Maintenance of villus height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning

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The aims of the present study were (a) to maintain the structure and function of the small intestine of the piglet after weaning, and (b) to compare the capacity *in vivo* of sucking and weaned piglets to digest oral boluses of lactose and sucrose and absorb their monosaccharide products. Piglets were fed on cows' whole milk *ad libitum* every 2 h for 5 d after weaning. Physiological doses of lactose plus fructose (treatment LAC + FRU) and sucrose plus galactose (treatment SUC + GAL) were administered on day 27 of lactation and on the fifth day after weaning, after which time piglets were killed. Villus height and crypt depth were maintained ($P > 0.05$) by feeding cows' milk after weaning. The areas under the curves (AUC) for galactose and glucose, adjusted for live weight and plasma volume, increased ($P < 0.05$) after weaning. Despite the enhancement of gut function after weaning, the galactose index (GalI: AUC for galactose ingested as lactose divided by the AUC for the same dose of galactose ingested as the monosaccharide) and fructose index (FruI: AUC for fructose ingested as sucrose divided by the AUC for the same dose of fructose ingested as the monosaccharide), which are indices of digestive and absorptive efficiency, both decreased after weaning. This apparent anomaly may be reconciled by increased growth, and hence surface area, of the small intestine between weaning and slaughter such that 'total' digestion and absorption most probably increased despite apparent decreases in GalI and FruI. Positive correlations ($P < 0.05$) between villus height and GalI are consistent with the maximum activity of lactase occurring more apically along the villus. Significant linear relationships ($P < 0.05$) were recorded between villus height at the proximal jejunum and adjusted AUC for galactose and glucose following treatment LAC + FRU, and between villus height at the proximal jejunum and adjusted glucose AUC following treatment SUC + GAL. These relationships suggest that maximum digestion and absorption occurs at increasing distances along the crypt:villus axis in the weaned pig.

Piglet: Sugars: Villus height: Crypt depth

Lactase (*EC* 3.2.1.23) activity in the small-intestinal mucosa of the newborn pig is high at birth and declines gradually during the first 2 months of life (Manners & Stevens, 1972; James *et al.* 1987). By comparison, the activity of sucrase (*EC* 3.2.1.48) is generally absent, or present at very low levels, at birth but increases with age (Kidder & Manners, 1980). Gay *et al.* (1976) reported an apparent 'loss' of lactase and sucrase activity attributable to weaning, an effect that has both been confirmed (Hampson & Kidder, 1986; Miller *et al.* 1986) and refuted (Kelly *et al.* 1991 *b*, *c*; Pluske *et al.* 1996 *a*, *b*) since. Nevertheless, weaning is coincidental with a reduction in villus height and an increase in crypt depth (Hornich *et*

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al. 1973; Gay *et al.* 1976; Kenworthy, 1976; Hampson, 1986*a, b*; Miller *et al.* 1986; Cera *et al.* 1988; Kelly *et al.* 1990, 1991*b, c*; Nabuurs *et al.* 1993; Pluske *et al.* 1996*a, b*) that leaves fewer and less-differentiated enterocytes on villi available for the digestion of nutrients (Smith, 1984; Hampson, 1986*a*; Hampson & Kidder, 1986). A concomitant reduction in the capacity of the small intestine to absorb xylose (Miller *et al.* 1984; Hampson & Kidder, 1986; Hampson & Smith, 1986), alanine (Smith, 1984; Miller *et al.* 1986), and electrolytes (Nabuurs *et al.* 1994), has also been reported after weaning.

Our previous studies demonstrated that villus height and crypt depth could be maintained in piglets after weaning by feeding a milk diet at intervals of 2 h (Pluske *et al.* 1996*a, b*). Under this regimen piglets fed *ad libitum* grew at rates of empty-body-weight gain (i.e. 407–463 g/d) indicative of a gut that was not impaired in its digestive and absorptive function. However, digestive enzyme activity and assessment of absorptive capacity using xylose failed to ratify both the maintenance of villus height and the exemplary rate of growth displayed by piglets after weaning. Similarly, Kelly *et al.* (1991*b, c*) reported ambiguity between the histological structure and digestive and absorptive function of the small intestine using *in vitro* techniques, and concluded that digestive enzyme activities *in vitro*, regardless of their basis of expression, can only provide a crude assessment of digestive capacity *in vivo*. There is a need, therefore, to establish techniques that provide an assessment *in vivo* of the digestive and absorptive capacity of the young pig.

The recent development of sensitive biochemical assays has allowed disaccharide hydrolysis and monosaccharide metabolism to be studied *in vivo* in the small intestine of human infants (Kliegman & Sparks, 1985; Siegel *et al.* 1988), adults (Williams *et al.* 1983), lambs (Kaempf *et al.* 1988, 1990; Spedale *et al.* 1992) and foals (Rice *et al.* 1992) under natural nursing conditions. In sucking piglets, Holmes *et al.* (1990) investigated the metabolism of lactose and galactose following ingestion of milk during letdown, and Bird & Hartmann (1994, 1996) and Bird *et al.* (1995) extended these studies to gain a further understanding of the digestion of lactose and sucrose and the metabolism of galactose, glucose and fructose in sucking piglets. However, these *in vivo* methods of investigation have not been conducted in weaner pigs.

The purpose of the present study was (a) to maintain villus height and crypt depth after weaning and, having done this, (b) to compare the capacity *in vivo* of brush-border lactase and sucrase to digest oral boluses of lactose and sucrose, and of the small intestine to absorb their monosaccharides, in sucking and weaned piglets. Since piglets were maintained on cows' milk after weaning, we hypothesized that the capacity *in vivo* of lactase and sucrase to digest lactose and sucrose would be maintained after weaning. In addition, brush-border lactase activity in the young pig has a more apical distribution on the villus than does sucrase (Dahlqvist & Nordström, 1966; Nordström & Dahlqvist, 1973; Kelly *et al.* 1991*a*) and may therefore be expected to show a greater reduction in activity after weaning in response to a change in villus height. For this part of the study we hypothesized that lactase activity *in vivo*, but not that of sucrase, is correlated to villus height after weaning.

MATERIALS AND METHODS
Animals and housing

Healthy 27-d-old sucking piglets (*n* 6) and 33-d-old piglets (*n* 12) that were weaned at 28 (SE 0·4) d and weighed 7·9 (SE 0·34) kg were selected for the study. At weaning piglets were transported to an experimental room that contained twelve individual stainless-steel pens arranged in four banks of three pens each. Each pen had a measured floor area of 0·45 m². Ambient temperature was maintained at 25·6 (SE 0·44)°. Two galvanized troughs were placed in each pen, one for feed and the other for fresh water.

Feeding

After weaning piglets were offered cows' whole milk *ad libitum* every 2 h. The amount of milk fed was increased by 10% at each feed if the piglet consumed all the milk offered to it in the previous meal, ensuring that more milk was offered than could be drunk. Piglets were given 30–45 min to drink the milk placed in front of them, after which time troughs were removed from each pen and any residual milk was noted. Between feedings all troughs were washed and scrubbed thoroughly with hot water.

Oral doses and blood sampling

Two solutions were prepared. The first contained lactose and fructose (treatment LAC+FRU) and the second contained sucrose and galactose (treatment SUC+GAL). Both solutions were prepared using doubly-deionized water 12–24 h before they were administered. They were kept in glass bottles with an airtight seal at 4° before use. Before oral dosing, the solutions were equilibrated to room temperature (20–25°).

At 09.00 hours on day 27 of lactation six sucking piglets were selected and moved to a pen in a neighbouring room. Piglets were watched beforehand to ensure they had not drunk from the sow in the previous hour. Similarly, at 09.00 hours on the fifth day after weaning, six piglets were selected and moved to a pen in an adjacent room. These were fed in a manner that ensured they had not consumed any milk in the previous hour. All piglets were dosed orally (via stomach tube) with 25 ml of each solution containing either 4 g lactose plus 2 g fructose or 4 g sucrose plus 2 g galactose in succession. Both doses were of the same osmolality, and therefore would have led to similar rates of stomach emptying.

Administration time for each dose was between 0·5 and 2 min with zero time being taken as the time when half the volume of the solution had been administered. Ear veins of the piglets were pricked using a finger lancet, and blood samples (about 60 μl) were collected into heparinized capillary tubes and immediately placed on ice. Following blood collection each capillary tube was sealed at one end with a disposable micro-packed cell volume tube closure, and the plasma was separated by centrifugation, isolated, and stored at $-20°$ for subsequent analysis. Samples were taken at regular intervals (3–5 min) for 60 min and then again at 75 and 90 min after dosing. After samples from this first dose were completed, the other solution was administered and blood samples were collected again for 90 min.

Post-mortem procedure

On the day of weaning (*n* 4), and on the fifth day after weaning (*n* 12), piglets were killed and samples of small intestine at sites 25, 50 and 75% along the gut were taken for subsequent histological examination. The slaughter, post-mortem procedure and preparation of gut samples for measurement of villus height and crypt depth have been described previously (Pluske *et al.* 1996*a*).

Biochemical analysis

Plasma (20 μl) was deproteinized using 200 μl 0·6 M-perchloric acid (Arthur *et al.* 1989). The concentration of glucose was measured by the glucose oxidase (*EC* 1.1.3.4) method of Bergmeyer & Bern't (1974) as modified by Holmes *et al.* (1990). Galactose concentration was determined by the bioluminescence method of Arthur *et al.* (1989), with adaptations, and fructose concentration was determined according to the method of Guppy *et al.* (1990).

Data analysis

The concentrations of glucose, galactose and fructose in the blood *v.* time after oral dosing were plotted and the area under the curve (AUC) for each piglet was calculated by the

trapezoid method reported by Williams *et al.* (1983), which was derived from Yeh & Kwan (1978). Since piglets of different live weights were administered the same amount of LAC+FRU and SUC+GAL, heavier piglets would have distributed the absorbed monosaccharides in a larger volume of plasma. It was necessary, therefore, to adjust the AUC to account for the different dilution of the monosaccharides in piglets differing in live weight. Bird & Hartmann (1994) provide a full description of the methods and calculations used to allow comparisons of AUC between piglets of different live weights.

Galactose and fructose indexes both before and after weaning were calculated from the adjusted AUC. By dividing the adjusted AUC values for galactose and fructose (ingested as components of lactose and sucrose, respectively) by the values obtained for galactose and fructose ingested as monosaccharides respectively, and then expressing the ratio as a percentage, an index equivalent to the glycaemic index used in human nutrition (Brand Miller *et al.* 1994) was obtained. The galactose index (GalI) and the fructose index (FruI), therefore, provide an index of efficiency *in vivo* of disaccharide digestion and monosaccharide absorption in the small intestine. Thus if, for example, the GalI was 50%, then the AUC for galactose consumed as lactose was half that observed when the same quantity of galactose was ingested as the monosaccharide.

Statistical analysis and presentation of results

Performance data after weaning are presented as mean, SEM, CV and the range of values encountered. Weight of the empty body was determined by multiplying the starting live weight of each piglet by the percentage of the carcass that was empty body in the piglets killed at weaning (Noblet & Etienne, 1987). This percentage was 98·2 (SE 0·26)% (CV 0·27%). Empty-body-weight gain was calculated, therefore, as the difference between the recorded weight of the empty body at slaughter 5 d after weaning and the estimated weight of the empty body at weaning.

Significant differences in adjusted AUC for glucose, galactose and fructose between sucking and weaned piglets were determined using unpaired Student's *t* test. Results are expressed as means with their standard errors. Simple linear regressions were performed to describe relationships between gut structure and adjusted AUC for products of lactose and sucrose hydrolysis. One-way ANOVA was used to study differences in structure of the small intestine between piglets killed before and after weaning.

RESULTS

Villus height and crypt depth after weaning

Feeding cows' whole milk to piglets for 5 d after weaning maintained ($P > 0.05$) villus height at each site along the small intestine compared with piglets killed at weaning. Villus height in weaned piglets ranged from 570 μm at the proximal jejunum to 342 μm at the distal ileum (Table 1). Crypt depth was similar ($P > 0.05$) between weaned and unweaned pigs at sites 25 and 50% along the gut, but at the distal ileum, crypts were 24% deeper (137 *v.* 110 μm, $P < 0.05$) in piglets fed on cows' milk than in piglets killed at weaning. The villus height:crypt depth ratio was similar ($P > 0.05$) for both groups of animals (Table 1).

Adjusted areas under the curves

The adjusted AUC for galactose, glucose and fructose following treatment LAC+FRU were highest in piglets assessed 5 d after weaning. For example, the adjusted AUC for galactose was 65% higher in weaned piglets (5600 *v.* 3399, $P = 0.016$) than that recorded in piglets before weaning. When piglets were dosed with treatment SUC+GAL the adjusted AUC for galactose and glucose were 107% ($P = 0.007$) and 31% ($P < 0.001$)

Table 1. *Villus height (μm), crypt depth (μm) and villus height:crypt depth ratio (VH:CD) of piglets killed either at weaning (n 4) or 5 d later (n 12)†*

Variable	% of intestine	Weaning		After weaning		Statistics (15 edf)
		Mean	SE	Mean	SE	
Villus height	25	550	66·1	570	24·4	NS
	50	523	82·2	513	27·1	NS
	75	427	45·1	342	29·4	NS
Mean‡		500	54·0	476	8·5	NS
Crypt depth	25	130	7·6	126	4·4	NS
	50	140	14·5	128	3·2	NS
	75	110[a]	9·7	137[b]	9·5	*
Mean‡		127	22·2	130	12·8	NS
Mean‡ VH:CD ratio		4·0	0·60	3·7	0·25	NS

[a,b] Mean values within a row with unlike superscript letters were significantly different, $P < 0.05$.
* $P < 0.05$.
† For details of procedures, see pp. 410–411.
‡ Mean of all three sites along the small intestine.

Table 2. *Adjusted area under the curve values (arbitrary units) for galactose, glucose and fructose for pigs dosed before weaning and on the fifth day after weaning with lactose plus fructose (treatment LAC + FRU) and sucrose plus galactose (treatment SUC + GAL)†*

(Mean values with their standard errors for six piglets)

	Before weaning (day 27)		After weaning (day 33)		Statistics (11 edf)
	Mean	SEM	Mean	SEM	
Treatment LAC + FRU					
Galactose	3399[a]	426·8	5600[b]	625·9	*
Glucose	239[a]	11·3	465[b]	15·0	***
Fructose	18021[a]	1564·5	39462[b]	4594·1	***
Treatment SUC + GAL					
Galactose	4792[a]	902·5	12017[b]	1627·0	**
Glucose	309[a]	11·3	404[b]	18·3	***
Fructose	15199	1559·2	17809	4833·7	NS

[a,b] Mean values within a row with unlike superscript letters were significantly different.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† For details of procedures, see pp. 410–412.

higher respectively, for piglets assessed 5 d after weaning compared with those assessed on day 27 of lactation. There was no difference ($P = 0.618$) in the adjusted AUC for fructose before and after weaning following treatment SUC + GAL (Table 2).

Galactose index and fructose index

The GalI on the day before weaning was 58·7 % and had declined to 46·6 % by the fifth day after weaning, whereas the FruI on day 27 was 84·3 % but had declined to 45·1 % when assessed after weaning.

Villus height at a site 25 % along the gut explained 87 % ($P = 0.007$) of the variation in GalI (Fig. 1(a)), and mean villus height along the entire length of the gut accounted for 70 % ($P = 0.037$) of the GalI (Fig. 1(b)). In contrast, no significant relationships (R^2 0.01–0.15, $P > 0.05$) were found between villus height at any site along the gut, and mean villus height, and FruI after weaning.

Relationships between villus height and adjusted areas under the curves

Correlations between villus height at the proximal jejunum and the adjusted AUC for galactose, glucose and fructose following treatments LAC+FRU (graphs a–c) and SUC+GAL (graphs d–f) are depicted in Fig. 2. Villus height at the proximal jejunum explained 70 % ($P = 0.039$) and 81 % ($P = 0.015$) of the adjusted AUC for galactose (Fig. 2(a)) and glucose (Fig. 2(b)) respectively, following treatment LAC+FRU on the fifth day after weaning. The adjusted AUC for fructose was not related (R^2 0.19, $P > 0.05$) to villus height following treatment LAC+FRU (Fig. 2(c)).

Following treatment SUC+GAL, only the adjusted AUC for glucose was correlated with villus height at a site 25 % along the gut (R^2 0.74, $P = 0.027$; Fig. 2(e)). The adjusted AUC for galactose and fructose (Fig. 2(d) and (f)) were not related (R^2 0.02 and 0.22 for galactose and fructose respectively; $P > 0.05$) to villus height after weaning. No significant correlations were found for sites 50 and 75 % along the small intestine, nor for mean villus height, for the adjusted AUC of any monosaccharides following treatments LAC+FRU or SUC+GAL.

Significant negative relationships were found for correlations between crypt depth at sites 25 and 50 % along the small intestine and the adjusted AUC for glucose following treatment LAC+FRU. Crypt depth (x) at the proximal jejunum explained 85 % of the variation in the adjusted glucose AUC (y):

$$y = 769.2 - 2.29x, \ F_{1,4} \ 22.10, \ P = 0.009; \ R^2 \ 0.85,$$

while crypt depth (x) at the mid jejunum explained 82 % of the total variation in the adjusted glucose AUC (y):

$$y = 836.9 - 2.82x, \ F_{1,4} \ 18.79, \ P = 0.012; \ R^2 \ 0.82.$$

Following treatment SUC+GAL, significant negative relationships were found also between crypt depth at the proximal and mid jejunum, and with mean crypt depth along the entire length of the small intestine, as follows:

(a) crypt depth at 25 % (x) and adjusted glucose AUC (y):

$$y = 783.8 - 2.86x, \ F_{1,4} \ 30.06, \ P = 0.005; \ R^2 \ 0.88;$$

(b) crypt depth at 50 % (x) and adjusted glucose AUC (y):

$$y = 859.2 - 3.46x, \ F_{1,4} \ 20.95, \ P = 0.012; \ R^2 \ 0.83;$$

(c) mean crypt depth (x) and adjusted glucose AUC (y):

$$y = 668.4 - 1.91x, \ F_{1,4} \ 11.66, \ P = 0.027; \ R^2 \ 0.74.$$

Piglet performance after weaning

The general health of all piglets during the experiment was excellent. Animals averaged an empty-body-weight of 268 g/d (live-weight gain 315 g/d) from weaning and consumed, on average, 238 g milk DM/d, or 5.5 MJ gross energy/d, over the duration of the study. Piglets converted milk DM to empty-body-weight gain at an efficiency of 0.8 and used, on average, 23 kJ digestible energy to deposit 1 g empty-body-weight gain (Table 3).
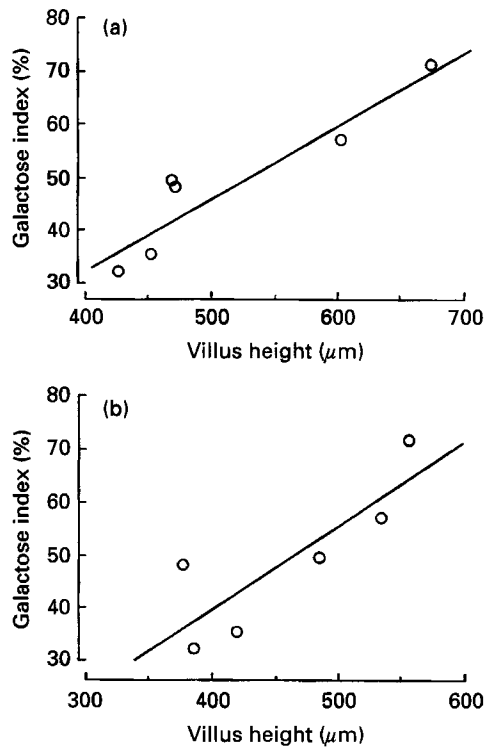
Fig. 1. Relationships between (a) villus height at a site 25% along the small intestine ($y = -20\cdot8 + 0\cdot14x$, $P = 0\cdot007$, $R^2\ 0\cdot87$) and (b) mean villus height along the entire length of the small intestine ($y = -23\cdot3 + 0\cdot16x$, $P = 0\cdot037$, $R^2\ 0\cdot70$), and the galactose index assessed 5 d after weaning. Each point represents an individual pig.

DISCUSSION

Villus height and crypt depth

Feeding cows' whole milk at intervals of 2 h for 5 d after weaning maintained villus height compared with piglets killed at the time of weaning. This concurs with results found in earlier experiments (Pluske et al. 1996a, b) and provides further evidence that gut structure can be maintained if piglets are coaxed to drink milk immediately after weaning to avoid the period of temporary 'starvation' that commonly occurs. Since villus height is correlated with the number of enterocytes on the villus column in 'normal' villi (Wright, 1982; Hampson, 1986a), the attenuation of voluntary feed intake after weaning must have preserved enterocyte number commensurate with a maintenance, or even enhancement (e.g. James et al. 1988), of digestive and absorptive capacity of each enterocyte. The lack of any increase in crypt-cell production rate after weaning supports this notion. An alternative reason for preservation of villus height, as suggested by Goodlad et al. (1988), is that villus cell population might increase by means of cell migration from the crypts without a concomitant increase in crypt-cell production rate. However, this has only been observed in rats re-fed after a period of short-term starvation (Altmann, 1972; Clarke, 1975), and so this mechanism is unlikely to be responsible for the maintenance of villus height seen after weaning in the present investigation.
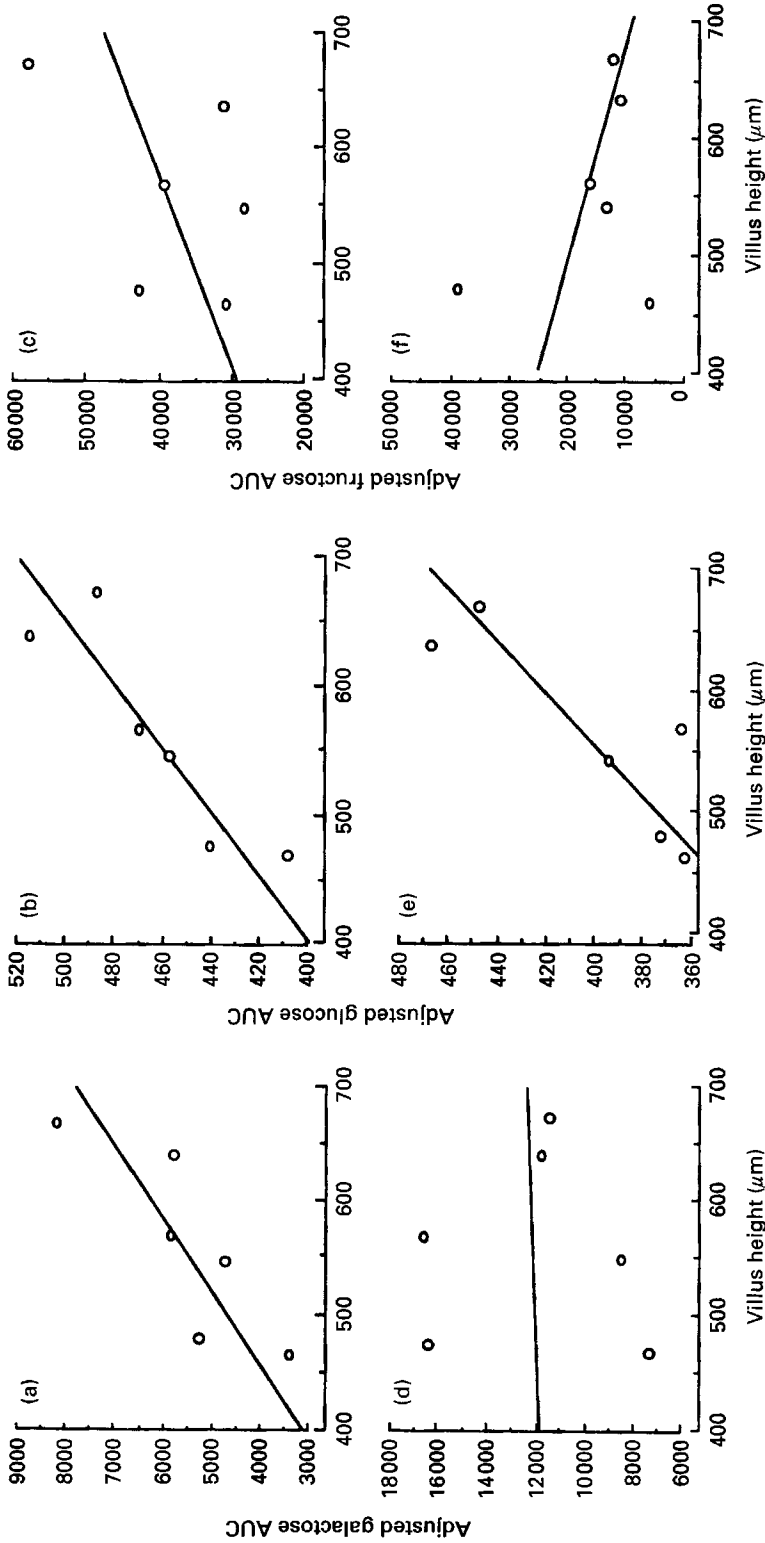
Fig. 2. Relationships between villus height at the proximal jejunum and (a) adjusted galactose area under the curve (AUC), (b) adjusted glucose AUC, and (c) adjusted fructose AUC following administration of lactose and fructose, and between villus height at the proximal jejunum and (d) adjusted galactose AUC, (e) adjusted glucose AUC, and (f) adjusted fructose AUC following administration of sucrose and galactose. Each point represents an individual pig. For coefficients of determination and P values, see p. 414.

Table 3. *Empty body weight (EBW), voluntary feed intake (VFI), feed conversion ratio (FCR), and the energy cost of body deposition of pigs fed on cows' whole milk* ad libitum *for 5 days after weaning* (n *12*)

	Mean	SEM	CV (%)	Range
EBW at weaning (kg)	7·7	0·22	9·7	6·4–8·7
EBW after 5 d (kg)	9·1	0·30	11·5	7·4–10·7
Live-weight gain (g/d)	315	33·4	36·7	114–456
EBW gain (g/d)	268	31·9	41·2	59–406
VFI (g DM/d)	238	17·7	25·7	130–333
Energy intake (MJ GE/d)	5·5	0·41	25·7	3·8–7·6
FCR (g DM:g EBW gain)	0·8	0·06	27·1	0·5–1·1
Energy cost/g EBW gain (kJ DE/g)	23·0	2·85	42·8	14·5–48·8

GE, gross energy; DE, digestible energy.

Adjusted areas under the curves, and the galactose and fructose indexes

The GalI and FruI provide a qualitative index of the capacity of lactase and sucrase *in vivo* to hydrolyse lactose and sucrose, and the capacity *in vivo* of enterocytes to absorb their monosaccharide products. There was an increase in GalI after weaning, indicating that more of the monosaccharide appeared in the peripheral circulation (Table 2). Since the values were corrected for differences in live weight, the increased GalI could have been caused by an enhancement in the digestive and/or absorptive capacity of the small intestine. Despite increases in the amount of monosaccharide entering the peripheral circulation, there were apparent decreases in GalI (from 58·7 to 46·6 %) and FruI (from 84·3 to 45·1 %) between weaning and slaughter 5 d later suggesting a decrease in the efficiencies of lactase and sucrase respectively. One explanation for this apparent anomaly between adjusted AUC and both GalI and FruI is the increased growth (and hence surface area) of the small intestine that occurred between weaning and slaughter such that 'total' digestion and absorption most probably increased despite apparent decreases in the activity of lactase and sucrase *in vivo*. In the present study the absolute (g) and relative (g/kg empty-body weight) weights of the small intestine increased from 171 to 285, and from 24·6 to 31·5 respectively, in the 5 d after weaning. Similarly, small-intestine length increased from 6·31 to 8·05 m.

The GalI on day 27 of lactation was 58·7 %, and is in good agreement with the value of 66 % for 'relative digestive capacity' of lactase reported by Bird *et al.* (1995) in piglets from 2 to 18 d of age. The decline in GalI after weaning is consistent with the observations of Walker (1959) and Ekstrom *et al.* (1975) who showed that, based on the calculation of total intestinal lactase, the capacity of brush-border lactase to hydrolyse lactose was maximum between 15 and 21 d of age and declined thereafter. Similarly, Kelly *et al.* (1991*a*) reported that biochemically active lactase declined significantly in the brush border of piglets in their first 8 weeks of life. Thus, lactase activity measurements reported by other workers are consistent with the changes observed for GalI.

Also in agreement with the data of Kelly *et al.* (1991*a*) was our finding that maximum lactase activity occurred further along (i.e. more apically) the crypt:villus axis (Fig. 1). Kelly *et al.* (1991*a*) reported that in normally suckled piglets, lactase activity at 5 weeks of age continued to increase during enterocyte migration up the villus, although in piglets killed at 7 and 8 weeks of age maximal activity of lactase was expressed at decreasing distances from the villus:crypt junction. These authors concluded that lactation products

(i.e. substances in milk such as hormones and growth factors) can accelerate the rate of decline of brush-border lactase. It is likely that similar compounds were present in the cows' milk we fed in this experiment and hence may also have contributed to the apparent decline in GalI. It would appear, therefore, that in the young piglet the functional limitation to its capacity to utilize lactose as an energy source is the hydrolytic activity of brush-border lactase. Bird *et al.* (1995) consistently recorded a lower adjusted galactose AUC following a dose of lactose compared with an iso-osmotic dose of glucose plus galactose in sucking piglets. Evidence of this occurred in the present experiment because the adjusted galactose AUC was higher when administered as a monosaccharide (treatment SUC + GAL) than when given as the disaccharide in treatment LAC + FRU (Table 2).

The aetiology of lactase decline is not clear but may be related to a reduction in enterocyte lifespan (Tsuboi *et al.* 1981, 1985), a suppression in the rate of enzyme synthesis (Jonas *et al.* 1985; Kelly *et al.* 1991 *a*), and/or a two-stage decline in the regulation of lactase expression involving both a shortening of the time allowed for enzyme expression on the villus and an inhibition in the rate at which lactase is expressed (Smith & James, 1987).

We calculated that the FruI in piglets aged 27 d of age was 84·3 % and that this declined to 45·1 % by the fifth day after weaning. Bird & Hartmann (1996) examined the relative digestive capacity for sucrase activity in piglets from 2 to 15 d of age and recorded a positive linear correlation between piglet age and the capacity to hydrolyse sucrose (R^2 0·96, $P < 0·001$). The oldest piglets examined (15 d) had a digestive capacity of 97 %, considerably higher than the 84 % recorded in the present experiment for pigs 27 d of age. What is unclear is whether the relationship described by Bird & Hartmann (1996) can be applied to piglets older than 15 d. Sucrase activity increases with time after birth (Manners & Stevens, 1972; Kidder & Manners, 1980), so it would be predicted that the digestive capacity of sucrase would also continue to increase. However in the present experiment the capacity for sucrase hydrolysis decreased almost 2-fold after weaning.

The primary reason for the decrease in FruI after weaning was the large fructose adjusted AUC following treatment LAC + FRU (Table 2), which is the denominator in the calculation of FruI. This is an apparent 220 % increase in the amount of fructose present in the peripheral circulation compared with that before weaning. It is difficult to imagine an increase in transport rate *per se* of this magnitude occurring in just 5 d. For example, Puchal & Buddington (1992) reported that at a physiological concentration of 5 mM (11 mM was given in the present study), fructose transport in the proximal small intestine increased from 11 % of glucose transport at birth to 15 % at 30 d of age. It is recognized that glucose and galactose are absorbed at a considerably faster rate than fructose, consistent with different mechanisms of transport across the epithelium (Williams, 1986). Glucose and galactose share one or more Na^+-dependent carriers located in the brush-border membrane of apical villi that allows these two monosaccharides to be actively transported across the mucosa. They are also transported by facilitated and passive diffusion. In contrast, fructose moves into the lamina propria by means of a Na^+-independent facilitated diffusion transporter having a low affinity for fructose (Puchal & Buddington, 1992). Differences in the concentration of fructose in the lumen may cause differential changes in the rate of fructose absorption across the epithelium but, since the amount of fructose given was the same before and after weaning (2 g) in the present experiment, it is unlikely that differences in transport rate are responsible for this disparity.

Another possibility is that the quantities of sucrose (4 g) and fructose (2 g) given in the present study may have exceeded the capacity of the enterocytes to digest sucrose and absorb fructose. If this was the case then the fructose adjusted AUC value after weaning may be a reflection of its longer half-life in the plasma and slower clearance after

absorption. This might have been caused by an unexpected 'carry-over' of fructose from the first dose (e.g. treatment SUC + GAL) that was not completely cleared in the 90 min sampling period before the next dose (e.g. treatment LAC + FRU) was administered.

Adjusted areas under the curves and villus height

Our hypothesis that lactase activity, but not that of sucrase, would exhibit a positive relationship with villus height after weaning because maximal activity of lactase is present in enterocytes located more apically on the villus was only partly supported by the present study. Even though the adjusted AUC for both galactose and glucose following treatment LAC + FRU were highly correlated (R^2 0·70 and 0·81 respectively) with villus height at the proximal jejunum, the adjusted AUC for glucose was also correlated with villus height at the same site following treatment SUC + GAL (R^2 0·74, $P = 0·027$). This suggests that within the range of villus heights encountered in this study (464–671 μm), lactase and sucrase showed increased activity in the upper part of the villus.

Correlations between villus height and the adjusted AUC for galactose and glucose following administration of both solutions were found at a site 25% along the small intestine only. The failure to detect relationships between lactose and sucrose digestion and villus height at sites 50 and 75% along the small intestine supports earlier research which suggests that lactase and sucrase activity in the young pig is maximal in the more proximal region of the small intestine (Manners & Stevens, 1972; Kidder & Manners, 1980; Shulman et al. 1988; Kelly et al. 1991 a–c). These results also support the findings of Puchal & Buddington (1992) who reported proximal to distal gradients in the rate and extent of galactose and glucose transport in sucking piglets. These findings illustrate the role of 'luminal nutrition' in determining gradients of disaccharidase activity along the small intestine of the young pig, a conclusion reached also by James et al. (1988) who examined lactase and sucrase activities in the weaned mouse.

Adjusted areas under the curves and crypt depth

Negative correlations were recorded between crypt depth at various sites along the gut and the adjusted AUC for glucose following both dosing treatments. An increase in crypt depth is indicative of an increased rate of enterocyte production and migration up the villi (Koldovsky et al. 1966; Al-Mukhtar et al. 1982). This results in proportionately more immature cells with reduced enzyme activity occupying the villus column because the rate of cell turnover is accelerated. The negative relationships observed in the present experiment are consistent with the proposition that pigs having a faster rate of enterocyte migration have a decreased capacity for lactose and sucrose hydrolysis. However, similar relationships were not observed for either galactose after lactose dosing (treatment LAC + FRU) or fructose after sucrose dosing (treatment SUC + GAL).

The decline in lactase and sucrase activities after weaning is generally associated with a decrease in villus height and in increase in crypt depth (Hampson & Kidder, 1986). The faster rate of cell migration and reduction in villus height results in the remaining villus enterocytes having insufficient time to differentiate fully and express maximum catalytic capacity before being extruded from the villus tip (Rey et al. 1971; Tsuboi et al. 1981; Smith, 1984; Miller et al. 1986). In the present experiment we demonstrated that the AUC for galactose and glucose were positively correlated with villus height at the proximal jejunum because of the existence of maximal lactase (and sucrase) activity further away from the crypt:villus junction. Further analysis of the histological data revealed a significant negative relationship between villus height and crypt depth at a site 25% along

the small intestine ($y = 220 \cdot 7 - 0 \cdot 16x$, $P = 0 \cdot 018$; R^2 $0 \cdot 79$), such that pigs having longer villi had a slower rate of cell turnover, or shorter crypts. This would result in a greater concentration of lactase and sucrase on the villus column because more mature enterocytes expressing maximum digestive capacity would be present. Although this association was only evident at the proximal jejunum, it accommodates both the negative relationship between crypt depth and the adjusted AUC for glucose, and the positive dependence between villus height and the amount of glucose present in the peripheral circulation following lactose and sucrose hydrolysis. However, why this should happen only with the adjusted AUC for glucose, and not for galactose or fructose, is unknown.

Conclusions

Villus height and crypt depth were maintained after weaning by the regular feeding of cows' whole milk. The capacity of the weaned pig to digest physiological boluses of lactose and sucrose and absorb their monosaccharide products increased between weaning and when pigs were killed 5 d later, as evidenced by an increased adjusted AUC for all monosaccharides. The GalI and FruI decreased after weaning despite increases in adjusted AUC for glucose, galactose and fructose following the digestion of lactose and sucrose. This incongruity may be explained by the increased growth (and hence surface area) of the small intestine such that total absorption *per se* may increase despite an apparent decrease in the enzymic activity *in vivo* of lactase and sucrase in the small intestine. Positive linear relationships between villus height and adjusted AUC for galactose and glucose following treatment LAC+FRU and adjusted AUC for glucose following treatment SUC+GAL confirm maximum disaccharidase activity occurring more apically along the crypt:villus axis. Finally, we believe that this technique may be suitable for estimating the digestive and absorptive capacity of the small intestine *in vivo* in the weaned piglet, but suggest that the methodology requires validation using conventional starter diets rather than diets based on milk.

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