Small Intestine Epithelial Barrier Function Is Compromised in Pigs with Low Feed Intake at Weaning$^{1,2}$

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ABSTRACT Compromising alterations in gastrointestinal architecture are common during the weaning transition of pigs. The relation between villous atrophy and epithelial barrier function at weaning is not well understood. This study evaluated in vitro transepithelial transport by Ussing metabolic chambers, local alterations in T-cell subsets and villous architecture at low energy intake level and their relation with lactose/protein ratios in the diet. Pigs ($n = 66, 26$ d old) were sampled either at weaning ($d = 0$), $d = 1, 2$ or $4$ postweaning. Piglets received one of three diets at a low energy intake level, which differed in lactose and protein ratio as follows: low lactose/high protein (LL/HP), control (C), or high lactose/low protein (HL/LP). Mean digestible energy intake was $648$ kJ/pig on $d = 1$, $1668$ kJ/pig on $d = 2$, $1995$ kJ/pig on $d = 3$ and $1990$ kJ/pig on $d = 4$ postweaning. The CD4$^+$/CD8$^+$ T-lymphocyte ratio decreased after weaning ($P < 0.05$). Decreased paracellular transport ($P < 0.01$), greater villous height ($P < 0.01$), shallower crypts and lower villus/crypt ratios ($P < 0.01$) were observed on $d = 2$ compared with $d = 0$. Piglets consuming the HL/LP diet tended to have less paracellular transport ($P < 0.10$) and greater villous height ($P < 0.10$) compared with piglets fed the other diets. During the first $4$ d postweaning, the effect of diet composition on mucosal integrity was not as important as the sequential effects of low energy intake at weaning. Stress and diminished enteral stimulation seem to compromise mucosal integrity as indicated by increased paracellular transport and altered T-cell subsets. J. Nutr. 131: 1520–1527, 2001.

KEY WORDS: • piglets • weaning • energy intake • epithelial barrier function • T lymphocytes

Pigs are confronted by multiple stressors at weaning. Under commercial conditions, weaning may involve complex social changes, including separation from the sow, a new housing system, separation from littermates and exposure to unfamiliar pigs (1). Diet composition also changes at weaning; the liquid milk from the sow is replaced by pelleted dry feed with carbohydrates instead of fat as the main energy source.

Abrupt weaning is typically accompanied by low feed intake, which seems to be the main reason for the growth stasis after weaning (2). Weaning also causes morphologic and histologic changes of the small intestine of pigs (3–12). These changes include reduction in villous height and an increased crypt depth. The magnitude of the intestinal responses seems to be related to feed intake of the piglets (7, 12), independent of diet composition (9,10). Beers-Schreurs (13) found that the weaning transition itself explained part of the reduction in villous height and increased crypt depth. Villous height decreased and crypt depth increased in weaned piglets compared with unweaned piglets given sow’s milk at a high energy level after weaning. The reduction in villous height was even more pronounced when the piglets were fed a weaning diet or sow’s milk at a comparable low energy level (13). Starvation itself decreased jejunal villous height and increased paracellular permeability in the ileum and jejunum of adult rats (14). An inverse relationship was found between ATP concentrations in jejunal mucosa and permeability (15), indicating that at a low energy level, permeability is increased.

The relationship between epithelial barrier function and villous atrophy at weaning is not understood. A compromise in epithelial barrier function possibly increases paracellular permeability. With increased paracellular permeability, toxins, allergenic compounds or bacteria may enter systemic tissues, resulting in inflammatory or immunologic responses (16,17).

Providing piglets sow’s milk after weaning resulted in less villous atrophy compared with a weaning diet (13); thus milk components seem to be favorable. Sow’s milk is composed mainly of fat (40.6 g/100 g), protein (29.4 g/100 g) and lactose (28.3 g/100 g) (18). Lactose is converted by lactase to galactose and glucose; glucose can be an energy source for epithelial cells (19). Lactose seems, therefore, a key energy source for intestinal epithelial cells in young piglets. Some amino acids in the milk protein can be used as an energy source for...
epithelial cells (e.g., glutamine), as well as contribute to protein synthesis.

This experiment investigated mucosal variables over time in response to low energy intake and compared the effectiveness of lactose vs. protein in preserving mucosal integrity during the weaning transition. We postulated that the energy supply is more limiting than the protein supply for epithelial cells in contributing to mucosal integrity, i.e., a diet with a high lactose/protein ratio would better preserve mucosal integrity in response to low energy intake and compared the effectiveness of lactose vs. protein in preserving mucosal integrity.

**MATERIALS AND METHODS**

**Animals and weaning.** Barrows (n = 66) procured from a commercial maternal line herd [Great York × (Dutch Landrace × Finnish Landrace)] were used. The piglets were weaned at 25.9 ± 2.01 d of age. Creep feed was not provided during the suckling period to avoid adaptation to experimental diets and to make the piglets' treatment uniform. At weaning, pigs were removed from the sow and transported 10 km to the TNO Nutrition research facility in Wageningen (The Netherlands). Upon arrival from the source farm, sows were weighed and housed individually in 90 cm² floor pens.

**Feeds, feeding and experimental design.** The experiment was conducted in two consecutive batches. On the day of weaning, dissection was performed on 12 randomly chosen piglets to collect reference values. Additionally, the remaining 54 piglets were assigned to 3 x 3 experimental groups on the basis of body weight (BW); the groups differed in diet and day of dissection. The experimental groups were given one of three experimental diets that differed in the ratio of lactose to protein (Table 1). A control liquid milk replacer (C) was compared with a liquid milk replacer with a low lactose/high protein (LL/HP) ratio, and a high lactose/low protein (HL/LP) ratio. The percentage of fat was the same in each experimental diet.

Piglets were fed at a relatively low energy level; the digestible energy (DE) offered was one third of the calculated energy intake according to formula 1. This formula describes the voluntary DE intake of weaned piglets from 5 to 15 kg based on BW (20, 13).

DE = [(455.5 × BW) – (9.46 × BW²) – 1531] × 4181 (1)

where DE is the digestible energy intake (kJ/d) and BW is body weight (kg).

The amount of milk replacer offered to the piglets was calculated daily. Body weight was calculated on the basis of BW upon arrival and the expected growth of 60 g/d [based on Pluske et al. (12)]. The milk replacer was fed at a concentration of 62 g/L of water. The pigs were fed 4 times per day at 0800, 1230, 1700 and 2130 h. Feed refusals were collected, weighed and subtracted from the amount of milk offered to calculate daily feed intake.

**Growth and health.** Piglets were weighed upon arrival and on the day of dissection to determine individual growth curves. Feces consistency and shape were scored twice a day from 0 to 3 where 0 = normally shaped feces, 1 = shapeless feces, 2 = thick liquid (soft) feces, and 3 = thin liquid feces (watery diarrhea).

**Sampling of gut for histology and permeability.** At d 0, 1, 2 and 4 postweaning, piglets to be killed were weighed and anesthetized by

**TABLE 1**

Diet composition of milk replacers that differ in the lactose and protein ratio: low lactose/high protein (LL/HP), control (C) or high lactose/low protein (HL/LP)

<table>
<thead>
<tr>
<th>Item</th>
<th>LL/HP</th>
<th>C</th>
<th>HL/LP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, g/kg</td>
<td>Calculated</td>
<td>Analyzed</td>
<td>Calculated</td>
</tr>
<tr>
<td>Calcium caseinate</td>
<td>265.0</td>
<td>175.0</td>
<td>85.0</td>
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<tr>
<td>Whey protein concentrate</td>
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</tr>
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<td>13.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Vitamin + mineral mix</td>
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<tr>
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<tr>
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<td>24.0</td>
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<td>23.1</td>
</tr>
<tr>
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<td>56.5</td>
<td>61</td>
<td>54.5</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>164.4</td>
<td>324.2</td>
<td>483.4</td>
</tr>
</tbody>
</table>

1 Esprion 580, DMV International, Veghel, The Netherlands. Crude protein, 780 g/kg; fat, 75 g/kg.
2 VanaGrasa 80 C, De Kievet bv, Meppel, The Netherlands. Butter oil, 800 g/kg.
3 Vitamin and mineral inclusion supplies (mg/kg milk replacer): retinol acetate, 6.9; cholecalciferol, 0.1; tocopherol, 50; thiamine, 6; riboflavin, 10; pyroxine, 4; cyanocobalamin, 0.25; d-pantothenic acid, 25; niacinamide, 40; L-ascorbic acid, 80; menadione, 4; folic acid, 1; biotin, 0.5; choline chloride, 1000; zinc oxide, 100; potassium iodate, 0.65; disodium selenium oxide pentahydrate, 0.5; copper sulfate pentahydrate, 80; ferrous sulfate heptahydrate, 400; manganous sulfate tetrahydrate, 60; cobalt sulfate heptahydrate, 10; magnesium oxide, 2500; dicalcium phosphate, 7500; sodium chloride, 5000.
4 Carbohydrates = dry matter – crude protein – crude fat – ash – crude fiber (=0).
inhalation of a mixture of N₂O/O₂ (ratio 2:1) and isoflurane. The
inlet of the Ussing chamber was 0.196 cm². The radiolabeled GlySar
(0.2 cm²) separate a 1.5 mL mucosal and a 1.5 mL serosal compart-
mont of the crypts using light microscopy.

To measure transepithelial transport, mid-small intestinal tissue
samples (5 cm) were taken. Transepithelial transport of two com-
ponents was measured in TNO transport chambers, i.e., [³H]GlySar
(Cambridge Research Biochemicals, Northwich, UK) and [¹²⁵I]manni-
tol (ICN Biomedicals, Zoetermeer, NL). GlySar is a small hydrophilic compound with a molecular weight of 146 Da. It is
transported mainly via a transcellular route with a H⁺-coupled di-
peptide carrier (21). Mannitol has a molecular weight of 182 Da
and is transported mainly via a paracellular route (21). Intestinal
tissues were rinsed with an ice-cold buffer solution of HEPES-buffered
medium. Both compartments were aerated (O₂/CO₂, 95:5) at a tem-
perature of 37°C and stirred by gas lift. At indicated time points (15,
30, 45, 75 and 105 min), 0.5-mL samples were taken from the serosal
side and the volume was reconstituted with DMEM without phenol
red. ³H and ¹⁴C radioactivity was determined in the samples and the
tissue (at the end of the experiment) by liquid scintillation counting
with the Digital Overlay Technique using the Spectrum Library and
the External Standard Spectrum for quench correction. Permeability
coefficients (Pₑₑₑ) were determined on the basis of the appearance of
the probe at the serosal side according to the following equation:

\[ Pₑₑₑ = \frac{R}{A \cdot C₀} \]  

(2)

where \( Pₑₑₑ \) is the permeability coefficient from mucosal to serosal side
(cm/s); \( R \) is the permeability rate (mol/s); \( A \) is the exposed intestinal
area (cm²); and \( C₀ \) is the initial mucosal concentration of the test
substance (mol/mL).

Statistical analysis. The variables measured met the normality
criterion. A General Linear Models procedure (SAS version 6.12,
SAS Institute, Cary, NC) was used to estimate the least-square means
of the three different treatments. The effect of day postweaning was
evaluated across diets. Day postweaning, batch and the two-ways
interaction were the independent variables in the statistical model.
The final model was as follows:

\[ y_{ijkl} = \mu + B_i + S_j + (B \times S)_k + e_{ijkl} \]  

(3)

where \( y_{ijkl} \) represents the independent variables; \( \mu \) is the overall
mean; \( B_i \) is batch (i = 1, 2); \( S_j \) is the fixed effect of day postweaning
(\( j = 1, 2, 3 \) and 4); \( (B \times S)_k \) is the interaction of batch (B) and
day postweaning (S); and \( e_{ijkl} \) is the error term.

The effect of diet composition was evaluated by including diet
composition, day postweaning and batch as independent variables in
the statistical model. All two-way interactions were examined, but
because these dependent variables appeared not to be significant,
these were excluded from the final model. The final model therefore
was as follows:

\[ y_{ijkl} = \mu + B_i + S_j + D_k + e_{ijkl} \]  

(4)

where \( y_{ijkl} \) represents the independent variables; \( \mu \) is the overall
mean; \( B_i \) is batch (i = 1, 2); \( S_j \) is the fixed effect of day postweaning
(\( j = 1, 2, 3 \) and 4); \( D_k \) is the fixed effect of diet composition (\( k = 1, 2, 3 \))
and \( e_{ijkl} \) is the error term.

\( \chi^2 \) analysis was used to analyze the diarrhea scores. Pearson cor-
relation analysis was performed to evaluate functional correlation
among mean energy intake, histologic parameters and epithelial
transport. Significance was assigned at \( P < 0.05 \); tendencies were
assigned at 0.05 < \( P < 0.10 \).

RESULTS

General. BW at weaning was 7.8 ± 0.13 kg. Daily weight
loss [g/(pig · d)] through the 4-d treatment period was 97.25,
128.59, 81.60, and 105.86 for LL/HP, 65.3 ± 127.23 for C,
and 69.4 ± 146.17 g/d for HL/LP. None of the piglets developed watery feces during
the experimental period (score 3). Two had thick liquid feces
(score 2); of these, 1 piglet received the C treatment and 1 the
HL/LP treatment. Eight piglets had shapeless feces (score 1).
Of these, 2 piglets received the C treatment, 1 piglet received
LL/HP and 5 received HL/LP. The diarrhea scores were not
significantly different among groups (\( P > 0.10 \)). Inclusion of an
independent binomial variable in the statistical model
indicating the occurrence/absence of diarrhea, or exclusion of
the piglets with diarrhea from the data did not affect the
results and conclusions; therefore, the piglets with a diarrhea
score were left in the database. None of the piglets received
medical treatment during the experimental period.

Energy intake. Figure 1 shows the DE intake of pigs fed
the three milk replacers for 4 d postweaning. The number of
piglets for the calculation of the mean DE intake decreased
from 54 piglets at d 1, to 36 at d 2 and to 18 at d 3 and 4, due
to dissection. DE intake did not differ among diet groups on
the different sampling days. DE intake was 648 ± 388.93
kcal/kilogram dry matter.
COMPROMISED INTESTINAL BARRIER FUNCTION AT WEANING

Crypt depth of the three sampled sites decreased during the first 2 d postweaning ($P < 0.05$) followed by an increase at d 4 postweaning. At d 0, the mean crypt depth ($\mu m$) was 170, 157 at d 1, 157 at d 2 and 175 at d 4 ($\text{SEM, 5.4}$). At the proximal small intestine, crypt depth tended also to decrease during the first 2 d postweaning, followed by an increase during d 2 to 4 postweaning ($P < 0.10$). Mid-intestinal crypts were significant deeper at d 4 (183 $\mu m$) compared with d 1 (163 $\mu m$) and d 2 (162 $\mu m$; $P < 0.05$).

The villus/crypt ratio of the three sampled sites was significantly lower ($P < 0.01$) at d 2 (1.7) and d 4 (1.9) compared with the d 0 (2.2) and d 1 (2.3). The ratio between villous height and crypt depth also decreased significantly over time postweaning at the proximal and mid-small intestine ($P < 0.05$), with the lowest ratio on d 2.

The weight of the small intestine per kg BW decreased significantly over time postweaning with the lowest weight at d 2 (23.6 g/kg body) (Table 2). The weight (g) per cm of the small intestine did not change during time postweaning and was, on average, 7.7 ± 1.08 g/cm.

Figure 2 shows the villous height and crypt depth of the proximal small intestine, mid-small intestine, distal small intestine and the mean value of those three sites of piglets fed LL/HP, C or HL/LP milk replacers. In the proximal small intestine, the villi of the piglets receiving the HL/HP diet tended to be shorter (347 $\mu m$) than the villi of the piglets receiving the LL/HP diet (419 $\mu m$; $P < 0.10$). In the proximal small intestine, the villus/crypt ratio was significantly higher ($P < 0.05$) in piglets fed the HL/LP diet (2.6) compared with those fed the LL/HP (2.0) and the C (2.2) diets ($\text{SEM, 0.16}$, data not shown).

Pearson correlation analysis indicated that the villous lengths in the proximal small intestine were correlated with those at mid- ($R = 0.47$, $P < 0.01$) and distal small intestine ($R = 0.28$, $P < 0.05$). The villous lengths at the mid- and distal small intestines were not correlated. The crypt depth and the ratio between villus and crypt were significantly correlated ($P < 0.05$) among the three sampling sites in the small intestine. At a low energy intake level, the mean energy intake per piglet was significantly correlated with the mean villous height only in the mid-small intestine ($R = 0.34$, $P < 0.05$), but not with the crypt depth or with the villus/crypt ratio.

### Table 2

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<th>Day postweaning</th>
<th>n</th>
<th>Proximal</th>
<th>Mid-</th>
<th>Distal</th>
<th>Mean</th>
<th>Proximal</th>
<th>Mid-</th>
<th>Distal</th>
<th>Mean</th>
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<th>Mid-</th>
<th>Distal</th>
<th>Mean</th>
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<tr>
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<td>351&amp;ab</td>
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<td>376a</td>
<td>231</td>
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<td>153</td>
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</tbody>
</table>

1 The histologic variables, villous height, crypt depth and ratio between villous height and crypt depth, were determined at proximal, mid- or distal part of the small intestine.

2 Means with different letters in a column are significantly different; the level of significance is identified by the P-value of the model; NS, $P > 0.05$.

3 Mean of three segments.
relative weight of the small intestine was significantly correlated with the crypt depth at all three sampling sites, but not with the villous height.

**Crypt goblet cells.** Overall, the number of goblet cell per 100 μm of crypt was not different over time postweaning or across dietary treatments (data not shown). The number of crypt goblet cells was 5.5 ± 0.6 cm/s n/10 crypts across dietary treatments (data not shown). Furthermore, the percentage of sulfomucin-containing cells in intestinal crypts was not different over time postweaning or across dietary treatments (data not shown). The percentage of crypt sulfomucin-containing cells was 35.4 ± 24.73% at the proximal, 27.2 ± 25.56% at the mid-, and 32.8 ± 25.04% at the distal small intestine (data not shown).

**T lymphocytes.** The numbers of CD4⁺ and CD8⁺ T cells (per 10⁶ μm² crypt) at the mid-small intestine d 0, 1, 2 or d 4 postweaning are shown in Table 3. The number of CD4⁺ T cells tended to be lower at d 1 compared with d 0 and 4 (P < 0.10). The number of CD8⁺ T cells at d 0 or 1 postweaning was numerically lower than at d 2 and 4 postweaning, but this difference was not significant. The CD4⁺/CD8⁺ ratio was significantly lower on d 1 and 2 compared with d 0 (P < 0.05), with the lowest ratio on d 1. The ratio of CD4⁺/CD8⁺ T cell lymphocytes had increased significantly by d 4 compared with d 1 postweaning. Diet composition did not affect the number of CD4⁺ and CD8⁺ T cells or the CD4⁺/CD8⁺ ratio (data not shown). A positive correlation was found between the number of CD4⁺ and CD8⁺ T cells (Table 4; R = 0.49, P < 0.01). The number of CD4⁺ T cells tended to be negatively correlated with villous height (R = −0.23, P < 0.10) and the villus/crypt ratio (R = −0.22, P < 0.10) at the mid-small intestine. The number of CD8⁺ T cells was negatively correlated with villous height (R = −0.27, P < 0.05) and the villus/crypt ratio (R = −0.25, P < 0.05) at the mid-small intestine. The mean DE intake tended to be positively correlated with CD4⁺ T cells (R = 0.25, P < 0.10) and the CD4⁺/CD8⁺ ratio (R = 0.22, P < 0.10).

**Permeability.** Table 3 presents transepithelial transport by GlySar (transcellular transport) and mannitol (paracellular transport) as affected by days postweaning. Figure 3 shows the effect of diet composition on the transepithelial transport. Transepithelial transport did not differ among days postweaning or the different weaning diets. Paracellular transport, however, was significantly higher at d 2 and 4 compared with d 0 and 1 postweaning (P < 0.01). Paracellular transport tended to be reduced for piglets consuming the HL/LP milk replacer diets (9.2 × 10⁻⁶ cm/s) compared with those fed the control diet (12.1; P < 0.10).

A significant positive correlation was observed between the concentration of mannitol and GlySar in the serosal fluid (R = 0.32; P < 0.05). Villous height, crypt depth and the villus/crypt ratio were not correlated with trans- or paracellular permeability. The number of CD8⁺ T cells was positively correlated with paracellular transport (R = 0.42, P < 0.01) and with tranacellular transport (R = 0.32, P < 0.05).

**DISCUSSION**

These data demonstrate an acute and sequential decline of mucosal barrier function in the pig small intestine during the first 4 d postweaning. The piglets were weaned abruptly at 26 d of age and fed one of three liquid milk replacers. For each of the three diets, the piglets consumed only 648 kJ/pig on d 1 postweaning; this corresponded to 43% of the amount offered. Voluntary milk consumption before weaning was not measured, but averages 5 MJ ME/(pig d) according to Harrell and Zandstra.

**TABLE 3**

<table>
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<tr>
<th>TRANSEPIHELIAL TRANSPORT</th>
<th>CD4⁺</th>
<th>CD8⁺</th>
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<td>Mannitol</td>
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</tbody>
</table>

1 Means with different letters in a column are significantly different; the level of significance is identified by the P-value of the model; NS, P > 0.05.
Thus, the small intestine was subject to a brief but substantial decrease in enteral stimulation at weaning. The importance of enteral stimulation for mucosal homeostasis is well documented (7,9,10,23–25), although the functional consequences of diminished enteral stimulation for the gut wall during the weaning transition in pigs are not clear. These data demonstrate a temporal relationship between low feed intake, increased paracellular transport, decreased ratio of CD4 and CD8 T-cell subsets and compromised epithelial architecture.

Stress and starvation both precede an acute temporal increase in paracellular transport and thereby affect mucosal integrity (14,15,26,27). Weaning may be regarded as a stressor as indicated by an increase of plasma cortisol concentration and certain behavioral modifications (28). Plasma cortisol concentrations were 258% greater in weanling pigs on d 2 postweaning compared with unweaned pigs (29). Kiliaan and coworkers (27) demonstrated that macromolecular protein uptake (horseradish peroxidase) increased in rats after exposure to restraint stress at 8°C, via both the transcellular and paracellular pathways. They found that acetylcholine release during the stress response was critical in the enhanced uptake of the macromolecules across the epithelium. Starvation also increases paracellular transport across intestinal epithelium (14,15). Moreover, Spitz and others (26) demonstrated that the combination of starvation and stress (by glucocorticosterone injection) resulted in a larger decrease in transepithelial resistance, indicating decreased tight junction resistance, compared with animals either starved or stressed. An increase in intestinal permeability can occur quickly. For example, within 12 h after administration of nonsteroidal anti-inflammatory drugs (NSAID), intestinal permeability to $^{51}$Cr-EDTA was increased (30).

By increased paracellular permeability, luminal antigens rather than bacteria may enter the lamina propria, resulting in inflammation. This is suggested by the fact that starvation alone does not appear sufficient for bacterial translocation, but after endotoxin challenge, starvation predisposes to bacterial translocation (31–33). Locally increased intestinal permeability...
ity leads to an imbalance in normal interactions between luminal aggressive factors (in the small intestine, mainly bile, pancreas secretion, bacteria and their degradation products) and intestinal mucosa, resulting in low grade inflammation perhaps similar to that observed with NSAID-induced enteropathy (30). Although a significant difference in paracellular transport was not observed between d 0 and 1 in this experiment, a numeric increase was noted ($P = 0.05$). The positive correlation, however, between either paracellular transport and the CD8⁺ T-cell subset predicts the direct involvement of acute inflammation in small intestinal permeability. We postulate that initial translocation of luminal antigens due to increased paracellular transport might have contributed to the alteration in CD4⁺ and CD8⁺ T-cell populations, which might have led to a further increase in paracellular transport during the following days. These data demonstrate a brief decline in the number of CD4⁺ T cells at d 1, followed by an expansion of CD8⁺ T cells at d 2 and 4 postweaning. The changes in T-cell subsets resulted in a significant decrease in the ratio of CD4⁺ to CD8⁺ T cells at d 1 and 2 compared with d 0. The ratio of the number of CD4⁺ to CD8⁺ T cells seems critical. The number of crypt goblets in cells was not affected by time postweaning or diet composition in this trial and was similar to that observed in an earlier piglet study (34). Dunsford and co-workers (35) showed incidentally a decrease in the number of goblet cells in the crypts after weaning. The results, however, were inconsistent across the small intestinal sites or across diets. In piglets administered total parenteral nutrition (TPN), the number of goblet cells increased in the villi but did not change in the crypts compared with baseline and orally fed piglets. The chemical composition of mucins was also altered in piglets administered TPN compared with baseline and orally fed piglets (25). A possibly adaptive response of goblet cells in the crypts to compromised integrity of the mucosal barrier at low feed intake level was not observed in the present study, although villous goblet cells were not evaluated.

Cytokine profiles were not measured here. In a study of De Winter and colleagues (36), however, downregulation of CD4⁺ T cells altered interleukin 10 and transforming growth factor β. Regulatory CD4⁺ T cells normally antagonize the expansion, localization, differentiation or effector function of T cells involved in inflammatory responses (36). Expansion of CD8⁺ cells likely results in the secretion of proinflammatory cytokines (e.g., tumor necrosis factor-α and interferon-γ), which further compromises barrier function (37,38). A systemic increase of proinflammatory cytokines decreases feed intake, resulting in starvation (39). The T-cell alterations affected the villi more than the crypts, indicated by the negative correlation between the number of CD8⁺ T cells and villous height. The relationship between DE intake and the ratio of CD4⁺ to CD8⁺ T-cell numbers tended to be positive, indicating that after weaning, DE intake might be important. The CD4⁺ and CD8⁺ T-cell subsets did not differ among dietary treatments. This is in agreement with the results of McCracken (10), who also showed that a low feed intake rather than diet composition contributes to local inflammation and affects the mucosal architecture after weaning.

The data demonstrate the onset of repair at d 4 postweaning for villous height, crypt depth, CD4⁺ T-cells and the ratio of CD4⁺ to CD8⁺ T-cell subsets. McCracken and co-workers (9) reported the lowest villus/crypt ratio at d 5 instead of d 2, in contrast to the sequential effect of the villus/crypt ratio of a liquid milk replacer on d 0, 1, 2, 5 and 7 postweaning. The resolution of inflammation is dependent on full restoration of epithelial barrier function, and the data indicate that paracellular transport remains elevated at d 4 postweaning. Plasma cortisol returned to preweaning levels on d 8 postweaning, comparing preweaned piglets and piglets at d 2 and 8 postweaning (29). Cessation of the stress likely corresponds with the observation that repair has begun at d 4.

Interestingly, despite the wide range of protein and lactose, diet effects were generally less pronounced than the sequential effects of low feed intake at weaning. A high lactose/protein ratio in the diet tended to result in greater villous length and less paracellular transport compared with the other diets. This observation is consistent with the hypothesis that energy from lactose is more limiting than protein for epithelial cells in contributing to mucosal integrity during the first days after weaning. However, diminished feed intake seems to override the effect of diet composition. Nutrient composition and availability may be more important in a reparative phase.

In summary, the effect of diet composition on mucosal integrity is not as important as the sequential effects of low feed intake during the first 4 postweaning. Low feed intake and stress seem to predispose to decreases in mucosal integrity. The data demonstrated an increase in paracellular transport, an alteration in T-cell subsets and a decrease in villous height. Diet composition did not have a pronounced effect on the variables measured. In a reparative stage, diet effects might be more pronounced, which will be investigated further.

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LITERATURE CITED


