Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning

J. R. Pluske†, I. H. Williams1 and F. X. Aherne2

1Animal Science, Faculty of Agriculture, University of Western Australia, Nedlands, WA 6907, Australia
2Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton AB T6G 2P5, Canada

Abstract

The hypothesis tested in this experiment was that the structure and function of the small intestine of piglets given a milk liquid diet after weaning depends on their level of energy intake. At weaning (28 days), 42 piglets were allocated to one of five treatments: (1) control group killed at weaning; (2) piglets offered a dry starter diet ad libitum; (3) piglets given cow’s fresh milk at maintenance energy intake (Ma); (4) piglets given cow’s fresh milk at 2.5 Ma; and (5) piglets given cow’s fresh milk ad libitum. On the 5th day all piglets were killed and samples of gut were taken for histological and biochemical examination. Piglets given milk ad libitum grew faster (P < 0.001) than piglets on all other treatments. Piglets offered the dry starter diet ingested similar quantities of dry matter and energy, and grew at the same rate as piglets given cows' milk at 2.5 Ma. As predicted, piglets given milk at maintenance energy intake grew slower (P < 0.001) and consumed less food (P < 0.001) than piglets in all other treatments. For piglets given both cows' fresh milk and the dry starter diet, there were significant linear relationships (r = 0.72 to 0.82, P < 0.05) between villous height and crypt depth with dry matter (energy) intake after weaning. In turn, mean villous height in both milk-fed and starter-fed piglets was significantly correlated (r = 0.68 to 0.79, P < 0.05) to empty body-weight gain in the first 5 days after weaning. Estimates of digestive enzyme activity and absorptive capacity of the gut did not corroborate the large differences seen between treatments in villous height and crypt depth, and possible reasons are discussed. These data illustrate the interdependence between voluntary food intake and mucosal architecture in determining piglet performance after weaning.

Keywords: crypt depth, energy intake, piglets, villous height.

Introduction

In our previous experiment (Pluske, Williams and Aherne, 1996) we demonstrated that the regular feeding of a milk diet prevented the decrease in villous height and increase in crypt depth generally associated with weaning. In contrast, piglets consuming a dry starter diet displayed villous atrophy, an increase in crypt depth, and a reduced concentration of mucosal protein. A dry-matter (DM) intake of 296 g/day supplying 7.4 MJ gross energy (GE) per day was sufficient to maintain the structure and function of the gut in piglets given ewes' fresh milk. However what could not be resolved was whether the maintenance of villous height and crypt depth after weaning was due to this particular level of milk intake or to some protective and/or stimulatory effect on the gut mucosa afforded by milk per se.

Prolonged lactation does not prevent the normal loss of lactase activity that occurs with increasing age (Lebenthal, Sunshine and Kretchmer, 1973; Kelly, King, McFadyen and Travis, 1991a). In addition, several authors (Hampson, 1986b; Kelly, O'Brien and McCracken, 1990b; Kelly, Smyth and McCracken, 1990a) have proposed that villous atrophy and crypt hyperplasia may be related to the withdrawal of 'intrinsic factors', such as IgA, in sows' milk. Mammalian milks, including that of the sow, also contain an array of biologically active peptides (e.g. epidermal growth factor) and compounds (e.g. polyamines) that may stimulate protein and DNA synthesis and have functional roles in intestinal
differentiation. Consumption of ewes' milk after weaning in our previous study (Pluske et al., 1996), especially as it was offered in an un-homogenized and unpasteurized state, may have attenuated the activity of enzymes, peptides and growth factors, and hence contributed to the integrity of the small intestine.

Studies with rodents (Stevens Hooper and Blair, 1958; Steiner, Bourges, Freedman and Gray, 1968; McManus and Isselbacher, 1970; Altman, 1972; Hopper, Rose and Wannemacher, 1972; Al-Dewachi, Wright, Appleton and Watson, 1975; Rudo, Rosenberg and Wissler, 1976; Goodlad and Wright, 1984; Goodlad, Plumb and Wright, 1988), however, have demonstrated a hypoproliferative effect of fasting on the intestinal mucosa that causes a decrease in villous height, crypt depth and/or the protein content of the mucosa. Our intention in this experiment was to feed milk to piglets at different levels of energy intake after weaning, ranging from maintenance through to ad libitum-intake, so that a graded response between energy intake and the structure and function of the small intestine could be established. This would allow an effect of voluntary food intake to be demonstrated that was not confounded with feeding a milk diet per se. Therefore, the hypothesis tested in this experiment was that the structure and function of the small intestine of piglets fed a meal diet after weaning depends on their level of energy intake. Piglets consuming a milk diet at their maintenance energy requirement would be expected to have shorter villi and shorter crypts than those on energy intakes above maintenance.

Material and methods

Animals and housing

Forty-two piglets from six primiparous gilts (Large White × Landrace) were used in the study. The genotype, management, feeding and housing of gilts and piglets have been described previously (Pluske et al., 1996). Creep food was not offered during suckling. Piglets were weaned at 29 (s.e. 0.2) days of age when they weighed 9.1 (s.e. 0.29) kg, and were housed in individual accommodation as already described (Pluske et al., 1996).

Experimental treatments

The experiment was a completely randomized block design. Piglets were allocated randomly on the basis of litter, sex and live weight to one of five treatment groups as follows: (1) sow-reared, control group killed on the day of weaning (SR; no. = 10); (2) piglets offered a pelleted starter diet (starter; no. = 8); (3) piglets offered cows' fresh milk at maintenance energy intake (Ma; no. = 8); (4) piglets offered cows' fresh milk at 2.5 times maintenance energy intake (2.5 Ma; no. = 8); (5) piglets offered cows' fresh milk ad libitum (AL; no. = 8).

Diets, feeding regimen and experimental procedures

Piglets were offered cows' fresh milk every 2 h according to the general feeding pattern outlined previously (Pluse, et al., 1996). Cows' milk was used in this study because it was readily available in large quantities. Food intake in treatments Ma and 2.5 Ma was determined on a metabolic live-weight basis for calculating the daily maintenance requirement of young piglets: $\text{ME}_{m} = 0.485 M^{0.75} \text{MJ ME per day}$, where $\text{ME}_{m}$ is the amount of metabolizable energy required by the pig for daily maintenance, and M is the live weight (kg) of the pig (after Close and Fowler, 1985).

At 14.00 h each day all piglets were weighed and, for piglets given food at Ma and 2.5 Ma, their energy requirement for the next 24 h was calculated. This was then divided into 12 equal feeds and offered every 2 h. For piglets that were given milk every 2 h, the amount offered was recorded and, after 10 to 15 min, troughs were removed from each pen and any residual milk was weighed in tared containers and recorded. For piglets offered milk ad libitum, troughs were left in the pens for longer (30 to 45 min) because some animals drank their milk in two episodes. Between feedings all troughs were washed and scrubbed thoroughly with hot water. For piglets given milk ad libitum, the amount offered was increased proportionately by 0.1 at each feed if the piglet consumed all the milk offered to it in the previous meal. This ensured that more milk was offered than could be drunk.

The dry starter diet used in this experiment has been described previously (Pluske et al., 1996). For piglets given this pelleted diet, any food that had spilled into the collection trays was collected and the weight recorded. Piglets sometimes urinated or defecated in their troughs and, when this happened, the food was removed, weighed, and an equivalent amount of fresh pellets replaced in the feeder.

One hour before slaughter on the 5th day after weaning the absorptive function of the small intestine was assessed using $(\text{D})$-xylose. A xylose solution (equivalent to $2 \text{ ml of } 5\% \text{ (w/v) per kg live weight}$) was either added to the troughs of piglets receiving milk or, in the case of piglets receiving solid food, was administered using a stomach tube.

Post-mortem procedure

The slaughter and post-mortem procedure for the processing of all piglets has been described in our...
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previous publication (Pluske et al., 1996). The pH of caecal contents was measured directly using a digital pH meter (Suntex SP-32, Stansens, Taiwan).

Analysis
Samples of cows’ liquid milk were collected in plastic vials and frozen at −20°C for analysis of fat, protein, and total solids. Milk was analysed using a MilkoScan® (Foss Electric, Denmark). The proportion of fat, protein and total solids contained in cows’ whole milk (no. = 64) was 33.4 (s.e. 1.3), 33.2 (s.e. 0.4) and 121.4 (s.e. 2.6) g/kg. The calculated GE content of the milk was 2.69 MJ GE per kg, or 22.16 MJ GE per kg DM, according to the equation of Perrin (1958).

Plasma glycerol was determined by capillary gas chromatography according to the technique developed by Fenton and Aherne (1987). Plasma alkaline phosphatase and urea were determined (in duplicate) using a biochemical analyser (COBAS MIRA, Roche Diagnostica, Switzerland). The level of free thyroxine (T4) in plasma was determined using an Amerlex-MAB FT4 kit (Amersham International, UK). This was a direct, competitive assay for free T4 using a high specific-activity, [125I]-labelled monoclonal antibody raised to T4. Plasma xylose was analysed according to the method of Merritt and Duely (1983).

Histology and brush-border lactase and sucrase specific activity
Villous height, crypt depth and the specific activities of lactase and sucrase were measured at three sites along the small intestine, as described previously (Pluske et al., 1996).

Statistical analysis and presentation of results
All data were subjected to least-squares, one-way analysis of variance for treatment effects using SYSTAT® (Wilkinson, 1990). Since this experiment was conducted in three parts, time was included as an independent variable in the initial analysis of variance. The effect of time was not significant for any variables analysed and the data were reanalysed with treatment group being the only independent variable. Pairwise comparisons between treatment means were made using Fisher’s-protected least significant difference (LSD) procedure (Maindonald, 1992).

Weight of the empty body was determined by multiplying the starting live weight of each piglet by the proportion of the carcass that was empty body in the piglets killed at weaning (Noblet and Etienne, 1987). This was 981 (s.e. 6.8) g/kg (CV = 0.0069). Empty body-weight gain was calculated, therefore, as the difference between the recorded weight of the empty body at slaughter 5 days after weaning and the estimated weight of the empty body at weaning.

Data were combined from the three milk treatments to test the hypothesis that there is a linear increase in villous height with increasing levels of voluntary food intake. Simple linear regression analysis was conducted using SYSTAT® (Wilkinson, 1990). To analyse the response of intestinal structure to food intake, both linear \( y = a + bx \) and quadratic regressions \( y = a + bx + cx^2 \) were calculated.

The protein content of the mucosa and specific enzyme activity are presented as the means of all three sites sampled proportionately 0.25, 0.50 and 0.75 along the small intestine.

Results
All piglets offered cows’ fresh milk began drinking within 8 h of weaning. Piglets receiving milk at Ma or 2.5 Ma finished their meal within 4 min of placing the trough in the pen, whereas those given food ad libitum took longer. The health of all piglets was excellent and no animals developed diarrhoea during the 5-day period.

Villous height and crypt depth after weaning
Feeding cows’ milk ad libitum after weaning maintained villous height at the proximal jejunum at a level similar to that of piglets killed at weaning. At the mid jejunum and distal ileum, villous height in piglets given food ad libitum was proportionally 0.23 \( (P < 0.05) \) and 0.33 \( (P < 0.01) \) higher relative to piglets at weaning. Piglets given cows’ milk at Ma had shorter villi \( (P < 0.001) \) at all sites along the small intestine than did piglets given milk ad libitum. Differences in villous height between piglets given milk at Ma and 2.5 Ma were less distinct, with only values at the mid jejunum being statistically significant \( (P < 0.05) \). Villous height in animals given milk at 2.5 Ma and ad libitum was similar at all sites except at the distal ileum. For piglets offered the dry starter diet, villous height was similar \( (P < 0.05) \) to those given milk at Ma but lower \( (P < 0.01) \) at all sites along the gut than in piglets given milk ad libitum. Relative to animals given milk at 2.5 Ma, villous height differed only at the mid jejunum \( (P < 0.051; \) Table 1).

Mean villous height was similar in piglets given the starter diet and those offered cows’ milk at Ma, despite there being nearly a three-fold difference in DM intake (Table 2). Mean villous height in piglets given cows’ milk at 2.5 Ma was proportionately 0.13 lower \( (P < 0.05) \) than those offered milk ad libitum,
Table 1 Villous height and crypt depth of piglets killed either at weaning or 5 days later

<table>
<thead>
<tr>
<th>Proportion of intestine</th>
<th>Treatment‡</th>
<th>s.e.d.</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR</td>
<td>Starter</td>
<td>Ma</td>
</tr>
<tr>
<td>Villous height (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>569 a</td>
<td>413 b</td>
<td>379 b</td>
</tr>
<tr>
<td>0.50</td>
<td>445 bc</td>
<td>384b</td>
<td>363 a</td>
</tr>
<tr>
<td>0.75</td>
<td>333a</td>
<td>300ab</td>
<td>249b</td>
</tr>
<tr>
<td>Mean‡</td>
<td>449a</td>
<td>366 b</td>
<td>330b</td>
</tr>
<tr>
<td>Crypt depth (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>122a</td>
<td>161b</td>
<td>122a</td>
</tr>
<tr>
<td>0.50</td>
<td>116a</td>
<td>169b</td>
<td>128bc</td>
</tr>
<tr>
<td>0.75</td>
<td>102a</td>
<td>141b</td>
<td>107a</td>
</tr>
<tr>
<td>Mean‡</td>
<td>114a</td>
<td>157c</td>
<td>119a</td>
</tr>
</tbody>
</table>

a,b,c Within rows, means not followed by a common superscript differ significantly.
† SR: piglets killed at weaning; starter: piglets given dry starter diet ad libitum; Ma: piglets given cows' fresh milk at maintenance; 2-5 Ma: piglets given cows' fresh milk at 2-5 times maintenance; ad libitum: piglets given cows' fresh milk ad libitum.
‡ Mean of all three sites.

was proportionately 0.18 higher (P < 0.05) than those offered the starter diet, but was comparable with those killed at weaning. Mean villous height in piglets given milk ad libitum was higher (P < 0.001) than in all other treatment groups, including those killed at weaning (Table 1).

Feeding the starter diet or cows' milk at either 2.5 Ma or ad libitum after weaning increased crypt depth (P < 0.05) at all sites along the gut compared with piglets killed at weaning, or piglets offered milk at Ma. Crypt depth at all sites was similar for piglets offered either the starter diet or cows' milk at the two higher levels of intake. Feeding milk to piglets at Ma did not alter mean crypt depth compared with piglets killed at weaning, whereas mean crypt depth in the remaining three groups (starter, 2.5 Ma and ad libitum) was proportionately from 0.21 to 0.35 deeper (P < 0.001) than in piglets given milk at Ma. Mean crypt depth in piglets given milk at 2.5 Ma was lower (P < 0.05) than in piglets offered the starter diet (Table 1).

Table 2 Performance of piglets after weaning

<table>
<thead>
<tr>
<th>Treatment‡</th>
<th>s.e.d.</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR</td>
<td>Starter</td>
</tr>
<tr>
<td>Live weight (kg)</td>
<td>8.9</td>
<td>9.0</td>
</tr>
<tr>
<td>after 5 days</td>
<td>10.5</td>
<td>9.4</td>
</tr>
<tr>
<td>Empty body weight (kg)</td>
<td>8.7</td>
<td>8.8</td>
</tr>
<tr>
<td>after 5 days</td>
<td>10.0</td>
<td>9.2</td>
</tr>
<tr>
<td>Daily gain (g/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>live weight</td>
<td>288a</td>
<td>58a</td>
</tr>
<tr>
<td>empty body weight</td>
<td>231a</td>
<td>49a</td>
</tr>
<tr>
<td>Voluntary food intake (g DM per day)</td>
<td>286a</td>
<td>102b</td>
</tr>
<tr>
<td>Energy intake (MG GE per day)</td>
<td>5.1a</td>
<td>2.3b</td>
</tr>
<tr>
<td>Food conversion ratio (g DM : g EBWG‡)</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Energy cost/g EBWG‡ (kJ DE per g)</td>
<td>21.1</td>
<td>23.8</td>
</tr>
</tbody>
</table>

a,b,c Within rows, means not followed by a common superscript differ significantly.
† See Table 1.
‡ EBWG: empty body-weight gain.
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Figure 1 The daily pattern of dry-matter intake (g) in pigs weaned at 29 (s.e. 0.2) days of age and fed for 5 days after weaning with either a pelleted starter diet (■) or cows' liquid milk at three levels of energy intake: maintenance (●), 2.5 maintenance (○), and ad libitum (□). Values are mean ± s.e. for eight pigs per treatment.

P < 0.01) of piglets offered the dry starter diet ad libitum in the 5 days after weaning. During this time piglets given milk ad libitum consumed proportionately 0.40 more DM (400 v. 286 g/day, P = 0.010) and 0.74 more energy (8.9 v. 5.1 MJ GE per day, P < 0.010) than piglets given dry pellets. Piglets offered the starter diet ingested proportionately 0.20 more DM (P > 0.05) but similar amounts of energy, and grew at the same rate, as piglets given cows' milk at 2.5 Ma. Animals given milk at Ma grew slower (P < 0.001) and were given less food (P < 0.001) than piglets in all other treatment groups (Table 2). The CV in daily empty body-weight gain ranged from 1.10 for piglets offered the starter diet to 0.15 for piglets given cows' milk ad libitum. Piglets drinking cows' milk ad libitum consumed around 3.5 times their maintenance level of energy intake over the duration of the experiment. In comparison, piglets consuming the starter diet ate the equivalent of 1.6 times their estimated maintenance requirement for energy.

Food conversion ratio was similar (P = 0.098) for all treatment groups except those given milk at Ma. Similarly, the energetic cost of 1 g of empty body-weight gain did not differ (P = 0.098) between piglets given milk at 2.5 Ma or ad libitum and piglets given the pelleted diet (Table 2).

Pattern of voluntary food intake after weaning
DM intake was highest (P < 0.001) on all days after weaning for piglets offered cows' milk ad libitum. Piglets given the starter diet consumed only 110 g on the 1st day after weaning but increased their intake rapidly and consumed 365 g by day 5. This was still proportionately 0.20 less DM than for piglets offered cows' milk ad libitum (Figure 1). DM intake in piglets offered the starter diet and those offered cows' milk at 2.5 Ma was similar over the duration of the study. The CV of DM intake was greatest in piglets offered the starter diet, decreasing from 0.96 on the 1st day after weaning to 0.54 on the final day of the experiment. In contrast, the CV for piglets offered cows' milk ad libitum decreased from 0.21 on day 1 to 0.14 by day 5.

Relationships between intestinal structure and performance after weaning
Data from the three groups given cows' milk were combined and the following relationships between food intake, intestinal structure, and the rate of empty body-weight gain were established. For all relationships, the inclusion of the quadratic term (x²)

Figure 2 Relationship between total dry-matter intake and (a) mean villous height ([y = 279.7 + 0.12x, r = 0.82, P < 0.001; no. = 22] and (b) mean crypt depth ([y = 114.3 + 0.02x, r = 0.44, P < 0.05; no. = 22]) along the entire length of the small intestine for pigs receiving cows' liquid milk.
failed to explain any more variation than the linear regression, so the response was assumed to be linear rather than quadratic.

Total DM intake in the first 5 days after weaning was highly correlated to mean villous height along the small intestine for all piglets fed cows’ liquid milk ($r = 0.82, P < 0.001$) (Figure 2a). Significant relationships were also found between villous height and energy intake for all piglets given milk ($r = 0.47 (P < 0.05), r = 0.53 (P < 0.01)$ and $r = 0.73 (P < 0.001)$, for sites 0.25, 0.50 and 0.75 along the gut, respectively). For crypt depth, only proportionately 0.19 of the total variation ($P < 0.05$) along the length of the small intestine was related to milk energy intake after weaning (Figure 2b). For individual sites along the gut, only crypt depth at the distal ileum showed any correlation ($r = 0.47, P < 0.010$) with DM intake in the first 5 days after weaning. For piglets given the starter diet, total DM intake was significantly correlated to villous height at sites 0.50 ($r = 0.72, P = 0.069$) and 0.75 ($r = 0.76, P < 0.05$) along the small intestine. Crypt depth at these two sites was also associated with the amount of food piglets consumed in the 5 days after weaning, although correlations were weaker ($r = 0.71, P = 0.078$ for mid jejunum, and $r = 0.62, P = 0.096$, for distal ileum). DM intake was correlated to mean crypt depth ($r = 0.69, P = 0.058$), but was not related to mean villous height.

When used as a predictor of growth after weaning, mean villous height along the length of the small intestine was highly correlated to empty body-weight gain for milk-fed piglets ($r = 68, P < 0.01$) (Figure 3a). Significant relationships were also found between villous height and total body gain for all

Figure 3 Relationship between (a) mean villous height and total empty body-weight gain [$y = -1788.3 + 7.24x, r = 0.68, P = 0.002; \text{no.} = 22$] and (b) mean crypt depth and total empty body-weight gain [$y = -919.3 + 15.86x, r = 0.45, P < 0.05; \text{no.} = 22$] for pigs receiving cows' liquid milk.

Table 3 Protein content of the mucosa, the specific activity of lactase (EC 3.2.1.23) and sucrase (EC 3.2.1.48), and plasma xylose concentration of piglets killed at weaning or 5 days later

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>SR</th>
<th>Starter</th>
<th>Ma</th>
<th>2-5 Ma</th>
<th>Ad libitum</th>
<th>s.e.d.</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosal protein content (mg/g mucosa)‡</td>
<td>129&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>106&lt;sup&gt;b&lt;/sup&gt;</td>
<td>133&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.4</td>
<td>***</td>
</tr>
<tr>
<td>Lactase activity (µmol/min per g protein)‡</td>
<td>77&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>105&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.4</td>
<td>*</td>
</tr>
<tr>
<td>Sucrase activity (µmol/min per g protein)‡</td>
<td>65&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.6</td>
<td>*</td>
</tr>
<tr>
<td>Xylose (mg/100 ml)</td>
<td>22</td>
<td>16</td>
<td>20</td>
<td>18</td>
<td>16</td>
<td>2.6</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Within rows, means not followed by a common superscript differ significantly.

† See Table 1.
‡ Mean of all three sites.
Gut morphology and energy intake

Mucosal protein and digestive enzyme activity
Mean protein content of the mucosa of the small intestine was proportionately from 0.27 to 0.36 higher ($P < 0.001$) in piglets that were killed either at weaning or received milk at 2.5 Ma or ad libitum compared with piglets receiving milk at Ma or the starter diet. Specific lactase activity was greatest ($P < 0.05$) for piglets offered the pelleted starter diet, and this was higher ($P < 0.05$) than for piglets given milk at 2.5 Ma or ad libitum. Lactase activity was similar in the three groups of piglets given cows’ milk ($P > 0.05$) and piglets killed at weaning. The specific activity of sucrase was highest for piglets given the pelleted diet and for those animals killed at weaning ($P = 0.012$). All groups given milk recorded a similar activity for sucrase (range: 45 to 57 μmol/min per g protein; Table 3). Mean lactase activity averaged over all treatments was highest ($P < 0.05$) at the proximal jejunum and declined ($P > 0.05$) to the distal ileum. In contrast, sucrase activity was highest ($P = 0.09$) towards the distal part of the small intestine. The amount of xylose absorbed by piglets on the 5th day after weaning was similar in all treatment groups (Table 3).

Organ weights and pH values
Significant differences between treatments were found for all organ weights when expressed as grams or g/kg empty body weight (EBW). Piglets given milk at Ma had similar values ($P > 0.05$) to those killed at weaning, but values were generally lower ($P < 0.001$) than for other treatments for all organs when expressed on an absolute weight basis. When corrected for empty body weight (g/kg), piglets given milk at Ma had values similar to the other groups given milk, but these were lower ($P < 0.001$) than in piglets given the starter diet. The length of the small intestine was similar in groups of piglets given food after weaning but was shorter in piglets killed at weaning ($P < 0.05$). Expressed as g/kg, the small intestine was identical (0.9 m/kg EBW) between groups.

The large intestine was heaviest ($P < 0.001$) in piglets given the starter diet but similar to that of piglets given milk ad libitum (172 vs. 159 g, $P < 0.068$). When expressed on a g/kg basis, the large intestine was proportionately from 0.18 to 0.41 heavier ($P < 0.01$) in piglets given the starter diet than in piglets from other treatments. Piglets given the starter diet after weaning had more acidic contents in their caeca (pH = 6.0; $P = 0.056$) (Table 4).

Plasma metabolites
The concentration of glycerol in plasma did not differ between treatment groups. Blood urea concentration was highest ($P < 0.01$) in piglets given cows’ milk ad libitum and lowest in piglets given milk.
Table 4  Organ weights and caecal pH of piglets killed at weaning or 5 days later

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>SR</th>
<th>Starter</th>
<th>Ma</th>
<th>2-5 Ma</th>
<th>Ad libitum</th>
<th>s.e.d.</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of small intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m</td>
<td>7.7a</td>
<td>8.8b</td>
<td>8.3ab</td>
<td>8.7b</td>
<td>8.7b</td>
<td>0.40</td>
<td>*</td>
</tr>
<tr>
<td>m/kg EBW‡</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
<td>0.07</td>
<td>*</td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>40a</td>
<td>60b</td>
<td>46ac</td>
<td>53bc</td>
<td>58b</td>
<td>3.8</td>
<td>***</td>
</tr>
<tr>
<td>g/kg EBW‡</td>
<td>4.6a</td>
<td>6.0b</td>
<td>5.1a</td>
<td>5.3bc</td>
<td>5.2b</td>
<td>0.41</td>
<td>**</td>
</tr>
<tr>
<td>Small intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>218a</td>
<td>305b</td>
<td>221a</td>
<td>294b</td>
<td>328b</td>
<td>25.0</td>
<td>***</td>
</tr>
<tr>
<td>g/kg EBW‡</td>
<td>25.3a</td>
<td>30.4b</td>
<td>25.6ac</td>
<td>29.2ab</td>
<td>29.9bc</td>
<td>2.23</td>
<td>*</td>
</tr>
<tr>
<td>Large intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>111a</td>
<td>172b</td>
<td>132ac</td>
<td>124a</td>
<td>159bc</td>
<td>14.4</td>
<td>***</td>
</tr>
<tr>
<td>g/kg EBW‡</td>
<td>12.8a</td>
<td>17.3b</td>
<td>13.2a</td>
<td>12.3a</td>
<td>14.6a</td>
<td>1.30</td>
<td>**</td>
</tr>
<tr>
<td>pH caecum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.5a</td>
<td>6.0b</td>
<td>6.9a</td>
<td>6.6a</td>
<td>6.6a</td>
<td>0.28</td>
<td>*</td>
</tr>
</tbody>
</table>

a,b,c Within rows, means not followed by a common superscript differ significantly.
† See Table 1.
‡ EBW: empty body weight.

at Ma. The level of free thyroxine was similar in piglets receiving cows' fresh milk at 2-5 Ma and ad libitum, but was higher (P = 0.012) in piglets given milk ad libitum than in animals killed at weaning, offered milk at Ma, or given the starter diet (Table 5).

Discussion

Villous height and crypt depth after weaning
In this experiment we tested the hypothesis that the structure and function of the small intestine of piglets given a milk diet after weaning would vary in direct response to increasing levels of milk energy intake. Positive correlations between voluntary food intake and both villous height and crypt depth clearly demonstrated that a component of the mucosal response of the small intestine after weaning is driven by the amount of food piglets eat. Similar relationships were recorded for piglets eating a dry starter diet. Furthermore, both villous height and crypt depth explained a significant proportion of the total variation in EBW gain in piglets given both cows' fresh milk and the starter diet. Whilst we cannot state definitively that cause and effect relationships exist between gut structure and weight gain, these data illustrate the apparent interdependence between voluntary food intake and mucosal structure in determining the performance of piglets after weaning, and concur with similar relationships found in our previous study (Pluske et al., 1996).

The dramatic effect of a reduction in voluntary food intake on mucosal growth was seen in piglets offered milk at maintenance. These piglets had a shorter small intestine, a reduced concentration of protein in the mucosa, and possessed shorter villi and crypts than piglets offered milk at the two higher levels of intake. These data also demonstrate that when piglets consumed a conventional starter diet at the

Table 5  The concentration of metabolites in the plasma of piglets killed at weaning or 5 days later

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>SR</th>
<th>Starter</th>
<th>Ma</th>
<th>2-5 Ma</th>
<th>Ad libitum</th>
<th>s.e.d.</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol (mg/l)</td>
<td>10.4</td>
<td>8.4</td>
<td>12.3</td>
<td>8.6</td>
<td>11.3</td>
<td>2.79</td>
<td>*</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>1.9b</td>
<td>2.1b</td>
<td>1.2a</td>
<td>1.9b</td>
<td>3.0c</td>
<td>0.66</td>
<td>**</td>
</tr>
<tr>
<td>Insulin (µU/l)</td>
<td>6.6</td>
<td>12.4</td>
<td>8.4</td>
<td>9.4</td>
<td>9.1</td>
<td>1.21</td>
<td>*</td>
</tr>
<tr>
<td>Free thyroxine (T₄) (pmol/l)</td>
<td>11.5a</td>
<td>12.2ab</td>
<td>12.6ab</td>
<td>16.4bc</td>
<td>16.8c</td>
<td>1.04</td>
<td>**</td>
</tr>
</tbody>
</table>

a,b,c Within rows, means not followed by a common superscript differ significantly.
† See Table 1.
same level of energy intake as piglets drinking a liquid milk diet (i.e. > 2.5 Ma), villous atrophy, an increase in crypt depth, and a decrease in mucosal protein content occurred. Despite these marked changes in gut morphology, piglets given the starter diet grew at the same rate after weaning as piglets given milk.

The absence of nutrients from the lumen caused, for example, by starvation (Altman, 1972; Hampson, 1983; Goodlad et al., 1988) or parenteral administration of the diet (Feldman, Dowling, McNaughton and Peters, 1976; Goldstein, Hebiguchi, Luk, Taqi, Guilarte, Franklin, Niemiec and Dudgeon, 1985; Castillo, Feng, Stevenson, Kerner and Kwong, 1990), causes villous atrophy and a decrease in the rate of cell production in the crypts of Lieberkühn (Goodlad and Wright, 1984). This is mediated by an increase in cell-cycle time (Al-Dewachi et al., 1975; Goodlad et al., 1988) that respectively increases during starvation and decreases after refeeding. A temporary reduction in crypt-cell production rate was reported by Hampson (1986a) and Hall and Byrne (1989) following weaning onto a solid diet in both conventional and gnotobiotic piglets. Hampson (1986a) considered that this brief respite in crypt-cell production may be a possible mediator of the reduction in enterocyte number on the villus after weaning. However the decrease in both villous height and crypt-cell production rate reported in these studies may also be explained by the period of temporary starvation that occurs after weaning.

Support for this notion comes from the studies of Koga and Kimura (1978, 1979 and 1980). Restricting adult mice to proportionately 0·60 of ad libitum intake caused a decrease in cell migration rate from the crypt onto the villus and a reduction in villous height (Koga and Kimura, 1978), a lower mitotic activity in the crypts (Koga and Kimura, 1979) and, in their final study, Koga and Kimura (1980) found that the cell-cycle time in the crypts of duodenal and jejunal cells, in particular the duration of the G1 phase, was prolonged under dietary restriction. The shorter crypt depth seen in this experiment in piglets given cows' milk at Ma is consistent with these mechanisms.

It is not possible to discern the precise aetiology whereby low food intake effects these changes to the mitotic cycle of undifferentiated cells in the crypts. Feldman et al. (1976) listed four ways in which the absence of nutrients from the lumen of the small intestine causes mucosal hypoplasia, and it is likely that some, if not all, of these mechanisms were implicated in the results observed in this experiment: (1) the absence of food may have changed the balance or numbers of bacterial flora in the gut, leading to secondary changes in the small intestine mucosa; (2) ingested food may have been used directly by the intestinal mucosa during transport, either as an energy source or as substrates for cell synthesis; (3) the absence of luminal contents could have led to smaller than normal release of gastrointestinal polypeptide hormones which, themselves, may be trophic to the intestine (e.g. enteroglucagon) (Williamson, 1978); and (4) the absence of food in the gut may have failed to trigger cholecystokinin (Kanayama and Liddle, 1991) and secretin release from the intestinal mucosa and, in turn, the resultant exocrine pancreatic hyposecretion may have led to hypoplasia (Altman and Leblond, 1970; Altman, 1971; Tivey and Shulman, 1991).

Hampson (1986b) also conducted a study in which the amount of a liquid milk diet offered after weaning was quantified, and then the structure and function of the small intestine assessed. In his study piglets achieved an average intake in the first 5 days after weaning of 400 g/day. The diet offered was a sow-milk replacer made up in a ratio of 1:3 in water (i.e. 330 g/kg DM), and this equated to an average DM intake of 133 g per pig per day, or 2·9 MJ GE per pig per day. Since piglets still displayed villous atrophy and reductions in brush-border enzyme activity, Hampson (1986b) concluded that 'neither poor and irregular intake of food after weaning nor physical trauma from the weaning diet are normally involved in these changes'. In the present experiment, piglets given cows' milk at Ma consumed 102·4 g DM, or 2·3 MJ GE, per day. These piglets displayed villous atrophy and no increase in crypt depth but, in contrast to the study of Hampson (1986b), showed no decline in the specific activity of lactase and sucrase. Since the amount of milk consumed by piglets at maintenance was only >30 g DM per day less than the intakes reported by Hampson (1986b), it is likely that the villous atrophy reported by Hampson (1986b) was due to nutrient deprivation in the small intestine. The similarity in consumption between the two studies provides further support for our hypothesis, and embraces the notion that low voluntary food intake after weaning is most likely limiting the development of digestive and absorptive processes in the small intestine. This comparison also reinforces the likelihood that changes to the structure and function of the gut that, in the past, have been attributed to factors such as chronic stress (Björk, 1989), dietary antigens (Miller, Newby, Stokes and Bourne, 1984), and a decrease in digestive and absorptive capacity (Gay, Barker and Moore, 1976; Hampson, 1983), are most likely confounded with the amount of food piglets eat.

Only Kelly, Smyth and McCracken (1991c) have studied the effects of different levels of voluntary
food intake on the digestive and absorptive development of the weaned piglet. These authors offered (by stomach tube) a restricted (0, 25, 50, 75, 100 g/day) or continuous (150, 175, 200, 225, 250 g/day, on average) amount of cereal-based diet to piglets weaned at 14 days of age for the first 5 days after weaning. Piglets given the restricted diet suffered a 0.19 proportional reduction in mean villous height and 0.13 decrease in mean crypt depth. Data from this and our previous study (Pluske et al., 1996) corroborate the work of Kelly et al. (1991c), and highlight the marked effect luminal nutrition has on gut morphology.

**Digestive enzyme activity after weaning**

*In vitro* estimates of specific lactase and sucrase activity failed to reflect large differences in villous height and empty body-weight gain recorded in these studies. Lactase and sucrase activities were generally higher in piglets given the starter diet and those given milk at maintenance, although statistical difference was not always achieved because of the large variation observed between values. The findings from this and the previous experiment (Pluske et al., 1996) show an anomaly between the histological structure and biochemical function of the small intestine that is not related to the observed growth rate. For example, piglets given ewes’ milk or ewes’ milk plus glutamine in our previous report converted DM to body gain (food conversion ratio of 0.7 to 0.8) with an efficiency analogous to that of sucking piglets (Lucas and Lodge, 1961; Noblet and Etienne, 1987). In this study piglets given cows’ milk *ad libitum* grew almost 10 times faster and had villi proportionately 0.51 higher than piglets given Ma but had similar levels of lactase and sucrase.

The disaccharidase enzymes lactase and sucrase were chosen as ‘markers’ of enteroocyte maturity and functional capacity since Berg, Dahlqvist, Lindberg and Nordén (1973) reported significant correlations between the activity of these two enzymes and villous architecture in biopsies of human small intestine. Gay et al. (1976), Hampson and Kidder (1986) and Miller, James, Smith and Bourne, (1986) reported large reductions in the specific activity of lactase and sucrase with minimum values at 4 to 5 days after weaning. These reductions may in fact be underestimates of the real loss of disaccharidase activity since Miller et al. (1986), using cytochemical techniques that can measure enzyme activity among a single villus, reported an additional three- to fivefold fall in the ability of individual enteroocytes to express lactase activity, and a two-fold reduction in sucrase expression. Since these changes were coincidental with villous atrophy and crypt hyperplasia, Hampson and Kidder (1986) postulated that an increased rate of enteroocyte production in the crypts, coupled with an increased rate of cellular migration up the villus (Smith, 1984), resulted in enteroocytes having insufficient time to differentiate fully and express maximum digestive and absorptive capacity before being extruded from the villous apex into the lumen (Rey, Schmitz, Rey and Jos, 1971). This is generally thought to result in cell loss before maximum digestive and absorptive capacity can be expressed (Smith, 1984). The greater reduction in lactase activity than sucrase activity found in these studies was most likely due to the more apical distribution of lactase on the villus (Nordström and Dahlqvist, 1973; Nichols and Nichols, 1988; Kelly et al., 1991a). An increase in crypt depth, therefore, would be expected to reduce the activity of lactase more than sucrase after weaning.

Our data agree with those found by Kelly, Smyth and McCracken (1991b and c). Kelly et al. (1991b) found a 0.55 proportional reduction in villous height and a 0.23 increase in crypt depth by the 5th day after weaning, but found no statistical decrease in either the specific or total activity of lactase or the specific activity of sucrase, that might have been predicted from such large changes in gut structure. However on the 7th day after weaning, lactase activity was less than half that of unweaned piglets killed at 22 days of age, and this coincided with a significant increase in crypt depth. As suggested by Kelly et al. (1991b), the delay in the loss of lactase activity may have been attributable to a higher supply of nutrients to the gut and/or be an artefact of the clean environment that the piglets were housed, since it is known that exposure of weaned piglets to a ‘dirty’ as opposed to a ‘clean’ environment causes a significant increase in crypt depth (Miller et al., 1986). In the present experiment, crypt depth in piglets offered the starter diet had increased significantly by the 5th day after weaning, but the specific activity of lactase and sucrase were not different from those given either of the milk diets. Furthermore, Kelly et al. (1991c) reported a 0.19 proportional decrease in mean villous height when piglets were offered a reduced quantity of food after weaning, yet the specific activity of lactase and sucrase increased compared with piglets given four times as much diet over the 5-day period. Piglets given a restricted basis had shorter crypts characteristic of a reduction in crypt-cell production rate (Al-Dewachi et al., 1975; Goodlad and Wright, 1984; Goodlad et al., 1988) that, according to the literature, would be expected to reduce the loss of enzyme activity from the villous tip. Expression of enzyme activity, either as specific or total activity, appears to provide only a crude appraisement of *in vivo* digestive activity (Kelly et al., 1991c). The most probable reasons for differences between the various reports are in the level of food intake achieved by...
piglets between experiments and variations in the methodology for determining activity.

Absorptive capacity of the small intestine after weaning

Villous atrophy at 3 to 4 weeks of age not only leaves fewer absorptive cells on the villi after weaning but is also associated with a reduction in the relative maturity of enterocytes because of faster rates of production in the crypts (Hampson, 1986a). In this instance enterocytes may be extruded from the villous apex before they express full absorptive capacity (Rey et al., 1971; Smith, 1984; Miller et al., 1986). However evidence to support this notion in the weaner is equivocal. Numerous workers have shown a reduced ability of piglets to absorb xylose (Miller et al., 1984; Hampson and Kidder, 1986; Hampson and Smith, 1986) and alanine (Smith, 1984; Miller et al., 1986) after weaning. In contrast, Kelly et al. (1991c) showed no decrease in the ability of piglets to absorb a standard xylose dose 5 days after weaning.

In the present experiment there was no statistical difference between treatment groups in their capacity to absorb xylose after weaning despite appreciable differences in villous height. Lowest values were recorded in piglets offered cows' milk at 2.5 Ma and ad libitum, so perhaps animals in these treatments metabolized xylose to a greater extent and/or excreted more xylose in the urine since they were growing faster. This would have resulted in a lower level of xylose in the blood. Alternatively, the enterocytes in piglets given milk at maintenance may have exhibited an adaptive increase in absorptive capacity, since there are numerous reports, mainly in the adult rat, where starvation causes a transient increase in absorption (Kershaw, Neame and Wiseman, 1960; Dowling, 1967; Lifshitz, Hawkins, Diaz-Bensussen and Wapnir, 1972; Wapnir and Lifshitz, 1974; Esposito, 1967; Bardocz, Grant, Brown, Ewen, Stewart and Puszta, 1991). Gupta and Waheed (1992) reported that the surface area of the microvilli, the fluidity of the brush-border membrane and D-glucose transport through intestinal epithelial membranes all showed an increase during starvation compared with well fed controls. The increase in xylose absorption seen in this study in piglets given cows' milk at maintenance provides some support for this finding, but more precise techniques than the 'xylose absorption test' would have to be implemented to confirm this possibility.

Compensatory mechanisms of growth for piglets fed a pelleted starter diet

Piglets given the starter diet grew at the same rate as those given cows' milk at 2.5 Ma in the 5 days after weaning despite suffering a 0.18 proportional decrease in villous height and an 0.11 increase in crypt depth. The decrease in villous height suggests a reduction in the surface area available for digestion and absorption of nutrients in the gut (Miller et al., 1986). It is likely that piglets given the pelleted diet compensated for this apparent loss of digestive and absorptive capacity by one of two mechanisms, or both: (i) an increase in the activity of starch-reducing enzymes, such as maltases and glucoamylase, located in the brush-border membrane, and (ii) production of short-chain fatty acids in the caecum.

Results from Kelly et al. (1990a and b) and Kelly et al. (1991b and c) showed increases in brush-border maltase and glucoamylase activity for piglets given a solid diet, even by 3 days after weaning, and support McCracken's (1984) notion of rapid substrate induction of brush-border enzymes. Kelly et al. (1991b) found maximum activity of these enzymes on the 5th day after weaning, yet activity did not differ between piglets that differed in their level of food intake (Kelly et al., 1991c). This suggests that by day 5 after weaning the limit to growth may be the absorption of these hydrolytic products. As suggested by Kelly et al. (1991b), it is probable that the levels of these two enzymes may be more relevant in limiting absorption of a weaner diet than either lactase of sucrase.

The increase in both the absolute and relative weight (g/kg EBW) of the large intestine following consumption of the pelleted solid diet after weaning concurs with the findings of Kelly et al. (1991b), and suggests a trophic effect of the starter diet on mucosal growth in the caecum and colon. The large intestine is the major site of water and electrolyte absorption in the weaned piglet (Hamilton and Roe, 1977; Buddle and Bolton, 1992) and has considerable propensity to produce volatile fatty acids. The acidification of the caecum (pH = 6.0) and increased growth of the large intestine of piglets given the starter diet (Table 5) is consistent with this proposition, and suggests a role for these acids in the supply of energy to the newly weaned piglet. In a biological system where renewal of villous enterocytes may take 3 to 4 days following villous atrophy (Moon, 1971), the production of volatile fatty acids in the hind gut may be an important 'reservoir' of energy for the young pig. However a different conclusion was reached by Hampson (1987), who reported that in 26-day-old piglets weaned for 5 days, microbial activity in the large intestine is not likely to be developed sufficiently to have a major influence on absorptive processes and may predispose the weaned pig to diarrhoea. Ultimately, the extent of diarrhoea will depend on digestive and absorptive processes in the small intestine and the
osmolality of active particles in the lumen of the large intestine.

Plasma metabolites
Metabolites were measured in this experiment to give an indication of the metabolic status of piglets under controlled conditions of voluntary food intake. Despite large differences in intake and the rate of empty body-weight gain, only the plasma levels of urea and thyroxine differed between treatment groups. In piglets given cows' milk from Ma through to ad libitum intake, blood urea increased from 1·2 to 1·9 to 3·0 mmol/l (P < 0·01) in line with an increase in milk protein intake. In the DM, nitrogen intake of piglets on a daily basis was 4·5 g, 10·2 g and 17·5 g for piglets given milk at Ma, 2·5 Ma and ad libitum respectively. The higher levels of urea found in piglets consuming more energy may reflect an intake of dietary protein in excess of that required by piglets for protein deposition. The higher level of free thyroxine present in piglets given milk at 2·5 Ma and ad libitum is most likely a reflection of the higher anabolic state of these animals. Support for this notion comes from a review by Aherne, Williams and Head (1991) showing that tissue anabolism in the lactating sow is associated with an increase in the level of circulating thyroxine.

Conclusions
This experiment showed that, when piglets are given milk above maintenance and the period of temporary starvation after weaning is avoided, the balance between cell loss from the villous tip and cell division in the crypts can be maintained compared with piglets killed at weaning. The linear response in villous height and crypt depth with an increase in DM intake in piglets given cows' fresh milk supports the theory of 'luminal nutrition' and its effects on gut morphology after weaning. The biological significance of these relationships was strengthened by the highly significant correlations found between villous height, crypt depth and the rate of empty body-weight gain. These relationships support the direct biological link between voluntary food intake and piglet growth after weaning. The dramatic effect of a reduction in voluntary food intake on mucosal morphology was observed in piglets given food at Ma. Since piglets suffer a period of 'temporary starvation' immediately after weaning, these results provide evidence that the villous atrophy observed in many previous studies, and thought attributable to a variety of causes, is likely to be confounded with a failure to control and quantify the amount of food consumed after weaning.

Villous height and crypt depth in piglets given the starter diet after weaning were also correlated to the level of voluntary food intake. However piglets in this group suffered a decrease in villous height and an increase in crypt depth compared with piglets given milk at similar levels of energy intake. Despite these changes to the histological appearance of the gut, piglets given the starter diet grew at the same rate as those given milk (at 2·5 Ma). In the absence of supporting data, we can only speculate that compensatory mechanisms, including an increase in the hydrolytic activity of maltase and glucoamylase and the production of volatile fatty acids in the caecum, must have provided more substrate for growth and been responsible for the similar rate of growth observed. Since there was no disparity in food intake, the exact aetiology of alterations to gut morphology cannot be resolved from this study.

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