The intestinal microflora of piglets around weaning - with emphasis on lactobacilli

R. Pieper¹, P. Janczyk¹, Rhena Schumann², W. B. Souffrant¹

¹“Oskar Kellner” Research Unit for Nutritional Physiology, Research Institute for the Biology of Farm Animals, D-18196 Dummerstorf, Germany
²Applied Ecology, Institute of Biological Sciences, Faculty of Mathematics and Natural Sciences, University of Rostock, D-18059 Rostock, Germany

ABSTRACT

This study was aimed at describing the dynamics of microflora colonizing different parts of gastrointestinal tract of piglets around weaning transition with emphasis on ileal lactobacilli, as they are known for their health promoting properties. Eight piglets were slaughtered at day of weaning and four on 1st, 2nd, 5th and 11th day post weaning (pw), respectively. Digesta samples were taken from ileum (1/3 of distal small intestine), caecum and colon (40-50 cm of proximal colon) for microbial examination using selective growth media. Results revealed dramatic and time dependent changes with all observed groups and more pronounced effects in ileum than in caecum or colon. Enterobacteriaceae count was in a steady state from day of weaning until 5th day pw whereas significantly lower counts were found on 11th day pw. Enterococci also showed no differences during first two days but dramatically decreased levels were observed on 5th and 11th day pw. Yeasts counts decreased 5 days pw but recovered until 11th day whereas number of lactobacilli decreased (p<0.01) on 1st day and recovered within 5 days to initial level.

A total amount of 72 lactobacilli colonies was picked up from counting plates, purified and identified by means of their carbohydrate fermentation ability. Results highlight L. acidophilus (44.4 %) followed by L. fermentum (35.7 %) and L. salivarius (15.3 %) being predominant species. Additionally, there was a time dependent shift with L. salivarius, L. fermentum and L. acidophilus being most abundant species before weaning whereas predominance of L. fermentum and L. salivarius on 1st, 2nd and 5th day pw and L. acidophilus on 11th day pw was observed. Our results provide a general overview of occurring changes with major groups of intestinal microbial community in weaning piglets. This could be helpful in current research for alternatives for in-feed-antibiotics. As demonstrated with lactobacilli, changes may also occur within these microbial groups. Further research is needed to elucidate impact of intestinal microbial population dynamic on piglet health status during weaning transition.

Keywords: intestinal microflora, weaning piglets, lactobacilli, Enterobacteriaceae, Enterococci, yeasts, gastrointestinal tract, API
INTRODUCTION

The weaning process in modern swine production is probably the most dramatically occurrence in pigs life and is associated with environmental, social and nutritional stressors resulting in depression of feed intake as well as decreased (and even negative) weight gain. In the European community, piglets are commonly weaned between 21st and 28th day of life, a time point when the porcine gastrointestinal tract (GIT) is still underdeveloped. This could lead to proliferation and overgrowth of enterotoxigenic bacteria such as *E. coli* and *Salmonella* (Hopwood and Hampson, 2003). Additionally, a decreased absorptive capacity due to morphological changes during weaning transition was reported (Spreeuwenberg et al., 2001; Boudry et al., 2004). Prevention of overgrowth of pathogenic bacteria within the weaning period was commonly achieved by addition of antimicrobials to starter diets in past decades. Increasing reports on developing cross resistances among *Escherichia coli* and *Enterococcus faecium* of pig origin (Mathew et al., 1998a; Aarestrup et al., 2000) and the EU-wide ban on antimicrobials as feed additives in swine diets recently highlighted the need to search for alternatives for in-feed-antibiotics. The microbiota colonising the GIT of pigs play an essential role for animal health, animal nutrition and performance. The insight into this ecosystem could become important in the current search for alternatives to antibiotics, such as probiotics and prebiotics. However, the microbial ecosystem in the GIT is unstable during the first week after weaning and it takes 2-3 weeks before hindgut fermentative capacity has developed (Jensen, 1998). Lactic acid bacteria are an indigenous and major bacterial group to intestinal tract of rodents, pigs and crops of poultry (Savage, 1977). A range of potential benefits have been described to be linked to either ingested as well as autochthonous lactobacilli including modulation of immune system and antagonism against bacterial pathogenic species (Blomberg et al., 1993; Tannock, 2004). However, recent data highlighted transient alteration of lactobacillus population during weaning period (Klüß et al., 2003). This raises the necessity of exploring and understanding the “normal” microflora along the GIT of piglets and occurring changes during the weaning transition, especially among bacterial groups known to have health promoting properties such as lactobacilli. This study was aimed at determining changes of microbial composition within GIT of piglets by means of classical microbiological methods when a commercial weaning diet without supplementation of antimicrobial agents was fed during the first two weeks post weaning. Additionally, we focussed on identification of ileal Lactobacilli, as they contribute to host’s health and display a major fraction in this part of the GIT.

MATERIAL AND METHODS

Twenty four purebred German Landrace piglets were used for this study; they were reared in our institute’s experimental station without access to creep
feed until day of weaning. They were weaned on the 28th day of life, weighed, randomly allocated to group pens (8 piglets each) and kept under constant conditions at 26-28°C and natural light system. The diet (Table 1) was formulated to meet NRC (1998) requirements and offered ad libitum. On 1st, 2nd, 5th and 11th day post weaning (pw) 4 piglets were weighed and subsequently slaughtered by intracardial injection of 1 ml T61® (Intervet, Germany). Additional 8 piglets were slaughtered on day of the weaning to determine the immediately pre weaning microbial composition in the GIT. Entire GIT was removed and digesta samples were taken from its three parts (ileum defined as distal 1/3 of small intestine, caecum and app. 40-50 cm of proximal colon), placed on ice and immediately transferred to our laboratory. For microbiological investigations, homogenized digesta was serially diluted in 0.9 % NaCl solution and poured with two parallels on agar plates containing different selective media. Plates for enumeration of Enterobactericeae (Violet-Red Bile Dextrose-agar containing in g/L: peptone from meat 7.0; yeast extract 3.0; NaCl 5.0; D(+)-glucose 10.0; bile salt mixture 1.5; neutral red 0.03; crystal violet 0.002; agar-agar 13.0), and Enterococci (membrane-filter enterococcus selective agar according to Slanetz and Bartley (1957) containing in g/l: tryptose 20.0; yeast extract 5.0; D(+)-glucose 2.0; K2HPO4 4.0; sodium azide 0.4; 2,3,5-triphenyltetrazolium chloride 0.1; agar-agar 10.0) were incubated aerobically at 37 °C for 24 h. Yeasts were grown for 5 days at 37 °C on SABOURAUD-Agar containing in g/l peptone 10.0; D(+)-glucose 40.0; agar-agar 15.0 and chloramphenicol (50 mg/l) to prevent bacterial growth. Lactobacilli (LAB) were grown on MRS-agar (according to De Man et al., (1960), containing in g/l: peptone from casein 10.0; meat extract 8.0; yeast extract 4.0; D(+)-glucose 20.0; K2HPO4 2.0; Tween® 80 1.0; (NH4)2HC6H5O7 2.0; NaCH3COOH 5.0; MgSO4 0.2; MnSO4 0.04; agar-agar 14.0) for 72 h at 37°C in anaerobic jars (Merck®, Germany) using Anaerocult A (Merck®, Germany). Colonies from the highest dilution rate of both parallels were counted and mean results given as colony forming units (cfu)/g digesta.

Table 1 Ingredients and chemical composition of the diet in this study

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
<th>Nutrient</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley meal</td>
<td>30.0</td>
<td>DM</td>
<td>888</td>
</tr>
<tr>
<td>Wheat meal</td>
<td>29.7</td>
<td>CP</td>
<td>191</td>
</tr>
<tr>
<td>Peas (44% starch)</td>
<td>5.0</td>
<td>Ash</td>
<td>55</td>
</tr>
<tr>
<td>Whey powder</td>
<td>8.0</td>
<td>Crude fibre</td>
<td>34</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>2.5</td>
<td>Crude fat</td>
<td>50</td>
</tr>
<tr>
<td>Soycomil (soy concentrate)</td>
<td>4.0</td>
<td>Starch + sugar</td>
<td>455</td>
</tr>
<tr>
<td>Maize starch</td>
<td>4.0</td>
<td>Lysine</td>
<td>12.5</td>
</tr>
<tr>
<td>Potato protein, purified</td>
<td>5.0</td>
<td>Methionine</td>
<td>4.4</td>
</tr>
<tr>
<td>Maize gluten meal</td>
<td>2.2</td>
<td>Methionine+Cysteine</td>
<td>7.8</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>2.5</td>
<td>Tryptophan</td>
<td>2.5</td>
</tr>
<tr>
<td>Ingredient</td>
<td>Value</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.02</td>
<td></td>
<td>Threonine</td>
</tr>
<tr>
<td>Mono calcium phosphate</td>
<td>0.78</td>
<td>Ca</td>
<td></td>
</tr>
<tr>
<td>Trace min.-vit. premix(^{2})</td>
<td>0.4</td>
<td>Total P</td>
<td>Digestible P</td>
</tr>
<tr>
<td>Methionine (99%)</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-lysine-HCl (79%)</td>
<td>0.34</td>
<td>Na</td>
<td></td>
</tr>
<tr>
<td>Tryptophan (99%)</td>
<td>0.031</td>
<td>K</td>
<td></td>
</tr>
<tr>
<td>Threonine (98%)</td>
<td>0.03</td>
<td>Cl</td>
<td></td>
</tr>
<tr>
<td>Palm oil + soybean oil</td>
<td>3.1</td>
<td>Cu, mg</td>
<td></td>
</tr>
<tr>
<td>Molasses</td>
<td>1.009</td>
<td>Zn, mg</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>0.28</td>
<td>NEF, MJ/kg</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{1}\) The diet was aligned by the consortium of EU-project FEED FOR PIG HEALTH (FOOD-CT-2004-506144)  
\(^{2}\) This trace mineral-vitamin premix (0.4%) supplies per kg diet as follows: retinol 1750 IU, cholecalciferol 200 IU, tocopherol 11 IU, phylloquinone 0.5 mg, thiamin 1.0 mg, riboflavin 4 mg, d-pantothenic acid 9 mg, nicotinic acid 12.5 mg, biotin 50 μg, cyanocobalamin15 μg, folic acid 0.3 mg, pyridoxine 1.5 mg, choline 400 mg, Fe 80 mg, Zn 54 mg, Mn 30 mg, Co 0.15 mg, I 0.14 mg, Se 0.25 mg, antioxidants (E310,320,321) 50 mg

Typical colonies of lactobacilli from MRS-agar plates at the highest dilution rate were picked up and cultivated in MRS-broth overnight. Subsequently they were purified by fractional plating 3-4 times on MRS-agar plates and cultivated as described previously. Strain purification was guaranteed by 3 separations spreading of colonies. Cell morphology was verified by microscopic observations as well as Gram-staining. Lactobacilli were determined using identification kits based on carbohydrate fermentation profiles (API 50 CHL, Biomerieux, Nuertingen, Germany). Briefly, test strips were inoculated with the respective strains and covered with paraffin-oil according to contributor’s instructions. Fermentation profiles were documented after incubation for 48 h at 30°C and strains identified using online-identification software (https://apiweb.biomereux.com). Log phase cells of selected strains were fluorescently labelled by LIVE BacLight™ Bacterial Gram Stain Kit (Molecular Probes, Leiden, Netherlands) for 5 min according to manufacturer's protocol. Cells were filtered onto irgalan-black stained polycarbonate filter membranes (Sigma-Aldrich, pore size 0.2 μm). Cells were visualized by epifluorescence microscopy (Olympus BX51, Hamburg, Germany) at magnification of 1000x (objective UPlan FL 100 NA 1.3 Oil) under blue excitation (U-MWB2). Microphotographs were taken with a SIS Colour View 12 digital camera and stored using AnalySIS Pro software 3.2 (Olympus Soft-Imaging Solutions GmbH, Münster, Germany). Statistical analysis of results was accomplished by ANOVA with following post-hoc (HSD-Tukey test) using software package (STATISTICA Vers. 6.0, StatSoft Inc. 2001, Tulsa, USA). Significance was considered at \( p < 0.05 \).
RESULTS AND DISCUSSION

The microbial community in the GIT of piglets plays an essential role for animal health, nutrition, performance and quality of animal products. The weaning process in modern pig production frequently involves sudden dietary changes, likely enhancing susceptibility to diseases due to instability of intestinal microbial ecosystem. This study was aimed at exploring microbial population dynamics associated with weaning in “healthy” piglets. Microbial counts in different sections of the GIT in piglets during early pw period obtained by classical cultivation are presented in Figures 1-3.

Cultivable cell counts of the four observed microbial groups increased from proximal to distal part of GIT which was also found by Decuypere and van der Heyde (1972), Kovacs et al. (1972) and Jensen (1998) investigating the cultivable microflora of suckling and weaning piglets. This effect is due to velocity of digesta flow being higher in small intestine compared to more distal segments. Enterobacteriaceae (including species such as E. coli, Salmonella, etc.) in ileal digesta increased after weaning from approximately 6.4 log cfu/g to > 7.0 log cfu/g on the 5th day but decreased significantly below the initial level at 11th day of pw period. Similar effects, but at generally higher cell numbers, were found in digesta from caecum and colon which supports results given by Jensen (1998). The same author also found an inverse relationship of lactobacilli and coliform counts during first week pw making piglets more susceptible to disorders and thereby a reduced performance during this period. We observed no overgrowth of Enterobacteriaceae, including E. coli and Salmonella, the main contributors to pw diarrhoea, which was consistent with health status of piglets free of diarrhoea during the experiment. Only minor shifts were reported in ileal microbial communities during weaning transition (Decuypere and van der Heyde, 1972; Kovacs et al., 1972). Mathew et al. (1997) reported a numerical increase in E.coli during first week pw but counts recovered rapidly to pre weaning levels. In a following study, investigating the effect of a life yeast supplementation, a steady state of E. coli during weaning period was observed (Mathew et al., 1998b). In a recent study, Klüß (2004) detected no change in Enterobacteriaceae after weaning but decreasing number 15 days pw when a high fibre diet was fed. In contrast, no change in ileal counts of Enterobacteriaceae was observed by the same author when low fibre diets as well as a diet containing antimicrobials were fed. The data suggest that changes in Enterobacteriaceae counts during the pw period depend on factors such as environment, weaning age and diet.

Number of Enterococci (including E. faecalis, E. faecium,) in ileal digesta was lower on 1st day pw than in unweaned piglets but reached the highest levels 2 days pw. We observed a decrease in numbers of Enterococci to almost below the detection limit of the method (10^2 cfu/g) on the 5th day pw in all parts of the GIT. The effect was again more pronounced in ileum compared with caecum and colon. As few data on adhesion properties of Enterococci to epithelial cells are available, this may be due to an increasing feed intake and, thereby, a higher
flow rate of digesta in the upper part of the GIT (“wash-out-effect”). Generally, our results are in good agreement with results of Klüß (2004), who also found decreasing numbers of Enterococci in ileal digesta 5 days after the weaning when diets with high or low fibre content were given.

![Figure 1](image1.png)

**Figure 1** Mean (± SD) counts of major microbial groups in ileum of weaning piglets at different time point after weaning as revealed by classical plating methods

![Figure 2](image2.png)

**Figure 2** Mean (± SD) counts of major microbial groups in caecum of weaning piglets at different time point after weaning as revealed by classical plating methods

Numbers of yeasts were unchanged one day after weaning compared to pre-weaning levels but increased 2 days after the weaning in all parts of the GIT. The lowest levels were found on the 5th and the highest on the 11th day pw.
Causes for these effects could not be clarified as only few data are available about succession and dynamics of yeasts in GIT of pigs. Generally, the range of counts in our study are in good agreement with values reported by Mikkelsen and Jensen (1998) and Mathew et al. (1998b) but lower than values given by Scholten et al. (2002). Yeasts such as *S. cerevisiae* and *S. cerevisiae* ssp. *boulardii* are considered to have probiotic properties. Both species were reported to increase daily weight gain in piglets when given orally after weaning (Mathew et al., 1998b; Bontempo et al., 2006) but had no significant influence on intestinal microflora (Mathew et al., 1998b). On the other hand, Scholten et al. (2002) reported an interrelationship between yeast and lactobacilli in small intestinal samples with decreasing yeast counts when lactobacilli increased. This could be one explanation for decreased values of yeast 5 days after weaning in our study as number of lactobacilli reached initial pre-weaning levels of >8.5 log cfu/g.

Lactobacilli were the most abundant species in all compartments of the gut before weaning with average counts of > 8.0 log cfu/g but the numbers decreased dramatically within 1 day. On 5th day pw lactobacilli counts reached initial pre-weaning levels and the highest levels on 11th day (8.96 cfu/g for ileum and > 9.0 log cfu/g, for caecum and colon). Decuypere and van der Heyde (1972) and Jensen (1998) reported lactobacilli as well as streptococci being the major microbial groups in small intestinal digesta. Additionally, our results show that lactobacilli also play a major role in distal parts of GIT (i.e. caecum and colon). Type of the diet and, thereby, composition of carbohydrates, being the major energy source for lactobacilli, may affect lactobacilli succession in GIT of weaning piglets. Numerous studies were carried out on changes of

![Figure 3. Mean (± SD) counts of major microbial groups in colon of weaning piglets at different time point after weaning as revealed by classical plating methods.](image-url)
microbial community composition by fermented liquid feeds. Van Winsen et al. (2001) showed that this type of diet reduced Enterobacteriaceae and increased total lactobacilli counts indicating a possible relationship between these microbial groups. Increased numbers of lactobacilli due to feeding liquid diets was also reported by Mikkelsen and Jensen (1998), Scholten et al. (2002) and Hojberg et al. (2003). Mathew et al. (1994) found a non-significant time dependent impact of creep-feeding on intestinal microflora. This was also reported by Franklin et al. (2002) who focussed specifically on weaning age (17 vs. 24 days). Numbers of ileal lactobacilli declined in both groups but to a greater extend in the early weaned group making piglets more susceptible to disorders than those weaned at a later stage suggesting impact of many different factors (i.e. dietary carbohydrates, type of diet, weaning age) for lactobacilli succession in GIT of weaning piglets.

To receive a deeper insight into the lactobacilli community in the ileum, typical colonies were taken from agar plates with the highest countable dilution rate, purified and subsequently checked for their carbohydrate fermentation ability which allows identification of species. Identification results at different time points pw are presented in Table 2. A total of 72 Lactobacillus spp. were identified. The predominant species were L. acidophilus (44.4 %) followed by L. fermentum (35.7 %) and L. salivarius (15.3 %), to a lesser extent L. brevis, L. crispatus, L. cellobiosus as well as Lc. raffinolactis (1.4 %, respectively). Several authors highlighted the role of L. acidophilus, L. reuteri, L. salivarius and L. fermentum as predominating species in the intestines of piglets. For example, Smith et al., (1999) identified several Lactobacillus spp. by their carbohydrate fermentation profiles and reported L. acidophilus, L. fermentum and L. salivarius being most abundant in faeces of pigs. The same species were found by Fuller et al. (1978) in stomachs of neonatal piglets while L. acidophilus and L. reuteri dominated in small intestinal samples.

<table>
<thead>
<tr>
<th>day of life (day post weaning)</th>
<th>28 (0)</th>
<th>29 (1)</th>
<th>30 (2)</th>
<th>33 (5)</th>
<th>39 (11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>L. salivarius</td>
<td>4</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>L. fermentum</td>
<td>11</td>
<td>1</td>
<td>5</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>L. brevis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>L. crispatus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>L. cellobiosus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Lc. raffinolactis</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>5</td>
<td>15</td>
<td>11</td>
<td>17</td>
</tr>
</tbody>
</table>

\(^1\) 8 piglets pre weaning and 4 piglets on each day post weaning
In our study, there was an apparent and time-dependent shift in lactobacilli community after the weaning. Before weaning \textit{L. salivarius}, \textit{L. fermentum} and \textit{L. acidophilus} were the most abundant lactobacilli whereas a shift towards \textit{L. fermentum} and \textit{L. salivarius} was observed, being the most abundant isolated species on the 2\textsuperscript{nd} and 5\textsuperscript{th} day pw. On the 11\textsuperscript{th} day after weaning, \textit{L. acidophilus} was the predominant species (14 out of 17 isolates) in ileal digesta. Figure 4 gives an example of typical lactobacilli in the GIT of weaning piglets. They are all Gram-positive non-spore forming rods occurring solitary or in short chains. \textit{L. acidophilus} cells are considerably larger with 1.3-3.3 µm length and 0.5-0.7 µm width in contrast to the short rods of \textit{L. fermentum} with 0.9-2.6 µm length and 0.6-0.8 µm width. Since bacteria react with changes in size to many environmental conditions, especially with a dramatic shrinkage to "dwarf" cells at starvation, cell sizing after fluorescence staining is a useful tool for microbial biomass estimation. This may be of special interest in the case of changing organic substrates (piglet diet), since an insufficient nutrient supply may reduce cultivability but not bacterial viability and survival \textit{in situ}. That may cause a substantial underestimation of abundance when classical microbiological methods are applied. Identification and differentiation of lactobacilli are also possible without cultivation, when novel 16S rRNA based techniques, such as fluorescence \textit{in situ} hybridization (FISH), and flow cytometry are applied. All these tools are applied by our group in outgoing studies, but corresponding results will be shown in separate publications.

![L. acidophilus](image1.png) ![L. fermentum](image2.png)

Figure 4  Abundant lactobacilli from ileal samples of weaning piglets stained with LIVE BacLight\textsuperscript{TM} Bacterial Gram Stain Kit (Molecular Probes, Leiden, Netherlands).

Succession of lactobacilli in the ileum largely depends on their growth rate and ability to adhere the intestinal mucus layer, thus avoiding the “wash-out” effect of the digesta flow (Tannock, 1995). Gastric emptying regulates digesta flow from stomach to small intestine and digesta moves at higher velocity through the small intestine compared with distal parts. Krause et al. (1997)
investigated lactobacilli that adhere to the intestinal epithelium of suckling and weaning piglets fed either corn-soy or a corn-soy-lactose diet and reported *L. fermentum* being the most abundant species in ileum as well as colon. A recent study by Maré et al. (2006) was aimed at determining the site of adhesion in the GIT and inhibitory effects of strain *L. plantarum* 423 and *L. salivarius* 241 on *Enterococcus faecalis* in pre and post weaned piglets. Whereas *L. plantarum* 423 adhered strongly to the ileum and distal colon and *L. salivarius* 241 to the duodenum in pre weaned piglets, high numbers of strain 241 were found in duodenum and distal colon of post weaned piglets. In contrast, strain 423 remained localized to the ileum. Lowering in *E. faecalis* cell numbers were recorded after challenge with strain 241 pre weaning. The authors concluded that this effect was likely due to production of antimicrobial compounds by the strains. Production of antimicrobial compounds by lactobacilli and, therefore, prevention of pathogenic micro-organisms such as *Salmonella*, *E. coli* and *Clostridia* was also reported in numerous studies (Blomberg et al., 1993, Bomba et al., 1997, Naaber et al., 2004, Coconnier-Polter et al., 2005, Bernbom et al., 2006). These studies also included lactobacilli such as *L. acidophilus* and *L. fermentum*. Therefore, a reduction of *Enterococci* and *Enterobacteriaceae* on 5th and 11th day in our study could be due to inhibitory effects by lactobacilli as their counts reached the highest values at these time points pw. Adhesion to intestinal cells by lactobacilli and thereby competition with other micro-organisms for adhesion sites could be a supporting factor for this effect. Also, analysis of lactic acid and volatile fatty acids can describe the fermentative abilities and actual activities of lactobacilli. According to current taxonomic knowledge on lactobacilli, *L. acidophilus* and *L. salivarius* are obligate homofermenters that ferment glucose exclusively to lactic acid whereas *L. fermentum* and *L. brevis* are obligate heterofermenters that ferment glucose to lactic acid, acetic acid and/or ethanol and carbon dioxide (Stiles and Holzapfel, 1994). Energy loss is generally greater with hetero- rather than homofermentative fermentation and lactic acid was initially assumed to have promotional and balancing effects on gut flora (Roth and Kirchgessner, 1998). Thus, presence of high numbers of homolactic acid bacteria such as *L. acidophilus* and *L. salivarius* may have a beneficial effect on the gut environment.

**CONCLUSIONS**

In conclusion, results obtained in this study provide insight into the dynamics of intestinal microflora and dynamics of the major bacterial groups during the weaning period. This will be helpful in current research on alternatives for in-feed-antibiotics, considering the ban on their usage as growth promoters in the EU. As demonstrated with lactobacilli, changes may also occur within these microbial groups. Further research is needed to elucidate the impact of intestinal microbial population dynamics on piglet health status.
during weaning transition. In our ongoing studies we especially focus on factors contributing to succession of lactobacilli in ileum of piglets during weaning transition by means of molecular biological methods such as FISH and flow cytometry.

ACKNOWLEDGEMENTS

We are grateful to our technicians Mrs. Aenne Koepnick, Ingetraud Prignitz and Mr. Walter Booth for laboratory assistance. The European Union is greatly acknowledged for financial support of the project FEED FOR PIG HEALTH (FOOD-CT-2004-506144). The authors are solely responsible for this text which does not represent the opinion of the EC, and the EC is not responsible for the information delivered.

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