinyl-iminomethyl) rifamycin SV]) was prepared as a stock solution (10 mg/ml) in dimethyl sulfoxide (DMSO) and used at a final concentration of 100 μg/ml. A parent strain of *L. plantarum* was spread-plated onto rifampicin gradient plates and incubated for 48 h at 30 °C. The colonies growing at higher concentrations were *L. plantarum*[rif]. Finally, by using single-colony isolation method, a rifampicin-resistant *L. plantarum*

was isolated and propagated in MRS broth at 30 °C for 24 h. In order to ensure that the only difference between the parent strain and the rif-mutant strain was rifampicin resistance, a number of biochemical and physiological parameters of both strains were compared. Stock *L. plantarum*[rif] cultures were maintained at − 80 °C in 1 ml aliquots of MRS broth with 20% v/v glycerol. The strain was always subcultured twice in 10 ml MRS broth (Oxoid) at 30 °C before being used to inoculate feed.

2.4. Monitoring of feed

The dry matter of fermented and nonfermented feed was determined daily by oven drying at 103 °C for 3–4 days (Method: ISO 6469/NEN 3332). The results were expressed as percentage dry matter.

Samples (∼ 20 ml) of liquid feed were removed daily from each batch for pH measurement and for microbial and chemical analyses.

The pH of liquid feed was measured using an electronic pH meter (W.G. Pye & Co., Cambridge, UK).

Microbial analyses of the feed samples were conducted after sanitization of NFLF and FLF with ClO_2 and at the end of the fermentation process. All selective media used were obtained from Oxoid. Representative samples were serially diluted 10-fold in Maximum Recovery Diluent (MRD). Relevant dilutions were plated onto selective media and plates were incubated at the recommended temperature. Coliforms were enumerated on VRBA agar using the double-layered pour-plate technique and incubated aerobically for 24 h at 37 °C. Lactic acid bacteria were enumerated on Rogosa agar and incubated anaerobically for 72 h at 30 °C. *L. plantarum* starter culture was selectively enumerated on Rogosa agar containing 100 μg/ml rifampicin and incubated anaerobically for 72 h at 30 °C. Yeasts were enumerated on Rose Bengal Chloramphenicol agar (RBCA) and incubated aerobically for 72 h at 30 °C.

2.5. Culture from faecal samples

Fresh faecal samples (approximately 100 g) were collected from the rectum of each sow 7, 5 and 2 days before anticipated farrowing date, at parturition (Day 0) and 2, 5, and 7 days after parturition. Dry matter concentration of each faecal sample was determined

by oven drying at 103 °C for 3 days. Microbiological analyses were performed immediately upon receipt of the samples. Faeces were suspended in sterile MRD as 10-fold dilutions (wt/vol) from which further 10-fold dilutions (vol/vol) were made. To evaluate LAB populations, relevant dilutions were plated onto Rogosa agar by double-layered pour-plate technique and plates were incubated anaerobically at 37 °C for 48 h. To evaluate the coliform populations, relevant dilutions were plated onto VRBA agar by the double-layered pour-plate technique. Plates were incubated at 37 °C for 24 h. To screen for the presence of *L. plantarum*[rif], appropriate dilutions were plated onto Rogosa agar containing rifampicin (100 μg/ml). Plates were incubated anaerobically for 72 h at 37 °C. Random colonies, selected daily from antibiotic plates, were checked by light microscopy for cell morphology and Gram reaction to confirm recovery of *L. plantarum*.

2.6. Quantification of short-chain fatty acids (SCFA) and lactic acid

SCFA analyses of feed and faecal samples were conducted by the HPLC method. Faecal samples were homogenized and diluted 1:10 with sterilized distilled water. After the addition of sulphuric acid (at final concentration of 0.1 g l^{-1}) as the internal standard, a proportion of the processed sample was centrifuged ($10\,000 \times g$ for 15 min) and filtered (filter pore size 0.45 μm). HPLC separations were performed using a P580 HPG pump and GINA 50 autosampler (Dionex, Cheshire, UK) with an Animex HPX-87H (30 cm × 6.8 mm) cation exchange column (Bio-rad, Herts, UK) and refractive index (RI) detector. The column temperature was maintained at 55 °C and the flow rate at 0.7 ml/ min. Determinations were based upon retention time in relation to authentic reference compounds. All calibrations were produced from peak height determinations utilising integration software (Chromeoleon, Dionex-Softron, Gemering, Germany).

2.7. Collection and processing of colostral samples

Colostrum samples were collected on the day of parturition by manual milking. These samples were centrifuged at $17\,000 \times g$ for 15 min to remove fat. The aqueous phase of whey samples was separated and stored at -20 °C until analyzed.

2.8. Mitogenic activity on intestinal epithelial cells (IEC-6)

2.8.1. Cell culture

The rat intestinal epithelial crypt cell line IEC-6 was obtained from the European Collection of Animal Cell Cultures (EAACC, Salisbury, UK). Cells (passage 36) were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in Dulbeco's Modified Eagle Medium (DMEM) (Gibco-BRL, Life Technologies, Paisley, UK) supplemented with 2 mM L-glutamine and 5% foetal calf serum (FCS) (Sigma, Poole, UK).

2.8.2. Mitogenic assay

IEC-6 cells were cultured in 24-well-plates (Corning Costar, High Wycombe, UK) at a density of 1×10^4 cells/well. Cells were allowed to adhere for 18 h and were then washed twice in Hank's Balanced Salt Solution (HBSS) (Sigma). Twenty-eight hours prior to colostrum stimulation the complete medium was replaced with DMEM without serum. The cells were then treated for 24 h with 100 μl colostrum. Cell culture with added foetal calf serum (100 μl) was used as a positive control, and cell culture with no added stimulants represented the negative control. After 18 h of culture, cells were labelled for the final 6 h with ^{3}H-thymidine (1 μCi/well). Plates were then washed with HBSS, fixed with methanol for 5 min at 4 °C, then with 10% Trichloroacetic acid (TCA) for 30 min and solubilized with 0.4N NaOH for 1 h at 50 °C. IEC-6 cells were transferred to scintillation vials and radioactivity was counted by scintillation spectrometer (Packard BioScience).

2.9. Mitogenic activity on blood lymphocytes

Lymphocyte cells were isolated from pig blood by Histopaque®-1077 procedure (Sigma-Aldrich, Procedure No.1077). The blood lymphocytes were diluted to a density of 5×10^6 cells/ml in the culture medium, and 100 μl volumes were dispensed into the wells of microtitre plates. Lymphocyte transformation was tested by the addition of 1 and 10 μg/well of Phytohemaglutinin (PHA) (nonspecific stimulation of lymphocyte replication; positive control) in 200 μl of culture medium. Some of the lymphocytes did not receive any stimulants except for 100 μl of culture medium (unstimulated lymphocytes; negative control). After a 48-h

incubation period in a humidified, CO_2 atmosphere (5%) at 37 °C, 0.5 μCi of tritiated thymidine was added to each well. The culture was incubated for further 16 h. The cultured cells were then harvested and the radio-activity (counts per minute) associated with the harvested cells was measured in a scintillation counter after the addition of liquid scintillation fluid.

2.10. Statistical analyses

All the original dilution and colony count data were recorded and calculated. The bacterial count per gram of faeces was further log transformed, tabulated, and statistically analysed by ANOVA. Arcsine transformation was used for all data presented in percentages.
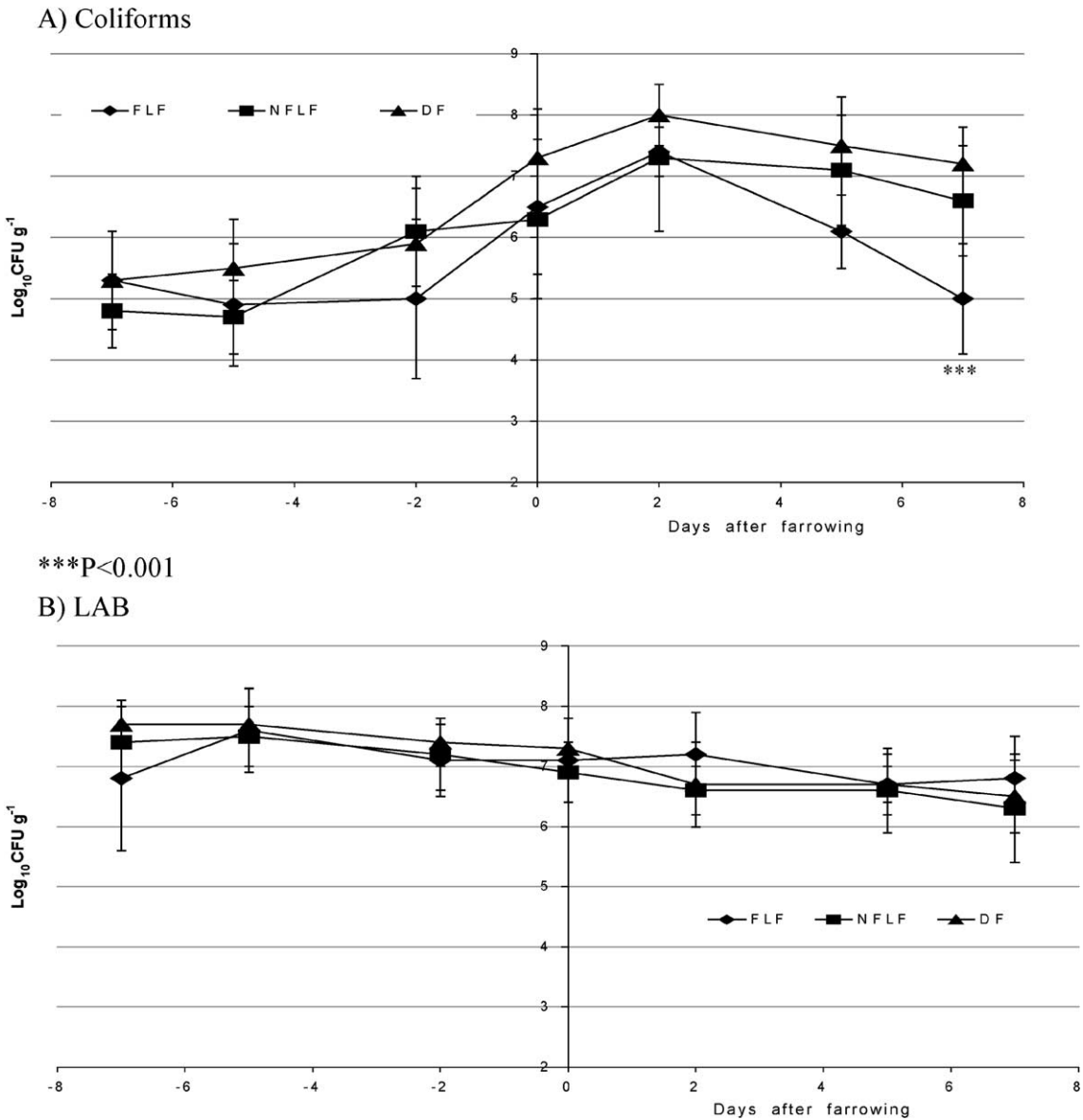

Fig. 1. Faecal counts of coliforms (A) and LAB (B) in the sows fed different diet fermented liquid feed (FLF), nonfermented liquid feed (NFLF) and dry feed (DF) for the period of 1 week before farrowing till 1 week after parturition.

Mitogenic experiments were carried out in triplicate determinations and repeated at least twice. Significant differences between treatment means were compared by Tukey's HSD test (Zar, 1999). The statistical analyses were undertaken using Minitab v.10.2 (Minitab, Pennsylvania, USA, 1994).

3. Results

3.1. Microbiology and analyses of the feed

While sanitization of liquid feed with chlorine dioxide eliminated coliforms successfully, it did not always eliminate LAB and yeasts. Typically, there were approximately 10^3 to 10^4 CFU/ml LAB and yeasts still remaining after 24-h steeping. At the end of the sanitization process, the pH of the liquid feed was around 5.2. After 72-h fermentation at 30 °C, the pH of the feed dropped to 3.9 ± 0.2 and remained around this value for the rest of the experiment. Microbial analyses of fermented feed showed the presence of high numbers of LAB (around 10^9 CFU/ml) while coliform levels were below detectable limits in each feed sample tested.

The dry matter (DM) concentrations of the FLF and NFLF were stable over the 28-day period with no significant differences between replicates. The average DM concentration for FLF was $27.8 \pm 2.2\%$, which was significantly lower ($P < 0.001$) than the average DM concentration of NFLF ($29.6 \pm 3.1\%$). That means that approximately 6.1% of the DM was the fermentation process.

3.2. Microbiology of the sows' and piglets' faeces

Numbers of faecal LAB and coliforms in the faeces of sows fed FLF, NFLF, and DF are shown in Fig. 1.

Table 2
Lactic acid bacteria (LAB) and coliforms ($\log_{10}$ CFU g^{-1}) in the faeces of 7-day-old piglets born to sows fed fermented liquid feed (FLF), nonfermented liquid feed (NFLF) and dry feed (DF)

	FLF	NFLF	DF	SE_D	P
LAB	7.70[a]	7.65[a]	7.30[b]	0.145	**
Coliforms	7.52[d]	7.80[cd]	8.07[c]	0.139	***

Means with different superscript are significantly different; ** $P < 0.01$; *** $P < 0.001$.

Table 3
Dry matter content (g kg^{-1}) of faeces of sows fed fermented liquid feed (FLF), nonfermented liquid feed (NFLF) and dry feed (DF)

Days	FLF	NFLF	DF	P
14 before farrowing	28.33 ± 1.97	30.67 ± 7.73	30.54 ± 7.35	ns
7 before farrowing	29.55 ± 5.64	25.28 ± 3.08	28.35 ± 4.51	ns
7 after farrowing	31.02 ± 3.61[b]	30.38 ± 3.87[b]	38.03 ± 3.98[a]	**

Data are expressed as a mean $\pm$ SD; ** $P < 0.01$; ns = not significant. Means with the same superscript are not significantly different.

While the LAB population was not significantly affected by dietary treatments, the most significant differences in coliform population were observed in the sows' faecal samples taken 7 days after parturition. Faeces excreted from sow fed FLF had significantly ($P < 0.001$) lower numbers of coliforms compared with faeces from sows fed NFLF and DF (Fig. 1). Although none of the dietary treatments were able to prevent a high coliform bloom (10^7–10^8 CFU g^{-1}) around farrowing time (Fig. 1A), FLF reduced this rapid coliform increase more rapidly than the other two treatments. By the 5th day after farrowing there was already a significant ($P < 0.05$) decrease in coliform excretion by sows fed FLF compare to DF-fed sows (Fig. 1A). Two days later the coliform population in faeces of FLF-fed sows dropped to approximately 10^5 CFU g^{-1}, which was significantly lower ($P < 0.001$) than the coliform numbers in the faeces of sows fed either NFLF or DF. The large increase of coliforms around farrowing was usually accompanied by a slight decrease of LAB population (Fig. 1). Although this reduction was not significant between treatments, the situation was not the same when LAB numbers were compared within treatments at 1 week before and during the period after farrowing. There was a significant drop in the numbers of LAB in the faeces of sows fed NFLF or DF after farrowing (days 2, 5, 7) compared with the numbers at 1 week before farrowing. Mean LAB counts for these two groups of sows ranged around 5×10^7 CFU g^{-1} of faeces until the day of farrowing, when the levels decreased to approximately 3×10^6 CFU g^{-1} of faeces. The LAB population in the faeces of sows fed FLF did not differ significantly at any sampling time during the 2-week examination period.

Table 4
Short-chain fatty acid (SCFA) concentrations (mmol g^{-1}) of sows'
faeces after 1 and 21 days of feeding with fermented liquid feed
(FLF), nonfermented liquid feed (NFLF) and dry feed (DF)

SCFA		FLF	NFLF	DF	P
Acetate	Day 1	77.2 [2]	38.8 [2]	54.8 [1]	ns
	Day 21	137.2 [a,1]	69.1 [b,1]	64.7 [b,1]	*
Propionate	Day 1	37.5 [2]	22.9 [2]	33.8 [1]	ns
	Day 21	64.6 [a,1]	35.2 [b,1]	46.1 [a,b,1]	*
Butyrate	Day 1	0.8 [b,2]	4.0 [a,b,1]	4.4 [a,1]	*
	Day 21	5.16 [1]	8.0 [1]	5.1 [1]	ns

ns = Not significant, * $P < 0.05$.
Means with the same letter superscript are not significantly different.
Means with the same number superscript are not significantly
different.

Consequently, piglets from FLF-fed mothers excreted faeces that were significantly higher ($P < 0.01$) in LAB and significantly lower ($P < 0.001$) in coliforms than faeces from the piglets of sows fed DF (Table 2). No significant differences were observed between the piglets of FLF and NFLF. These results were reflected in more favourable faecal LAB:Coliform ratios in the piglets of sows fed liquid diets.

Antibiotic plate counts from sows' and piglets' faecal samples confirmed that the antibiotic-resistant *L. plantarum* strain, used to ferment the feed, survived passage through the intestinal tract. No rifampicin-resistant colonies were detected in any group of sows prior to introduction of liquid feed fermented with

rifampicin-resistant *L. plantarum*. After 7 days of feeding, the *L. plantarum*[rif] strain maintained a population of about 3.7×10^4 CFU g^{-1} of faeces. During a further 7 days, the population of the introduced strain increased significantly ($P < 0.05$) up to 2.5×10^5 CFU g^{-1} and fluctuated about this value for the rest of the 2-week testing period. The presence of the test strain was confirmed also in piglets' faeces on the 7th day of suckling but the counts constituted 1% or less of the total LAB.

3.3. Dry matter and SCFA concentration of the sows' faeces

The dry matter of the sows' faeces was not significantly affected by any dietary treatment during the 2-week period before farrowing. A significant increase ($P < 0.01$) in dry matter concentration of faeces was observed only in the sows fed DF at 1 week after farrowing (Table 3).

The mean values of SCFA concentrations in the sow faeces are presented in Table 4. Significant increases in the total faecal concentration of acetic acid ($P < 0.05$) and propionic acid ($P < 0.05$) were recorded in the FLF group after 3 weeks of intake compared with sows fed NFLF or DF. There was also a significant increase ($P < 0.05$) in butyric acid in sows fed FLF after 3 weeks of feeding. No significant changes in the SCFA acid concentration were observed
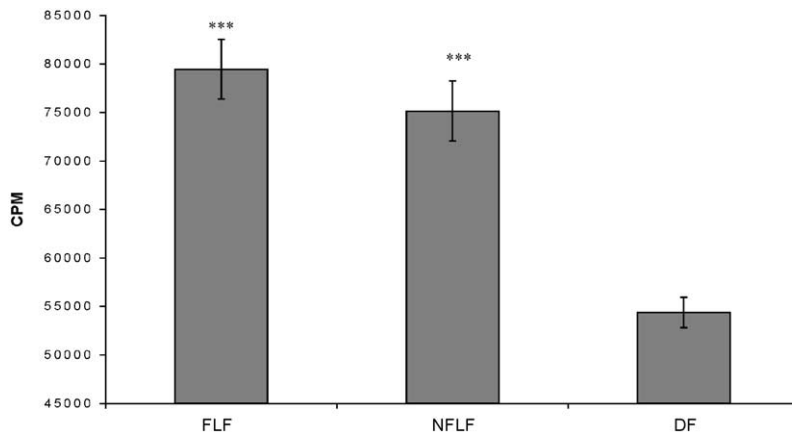

Fig. 2. Mitogenic activity of sow colostrum on intestinal epithelial cells (IEC-6). Data are expressed as mean counts per minute (CPM). Error bars represent the standard error of the mean; * * * $P < 0.001$. FLF: fermented liquid feed; NFLF: nonfermented liquid feed; DF: dry feed.
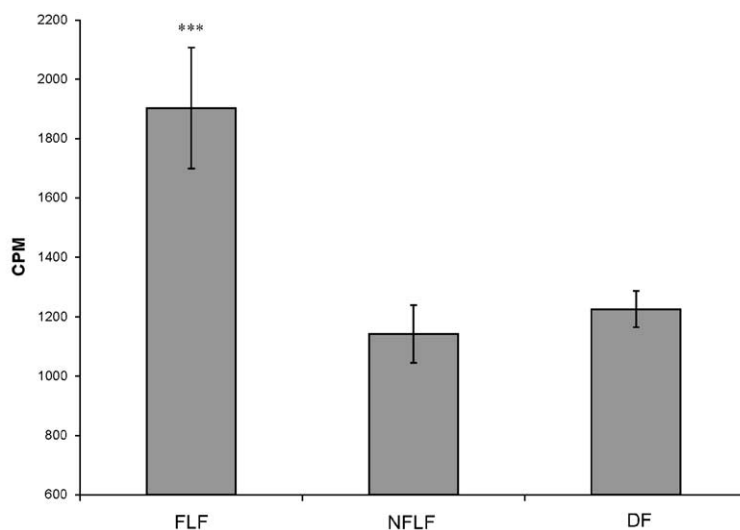
Fig. 3. Mitogenic activity of sow colostrum on blood lymphocytes. Data are expressed as mean counts per minute (CPM). Error bars represent the standard error of the mean; $***P < 0.001$. FLF: fermented liquid feed; NFLF: nonfermented liquid feed; DF: dry feed.

in the sows fed DF. In all experimental groups, acetate was the predominant SCFA, propionate was the second most predominant acid and butyrate was usually in the third position in terms of concentration. Neither lactic nor valeric acid was detected in the faeces of any experimental group.

3.4. Mitogenic analyses of colostral samples on IEC-6 and blood lymphocytes

The mitogenic response (uptake of tritiated thimidine measured as counts per minute of harvested cells) of intestinal epithelial cells (IEC-6), exposed to the colostrum samples taken from the sows on different diets, is presented in Fig. 2. Although all colostrum samples stimulated proliferation of IEC-6 compared to the control, colostrum from sows fed liquid feed had a significantly greater ($P < 0.001$) mitogenic activity than colostrum from DF-fed sows.

Lymphocyte mitogenic activity confirmed significantly ($P < 0.001$) higher stimulation potential of colostrum produced by sows fed FLF (Fig. 3). In this case, there were no significance differences between the colostrum samples from sows fed NFLF or DF.

4. Discussion

4.1. Microbiology and analyses of the feed

Using chlorine dioxide in a concentration of 300 ppm, successfully eliminated coliforms to below detectable levels in the feed after 24 h of steeping in each feed sample during the whole experimental period. This confirmed previous studies performed in laboratory conditions (Demeckova et al., 2001). However, under farm conditions it is not easy to maintain a sterile environment, which explains the low numbers of LAB and yeasts still remaining in the majority of feed samples tested. In general, sanitization was quite successful and allowed the starter culture *L. plantarum*[rif] to become the predominant LAB strain in each replicate.

The loss of dry matter (6%) in FLF in the current study was less than in the study of Moran (2001) (12%) but higher than in the studies of Geary (1997) and Jensen et al. (1998) (3% DM). However, because the total organic acids and ethanol were not quantified in the FLF their loss during the drying process could not be estimated. Therefore, no corrections were made in the dry matter calculations for the volatile components of the FLF diet during this trial. According to

CVB (1999), 8% of lactic acid, 50% of acetic acid and 100% of ethanol is lost due to volatilisation during the procedure to determine dry matter content.

4.2. Microbiology of the sows' and piglets' faeces

Coliforms are present in the intestine of the sow in large numbers and increase dramatically just prior to farrowing due to stress induced by movement and parturition (Maclean and Thomas, 1974; Arbuckle, 1968). Therefore, the newborn piglet is exposed to a high risk of infection.

Diet represents one of the most studied external factors that influences the establishment or beneficial changes of the intestinal microbiota. It has been reported previously that FLF reduces coliform numbers in the gut (Jensen and Mikkelsen, 1998; Hansen et al., 2000; van Winsen et al., 2001; Moran, 2001). Although FLF did not prevent the coliform bloom around farrowing, FLF did reduce it compared with NFLF and DF. A number of factors may account for the beneficial effect of FLF, and these may act independently or synergistically. The low pH of the diet, the high numbers of LAB and high concentration of lactic acid represent the most important characteristics of FLF in terms of its protective effect. The ability of LAB to inhibit the growth of various gram-negative bacteria, especially pathogenic *E. coli*, is well documented for both in vitro (Hillman et al., 1995; Drago et al., 1997; Jin et al., 2000; Gopal et al., 2001) and in vivo conditions (Perdigon et al., 1990; O'Mahony et al., 2001). Establishment of low pH due to production of lactic acid and short-chain fatty acids (SCFA) plays a very important role in the elimination of many pathogens, which are not able to tolerate low pH conditions (Muralidhara et al., 1977; Jin et al., 2000). Sows fed FLF were exposed to a large intake of lactic acid (288 mM) and acetic acid (25.4 mM). In addition, significant increases in the concentration of acetic, propionic, and butyric acids were obtained in the faeces of the sows fed FLF, indicating increased fermentation in the colon. A study by Canibe and Jensen (2000) showed significantly lower pH in the stomach of growing pigs fed FLF compared with pigs fed NFLF and DF. In contrast, Moran (2001) reported no significant effect of FLF on the pH of the pig lower GI tract. However, taking into account the different diet (weaning diet vs. lactation diet) and the consid-

erably lower amount of ingested lactic acid in Moran's experiment, further work is needed before the possibility that FLF has no influence on the pH of the lower GI tract can be rejected.

Despite feeding high concentrations of lactic acid to the sows fed FLF, there was no lactic acid present in their faeces. Lactic acid is very efficiently utilised by pigs (Everts et al., 2000).

In spite of feeding very large numbers of LAB, the faecal LAB counts did not increase significantly. This is in agreement with results obtained by Paul and Hoskins (1972), Muralidhara et al. (1977), Jensen and Mikkelsen (1998), du Toit et al. (1998) and Donnet-Hughes et al. (1999). The reason for this is not known and it was not described in any of the studies mentioned.

Many authors have suggested that survival of LAB in the faeces following oral administration reflects large bowel colonization. This would indicate that enumeration of faecal bacterial strains may not be an accurate reflection of those in the gut. For example, Murphy et al. (1999) administered a *L. salivarius* strain to mice and found that it was not detectable in mouse faeces, even though the marked strain was found to have persisted in the ileocecal region of the small intestine for 7 days after termination of dosing.

In order to determine whether the *L. plantarum* in the starter culture can be transferred from the feed to the sow and from the sow to the piglets, a rifampicin-resistant mutant of the parent *L. plantarum* (PC-81-11-02, Alltech) was generated. This technique not only facilitated enumeration of the administered *L. plantarum* from faeces but also allowed its easy differentiation from the indigenous population. The power and stability of antibiotic selection in tracking exogenous organisms introduced into the host gastrointestinal tract has been demonstrated by many other workers (Pedersen et al., 1992; Pedersen and Jørgensen, 1992; Rada and Rychlý, 1995; Murphy et al., 1999; Simpson et al., 2000; Fujiwara et al., 2001a,b). Naturally occurring resistance to rifampicin among porcine lactobacilli and enterococci is negligible (Pedersen et al., 1992). This was also confirmed by our preliminary experiments where no naturally rifampicin-resistant LAB were found in sows' faeces. The strain was easily detectable on antibiotic-selective plates, at a level of 10^4–10^5 CFU g^{-1} (wet weight). This count indicates that despite ingesting high numbers of rifampicin-resistant

L. plantarum from the feed (10^8–10^9 CFU/ml), the faecal counts of the introduced strain constituted less than 1% of the total LAB. In our studies the *L. plantarum* was selected on the basis of very good fermentative abilities and no screening was performed to investigate its colonization potential.

Colonization of the piglet's gut occurs very rapidly after birth. Naito et al. (1995) reported that total counts of faecal bacteria of all newborn piglets tested in their study reached $10^{9.4}$–$10^{10.6}$ g^{-1} faeces (wet weight) 24 h after birth. In the study reported here, 7-day-old piglets in all experimental groups excreted considerable numbers of coliforms and LAB (10^8–10^9 CFU g^{-1}). However, the piglets from FLF-fed sows excreted significantly less coliforms and more LAB compared with the other two treatments, which was reflected in a higher LAB:Coliforms (L:C) ratio. According to Muralidhara et al. (1977), a higher L:C ratio is usually associated with a bacterial flora that contributes to improved animal growth and performance.

The test strain *L. plantarum*[rif] was detected in all faecal samples collected from the piglets, but it constituted less then 1% of the total LAB. Despite such low numbers we can conclude that the transfer of the strain from the feed to the mother and from the mother to the piglet did occur, which is in accordance with the results of other workers (Tannock et al., 1990; Pedersen et al., 1992) and means that the mother represents a source of piglets' LAB flora. Hence, by appropriate nutritional regimes there is a real opportunity to beneficially influence bacterial excretion from sows, which could be reflected in a more 'friendly' bacterial flora in the neonate GI tract. This approach would represent a very natural way of protecting piglets during the critical period after birth.

4.3. Mitogenic activity of sow colostrum on IEC-6 and blood lymphocytes

The growth promoting effect of sow colostrum and/or milk on the gastrointestinal tissue development, especially in the small intestine, has been well documented (Cera et al., 1987; Simmen et al., 1990; Kelly and King, 1991; Wang and Xu, 1996; Zhang et al., 1997, 1998). This was confirmed also in the current study. All colostrum samples had mitogenic potential on the intestinal epithelial cells (IEC) com-

pared with the control (cells without colostrum), but there were significant differences between the experimental groups. Colostrum produced by sows fed liquid feed had significantly higher growth promoting activity compared with DF fed sows. However, mechanisms regulating this process are unclear. Porcine colostrum contains trophic factors such as epidermal growth factor (EGF), insulin-like growth factor I and II (IGF-I, IGF-II) and insulin (Jaeger et al., 1987; Xu et al., 1994; Donovan et al., 1994). All these factors are present in much higher quantity (3- to 100-fold) in colostrum than in mature milk (Cera et al., 1987; Xu et al., 1994) and despite their protein structure, many are stable in the gastrointestinal lumen of sucking piglets (Shen and Xu, 1998, 2000a,b). In addition to the growth factors mentioned above, there are other specific molecules present in colostrum that may have a potent growth stimulating effect on the gut epithelium. They include nitrogen compounds-polyamines (spermidine and spermine) (Kelly et al., 1991; Motyl et al., 1995), iron binding protein-lactoferrin (Hashizume et al., 1985; Hagiwara et al., 1995), glutamine (Ko et al., 1993), platelet-derived growth factor (PDGF) and cytokines (Hagiwara et al., 2000).

Intestinal epithelium represents a tissue with rapid cellular turnover. An increase of the cell proliferation rate in the crypts will result in an overall increase of the epithelial cell population and associated increases in villus height (Lipkin, 1985). Thus, colostrum with higher mitogenic activity has the potential both to accelerate the maturation of the newborn's GI tract and provide the piglet with better protection by maintaining the integrity of the intestinal mucosa. In addition, it is well known that intestinal epithelial cells produce a variety of innate antimicrobial substances such as lysozyme and defensins, acting directly against bacterial growth by disrupting their cell membranes (Quellette, 1997). Finally, by having the ability to produce different types of cytokines (Seydel et al., 1997; Haller et al., 2000; Ohtsuka et al., 2001), intestinal epithelial cells can no longer be considered as only a mechanical barrier, but 'as an integral component of a communication network that involves interactions between epithelial cells, luminal microbes and host local mucosal immune system' (Kagnoff and Eckmann, 1997). By realising the importance of proper function of epithelial cells, the significance of having colostrum of higher mitogenic activity

becomes very clear. Therefore, good management strategies for efficient pig production should aim to achieve not only to produce a greater quantity of colostrum but also, and this is even more important, colostrum of improved quality.

The increased mitogenic activity of colostrum produced by sows fed FLF was confirmed also in our next experiment with pig blood lymphocytes. However, the fact that there was also a significant difference between FLF and NFLF colostral samples leads us to speculate that it is most likely the fermentation process that gives the feed some factors the presence of which are needed to produced colostrum of higher mitogenic potential in terms of lymphocyte proliferation. Immune responses depend not only on the activation of lymphocytes, but also on the ability of lymphocytes to proliferate. However, in the current experiment it is not possible to conclude which type of lymphocytes proliferated, as there was a mixture of both B and T lymphocytes in the blood samples.

According to Donnet-Hughes et al. (1999), the minimum daily dose of viable *Lactobacillus* strains, which will significantly modulate nonspecific immunity, is 10^9 CFU. This level also represents a daily dose of *L. plantarum* in FLF. However, more detailed studies are needed to confirm the immunostimulation effect of the *L. plantarum* used in this experiment.

Therefore, this study gives further evidence of the importance and benefits of suckling, indicating that enhanced neonatal defence can be made available by manipulating the immune status of farrowing sows. This in turn will be reflected in the production of colostrum/milk of higher immunological and nutritional quality.

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References

Arbuckle, J.B.R., 1968. The distribution of certain *Escherichia coli* strains in pigs and their environment. Br. Vet. J. 124, 152–159.

Brooks, P.H., Geary, T.M., Morgan, D.T., Campbell, A., 1996. New developments in liquid feeding. Pig Vet. J. 36, 43–64.

Canibe, N., Jensen, B.B., 2000. Effect of nonfermented- and fermented-liquid feeding on the intestinal microbiota of growing pigs. Reprod. Nutr. Dev. 40, 203.

Cera, K., Mahan, D.C., Simmen, F.A., 1987. In vitro growth-promoting activity of porcine mammary secretions: initial characterization and relationship to known peptide growth factors. J. Anim. Sci. 65, 1149–1159.

Conway, P.L., 1996. Development of intestinal microbiota. Gastrointestinal microbes and host interactions. In: Mackie, R.I., Whyte, B.A., Isaacson, R.E. (Eds.), Gastrointestinal Microbiology. Chapman & Hall, London, pp. 3–39.

CVB, 1999. Veevoedertabel. Centraal Veevoederbureaau in Nederland, Lelystad, The Netherlands.

Demeckova, V., Moran, C.A., Cavenay, C., Campbell, A.C., Kuri, V., Brooks, P.H., 2001. The effect of fermentation and/or sanitization of liquid diets on the feeding preferences of newly weaned pigs. In: Lindberg, J.E., Ogle, B. (Eds.), Digestive physiology of pigs. CABI Publishing, Wallingford, UK, pp. 291–293.

Donnet-Hughes, A., Rochat, F., Serrant, P., Aeschlimann, J.M., Schiffrin, E.J., 1999. Modulation of nonspecific mechanisms of defence by lactic acid bacteria: effective dose. J. Dairy Sci. 82, 863–869.

Donovan, S.M., Zijlstra, R.T., Odle, J., 1994. Use of the piglet to study the role of growth factors in neonatal intestinal development. Endocr. Regul. 28, 153–162.

Drago, L., Gismondo, M.R., Lombardi, A., de Haen, C., Gozzini, L., 1997. Inhibition of in vitro growth of enteropathogens by new *Lactobacillus* isolates of human intestinal origin. FEMS Microbiol. Lett. 153, 455–463.

Du Toit, M., Franz, C.M.A.P., Dicks, L.M.T., Schillinger, U., Haberer, P., Warlies, B., Ahrens, F., Holzapfel, W.H., 1998. Characterisation and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. Int. J. Food Microbiol. 40, 93–104.

Everts, H., Salden, N., Lemmens, A.G., Wijers, J., Beynen, A.C., 2000. High intakes of L^+ and D^- lactic acid are efficiently metabolized by pigs and rats. J. Anim. Physiol. Anim. Nutr. 83, 224–230.

Fujiwara, S., Seto, Y., Kimura, A., Hashiba, H., 2001a. Intestinal transit of an orally administered streptomycin–rifampicin-resistant variant of *Bifidobacterium longum* SBT2928: its long-term survival and effect on the intestinal microflora and metabolism. J. Appl. Microbiol. 90, 43–52.

Fujiwara, S., Seto, Y., Kimura, A., Hashiba, H., 2001b. Establishment of orally-administered *Lactobacillus gasseri* SBT2055SR in the gastrointestinal tract of humans and its influence on intestinal microflora and metabolism. J. Appl. Microbiol. 90, 343–352.

Geary, T.M., 1997. Improving the performance of weaner pigs through developments in liquid feeding. PhD Thesis, University of Plymouth, Plymouth, UK.

Geary, T.M., Brooks, P.H.M., Morgan, D.T., Campbell, A.C., Russell, P.J., 1996. Performance of weaner pigs fed ad libitum with

liquid feed at different dry matter concentrations. J. Sci. Food Agric. 72, 17–24.

Gill, H.S., Rutherfurd, K.J., 2001. Viability and dose-response studies on the effects of the immunoenhancing lactic acid bacterium *Lactobacillus rhamnosus* in mice. Br. J. Nutr. 86, 285–289.

Gopal, P.K., Prasad, J., Smart, J., Gill, H.S., 2001. In vitro adherence properties of *Lactobacillus rhamnosus* DR20 and *Bifidobacterium lactis* DR10 strains and their antagonistic activity against an enterotoxigenic *Escherichia coli*. Int. J. Food Microbiol. 67, 207–216.

Grangette, C., Müller-Alouf, H., Goudercourt, D., Geoffroy, M.-C., Turneer, M., Mercenier, A., 2001. Mucosal immune responses and protection against tetanus toxin after intranasal immunization with recombinant *Lactobacillus plantarum*. Infect. Immun. 69, 1547–1553.

Hagiwara, T., Shinoda, I., Fukuwatari, Y., Shimamura, S., 1995. Effects of lactoferrin and its peptides on proliferation of rat intestinal epithelial cell line, IEC-18, in the presence of epidermal growth factor. Biosci. Biotechnol. Biochem. 59, 1875–1881.

Hagiwara, K., Kataoka, S., Yamanaka, H., Kirisawa, R., Iwai, H., 2000. Detection of cytokines in bovine colostrum. Vet. Immunol. Immunopathol. 76, 183–190.

Haller, D., Bode, C., Hammes, W.P., Pfeifer, A.M.A., Schiffrin, E.J., Blum, S., 2000. Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures. Gut 47, 79–87.

Hansen, L.L., Mikkelsen, L.L., Agerhem, H., Laue, A., Jensen, M.T., Jensen, B.B., 2000. Effect of fermented liquid food and zinc bacitracin on microbial metabolism in the gut and sensoric profile of m. longissimus dorsi from entire male and female pigs. Anim. Sci. 71, 65–80.

Hashizume, S., Kuroda, K., Murakami, H., 1985. Identification of lactoferrin as an essential growth factor for human lymphocytic cell lines in serum-free medium. Biochim. Biophys. Acta 763, 377–382.

Hillman, K., Spencer, R.J., Murdoch, T.A., Stewart, C.S., 1995. The effect of mixtures of *Lactobacillus* spp. on the survival of enterotoxigenic *Escherichia coli* in vitro continuous culture of porcine intestinal bacteria. Lett. Appl. Microbiol. 20, 130–133.

Jaeger, L.A., Lamar, C.H., Bottoms, G.D., Cline, T.R., 1987. Growth stimulating substances in porcine milk. Am. J. Vet. Res. 48, 1531–1533.

Jensen, B.B., Mikkelsen, L.L., 1998. Feeding liquid diets to pigs. In: Garnsworthy, P.C., Wiseman, J. (Eds.), Recent Advances in Animal nutrition. Notthingam Univ. Press, Nottingham, pp. 107–126.

Jin, L.Z., Marquardt, R.R., Baidoo, S.K., 2000. Inhibition of enterotoxigenic *Escherichia coli* K88, K99 and 987P by the *Lactobacillus* isolates from porcine origin. J. Sci. Food Agric. 80, 619–624.

Kagnoff, M.F., Eckmann, L., 1997. Epithelial cells as sensors for microbial infection. J. Clin. Invest. 100, 6–10.

Kelly, D., King, T.P., 1991. The influence of lactation products on the temporal expression of histo-blood group antigens in the intestines of suckling pigs: lectin histochemistry and immunohistochemical analysis. Histochem. J. 23, 55–60.

Kelly, D., King, T.P., Brown, D.S., McFadyen, M., 1991. Polyamide

profiles of porcine milk and of intestinal tissue of pigs during suckling. Reprod. Nutr. Dev. 31, 73–80.

Klobasa, F., Werhahn, E., Butler, J.E., 1981. Regulation of humoral immunity in the piglet by immunoglobulins of maternal origin. Res. Vet. Sci. 31, 195–206.

Ko, T.C., Beauchamp, R.D., Townsend, C.M., Thompson, J.C., 1993. Glutamine is essential for epidermal growth factor-stimulated intestinal cell proliferation. Surgery 114, 147–153.

Lipkin, M., 1985. Growth and development of gastrointestinal cells. Annu. Rev. Physiol. 47, 175–197.

Maclean, C.W., Thomas, N.D., 1974. Faecal and vaginal bacteriology of sows during the reproductive cycle. Brit. Vet. J. 130, 230–237.

Madsen, K., Cornish, A., Soper, P., McKaigney, C., Jijon, H., Yachimec, C., Doyle, J., Jewell, L., de Simone, C., 2001. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology 121, 580–591.

Mikkelsen, L.L., Jensen, B.B., 1997. Effect of fermented liquid feed (FLF) on growth performance and microbial activity in the gastrointestinal tract of weaned piglets. In: Laplace, J.-P., Fevrier, C., Barbeau, A. (Eds.), Digestive Physiology in Pigs, vol. 88. EAAP Publication INRA, Paris, pp. 639–642.

Moran, C.A., 2001. Developments and benefits of liquid feeding for the post-weaned pig. PhD Thesis, University of Plymouth, Plymouth, UK.

Motyl, T., Płoszaj, T., Wojtasik, A., Kukulska, W., Podgurniak, M., 1995. Polyamines in cow's and sow's milk. Comp. Biochem. Physiol., Part B Biochem. Mol. Biol. 111, 427–433.

Muralidhara, K.S., Sheggeby, G.G., Elliker, P.R., England, D.C., Sandine, W.E., 1977. Effect of feeding lactobacilli on the coliform and lactobacillus flora of intestinal tissue and feces from piglets. J. Food Prot. 40, 288–295.

Murphy, L.C., Dunne, C., Kiely, B., Shanahan, F., O'Sullivan, G., Collins, K., 1999. In vivo assessment of potential probiotic *Lactobacillus salivarius* strains: evaluation of their establishment, persistence, and localisation in the murine gastrointestinal tract. Microb. Ecol. Health Dis. 11, 149–157.

Naito, S., Hayashidani, H., Kaneko, K., Ogawa, M., Benno, Y., 1995. Development of intestinal lactobacilli in normal piglets. J. Appl. Bacteriol. 79, 230–236.

Ohtsuka, Y., Lee, J., Stamm, D.S., Sanderson, I.R., 2001. MIP-2 secreted by epithelial cells increases neutrophil and lymphocyte recruitment in the mouse intestine. Gut 49, 526–533.

O'Mahony, L., Feeney, M., O'Halloran, S., Murphy, L., Kiely, B., Fitzgibbon, J., Lee, G., O'Sullivan, G., Shanahan, F., Collins, J.K, 2001. Probiotic impact on microbial flora, inflammation and tumour development in IL-10 knockout mice. Aliment. Pharmacol. Ther. 15, 1219–1225.

Paul, D., Hoskins, L.C., 1972. Effect of oral *Lactobacillus* feedings on fecal *Lactobacillus* counts. Am. J. Clin. Nutr. 25, 763–765.

Pedersen, K., Jørgensen, M., 1992. Lactic acid bacteria for mink. Colonization and persistence of Enterococcus faecium Cernelle 68 in the digestive tract of mink. Acta Vet. Scand. 33, 95–103.

Pedersen, K., Christensen, G.W., Steffensen, M., Schyum, P., Johansen, A.K., 1992. Transfer of lactic acid bacterial strains from the feed to the sows, the environment, and the piglets. Acta Vet. Scand. 33, 297–303.

Perdigon, G., Nader de Macias, M.A., Alvarez, S., Oliver, G., Pesce de Ruiz Holgado, A.A., 1990. Prevention of gastrointestinal infection using immunological methods with milk fermented with *Lactobacillus* casei and *Lactobacillus acidophilus*. J. Dairy Res. 57, 255–264.

Quellette, A.J., 1997. Paneth cells and innate immunity in the crypt microenvironment. Gastroenterology 113, 1779–1784.

Rada, V., Rychlý, I., 1995. The effect of *Lactobacillus salivarius* administration on coliforms and enterococci in the crop and caeca of chicken broilers. Vet. Med. (Czech.) 40, 311–315.

Sansom, B.F., Gleed, P.T., 1981. The ingestion of sow's faeces by suckling piglets. Br. J. Nutr. 46, 451–456.

Seydel, K.B., Li, E., Swanson, P.E., Stanley, S.L., 1997. Human intestinal epithelial cells produce proinflammatory cytokines in response to infection in SCID mouse-human intestinal xenograft model of amebiasis. Infect. Immun. 65, 1631–1639.

Shen, W.H., Xu, R.J., 1998. Stability and distribution of orally administered epidermal growth factor in neonatal pigs. Life Sci. 63, 809–820.

Shen, W.H., Xu, R.J., 2000a. Gastrointestinal stability and absorption of insulin in suckling pigs. Comp. Biochem. Physiol., Part A Mol. Integr. Physiol. 125, 389–401.

Shen, W.H., Xu, R.J., 2000b. Stability of insulin-like growth factor I in the gastrointestinal lumen in neonatal pigs. J. Pediatr. Gastroenterol. Nutr. 30, 299–304.

Simmen, F.A., Cera, K.R., Mahan, D.C., 1990. Stimulation by colostrum or mature milk of gastrointestinal tissue development in newborn pigs. J. Anim. Sci. 68, 3596–3603.

Simpson, J.M., McCracken, V.J., Gaskins, R.H., Mackie, R.I., 2000. Denaturing gradient gel electrophoresis analysis of 16S ribosomal DNA amplicons to monitor changes in fecal bacterial populations of weaning pigs after introduction of *Lactobacillus reuteri* strain MM53. Appl. Environ. Microbiol. 66, 4705–4714.

Smith, H.W., Crab, W.E., 1961. The faecal bacterial flora of animals and man: its development in the young. J. Pathol. Bacteriol. 82, 53–66.

Tannock, G.W., Fuller, R., Pedersen, K., 1990. *Lactobacillus* succession in the piglet digestive tract demonstrated by plasmid profiling. Appl. Environ. Microbiol. 56, 1310–1316.

van Winsen, R.L., Urlings, B.A., Lipman, L.J., Snijders, J.M., Keuzenkamp, D., Verheijden, J.H., van Knapen, F., 2001. Effect of fermented feed on the microbial population of the gastrointestinal tracts of pigs. Appl. Environ. Microbiol. 67, 3071–3076.

von der Weid, T., Bulliard, C., Schiffrin, E.J., 2001. Induction by a lactic acid bacterium of a population of $CD4^+$ T cells with low proliferative capacity that produce transforming growth factor β and Interleukin-10. Clin. Diagn. Lab. Immunol. 8, 695–701.

Wang, T., Xu, R.-J., 1996. Effects of colostrum feeding on intestinal development in newborn pigs. Biol. Neonate 70, 339–348.

Xu, R.-J., Mellor, D.J., Birtles, M.J., Breier, B.H., Gluckman, P.D., 1994. Effects of oral IGF-I or IGF-II on digestive organ growth in newborn piglets. Biol. Neonate 66, 280–287.

Zar, J.H., 1999. Biostatistical analysis, 4th ed. Prentice Hall (UK), London, UK.

Zhang, H., Malo, C., Buddington, R.K., 1997. Suckling induces rapid intestinal growth and in brush border digestive functions of newborn pigs. J. Nutr. 127, 418–426.

Zhang, H., Malo, C., Boyle, C.R., Buddington, R.K., 1998. Diet influences development of the pig (Sus scrofa) intestine during the first 6 hours after birth. J. Nutr. 128, 1302–1310.