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Changes in caecal microbiota and mucosal morphology of weaned pigs

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Abstract
An experiment was designed to monitor the changes in caecal microbiota associated with early-weaning. Twelve piglets (20 ± 2 days) from six different litters were selected from a commercial source. For the two experimental groups, one animal from each litter was weaned onto a post-weaning diet (W) and the other remained with the sow (S). After 1 week, animals were sacrificed and caecal samples taken. Microbial counts for total bacteria, enterobacteria and lactobacilli populations were determined by quantitative PCR using SYBR Green® dye. Microbial profiles were assessed by terminal restriction fragment length polymorphism (t-RFLP). Weaning promoted an increase in the enterobacteria:lactobacilli ratio (0.27 vs. 1.67 log/log 16S rRNA gene copy number, \( P = 0.05 \)). Total bacteria and richness of the caecal microbial ecosystem (number of peaks) were similar in both experimental groups (49.3 for S and 53.4 for W, respectively, \( P = 0.22 \)), although the band patterns were clearly grouped in two different clusters by dendogram analysis. Weaning was also associated with a decrease in crypt density, an increase in mytotic index and a decrease in the number of goblet cells. A reduced immunological response was also observed and was manifested by an increase in intraepithelial lymphocytes and lymphocyte density in the lamina propria. Weaning appears to be critical in the establishment of the caecal microbiota in pigs with important changes, particularly in microbial groups and in caecal mucosal architecture.

Keywords: caecal histology, microbiota, pig, real-time PCR, t-RFLP, weaning.

1. Introduction
At early-weaning, the pig is affected by a high number of stressors that increase its susceptibility to disease. As a result, lower growth rates and post-weaning diarrhoea are significant problems in pig production after early-weaning.

During natural weaning, the piglet is progressively introduced to several external feed sources and its fermentative capacity is progressively developed for approximately 10 weeks, at which time the animal is completely weaned. In commercial weaning, this process happens in a couple of days and post-weaning diarrhoea is mainly related to intestinal disbiosis during this rapid adaptation to the new diet. It is generally recognised that the establishment of a diverse bacterial microbiota, characteristic and dynamic for each individual (Simpson et al., 2000), plays a key role in the maintenance of the gastrointestinal health by preventing colonization by pathogens (Van Kessel et al., 2004). This beneficial microbiota is especially important at periods such as weaning, when the animal still has an immature immune system and depends on certain compounds in the sow’s milk to prevent the growth of opportunistic bacteria (Edwards and Parret, 2002). There are several studies suggesting that early weaning causes substantial changes in the intestinal bacterial community (Franklin et al., 2002, Konstantinov et al., 2006), but more research is needed regarding the specific changes of the microbial ecosystem during this critical phase.

The gastrointestinal tract also needs to adapt to this new situation along with the microbial population. It is known that adaptation of the small intestine epithelium at weaning is greatly conditioned by bacterial colonization (Pluske, 1997). However, information on changes in caecal epithelium at weaning is scarce.
The objective of this study was to assess microbial shifts in the caecum of commercially weaned pigs using t-RFLP and real-time PCR, and to monitor changes in caecal mucosa after weaning.

2. Material and methods

2.1. Animals and housing

The experiment was performed at the Experimental Unit of the Universitat Autònoma de Barcelona and received prior approval from the Animal Protocol Review Committee of the institution. Management, housing and slaughtering conditions conformed to the European Union Guidelines. A total of 12 piglets (4.4 ± 0.36 kg; 20 ± 2 days, mixed males and females) were selected from six commercial litters, taking initial body weight into account. One piglet from each litter was weaned and fed a commercial post-weaning diet (weaned group, W) for 1 week (Table 1); the other piglet remained on the original commercial farm with the sow and littermates (control group, S).

2.2. Sacrifice and sampling

On day 28, the animals were weighed and sacrificed with an intravenous injection of sodium pentobarbitone (Dolethal, Vetoquinol, S.A., Madrid, Spain; 200 mg/kg BW). Animals were bled and the abdomen immediately opened from sternum to pubis. The whole gastrointestinal tract was removed, weighed and sampled. pH in four segments of the gastrointestinal tract was measured by insertion of a unipolar electrode through a small incision made in the wall (penetration pH meter CRISON 507, electrode Crison 52-32, Net Interlab S.A.L., Madrid, Spain). The pH measurements were performed in the middle of the caudal portion of the stomach, 15 cm proximal to the ileocecal valve, in the lowest part of the caecum and in the colon, 20 cm distal to the caecum. Samples
(1 g) of the caecal contents were taken and kept in tubes with 3 ml of ethanol (96%) as a preservative. Stomach, small intestine and large intestine were emptied and weighted. For histological study, samples from the middle caecum were opened longitudinally and fixed by immersion in 10% (v/v) buffered formalin immediately after slaughter.

2.3. DNA extraction

Samples of equivalent volume to 400 mg of digesta were preserved in ethanol and precipitated by centrifugation (13,000 g for 5 min). DNA in the precipitate was extracted and purified using the commercial QIAamp DNA Stool Mini Kit (Qiagen, West Sussex, UK). The recommended lysis temperature was increased to 90 °C and an incubation step with lysozyme was added afterwards (10 mg/ml, 37 °C, 30 min) to improve bacterial cell rupture. The DNA was stored at –80 °C until analysis.

2.4. Real-time PCR (qPCR)

Total bacteria, lactobacilli and enterobacteria were quantified using real-time PCR following procedures and using primers described by Castillo et al. (2006). The oligonucleotides used were based on regions of identity within the 16S rRNA gene and were adapted from published specific primers or probes using the Primer Express Software (Applied Biosystems, CA, USA). Primers used for total bacteria were: F-tot (forward) 5’GCAGGCCTAACACATGCAAGTC3’ and R-tot (reverse) 5’CTGCTGCCTCCCGTAGGAGT3’. For lactobacilli: F-lac 5’GCAGCAGTAGGGAATCTTCCA3’ and R-lac 5’GCATTYCACCGCTACACATG3’, and for enterobacteria F-ent 5’ATGGCTGTCGTCAGCTCGT3’ and R-ent 5’CCTACTTCTTTTGCAACCCACTG3’. Amplification and detection of DNA by quantitative real-time PCR was performed with the ABI 7900 HT Sequence Detection
System using optical-grade 96-well plates and SYBR Green dye (PE Biosystems, Warrington, UK). For absolute quantification, PCR products obtained from the amplification of the whole 16S rRNA gene of *Escherichia coli* (CECT 515NT) and *Lactobacillus acidophilus* (CECT 903NT) were used to construct the standard curves. The PCR conditions corresponded to those published by Leser et al. (2002). The amplified gene from *E. coli* was used for absolute quantification of the total bacteria and enterobacteria and the amplified gene from *L. acidophilus* for quantification of the lactobacilli. Quantitative values were expressed as log of 16S rRNA gene copies/g fresh matter (FM). Enterobacteria:lactobacilli ratio were expressed as the difference of logarithms.

2.5. Terminal-Restriction Fragment Length Polymorphism (t-RFLP)

T-RFLP analysis of bacterial community was performed following the procedure described by Höjberg et al. (2005). Briefly, a 1497-bp fragment of the 16S rRNA gene was amplified using a 6-carboxy-fluorescein-labeled forward primer: S-D-Bact-0008-a-S-20 (5’-6-FAM-AGAGTTTGATCMTGGCTCAG-3’) and reverse primer PH1552 (5’-AAGGAGGTGATCCAGCCGCA-3’). Duplicate PCR analyses were perform for each sample. Fluorescent-labeled PCR products were purified on QIAquick PCR purification kit columns (Qiagen, West Sussex, UK,) and eluted in a final volume of 30 µl of Milli-Q water. Then, the resultant PCR product was subjected to a restriction with *HhaI* (2000 U/µl) (Biolabs Inc. New England, USA). Fluorescent-labeled terminal restriction fragments (TRF) were analyzed by capillary electrophoresis on an automatic sequence analyzer (ABI 3100 Genetic Analyzer, PE Biosystems, Warrington, UK) in Gene-Scan mode with a 25-U detection threshold. Determination of the TRFs sizes in the range 50–
700 base pairs (bp) were performed with the size standard GS-1000-ROX (PE Biosystems).

2.5.1. Analysis of t-RFLP data

Sample data consisted of size (base pairs) and peak area for each TRF. To standardize the DNA loaded on the capillary, the sum of all TRF peak areas in the pattern was used to normalize the peak detection threshold in each sample. Following the method of Kitts (2001), a new threshold value was obtained by multiplying a pattern’s relative DNA ratio (the ratio of total peak area in the pattern to the total area in the sample with the smallest total peak area) by 323 area units (the area of the smallest peak at the 25 detection threshold in the sample with the smallest total peak area). For each sample, peaks with a lower area were deleted from the data set. New total area was obtained by the sum of all the remaining peak areas in each pattern.

Richness was considered as the number of peaks in each sample after standardization. For pair-wise comparisons of the profiles, a Dice coefficient was calculated and dendograms were constructed using Fingerprinting II (Informatix, Bio-Rad, CA, USA) software and an unweighted pair-group method with averaging algorithm (UPGMA).

To deduce the potential bacterial composition of the samples, in silico restrictions for the major pig gut bacteria with the primers and the enzyme used were obtained using the analysis function TAP-tRFLP from the Ribosomal Database Project II software (Table 2).

2.6. Morphometric analysis

Tissue samples for histological study were dehydrated and embedded in paraffin wax, sectioned at 4 µm and stained with haematoxylin and eosin. Morphometric measurements were performed with a light microscope (BHS, Olympus, Spain). Crypt
depth (CD), intraepithelial lymphocytes (IEL), the index of mitosis, lamina propria cell
density and goblet cell numbers in crypts were measured (Nofrarias et al., 2006).
Measurements were performed in 10 well-oriented crypts from each animal. CD was
measured using a linear ocular micrometer (Olympus, REF.209-35040, Microplanet,
Barcelona, Spain). The same crypt columns were used to determine the number of IEL,
goblet cells and index of mitosis (meta- and anaphases); these variables were expressed
per 100 enterocytes. On the basis of the cellular morphology, differences between the
nuclei of enterocytes, mitotic figures, goblet cells and lymphocytes were clearly
distinguishable at 400× magnification. Lamina propria cell density was determined by
counting total visibly stained nuclei and total lymphocytes in 10 fields (total area of
4000 µm$^2$) from each section using an ocular grid (Olympus, REF. 209-35046,
Microplanet, Barcelona, Spain). Cell density was expressed as the number of total
stained cells and the number of lymphocyte-like cells per 1000 µm$^2$ area. The number of
lymphocytes in relation to the number of total cells was also calculated. Crypt density
was also determined (Brunsgaard, 1997). All morphometric analyses were done by the
same person, who was blind to treatment modality.

2.7. Statistical Analysis

The effect of weaning on body weights, pH, total bacteria, lactobacilli, enterobacteria,
richness and histological measurements was tested with ANOVA using the GLM
procedure of SAS statistic package (SAS Inst., Inc. 8.1, Cary, NC, USA). The pig was
used as the experimental unit. Statistical significance was accepted at $P \leq 0.05$.

3. Results and discussion
Diarrhoea was not detected in the pigs and there was only one case of liquid faeces (W group). Initial body weight (BW) was similar for both groups, at 4.4 ± 0.16 kg for S and 4.4 ± 0.15 kg for W. As expected, at the end of the experimental period, BW was higher for piglets that remained with the sow than for weaned pigs (6.1 ± 0.25 versus 5.05 ± 0.27 kg for S and W, respectively, \( P < 0.001 \)). Growth rate, expressed as average daily gain (ADG), was higher for S than for W pigs (0.25 ± 0.02 versus 0.10 ± 0.02 kg for S and W, respectively, \( P < 0.001 \)).

### 3.1. Bacterial quantitative change measured by real-time PCR

The total microbial population, lactobacilli and enterobacteria were quantified in caecum digesta using qPCR (Figure 1).

The total bacteria counts, expressed as log 16S rRNA gene copies/g fresh matter (FM), did not differ between groups (12.84 and 12.81 log gene copy number/g FM for S and W, respectively, \( P > 0.05 \)). Similar total faecal anaerobic counts after weaning were found by Franklin et al. (2002) in piglets weaned at 24 days.

Lactobacilli and enterobacteria have been traditionally selected as microbial groups with a particular significance for gut health. The ratio between these two bacterial groups has been routinely used as a gut-health indicator and it is desirable that lactobacilli outnumber enterobacteria to improve robustness against opportunistic pathogens. In our case, this ratio was higher in W than in S pigs, reflecting the negative effect of weaning on lactobacilli and enterobacteria populations (0.27 versus 1.76 for S and W group, respectively, \( P = 0.05 \)). An inverse correlation between lactobacilli and enterobacteria during the first week post-weaning has been reported previously (Risley et al., 1992; Jensen, 1998; Franklin et al., 2002).

### 3.2. Ecological bacterial changes, t-RFLP results.
To evaluate global changes in the microbial ecosystem, the t-RFLP method was employed; a technique recently used to characterize the effect of different dietary treatments on pig gut microbiota (Höjberg et al. 2005).

The similarity indexes of the t-RFLP profiles illustrated in the form of a dendogram are shown in Figure 2. It only shows the microbial profiles of 11 pigs due to the fact that one pig had no digesta present in the caecum at the time of sacrifice. The effect of weaning on the ecological composition of microbiota is clear, compared to other possible factors, such as litter or individual effects. This was reflected in two clearly separate clusters, one for each experimental group. There was one exception: a weaned piglet was grouped in the suckling branch of the dendogram and, interestingly, was the animal that showed liquid faeces. Separation of this piglet in the dendogram might reflect the beginning of some kind of enteric disbiosis in this animal and the failure of its microbial ecosystem to adapt to solid, dry food.

Microbial profiles of S pigs showed a higher similarity to one another (54–78%) than those of W pigs, which showed more heterogeneous microbial profiles (25–76%). The higher variability in microbial profiles in the W group suggests that the pigs suffered stress at weaning and that each individual responded differently to that stress.

Microbiota richness was measured as the number of similar bands between both experimental groups (49.34 for S and 53.40 for W, $P = 0.22$). Various studies have described a marked decrease in biodiversity just after piglet weaning (Katouli et al., 1997; Jensen-Waern et al., 1998), showing that early weaning involves a obvious disruption in normal pig microbiota evolution. Thereafter, there is a re-establishment process which can vary with time depending on a number of factors. In this study, the
weaned pigs were probably in the process of re-establishing a new microbial equilibrium.

_in silico_ restriction, using Ribosomal Database Project II, was used to deduce potential ecological changes in the samples. However, it should be noted that dispersed phylogenetic groups of bacteria may produce terminal restriction fragments (TRFs) of identical size and that a single TRF in a profile may represent more than one organism in the sample. Results are, therefore, presented as potential compatible bacterial species. Note also that direct attribution of species to individual peaks is not unequivocally possible unless fingerprinting is complemented with sequence analysis of clone libraries.

In this study, compatible bacteria species were assigned to recently described, major pig-gut bacterial groups (Leser et al., 2002). Table 2 shows the frequency of detection of compatible bacteria that were represented in at least three animals.

Analysis of electropherograms revealed compatible TRFs with different lactic acid bacteria including _L. acidophilus, L. bifidum, L. brevis, L. casei, L. rhamnosum, L. vaginalis, Lactococcus lactis, L. delbruekii sp. lactis, L. delbruekii sp. delbruekii, and L. fructivorans_. Interestingly, the fragment of 62 base pairs, compatible with both _L. lactis_ and _L. vaginalis_, was present in all the animals, with a mean contribution of 15–20% of total area. Although mean area for total lactobacilli was similar in both groups (23.1 and 21.5 % for S and W pigs, respectively), S pigs showed higher diversity in compatible TRFs with different lactobacilli species than W pigs. In particular, _L. delbruekii sp. lactis_ was present in five animals from this group, representing nearly 5% of total area, whereas no animal of the W group showed any fragment with compatible size. Similarly, _L. delbruekii sp. delbruekii_ was present in four S pigs but in only one W
The presence of a higher diversity of lactobacilli in S pigs has been reported previously (Krause et al., 1995; Konstantinov et al., 2006).

In the case of Enterococcus sp., five of six pigs in the S group showed a peak compatible with this group (2.18%), but only appeared in two pigs from the W group (1.35%). This is in agreement with Jensen (1998), who found a decrease in enterococci in weaned piglets.

Different species from the Bacteroidetes phylum can be compatible with a series of TRFs of similar size, ranging 89–104 bp (Table 2). Summed areas of these peaks represent 4.0 and 5.2% of total peak area for suckling and weaned pigs, respectively, and are, therefore, the second most importance group after lactobacilli. Adami and Cavazzoni (1999) have also described the importance of this group of bacteria in young piglets.

In our study, a peak compatible with Clostridium coccoides only appeared in three suckling pigs, representing 1.67% of total peak area. Similarly, a peak compatible with C. butyricum (0.86 %) was only found in the S group. Other studies have described Clostridium as one of the main anaerobic bacteria during the suckling period, declining progressively in abundance with the age (Swords et al., 1993). The presence of C. coccoides may be considered beneficial for the piglets due to its production of short-chain fatty acids. In fact, it has been used as a probiotic both in animals and humans (Han et al., 1984: Seki et al., 2003). Other peaks, compatible with different species from the Clostridium clusters I, IV and XIVa and XVIII, were found in both groups of animals, representing a mean of 2.49 and 1.99% of total area for S and W pigs, respectively.
Peaks compatible with *Fibrobacter succinogenes* and *Fibrobacter intestinalis* were found in both groups (5.3% of total peak area for suckling and 3.4% for weaned pigs). Bacteria belonging to this genus, which show high cellulolytic and hemicellulolytic enzyme activities, have been reported previously from the porcine gastrointestinal tract (Varel and Yen, 1997). Although fibre was absent in the milk diet and at a very low level in the dry feed, the presence this genus could indicate the ability of the pigs to select a particular indigenous microbiota, allowing effective digestion of fibre with growth and change in diet.

Potential compatible peaks with species from the *Enterobacteriaceae* family, such as *E. coli*, were found in only four pigs, even though enterobacteria counts were determined by qPCR in all animals. A bias in the amplification of particular sequences caused by preferential annealing of particular primer pairs to certain templates and the difficulty of amplifying bacteria in lower proportions in complex samples, such as digesta, might explain the absence of compatible TRFs.

### 3.3. Intestinal morphology

Results of empty weight, pH in different gastrointestinal compartments and histological measurements in caecal samples are shown in Table 3. Weight of stomach and small intestine did not differ between groups; however, the empty weight of the large intestine was significantly higher in the W than in the S group. This increase in large intestine weight could correspond to the beginning of fermentative activity in this compartment due to an increase in the amount of dietary fermentable material arriving in the caecum. Moreover, pH values confirm this increase in fermentation, with significant decreases in caecum and colon pH. The relationship between the beginning of fermentative activity and the development of the large
intestine has been described previously and could be mediated by a trophic effect of an increasing amount of fermentation products, such as SCFA (Frankel et al., 1994).

Regarding caecal histology, differences in crypt depth were not detected, but the crypts were further apart and showed a significant increase in mitotic index in W animals. Brunsgaard (1997) also found increases in the proliferative activity of crypts in the hindgut of pigs at weaning, with a corresponding increase in crypt size. In the current study, increased mitotic activity was observed without crypt depth variation, suggesting both increased renewal and apical epithelial loss in the crypt.

Concerning the intestinal barrier, both cells producing the mucous layer and immune cells were studied. A decrease in the number of goblet cells was found in the W group compared to the S group. Mucus production and breakdown, as well as the number of goblet cells, are regulated by both microbial and host-related factors (Deplancke and Gaskins, 2001). However, little information is available on the effect of weaning and diet on mucin secretion. Pestova et al. (2000) found increased concentrations of intestinal mucins in ileal digesta after weaning, but no measurements were taken in the hindgut.

Immune cells, another important component of the intestinal barrier, also showed differences between groups. Both intraepithelial and lamina propria lymphocytes were higher in weaned pigs, suggesting an increase in activity of the immune system.

An increase in intraepithelial lymphocytes, the first cell line of immune defence, (Cheroutre 2004) is related to changes in intestinal microbiota rather than changes in nutrition (Rothkötter et al., 1999). The changes in intestinal microbiota could also be responsible for the variations observed in the caecum, but the effect of fresh nutrients coming from the solid feed cannot be ruled out.
4. Conclusions

The caecum undergoes significant microbiological and morphological changes at weaning to develop fermentative activity. Commercial weaning produced marked changes in pig caecum microbiota, with an increase in the enterobacteria:lactobacilli ratio. t-RFLP showed differences in bacterial profiles between groups. Though only assumptions can be made, suckling pigs showed a higher diversity in lactic acid bacteria species than the weaned group and showed peaks, compatible with *C. coccoides* and *C. butyricum* species, that were absent in weaned pigs. These changes in microbiota are accompanied by significant changes in caecal histology, with increased proliferative activity in crypts and increased activity of the mucosal immune system.

Acknowledgements

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References


**Tables**

Table 1. Control diet composition (as fed basis).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg</th>
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</thead>
<tbody>
<tr>
<td>Corn</td>
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<tr>
<td>Full fat extruded soybeans</td>
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<tr>
<td>Lactose</td>
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<td>Potato protein</td>
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<td>Whey powder</td>
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<td>DL-Methionine</td>
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<tr>
<td>Bicalcium phosphate</td>
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<tr>
<td>Salt</td>
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<td>Vit-Mineral premix</td>
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<tr>
<td>Sepiolite</td>
<td>1.26</td>
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Table 2. Theoretical restriction 5’-fragment length predicted for the major pig gut bacteria. Results were obtained from the TAP-RFLP tool of the Ribosomal Database II Project software.

<table>
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<tr>
<th>Bacteria groups</th>
<th>Compatible bacteria a</th>
<th>In silico restriction b</th>
<th>Real restriction c</th>
<th>Frequency d</th>
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<td></td>
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<tr>
<td></td>
<td>L. acidophilus, L. brevis, L. bifermentum, rhamnosum, casei</td>
<td>597, 598, 599</td>
<td>597, 599</td>
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<td></td>
<td>L. delbruekii sp. delbruekii</td>
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<td>254</td>
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<td>Lactic Acid Bacteria</td>
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<td>68</td>
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<td></td>
<td>Lactococcus lactis, Lactobacillus vaginalis</td>
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<td>Enterococcus sp.</td>
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<tr>
<td>Weaned</td>
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<td>Bacteroides and relatives</td>
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<td>Fibrobacter</td>
<td>Fibrobacter succinogenes</td>
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<td>Fibrobacter intestinales</td>
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<td>Clostridium cocoides</td>
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<td>3 (1.67)</td>
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<td>C. butyricum</td>
<td>544</td>
<td>544</td>
<td>5 (0.86)</td>
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</tr>
<tr>
<td></td>
<td>Ruminococcus</td>
<td>189</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clostridium clostriforme, C. symbiosum</td>
<td>190</td>
<td>188-193</td>
<td>2 (1.19)</td>
</tr>
<tr>
<td></td>
<td>Roseburia</td>
<td>192</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Butyrivibrio</td>
<td>193</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clostridium spp.</td>
<td>229, 231, 233, 237</td>
<td>229-232, 237</td>
<td>6 (1.30)</td>
</tr>
<tr>
<td></td>
<td>Escherichia sp</td>
<td>371, 372, 373, 374</td>
<td>376-377</td>
<td>3 (0.51)</td>
</tr>
<tr>
<td></td>
<td>Other enteric bacteria (Salmonella, Citrobacter, K. bksiella)</td>
<td>(367, 370, 372), 373, 371</td>
<td></td>
<td>1 (0.55)</td>
</tr>
</tbody>
</table>

*Major pig gut bacteria, based on the work of Leser et al., 2002, with a potential compatible fragment found in at least three animals. Other peaks with 58, 59, 69, 111-120, 123, 133, 162, 211, 278 and 279 did not correspond with any 16S rRNA sequences in the database from the Ribosomal Database Project 8.1 software.

b In silico restriction was performed using the tap-tRFLP tool from the Ribosomal Database Project II.

c Terminal fragment length obtained after PCR product restriction with Hha I.

d Number of animals that showed the peak in each experimental group. The mean peak area (%) value in these animals with the peak is shown in brackets.
Table 3. Weights and pH of the stomach, small and large intestine, and caecal morphology of the early-weaned pigs receiving sow milk or dry feed\(^1\).

<table>
<thead>
<tr>
<th></th>
<th>Suckling</th>
<th>Weaned</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty weight, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>37.4 ± 7.54</td>
<td>38.7 ± 4.88</td>
<td>0.737</td>
</tr>
<tr>
<td>Small intestine</td>
<td>330 ± 56.4</td>
<td>370 ± 98.1</td>
<td>0.393</td>
</tr>
<tr>
<td>Large intestine</td>
<td>120 ± 31.2</td>
<td>195 ± 98.1</td>
<td>0.027</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>3.2 ± 0.84</td>
<td>4.4 ± 0.67</td>
<td>0.019</td>
</tr>
<tr>
<td>Ileum</td>
<td>7.0 ± 0.44</td>
<td>6.7 ± 0.37</td>
<td>0.371</td>
</tr>
<tr>
<td>Cecum</td>
<td>6.3 ± 0.13</td>
<td>5.8 ± 0.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Colon</td>
<td>6.9 ± 0.29</td>
<td>6.03 ± 0.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Caecum histological study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crypt depth, µm</td>
<td>373 ± 48.6</td>
<td>359 ± 30.6</td>
<td>0.566</td>
</tr>
<tr>
<td>Crypt density(^2)</td>
<td>12.4 ± 0.36</td>
<td>10.5 ± 0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mitoses / 100 cells</td>
<td>1.0 ± 0.47</td>
<td>1.8 ± 0.47</td>
<td>0.021</td>
</tr>
<tr>
<td>Goblet cells / 100 cells</td>
<td>25.5 ± 3.48</td>
<td>19.4 ± 3.30</td>
<td>0.011</td>
</tr>
<tr>
<td>Intraepithelial lymphocytes / 100 cells</td>
<td>1.4 ± 0.67</td>
<td>2.6 ± 0.91</td>
<td>0.024</td>
</tr>
<tr>
<td>Lymphocyte density(^3)</td>
<td>1.7 ± 0.45</td>
<td>2.3 ± 0.40</td>
<td>0.045</td>
</tr>
<tr>
<td>Total cell density(^3)</td>
<td>8.7 ± 0.90</td>
<td>8.9 ± 0.47</td>
<td>0.665</td>
</tr>
<tr>
<td>Lymphocyte / Total cells</td>
<td>0.2 ± 0.04</td>
<td>0.3 ± 0.07</td>
<td>0.109</td>
</tr>
<tr>
<td>Mucosal thickness, µm</td>
<td>470 ± 74.8</td>
<td>528 ± 70.8</td>
<td>0.243</td>
</tr>
<tr>
<td>Muscular layer thickness, µm</td>
<td>370 ± 49.5</td>
<td>328 ± 37.0</td>
<td>0.111</td>
</tr>
<tr>
<td>Total intestinal wall thickness, µm</td>
<td>848 ± 98.1</td>
<td>856 ± 95.1</td>
<td>0.899</td>
</tr>
</tbody>
</table>

\(^1\) Values are means, n=6.
\(^2\) Number of crypts appearing over a 1mm distance across the basal part of the mucosa.
\(^3\) Number of lymphocytes like cells or total cells per 1000 µm\(^2\) area in the lamina propria.
Figure captions

Figure 1. Bacterial loads in caecum measured by quantitative PCR (log 16S rRNA gene copies/g FM) in early-weaned pigs receiving sow milk or dry feed.

Figure 2. Dendogram illustrating the correlation between experimental diets in t-RFLP banding patterns. The dendogram represents results from 11 piglets sacrificed on day 28. There was no digesta content in one of the weaned piglet’s caecum. The dendogram distances are in percentage of similarity.
Figure 1

Log 16S rDNA gene copies /g FM

<table>
<thead>
<tr>
<th></th>
<th>Suckling</th>
<th>Weaned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>12.84</td>
<td>12.81</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>9.70</td>
<td>9.01</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>9.97</td>
<td>10.78</td>
</tr>
<tr>
<td>Ratio E:L</td>
<td>0.27</td>
<td>1.76</td>
</tr>
</tbody>
</table>

* indicates a significant difference.
Percentage of similarity

W1
W2
W3
W4

S1
S2
S3
S4
S5
S6

W5

Weaned

Suckling