Effect of feeding level on the composition of the intestinal microbiota in weaned piglets

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ABSTRACT: In piglets, the development and composition of intestinal microbiota is influenced by dietary factors. A considerable reduction in feed intake during the transition from liquid to solid feed is often accompanied by postweaning diarrhea. Therefore, it is hypothesized that variations in feeding level during weaning may affect intestinal microbial composition. Forty-eight piglets fitted with simple ileal T-cannulas were used to examine the effects of a high (60 g/kg BW) and a low (30 g/kg BW) feeding level on the composition of the ileal and fecal microbiota. The assay diets contained graded inclusion levels of soybean (Glycine max) meal or casein. Bacterial cell numbers of total eubacteria, Lactobacillus spp., and the mainly proteolytic Clostridium leptum, Clostridium coccoides, Enterobacteriaceae, and Bacteroides–Prevotella–Porphyromonas group (Bacteroides group) of feces and ileal digesta were determined by use of real-time PCR. There were no interactions between feeding level and protein source except for the Bacteroides group in ileal digesta. Ileal cell numbers of lactobacilli were increased (P < 0.001) at the higher feeding level. In contrast, ileal cell numbers of Clostridium coccoides were lower (P < 0.001) at the higher feeding level. There were no differences of bacterial cell numbers in feces. Results indicate that feeding level affects microbial composition in the small intestine. Also, sufficient feed intake during weaning encourages proliferation of beneficial bacteria, thereby contributing to improved gut health.

Key words: feeding level, intestinal microbiota, weaned piglets


INTRODUCTION

In piglets, the composition of the intestinal microbiota is influenced by various factors such as diet composition and feeding regime. Profound changes of the intestinal microbiota are observed after weaning when adaptation to solid feed and social and environmental changes challenge the animal. Extreme low voluntary feed intake after weaning is often associated with postweaning diarrhea, and variations in feed intake level during weaning have been shown to affect morphological and histological structures in piglets’ intestine (Spreeuwenberg et al., 2001). Furthermore, feeding level could have an impact on factors that may influence intestinal microbiota, such as digesta passage, retention times, and nutrient availability (Rodríguez et al., 2003). Potential effects of feeding level on intestinal microbiota have been shown in piglets’ stomachs (Verdonk et al., 2003). Therefore, it was hypothesized that variations in feeding level might affect the composition of the intestinal microbiota of weaning piglets in the small and large intestine, thereby eventually affecting intestinal health. Therefore, the present study aimed to examine in weaned piglets the effect of a high and a low feeding level on the composition of the microbial community at the ileal and fecal level.

MATERIALS AND METHODS

Animals, Experimental Design, Diets, and Analyses

The research protocol and care of animals was approved by the German Ethical Commission for Animal Welfare. A total of 48 piglets plus 3 for replacement (weaned at 17 d of age with an initial BW of 5.9 ± 0.94 kg) fitted with simple T-cannulas at the distal ileum was
used in 4 consecutive experiments consisting of 2 periods each. To avoid repeated measurement structures, 1 of 4 optional sequences of combinations between feeding level and protein source were realized for a piglet in 2 periods. The piglets were fed semisynthetic assay diets consisting of either graded levels of soybean meal (SBM; 213, 338, 464, 589, 715, and 840 g/kg as-fed basis) or casein (99, 157, 215, 274, 332, and 390 g/kg as-fed basis) at the expense of cornstarch. Diets were supplemented with 45 g/kg dextrose, 50 g/kg lactose, 30 g/kg cellulose, 29 g/kg oil and 36 g/kg mineral premix, and, in case of the casein diet, 5 g/kg KCl were added. The pigs received their diets at 2 feeding levels, referred to as low (30 g/kg BW) and high (60 g/kg BW; n = 24 for feeding level × protein source). Each experimental period included 7 d for adaptation to the diets and feed allowances. On day 8, fresh feces of each piglet were sampled once during the morning feeding immediately after defecation and ileal digesta were sampled at the time of highest digesta flow rate at 1200 h. Genomic DNA of ileal and fecal samples was extracted using the QIAamp DNA Stool mini Kit (Qiagen, Hilden, Germany) according to the manufacturers’ protocol for stool pathogen detection. The quantification was performed by means of an iCycler iQ 5 realtime Detection System (Bio-Rad, Munich, Germany) using previously described primer sets for total eubacteria (Lee et al., 1996), Lactobacillus spp. (Walter et al., 2001; Heilig et al., 2002), Clostridium leptum (Matsuki et al., 2004), Clostridium coccoides (Matsuki et al., 2002), Enterobacteriaceae (Bartosch et al., 2004), and the Bacteroides–Prevotella–Porphyromonas group (Bacteroides group; Rinttilä et al., 2004). Results were reported as log_{10} 16S ribosomal DNA gene copies/g fresh matter. The data were analyzed using the PROC MIXED procedure of SAS (2008). The linear model included the fixed effects of feeding level, protein source, and protein level and their interactions. Experiment × period and experiment × animal were considered as random effects. The significance level was set at α = 0.05.

RESULTS AND DISCUSSION

There were no interactions between the effect of feeding level and the protein source on intestinal microbiota in ileal digesta, except for the Bacteroides group (P < 0.05), where the effect of feeding level was more pronounced in piglets fed the SBM diet rather than the casein diet (data not shown). According to Etheridge et al. (1984), numbers of other proteolytic bacteria, for example coliforms, have also been shown to be lower in piglets’ feces fed SBM based diets compared to a milk protein based diet, but the reasons for this phenomenon need to be examined. The high feeding level resulted in increased (P < 0.001) bacterial cell numbers of lactobacilli at the ileal level (Table 1). Verdonk et al. (2003) found higher lactobacilli numbers in the stomach but no changes in jejunal cell counts of lactobacilli when weaned piglets were fed ad libitum rather than restricted. It remains speculative if these differences between studies can be attributed to the different sampling sites for digesta. The results of the present study indicate that the observed increase in beneficial lactobacilli in the small intestine at the high feed intake level might be due to the higher flow of dietary energy and nutrients into the piglets’ small intestine, thereby enhancing the availability of energy and nutrients to the microbes. Bacterial cell numbers of Clostridium coccoides (P < 0.001) were decreased for the high feeding level (Table 1). As it has been shown in sheep, feeding level was negatively correlated with intestinal retention time of digesta (Rodríguez et al., 2003). Furthermore, in a human in vitro gut model, reduced retention times were associated with suppressed microbial proteolytic metabolism (Smith and Macfarlane, 1996). Accordingly, in the present study, although no metabolites of proteolytic metabolism were determined, the high feed intake level may have reduced retention time, thereby possibly suppressing growth and activity of proteolytic bacteria. At the fecal level, no significant differences between treatments could be observed, although numeric differences for Lactobacillus spp. and Bacteroides group were at the same range as observed at the ileal level (data not shown).

Ensuring a sufficient feed intake around weaning has been proven to exert positive effects on morphological and histological structures, for example villous height, in piglets’ small intestine (Spreeuwenberg et al., 2001). It appears, from the results of the present study, that a high feed intake may also stimulate the proliferation of beneficial bacteria associated with a suppression of potential pathogenic bacteria, thereby contributing to improved gut health in piglets during the critical time of weaning.

Table 1. Bacterial numbers (log_{10} gene copies/g fresh matter) in ileal digesta as influenced by feed intake level

<table>
<thead>
<tr>
<th>Item</th>
<th>Level of feed intake, g/kg as-fed basis</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total eubacteria</td>
<td>7.9</td>
<td>7.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>5.3</td>
<td>5.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>7.6</td>
<td>7.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Bacteroides–Prevotella–Porphyromonas</td>
<td>7.2</td>
<td>7.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Clostridium leptum</td>
<td>5.5</td>
<td>5.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Clostridium coccoides</td>
<td>6.4</td>
<td>6.0</td>
<td>0.3</td>
</tr>
</tbody>
</table>

1Least squares means.
Feeding level and pigs' intestinal microbiota

LITERATURE CITED


