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Interrelationships between gut morphology and faeces consistency in newly weaned piglets

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Abstract

A total of 104 weanling piglets was used to study the interrelationships between faeces consistency and mucosal integrity, as assessed by specific aminopeptidase and isomaltase-sucrase activity, villus height and crypt depth. Piglets were weaned at 26 (s.d. 1·4) days of age, weighing 8·4 (s.d. 0·70) kg. On the day of weaning (day 0), dissection was performed on one group of eight piglets. The remaining piglets were given restricted amounts of diets containing different protein sources. However, during the first 7 days post weaning 72% of the piglets ate on average less than 0·9 of the amount offered and thus actually had ad libitum *access to food. On days 3 or 7 post weaning pigs were weighed and euthanased. Diet composition did not effect small intestine integrity and the data were pooled for further analysis. The weight of the stomach, large intestine and pancreas increased with time post weaning (*$P < 0·001$*). Small intestine weight decreased from day 0 to 3 and was increased again on day 7, exceeding the pre-weaning value (*$P < 0·001$*). Isomaltase-sucrase and aminopeptidase activities were decreased on days 3 and 7 when compared with day 0. Villus height was decreased after weaning, followed by an increase on day 7 post weaning at the proximal small intestine, but by a further decrease at the mid small intestine (*$P < 0·001$*). Crypt depth was increased after weaning (*$P < 0·001$*). Faeces consistency was scored twice a day on a scale from 0 to 3 with increasing liquid nature. The average percentage of days during which piglets had more-liquid faeces was 26%. During the 1st week post weaning, 73% of the piglets showed a faeces score of 2 during at least 1 day. Villus height was positively correlated with food intake level, brush-border enzyme activity and dry matter content of the chyme. Villus height was negatively correlated with more-liquid faeces. Crypt depth was positively associated with the weight of various parts of the gastro-intestinal tract. It is concluded that this study supports the concept that food intake by weaned piglets determines villus height in the small intestine and brush-border enzyme production which in turn determine the risk of diarrhoea development.*

Keywords: *diarrhoea, piglets, small intestine morphology.*

Introduction

Abrupt weaning of piglets around 4 weeks of age involves social, environmental and nutritional changes (Fraser *et al.*, 1998). As a consequence, weanling piglets refrain from eating (Le Dividich and Herpin, 1994) which leads to growth depression (Leibbrandt *et al.*, 1975). The average food intake after weaning is highly variable between piglets and the latency time to the first solid-food intake can take up to 3 days (Bruininx *et al.*, 2001). Thus, most pigs show a temporary depression in food intake, some do not, but all of them eventually start to eat. The low food intake after weaning causes a reduction in villus height (Kelly *et al.*, 1991b; Pluske *et al.*, 1996; Verdonk *et al.*, 2001) and a decrease in total brush border enzyme activity (Kelly *et al.*, 1991b; Núñez *et*

al., 1996; Lopez-Pedrosa *et al.*, 1998). The alterations in morphology and function of the small intestine may impair the ability to digest and absorb nutrients and to predispose the weanling piglet to development of malabsorption and diarrhoea. Indeed, in practice, post-weaning diarrhoea occurs frequently. However, as far as we know, there are no published studies describing in quantitative terms the associations in weanling pigs between diarrhoea on the one hand and either integrity of the small intestine or growth performance on the other hand. In the experiment described here, the weanling piglets had more-liquid faeces during on average one quarter of the 7 post-weaning days. On day 7 after weaning, 75% of the piglets produced higher-liquid faeces. Due to the relatively high incidence of more-liquid faeces and considerable variation between piglets, the data were considered suitable to assess the associations between faeces consistency, small intestine morphology, enzyme activity, food intake and growth during the 1st week post weaning. It was anticipated that the information thus obtained would provide insight into the determinants of post-weaning diarrhoea and provide clues as to the prevention of diarrhoea given the current management of weanling piglets.

Material and methods

Piglets and weaning

The experiment, in the form of two consecutive batches, was performed at the Swine Research Centre (SRC) of Nutreco (Boxmeer, The Netherlands). Batch 1 and 2 consisted of 48 and 56 barrows, respectively. Piglets [(Duroc × Yorkshire synthetic) × (Yorkshire × Dutch Landrace synthetic)] were weaned at 26 (s.d. 1·4) days of age; they had an average body weight of 8·4 (s.d. 0·70) kg. At weaning (day 0), piglets were removed from the sow, weighed and housed individually in pens (77×76 cm^2). Each pen was equipped with a trough and a water nipple. Environmental temperature was maintained at 27ºC. Lights were on from 06:00 to 22:00 h. Creep food was not provided during the suckling period. The experimental protocol was approved by the Animal Care and Ethics Committee of the University of Nijmegen (The Netherlands).

Foods, feeding and experimental design.

On the day before weaning (day –1), piglets were blocked by body weight (BW) and randomly allocated to one of 12 groups (batch 1) consisting of four piglets each or to 13 groups (batch 2) consisting of four piglets each for 12 groups and having eight piglets for one group. Littermates were divided evenly among the groups. The groups were to differ in diet type and/or day of dissection. On day 0, dissection was performed on the group of eight piglets in batch 2. The 12 groups with four piglets each per batch were dissected on day 3 or 7 post weaning and received one of six experimental diets. The diets were pelleted and then crumbled prior to feeding. The experimental diets were isonitrogenous, but differed with respect to protein or amino acid composition. The variable protein sources were wheat gluten, soya-bean meal and potato protein. One diet contained extra glutamine and another arginine. The composition of the diets is shown in Table 1. Calculated net energy (NE) content of all diets was 10·7 MJ NE per kg food.

After weaning (days 0 to 7) the piglets were offered restrictedly a maximum amount of dietary energy. The following formula describes the amount of net energy requirement for maintenance (NE_m) of the piglets according to their metabolic weight on the day of weaning (National Research Council (NRC), 1998) : NE_m (kJ/day) = $326{\cdot}4 \times M_0^{0{\cdot}75}$, where NE_m is the net energy requirement at maintenance level (kJ/day) and M_0 is body weight at day 0 (kg). The piglets received $0{\cdot}25 \times NE_m$ on day 0, $0{\cdot}5 \times NE_m$ on day 1, $0{\cdot}75 \times NE_m$ on day 2, $1 \times NE_m$ on day 3, $1{\cdot}5 \times NE_m$ on day 4, $2 \times NE_m$ on day 5, $2{\cdot}5 \times NE_m$ on days 6 and 7. Piglets were given equal portions of their allowance four times per day at 09 : 00, 11 : 30, 14:00 and 17:00 h. Food refusals were collected, weighed and subtracted from the amount of food offered to calculate actual daily food intake which was expressed as g /kg $M_0^{0{\cdot}75}$.

Growth and faeces consistency

Piglets were weighed on days –1, 0, 3 and 7 post weaning. Average daily gain (ADG) was calculated for the periods : days –1 to 0, days 0 to 3 and days 3 to 7. Faecal consistency was monitored twice a day and quantified using a scale ranging from 0 to 3 with 0 = normally shaped faeces, 1 = shapeless (loose) faeces, 2 = thick, liquid (soft) faeces, and 3 = thin, liquid faeces. Scoring was done by experienced animal carers who were blind to treatment modality.

Sampling of small intestine

On days 0, 3 and 7 post weaning, piglets to be killed were weighed and euthanased with a 5-ml intra-cardiac injection of Euthestate® (pentobarbital sodium, 200 mg/ml; Ceva Sante Animale B. V. Maasluis, The Netherlands). The piglets were killed between 08:00 and 16:00 h on days 3 and 7, the order being stratified according to the type of diet given. At 30 min before killing on days 3 and 7, each piglet was given access to its food. A mid-line laparotomy was performed and a jejunal segment was taken just distal to the ligament of Treitz (proximal small intestine) and a second segment at 3 m distal of this ligament (mid small intestine). Liver and pancreas

Table 1 *Ingredient composition of the experimental diets* (*g/kg food*)

	Diet †					
Ingredients (g/kg food)	SBM WG	SBM HWG	HSBM WG	SBM PP	SBM PP gln	SBM WG arg
Wheat gluten (CP ‡, 815 g/kg)	100·0		100·0			100·0
Hydrolysed wheat gluten §		98·8				
Soya-bean meal (CP, 512 g/kg)	160·0	160·0	160·0	160·0	160·0	
Hydrolysed soya-bean meal ‖			162·2			
Potato protein (CP, 792 g/kg)				102·9	102·9	
Pre-gelatanized maize starch	192·9	190·8	199·0	195·6	174·3	181·9
Limestone	10·8	10·6	11·1	11·2	11·2	10·8
Monobasic calcium phosphate	16·3	16·5	15·8	15·6	15·6	16·3
Salt	9·4	8·1	5·2	9·5	9·5	9·4
Soya oil	43·0	45·5	39·0	45·0	46·3	44·0
Lysine	5·6	6·0	5·2			5·6
Methionine	–	1·0	0·1	0·4	0·4	
Threonine	2·2	2·4	2·1			2·2
Trytophan	0·1	0·6	0·6			0·1
L-Glutamine ¶					20·0	
L -Arginine ¶						10·0
Constant components ††	459·8	459·8	459·8	459·8	459·8	459·8

† arg = arginine, gln = glutamine; HSBM = hydrolysed soya-bean meal; HWG = hydrolysed wheat gluten; PP = potato protein; SBM = soya-bean meal; WG = wheat gluten.
‡ CP = crude protein.
§ DMV International, Veghel, The Netherlands: CP, 825 g/kg; average molecular weight (MW), 800 D; degree of hydrolysis (DH; is the number of peptide bonds broken upon hydrolysis as% of total number present in intact protein) 9%; free amino acids (FAA), 2%.
‖ DMV International, Veghel, The Netherlands: CP, 505 g/kg; MW, 320 D; DH, 27%; FAA, 7%.
¶ Purity ≥ 99%.
†† The constant components consisted of (g/kg food): maize, 250; barley, 150; lactose, 50; choline chloride (purity 50%), 2·8; titanium oxide, 5; vitamin and trace element pre-mix, 2. The vitamin and trace element inclusion supplied (mg/kg food): retinol 3; cholecalciferol 0·050; alpha-tocopherol 43000; phytylmenaquinone 2; thiamine 1; riboflavin 3; pantothenic acid 10; niacin 20; biotin 0·030; cyanocobalamin 0·020; folic acid 0·2; pyridoxine 4; Fe 160; Cu 160; Zn 100; Mn 30; I 10; Se 0·2; antioxidants (E130, E320, E321) 60.

were removed and weighed. Stomach, small intestine and large intestine were removed and their empty weights determined. Chyme present in the last 2 m of the small intestine tract was collected to determine its dry matter content.

To characterize the jejunal mucosa morphologically, the tissue samples of the proximal and mid small intestine were cut open longitudinally at the anti-mesenteric attachment. After attachment to dental wax, the tissue was fixed in phosphate buffered (0·1 mol/l) formalin (40 ml formalin per l buffer). A 3-mm wide zone from the mesenteric site was cut at right angles to the surface of the mucosa and embedded in paraffin wax. Sections (5 µm) were cut and stained with the periodic acid/Schiff procedure (PA/S). These PA/S-stained sections were subsequently used to determine crypt depth (µm) and villus length (µm). One slide per piglet was used and the average values taken for a minimum of five villi and crypts.

To measure the specific activity of brush-border-membrane associated activity of isomaltase-sucrase and aminopeptidase, a proximal small intestine tissue sample (length = 15 cm) was taken and rinsed with phosphate-buffered saline (PBS), pH 7·2 (0·01 mol/l NaH_2PO_4, 0·01 mol/l Na_2HPO_4, 0·9% (w/v) NaCl). The mucosal layer was carefully scraped off from the muscle layer, quickly frozen in liquid nitrogen and stored at –80ºC until analysis. The enzyme activity was measured as described by Pusztai *et al.* (1996). The mucosal scrapings were homogenized in ice-cold, twice-distilled water using a Virtis blender (The Virtis Company, Gardiner, NY, USA) at full speed for 1 min at 0ºC to give a final concentration of 5% (v/w). Subsequently, the homogenates were sonicated twice at 0ºC for 15 s,

separated by an interval of 30 s, at an amplitude of 24 μm with a MSE Soniprep 150 (Beun de Ronde B. V., Abcoude, The Netherlands). The protein content of the sonicates was determined (Smith *et al.*, 1985), adjusted to approximately 350 μg protein per ml and used to calculate enzyme activities. Enzyme activities were tested under conditions of linearity with regard to amount of enzyme and incubation time. The reactions were done in triplicate on each intestinal segment per piglet. The activity of isomaltase-sucrase (EC 3·2·1·48) was measured with saccharose (Messer and Dahlqvist, 1966) as substrate (1 unit = 1 μmol disaccharide hydrolysed per min), and the activity of aminopeptidase (EC 3·4·11·2) using L-alanine-p-nitroanilide (Marouz *et al.*, 1973) as substrate (1 unit = 1 nmol substrate hydrolysed per min) and expressed as enzyme units per g protein.

Stastistical analysis

A GLM procedure (SAS version 6·12, SAS Institute, Cary, NC) was used to estimate the least-square means of the different treatments. The effect of day post weaning was evaluated across diets. Day post weaning and batch were the independent variables in the final model: $y_{ijk} = \mu + B_i + D_j + e_{ijk}$, where y_{ijk} = dependent variable; μ = overall mean; B_i = fixed effect of batch (i = 1, 2); D_j = fixed effect of day post weaning (j = 1, 2, 3); e_{ijk} = error term.

The effect of level of intake and severity of diarrhoea was evaluated for the piglets dissected on day 7 with the following model: $y_{ijk} = \mu + E_i + F_j + (E \times F)_{ij} + e_{ijk}$, where y_{ijk} = dependent variable; μ = overall mean; E_i = fixed effect of eating (i = 1, 2), a piglet was regarded as an eater if the average daily net energy intake for the 3-day period just before dissection (days 5 to 7) was above the energy level for maintenance (NE_m); F_j = fixed effect of diarrhoea (j = 1, 2), diarrhoea was equivalent to a faecal consistency score of either 1, 2 or 3 as measured during at least 2 days of the 3-day period just before dissection (days 5 to 7); $(E \times F)_{ij}$ = interaction between eating and diarrhoea; e_{ijk} = error term.

A multivariate analysis (Simca-P version 3·01, Umetri AB and Ericsson Erisoft AB, Umeå, Sweden) was performed using partial least squares (PLS) regression analysis. PLS has two primary objectives, namely to approximate X and Y and to model the relationship between X and Y. In total, 24 variables were taken into account: weight at weaning; food intake per kg metabolic weight from days 1 to 3 (MFI_{13}) and from days 3 to 7 (MFI_{37}); organ weights, expressed as g/kg empty body weight (EBW), i.e. stomach, small intestine (SI), large intestine (LI); total weight of gastro-intestinal tract (GIT); weight of small intestine per cm (SI g/cm); day of dissection i.e. day 0, 3 or 7; occurrence of more-liquid faeces for each day (faeces 1 to 7); villus height and crypt depth at proximal (prox) and mid small intestine; specific isomaltase-sucrase (IMS) and aminopeptidase (AMP) activity. In the first, second and third PLS analysis, respectively, villus height, crypt depth and enzyme activity were used as *Y* variable and the remaining variables as *X* variables.

Results

The calculated and analysed (Table 2) nutrient compositions agreed well. Diet composition after weaning did not differentially affect small intestine integrity as measured 3 and 7 days post weaning (Spreeuwenberg, 2002). Therefore, the data for the various diets were pooled and selected correlations calculated.

Piglets weighed on average 8·4 (s.d. 0·70) kg at weaning (M_0). Daily food intake (g/kg $M_0^{0.75}$) increased from 1 (s.d. 1·4, no. = 96) on day 1, to 7 (s.d. 6·2, no. = 96) on day 2, 16 (s.d. 8·3, no. = 96) on day 3, 24 (s.d. 9·1, no. = 48) on day 4, 35 (s.d. 12·7, no. = 48) on day 5, 41 (s.d. 16·8, no. = 48) on day 6 and 40 (s.d. 20·7, no. = 48) on day 7. Seventy-two percent of the piglets ate on average less than 0·9 of the amount offered during the first 7 days post weaning and thus actually had *ad libitum* access to food. Based on the energy content of the foods (10·7 MJ NE per kg food) and assumed maintenance requirement (NRC, 1998), the food intake to meet the net energy requirement for maintenance was estimated to be about 31 g/kg $M_0^{0.75}$ per day. Of the piglets dissected on day 7, 31% ate below their maintenance requirements during the 3-day period before dissection. Average daily gain (g/day per piglet) was 280 (s.d. 145·7) from days -1 to 0 (no. = 104), -39 (s.d. 84·0) from days 0 to 3 (no. = 96) and 119 (s.d. 109·4) from days 3 to 7. Food intake and average daily gain within the periods of days 1 to 3 and days 3 to 7 were positively correlated ($P < 0·001$)

Table 2 *Analysed nutrient composition of the experimental diets*

	Diet †					
	SBM WG	SBM HWG	HSBM WG	SBM PP	SBM PP gln	SBM WG arg
Macronutrients (g/kg food)						
Moisture	98	92	86	98	99	97
Crude protein	199	209	203	199	223	219
Crude fat	59	54	55	56	59	61
Crude fibre	19	18	19	21	25	25
Ash	50	52	52	51	49	51
Starch	453	428	446	452	428	442

† See Table 1.

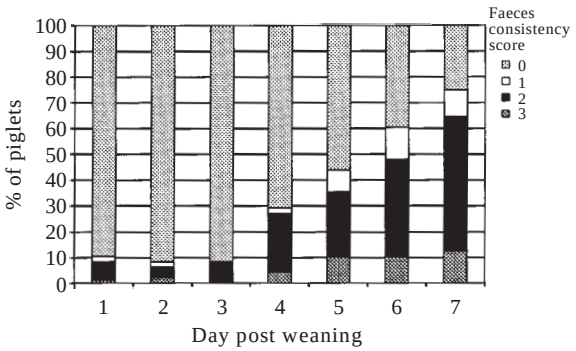

Figure 1 The distribution of faeces consistency score of piglets for each day during the 1st week post weaning (days 1 to 3, no. = 96; days 4 to 7, no. = 48).

and explained 0·59 (no. = 96) and 0·66 (no. = 48) of the variation (R^2), respectively. Average food intake during days 1 to 3 explained 0·17 of the variation (R^2) of the growth from days 3 to 7.

None of the piglets showed clinical signs of illness. The average incidence of more-liquid faeces than normal, expressed as a percentage of days with a faecal consistency score of either 1, 2, or 3, was 10% (s.d. 24·0) for the piglets dissected on day 3 and 33% (s.d. 26·7) for the piglets dissected on day 7. Figure 1 shows the distribution of faeces scores for each day. During the 1st week post weaning, 73% of the piglets showed a faeces score of 2 during at least 1 day.

The time course of body weight, organ weights and small intestine characteristics in weaned piglets is shown in Table 3. Body weight tended to be higher on day 7 post weaning than on day 3 ($P < 0·10$). Organ weights are expressed per kg of EBW. Relative weights of the stomach, large intestine and pancreas increased with time post weaning ($P < 0·001$). The small intestine weight was lower on day 3 when compared with either day 0 or 7. On day 7, the weight of the small intestine per cm exceeded pre-weaning levels ($P < 0·001$). Although organ weights were generally higher on day 7 than on day 0, villus height at the proximal and mid small intestine did not reach pre-weaning levels on day 7. At the proximal small intestine, villus height on day 7 was higher than on day 3 but, at the mid small intestine,

Table 3 *Body weights, organ weights and small intestine characteristics in weaned piglets in relation to day post weaning*†

	Day post weaning				
	0 (no. = 8)	3 (no. = 48)	7 (no. = 48)	Residual s.d.	Significance
General					
Body weight (kg)	8·5	8·3	8·7	0·80	
Empty body weight (EBW; kg)	7·6	7·3	7·3	0·72	
Organ weights (g / kg EBW)					
Stomach	5·3^c	6·2^b	8·0^a	1·24	***
Small intestine	32·2^b	27·0^c	39·0^a	5·00	***
Large intestine	11·6^c	13·9^b	20·2^a	2·53	***
Liver	29·8	29·5	30·4	3·15	
Pancreas	0·9^c	1·3^b	2·2^a	0·38	***
Small intestine (SI) characteristics					
Length / EBW (cm / kg)	96·6 ^{b}	102·8^b	111·7^a	13·75	**
Weight / length (g / cm)	0·3^a	0·3^b	0·4^a	0·03	***
Protein content of mucosa (g / g)	0·7^a	0·5^b	0·7^a	0·11	***
Villus height (μm)					
proximal SI	560^a	280^c	324^b	101·8	***
mid SI	522^a	313^b	259^c	77·4	***
Crypt depth (μm)					
proximal SI	220^b	240^b	313^a	42·2	***
mid SI	166^b	180^b	251^a	33·9	***
Villus / crypt ratio					
proximal SI	2·7^a	1·2^b	1·1^b	0·41	***
mid SI	3·2^a	1·8^b	1·0^c	0·45	***
Isomaltase-sucrase (units per g CP‡)	63·9^a	17·2^b	16·2^b	9·58	***
Aminopeptidase (units per g CP‡)	587^a	359^b	326^b	132·5	***

† Data in the table are presented as least-square means (LS Means). LS Means within a row with different superscript letters are significantly different ($P < 0·05$).
‡ CP = crude protein.

Table 4 *Gut morphology at the proximal and mid small intestine (SI) and specific enzyme activities at the proximal small intestine in piglets in relation to eating and diarrhoea*†

Eating (E)	Eater		Non-eater			Significance ‖	
Diarrhoea (F)§	No (no. = 16)	Yes (no. = 19)	No (no. = 4)	Yes (no. = 9)	Residual s. d.	E	F
Villus height (μm)							
Proximal SI	386	321	287	236	80·6	**	*
Mid SI	303	259	241	190	58·4	**	*
Crypt depth (μm)							
Proximal SI	332	315	312	277	40·7	*	
Mid SI	260	254	240	234	35·5		
Villus crypt ratio							
Proximal SI	1·2	1·0	1·0	0·9	0·32		
Mid SI	1·2	1·0	1·0	0·8	0·26		
Isomaltase-sucrase (units per g CP¶)	19·8	15·3	18·8	10·5	9·09		*
Aminopeptidase (units per g CP¶)	377	321	311	252	88·0	*	

† Data in the table are presented as least-square means (LS means) and refer to day 7 post weaning.
‡ A piglet was regarded as an eater if the average daily net energy intake during the 3-day period just before dissection (days 5 to 7) was above the net energy level for maintenance (NE_m), i. e. > 326·4 kJ / kg $M_0^{0.75}$, where M_0 is body weight at weaning (kg).
§ Diarrhoea was defined as less-consistent faeces, i. e. a faecal consistency score of either 1, 2, or 3 during 2 days of the 3-day period just before dissection (days 5 to 7). The average score during this period was 0·3 for piglets without and 1·9 for piglets with diarrhoea.
‖ There were no significant E × F interactions ($P > 0·05$).
¶ CP = crude protein.

villus height on day 7 was lower than on day 3 ($P < 0·001$). Crypt depth increased with time post weaning, both at the proximal and mid small intestine ($P < 0·001$). Villus / crypt ratio decreased with time post weaning ($P < 0·001$). Specific isomaltase-sucrase and aminopeptidase activities were lower on days 3 and 7 when compared with day 0 ($P < 0·001$). Group mean activity of the two enzymes was lower on day 7 than on day 3.

Table 4 shows villus architecture and enzyme activity in piglets at 7 days post weaning in relation to eating and diarrhoea. The average faecal score during this period was 0·3 for piglets without and 1·9 for piglets with diarrhoea. There was no significant interaction between eating and diarrhoea. Villus height at both the proximal and mid small intestine was associated with both eating and diarrhoea. Eaters had longer villi than non-eaters ($P < 0·01$) and piglets without diarrhoea had longer villi than piglets with diarrhoea ($P < 0·05$). Piglets that were labelled non-eaters with diarrhoea had the lowest group-mean villus height at the proximal and mid small intestine, whereas eaters without diarrhoea had the longest villi. At the proximal, but not at the mid small intestine, eaters had deeper crypts than non-eaters ($P < 0·05$) and piglets without diarrhoea tended to have deeper crypts than piglets with diarrhoea ($P < 0·10$). Isomaltase-sucrase activity was not affected by eating, whereas piglets without diarrhoea had a

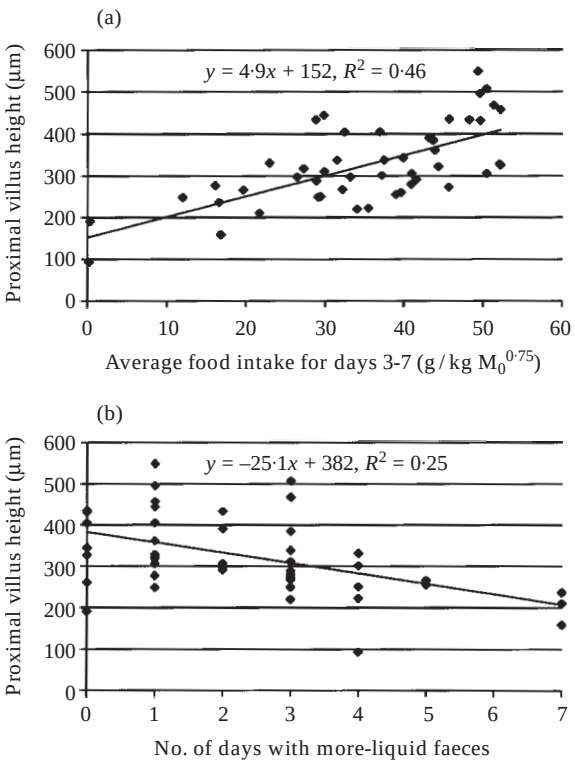

Figure 2 Villus height on day 7 post weaning in the proximal small intestine of individual piglets in relation to (a) average food intake for days 3 to 7 and (b) the number of days with more-liquid faeces than normal days 0 to 7.

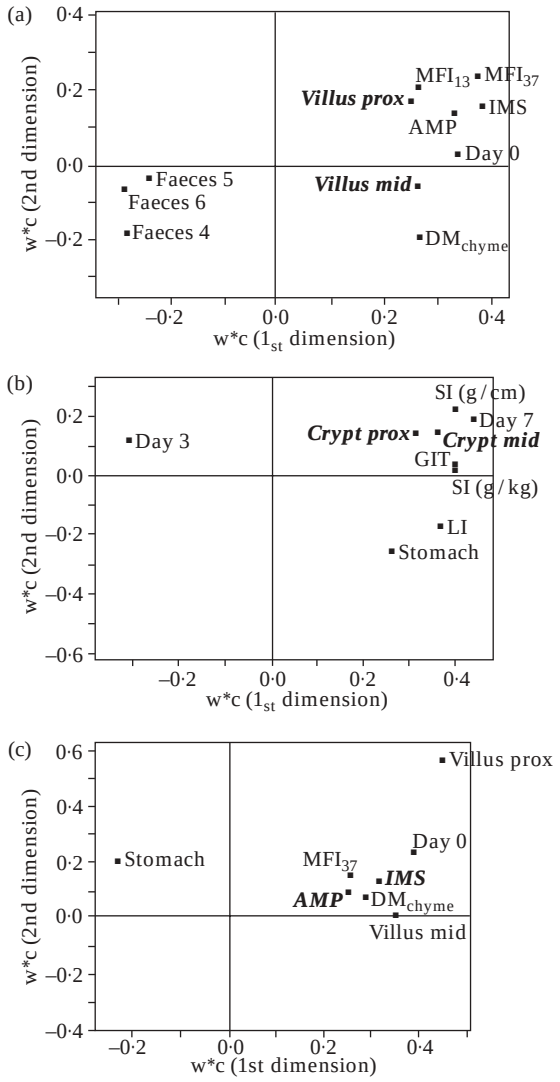

Figure 3 Partial least square (PLS) regression analysis and the relationship between the weights of the *X* variables (w*) and *Y* variables (c) in the first and second dimension, respectively. The *Y* variables (shown in bold italic) differ in each graph: (a) villus height at proximal (villus prox) and mid (villus mid) small intestine, (b) crypt depth at the proximal (crypt prox) and mid (crypt mid) small intestine, (c) aminopeptidase (AMP) and isomaltase-sucrase (IMS) activity. Only the *X* variables that contributed to the prediction of the *Y* variables are shown, where MFI_{13}, MFI_{37} = food intake per kg metabolic weight from days 1 to 3 and days 3 to 7; organ weights are expressed per kg empty body weight, i.e. stomach (g/kg); SI (g/kg) = small intestine weight, LI (g/kg) = large intestine weight; GIT = total weight of gastro-intestinal tract (g); SI (g/cm) = weight of small intestine per cm; day 0, 3 or 7 = dissection on day 0, 3, 7; faeces 4, 5 or 6 = occurence of less-consistent faeces (faeces score either 1, 2, or 3) on day 4, 5, or 6.

higher activity than those with diarrhoea ($P < 0{\cdot}05$). Aminopeptidase activity was higher in eaters than in non-eaters ($P < 0{\cdot}05$) and tended to be higher in piglets without diarrhoea when compared with those with diarrhoea ($P < 0{\cdot}10$). On day 7 post weaning, the average food intake from day 3 to 7 and the number of days with more-liquid faeces, respectively, explained proportionately 0·46 and 0·25 of the variation (R^2) in villus height in the proximal small intestine (Figure 2). Food intake and villus height were positively correlated, whereas the occurrence of more-liquid faeces on days 4 to 6 and villus height were negatively correlated. The average food intake and the number of days with more-liquid faeces explained 0·20 and 0·22, respectively, of the variation (R^2) in villus height, in the mid small intestine (data not shown).

Figure 3 shows the association between the variables as based on multivariate analysis. Only those variables that predict villus height, crypt depth or specific enzyme activity (Figure 3a, b and c respectively) are shown. The variables shown on the right side of the vertical zero line are positively associated and those on the left side are negatively associated. Variables with higher w*c-values contribute more to predicting villus height, crypt depth or enzyme activity. Villus height was positively associated with the average food intake, enzyme activities and dry matter content of the chyme and negatively associated with faeces consistency score on either days 4, 5 or 6. Crypt depth was positively associated with organ weights. Piglets dissected on day 7 generally had deeper crypts and those dissected on day 3 had shallower crypts. Specific enzyme activity was positively associated with villus height, average food intake and dry matter content of the chyme, and was negatively associated with stomach weight.

Discussion

This study provides new information as to the associations between faeces consistency, small intestine integrity, energy intake and organ weights. Weaning is usually associated with a dramatic reduction in food intake, resulting in growth stasis and altered structure of the small intestine. Indeed, the piglets showed weight loss during the first 3 days post weaning. In agreement with previous work, the weaning transition was also associated with an increase in organ weight (Efird *et al.*, 1982; Kelly *et al.*, 1991a; Pluske *et al.*, 1996), a decrease in villus height and an increase in crypt depth (Hampson, 1986a; Miller *et al.*, 1986; Cera *et al.*, 1988; Dunsford *et al.*, 1989; Hall and Byrne, 1989; Kelly *et al.*, 1991a; Nabuurs, 1991; Pluske *et al.*, 1996; Spreeuwenberg *et al.*, 2001) and a decrease in isomaltase-sucrase and

aminopeptidase activity (Hampson, 1986a; Miller *et al.*, 1986; Kelly *et al.*, 1991a). Food intake by individual piglets was positively correlated with villus height and brush border enzyme activity, as shown previously (Kelly *et al.*, 1991b; Núñez *et al.*, 1996; Pluske *et al.*, 1996; Lopez-Pedrosa *et al.*, 1998; Verdonk *et al.*, 2001).

Regarding the temporal changes in small intestine integrity during the weaning transition a de- and regenerative phase can be distinguished. Compared with the day of weaning, both the weight of the small intestine (g/kg EBW), the segmental weight of the small intestine (g/cm) and the protein content of the mucosa (g/g) were decreased on day 3, followed by an increase on day 7 post weaning. On day 7, the weight of the small intestine exceeded the pre-weaning value. Although small intestine weight had fully recovered on day 7 post weaning, the morphology of the gut wall and the enzyme activity of the brush border had not reached pre-weaning levels. Crypt depth was greater on day 7 when compared with both days 3 and 0, pointing to increased crypt cell production on day 7 (Pluske *et al.*, 1997). The multivariate analysis indicates that crypt depth was positively associated with organ weight, but not with villus height. Increased proliferation might not only occur in the crypts of the small intestine, but also in other parts of the gastro-intestinal tract as indicated by the increased weight of the stomach, small intestine and large intestine. In general, the brush border enzyme activity increases markedly when going from the bottom of the crypt to the tip of the villus (Miller *et al.*, 1986; Fan *et al.*, 2001). The high enzyme activity at the villus tip is associated with enterocyte differentiation (Fan *et al.*, 2001), which agrees with the positive association between either isomaltase-sucrase or aminopeptidase activity and villus height in the current study. Faeces consistency was not found to be associated with crypt depth and enzyme activity.

Surprisingly, villus height on day 7 *versus* day 3 was increased at the proximal small intestine but was decreased at the mid small intestine ($P < 0{\cdot}001$). Thus, villus height seemed to be in the regenerative phase at the proximal small intestine, but was still in the degenerative phase at the mid small intestine. In agreement with our results, Marion *et al.* (2002) found in piglets weaned at 7 days of age that villus height was numerically lower on day 3 post weaning at the proximal small intestine than it was on day 7 at the mid small intestine. Normally, macronutrients are degraded by enzymatic hydrolysis and their breakdown products are subsequently absorbed. As a consequence, the amount of nutrients in the lumen of the gut decreases in the distal direction. With energy intake being below maintenance in 45% of the piglets dissected on day 7, nutrient availability for the proximal small intestine might have been just sufficient, but maybe it was insufficient for the mid small intestine. This reasoning might explain the opposite difference in villus height on days 3 and 7 post weaning when the proximal and mid small intestine were compared.

During the 1st week post weaning, 73% of the piglets had a faeces score of 2 during at least 1 day. The reported incidences of diarrhoea are 32% (Ball and Aherne, 1982) and 39% (Hampson, 1986b) for the period of weaning to 14 days post weaning. Nabuurs (1991) reported an incidence of diarrhoea of 40% during the first, 69% during the second and 50% during the 3rd week post weaning of piglets reared under commercial conditions. It would appear that the piglets in this study showed extensive diarrhoea, but it should be noted that a faeces score of 2 is not equivalent to overt diarrhoea. Piglets with more-liquid faeces had shorter villi, which may reflect that shorter villi result in decreased faeces consistency. Nabuurs *et al.* (1993) showed on a herd level that mean villus height in diarrhoeic herds was relatively lower than in specific pathogen free (SPF) herds. Low food intake may lead to shorter villi which in turn causes diarrhoea through maldigestion, malabsorption and increased fermentation. Indeed, the variation in villus height ($R_{\text{Pearson}} = -0{\cdot}38$, $P < 0{\cdot}01$) explained more variation in faeces consistency score than did the variation in food intake ($R_{\text{Pearson}} = -0{\cdot}14$, $P > 0{\cdot}10$). Crypt depth was not associated with faeces consistency. Villus height and crypt depth may influence the pathogenesis of post-weaning diarrhoea, as suggested by Nabuurs *et al.* (1993), through the absorptive and secretive properties of small intestine enterocytes (Powell, 1987). Other factors also play a rôle in the onset of diarrhoea. The pathogens *E. coli* and rotavirus are frequently detected in piglets with post-weaning diarrhoea (Hampson, 1986a; Van Beers-Schreurs *et al.*, 1998; Nabuurs *et al.*, 1993).

In conclusion, the results of this study support the concept that food intake by weaned piglets determines villus height and brush-border enzyme production in the small intestine, which in turn determine the risk of diarrhoea development. The negative correlation between villus height and the excretion of more-liquid faeces has not been reported before. It is clear that, under the conditions of the present study, the relatively simple measure, faeces consistency, reflected the more complex measure, small intestinal villus height. Given the current means of raising piglets, it is a challenge to formulate diets that stimulate post-weaning food intake.

References

Ball, R. O. and Aherne, F. X. 1982. Effect of diet complexity and feed restriction on the incidence and severity of diarrhoea in early-weaned pigs. *Canadian Journal of Animal Science* **62:** 907-913.

Bruininx, E. M. A. M., Peet-Schwering, C. M. C. van der, Schrama, J. W., Vereijken, P. F. G., Vesseur, P. C., Everts, H., Hartog, L. A. den and Beynen, A. C. 2001. Individually measured feed intake characteristics and growth performance of group-housed weanling pigs: effects of sex, initial body weight, and body weight distribution within groups. *Journal of Animal Science* **79:** 301-308.

Cera, K. R., Mahan, D. C., Cross, R. F., Reinhart, G. A. and Whitmoyer, R. E. 1988. Effect of age, weaning and postweaning diet on small intestinal growth and small intestinal morphology in young swine. *Journal of Animal Science* **66:** 574-584.

Dunsford, B. R., Knabe, D. A. and Haensly, W. E. 1989. Effect of dietary soybeanmeal on the microscopic anatomy of the small intestine in the early weaned pig. *Journal of Animal Science* **67:** 1855-1863.

Efird, R. C., Armstrong, W. D. and Herman, D. L. 1982. The development of digestive capacity in young pigs: effects of age and weaning system. *Journal of Animal Science* **55:** 1380-1387.

Fan, M. Z., Stoll, B., Jiang, R. and Burrin, D. G. 2001. Enterocyte digestive enzyme activity along the crypt-villus and longitudinal axes in the neonatal pig small intestine. *Journal of Animal Science* **79:** 371-381.

Fraser, D., Milligan, B. N., Pajor, E. A., Philips, P. A., Taylor, A. A. and Weary, D. M. 1998. Behavioural perspectives on weaning in domestic pigs. In *Progress in pig science* (ed. J. Wiseman, M. A. Varley and J. P. Chadwick), pp. 121-138. Nottingham University Press, Nottingham.

Hall, G. A. and Byrne, T. F. 1989. Effects of age and diet on small intestinal structure and function in gnotobiotic piglets. *Research in Veterinary Science* **47:** 387-392.

Hampson, D. J. 1986a. Attempts to modify changes in the piglet small intestine after weaning. *Research in Veterinary Science* **40:** 313-317.

Hampson, D. J. 1986b. Influence of creep feeding and dietary intake after weaning on malabsorption and occurrence of diarrhoea in newly weaned pig. *Research in Veterinary Science* **41:** 63-69.

Kelly, D., Smyth, J. A. and McCracken, K. J. 1991a. Digestive development of the early weaned pig; effect of continuous nutrient supply on the development of the digestive tract and on changes in digestive enzyme activity during the first week post-weaning. *British Journal of Nutrition* **65:** 169-180.

Kelly, D., Smyth, J. A. and McCracken, K. J. 1991b. Digestive development of the early weaned pig; effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. *British Journal of Nutrition* **65:** 181-188.

Le Dividich, J. and Herpin, P. 1994. Effects of climatic conditions on the performance, metabolism and health status of weaned piglets: a review. *Livestock Production Science* **38:** 79-90.

Leibbrandt, V. D., Ewan, R. C., Speer, V. C. and Zimmerman, D. R. 1975. Effect of weaning and age at weaning on baby pig performance. *Journal of Animal Science* **40:** 1077-1080.

Lopez-Pedrosa, J. M., Torres, M. I., Fernandez, M. I., Rios, A. and Gill, A. 1998. Severe malnutrition alters lipid composition and fatty acid profile of the small intestine in newborn piglets. *Journal of Nutrition* **128:** 224-233.

Marion, J., Biernat, M., Savaray, G., Thomas, F., Zabielski, R., Le Huërou-Luron, I. and Le Dividich, J. 2002. Effect d'un sevrage à l'âge de 7 jours et du niveau alimentaire après le sevrage sur les modifications structurales de l'intestine grêle chez le porcelet. *Journées de la Recherche Porcine en France* **34:** 89-95.

Marouz, S., Louvard, D. and Barratti, J. 1973. The aminopeptidase of high intestinal brush border. *Biochemical and Biophysical Acta* **321:** 282-295.

Messer, M. and Dahlqvist, A. 1966. A one step ultramicromethod for the assay of intestinal disaccharidases. *Analytical Biochemistry* **14:** 376-392.

Miller, B. G., James, P. S., Smith, M. W. and Bourne, F. J. 1986. Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. *Journal of Agricultural Science, Cambridge* **107:** 579-589.

Nabuurs, M. 1991. Etiologic and pathogenic studies on postweaning diarrhoea. *Ph. D. thesis, University of Utrecht.*

Nabuurs, M. J. A., Hoogendoorn, A., Molen, E. J. van der and Osta, A. L. M. van. 1993. Villus height and crypt dept in weaned and unweaned pigs, reared under various circumstances in the Netherlands. *Research in Veterinary Science* **55:** 78-84.

National Research Council. 1998. *Nutrient requirements of swine, 10th edition*. National Academy Press, Washington, DC.

Núñez, M. C., Bueno, J. D., Ayudarte, M. V., Almendros, A., Rios, A., Suarez, M. D. and Gil, A. 1996. Dietary restriction induces biochemical and morphometric changes in the small intestine of nursing pigs. *Journal of Nutrition* **126:** 933-944.

Pluske, J. R., Hampson, D. J. and Williams, I. H. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livestock Production Science* **51:** 215-236.

Pluske, J. R., Williams, I. H. and Aherne, F. X. 1996. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. *Animal Science* **62:** 145-158.

Powell, D. W. 1987. Intestinal water and electrolyte transport. In *Physiology of the gastrointestinal tract, second edition* (ed. J. R. Johnson), pp. 1267-1305. Raven Press, New York.

Pusztai, A., Koninkx, J., Hendriks, H., Kok, W., Hulshcer, S., Van Damme, E. J. M., Puemans, W. J., Grant, G. and Bardocz, S. 1996. Effect of the insecticidal *Galanthus nivalis* agglutinin on metabolism and the activities of brush border enzymes in the rat small intestine. *Nutritional Biochemistry* **7:** 677-682.

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke,

N. M., Olson, B. J. and Klenk, D. C. 1985. Measurements of protein using bicinchoninic acid. *Analytical Biochemistry* **150:** 76-85.

Spreeuwenberg, M. A. M. 2002. Diet composition and gut integrity in weaned piglets. *Ph. D. thesis, Wageningen University, Wageningen.*

Spreeuwenberg, M. A. M., Verdonk, J. M. A. J., Gaskins, H. R. and Verstegen, M. W. A. 2001. Small intestine epithelial barrier function is compromised in pigs with low feed intake at weaning. *Journal of Nutrition* **131:** 1520-1527.

Van Beers-Schreurs, H. M. G., Nabuurs, M. J. A., Vellenga, L., Wensing, Th. and Breukink, H. J. 1998. Role of the large intestine in the pathogenesis of diarrhoea in weaned pigs. *Journal of Nutrition* **59:** 696-703.

Verdonk, J. M. A. J., Spreeuwenberg, M. A. M., Bakker, G. C. M. and Verstegen, M. W. A. 2001. Effect of protein source and feed intake level on the small intestine in newly weaned piglets. In *Digestive physiology of pigs* (ed. J. E. Lindberg and B. Ogle), pp. 347-349. CABI Publishing, Wallingford, UK.

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