Lactulose as a marker of intestinal barrier function in pigs after weaning

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ABSTRACT: Intestinal barrier function in pigs after weaning is almost exclusively determined in terminal experiments with Ussing chambers. Alternatively, the recovery in urine of orally administered lactulose can be used to assess intestinal permeability in living animals. This experiment was designed to study the barrier function of the small intestine of pigs over time after weaning. The aim was to relate paracellular barrier function (measured by lactulose recovery in the urine) with macromolecular transport [measured by horseradish peroxidase (HRP) using Ussing chambers] and bacterial translocation to assess whether lactulose recovery is related to possible causes of infection and disease. Forty gonadectomized male pigs (6.7 ± 0.6 kg) were weaned (d 0) at a mean age of 19 d, fitted with urine collection bags, and individually housed. Pigs were dosed by oral gavage with a marker solution containing lactulose (disaccharide) and the monosaccharides l-rhamnose, 3-O-methylglucose, and d-xylose at 2 h and at 4, 8, and 12 d after weaning. The recovery of sugars in the urine was determined over 18 h after each oral gavage. The day after each permeability test, the intestines of 10 pigs were dissected to determine bacterial translocation to the mesenteric lymph nodes and jejunal permeability for HRP in Ussing chambers. Recovery of l-rhamnose in urine was affected by feed intake and by the time after weaning (P ≤ 0.05). Recovery of lactulose from the urine was greater (P ≤ 0.05) at 4, 8, and 12 d after weaning compared with the first day after weaning and was negatively correlated with feed intake (r = −0.63, P ≤ 0.001). The mean translocation of aerobic bacteria to the mesenteric lymph nodes was greater at 5 and 13 d after weaning compared with d 1 (P ≤ 0.05). Lactulose recovery showed no correlation with permeability for HRP nor with bacterial translocation (P > 0.05). Although both lactulose recovery and bacterial translocation increased over time after weaning, lactulose recovery did not correlate with the permeability for HRP nor bacterial translocation within a pig (P > 0.05). Therefore, we conclude that lactulose recovery in the urine of pigs after weaning is not associated with risk factors for infections. However, it appears to be possible to measure paracellular barrier function with orally administered lactulose in pigs shortly after weaning. Further studies will reveal whether this variable is relevant for the long-term performance or health of pigs after weaning.

Key words: bacterial translocation, horseradish peroxidase, intestinal barrier function, lactulose, pig, weaning

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INTRODUCTION

Decreased feed intake after weaning results in atrophy of the villi of the small intestine within a few days postweaning (Pluske et al., 1996; van Beers-Schreurs et al., 1998; Lallès et al., 2004). Additionally, wean-
charides and lactulose have been used to assess transcellular absorption and paracellular barrier function, respectively (Bjarnason et al., 1995). Orally administered marker probes in pigs are more advantageous than Ussing chambers because multiple measurements can be obtained from the same animal over time. This technique also allows one to determine when barrier function is least effective and, therefore, determine the time at which each pig is most vulnerable. Moreover, a correlation of intestinal function with health and performance variables later in life can be determined. We sought to study the changes in small intestine barrier function over time as measured with lactulose and to test its relationship with putative causes of infection and disease. Lactulose recovery in urine was compared with bacterial translocation, as well as with macromolecular transport of horseradish peroxidase (HRP) in Ussing chambers. This study aimed to improve our understanding of health disorders after weaning and may aid in the development of strategies that prevent or cure postweaning disorders.

**MATERIALS AND METHODS**

The study was approved by the Ethical Committee of the Animal Science Group of Wageningen University and Research Centre, Lelystad, the Netherlands.

**Animals and Housing**

Forty Piétrain × (Large White × Dutch Landrace) gonadectomized male pigs were selected from 18 litters of our institutional herd (Provimi Research Centre "De Viersprong," Valkenswaard, The Netherlands). The pigs were assigned to 4 groups of 10 pigs each with a similar mean BW (6.7 ± 0.6 kg). Animals had free access to creep feed before weaning from 4 d of age onward. The pigs were weaned at a mean age of 19.3 ± 0.5 d and subsequently housed individually. All pigs had free access to water through nipple drinkers, but had limited access to a standard nursery diet during the first 4 d after weaning (5, 30, 75, and 115 g/pig for each of the 4 subsequent days, respectively). The feed supply corresponded to typical intakes of pen-housed pigs in the same facility on the same diet. After 4 d of dietary restriction, pigs had free access to the diet. Feed intake was determined daily, and pigs were weighed (without feed and water restriction) at weaning and subsequently at 4, 8, and 12 d postweaning.

**Experimental Procedures**

Permeability of the small intestines of pigs was assessed in vivo at d 0, 4, 8, and 12 after weaning (Figure 1). Pigs were initially fasted for 2 h and then each pig was subsequently dosed by oral gavage (gastro-duodenal feeding tube, Levin type, Vygon Nederland B.V., Valkenswaard, the Netherlands) with a marker solution containing 2.83 g of lactulose, 0.19 g of L-rhamnose, 0.04 g of 3-O-methylglucose (3OMG), and 0.09 g of D-xyllose (Sigma, Zwijndrecht, The Netherlands) dissolved in deionized water for a total dose of 15 g of sugar mixture per pig. The amount of L-rhamnose, 3OMG, and D-xyllose was derived from a human study (Blijlevens et al., 2004) and adapted for pigs based on metabolic BW. The dose of lactulose used was similar to the dose used in a previous study with neonatal piglets (Kansagra et al., 2003). Total voided urine was collected in a pouch glued and taped to the belly of each pig. At 2, 8, and 18 h after marker administration, urine from the pouch was collected and refrigerated at 6°C in a jar containing 100 μL of a thimerosal solution (100 g/L; Sigma) for preservation. For pigs that did not urinate between 2 and 18 h after marker administration, the urine collection period was prolonged until urine was voided, or urine was directly collected from the bladder if pigs were dissected the next day (see below). At d 0, 4, 8, and 12 after weaning, 25, 57, 20, and 10% of the pigs were subjected to prolonged urine collection, respectively.

**Dissection**

Two hours after marker administration (after 4 h of feed deprivation), access to feed was restored. From about 18 through 22 h after marker administration, 10 previously assigned pigs were dissected as described below. The experimental design allowed for repeated measurements over time of the recovery of permeability markers. However, the number of measurements per pig ranged from 1 for the pigs dissected at d 1, to 4 for the pigs dissected at d 13. Pigs were anesthetized with 24 mg of sodium pentobarbital/kg of BW (Euthasol, AST-farma, Oudewater, the Netherlands). The abdominal cavity was opened under sterile conditions, and samples of the mesenteric lymph nodes (MLN) of the proximal and distal small intestines and the liver were isolated to determine bacterial counts. Twenty centimeters of the small intestine (between 100 and 150 cm distal from the stomach) was then removed to measure the permeability to HRP using Ussing chambers. The total urine in the bladder was also collected. The urine was used to analyze marker sugars if the pigs did not urinate from 2 h after marker administration to dissection. Pigs were subsequently euthanized by exsanguination.

**Sample Handling and Analyses**

Aliquots of urine were stored at −20°C before the analyses for marker sugars. For these analyses, urine samples were diluted 10-fold using deionized water. Chloride was subsequently precipitated by adding silver nitrate. After centrifugation (13,000 × g, 10 min at 21°C), the supernatant was separated on an OnGuard Ba and H column (Dionex, Amsterdam, the Netherlands) followed by analysis using high-performance anion-exchange chromatography with a pulsed amperometric detector (Dionex). The method was performed...
according to the manufacturer’s instructions (Dionex, 2000, 2004). The limits of detection for lactulose and the monosaccharides were 100 and 60 μM, respectively. Sugars that were recovered below the limit of detection were assumed to be 50% of the detection limit. In those cases, the estimated amounts were applied in the statistical analyses.

Liver and MLN samples for bacterial counts were stored in transport medium [buffered peptone water supplemented with 0.5% cysteine-HCl (Oxoid Ltd., Cambridge, UK)]. Before analysis, the samples were homogenized using an UltraTorax in an anaerobic cabinet (Bactron X, Shellab, Cornelius, OR). Ten-fold dilutions of the homogenized samples were made in reduced peptone physiological salt solution (1 g of peptone and 8.5 g of NaCl per liter). One hundred microliters of each solution was plated in duplicate on either brain heart infusion agar plates or blood-reinforced clostridial agar plates. The brain heart infusion plates were incubated for 24 h at 37°C in normal air to determine total aerobic counts. The blood reinforced clostridial agar plates were incubated for 3 d under anaerobic conditions to determine total anaerobic counts.

The jejunal segment of the intestine was analyzed using an Ussing chamber. The tissue was placed in Ringer buffer containing 25 mM NaHCO₃, 117.5 mM NaCl, 2.5 mM CaCl₂, 5.7 mM KCl, 1.2 mM NaH₂PO₄, 1.2 mM MgSO₄, and 27.8 mM d-glucose at room temperature that had previously been oxygenated with 95% O₂/5% CO₂. Smooth muscle layers were stripped off, and 3 adjacent sections per segment, which were free of Peyer’s patches, were mounted in Ussing chambers (TNO, Zeist, the Netherlands). Tissues were bathed on their serosal and mucosal sides with 1.5 mL of Ringer’s solution, oxygenated, and maintained at 37°C. After equilibration for 30 min, the permeability was assessed by placing 10⁻⁵ M HRP (Sigma) on the mucosal side of the Ussing chamber. Serosal samples (20 μL) were taken at 30, 60, 90, and 120 min and replaced with 20 μL of fresh Ringer’s solution to keep the volume constant. Enzymatic activity of HRP was analyzed using 3,3',5,5'-tetramethylbenzidine substrate (Pierce, Rockford, IL) and 10% H₂SO₄ for the stop reagent as published by Gallati and Pracht (1985). The reaction was assessed by measuring optical density at 450 nm.

Statistical Analyses

Feed intake after weaning has been shown to affect intestinal barrier function (Verdonk, 2006). To account for substantial differences in feed intake, the animals were classified as eaters or noneaters. Pigs were defined as eaters if they had eaten at least 30 g/d during 1 of the first 5 d after weaning. Marker recovery, the lactulose:L-rhamnose ratio (L:R), and BW were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) to allow for correlations between repeated observations over time using pig as the experimental unit. Mixed linear models were fitted with fixed effects for group (eaters or noneaters), day, and their interaction, and BW at weaning as the covariate. Different correlation structures were compared for the repeated measurements over days, applying a compound symmetric, first-order autoregressive, and an unstructured covariance structure (Littell et al., 1998), and then subsequently selecting the model with the least Akaike’s information criterion (Table 1). Horseradish peroxidase flux and bacterial counts (in the proximal and distal MLN and in the liver) were analyzed using day as the only factor and BW as a covariate in the model. In addition, HRP flux and bacterial counts were analyzed using group (eaters or noneaters) and group × day interactions in the model, but without the inclusion of data obtained from d 1 (grouping was not yet possible
as these pigs were dissected at d 1). Due to difficulties in marker administration for 2 pigs on d 0 and 4, and for 1 pig on d 8, these were considered outliers and were omitted from the analysis of intestinal permeability for that day. Moreover, the data for 3 samples of bacterial counts were also omitted from statistical analyses due to incorrect sample handling. Residuals were plotted to evaluate normality of distribution and homogeneity of variance. Based on these data, some variables were log-transformed (lactulose recovery, L∶R, and HRP flux using the natural logarithm and by convention, bacterial counts using \( \log_{10} \)), and monosaccharide recoveries were square root-transformed before the final statistical analyses. All results were presented as least squares means, and if transformed, the means of the data before transformation were also presented (with the exception of bacterial counts). Differences between day × group combinations or only differences between days were analyzed for significance \( (P \leq 0.05) \) using the Tukey procedure to account for multiple comparisons. Pearson correlation coefficients were calculated between HRP flux, bacterial counts, marker recoveries, L∶R, and feed intake the day before dissection using the CORR procedure of SAS. Partial correlations were calculated between the residuals of the variables after correction of the variables for the day by ANOVA using the GLM procedure of SAS.

**RESULTS**

The noneaters ate almost nothing for the first 5 d after weaning and ate only minor amounts thereafter (Figure 2). The mean BW decreased gradually after weaning for the noneaters but increased \( (P < 0.001) \) for the eaters (Figure 3). The noneaters and eaters were evenly distributed over the day of sample collection.

Lactulose recovery in the urine was greater at d 4, 8, and 12 after weaning compared with the first day after weaning \( (P < 0.001) \). Moreover, lactulose recovery after weaning was greater for the noneaters than for the eaters (Table 1; \( P = 0.05 \)). Although no significant association between the group and day was found \( (P = 0.29) \), the overall difference between the groups seemed to be due to the difference at d 4 and 8 (Table

<table>
<thead>
<tr>
<th>Variable</th>
<th>Covariance structure</th>
<th>BW d 0 P</th>
<th>Group¹</th>
<th>Day</th>
<th>Group¹ × day</th>
</tr>
</thead>
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<td>Repeated measurements over days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactulose (L)</td>
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<td>0.05</td>
<td>27</td>
<td>&lt;0.001</td>
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<tr>
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<td>0.02</td>
<td>20.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>0.36</td>
<td>27</td>
<td>0.90</td>
</tr>
<tr>
<td>3OMG³</td>
<td>Compound symmetry</td>
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<td>0.45</td>
<td>27</td>
<td>0.85</td>
</tr>
<tr>
<td>d-Xylose</td>
<td>Compound symmetry</td>
<td>0.30</td>
<td>0.45</td>
<td>27</td>
<td>0.85</td>
</tr>
<tr>
<td>BW</td>
<td>First order autoregressive</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>27</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

| No repeated measurements⁴     |                      |         |        |     |             |
| HRP                          | —                    | 0.99    | —      |    | 0.73        |
| Aerobic liver                | —                    | 0.04    | —      |    | 0.13        |
| Aerobic prox. MLN            | —                    | 0.32    | —      |    | 0.03        |
| Aerobic dist. MLN            | —                    | 0.05    | —      |    | 0.02        |
| Anaerobic liver              | —                    | 0.55    | —      |    | 0.15        |
| Anaerobic prox. MLN          | —                    | 0.41    | —      |    | 0.10        |
| Anaerobic dist. MLN          | —                    | 0.19    | —      |    | 0.15        |

¹Group = eater (pigs that had eaten at least 30 g/d during 1 of the first 5 d after weaning) or noneater (all other pigs).
²Degrees of freedom were estimated with the Kenward-Roger method with an unstructured covariance structure.
³3OMG = 3-O-methylglucose.
⁴HRP = horseradish peroxidase; MLN = mesenteric lymph nodes (of the small intestine); prox. = proximal; dist. = distal.
For l-rhamnose recovery in the urine, the feed intake group was associated with the test day (Table 1; \( P = 0.03 \)). This association was the consequence of a constant l-rhamnose recovery over time for the noneaters and a decrease in recovery over time for the eaters (Figure 4). Recovery of 3OMG and d-xylose in the urine did not differ over time (Figure 5; \( P = 0.90 \) and \( P = 0.85 \), respectively).

Feed intake differences between groups (eaters or noneaters) had no effect on small intestinal HRP flux (\( P = 0.80 \)). Therefore, the HRP flux was not presented separately for eaters and noneaters (Figure 6). In addition, the HRP flux was not affected over time (Figure 6; \( P = 0.73 \)). Feed intake differences between groups (eaters or noneaters) had no effect on the bacterial counts in the liver or in the proximal and distal MLN (\( P \geq 0.24 \)). Therefore, the bacterial counts from the 3 sample locations were not separately presented for eaters and noneaters (Figure 7). The anaerobic bacteria counts were not different over time for any of the sample locations (Figure 7; \( P \geq 0.10 \)). The aerobic bacteria counts in the proximal MLN at d 5 and in the proximal and distal MLN at d 13 were increased compared with

Figure 3. Body weight of eaters (■) and noneaters (∆). a–d Different letters indicate differences between days (\( P \leq 0.05 \)), and * indicates differences within days between groups (\( P \leq 0.05 \)). The number of pigs for eaters was 19, 19, 13, and 7 on d 0, 4, 8, and 12, respectively, and for noneaters was 11, 11, 7, and 3, respectively. Error bars represent SE of the least squares means.

Figure 4. Recovery of lactulose and l-rhamnose from urine and lactulose:l-rhamnose ratio (L:R) of eaters (■) and noneaters (∆). Panels A to C represent untransformed data, and panel D to F represent the natural logarithm (lactulose and L:R) or square root (l-rhamnose) transformed data. a–c Different letters indicate differences between days (\( P \leq 0.05 \)), and * indicates differences within days between groups (\( P \leq 0.05 \)). The number of pigs for eaters was 17, 17, 12, and 7 on d 0, 4, 8, and 12, respectively, and for noneaters was 11, 11, 7, and 3, respectively. Error bars represent SE of the mean (panels A to C) or of the least squares means (panels D to F).
the counts at d 1 after weaning ($P = 0.04$, $P = 0.04$ and $P = 0.03$, respectively).

The lactulose recovery in the urine was negatively correlated with feed intake (Table 2; $r = −0.63$, $P \leq 0.001$). In contrast, bacterial translocation to the MLN and liver and HRP flux in the small intestine were not correlated with feed intake (Table 2; $P \geq 0.31$). Moreover, lactulose recovery was not correlated with HRP flux or with bacterial translocation ($P \geq 0.05$). Lactulose recovery was negatively correlated with the translocation of aerobic bacteria to the MLN of the distal small intestine ($P = 0.04$).

**DISCUSSION**

Animals were divided into groups of eaters and noneaters because feed intake after weaning has been shown to be related to intestinal barrier function (Verdonk, 2006). The noneaters ate almost nothing for the first 5 d after weaning and ate only minor amounts thereafter. This was also reflected in the change in BW, which decreased gradually after weaning for the noneaters but increased for the eaters. The noneaters and eaters were evenly distributed over the day of sample collection. The total number of noneaters (11 out of 30) was large. It has been reported that about 90% of pigs start eating during the first 48 h after weaning (Brooks et al., 2001). The large number of noneaters observed in the present study may be related to the individual housing of the pigs, as well as to the experimental protocol that was used. In other experiments in this facility, we have also observed a large number of noneaters, although this is highly variable among experiments. The stresses that were associated with the intensive handling of the pigs during this study (oral gavage, connection of the pouch, and urine collection) may also have been contributing factors that increased the number of noneaters. It is not likely that the oral marker probes themselves were the cause of the large number of noneaters because the total sugar dose
per oral gavage was relatively small (about 3 g) compared with the normal feed intake of pigs.

In this discussion section, we distinguish between the paracellular barrier function and absorption function of the small intestine. The paracellular barrier function was measured by lactulose recovery in the urine, and the absorption function was measured by monosaccharide recovery in the urine.

**Paracellular Barrier Function**

Lactulose is a marker that can be used as an orally administered probe to assess paracellular barrier function of the small intestine by measuring the recovery of lactulose in the urine (Bjarnason et al., 1995). However, the test results with this technique can be influenced by many premucosal (e.g., bacterial degradation) and
postmucosal (e.g., completeness of urine collection) factors (Bjarnason et al., 1995). To reduce the effects of those premucosal and postmucosal factors, the dual sugar test was introduced by Menzies (1974). The principle of the test is based in the fact that orally administered disaccharides (e.g., lactulose) will pass the intestinal epithelium through the paracellular route when the barrier function is compromised. In addition, a monosaccharide, such as l-rhamnose, is also administered, which passes the intestinal epithelium by unmediated diffusion either through paracellular or transcellular routes, and therefore provides a measure of the absorptive surface. After absorption, the sugars are not metabolized and the majority of the absorbed sugar is excreted in the urine. The assumption of this test is that both probes are affected by the premucosal and postmucosal factors to a similar degree, and therefore their ratio is not disturbed by those factors (Uil et al., 1997). The ratio between the lactulose and l-rhamnose excreted in the urine provides information regarding the intestinal barrier function (Bjarnason et al., 1995). For example, an increase in the L:R indicates a decrease of the intestinal paracellular barrier function, whereas a decrease in the L:R indicates improved intestinal paracellular barrier function.

In the current study, l-rhamnose recovery decreased after weaning in the eaters group but was not affected over time in the noneaters group. This unexpected result makes it difficult to interpret the L:R for evaluating intestinal barrier function after weaning because an increase in the ratio may reflect a decreased l-rhamnose recovery, as well as an increased lactulose recovery. Therefore, in the present study with pigs, it was better to assess paracellular barrier function solely based on lactulose instead of the L:R. Lactulose recovery in the urine was greater at d 4, 8, and 12 after weaning compared with the first day after weaning. Moreover, lactulose recovery after weaning was greater for the noneaters than the eaters. It cannot be excluded that the differences in lactulose recovery in the urine over time or between eater groups were influenced by differences in bacterial degradation. However, this possibility was not likely for 2 reasons. First, the total number of bacteria per gram of digesta in the lumen of the small intestine has been shown to decrease during the first 2 d after weaning and were similar or increased during the second week after weaning compared with the numbers before weaning (Montagne et al., 2007; Pieper et al., 2008). Therefore, if lactulose recovery over time were markedly influenced by bacterial degradation in the current study, we would expect a decrease in recovery over time instead of an increase, which was not observed. Second, the recovery of l-rhamnose, D-xylose, and 3OMG in the urine was either constant or decreased over time in the current experiment, which is opposite to the recovery of lactulose over time. Therefore, if the monosaccharides and lactulose were markedly influenced by bacterial degradation over time, we would expect a similar change over time for all saccha-

<table>
<thead>
<tr>
<th>Item</th>
<th>Lactulose</th>
<th>l-Rhamnose</th>
<th>L:R</th>
<th>3OMG</th>
<th>d-Xylose</th>
<th>HRP</th>
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<td>3OMG</td>
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</table>

1Pearson correlations were calculated for the residuals from ANOVA analyses with only day in the model. Correlations were calculated between the dissection variables and the marker recoveries in the urine and feed intake of the pigs the day before the dissection.
2L:R = lactulose:l-rhamnose ratio.
33OMG = 3-O-methylglucose.
4HRP = horseradish peroxidase.
5MLN = mesenteric lymph nodes; Prox. = proximal; Dist. = distal.
6ns = nonsignificant.
*P < 0.05; **P < 0.01; ***P < 0.001.
Absorption Function

In the current study, the HRP flux was not affected by time. In contrast, the HRP flux in the proximal jejunum significantly decreased at different days between 2 and 15 d after weaning compared with preweaning stage (Boudry et al., 2004; Verdonk et al., 2007). We did not measure the HRP flux before weaning in the current study, which may be why a decrease over time was not observed. Horseradish peroxidase is used as a marker of antigen uptake through endocytosis (Keita et al., 2010). Therefore, a reduced HRP flux is an indication of improved intestinal barrier function. However, it is generally believed that the intestinal barrier function is disturbed after weaning. This is supported by the greater susceptibility of pigs to infections and edema disease after weaning than before weaning (Niewold et al., 2000; Lallès et al., 2004). Moreover, it was shown that the transepithelial electrical resistance (TER) of the proximal jejunum decreased after weaning, which indicates a disturbed barrier function (Boudry et al., 2004). In that study, the TER responded in the same direction as HRP flux after weaning, whereas these variables are supposed to respond in an opposite manner. One possible explanation for this observation is that in general, only the paracellular barrier function, which

Lactulose and intestinal barrier function

HRP Flux and Bacterial Translocation

In the current study, the HRP flux was not affected by time. In contrast, the HRP flux in the proximal jejunum significantly decreased at different days between 2 and 15 d after weaning compared with preweaning stage (Boudry et al., 2004; Verdonk et al., 2007). We did not measure the HRP flux before weaning in the current study, which may be why a decrease over time was not observed. Horseradish peroxidase is used as a marker of antigen uptake through endocytosis (Keita et al., 2010). Therefore, a reduced HRP flux is an indication of improved intestinal barrier function. However, it is generally believed that the intestinal barrier function is disturbed after weaning. This is supported by the greater susceptibility of pigs to infections and edema disease after weaning than before weaning (Niewold et al., 2000; Lallès et al., 2004). Moreover, it was shown that the transepithelial electrical resistance (TER) of the proximal jejunum decreased after weaning, which indicates a disturbed barrier function (Boudry et al., 2004). In that study, the TER responded in the same direction as HRP flux after weaning, whereas these variables are supposed to respond in an opposite manner. One possible explanation for this observation is that in general, only the paracellular barrier function, which
is represented by TER, is compromised after weaning. The barrier function for HRP is mainly related to endocytosis and represents transcellular barrier function (Bijlsma et al., 1996; Cameron and Perdue, 2007). This may only be compromised after the occurrence of additional stressors, such as transport stress or a bacterial challenge, as we have previously hypothesized (Wijtten et al., 2011).

In the current experiment, the aerobic bacteria counts in the proximal MLN at d 5 and in the proximal and distal MLN at d 13 were increased compared with d 1 after weaning. To our knowledge, there is no comparable study that has reported changes over time of bacterial translocation shortly after weaning in pigs. One study has assessed bacterial translocation 6 d after weaning in pigs that were weaned at 23 d of age (Broom et al., 2006). However, in contrast to the current study, the pigs in that study did not have access to creep feed before weaning, were housed in groups in fully slatted pig units, and received a weaner diet that had a relatively large fishmeal concentration (12.5%) compared with our study (3%). The average bacterial count in the distal MLN at 5, 9, and 13 d after weaning for aerobes (3.6 log_{10} cfu/g) and anaerobes (2.9 log_{10} cfu/g) in our study was also less than that reported by Broom et al. (2006). At 6 d after weaning, they found an average of 4.8 log_{10} cfu/g for aerobes and 5.5 log_{10} cfu/g for anaerobes. It has been shown that weaning deteriorates the paracellular barrier function of the small intestine (Spreeuwenberg et al., 2001; Verdonk, 2006; Moeser et al., 2007a,b; Verdonk et al., 2007). We previously hypothesized that the deteriorated paracellular barrier function may be an indication for an increased risk of bacterial translocation (Wijtten et al., 2011). Therefore, the increase over time of aerobic bacteria counts in the MLN after weaning is in agreement with this hypothesis.

Partial Correlations Between Variables

In the present study, lactulose recovery in the urine was negatively correlated with feed intake. This is in agreement with the previous observation that lactulose recovery is greater in noneaters than in eaters and shows that the intestinal permeability for lactulose is associated with feed intake at the day of the permeability test. In contrast, bacterial translocation to the MLN and liver, as well as HRP flux in the small intestine, did not correlate with feed intake in the current study. Moreover, in the current study, lactulose recovery did not positively correlate with HRP flux or with bacterial translocation. In agreement with these findings, Verdonk (2006) found no correlation between small intestinal HRP flux and mannitol flux in Ussing chambers for pigs at 4 and 7 d after weaning. In the present study, lactulose recovery was even negatively correlated with bacterial translocation to the MLN of the distal small intestine. In addition to the intestinal barrier function, the bacterial concentration in the intestinal lumen is also an important factor with respect to bacterial translocation (Berg, 1995). We hypothesize that greater bacterial concentrations in the intestinal lumen of specific pigs may have increased bacterial translocation. In addition, we hypothesize that in the same pigs, the greater bacterial concentration may have increased the intestinal fermentation of lactulose, which consequently may have decreased the recovery of lactulose in the urine. This may explain the negative correlation between lactulose recovery and bacterial translocation to the MLN of the distal small intestine. In previous studies with rats, an increased intestinal permeability for lactulose was associated with increased bacterial translocation (Ding et al., 2004; Song et al., 2006). In agreement with these findings, we found that the mean translocation of aerobic bacteria to the MLN and the recovery of lactulose in the urine showed a similar increase over time after weaning. However, we hypothesize that the Pearson correlation test in the current study may not have shown a relationship between the recovery of lactulose in the urine and translocation of aerobic bacteria to the MLN because of the variation in concentration of bacteria in the lumen between individual pigs.

Conclusions

Recovery of lactulose in the urine of pigs increased after weaning and was greater in noneaters than in eaters. These findings indicate that based on lactulose, the weaning process disturbs paracellular barrier function of the small intestine, which is more prominent in pigs with reduced feed intake. In contrast, HRP flux was not different between days after weaning or between eaters and noneaters, and showed no correlation with lactulose recovery. Bacterial translocation of aerobic bacteria to the different tissues was greater at 5, 8, and 13 d after weaning than at 1 d after weaning, which was in agreement with the lactulose data. However, bacterial translocation to the different tissues was not different for eaters and noneaters, and showed no relationship with lactulose recovery in a Pearson correlation test. Although both lactulose recovery and bacterial translocation increased over time after weaning, lactulose recovery did not correlate with permeability for HRP nor bacterial translocation. We conclude that lactulose recovery in the urine of pigs after weaning is not associated with risk factors for infections. However, it appears to be possible to measure paracellular barrier function with orally administered lactulose in pigs shortly after weaning. Further studies will reveal whether this variable is relevant for the long-term performance or health of pigs after weaning.

LITERATURE CITED
