Impact of diet composition on ileal digestibility and small intestinal morphology in early-weaned pigs fitted with a T-cannula

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Piglets, separated from their dam at 12 days of age and fed a milk substitute hourly, were used as a model for suckling. Animals were fitted with a terminal ileal T-cannula and a jugular vein catheter. At 28 days of age, half of the pigs had a dietary change to a cereal-based weaner diet fed as slurry, and the others remained on milk substitute. Animals were labelled by oral administration of ¹⁵N-labelled yeast for 10 days (days 15 to 25). Blood samples were taken twice a day to monitor ¹⁵N enrichment of the blood plasma. Diets included polyethylenglycol (PEG 4000) to allow calculation of apparent ileal digestibility of nitrogen and individual amino acids. Ileal bacterial nitrogen was calculated from D-alanine content of the digesta. Furthermore, small intestinal (SI) villus height and crypt depth were measured. Feed intake was increased by the dietary change. The total nitrogen flow was 3.2 ± 0.4 g/day and 5.9 ± 0.4 for the milk and weaner diet, respectively. Endogenous nitrogen flow at the terminal ileum was similar for both groups (milk diet 2.4 ± 0.4 v. weaner diet 2.2 ± 0.3 g/day), whereas the bacterial nitrogen content (0.08 ± 0.01 g/day milk diet v. 0.15 ± 0.01 g/day weaner diet, P < 0.01) and exogenous nitrogen flow (0.94 ± 0.16 g/day milk diet v. 3.29 ± 0.12 g/day weaner diet, P < 0.001) increased significantly in the weaner-diet group. The ileal apparent digestibility coefficient of protein was 0.81 ± 0.06 and 0.68 ± 0.01 for the milk replacer and the weaner diet, respectively. Morphology measurements made along the SI at 25%, 50% and 75% were similar between piglets fed milk replacer and those fed a cereal-based weaner diet. The only statistical effect (P < 0.01) of dietary change was an increase in crypt depth in the weaner-diet group. In conclusion, pigs, following a dietary change analogous to weaning, lack the capacity to fully digest a standard weaner diet. This may result in an increased nutrient content entering the large intestine and an altered microbiota. In the absence of a period of anorexia, often associated with traditional weaning, we saw no evidence of villous atrophy, but report here a significant crypt hyperplasia, especially at the 75% level, as a result of dietary change.

Keywords: ileal digestibility, endogenous nitrogen loss, microbial nitrogen, early-weaned piglet, crypt hyperplasia

Implications

This study has potential implications for the formulation of diets for early-weaned pigs, as it suggests that they lack the capacity to fully digest a standard weaner diet, which may predispose them to post-weaning disease.

Introduction

Early weaning of pigs involves a period of rapid adaptation to multiple stressors. These include removal from the sow, mixing with novel pigs, loss of protective anti-microbial milk components and a change of diet with its nutritional and immunological consequences. The latter involves a change of both dietary form and composition, often along with a period of anorexia. Finally, weaning conventionally involves moving animals to new accommodation associated with both environmental and microbial challenge. All of this occurs when the pig’s gastrointestinal (Spreeuwenberg et al., 2001) and immune system is poorly developed (McCracken et al., 1999), and when maternally derived passive immunity has waned. It is, therefore, not surprising that this is also a period of heightened sensitivity to disease and poor growth. Given the European Union (EU) ban on the prophylactic use of antibiotics, it is important to establish alternative strategies to support the piglet during this ‘at risk’ period. It is crucial to identify the relative

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Material and methods

The experiment was conducted at the University of Bristol, School of Clinical Veterinary Science. All procedures were carried out under a UK Home Office licence.

Animals, diets and experimental procedures

Seven piglets from each of two litters were collected at 12 to 14 days of age from a commercial pig farm and transported to the isolator unit at the University of Bristol’s School of Clinical Veterinary Science. The isolator unit consists of a suite of a high efficiency particulate air-filtered, positive pressure (50 pascals) rooms equipped with individual cages (80 × 70 × 45 cm), group pens, an automated feeding system for liquid diets and a separate operating room. The light regime was set to a cycle of 12-h light and 12-h darkness and ambient temperature to 30°C (Thorpe, 1999).

Upon arrival (experimental day 1), piglets were cleaned and disinfected thoroughly by passing them through a warm disinfectant bath (Virkon®, RS Biotech, Finedon, Northants, UK). Each pig received a 3-day prophylactic antibiotic course (7 mg/kg BW/day amoxicillin intramuscularly (Clamoxyl™, Pfizer Ltd, Sandwich, Kent, UK)). They were housed in a group pen for approximately 36 h in order to adapt them to consuming milk substitute from a trough. They were subsequently allotted to individual cages and fed hourly. The experimental design is depicted in Figure 1.

Piglets were fed hourly with a commercial milk substitute prepared at 200 g per litre (‘Piggi-Milk’, Parnutt Foods Limited, Sleaford, Lincolnshire, UK; 19.5 MJ/kg digestible energy (DE), 240 g/kg crude protein (CP)) at an initial rate of 0.5 g/kg BW/day amoxycillin intramuscularly (Clamoxyl™, Pfizer Ltd, Sandwich, Kent, UK)). They were housed in a group pen for approximately 36 h in order to adapt them to consuming milk substitute from a trough. They were subsequently allotted to individual cages and fed hourly. The experimental design is depicted in Figure 1.

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From 15 to 25 days of age, all piglets were fed 0.5 g 15N-labelled yeast suspended in 50 ml milk substitute twice daily. The 15N-yeast was produced according to the description of Kluess (2004).

Animals were fitted with a simple T-cannula at the terminal ileum and a permanent indwelling jugular catheter at 19 ± 2 days of age during the first experimental week (Thorpe, 1999; Kluess, 2004).

At 28 days of age, piglets were maintained on 90 ml hourly feeds and were allocated to one of two treatments, distributing litters equally: piglets in the control group

![Figure 1](https://www.cambridge.org/core/terms, https://doi.org/10.1017/S1751371109991455)

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**Table 1** Experimental design of a study investigating ileal digestibility and intestinal morphology in early-weaned piglets fed either milk substitute or a weaner diet.

<table>
<thead>
<tr>
<th>Age (d)</th>
<th>Prewearing phase</th>
<th>Milk replacer</th>
<th>Weaner phase</th>
<th>15N-labelled yeast orally (2x daily)</th>
<th>Digesta collection, daily</th>
<th>Surgery</th>
<th>Blood sampling, 2x daily</th>
<th>Slaughter</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
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<td>Arrival</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adaptation</td>
<td>15N-labelled yeast orally (2x daily)</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Prewearing/phase, milk replacer</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaner/phase, 2 treatments</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood sampling, 2x daily</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Slaughter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Table 1** Experimental design of a study investigating ileal digestibility and intestinal morphology in early-weaned piglets fed either milk substitute or a weaner diet.

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**Figure 1** Experimental design of a study investigating ileal digestibility and intestinal morphology in early-weaned piglets fed either milk substitute or a weaner diet.
Ingredients (%)  
Weaner diet

Wheat seeds, ground  24.66
Barley seeds, ground  24.70
Soybean meal  16.00
Maltodextrin  4.00
Whey, dehydrated  15.00
Soluble fish protein concentrate  8.25
Sunflower oil  2.80
CaCO₃  1.61
Ca₃(PO₄)₂·H₂O  2.10
Trace elements and vitamin premix†  0.50
L-lysine HCl  0.13
L-threonine  0.13
DL-methionine  0.09
L-tryptophan  0.03

WL-methionine  0.09
L-lysine HCl  0.13
CaCO₃  1.61
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DL-methionine  0.09
L-tryptophan  0.03

†Trace elements and vitamin premix: content/kg: 25.70 g ZnO; 16.00 g CuSO₄·5H₂O; 11.70 g MnO; 50.00 g FeCO₃; 0.32 Ca(IO₃)₂; 1.905 g CoSO₄·6H₂O; 6.00 g Na₂SeO₄ premix (1% Se); 0.72 g A; 600.000/120.000 IU vitamin A/D₃; 0.012 g vitamin B₆; 2.00 g biotine; 0.40 g folic acid; 10.00 g vitamin B₁₂; 20.00 g L-tryptophan; 0.03 g L-methionine; 0.09 g L-lysine HCl; 0.13 g CaCO₃; 1.61 g Sunflower oil; 2.80 g whey, dehydrated; 4.00 g soluble fish protein concentrate; and 8.25 g sunflower oil.

Calculation of digestibility

Apparent ileal nitrogen digestibility was determined on the basis of the indigestible marker PEG 4000 and its respective ratio in diet and ileal digesta (equation 1: Schneider and Flatt, 1975) for each daily sampling.

\[
\text{AID}_{\text{N,AA}} = \frac{100 - \frac{\text{PEG}_{\text{feed}}}{\text{PEG}_{\text{ileal digesta}}} \times \frac{\% \text{N}_{\text{AA,feed}}}{\% \text{N}_{\text{AA, ileal digesta}}}}{\frac{\text{PEG}_{\text{feed}}}{\text{PEG}_{\text{ileal digesta}}} \times \frac{\% \text{N}_{\text{AA, feed}}}{\% \text{N}_{\text{AA, ileal digesta}}}}
\]

(1)

Furthermore, the 15N enrichment was measured in ileal digesta and blood plasma in each animal, which enabled the estimation of endogenous nitrogen losses (ENL) at the terminal ileum (equation 2).

\[
\text{ENL} = \frac{[\text{at}\%]^{15} - \text{excess ileal digesta} \times 100}{[\text{at}\%]^{15} - \text{excess plasma}}
\]

(2)

The apparent ileal nitrogen digestibility was corrected on the basis of the estimated ENL and true ileal nitrogen digestibility (TID₊₆) was calculated according to equation 3.
Data, unless otherwise stated, were analysed using repeated measures ANOVA including treatment (control v. weaned) and either sampling occasion or site (25% v. 50% v. 75%) as fixed effects in the case of dietary or morphological measurements, respectively. Litter was included as a random effect; and the treatment × occasion or treatment × site interaction was also included. Suitable denominators for the calculation of F ratios were determined using an algorithm described by Zar (1996). The F ratios for treatment and litter were calculated using the mean sum of squares for piglet as the denominator; while the F ratios for all other effects used the error mean sum of squares as the denominator. Potential violations of the sphericity assumption were adjusted for using a combination of the methods of Huynh–Feldt and Greenhouse–Geisser as suggested by Stevens (1996).

### Table 2 Analysed nutrient content of milk substitute and weaner diet applied in the experimental period

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Milk substitute</th>
<th>Weaner diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (%)</td>
<td>96.01</td>
<td>90.13</td>
</tr>
<tr>
<td>Nitrogen (% DM)</td>
<td>4.15</td>
<td>3.79</td>
</tr>
<tr>
<td>CP (% DM)</td>
<td>25.94</td>
<td>23.69</td>
</tr>
<tr>
<td>Ash (% DM)</td>
<td>7.23</td>
<td>8.36</td>
</tr>
<tr>
<td>Crude fibre (% DM)</td>
<td>0.60</td>
<td>3.05</td>
</tr>
<tr>
<td>Crude fat (% DM)</td>
<td>17.73</td>
<td>5.18</td>
</tr>
<tr>
<td>Total amino acids (g/16 g N)</td>
<td>103.18</td>
<td>88.64</td>
</tr>
<tr>
<td>ASP</td>
<td>8.10</td>
<td>7.87</td>
</tr>
<tr>
<td>THR¹</td>
<td>4.66</td>
<td>3.93</td>
</tr>
<tr>
<td>SER</td>
<td>5.46</td>
<td>4.41</td>
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<tr>
<td>GLU</td>
<td>21.22</td>
<td>16.26</td>
</tr>
<tr>
<td>GLY</td>
<td>2.02</td>
<td>5.10</td>
</tr>
<tr>
<td>ALA</td>
<td>3.50</td>
<td>4.31</td>
</tr>
<tr>
<td>VAL¹</td>
<td>6.34</td>
<td>3.91</td>
</tr>
<tr>
<td>ILE¹</td>
<td>5.29</td>
<td>3.37</td>
</tr>
<tr>
<td>LEU¹</td>
<td>9.80</td>
<td>6.30</td>
</tr>
<tr>
<td>TYR</td>
<td>4.02</td>
<td>2.42</td>
</tr>
<tr>
<td>PHE¹</td>
<td>4.72</td>
<td>3.88</td>
</tr>
<tr>
<td>HIS¹</td>
<td>2.76</td>
<td>2.17</td>
</tr>
<tr>
<td>LYS¹</td>
<td>7.79</td>
<td>5.72</td>
</tr>
<tr>
<td>ARG¹</td>
<td>3.26</td>
<td>4.97</td>
</tr>
<tr>
<td>PRO</td>
<td>9.34</td>
<td>5.69</td>
</tr>
<tr>
<td>CYS</td>
<td>1.08</td>
<td>1.49</td>
</tr>
<tr>
<td>MET¹</td>
<td>2.11</td>
<td>1.92</td>
</tr>
<tr>
<td>TRP¹</td>
<td>1.71</td>
<td>1.11</td>
</tr>
</tbody>
</table>

**Calculation of true ileal nitrogen digestibility (TIDN, %)**

\[
\text{TIDN} = \frac{(N_{\text{intake}} \text{ (g/day) } - N_{\text{endogenous}} \text{ (g/day)) } \times 100}{N_{\text{intake}} \text{ (g/day)}}
\]

### Statistical analysis

Average daily gain (ADG) of piglets in the ‘pre-weaning’ phase, when both groups were fed milk substitute, was $150 \pm 30$ g (mean ± s.e.m.); this approximately doubled in the ‘post-weaning’ period in both experimental groups (Figure 2). There was no difference in ADG between the control and the weaned group ($P < 0.05$) after dietary change. Total ileal nitrogen, comprising of endogenous, microbial and exogenous, that is, non-digested nitrogen, was significantly higher in pigs fed the weaner diets compared with the milk substitute group. We observed a significant time effect, that is, a decline in total ileal nitrogen in the weaner diet group and considerable shifts in total ileal nitrogen over time in the milk substitute group (Figure 3).

### Results

- **Dietary change effects on gut structure and digestibility**

### Discussion

We conducted an experiment to investigate the impact of dietary composition on SI morphology and the digestion of protein and amino acids in piglets. During the experimental period, we could exclude stressors seen under natural weaning conditions such as weaning anorexia, social and environmental changes.

Our data show clearly that changing the diet of piglets abruptly to one based on cereals was associated with significantly lower ileal nitrogen and amino acid digestibility compared with the control animals fed milk substitute (Figure 4; Table 4). Absolute flow rates of total nitrogen at the terminal ileum were significantly higher in piglets fed the weaner diet, which was mainly caused by a higher microbial and non-digested dietary nitrogen contribution.
The nature of change in the microbiota is not entirely clear; changes may have occurred as a result of an increase in microbial diversity (Konstantinov et al., 2004; Kluess, 2004) as well as due to an increase in the number of pre-existing microorganisms.

The majority of the studies, investigating endogenous nitrogen losses, have been conducted in weaned and growing pigs receiving solid feed. Data about ileal digestibility of nitrogen and amino acids, endogenous and microbial nitrogen contribution in pre-weaning piglets fed sow’s milk or milk substitute are scarce. Wilson and Leibholz (1981) conducted various experiments to investigate the digestion of nitrogen and amino acids and endogenous nitrogen losses in artificially reared piglets fed either diets based on milk (pellets/liquid) or soybean protein (pellets). Data were obtained for animals between 14 and 35 days of age fed ad libitum and at two-hourly intervals (liquid milk diet). Initial body weight and growth performance were comparable to our own piglets and daily nitrogen intake in the 35-day-old animals equalled that of the pigs used in our experiment. Their estimate of ileal digestibility of nitrogen was similar to present data, with a clearly higher digestibility in pigs fed milk protein-based diets over those fed soybean meal. Apparent ileal digestibility of the individual amino acids (essential and non-essential) was considerably lower for the milk protein fed animals in the Wilson and Leibholz study as compared to our results for the control group, whereas data from the soybean-fed group were comparable with our group fed the weaner diet. With respect to the endogenous nitrogen losses, they reported different levels of ileal flow, which could in part be explained by the use of different methodology (nitrogen-free method in the Wilson and Leibholz study, in comparison with the $^{15}\text{N}$-tracer technique in our study).

Another study dealing with ENL, ileal nitrogen dynamics and amino acid digestibility in very young piglets was conducted by Mavromichalis et al. (2001). They determined AIDN in unweaned cannulated piglets fed sow’s milk and

![Figure 2](https://www.cambridge.org/core/core/terms. https://doi.org/10.1017/S1751731109991455)
Table 3 Ileal nitrogen flow (means ± s.e.m.) of early-weaned piglets fed either milk substitute or a weaner diet

<table>
<thead>
<tr>
<th>Milk substitute</th>
<th>Weaner diet</th>
<th>Significance</th>
<th>Milk substitute</th>
<th>Weaner diet</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ileal flow</td>
<td>g/day</td>
<td>g/day</td>
<td>g/100 g DMI</td>
<td>g/100 g DMI</td>
<td></td>
</tr>
<tr>
<td>N\text{total}</td>
<td>3.21 ± 0.18</td>
<td>5.86 ± 0.18</td>
<td>**</td>
<td>0.82 ± 0.05</td>
<td>1.18 ± 0.04</td>
</tr>
<tr>
<td>N\text{endogenous}</td>
<td>2.22 ± 0.14</td>
<td>2.42 ± 0.17</td>
<td>ns</td>
<td>0.49 ± 0.03</td>
<td>0.56 ± 0.04</td>
</tr>
<tr>
<td>N\text{microbial}</td>
<td>0.08 ± 0.004</td>
<td>0.15 ± 0.01</td>
<td>**</td>
<td>0.02 ± 0.001</td>
<td>0.03 ± 0.001</td>
</tr>
<tr>
<td>N\text{endogenous}</td>
<td>0.94 ± 0.16</td>
<td>3.29 ± 0.12</td>
<td>***</td>
<td>0.24 ± 0.03</td>
<td>0.66 ± 0.02</td>
</tr>
</tbody>
</table>

DMI = dry matter intake; ns = non-significant.

Table 4 Apparent ileal digestibility (%) of amino acids (mean ± s.e.m.) of early-weaned piglets fed either milk substitute or a weaner diet

<table>
<thead>
<tr>
<th></th>
<th>Milk substitute</th>
<th>Weaner diet</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amino acids</td>
<td>84.2 ± 2.2</td>
<td>64.3 ± 1.3</td>
<td>***</td>
</tr>
<tr>
<td>ASP</td>
<td>83.4 ± 2.3</td>
<td>57.9 ± 1.4</td>
<td>***</td>
</tr>
<tr>
<td>THR</td>
<td>76.2 ± 2.9</td>
<td>63.4 ± 1.3</td>
<td>***</td>
</tr>
<tr>
<td>SER</td>
<td>78.6 ± 3.0</td>
<td>61.0 ± 1.1</td>
<td>***</td>
</tr>
<tr>
<td>GLU</td>
<td>86.9 ± 2.1</td>
<td>68.4 ± 1.4</td>
<td>***</td>
</tr>
<tr>
<td>VAL\text{t}</td>
<td>85.7 ± 2.0</td>
<td>64.2 ± 1.2</td>
<td>***</td>
</tr>
<tr>
<td>ILE\text{t}</td>
<td>87.1 ± 1.9</td>
<td>66.4 ± 1.2</td>
<td>***</td>
</tr>
<tr>
<td>LEU\text{t}</td>
<td>89.4 ± 1.5</td>
<td>67.2 ± 1.1</td>
<td>***</td>
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<tr>
<td>TYR</td>
<td>88.8 ± 1.6</td>
<td>61.4 ± 1.4</td>
<td>***</td>
</tr>
<tr>
<td>PHE\text{t}</td>
<td>88.3 ± 1.6</td>
<td>65.4 ± 1.3</td>
<td>***</td>
</tr>
<tr>
<td>HIS\text{t}</td>
<td>87.7 ± 1.7</td>
<td>62.1 ± 1.4</td>
<td>***</td>
</tr>
<tr>
<td>LYS\text{t}</td>
<td>86.4 ± 1.8</td>
<td>68.4 ± 1.2</td>
<td>***</td>
</tr>
<tr>
<td>ARG\text{t}</td>
<td>82.4 ± 2.4</td>
<td>74.1 ± 1.1</td>
<td>**</td>
</tr>
<tr>
<td>PRO</td>
<td>87.6 ± 1.7</td>
<td>65.4 ± 2.2</td>
<td>***</td>
</tr>
<tr>
<td>CYS</td>
<td>71.8 ± 3.7</td>
<td>46.6 ± 2.2</td>
<td>***</td>
</tr>
<tr>
<td>MET\text{t}</td>
<td>93.2 ± 0.9</td>
<td>77.4 ± 0.5</td>
<td>***</td>
</tr>
<tr>
<td>TRP\text{t}</td>
<td>85.0 ± 2.5</td>
<td>61.7 ± 1.0</td>
<td>***</td>
</tr>
</tbody>
</table>

ASP = asparagine; THR = threonine; SER = serine; GLU = glutamine; GLY = glycine; ns = non-significant; ALA = alanine; VAL = valine; ILE = isoleucine; LEU = leucine; TYR = tyrosine; PHE = phenylalanine; HIS = histidine; LYS = lysine; ARG = arginine; PRO = proline; CYS = cysteine; MET = methionine; TRP = tryptophan.

Dietary change effects on gut structure and digestibility

Figure 4 Apparent (AID) and true (TID) ileal nitrogen digestibility (means ± s.e.m.) of early-weaned piglets fed either milk substitute or a weaner diet; effect of diet on digestibility (AID and TID), P < 0.001.

TID\text{N} by means of enzymatically hydrolysed casein-based diet and indigestible soluble markers (co-EDTA, YbCl\text{3}). They reported comparable values to the milk substitute group in our own study (92.8% TID\text{N}). Another interesting point was the use of sow’s milk in this study; the authors analysed the amino acid composition of the harvested sow’s milk and discussed the particular amino acid digestibility in relation to the main milk components, casein and whey. Casein is the main dietary source of amino acids in suckling piglets, whereas whey proteins include several immunoglobulins, in particular IgA. Approximately 20% of the IgA has been shown to be excreted undigested in human infants, which could explain why amino acids in sow’s milk are not 100% digestible, as one would assume in such a highly digestible diet. Comparing the nitrogen and amino acid content of sow’s milk (reported in Mavromichalis et al., 2001) with the substitute based on bovine milk as used in our study revealed a surprisingly similar composition, although nitrogen content was slightly lower (4.2% v. 4.8%), most amino acids were approximately equal, except for proline, arginine and methionine, which showed a markedly lower content (2.42%, 0.85% and 0.55% DM v. 3.59%, 1.37% and 0.64% DM) than in sow milk.

Besides ENL, we were also interested in the contribution of microbial nitrogen at the terminal ileum in young piglets. We estimated the contribution of microbial N at about 2.5% of total ileal nitrogen, and in absolute values...
and in one case miniature pigs (Bartelt et al., 1994) with an animals used. These authors used growing or fattening pigs. The main reason for this difference can be attributed to the considerably higher than those we obtained in our studies. Reported data on bacterial nitrogen in ileal digesta are investigations of Wünsche et al. (1991).

This assumption is supported by the variability between pigs with respect to the bacterial fraction. Such differences between studies using the same methods indicate that besides an age-dependent increase in bacterial mass, there also exists a high level of adaptation time to solid, cereal-based feed and a different experimental environment. Furthermore, the mentioned research groups applied diaminopimelic acid (DAPA) as a bacterial marker and determined it in an isolated bacterial fraction; in contrast to our study in which D-alanine was determined directly in digesta. However, Huang et al. (2001) reported lower values for bacterial nitrogen in ileal digesta of growing pigs, ranging between 14% and 21% of total ileal nitrogen, although applying DAPA in isolated bacterial fraction. Such differences between studies using the same methods indicate that besides an age-dependent increase in bacterial mass, there also exists a high level of variability between pigs with respect to the bacterial fraction in the SI. This assumption is supported by the investigations of Wünsche et al. (1991).

At present, the use of D-alanine as a bacterial marker is prevalent in the ruminant studies in which it is regarded as superior to DAPA (Garrett et al., 1987; Schoenhusen et al., 2008). There is only one short communication that refers to the use of D-alanine in pigs (Hennig et al., 1999). However, the authors used growing miniature and saddleback pigs with an average body weight above 25 kg and therefore reported higher bacterial N in ileal digesta. Literature dealing with the assessment of bacterial nitrogen contribution in the SI of weaning piglets is sparse. Our studies were a first attempt to establish D-alanine as a direct microbial marker in young monogastric animals, but further investigations are required.

Intestinal morphology was also assessed at three sites in the SI, at 25%, 50% and 75%. Small intestinal morphology of piglets receiving milk replacer constantly was very similar to that of suckling piglets, with respect to villous height and crypt depth, as reported in the literature (van Beers-Schreurs et al., 1998; Hedemann et al., 2003) and in our prior studies. In these studies conducted just before the present experiment in the same facilities (Harris, 2005), isolator-reared piglets were compared with those raised by their dam. The data showed that there was no difference for villous height and crypt depth between piglets from the isolator and those raised by their dam. This also supported the hypothesis that the use of artificially reared, milk substitute-fed piglets is a good model for the study of the suckling piglet.

Interestingly, villous height did not differ between the two treatments, but there was a significant increase in crypt depth at all three sites in the group fed the weaner diet. The observation that villous height was unaffected by dietary change is supportive of the view that the villous atrophy normally associated with weaning is due to the period of anorexia that occurs immediately post-weaning (Pluske, 2001). Weaning anorexia is a multifactorial phenomenon, which we tried to discern in our study in order to focus on the dietary aspect only. The decrease in feed intake was prevented by providing the weaner diet as slurry, which is in agreement with earlier publications (van Beers-Schreurs et al., 1998; Dunshea et al., 1999). Therefore, effects on intestinal morphology can be largely attributed to the

![Figure 5](https://www.cambridge.org/core/images/Figure_05.png)

**Figure 5** Villous height and crypt depth (±s.e.m.) at three sites of the small intestine (25%, 50% and 75%) of early-weaned piglets fed either milk substitute or a weaner diet; effect of diet on villous height, $P > 0.05$; crypt depth, $P < 0.01$; effect of site on villous height, $P < 0.001$; crypt depth, $P < 0.05$. 

at 0.08 ± 0.004 g/day (milk fed) and 0.15 ± 0.01 g/day (weaner diet).

There is little information about bacterial nitrogen in ileal digesta in piglets around weaning. Wünsche et al. (1991) determined the bacterial nitrogen contribution in ileal digesta of pigs, applying different methods for digesta collection: post-valve T-cannula and end-to-end or end-to-side ileorectal anastomosis. The authors assessed the average bacterial nitrogen as approximately 25% of total ileal nitrogen. They are in good agreement with studies from Schulze et al. (1994) and Bartelt et al. (1994). Reported data on bacterial nitrogen in ileal digesta are considerably higher than those we obtained in our studies. The main reason for this difference can be attributed to the animals used. These authors used growing or fattenning pigs and in one case miniature pigs (Bartelt et al., 1994) with an average body weight well above 25 kg. It can be assumed that the bacterial N contribution in such animals is much higher than in piglets aged 28 days due to a longer adaptation time to solid, cereal-based feed and a different experimental environment. Furthermore, the mentioned research groups applied diaminopimelic acid (DAPA) as a bacterial marker and determined it in an isolated bacterial fraction; in contrast to our study in which D-alanine was determined directly in digesta. However, Huang et al. (2001) reported lower values for bacterial nitrogen in ileal digesta of growing pigs, ranging between 14% and 21% of total ileal nitrogen, although applying DAPA in isolated bacterial fraction. Such differences between studies using the same methods indicate that besides an age-dependent increase in bacterial mass, there also exists a high level of variability between pigs with respect to the bacterial fraction in the SI. This assumption is supported by the investigations of Wünsche et al. (1991).

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change in diet composition, excluding the previously mentioned factors occurring during weaning transition. Crypt hyperplasia occurring in the absence of villous atrophy is, however, a novel observation. A possible explanation is that the pig’s immune system is directly responding to novel antigens (cereal, fish protein concentrate and soybean) being presented at the gut mucosa. Bailey et al. (1993) have reported that pigs show a systemic antibody response to antigens within the weaning diet and experience a transient period of sensitivity before the development of oral tolerance. The hyperplasia of the crypt could be indicative of a type III, cell-mediated hypersensitivity response (Miller et al., 1983). Another possibility is the impact of the gastrointestinal microbiota, as one can assume that the microorganisms differ between the milk-fed and cereal-fed animals. Shirkey et al. (2006) showed a great effect of bacterial inoculum in sterile piglets on villous height and crypt depth, with mono-associated animals displaying longer villi and shallower crypts in the distal SI as compared to animals associated with a mixed inoculum from porcine faeces. This shows clearly the effect of intestinal microbiota on gut morphology. However, as we did not investigate the microbiota in the scope of this study, we can only speculate about the difference between experimental groups.

Conclusion

We determined endogenous nitrogen losses, and subsequently true ileal digestibility, and investigated the intestinal morphology in a piglet model. Our data show that the imposed dietary change to a cereal-based diet resulted in a decrease in ileal digestibility of nitrogen and amino acids compared with a milk-based diet, which was mainly due to an increase in exogenous and microbial nitrogen flow at the terminal ileum. This is likely to have an impact on large intestinal fermentation as more protein can enter the hindgut.

Furthermore, we could show for the first time that dietary change without a decrease in feed intake (weaning anorexia) maintained villous height but resulted in a significant crypt hyperplasia. The latter is hypothesised to be related to a hypersensitivity response towards dietary antigens or to a shift in the gastrointestinal microbiota, but requires further research in order to elucidate this effect.

On the basis of our results, we can state that we developed an experimental model that enables us to discern the various factors associated with weaning, in particular weaning anorexia and dietary composition. This is a useful experimental setup to investigate the weaning transition of piglets more in depth.

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References


Harris CR 2005. Effect of environmental factors on the development of the mucosal immune system in the piglet. PhD, University of Bristol, Bristol, UK.


Isikawa S 1966. Reliability of polyethylene glycol as an indicator for digestion studies with swine, part I. Rate of passage of PEG through the digestive tract. Agricultural and Biological Chemistry 30, 278–284.


Schneider BH and Flatt WP 1975. The evaluation of feeds through digestibility experiments. University of Georgia Press, Athens, GA, USA.


Thorpe J 1999. The effects of two in-feed protease enzymes on the digestibility of soy proteins in the early weaned pig. PhD, University of Bristol, Bristol, UK.


