

# **Diet Composition and Gut Integrity in Weaned Piglets**

Promotoren:

Prof. Dr. Ir. M. W. A. Verstegen  
Hoogleraar in de Diervoeding  
Wageningen Universiteit

Prof. Dr. Ir. A. C. Beynen  
Hoogleraar Veterinaire Diervoeding  
Faculteit Diergeneeskunde  
Universiteit Utrecht

Promotiecommissie:

Prof. Dr. Ir. L. A. den Hartog, Wageningen Universiteit  
Dr. J. Pluske, Murdoch University, Australia  
Prof. Dr. Ir. G. J. Schaafsma, Wageningen Universiteit  
Prof. Dr. J. H. M. Verheijden, Universiteit Utrecht

# **Diet Composition and Gut Integrity in Weaned Piglets**

Maria Antoinette Martina Spreeuwenberg

Proefschrift  
ter verkrijging van de graad van doctor  
op gezag van de rector magnificus  
van Wageningen Universiteit,  
Prof. Dr. Ir. L. Speelman,  
in het openbaar te verdedigen  
op vrijdag 22 november 2002  
des namiddags te half twee in de Aula

Spreeuwenberg, M. A. M., 2002. Diet Composition and Gut Integrity in Weaned Piglets.  
PhD Thesis. Wageningen University, Wageningen, The Netherlands.  
ISBN: 90-5808-738-7

---

# CONTENTS

---

Chapter 1	General Introduction	1
Chapter 2	Diet-mediated Modulation of Small Intestinal Integrity in Weaned Piglets: a Review	7
Chapter 3	Small Intestine Epithelial Barrier Function is compromised in Pigs with Low Feed Intake at Weaning	57
Chapter 4	Effect of Dietary Protein Source on Feed Intake and Small Intestinal Morphology in Newly Weaned Piglets	77
Chapter 5	Dietary Protein Hydrolysates versus the Intact Proteins do not enhance Mucosal Integrity and Growth Performance in Weaned Piglets	93
Chapter 6	Villus Height and Gut Development in Weaned Piglets receiving Diets containing either Glucose, Lactose or Starch.	113
Chapter 7	Interrelationship between Gut Morphology and Faeces Consistency in Newly Weaned Piglets	127
Chapter 8	General Discussion	143
Summary		161
Samenvatting		167
List of Publications		173
Nawoord		175
Curriculum Vitae		177



---

# CHAPTER 1

---

## General Introduction

In modern pig husbandry in the Netherlands, piglets are abruptly weaned at 24-28 days of age. The weaning transition usually involves complex social, environmental and nutritional changes for the piglets (e.g. Fraser et al., 1998) and is generally accepted as a stressful event (e.g. Worsaae and Schmidt, 1980). The transition from suckling to eating solid food is associated with a critical period of underfeeding during which the piglet is adapting itself to dry food (Le Dividich and Herpin, 1994). It may take 4 (Le Dividich and Herpin, 1994; Pluske et al., 1996) to 7 days (Bruininx, 2002) after weaning before piglets reach on average a level of feed intake meeting the energy requirement for maintenance. Therefore the observed growth stasis after weaning is not surprising (Leibrandt et al., 1975). Another postweaning problem is the occurrence of diarrhoea (Nabuurs, 1991). The small intestine is thought to play an important role in the etiology of digestive disorders. The gastrointestinal tract not only provides for the digestion and absorption of nutrients, but also acts as a barrier for bacteria, toxins and allergic compounds that otherwise may reach the systemic organs and tissues. The marked changes that occur in gut structure and function after weaning, such as villous atrophy and crypt hyperplasia (Hampson, 1986; Miller et al., 1986; Kelly et al., 1991a; Nabuurs 1991; Pluske et al., 1996), are generally associated a temporary decrease in digestive and absorptive capacity of the small intestine. The concerted actions of the small intestine regarding absorption and exclusion of pathogenic compounds are addressed to as small intestinal integrity. Not only was small intestinal morphology investigated as a parameter for intestinal integrity, but also functionality (enzyme secretion and permeability across the gut wall) and indicators of inflammation (cell differentiation molecules on T-cell lymphocytes, i. e. CD4<sup>+</sup> and CD8<sup>+</sup> T-cell lymphocytes and haptoglobin levels in blood plasma). The generally assumed negative association between small intestinal integrity on one hand and digestive disorders on the other hand is not clear.

During the weaning transition of piglets, two successive phases in the small intestinal integrity can be distinguished: a de- and regenerative phase. Within the degenerative phase, McCracken and colleagues (1995; 1999) suggested that diet-independent and diet-dependent metabolic and morphologic changes occur during the weaning transition in pigs. The diet-independent changes result from stress induced by a change in environment and social surroundings at weaning. The diet-dependent changes largely reflect differences in feed intake (McCracken, 1995; 1999; Kelly et al., 1991b; Van Beers-Schreurs, 1996; Verdonk et al., 2001). Subsequently, in the regenerative phase, the mucosal reconditioning is suggested to be diet-dependent. To assess the effect of dietary components in weaned piglets independently of feed intake, the piglets were offered a pre-determined maximum amount of feed. A quick recovery on small intestinal integrity is likely to be critical for preventing secondary infections. The objective was to investigate the effect of diet composition on small intestinal integrity and digestive disorders in weaned piglets, attempting either to ameliorate the decrease in small intestinal integrity or to enhance its recovery.



For mucosal reconditioning, nutrient availability is thought to play an important role. An integrated concept of the pathogenesis of the post-weaning syndrome is described in Chapter 2. Briefly, the low feed intake after weaning results in a lack of enteral nutrition for the small intestinal enterocytes, which is followed by an impairment of mucosal function. This may result in maldigestion/malabsorption, and subsequently poor performance. The overall hypothesis tested was that increasing the availability of nutrients for the mucosa will support the intestinal integrity in both the de- and regenerative phase. The macronutrients protein and carbohydrates are most abundantly available in a weaner diet. Increasing their availability and/or quality may affect small intestinal integrity. The effect of lactose versus protein was investigated by changing the ratio lactose/protein in the diet (Chapter 3). The effect of protein source was investigated by supplying either poorly or highly digestible protein (Chapter 4), protein hydrolysates (Chapter 5) or the inclusion of glutamine (Chapter 5). The effect of carbohydrate source was investigated by feeding the piglets either glucose, lactose or native starch. Figure 1 shows the schematic outline of this thesis.

In summary, the scope of the present thesis was as follows:

- to investigate the effect of de- and regeneration of small intestinal integrity in time rather than only looking to a single point in time.
- to investigate the effect of diet composition on small intestinal integrity: not just measuring small intestinal morphology, but also investigating the functionality of the intestine (enzyme secretion and permeability across the gut wall) and indicators of inflammation (cell differentiation molecules on T-cell lymphocytes, i. e. CD4<sup>+</sup> and CD8<sup>+</sup> T-cell lymphocytes and haptoglobin levels in blood plasma)
- to investigate the effect of protein or carbohydrates on small intestinal integrity. The effect of changing the ratio of lactose to protein was investigated. The effect of protein was tested by feeding piglets (I) poorly or highly digestible protein, (II) protein hydrolysates versus the native protein sources, or (III) by supplementation of the diet with the single amino acid glutamine. For carbohydrates, the effect of feeding either the monosaccharide glucose, the disaccharide lactose or the polysaccharide starch was investigated
- to assess the association between feed intake, small intestinal integrity, growth performance and diarrhoea

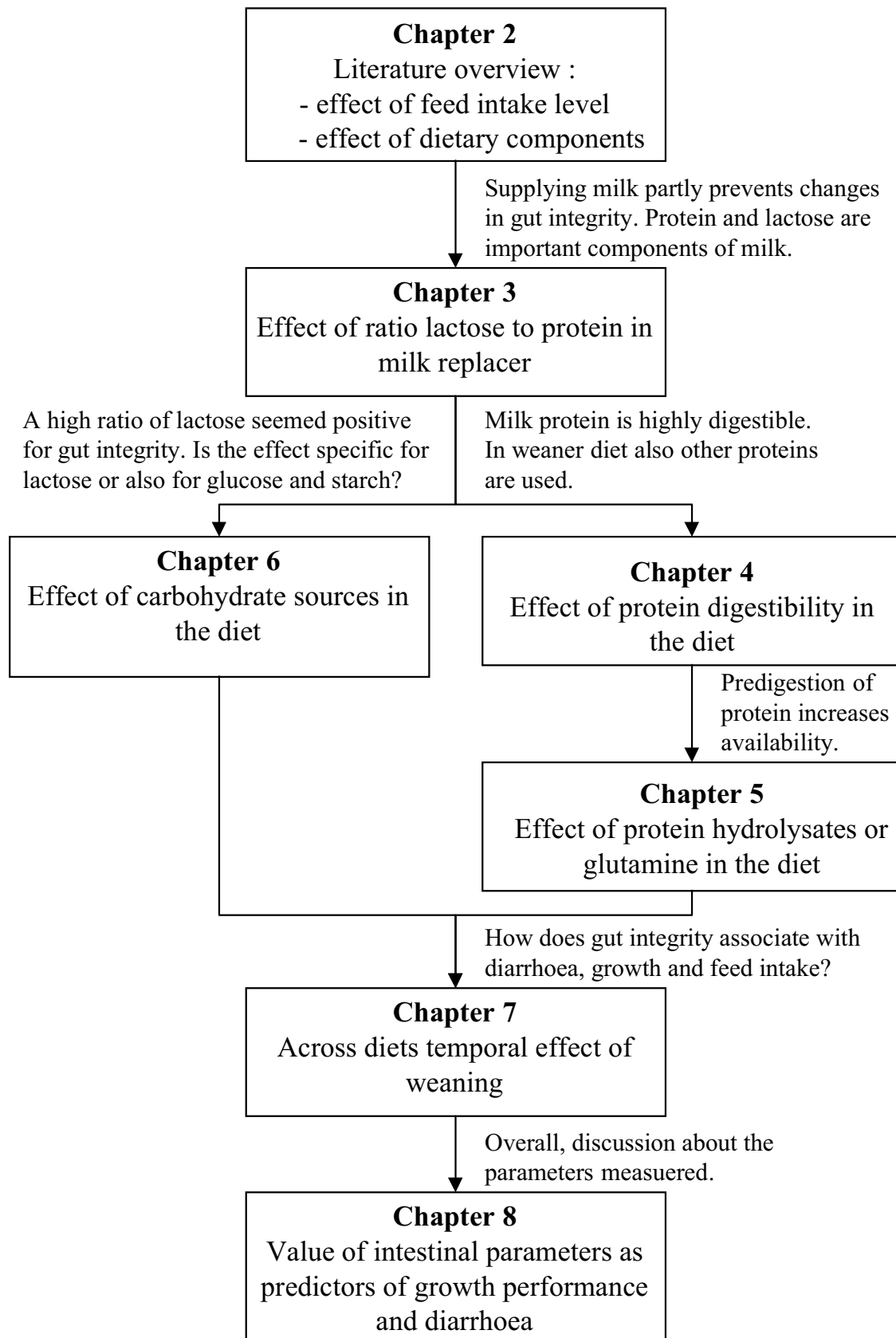


Figure 1 A schematic representation of the outline of this thesis.

## LITERATURE CITED

- Bruininx, E. M. A. M. 2002. Individually measured feed intake characteristics in group-housed weanling pigs. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- Fraser, D., B. N. Milligan, E. A. Pajor, P. A. Philips, A. A. Taylor, and D. M. Weary. 1998. Behavioural perspectives on weaning in domestic pigs. Pages 121-138 in *Progress in Pig Science*. J. Wiseman, M. A. Varley, and J. P. Chadwick, eds. Nottingham University Press, Nottingham, UK.
- Hampson, D. J. 1986. Attempts to modify changes in the piglet small intestine after weaning. *Res. Vet. Sci.* 40: 313-317.
- Kelly, D., J. A. Smyth, and K. J. McCracken. 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 weak post-weaning. *Brit. J. Nutr.* 65: 169-180.
- Kelly, D., J. A. Smyth, and K. J. McCracken. 1991b. Digestive development of the early weaned pig; effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. *Brit. J. Nutr.* 65: 181-188.
- Le Dividich, J., and P. Herpin. 1994. Effects of climatic conditions on the performance, metabolism and health status of weaned piglets: a review. *Livest. Prod. Sci.* 38: 79-90.
- Leibbrandt, V. D., R. C. Ewan, V. C. Speer, and D. R. Zimmerman. 1975. Effect of weaning and age at weaning on baby pig performance. *J. Anim. Sci.* 40(6): 1077-1080.
- McCracken, B. A., H. R. Gaskins, P. J. Ruwe-Kaiser, K. C. Klasing, and D. E. Jewell. 1995. Diet-dependent and diet-independent metabolic responses underlie growth stasis of pigs at weaning. *J. Nutr.* 125: 2838-2845.
- McCracken, B. A., M. E. Spurlock, M. A. Roos, F. A. Zuckermann, and H. R. Gaskins. 1999. Weaning anorexia may contribute to local inflammation in the piglet small intestine. *J. Nutr.* 129: 613-619.
- Miller, B. G., P. S. James, M. W. Smith, and F. J. Bourne. 1986. Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. *J. Agric. Sci. Camb.* 107: 579-589.
- Nabuurs, M. J. A. 1991. Etiologic and pathogenic studies on postweaning diarrhea. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- Pluske, J.R., I. H. Williams, and F. X. Aherne. 1996. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. *Anim. Sci.* 62: 145-158.
- Van Beers-Schreurs, H. 1996. The changes in the function of the large intestine of weaned pigs. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- Verdonk, J. M. A. J., M. A. M. Spreeuwenberg, G. C. M. Bakker, and M. W. A. Verstegen. 2001. Nutrient intake level affects histology and permeability of the small intestine in newly weaned piglets. Pages 332-334 in *Digestive physiology of pigs*. J. E. Lindberg, and B. Ogle, eds. Cabi Publishing, Wallingford, UK.
- Worsaae, H., and M. Schmidt. 1980. Plasma cortisol and behaviour in early weaned piglets. *Acta Vet. Scan.* 21: 640-657.



---

## CHAPTER 2

---

# **Diet-mediated Modulation of Small Intestinal Integrity in Weaned Piglets: a Review**

M. A. M. Spreeuwenberg <sup>1</sup>, and A. C. Beynen <sup>2</sup>.

<sup>1</sup> Nutreco Swine Research Centre, Boxmeer, The Netherlands; <sup>2</sup> Department of Nutrition, Faculty of Veterinary Medicine, University of Utrecht, The Netherlands.

Accepted for publication in:

J. R. Pluske, J. Le Dividich, and M. W. A. Verstegen. The Weaner Pig: Concepts and Consequences. Wageningen Pers, Wageningen, The Netherlands.

## ABSTRACT

Piglets are faced with multiple changes around the weaning transition. This generally results in low voluntary feed intake, sub-optimal growth rate, and diarrhoea may occur frequently. The small intestine not only digests and absorbs nutrients, but also excludes pathogens, toxins and allergic compounds. Small intestinal function depends on its integrity, which can be assessed on the basis of indicators such as villous length, crypt depth, number of goblet cells, transepithelial permeability, brush border enzyme activity and growth performance. Weaning of piglets negatively affects small intestinal integrity as indicated by a decrease in villous length, an increase in paracellular permeability and a decrease in total brush border enzyme activities. This review focuses on dietary modulation of the weaning-induced impairment of small intestinal integrity. It is concluded that the level of feed intake is the most important determinant of mucosal function and integrity. Thus, the temporal low feed intake immediately after weaning is the main cause of the decrease in small intestinal integrity. Furthermore, the actual amount of feed consumed is positively correlated with the development of the small intestine. Studies reviewed are those dealing with potential functional feed ingredients, including protein source, specific amino acids, fatty acids, fibres, non-digestible oligosaccharides, growth factors, polyamines, and nucleotides. It is concluded that the individual feed constituents have only marginal effects on small intestinal integrity of the weaned pig. Possibly, combinations of functional feed ingredients will be more successful. Further research should involve identification of determinants of feed intake immediately after weaning and functional feed ingredients to stimulate epithelial cell proliferation and differentiation, enhance immune function, and promote growth of beneficial bacteria.

## INTRODUCTION

At weaning, piglets are faced with changes of various nature. Under commercial conditions, weaning at 24-28 days of age usually involves complex social changes for the piglets, including their separation from the mother, separation from litter-mates and exposure to unfamiliar counterparts (Fraser et al., 1998). The composition of the piglets' diet changes drastically at weaning; the liquid milk from the sow is replaced by pelleted dry feed with starch instead of fat as the main energy source. The transition from suckling to eating solid food is associated with a critical period of underfeeding during which the pig is adapting itself to the dry food (Le Dividich and Herpin, 1994). The low feed intake during the first two days after weaning, which essentially is independent of diet composition (McCracken et al., 1995), causes growth stasis (Leibrandt et al., 1975; McCracken et al., 1995; 1999).

Diarrhoea frequently occurs after the weaning transition (Nabuurs, 1991). The gastrointestinal tract not only allows for the digestion and absorption of nutrients, but also acts as a barrier for bacteria, toxins and allergic compounds that otherwise may reach the systemic organs and tissues. For the small intestine, the level of feed intake is a critical determinant of its digestive and absorptive capacity (Pekas, 1991) and also of its barrier function (Bishop et al., 1992). The low feed intake caused by weaning often leads to maldigestion and malabsorption and also to reduced small intestinal barrier function. When feed intake increases, diarrhoea may occur. Enterotoxemic bacteria proliferate and release their toxins. An integrated concept of the response to weaning is given in Figure 1.

### Pathogenesis of the post-weaning syndrome

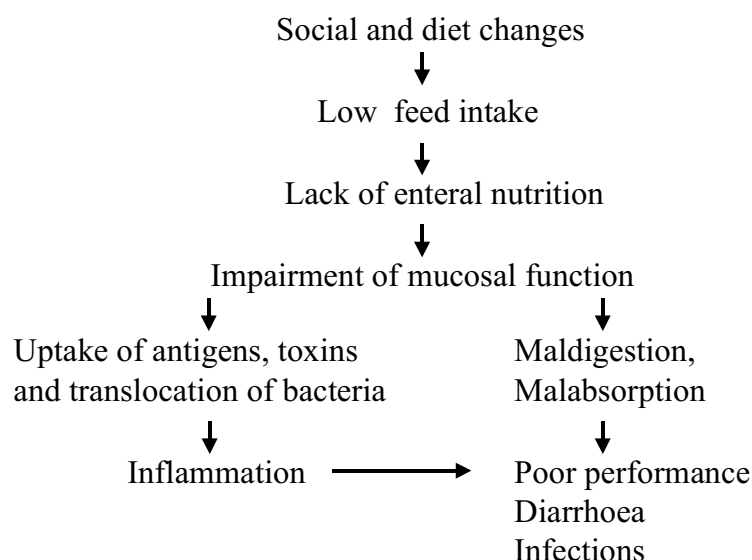


Figure 1 An integrated concept of the effect of weaning on mucosal barrier function, performance and health in piglets.

The problems associated with weaning are mainly a consequence of the commercial conditions. Weaning of piglets at an age as young as possible increases the number of piglets per sow per year. Under natural conditions, piglets gradually develop the capability to digest solid food and voluntarily reduce their intake of milk. Thus, the piglets themselves control the weaning process. Some nursing may still continue until the piglets are 12-16 weeks of age, this being considered the natural age of weaning (Jensen and Recén, 1989; Fraser et al., 1998).

Based on general knowledge of the influence of nutrition on gut function and health, diets may be formulated that alleviate or prevent the adverse effects of weaning at 4 weeks of age. The objective of this chapter is to highlight the nutritional opportunities to modulate the

intestinal barrier function after weaning, thus resulting in increased piglet performance. It is beyond the scope of this chapter to review the effect of hormones on small intestinal integrity. Prior to describing the effects of diet composition, the indicators of small intestinal integrity will be discussed briefly.

## SMALL INTESTINAL INTEGRITY

One important function of the gastrointestinal tract is to transform ingested food so that absorbable nutrients become available for the body. Morphologically, the small intestine represents a maximum absorptive surface. The presence of Kerckring's folds, villi and microvilli in the small intestine produces a large surface area compared with that of a cylindrical tube (Junqueira and Cerneiro, 1980; Caspary, 1987, 1992; Dyce et al., 1987). The small intestinal villi of healthy piglets are predominantly finger-shaped with few tongue-shaped villi (Mouwen, 1972). A 10-day old, 3-kg piglet has a relatively small intestine with a total absorptive surface area of 114 m<sup>2</sup> (Buddle and Bolton, 1992). The epithelial cells lining of the gastrointestinal tract renew rapidly. The small intestinal villus epithelium in 1-day-old pigs is replaced in 7-10 days, whereas this process in 3-week-old pigs takes 2-4 days (Moon, 1971). The epithelial cells have apical 0.5-2 µm intercellular attachment zones or junctional complexes, which join them together. These tight junctions regulate epithelial permeability by influencing paracellular flow of fluid and constituents. In general, the complexity, strand number and depth of the tight junction correlate inversely with the permeability of epithelia (Trier and Madara, 1981).

The gastrointestinal tract provides an extensive surface area with intimate contact between the host organism and dietary substances, microorganisms, parasites and exogenous toxins. The intestine permits the uptake of dietary substances into the systemic circulation, but at the same time excludes pathogenic compounds (Gaskins, 1997). The gastrointestinal tract has multiple non-specific and immunological defence mechanisms. The non-specific defence includes gastric acid production, peristaltis, mucus layer, tight junctions, epithelial desquamation, proteolysis, resistance against colonisation of pathogenic bacteria, and the gut-liver axis. The immunological defence of the small intestine includes the production of secretory immunoglobulins, M-cells, and lymphocytes (Madara et al. 1990; Walker and Owen, 1990; Deitch, 1993; Wang, 1995). Components of the intestinal barrier are shown in Figure 2.

Concurrent absorption of nutrients and exclusion of pathogenic compounds is achieved through concerted actions of the small intestine. For example, tight junctions are crucial for baseline intestinal barrier function, but regulation adapts them to the uptake of nutrients (Madara, 1989). That the small intestine has two functions is reflected in the difficulty to interpretate numerical values as to small intestinal integrity. Commonly used indicators of



small intestinal integrity are villus length, crypt depth, number of goblet cells, mucus production, transepithelial permeability, inflammation, brush border enzyme activity, and animal performance. These indicators and their relation to the process of weaning are discussed briefly below.

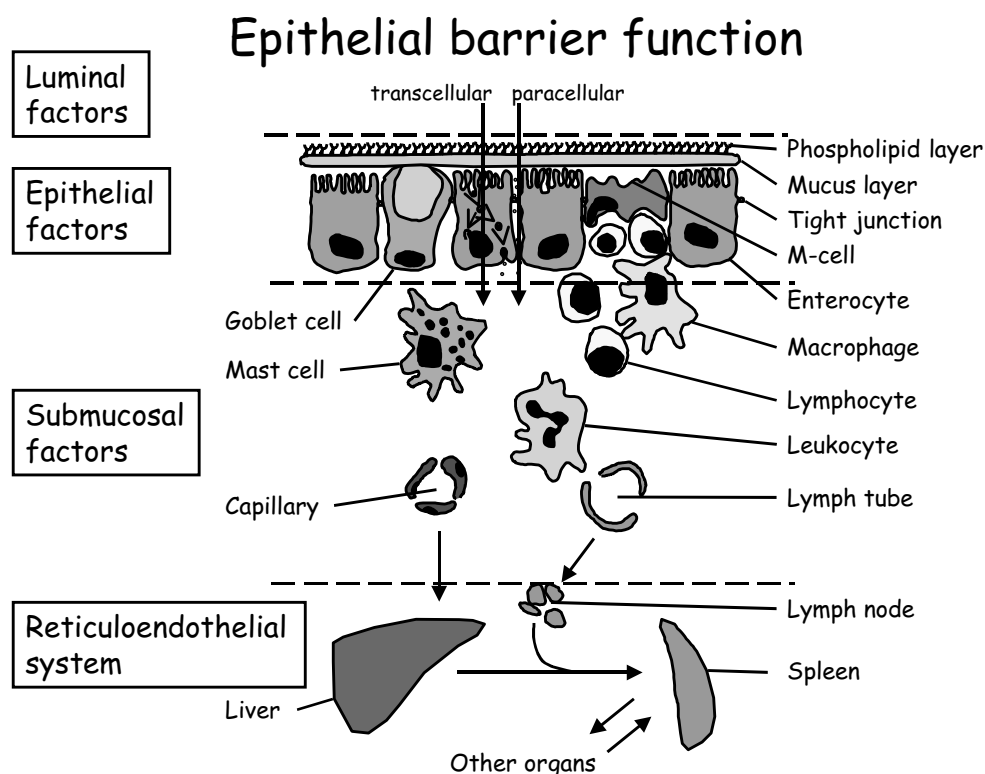


Figure 2 Schematic presentation of the gastrointestinal defence barrier and effector factors (After Wang, 1995).

## Morphology

The depth and shape of the crypts of Lieberkühn, the shape and height of the villi, and the number of goblet cells are indicators of intestinal integrity. The villus orientation and shape has been classified by Mouwen (1972), with classes including tongue-shaped, finger shaped, leaf-shaped, ridged-shaped and convoluted villi. Small intestinal integrity is most commonly assessed by histologic measurements of villus height and crypt dept. Weaning causes a reduction in villus height and an increase in crypt depth (Hampson, 1986; Miller et al., 1986; Cera et al., 1988; Dunsfort et al., 1989; Hall and Byrne, 1989; Kelly et al., 1991a; Nabuurs et al., 1993; Pluske et al., 1996a; 1996b). Villous atrophy after weaning is caused by a combination of increased rate of cell loss and reduced rate of cell renewal (Pluske et al., 1997). The histological changes are smaller with higher postweaning feed intakes (Kelly et al., 1991b; McCracken et al., 1995; Van Beers-Schreurs, 1996; Pluske 1996b). Ideally, specific diet formulations for weanling piglets should ameliorate the weaning-induced decrease in villus height.

## **Mucus production**

The mucus protects the mucosa against digestive secretions, pathogens and physico-chemical damage (Mantle and Allen, 1989; Stokes and Bourne, 1989; Forstner and Forstner, 1994). Binding of pathogens to mucins rather than to epithelial cells is generally regarded as an important host defence mechanism (Forstner and Forstner, 1994). Mucus gel is stored in the intestinal goblet cells and secreted by baseline or accelerated secretion (Lamont, 1992). Baseline secretion is continuous and provides renewal of the mucus coat that is lost due to erosion, digestion and luminal digesta flow. Accelerated secretion is characterised by rapid, massive goblet discharge in response to physiological or pathological stimuli (Lamont, 1992, Epple et al., 1997), including inflammatory mediators (Specian and Neutra, 1982; Cohen et al., 1991; Plaisancié et al., 1998) and bacterial toxins (Roomi et al., 1984; Cohen et al., 1991, Epple et al., 1997). The actual amount of mucus secreted cannot be measured. An increase in the number of goblet cells might point to increased mucus production. Weaning of piglets has been shown to result in either unchanged (Dunsford et al., 1991; McCracken et al., 1999) or decreased (McCracken et al., 1995) numbers of goblet cells in the villi, and unchanged (McCracken et al., 1995; Chapter 3) or decreased (Dunsford et al., 1991) numbers of goblet cells in the crypts. The importance of the number of goblet cells as an indicator of intestinal integrity seems limited due to the inconsistent response to weaning.

## **Transepithelial permeability**

Small intestinal integrity can be estimated on the basis of intestinal permeability for macromolecules, which can be measured as passive diffusion of a marker compound. Ideal markers cross the intestinal epithelium by non-mediated diffusion, are recovered quantitatively after oral administration, and can be reliably measured in blood or urine by a convenient technique (Uil et al., 1997). Various probe molecules have been used to measure intestinal permeability, including the sugars lactulose and mannitol (Uil et al., 1997), horseradish peroxidase, ova-albumin and chromium-labeled ethylene diamine tetra-acetate ( $^{51}\text{Cr}$ -EDTA) (Vellenga, 1989; Bjarnason et al., 1995). Transepithelial transport can also be measured with the use of Ussing chambers. An intestinal biopsy is placed in an Ussing chamber separating the mucosal and serosal site of the tissue. The marker is added at the mucosal site. At given time points the serosal fluid is sampled to measure the amount of marker that has crossed the epithelium. The trans-epithelial electrical resistance (TEER) and short circuit current (Isc) may also be measured. The TEER has been suggested to reflect tight junction function (Wirén et al., 1999), whereas the Isc reflects ion pump activity (Wirén et al., 1999). With increased paracellular transport of markers it is anticipated that mucosal integrity is diminished and that pathogens and toxins may cross the epithelial barrier. Weaning results in increased paracellular transport for mannitol in transport chambers (Verdonk et al., 2001). Plasma xylose concentration after oral administration was similar in weaned and unweaned

piglets (Pluske et al., 1996b). Thus, the formulation of diets for weanling piglets may aim at reducing the paracellular transport of an appropriate marker in an intestinal biopsy placed in a transport chamber.

## **Inflammation**

If and when bacteria or other deleterious agents cross the first line of defence and reach the connective tissue of the lamina propria, their metabolites or mediators liberated from epithelial cells may evoke an inflammatory response (Gaskins, 1997). The different T-cell subsets or major histocompatibility complex (MHC) classes indicate the status of small intestinal immunity. Class I MHC molecules interact with CD8-positive T-cells which usually have a cytotoxic function. Class II MHC molecules interact with CD4-positive T-cells which provide help to the antigenic peptide recognition (Shanahan, 1994). The measurement of pro-inflammatory cytokines provides information as to local inflammation. The production of interleukin-1 (Il-1), Il-6 and tumor necrosis factor (TNF) occurs rapidly following infection, tissue injury and trauma. The cytokines activate receptors on different target cells, leading to a wide range of effects, including anorexia, fever and acute phase protein production (Gruys et al., 1999), and also inhibition of growth (Johnson, 1997). Weaning results in an inflammatory response as measured by an increased production of Il-1 on day 1 and 2 postweaning (McCracken et al., 1995). However, the production of TNF is unchanged when compared to the production rates on the day of weaning (McCracken et al., 1995). With an average digestible energy intake of 1575 kJ during the first four days after weaning, the ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T-cell subsets decreased when compared to the ratio on the day of weaning, which might point to an inflammatory response (Chapter 3). Thus, the formulation of diets for weanling piglets may aim at reducing inflammation. Interleukins are indicators, which measure an inflammatory response directly. Decreased ratios of CD4<sup>+</sup> to CD8<sup>+</sup> T-cells or MHC II to MHC I classes are indirect indicators of an inflammatory response.

## **Brush border enzyme activity**

The enzyme activity of the brush border and pancreas may also serve as indicators of small intestinal function. The maturing enterocytes embedded in the apical membrane of the small intestine synthesise enzymes to hydrolyse disaccharides and small peptides (Caspary, 1992). Enzyme production of enterocytes during the weaning transition of piglets is determined by villus height and maturity of the enterocytes (Smith et al., 1985; Miller et al., 1986). 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 increased enzyme activity at the villus tip is consistent with enterocyte differentiation (Fan et al., 2001). Enzyme activity may be expressed in units produced per time interval (total enzyme activity), in units per gram of brush border membrane protein (specific activity) or units per cm of small

intestine. Weaned piglets have low specific activity of sucrase, lactase (Hampson, 1986; Miller et al., 1986; Kelly et al., 1991a) and isomaltase (Miller et al., 1986) when compared to unweaned piglets of the same age. The effect of weaning on disaccharidase activity is less pronounced when the pigs are weaned at an older age (Miller et al., 1986). Activities of maltase II and maltase III increase in response to weaning at six weeks of age when compared to unweaned piglets of the same age, but show no change in four-week-old pigs (Miller et al., 1986). Pluske and colleagues (1997) showed that maltase and glucoamylase activities increased with age (2 versus 4 weeks of age) and with day postweaning. Kelly and co-workers (1991a) reported increases in specific activities of maltase and amylase on 7 days postweaning when compared to sow-reared piglets of the same age. The discrepancy in response of various disaccharidases specific activities compared to weaning might be explained by substrate induction through the weaner diet. Efird and colleagues (1982) found an increased amount of trypsin and chymotrypsin ( $\text{g} \cdot (\text{kg body weight})^{-1}$ ) in the intestinal contents and a decreased amount of pancreatic trypsin and chymotrypsin ( $\text{g} \cdot (\text{kg body weight})^{-1}$ ). The sum of trypsin and chymotrypsin activities tended to be lower in weaned piglets compared to sow-reared piglets (Efird et al., 1982). Thus, the formulation of diets for weanling piglets may aim at stimulating the production of disaccharidase and pancreatic enzyme activity in order to maintain the digestive capacity.

### **Animal performance**

The length and weight of the small intestine, the weight of the digestive organs, the average daily gain (ADG) and the health status are indicators of digestive development and capacity, and thus of intestinal integrity. These indicators are positively influenced by feed intake, which is the most important determinant. As mentioned above, high feed intakes after weaning counteract the weaning-induced negative changes in indicators of gut integrity. A major goal of formulating diets for weanling piglets is to stimulate feed intake.

## **MODULATION OF INTEGRITY BY LUMINAL NUTRITION**

During periods of stress, such as weaning, the nutrients that are required for cell turnover and maintenance of barrier function are critically important. These nutrients can be supplied via the intestinal lumen or via the splanchnic blood flow. Factors in response to ingestion and digestion of food acting on mucosal growth include cell loss, local nutrients, bulk properties and pH. Additionally, gastrointestinal hormones and nerves also act on mucosal growth (Johnson and McCormack, 1994), but are outside the scope of this review. The effect on intestinal integrity of route of nutrient supplementation, energy intake level and specific dietary components will be discussed below.

## Modulation by route of administration

Exposure of the gastrointestinal tract to nutrients is essential for maintaining its integrity (Goldstein et al., 1985; Bishop et al., 1992; Park et al., 1998; Bertolo et al., 1999; Ganessunker et al., 1999; Burrin et al., 2000). The importance of the presence of food in the lumen of the gastrointestinal tract (luminal nutrition) on mucosal integrity can be assessed by intravenous (parenteral) feeding as the sole source of nutrition. Table 1 summarises studies comparing the effects of total parenteral nutrition (TPN) versus enteral nutrition (EN).

Despite similar body-weight gain in all studies, total intestinal mass, mucosal mass, villus height and villus surface area were all markedly reduced in piglets receiving TPN compared to their counterparts receiving EN (Goldstein et al., 1985; Park et al., 1998; Bertolo et al., 1999; Ganessunker et al., 1999; Burrin et al., 2000). This observation indicates that TPN can supply adequate nutrients to sustain somatic growth, but for intestinal integrity nutrients have to be provided from the luminal site. Interestingly, the intestinal length was not affected by TPN, pointing at selective inhibition of mucosal growth (Park et al., 1998). The lack of enteral stimulation associated with the administration of TPN may alter the intestinal immune cells as shown by an increased number of CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes (Ganessunker et al., 1999). Total mucosal disaccharidase activity was also decreased by TPN (Park et al., 1998). Park and co-workers (1998) showed that provision of enteral nutrition at  $\pm 1\%$  of normal intake was not sufficient for improvement of intestinal integrity compared to non-supplemented piglets. Total parenteral nutrition with enteral IGF-I ( $1000 \mu\text{g} \cdot \text{l}^{-1}$ ) had no effect on intestinal development relative to TPN alone, but the dosage of IGF-I could have been too low (Park et al., 1998).

Burrin and colleagues (2000) showed, in an elegant study, that the minimal enteral nutrient intake necessary for efficacy depends on the measure chosen. Piglets were fed by both intravenous and enteral nutrition, the contribution of the two routes to total feed intake being variable. Irrespective of the intestinal region studied, the amount of enteral nutrition required to increase mass and protein content was less than that required to stimulate proliferative activity as based on measurements of DNA content, crypt depth and BrdU (5-bromodeoxyuridine) incorporation. The protein mass of the proximal region of the intestine was more responsive to a decrease in enteral nutrition than that of the distal region. In contrast, the proportion of enteral nutrition needed to increase cell proliferation showed much less regional variation along the gastrointestinal tract. The daily feed intake in the study was approximately  $900 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ , corresponding with 2800 kJ for piglets of 3.1 kg (Burrin et al., 2000). Maintenance requirement for these piglets is approximately 1040 kJ ME  $\cdot \text{day}^{-1}$  (NRC, 1998) so that they were fed at  $\approx 2.7 \times$  maintenance. Sixty percent of total feed intake in the form of enteral nutrition was necessary to sustain normal mucosal proliferation and growth, which corresponds to  $1.6 \times$  maintenance requirement.

Table 1 Effect of route of feed administration on small intestinal integrity of weanling piglets.

Ref. <sup>1</sup>	Treatments <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
I	- EN (TPN solution) - EN (starter diet) - TPN-IV	- weaned piglets, 6 weeks of age, 10 kg - duration experiment: 0, 21 d - similar energy intake for all treatments: $\pm 711 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ - n=3 / treatment	Comparing TPN-IV vs. EN (TPN solution): - 0 ADG - ↓ intestinal weight - ↓ villus height, 0 crypt depth, ↓ number of epithelial cells - similar lactase, maltase and sucrose specific activity	- ↑ lactase, maltase and sucrose specific activity for EN (starter diet) when compared to EN (TPN solution)
II	- EN (MR) - TPN-IP + water - TPN-IP + EN (MR) - TPN-IP + EN (MR + IGF-I)	- piglets, 1 day postpartum - duration experiment: 0, 7 days postweaning - similar energy and protein intake for all treatments - n=4, 5 or 6 / treatment	Comparing mean of TPN-IP across treatments vs. EN: - 0 ADG - ↓ in intestinal weight (47 %), ↓ mucosal weight (49 %), ↓ mucosal protein content (17 %) - 0 intestinal length - ↓ villus height (24 %), ↓ crypt depth (16 %) - ↓ lactase and sucrose total activity	- no effect of TPN-IP+EN (MR) or TPN-IP+EN (MR+IGF-I) vs. TPN-IP
III	- EN (TPN solution) - TPN-IV - TPN- IP	- piglets, 2 - 4 days postpartum - duration experiment: 8 days postweaning - similar intake - n=5 / treatment	Comparing TPN (IV and IP) vs. EN - 0 ADG - ↓ intestinal weight (60 %), ↓ mucosal weight (41 %) - 0 intestinal length - ↓ villus height, ↓ crypt depth for TPN-IV, 0 crypt depth TPN-IP	
IV	- EN (MR) - TPN-IP	- piglets, 1 day postpartum - duration experiment: 0, 7 days postweaning - n=6	Comparing TPN-IP vs. EN - 0 ADG - ↓ in intestinal weight (50 %) - 0 intestinal length - 0 villus height, ↓ in crypt depth (30 %) - ↑ # goblet cells in villi (147 %), 0 in crypts - ↑ # CD4 <sup>+</sup> and CD8 <sup>+</sup> T-lymphocytes - ↓ in MHC-I (57 %), 0 MHC-II in jejunum, ↑ in MHC-II in ileum (455 %)	- ↑ energy and protein intake for EN

Table 1 Continued.

Ref. <sup>1</sup>	Treatments <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
V	Of diet supplied: - 100 % TPN-IV - 10 % EN + 90 % TPN-IV - 20 % EN + 80 % TPN-IV - 40 % EN + 60 % TPN-IV - 60 % EN + 40 % TPN-IV - 80 % EN + 20 % TPN-IV - 100 % EN	- piglets 7 days postpartum, 3.1 kg - duration experiment: 0, 7 days postweaning - n=5 / treatment - TPN solution either fed via TPN-IV or via EN - balanced for nutrient intake, energy intake for all treatments: 900 kJ · kg <sup>-1</sup> · day <sup>-1</sup>	Comparing increasing percentages of EN - proximal small intestine more sensitive to amount of EN then distal segment - 0 ADG - ↑ in wet weight and protein content in jejunum with from 40 % EN onwards, in ileum from 60 % EN onwards, ↑ in DNA content from 60 % EN onwards - ↑ in villus height from 40 % EN onwards, ↑ in crypt depth from 60 % EN onwards - ↑ in lactase activity from 80 % EN onwards	

<sup>1</sup> References: I: Goldstein et al., 1985; II: Park et al., 1998; III: Bertolo et al., 1999; IV: Ganessunker et al., 1999; V: Burrin et al., 2000

<sup>2</sup> Abbreviations: ADG: average daily gain; d: day; CD: cell differentiation molecules, surface markers of leukocyte subsets; EN: enteral nutrition; IGF-I: insulin like growth factor; MHC: major histocompatibility complex; MR: milk replacer; TPN-IP: total parenteral nutrition fed intraportally; TPN-IV: total parenteral nutrition fed intravenously

<sup>3</sup> 0: similar, ↑: increased, ↓: decreased, #: number

The piglets used in the experiments comparing the effects of TPN and EN were generally weaned at a very young age, i.e. 1 to 7 days postpartum. The young age relates to the fact that the piglets were used as model for low birth weight infants with low nutrient stores, high metabolic rate and immature gastrointestinal development. In piglets weaned at an older age (Goldstein et al., 1985) the results were comparable to those weaned at a younger age. Total parenteral nutrition is also used for critically injured patients (McCauley et al., 1996). The effect of early EN, in addition to TPN, on post-surgery infectious complications or bacterial translocation is not consistent. Some experiments show a reduction in infectious complications (Kudsk, 1994), but others show no effect on bacterial translocation (McCauley et al., 1996). A decreased mucosal integrity through lack of nutrients in the small intestine might reduce its immunological defence mechanisms. So, although TPN is generally used as a model for low birth weight infants or critically ill patients, it can very well be used to study the effect of enteral nutrition on small intestinal integrity.

The effect of short-term starvation immediately after hatching in chickens has been investigated. Under commercial conditions, newly hatched pullets are usually refrained from feed up to a maximum of 48 hours. The delay in access to feed results in decreased body weight when compared to immediate access (Pinchasov and Noy, 1993; Uni et al., 1998; Noy and Sklan 1999), and also leads to decreased villus height and shallower crypts (Uni et al., 1998). Access to a non-nutritious bulk material in the form of sawdust to provide gut fill overcame the loss of body weight during short-term starvation to a similar extent as did access to dry or liquid feed (Noy and Sklan, 1999). This outcome indicates that mechanical stimulation by non-nutritious gut fill is important in the early feeding process. It is not known whether mechanical stimulation per se has positive effects on intestinal integrity in weanling piglets.

### **Modulation by level of energy intake**

Table 2 summarises studies comparing the effect of level of feed intake on small intestinal integrity in early-weaned piglets. Underfed piglets show decreased daily gain, decreased intestinal and mucosal mass and decreased villus height (Kelly et al., 1991b; Núñez et al., 1996; Pluske et al., 1996b; Van Beers-Schreurs, 1996; Lopez-Pedrosa et al., 1998; Verdonk et al., 2001). These piglets also have lower numbers of goblet cells in the villi (Núñez et al., 1996) with low levels of mucin (Lopez-Pedrosa et al., 1998). The effect of low feed intake on crypt depth is inconsistent. Crypt depth was either increased (Núñez et al., 1996; Pluske et al., 1996b), similar (Van Beers-Schreurs, 1996; Verdonk et al., 2001), or decreased (Kelly et al., 1991b) for low versus high feed intake. Shallower crypts are thought to be associated with decreased cell renewal in the crypt and deeper crypts with increased cell proliferation (Pluske et al., 1997). The reason for the differences between studies as to the response of crypt cells to underfeeding is not known.



In general (Table 3), total enzyme activities were decreased and specific activities were increased in malnourished piglets. The increase in enzyme activity when expressed per gram of mucosal protein implies that the relative effect of malnutrition on total protein content of the small intestine is larger than that on enzyme activity. Alternatively, underfeeding leads to an increase in enzyme capacity per enterocyte. Because underfeeding is associated with a negative nitrogen balance it is likely that the increase in specific activity of digestive enzymes is caused by protein depletion of the intestine.

Verdonk et al. (2001) showed increased paracellular transport of mannitol across the small intestinal epithelium in underfed piglets. Wirén and colleagues (1999) investigated the influence of starvation, anesthesia and surgical trauma in rats. Starvation only caused a decrease in villous height in the jejunum and an increase in paracellular permeability in the ileum and jejunum (Wirén et al., 1999). Yang and coworkers (1999) found an inverse relation between the ATP levels in jejunal mucosa and permeability in rats, indicating that low ATP levels are associated with increased permeability. Starvation lowers the TEER, which also points at impaired tight junction function being associated with increased permeability. Starvation also produced a decrease in short-circuit current, indicating a decrease in the ion pump activity (Wirén et al., 1999). So, underfeeding leads to increased paracellular permeability, which is anticipated with diminished mucosal integrity, so that pathogens and toxins may cross the epithelial barrier.

It is possible to improve feed intake at weaning by the use of liquid feeding. In general, improvements in postweaning growth rates have been reported in most of the studies with piglets fed liquid feed versus dry feed. However, the efficiency of the feed utilisation is in general lower in piglets receiving liquid feed compared to those receiving dry feed, as reviewed by Jensen and Mikkelsen (1998). Water consumption also increased by supplying liquid feed (Russell et al., 1996; Schellingerhout et al., 2002b). Water and feed intake are positively correlated (Barber et al., 1989; Schellingerhout et al., 2002b). Deprez and colleagues (1987) observed smaller morphological change in the distal jejunum and in the ileum when a liquid diet (water: feed = 2:1; w:w) instead of a dry feed was offered to weaned piglets. Blanchard and colleagues (2000) studied in a 2 × 2 factorial design the effect of liquid or dry feed fed before and/or after weaning on villus architecture at 25, 50 or 75 % along the small intestinal tract. Piglets fed liquid feed before and after weaning showed increased villus height at 25 % of the small intestine when compared to the other treatments. Crypt depth and number of goblet cells were not affected by dietary treatment. However, in both studies investigating the effect of liquid versus dry feed on gut morphology, no information was given on actual dry matter intakes of the experimental groups. Therefore it is not clear whether the observed increased villus height is due to the liquid feed itself or due to the increased feed intake.

Table 2 Effect of level of feed intake on small intestinal integrity of weanling piglets.

Ref. <sup>1</sup>	Treatments <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
I	- continuous PD ( $\pm 200$ g · pig <sup>-1</sup> · day <sup>-1</sup> ) - 75 % restricted PD ( $\pm 50$ g · pig <sup>-1</sup> · day <sup>-1</sup> )	- newly weaned piglets, 14 days postpartum - duration experiment: 5 days postweaning - n=18 / treatment	Comparing restricted vs. continuous PD - ↓ in ADG - ↓ in small intestinal weight (51 %), 0 mucosal protein content - ↓ villus height (10 %), ↓ crypt depth (15 %) - 0 plasma xylose concentration	piglets were gavaged fed
II	- ad lib MR - 60 % restriction of ad lib	- newly weaned piglets, 5 days postpartum - duration experiment: 0, 30 days postweaning - n= 6 or 7 / treatment	Comparing restricted vs. ad lib - ↓ in ADG (42 %) - ↓ in small intestinal weight (51 %), ↓ mucosal weight (56 %), ↓ mucosal protein content (72 %) - ↓ villus height (47 %), ↑ crypt depth (7 %) - ↓ # goblet cells in villi (34 %) - ↑ # infiltrated cells in lamina propria (51 %)	- energy intake not given
III	- PD - MR at Ma - MR at 2.5 × Ma - MR ad lib	- newly weaned piglets, 29 days postpartum - duration experiment: 0, 5 days postweaning - n=8 / treatment	Comparing MR fed at Ma vs. 2.5×Ma and ad lib - ↓ in ADG - ↓ mucosal protein content (21 %) - ↓ villus height (29 %), ↑ crypt depth (18 %) - 0 plasma xylose concentration	
IV	- sow milk semi ad lib <sup>d</sup> - PD - sow milk pair fed with PD	- newly weaned piglets, 28 days postpartum - duration experiment 0, 4, 7 days postweaning - n=6 / treatment	Comparing PD and sow milk pair fed with PD vs. sow milk semi ad lib - ↓ in ADG - ↓ in villus height, 0 crypt depth	- ↑ crypt depth for PD when compared to sow milk pair fed with PD on day 4

Table 2 Continued.

Ref. <sup>1</sup>	Treatments <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
V	- ad lib MR - 80 % restriction of ad lib	- weaned piglets, 7 days postpartum - duration experiment: 30 days postweaning - n=6 / treatment	Comparing restricted vs. ad lib - ↓ weight · cm <sup>-1</sup> of the intestine, ↓ DNA, protein, triglyceride, cholesterol and phospholipid content in mucosa - ↓ villus height, ↑ in enterocyte losses - ↓ mucin levels in goblet cells	
VI	- semi ad lib MR - 67 % restriction of semi ad lib <sup>4</sup>	- newly weaned piglets, 26 days postpartum - duration experiment 0, 1, 2, or 4 days postweaning - n=6 / treatment	Comparing restricted vs. semi ad lib - ↓ in villus height in proximal small intestine (19 %), 0 crypt depth - ↑ in paracellular transport (48 %), 0 transcellular transport	- symposium paper
<sup>1</sup>	References: I: Kelly et al., 1991b; II: Núñez et al., 1996; III: Pluske et al., 1996b; IV: Van Beers- Schreurs, 1996; V: Lopez-Pedrosa et al., 1998; VI: Verdonk et al. 2001			
<sup>2</sup>	Abbreviations: ADG: average daily gain; d: day; Ma: maintenance; MR: milk replacer; PD: pelleted starter diet;			
<sup>3</sup>	0: similar, ↑: increased, ↓: decreased, #: number			
<sup>4</sup>	semi ad lib: according to formula describing voluntary feed intake of piglets (NRC, 1998)			

Table 3 Comparing restricted versus unrestricted feed intake on small intestinal brush border disaccharidase activity.

Reference <sup>1</sup> unit	I		II		III	IV	
	$\mu\text{mol} \cdot \text{min}^{-1} \cdot (\text{g protein})^{-1}$	$\text{mol} \cdot \text{day}^{-1}$	$\mu\text{mol} \cdot \text{min}^{-1} \cdot (\text{g protein})^{-1}$	$\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-1}$	$\mu\text{mol} \cdot \text{min}^{-1} \cdot (\text{g protein})^{-1}$	$\mu\text{mol} \cdot \text{min}^{-1} \cdot (\text{g protein})^{-1}$	$\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-1}$
Lactase	0 <sup>2</sup>	0	↑ 83 %	↓ 51 %	0	↑ 22 %	↓ 123 %
Sucrase	↑ 25 %	0	↑ 46 %	↓ 56 %	0	↑ 37 %	↓ 46 %
Maltase	0	↓ 55 %	↑ 22 %	↓ 60 %		↑ 39 %	↓ 38 %
Isomaltase	nd	nd	↑ 182 %	↓ 22 %	nd	nd	nd
Glucoamylase	0	↓ 47 %	nd	nd	nd	nd	nd
Aminopeptidase	nd	nd	↑ 31 %	↓ 60 %	nd	nd	nd
protein content	0 ( $\text{mg} \cdot \text{g}^{-1}$ )		↓ 72 % ( $\text{mg} \cdot \text{cm}^{-1}$ )		↓ 21 % ( $\text{mg} \cdot \text{g}^{-1}$ )	↓ 113 % ( $\text{mg} \cdot \text{cm}^{-1}$ )	

<sup>1</sup>: I: Kelly et al., 1991; II: Núñez et al., 1991; III: Pluske et al., 1996; IV: Lopez-Pedrosa et al., 1998

<sup>2</sup>: 0: similar enzyme activity; ↑: increased enzyme activity in restricted versus unrestricted-fed piglets; ↓: decreased enzyme activity in restricted versus unrestricted-fed piglets; nd: not determined

## Modulation by dietary components

It is clear that luminal nutrition and level of feed intake per se affect gut structure and function. Functional feed ingredients may indirectly, through enhanced feed intake, and/or directly, through specific effects, improve small intestinal integrity. In the following sections, the effects of specific nutrients on gut integrity are discussed with special attention given to actual feed intake as a possible confounder.

### Protein

As to the effect of dietary protein on small intestinal integrity, there is ample work on comparing the effect of native soy proteins with that of treated soy proteins or milk proteins. Table 4 summarises the reported effects of protein source on small intestinal integrity in weaned piglets. The inclusion in the diet of soybean meal instead of milk protein results in similar (Makkink, 1993; Makinde et al., 1996) or decreased ADG (Efird et al., 1982; Owsley et al., 1986; Dunsford et al., 1989; Li et al., 1991). Villus height after feeding soybean meal was either similar (Makkink, 1993; McCracken et al., 1999) or decreased (Dunsford et al., 1989; Li et al., 1991; Makinde et al., 1996). Zarkadas and Wiseman (2000a; 2000b) showed that the intake level of trypsin inhibitor as a component of soybean meal was negatively correlated to body-weight gain and villus height in weaned piglets. Feed conversion ratio (feed intake/weight gain) was positively correlated to the level of trypsin inhibitor intake (Zarkadas and Wiseman, 2000a). Crypt depth responded inconsistently and is either increased (Dunsford et al., 1989; Li et al., 1991), similar (McCracken et al., 1998; 1999; Makkink, 1993) or decreased (Makinde et al., 1996) by inclusion of soybean meal. The number of goblet cells was not affected by dietary soybean meal (Dunsford et al., 1991; McCracken et al., 1999).

Makkink (1993) compared, skimmed milk powder, soy protein concentrate, soybean meal and fish meal with regard to small intestinal morphology. In the proximal and distal jejunum, the type of protein source in the diet did not affect villus length, crypt depth and intestinal weight. Within the experimental treatments, the level of feed intake affected villus architecture. To assess the effect of protein source per se, feed intake should be comparable as may be achieved by a pair-feeding or restricted-feeding regimen. Newport and Keal (1983) reported a decrease in ADG when milk protein was replaced by fish protein in the diet. However, the piglets were weaned as young as 2 days of age and were fed a liquid milk replacer. The piglets might have been too young to tolerate high levels of fish meal and the practical relevance of this trial can be questioned. We have compared the effect of protein from feather meal and skimmed milk powder, which both are low in anti-nutritional factors (ANFs). The piglets fed the two protein sources and used for measurements were selected on the basis of comparable feed intake. Villus architecture and growth were measured on 4, 7 and 14 days postweaning. ADG was increased by 72 % during first two weeks postweaning when comparing piglets receiving the skimmed milk powder diet to those fed the feather meal diet. Across days, skimmed milk powder increased villus height (14 %) and crypt depth (10 %) compared to feather meal (Chapter 4).

Van Dijk and colleagues (2001) conducted a multiple regression analysis and concluded that dietary sprayed dried animal plasma (SDAP) levels up to 6 % in the diet increase both average daily gain and feed intake in the first 2 weeks after weaning in a dose-dependent fashion. The positive effect of SDAP was more pronounced in the first than in the second week after weaning. It is suggested that the positive effect of SDAP can be explained by increased feed intake, and possibly also by specific bioactive components preventing attachment of pathogenic *E. coli* to the intestine (Van Dijk et al., 2002). Villus height, crypt depth and cell proliferation were unaffected by SDAP (Jiang et al., 2000; Van Dijk et al., 2001). Due to health risks associated with the use of non-sterilised products of animal origin as feed ingredients, SDAP may be banned as an ingredient for animal feed. Unravelling the mechanism underlying the positive effect of SDAP would be important for further developing functional feeds. However, the positive effect of SDAP seems mainly to occur via stimulation of feed intake.

Table 4 Effect of protein source in the diet on small intestinal integrity of weanling piglets.

Ref. <sup>1</sup>	Dietary variables <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
I	Experiment 1 (dry feed) - MP - SBM Experiment 2 - MP (dry feed) - MP (liquid feed) - CSBM (dry feed)	- newly weaned piglets, 21 days postpartum - duration experiment 1: 7, 14 days postweaning (n=6 / treatment) - duration experiment 2: 7, 14, 21 days postweaning (n=5 / treatment) - balanced diets for protein delivered by test component (re = 24 %)	Experiment 1: Comparing SBM vs. MP - ↓ ADG (50 %) - 0 intestinal weight · cm <sup>-1</sup> , ↑ pancreas weight (19 %), ↑ intestinal length (28 %) - ↑ trypsin in intestine (63 %), 0 trypsin in pancreas, 0 chymotrypsin in intestine, ↓ chymotrypsin in pancreas (29 %) Experiment 2 Comparing CSBM vs. MP (dry feed) - ↓ ADG (49 %) - ↑ intestinal weight (15 %) and length (20 %), 0 intestinal weight · length <sup>-1</sup> , ↑ pancreas weight (31 %) - ↑ total trypsin in intestine (341 %), 0 total trypsin in pancreas, ↑ total chymotrypsin in intestine (95 %), ↓ total chymotrypsin in pancreas (61 %)	- no data on feed intake
II	- CSBM - CSBM + 20 % DW - CSBM + 5 % lard	- newly weaned piglets, 28 days postpartum - duration experiment: 1, 3, 14, 16, 28d postweaning - n=6 / treatment	Comparing CSBM vs. CSBM + DW - ↓ ADG (6 %) - ↓ total trypsin units in intestine (32 %), 0 total trypsin units in pancreas, ↑ chymotrypsin in intestine (31 %), 0 total chymotrypsin in pancreas	- diets not balanced on protein content (re = 23.82 vs. 21.45 vs. 22.64) or lysine (1.21 vs. 1.09 vs. 1.22) - no data on feed intake
III	- casein - SBM - CSBM	- newly weaned piglets, 21 days postpartum - duration experiment: 0, 3, 6, 9, 12, 15 postweaning - n=5 / treatment - balanced diets for protein delivered by test component (re = 20 %)	Comparing SBM and CSBM vs. casein - ↓ ADG for SBM (30 %) and CSBM (70 %), ↓ FI for SBM (8 %) and CSBM (51 %) - ↓ in villus height for SBM (14 %) and CSBM (9 %), ↑ crypt dept for SBM (16 %) and 0 for casein - 0 areas of Peyer's patches, 0 # goblet cells in villi and crypts	

Table 4 Continued.

Ref. <sup>1</sup>	Dietary variables <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
IV	- MP - SBM - SPC - extruded SPC - SPI	- duration experiment: 7 days postweaning - newly weaned piglets, 21 days postpartum - pigs were sensitised with the respective protein source from days 7 to 12 of age - n=8 / treatment - balanced diets for protein and energy	- ↓ ADG at week 1 and 2 for SBM and SPC compared to MP, ↓ FI for SBM vs. SPC in week 1, ↓ FI for MP vs. extruded SPC - ↓ villus height of all diets compared to MP, ↑ crypt dept for SBM compared to other diets - ↑ lymphocyte density for SBM compared to other diets - ↑ IgG titers to soy proteins for SBM compared to other diets - ↓ xylose concentration in plasma for SBM and extruded SPC compared to SPI or MP	
V	- MP - 15.5 % SBM - 31.5 % SBM - Cowpea meal	- newly weaned piglets, 28 days postpartum - duration experiment: 0, 7, 14, 21 days postweaning - before weaning, half of piglets received creep feed - n=5 / treatment	- ↓ ADG Cowpea compared to other diets. - ↓ villus height, ↓ crypt depth for SBM diets and cowpea diet compared to control on day 7. ↑ villus height and similar crypt depth for SBM diets on day 21. ↓ villus height for cowpea diet on day 21	- diets not balanced for raw materials - cowpea was fed as a single raw material - no data on feed intake
VI	- MP - SBM + SPC	- newly weaned piglets, 21 days postpartum - duration experiment: 0, 0.5, 1, 2, 4, 7 d - n=10 / treatment - balanced diets for protein delivered by test component (re = 20 %)	Comparing SBM + SPC vs. MP - 0 FI days 0-4, ↑ FI days 4-7 - 0 villus height and crypt depth - 0 # goblet cells - 0 # CD8 <sup>+</sup> and CD4 <sup>+</sup> T-cells, 0 concentration of prostaglandin 2	- weaning itself resulted in villus atrophy and intestinal inflammation
VII	- SMP - SPC - SBM - FM	- newly weaned piglets, 28 days postpartum - duration experiment: 0, 3, 6, or 10 days postweaning - n=5 / treatment - balanced diets for protein	- 0 ADG, ↑ FI for FM and SBM compared to SMP from days 0-3, 0 FI from days 3-10. - ↑ pancreatic weights for SMP and SPC - 0 villus length, 0 crypt depth on day 6	- villus height and crypt depth were affected by level of feed intake

Table 4 Continued.

Ref. <sup>1</sup>	Dietary variables <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
VIII	<ul style="list-style-type: none"> <li>of protein in diet:</li> <li>- 0 % FM + 100 % MP</li> <li>- 35 % FM + 65 % MP</li> <li>- 52.5 % FM + 47.5 % MP</li> <li>- 70 % FM + 30 % MP</li> </ul>	<ul style="list-style-type: none"> <li>- newly weaned piglets, 2 days postpartum</li> <li>- duration experiment: 0, 5, 26</li> <li>- n=7 / treatment</li> <li>- balanced diets for protein</li> </ul>	Comparing different ratios of FM and MP in diets - ↓ ADG and ↑ feed : gain with increasing FM content - ↓ pH, DM and total N in the stomach with increasing FM content, 0 total N in small intestine - 0 chymotrypsin and trypsin activity	- diets fed as milk replacer
IX	<ul style="list-style-type: none"> <li>- SMP</li> <li>- feather meal</li> </ul>	<ul style="list-style-type: none"> <li>- newly weaned piglets, 27 days postpartum</li> <li>- duration experiment: 0, 4, 7, or 14 days postweaning</li> <li>- n=6 / treatment</li> <li>- balanced diet for protein and lactose</li> </ul>	Comparing feather meal with SMP - ↓ ADG (46 %), ↓ feed efficiency (50 %) - ↓ in villus height (12 %), ↓ in crypt depth (9 %)	- piglets were selected for comparable feed intake to avoid entanglement between protein source and feed intake
X	<ul style="list-style-type: none"> <li>- DW + SBM</li> <li>- SDAP</li> <li>- SDAP pair fed to DW + SBM</li> </ul>	<ul style="list-style-type: none"> <li>- newly weaned piglets, 14 days postpartum</li> <li>- duration experiment: 0, 2, 4, 8, 16 days postweaning</li> <li>- n=8 / treatment</li> </ul>	Comparing SDAP and pair fed SDAP to DW+SBM vs. DW+SBM: - ↑ ADG days 0-16 for SDAP, ↑ FI days 0-16 SDP, 0 ADG and FI for days 0-4 and 0-8 - ↓ small intestine weight · (kg of body weight) <sup>-1</sup> on day 16, ↓ DNA and protein content on day 16 - 0 villus height, crypt depth, mucosal thickness - 0 5-bromo-2'-deoxyuridine labeling - ↓ intravillus lamina propria cell density in the proximal jejunum on days 4, 8, and 16	- diets not balanced for protein (re=24 vs. 22) - feed intake of pair fed animals is lower than feed intake of control
XI	<ul style="list-style-type: none"> <li>- control</li> <li>- SDAP</li> </ul>	<ul style="list-style-type: none"> <li>- weaned piglets</li> </ul>	Comparing SDAP vs. control: - ↑ ADG (26.8 %), ↑ FI (24.5 %), ↓ feed efficiency (3.2 %)	- review combining 15 published studies with SDAP



Table 4 Continued.

Ref. <sup>1</sup>	Dietary variables <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
XII	- MP	- newly weaned piglets, 4 days postpartum	- ↓ ADG with SPI, 0 with hydrolysis, 0 feed intake	- diets fed as milk replacer
	- hydrolysed MP	- adaptation for 3 days (= start trial)	- ↑ small intestinal weight per kg of body weight for piglets receiving SPI	
	- SPI	- duration experiment: 21 days	- ↓ specific activities of trypsin and chymotrypsin in the duodenum and pancreas by hydrolysis	
	- hydrolysed SPI	- n=8 / treatment		
XIII	- casein	- balanced diets for protein and lactose		
	- SPI	- newly weaned piglets, 2 d. postpartum	- tendency for ↑ ADG for hydrolysed compared to normal SPI, 0 FI	
	- hydrolysed SPI	- duration experiment: 0, 2, 5 and 10 days after adaptation for 5 d	- ↓ diarrhoea with hydrolysed SPI on day 2	
		- n=4 / treatment	- ↓ villus height at proximal jejunum on day 2 for hydrolysed and normal SPI. 0 villus height on day 5 and 10 at proximal jejunum, 0 villus height on all days at mid and distal jejunum, ↑ crypt depth at mid small intestine on day 2 for hydrolysed and normal SPI, 0 crypt depth other days and segments	
XIV	- casein	- balanced diets for protein	- 0 # CD8 <sup>+</sup> T-cells and prostaglandin concentration	
	- SPI			
	- hydrolysed SPI			
	- MP			
XV	- SPC			

<sup>1</sup> References: I: Efird et al., 1982; II: Owsley et al., 1986; III: Dunsford et al., 1989; 1991; IV: Li et al., 1991; V: Makinde et al., 1996; VI: McCracken et al., 1999; VII: Makkink, 1993; VIII: Newport and Keal, 1983; IX: Chapter 4; X: Jiang et al., 2000; XI: Van Dijk et al., 2001; XII: Leibholz, 1981; XIII: McCracken et al., 1998; XIV: Rooke et al., 1998

<sup>2</sup> Abbreviations: ADG: average daily gain; CD: cell differentiation molecules, surface markers of leukocyte subsets; CSBM: corn + soyabean meal, DW: dried whey, FI: feed intake; FM: fish meal, MP: milk protein, SBM: soyabean meal, SDAP: spray dried plasma protein, SMP: skimmed milk powder, SPC: soya protein concentrate, SPI: soya protein isolate

<sup>3</sup> 0: similar, ↑: increased, ↓: decreased, #: number

The early-weaned piglet has limited capacity to digest dietary proteins. By enzymatic hydrolysis of feed proteins, protein digestibility and availability for early-weaned piglets might be improved. It is difficult to draw general conclusions about the efficacy of hydrolysed proteins because the conditions of processing and enzymes used are variable, leading to different hydrolysis products. Treatment of soy proteins has been shown to ameliorate effects of ANFs and to decrease the serum antibody immunoglobulin G titers (Li et al., 1991). Rooke and co-workers (1998) showed lower antigenic protein contents in hydrolysed soybean meal, but no effect on antibody titers. When comparing soybean meal with hydrolysed soybean meal, ADG was either similar (Leibholz, 1981) or increased (McCracken et al., 1998; Rooke et al., 1998), and gut wall architecture was not different (McCracken et al., 1998; Rooke et al., 1998). McCracken and colleagues (1998) showed less postweaning diarrhoea after feeding diets with hydrolysed soy protein isolate instead of either soy protein isolate or milk protein. However, there was no diet effect on intestinal numbers of goblet cells, mast cells, T-cells, local production of prostaglandins and local expression of MHC genes, demonstrating that the type of protein did not influence inflammation when fed to piglets weaned 2 days postpartum (McCracken et al., 1998). Poullain and colleagues (1989) compared the effects of alimentary whole whey protein, whey protein oligopeptides and an amino acid mixture in rats. Growth and nitrogen retention after starvation followed by realimentation was highest for rats receiving the oligopeptides. Weanling rats recovering from severe starvation by feeding either a casein hydrolysate or the native protein had similar weight gain. However, intestinal permeability of ovalbumin remained increased only in the group refed with the casein diet (Boza et al., 1995). Possibly, the feeding of hydrolysed protein more effectively counteracts the weaning-induced impairment of gut integrity than does feeding of the intact protein.

### ***Amino Acids***

Amino acids taken up by the intestinal mucosa are derived from the blood and from the intestinal lumen. Stoll and colleagues (1998) conducted tracer balance studies with radioactive amino acids and measured amino acid incorporation into mucosal protein in piglets. The authors concluded that 60 % of the essential amino acids taken up from the intestinal lumen were catabolised by the intestine. The amount of catabolised amino acid was equivalent to at least 20 % of the essential amino acids consumed and was directly related to the mucosal mass (Stoll et al., 1998). This not only implies intestinal mass determines the efficiency of dietary protein utilization, but also that the availability of luminal amino acids is important for maintaining the mucosal mass and thus mucosal integrity.

Individual amino acids may have a specific role in regulating intestinal integrity and function (Wu, 1998). Glutamine, glutamate and aspartate are major fuels for small intestinal mucosa and support ATP-dependent metabolic processes such as active nutrient transport and high rates of intracellular protein turnover. Ornithine, which is derived from arginine, glutamine

and proline, is the immediate precursor for polyamine synthesis, which is essential for proliferation, differentiation and repair of intestinal epithelial cells. Arginine is the physiological precursor of nitric oxide (NO), which plays an important role in processes such as vasodilation, immune responses, neurotransmission and adhesion of platelets and leucocytes (Wu and Morris, 1998). Glutamate, glycine and cystine are precursors for the synthesis of glutathione, a tripeptide critical for defending the intestinal mucosa against toxic and peroxidative damage (Wu, 1998). Thus dietary glutamine is involved in the energy supply of the intestine, while the other amino acids through conversion have regulatory properties.

We are not aware of studies on the effect of dietary supplementation of aspartate, glycine, cystine or proline on small intestinal integrity of the weaned pig as measured by histology, specific enzyme activity and permeability. Supplementation to the diet of either 0.6 % or 0.93 % arginine did not affect growth performance and villus height (Touchette et al., 2000; Ewtushik et al., 2000). The effects of glutamine have been repeatedly studied. Whilst not considered to be an essential amino acid, L-glutamine is an abundant free amino acid in the plasma of animals (Wu et al., 1996) and in sow's milk (Wu and Knabe, 1994). As mentioned above, glutamine is a major energy source for the gut and supports nucleotide biosynthesis, but it also serves as an ammonia scavenger and preserves the immunological function during total parenteral nutrition (Windmueller, 1982; Alverdy, 1990; Souba 1993; Salway, 1995). Glutamine can be taken up with feed, but it can also be formed from glutamate and  $\text{NH}_4^+$  in an ATP-requiring reaction catalysed by glutamine synthetase. Hydrolysis of the terminal amide group of glutamine by glutaminase results in formation of glutamate and ammonia. As an energy source, glutamate readily enters the Krebs cycle following oxidative deamination by glutamate dehydrogenase into  $\alpha$ -ketoglutarate. Complete oxidation of 1 molecule of glutamate generates 12 molecules of ATP. A study by Houdijk and colleagues (1994) showed that feeding a glutamine-enriched diet increased the splanchnic blood flow in the rat. Thus extra glutamine provides energy in itself and indirectly by increasing the blood flow to the intestine. Glutamine, but not glutamate, plays a role in nucleotide metabolism as it donates the nitrogen atoms which form N-9 and N-3 of the purine ring (Salway, 1995). Depending on the activity of glutamine synthetase, glutamate can substitute for glutamine in purine metabolism.

A disadvantage of glutamine for dietary supplementation is its instability. Degradation of glutamine can be minimised by the addition of L-glutamine shortly before administration or by the use of a more stable form, e.g. L-alanyl-L glutamine or L-glycyl-L-glutamine. Dipeptides are rapidly hydrolyzed to their respective amino acids (Lacey and Wilmore, 1990), but are relatively expensive.

Table 5 Effect of glutamine on small intestinal integrity of early weaned piglets.

Ref. <sup>1</sup>	Dietary variables <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
I	Enteral nutrition - 4 % gln - 4 % gly	- newly weaned piglets, 21 days postpartum - duration experiment: 5 days - n=10 / treatment	Comparing addition of gln vs. gly - 0 ADG, 0 FI - 0 protein content ( $\text{mg} \cdot (\text{cm gut})^{-1}$ ), $\uparrow$ DNA content ( $\mu\text{g} \cdot (\text{cm gut})^{-1}$ ) - $\uparrow$ villus height and crypt depth in ileum and jejunum. - $\uparrow$ jejunal glutaminase ( $\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{cm}^{-1}$ )	symposium paper
II	Enteral nutrition - 0 % gln - 0.2 % gln - 0.6 % gln - 1.0 % gln	- newly weaned piglets, 21 days postpartum - duration experiment: 0, 7, 14 postweaning - n=5 / treatment	Comparing 1.0 % vs. 0 % gln - 0 ADG and FI during week 1 and 2, $\uparrow$ ADG and feed efficiency during week 2 - $\uparrow$ villus height on 7 days postweaning at jejunum, 0 villus height on 7 days postweaning in duodenum and on 14 days postweaning in duodenum and jejunum, $\downarrow$ crypt depth on 14 days postweaning at jejunum, 0 crypt depth on 14 days postweaning in duodenum and on 7 days postweaning in duodenum and jejunum	- no information on feed intake for piglets with morphology measurements - In growth trial piglets receiving 1 % gln had numeric lower feed intake
III	- 0 % glu, arg - 6.51 % glu - 0.93 % arg	- newly weaned piglets, 12 days postpartum - duration experiment: 0, 10 postweaning - n=7 / treatment	Comparing the addition of 0 vs. 6.51 % glu - 0 ADG and FI - 0 organ weights - 0 sucrose, lactase, maltase specific and total activity - $\uparrow$ villus height duodenum, 0 villus height proximal and mid jejunum and ileum. 0 crypt depth	- Piglets receiving arginine did not differ from control group
IV	- 0 % gln - 1.0 % gln	- newly weaned piglets, 18 days postpartum - duration experiment (postweaning): 0, 4 for histologic sampling. 0, 4, 7, 14, 21 for growth performance - n=4 / treatment	- 0 ADG and FI from 0-14, $\uparrow$ ADG and FI from 14-21 - 0 villus height	- Piglets used to measure growth performance or villus height were not the same

Table 5 Continued.

Ref. <sup>1</sup>	Dietary variables <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
V	- 0 % gln, arg - 1.2 % gln - 0.6 % arg	- newly weaned piglets, 17 days postpartum - duration experiment (postweaning): 0, 7, 14 for histologic sampling. 0, 7, 14, 28 for growth performance - n=6 / treatment	Comparing the addition of 0 vs. 1.2 % gln: - 0 ADG and FI - 0 villus height, ↑ crypt depth on day 14	- arg vs. gln had ↓ ADG from 0-7 and 14-28 - arg vs. control and gln showed either 0 and ↓ crypt depth
VI	TPN: - 0 % gln + 0 % glu - 0.35 % gln + 0 % glu - 0 % gln + 0.35 % glu	- newly weaned miniature piglets, 2 days postpartum - adaptation period: 5 days - duration experiment: 7 days post adaptation	- 0 ADG - 0 plasma and jejunal mucosa concentration of gln and glu - 0 intestinal weight, protein, DNA content or protein/DNA ratios - similar lactase, sucrase or maltase specific activities	
VII	perfused Using chambers: - newborn + HBBS (A) - weanling + HBBS (B) - A + 0.29 % gln (C) - B + 0.29 % gln (D) - C + E. coli - D + E. coli	- newborn piglets, 1 to 4 days postpartum - weanling piglets, 21 days postpartum - permeability measured with Using chamber - n=4-8 / treatment	Comparing weanling with gln vs. without - ↑ potential difference (=tissue viability) - 0 resistance (= tissue integrity) - no bacterial translocation	
VIII	perfused intestinal loops: - Ringer's lactate solution - Ringer's lactate solution + 2 % gln - oxygen-purged Ringer's lactate solution + 2 % gln	- piglets 21 days postpartum - permeability estimated by ratio of clearance of <sup>51</sup> Cr-EDTA and urea - administration of bacterial endotoxin - n=4 / treatment	- 0 permeability after endotoxin administration for gln perfused loops, ↑ permeability for loops perfused with only Ringer's lactate solution	

<sup>1</sup> References: I: Ayonrinde et al., 1995b; II: Wu et al., 1996; III: Ewtushik et al., 2000; IV: Kitt et al., 2001; V: Touchette et al., 2000; VI Burrin et al., 1991; VII: Smith et al., 1992; VIII: Dugan and McBurney, 1995

<sup>2</sup> Abbreviations: ADG: average daily gain; FI: average daily feed; glutamic acid: glu; glutamine: gln; glycine: gly; HBBS: Hanks Balanced Salt Solution; TPN: total parenteral nutrition

<sup>3</sup> 0: similar, ↑: increased, ↓: decreased

Table 5 summarises the reported effects of glutamine on the small intestine. In newly-weaned piglets plasma concentrations of glutamine are reduced when compared to unweaned, suckling piglets (Ayonrinde et al., 1995a). Some experiments with weaned piglets showed no effect on villus height with either 1 % (Kitt et al., 2001) or 1.2 % glutamine in the diet (Touchette et al., 2000). Some showed that 1 % glutamine (Wu et al., 1996) or 6.5 % glutamate (Ewtushik et al., 2000) had an effect on one site of the proximal small intestine but not further along the intestine. One study showed that 4 % glutamine increased villus height in both the duodenum and ileum (Ayonrinde et al., 1995b). Wu and colleagues (1996) showed improved feed efficiency but similar growth during the second week postweaning when 1 % glutamine was fed. In other studies, growth was either similar (Ewtushik et al., 2000) or increased by the addition of glutamine to the diet (Kitt et al., 2001). Lackeyram and colleagues (2001) noted increased growth with 0.8 % glutamine, but no effect with either 1.6 % or 2.4 %. It may be concluded that the effects of glutamine supplementation on villus architecture and growth performance are equivocal.

Perfusion of the epithelium of the ileum of weaned piglets with L-glutamine increased tissue viability as indicated by an increase in transmembrane potential difference (Smith et al., 1992). However, glutamine administration had no effect on tissue integrity as based on the TEER (Smith et al., 1992). Bacterial translocation of orally administered *E. coli* did not occur in either control or glutamine supplemented weanling piglets (Smith et al., 1992). Dugan and McBurney (1995) indicated that luminal glutamine is beneficial for the maintenance of normal mucosal permeability during endotoxemia. Ileal perfusion with a glutamine-containing solution effectively abolished endotoxin-induced increases in mucosal permeability in intestinal loops. In endotoxemic rats, glutamine-supplemented parenteral nutrition improved the morphology of the jejunal mucosa as based on increased villus height, crypt depth and wall thickness. In the glutamine group, the arterio – portal venous endotoxin difference after intravenous infusion of a lipopolysaccharide of *E. coli* was less negative, suggesting that the absorption of endotoxin across the gut was diminished through improved mucosal barrier function (Chen et al., 1994). Yoo and colleagues (1997) studied the proliferative response of lymphocytes to concanavalin A, which specifically activates T-cells via binding to specific membrane receptors (CD3). The proliferative response in lymphocytes from pigs infected with *E. coli* and fed a diet without glutamine was depressed, whereas lymphocytes from infected pigs fed a diet with 4 % glutamine responded similarly to those isolated from non-infected pigs. Both the control diet and the diet with extra glutamine contained 4.4 % glutamate. It may be concluded that glutamine supplementation supports immune function during critical states, but has no clear effect in non-challenged weanling piglets.

### ***Fat and poly-unsaturated fatty acids***

The addition of fat at the expense of corn to pig starter diets does not consistently enhance growth rates and feed/energy conversion during the initial weeks postweaning (Li et al., 1990; Cera et al., 1990b, Mahan, 1991). However, during the second phase of the nursery period, the addition of extra fat improves daily gain and feed efficiency (Li et al., 1990; Cera et al., 1990b; Mahan, 1991), but energy conversion is not or slightly improved. The most pronounced effects of added fat on daily gain during the second period are seen with coconut, soybean and corn oil (Li et al., 1990; Cera et al., 1990b; Mahan, 1991). Cera and co-workers (1990a) showed that luminal lipase activity is low during the initial postweaning period, but subsequently increases again. This observation confirms the increase in growth and feed efficiency with postweaning age.

Table 6 summarises the outcome of two studies on the influence of the fat source in the weaner diet on small intestinal morphology. Cera and colleagues (1988) showed that supplementation of the diet with 6 % corn oil at the expense of corn reduced villus height in the small intestine of weaned piglets. However, feed intake data were not shown. However, body weight was similar in the low and high fat diet. Li and colleagues (1990) compared diets supplemented with either soy oil, coconut fat or a 50/50 mixture of these two fat sources. The piglets that received either coconut or soybean oil had shorter villi than did the piglets that received the fat mixture, but when compared to the control diet, fat supplementation did not affect villus height. Fat supplementation at the expense of corn resulted in deeper crypts, irrespective of the type of fat (Li et al., 1990). Likewise, in rats, the addition of 8 % instead of 4 % corn oil to the diet increased crypt cell proliferation resulting in deeper crypts (Pell et al., 1992). It may be concluded that the addition of extra fat to the diet increases crypt depth and may lower villus height in weanling piglets without affecting growth performance.

Polyunsaturated fatty acids can belong to either the omega-3 ( $\omega$ -3) or omega-6 ( $\omega$ -6) family of fatty acids. Soybean, corn and sunflower oil are fat sources rich in the  $\omega$ -6 fatty acids. Linseed and fish oil are rich in the  $\omega$ -3 fatty acids  $\alpha$ -linolenic and eicosapentanoic acid, respectively. The  $\omega$ -3 polyunsaturated fatty acids have been investigated for use in the treatment of inflammatory diseases (Blok et al., 1996; Calder, 1998). Calder (1998) reviewed the effect of dietary fatty acids on the immune system and indicated that high-fat diets generally lower T-lymphocyte proliferation and natural killer cell activation when compared with low-fat diets. Among the fat sources in high-fat diets the order of potency was found to be: saturated fat (e.g. palm oil, coconut fat) < n-6 polyunsaturated rich oils (e.g. corn oil, soybean oil, sunflower seed oil) < olive oil < linseed oil < fish oil. Studies with experimental animals indicate that diets rich in  $\omega$ -3 polyunsaturated fatty acids are anti-inflammatory and immunosuppressive in vivo (Calder, 1998).

Table 6 Effect of fat and fatty acids in the diet on small intestinal integrity of early weaned piglets.

Ref. <sup>1</sup>	Dietary variables <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
I	- CSBM - CSBM + DW - CSBM + 5 % lard	- newly weaned piglets, 28 days postpartum - duration experiment: 0, 14, 27, 29, 31, 42, 44, 56 postpartum - n=6 / treatment	Comparing CSBM+ 5 % lard vs. CSBM - ↓ ADG (10 %) - 0 trypsin in intestine and in pancreas, ↑ trypsin per kg of pancreas (77 %), 0 chymotrypsin in the intestinal contents and pancreas	- diets not balanced on fat content (ether extract (%)) = 1.34 vs. 2.38 vs. 8.68) - no data on feed intake
II	- 0 % DW + 0 % corn oil - 25 % DW + 0 % corn oil - 0 % DW + 6 % corn oil - 25 % DW + 6 % corn oil	- newly weaned piglets, 21 days postpartum - duration experiment 3, 7, 14, 21, and 28 d - n=6 / treatment - diets balanced on kcal ME · (g lysine) <sup>-1</sup> , increased ME content for diets with oil	- ↓ lipase activity after weaning Comparing 6 % vs. 0 % corn oil - 0 ADG and intestinal weight, ↑ pancreas weight on day 28 - ↓ villus height - ↑ total lipase in pancreas, 0 lipase · (g pancreas) <sup>-1</sup> , 0 lipase in intestine	- no data on feed intake
III	4 trials with: - control - white grease - soybean oil - coconut oil - soybean oil + coconut oil	- newly weaned piglets, between 18 and 21 d - diets balanced for lysine/energy ratio	- 0 ADG, FE and ↓ FI with addition of 10 % fat during first 2 weeks, ↑ ADG with addition of fat from week 3-5 postweaning, especially with combination of soybean and coconut oil - ↓ ileal DM digestibility with addition of fat - ↑ villus height with combination of soybean and coconut oil compared to soybean or coconut oil alone, 0 villus height with addition of fat compared to control, ↑ crypt depth with addition of fat compared to control	
IV	- control - oil: ω3:ω6 = 10:1	- suckling piglets, 4 days postpartum - n= 5 or 6 / treatment	Comparing oil vs. control - 0 # leukocytes and lymphocytes, 0 migration index of lymphocytes, - 0 CD4 <sup>+</sup> , ↑ CD8 <sup>+</sup> , 0 CD2 <sup>+</sup> lymphocytes - ↓ level of archidonic acid (ω6), ↑ docosahexaenoic acid (ω6), ↑ gamma-linolenic acid (ω3), eicosapentaenoic acid (ω3) and docosahexaenoic acid (ω3) - ↑ growth factors - ↑ IgM (43 %)	- no data on feed intake and growth



Table 6 Continued.

Ref. <sup>1</sup>	Dietary variables <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
V	- Control - Control + PUFA - Malnourished - Malnourished + PUFA	- weaned piglets, 7 days postpartum - malnutrition (20 % of control) during 30 days followed by 10 days refeeding with or without fatty acids	Comparing PUFA vs. no PUFA - ↑ weight per length ratio of the intestine for malnourished piglets - ↑ recovery in the morphology in malnourished piglets - 0 disaccharidase and alkaline phosphatase activities - ↑ DNA, protein, cholesterol, phospholipid and triglyceride content in jejunal but 0 in ileal mucosa of malnourished piglets	diet was supplemented with a phospholipid concentrate of ω-6 and ω-3 long chain fatty acids also containing cholesterol.

<sup>1</sup> References: I: Owsley et al., 1986; II: Cera et al., 1988; 1990; III: Li et al., 1990; IV: Kastel et al., 1999; V: Lopez-Pedrosa et al. 1999

<sup>2</sup> Abbreviations: ADG: average daily gain; DM: dry matter; DW: dried whey; FE: feed efficiency; IgM: immunoglobulin M; PUFA: poly unsaturated fatty acids

<sup>3</sup> 0: similar, ↑: increased, ↓: decreased, #: number

The effect of  $\omega$ -3 and  $\omega$ -6 polyunsaturated fatty acids has not been extensively investigated in piglets (Table 6). Kastel and colleagues (1999) found that oral administration of  $\omega$ -3 polyunsaturated fatty acids to piglets affected the immune response. The production of  $\omega$ -3 derived docosahexanoic acid was significantly increased in the blood at the expense of  $\omega$ -6 derived arachidonic acid. The production of IgM by B lymphocytes and growth factor (somatomedin C) was increased after  $\omega$ -3 supplementation, but so was the production of cytotoxic T-lymphocytes (Kastel et al., 1999). Lopez-Pedrosa and co-workers (1999) investigated the effect of feed restriction and combined  $\omega$ -6 and  $\omega$ -3 polyunsaturated fatty acid supplementation in a 2×2 factorial design. Extra fatty acids enhanced small intestinal recovery after feed restriction, but had only limited effect in well-nourished piglets.

In weanling piglets, offering a diet containing linseed oil, which is rich in  $\alpha$ -linolenic acid, visually improved assessed body condition but not growth performance when compared with a diet containing corn oil, which is rich in linoleic acid (Schellingerhout et al., 2002a). It may be concluded that the addition of  $\omega$ -3 fatty acids to the diet of weanling piglets might have beneficial effects, especially when feed intake is low and hygiene status is suboptimal.

### ***Fibres and non-digestible oligosaccharides***

The term dietary fibre refers to plant carbohydrates, including pectins that resist hydrolysis by alimentary enzymes but can be fermented by the gastrointestinal flora. Dietary fibres cover a wide variety of substances with different physical properties and physiological effects. Some components are soluble, whereas others are insoluble; some have a high water-holding capacity, whereas others have a low or no water-holding capacity (Roberfroid, 1993). Soluble fibers may delay, whereas insoluble fibers may accelerate, small intestinal transit time, influencing contact time between digesta, enzymes and microbes. The major effect of soluble fibre is a reduction in starch hydrolysis and carbohydrate absorption, leading to a reduced and flattened glycemic response as well as reduced insulinemia (Bueno et al., 1981; Silk, 1989; Scheppach et al., 1990; Roberfroid, 1993; Mosenthin and Hambrecht, 1998). Soluble fibers may increase the thickness of the unstirred water layer covering the epithelial cells in the small intestine and thereby create a diffusion barrier that limits contact between intestinal enzymes and their substrates, and consequently reduces apparent enzyme activity. The increased unstirred layer may protect the mucosa against damage from particles.

The reported effects of dietary fibres on small intestinal integrity in weaned piglets are shown in Table 7. In general, inclusion of fiber in the diet did not affect growth (Moore et al., 1988; Jin et al., 1994; Longland et al., 1994; Lizardo et al., 1997; Hambrecht, 1998; Gill et al., 2000). Small intestinal weight was either unchanged (Jin et al., 1994, Lizardo et al., 1997) or increased after fibre consumption (Hambrecht, 1998). Hambrecht (1998) reported an increased incidence of diarrhoea during the first 2 weeks after weaning with the inclusion of

wheat bran in the diet, however over a 5-week period, there was no effect on the incidence of diarrhoea. Extra intake of fibre by weaned piglets increased total tract apparent digestibility of non-starch polysaccharides, but had no effect on total tract apparent digestibility of protein, dry matter and energy (Longland et al., 1994; Lizardo et al., 1997; Gill et al., 2000). Lizardo and colleagues (1997) showed in weanling piglets that faecal nutrient digestibility was similar for fibrous diets versus fibre-free diets, but apparent ileal nutrient digestibility was decreased.

Jin and colleagues (1994) investigated the effect of 10 % wheat straw in the diet on small intestinal architecture in weaned piglets. Villus height was not affected by dietary fibre, but the width of the villi and crypt depth were increased. Because the crypts are the principal site of cell proliferation in the intestinal mucosa, these data, in conjunction with the observed increase in cell proliferation and cell death, support the hypothesis that high fibre intake increases the rate of turnover of intestinal mucosal cells (Jin et al., 1994). Moore and coworkers (1988) showed no effect of dietary fibre on microscopic morphology. The effects seen in weaned piglets agree with those found in rats. In rats, supplementation of the diet with 10 % guar gum also increased crypt cell proliferation, resulting in deeper crypts. However insoluble wood cellulose had no effect on crypt cell proliferation, which may be due to its poor fermentability (Pell et al., 1992). In rats, dietary supplementation with either guar gum or pectin increased crypt depth, crypt cell proliferation and the migration rate of cells along the crypt villus axis when compared to either a fibre free diet or diets supplemented with either cellulose or retrograded starch. The effects of the soluble fibres were more pronounced in the proximal and mid small intestine than in the distal small intestine. Villus height was not affected by the type and amount of dietary fibre (Brunsgaard and Eggum, 1995).

Short chain fatty acids (SCFA) may be involved in increased proliferation of crypt cells caused by soluble fiber. In fistulated rats, SCFA infusion at a physiological dose increased the crypt cell production rate in the small and large intestine in a dose-dependent manner, the effectiveness being in the order n-butyric > propionic > acetic acid (Sakata, 1987). Fermentation of dietary soluble fibres by microbes leads to the generation of SCFA. The number of bacteria and SCFA production in the different segments of the small intestine are indicators of fermentative capacity. The stomach and proximal small intestine of the pig contain relatively low numbers of microbes ( $10^3$ - $10^5$  bacteria per ml of digesta). The distal small intestine (ileum), however, maintains a more diverse microbiota and higher bacterial numbers ( $10^8$  per ml of digesta) than the upper intestine. The large intestine is a major site of microbial colonization and is characterised by large numbers of bacteria ( $10^{10}$ - $10^{11}$  per ml of digesta) (Gaskins, 2000).

Table 7 Effect of fibers in the diet on small intestinal integrity of piglets.

Ref. <sup>1</sup>	Dietary variables <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
I	- CWR - W and B - CWR, W and B	- newly weaned piglets, 24 days postpartum - duration experiment: 14 or 35 days postweaning	Comparing W and B vs CWR during 2 weeks: - 0 ADG, FI and FE - ↑ incidence of diarrhoea - ↑ weight of proximal and distal small intestine - 0 total VFA production in distal small intestine, caecum and colon Comparing all three diets during 5 weeks: - ↓ FI in week 3, 4, 5 for CWR. ↑ FE for CWR in week 4, 5 - 0 in diarrhoea - ↑ weight of distal and similar weight of proximal small intestine for W/B - ↑ total VFA production in distal small intestine for W/B and CWR/WB and similar total VFA production in caecum and colon	
II	- CSBM - OH - SBH - AM	- piglets, 9.7 kg - duration growth trial 34 days post start of trial - n=3 / treatment - diets balanced for protein and energy	- ADG and FE tended to be lower for AM diet compared to others, 0 FI - 0 morphology (shape of villi)	
III	- 0 % WS - 10 % WS	- barrows, 14.3 kg - n=4 / treatment - duration experiment: 14 d - diets balanced for protein and energy	Comparing 10 vs. 0 % WS - 0 ADG, FI and FE - 0 weight of small intestine - 0 villus height, ↑ width of intestinal villi, ↑ crypt depth - ↑ rates of cell proliferation (5-bromo-2-deoxy-uridine) in jejunum and colon - ↑ rate of programmed cell death in jejunum and ileum	

Table 7 Continued.

Ref. <sup>1</sup>	Dietary variables <sup>2</sup>	Design	Observations <sup>3</sup>	Remarks
IV	- 0 % SBP - 15 % SBP	- weaned boars, 21 days postpartum - n=6 / treatment - diets balanced for protein and energy	Comparing 15 % with 0 % SBP - 0 ADG, ADFI and FE - ↑ TTAD of NSP (39 %), 0 TTAD of N and energy	
V	Diet composition - W - B - SBP Diet with or without enzymes	- weaned piglets, 28 days postpartum - growth trial (n=6 / treatment), duration experiment 4 weeks postweaning - digestibility trial (n=4 / treatment), duration experiment 11 days postweaning	- 0 ADG, FI, FE - 0 TTAD of N, DM, GE, ↑ TTAD of NSP	
VI	- SBM - SBM + SBP (12 %) - SFPC - SFPC + SBP (12 %)	- weaned piglets, 25 days postpartum - duration experiment 31 days - n=7 / treatment - diets are balanced for energy, protein and total lysin	Comparing 12 % with 0 % SBP: - 0 ADG and FI - 0 small intestinal weight and protein content. - ↑ TTAD of fibrous components, similar for other nutrients - ↓ ileal nutrient apparent digestibility - ↓ ileal N retention, ↑ faecal N retention, - ↑ dipeptidyl peptidase, N-aminopeptidase, alkaline phosphatase and similar maltase and $\gamma$ -glutamyl transferase in the ileum, 0 enzyme activities in jejunum, 0 $\alpha$ -amylase, trypsin, chymotrypsin activity, ↑ lipase activity	- for enzyme activities, piglets were 56 days of age - ileal digestibility measured by ileo-rectal anastomosis

<sup>1</sup> References: I: Hambrecht, 1998; II: Moore et al., 1988; III: Jin et al., 1994; IV: Longland et al., 1994; V: Gill et al., 2000; VI: Lizardo et al., 1997

<sup>2</sup> Abbreviations: ADG: average daily gain, AM: Alfalfa meal; B: barley; CSBM: corn soybean meal; CWR: cooked white rice; FE: feed efficiency; FI: feed intake; NSP: non starch polysaccharides; OH: oat hulls; SBH: soya bean hulls; SBM: soybean meal; SBP: sugar beet pulp; SFPC: soluble fish protein concentrate; TTAD: total tract apparent digestibility; W: wheat, WB: wheat bran; WS: wheat straw

<sup>3</sup> 0: similar, ↑: increased, ↓: decreased

In piglets weaned at 5 <sup>1</sup>/<sub>2</sub> weeks of age, SCFA production ( $\mu\text{mol} \cdot (\text{g dry matter of digesta})^{-1}$ ) in the distal small intestine was only 2 and 3 % of SCFA production in the caecum and proximal large intestine, respectively (Hambrecht, 1998). Although the number of bacteria and SCFA production indicate that only limited fermentation occurs in the small intestine, Houdijk (1998) showed that of the fructooligosaccharides (FOS) added to a weaner diet at a level of 40 g  $\cdot$  (kg feed)<sup>-1</sup> more than 90 % was degraded pre-caecally. This observation indicates that fermentation takes place in the small intestine. Thus, it is feasible that the observed effects of soluble fibres on small intestinal integrity are mediated by SCFA.

Non-digestible oligosaccharides (NDO) resist the hydrolysis by the alimentary enzymes. The pH of the ileal digesta decreased after addition to the diet of 4 % FOS when compared to a negative control. An effect on pH was not detected with 1 % FOS or either 1 or 4 % transgalacto oligosaccharide in the diet. Short chain fatty acid production and number of bacteria in the ileal digesta did not differ between piglets fed diets with or without dietary oligosaccharides (Houdijk, 1998). The inclusion in the diet of 0.2 % transgalactosylated oligosaccharide, 0.2 % glucooligosaccharide, 0.2 % lactitol (Gabert et al., 1995), 0.5 % galactosyl lactose (Mathew et al., 1997), either 1 or 2 % sucrose thermal oligosaccharide caramel (Orban et al., 1996) and 0.1 % mannoooligosaccharide (Kim et al., 2000) had no effect on the composition and activity of the microflora, the pH and the concentrations of SCFA and NH<sub>3</sub> in the small intestinal digesta of weaned piglets. The incidence of diarrhoea was not affected either. It follows that NDO's have no effect on small intestinal integrity in contrast to soluble fibres. The lack of effect of NDO consumption on SCFA concentration in the digesta may be explained by rapid absorption of SCFA. It could be suggested that soluble fibres not only act through generation of SCFA.

Fibres and SCFA have accessory effects in relation to the small intestine. The inclusion of fibre in orally or intravenously supplied TPN prevented bacterial translocation to the mesenteric lymph nodes even in the absence of oral nutrients (Spaeth et al., 1990). Dietary soluble fibre may enhance the faecal excretion of bile acids and render them unavailable for the formation of intra-luminal micelles so that fat and cholesterol absorption be reduced (Roberfroid, 1993). SCFAs are avidly absorbed and at the same time stimulate colonic sodium and water absorption, thereby acting as anti-diarrhoeal agents (Silk, 1989; Scheppach et al., 1990). SCFAs, especially butyric acid, are preferred energy sources for colonocytes (Roediger, 1982).

### ***Probiotics and lactic acid***

It is reasonable to suggest that dietary measures which enhance colonisation resistance and/or translocation resistance against enteropathogenic *E. coli* will have a positive effect on the performance of weanling piglets. Colonisation and translocation resistance may be influenced

by the feeding of antibiotics, probiotics, prebiotics and/or other ingredients that affect microbial ecology of the small intestine. In weanling piglets, antibiotics may be used therapeutically, but in the European Union most antibiotics have been banned for preventive use. In the weanling pig, the effect of feeding probiotics, i.e. live microorganisms with beneficial activity on the host, has been studied. The feeding of either  $10^6$  or  $10^7$  viable spores of *B. licheniformis* or  $10^6$  viable spores of *B. toyoi* when compared to a negative control improved growth performance in piglets with 31, 99, or 28 % respectively from 0 to 28 days postweaning (Kyriakis et al., 1999). However, the extremely high morbidity and mortality in the negative control group may have caused the lower growth performance in the negative control group. Mortality was 44 % in the negative control and on average 20 % in the probiotic treated groups. The administration of commercial preparations of probiotics to weanling piglets either showed no effect (Jost and Bracher-Jakob, 1998), or increased growth performance by 4 % when compared to a negative control (Inamoto and Waltanabe, 1998).

Prebiotics such as fructooligosaccharides have been shown to specifically stimulate the growth of lactobacilli and bifidobacteria in the intestine, but as mentioned previously there is no evidence that these probiotics influence gut integrity in weanling piglets. Lactobacilli produce lactic acid, which is known to have antibacterial activity. In weanling piglets dietary lactic acid concentrations of 0.8 - 2.4 % have been shown to stimulate feed intake and growth (Roth et al., 1993; Smolders et al., 2000). Likewise, the feeding of fermented feed, which is rich in lactic acid, also stimulated growth in weanling piglets (Jensen and Mikkeelsen, 1998; Scholten, 2001) and increased villus height (Scholten, 2001). Thus probiotics, lactic acid and fermented feed might be beneficial to weanling piglets, but it is not known whether there is a direct effect on gut integrity or that these compounds act through enhanced feed intake.

### **Growth factors**

Growth factors, especially epidermal growth factor (EGF) and insulin-like growth factors I and II (IGF-I and IGF-II), are present in the colostrum and milk of the sow. The concentration EGF per ml colostrum or milk is 1.5 µg and 0.15 - 0.25 µg, respectively (Xu, 1996). The concentration IGF-I per ml colostrum or milk is 0.07 - 0.35 µg and 0.004-0.014 µg, respectively (Xu, 1996). The growth factors stimulate growth, maturation and/or functional development of the intestinal tract (Kelly, 1994; Xu, 1996; Odle et al., 1996). Epidermal growth factor is a trophic peptide for the gastrointestinal mucosa and acts both from the lumen and the blood. Playford and colleagues (1993) showed that lumenally-supplied EGF is rapidly hydrolysed by proteases in the small intestine of human subjects while in the fasting state. Hydrolysis was blocked by the presence of casein or a soybean trypsin inhibitor. It was hypothesised that EGF is digested by pancreatic enzymes in the fasting state, but is preserved when food proteins act as competitive substrates and/or block the active sites of these enzymes (Playford et al., 1993). Oral supplementation of 372 µg · day<sup>-1</sup> EGF, but not

124  $\mu\text{g} \cdot \text{day}^{-1}$ , to weanling piglets partly counteracted the weaning-induced decrease in lactase specific activity. Small intestinal sucrase specific activity was increased on day 3 after weaning by a supplementation with the high dose of EGF. However, supplementation of EGF did not affect on the mucosal protein content and the villus/crypt ratio in the small intestine (Jaeger et al., 1990). Zijlstra and colleagues (1994) examined the effects of EGF given with a milk replacer (0, 500, or 1000  $\mu\text{g} \cdot \text{l}^{-1}$ ) on the recovery of piglets that were infected at 4 days of age with rotavirus enteritis. EGF increased villus length and lactase specific activity in a dose-dependent fashion. At the dose of 500  $\mu\text{g} \cdot \text{l}^{-1}$ , effects were seen only in the proximal portion of the small intestine, whereas with the higher EGF level there also were effects further down the tract (Zijlstra et al., 1994). Houle and colleagues (1997) looked at the effect of oral IGF-I administration (500  $\mu\text{g} \cdot (\text{l milk replacer})^{-1}$ ) in neonatal piglets until 7 and 14 days postpartum. Circulating concentrations of IGF-I did not change and growth, organ weights, mucosal RNA, mucosal DNA and mucosal protein content were not affected. Mean villus height in the proximal ileum tended to be higher and that in the terminal ileum was significantly higher in IGF-I-treated piglets. In other regions of the intestine, no effect of IGF-I on villus architecture was detected. By day 14 after birth, sucrase and lactase specific activities were increased throughout the jejunum and ileum in IGF-I-treated piglets. On day 7, enzyme specific activity was not affected by IGF-I administration (Houle et al., 1997). The addition of IGF-I to sow's milk so as to double the concentration of that present in sows' colostrum was found to increase the length of the tight junctions by 23 % in 36-hour old piglets. However, sows' milk with a IGF-I concentration similar to that in sows' colostrum did not affect tight junction structure. Thus at high intake levels IGF-I can modulate the tight junction structure and thereby influence intestinal permeability (Zarrinkalam et al., 1999). In rabbits intestinal transport of electrolytes and nutrients was measured with Ussing chambers. EGF supplementation to the perfusate up-regulated intestinal transport (Opleta-Madsen et al., 1991).

It may be concluded that dietary supplementation of IGF-I and EGF has only limited effects on body or organ weight. Within the intestine, IGF-I and EGF increased sucrase and lactase activities without significantly increasing intestinal weight, length, villus architecture, protein or DNA content. Thus, IGF-I and EGF may regulate disaccharidase activities through modifying the function or differentiation of individual enterocytes. The action of orally administered IGF-I and EGF seems to be limited to the intestine without exerting systemic effects. So far the role of growth factors on intestinal development has been studied in neonatal and not in weanling piglets. Applications might be restricted to prophylactic administration of growth factors to enhance recovery from gastrointestinal trauma.



### ***Polyamines***

Polyamines are characterised by multiple  $\text{NH}_2$  groups in the molecule, representatives being putrescine, spermidine and spermine (Halász and Baráth, 1998). Polyamines have been shown to play a role in regulating growth of the gastrointestinal mucosa and also postnatal maturation, turnover of intestinal mucosa, binding of the vitamin D receptor to DNA, postprandial intestinal motility, transport of D-glucose and mucosal hyperplasia during lactation (Johnson and McCormack, 1994; Blachier, 1997; Halász and Baráth, 1998). Polyamines are present in sow milk (Kelly et al., 1991c). For the biosynthesis of the polyamines in animal tissue the precursors ornithine, which is not found in proteins but is synthesised from arginine, or L-methionine, are required (McCormack and Johnson, 1991). Polyamines are synthesised from L-arginine in absorptive cells, secreted by exocrine pancreas and provided by extruded enterocytes at the top of villi (Blachier, 1997). Polyamines are also produced by intestinal bacteria (Blachier, 1997). Wu and colleagues (2000a) showed that intestinal polyamine synthesis is enhanced after weaning of piglets at 21 days of age. Grant and colleagues (1990) studied the effect of polyamine supplementation to a liquid milk replacer fed to piglets weaned at 2 days of age. An all-milk-protein milk replacer was compared with the same milk replacer in which 20 % of the protein was replaced with soy protein isolate without or with  $25 \text{ g} \cdot \text{l}^{-1}$  of either putrescine dihydrochloride or ethylamine hydrochloride. Daily gain, villus height and the kinetics of xylose absorption did not differ between dietary treatments. Crypt depth tended to be lowest in the milk-soy diet without polyamines, but mitotic index was altered. Specific and total activities of sucrase in the brush border were highest for the piglets fed the all-milk diet. Specific activity of cytosolic dipeptidase was lowest for piglets fed the milk replacer with putrescine. Total dipeptidase activity was lower in piglets fed the diets with putrescine or ethylamine when compared to the milk diet. Grant and colleagues (1989) applied the same dietary treatments to 3-day old preruminant calves as well. The plasma xylose concentration was highest in calves receiving the milk diet. Enterocyte proliferation was decreased in calves fed the soy-milk diet without added polyamines when compared to the other diets. Thus supplementation of the milk-soy protein diet with either putrescine or ethylamine enhanced enterocyte proliferation. Villus architecture was not affected by any dietary treatment (Grant et al., 1989). Oral daily supplementation of rats with  $6 \text{ } \mu\text{mol}$  spermine or  $10 \text{ } \mu\text{mol}$  spermidine in rats increased sucrase and maltase specific activity and decreased lactase specific activity. Ileal villus enterocytes were maturer in either spermine or spermidine treated rats, when compared to control animals, as based on changes in enterocytes structure and disaccharase activities (Dufour et al., 1988). Osman and colleagues (1998) investigated the effect of spermine on intestinal permeability in rats by Ussing diffusion chambers. High spermine concentrations ( $10\text{--}50 \text{ mM}$ ) enhanced transcellular permeability, whereas low concentrations ( $0.5\text{--}1 \text{ mM}$ ) either had no effect or produced a decrease. Thus, spermine concentration has no

straightforward action on epithelial barrier function. It is clear that administration of polyamines to rats induces intestinal maturation and increases proliferation. We are not aware of any studies on polyamine supplementation in piglets weaned at 3 weeks of age. However, polyamines added to a liquid milk replacer for either neonatal piglets or calves, did neither affect performance nor intestinal integrity.

### ***Nucleotides***

Nucleotides are building blocks of RNA and DNA, which can be either purine or pyrimidine nucleosides. Nucleotides may also function as energy source in cellular metabolism, influence lipid metabolism and serve as intermediates in biosynthetic and oxidative pathways. Nucleotides are important for immunity and gut development and repair (Boza et al., 1992; Carver and Walker, 1995; LeLeiko and Walsh, 1996; Nagafuchi et al., 1997). Cellular proliferation requires nucleotides derived either from glutamine, glycine and ribosylphosphates or from reuse of digested desquamated mucosal cells (LeLeiko et al., 1996). Bueno and colleagues (1994) fed weanling rats diets containing either corn starch or lactose for two weeks, followed by a 4-week period during which the corn starch diet with or without a nucleotide mixture was given. The lactose diet was used to induce diarrhoea. Rats that recovered from diarrhoea and received the diet with nucleotides showed increased villus height when compared to the rats not supplemented with nucleotides. However, rats that received the corn starch diet throughout did not benefit from nucleotide supplementation. This observation suggests that dietary nucleotides may improve intestinal healing after injury as induced by chronic diarrhoea. Adjei and colleagues (1996) fed mice either a casein diet, a protein-free diet, the protein-free diet with individual components of nucleotides/nucleosides or the protein-free diet with a nucleotide/nucleosides mixture to investigate the effect of diet on endotoxin-induced (*E. coli* O26:B6) bacterial translocation and small intestinal injury. Compared to the protein-deficient mice, dietary supplementation of a mixture of nucleotides and nucleosides or the individual component cytidine increased villus height and reduced the incidence of bacterial translocation. However, preventing protein malnutrition by feeding the casein diet resulted in higher villi and less bacterial translocation than did protein-free diet with a mixture of nucleotides and nucleosides (Adjei et al., 1996). The authors do not know published studies on dietary supplementation with nucleotides of weaner diets for piglets. However, in specific rodent models nucleotide supplementation may improve intestinal recovery after chronic diarrhoea or malnutrition.

## **CONCLUDING REMARKS**

Weaning is a stressful event as indicated by an increase of plasma cortisol concentration and behavioral changes (Worsaae and Schmidt, 1980). Plasma cortisol concentrations were more

than 2.5 times higher in weanling pigs on day 2 postweaning when compared to unweaned pigs (Wu et al., 2000a; 2000b). Inappetance and low feed intake, lethargy, reduced activity and fever are prevalent during many types of stress (Elsasser et al., 2000). The transition from suckling to eating solid food is typically associated with a critical period of underfeeding (Leibbrandt et al., 1975, Okai et al., 1976, Le Dividich and Herpin, 1994). Le Dividich and Herpin (1994) and Pluske and colleagues (1995) used various data sets and concluded that the daily metabolisable energy (ME) intake necessary for maintenance was not met until the fifth day after weaning. The level of preweaning ME intake was not attained until the end of the second week following weaning. Clearly, the weaning transition of piglets causes underfeeding.

The low feed intake after weaning and the associated decreased mucosal integrity both negatively affect growth performance and health of the early-weaned pig. There generally is a high incidence of diarrhoea after weaning (Nabuurs, 1991). With early weaning being fundamental, nutritional interventions to counteract the weaning-induced decrease in mucosal barrier function should aim at increasing feed intake and/or the formulation of specific diet compositions. Experiments indeed confirm that feed intake level is critically important. Low feed intake is associated with decreased absorptive and digestive capacity as indicated by the decreased mucosal surface area and often low total brush border enzyme activities. Permeability of macromolecules, an indicator of small intestinal integrity, is increased by low feed intake. In contrast to feed intake level, dietary constituents studied thus far only have marginal effects on small intestinal integrity in the weaned piglet. The effect of dietary constituents generally is more pronounced in malnourished/diseased piglets when compared to apparently healthy weanling piglets. There are potential functional ingredients to improve the mucosal integrity, but data for weanling pigs are relatively scarce, even though the weaned piglet is a good model for human infants (Reeds et al., 1997). Most studies on potential functional dietary ingredients have been conducted with rodents or neonatal piglets instead of piglets weaned at 3 weeks of age. In the nutrition of monogastric farm animals, emphasis has been on anti-nutritional factors (Van Weerden and Huisman, 1989) and only recently researchers have started to explore the functional properties of certain feed constituents.

Regarding the diet of weanling piglets, research should focus on critical determinants of feed intake immediately after weaning and functional feed ingredients to stimulate epithelial cell proliferation and differentiation, enhance immune function, and promote growth of beneficial bacteria. Combinations of functional feed ingredients may be more successful than the use of single ingredients. The cost-efficiency of the ingredients will determine their application in practice.

## LITERATURE CITED

- Adjei, A. A., K. Yamauchi, Y. C. Chan, M. Konishi, and S. Yamamoto. 1996. Comparative effects of dietary nucleoside-nucleotide mixture and its components on endotoxin induced bacterial translocation and small intestinal injury in protein deficient mice. *Gut*. 38: 531-537.
- Alverdy, J. C. 1990. Effects of glutamine supplemented diets on immunology of the gut. *JPEN – Parenter Enter.* 14: 109S-113S.
- Ayonrinde, A. I., I. H. Williams, R. McCauley, and B. P. Mullan. 1995a. Reduced plasma concentrations of glutamine and its metabolites in weaned piglets. Page 179 in *Manipulating Pig Production V.*, D. P. Hennessy, and P. D. Cranwell, eds. Australasian Pig Science Association, Werribee, Australia.
- Ayonrinde, A. I., I. H. Williams, R. McCauley and B. P. Mullan. 1995b. Glutamine stimulates intestinal hyperplasia in weaned piglets. Page 180 in *Manipulating Pig Production V.*, D. P. Hennessy, and P. D. Cranwell, eds. Australasian Pig Science Association, Werribee, Australia.
- Barber, J., P. H. Brooks, and J. L. Carpenter. 1989. The effects of water delivery rate on the voluntary food intake, water use and performance of early-weaned pigs from 3 to 6 weeks of age. Pages 103-104 in *The Voluntary Food Intake of Pigs*. J. M. Forbes, M. A. Varley, and T. L. J. Lawrence, eds. British Society of Animal Production, Occasional publication No. 13, Edinburgh, UK.
- Bertolo, R. F. P., C. Z. L. Chen, P. B. Pencharz, and R. O. Ball. 1999. Intestinal atrophy has a greater impact on nitrogen metabolism than liver by-pass in piglets fed identical diets via gastric, central venous or portal venous routes. *J. Nutr.* 129: 1045-1052.
- Bishop, W. P., S. I. Kim, M. Yamazato, H. Yoshino, and K. Kimura. 1992. Mucosal morphology in isolated bowel segments: importance of exposure to luminal contents. *J. Pediatr. Surg.* 27(8): 1061-1065.
- Bjarnason, I., A. Macpherson, and D. Hollander. 1995. Intestinal permeability: an overview. *Gastroenterology*. 108: 1566-1581.
- Blachier, F. 1997. Intestinal Polyamines. Pages 222-223 in *Digestive Physiology in Pigs*. J. P. Laplace, C. Fevrier, and A. Barbeau, eds. EAAP publication no. 88, Saint Malo, France.
- Blanchard, P. J., H. Miller, D. Perris, and P. Toplis. 2000. Benefits of gruel feeding to gut integrity of newly weaned pigs. Page 17 in *The Weaner Pig*. M. Varley, and J. Wiseman, eds. British Society of Animal Science Occasional Meeting, Univ. of Nottingham, Nottingham, UK.
- Blok, W. L., M. B. Katan, and J. W. M. van der Meer. 1996. Modulation of inflammation and cytokine production by dietary (n-3) fatty acids. *J. Nutr.* 126: 1515-1533.
- Boza, J., J. Jimenez, M. Jose Faus, and A. Gil. 1992. Influences of postnatal age and dietary nucleotide on plasma fatty acids in the weanling rat. *JPEN – Parenter Enter.* 16: 322-326.
- Boza, J., O. Martinez, L. Baro, M. D. Suarez, and A. Gu. 1995. Influence of casein and casein hydrolysate diets on nutritional recovery of starved rats. *JPEEN – Parent Enter.* 19: 216-221.
- Brunsgaard, G., and B. O. Eggum. 1995. Small intestinal tissue structure and proliferation as influenced by adaptation period and indigestible polysaccharides. *Comp. Biochem. Physiol.* 1112A (3/4): 365-377.
- Buddle, J. R., and J. R. Bolton. 1992. The pathophysiology of diarrhoea in pigs. *Pig News and Information*. 13(1): 41N-45N.
- Bueno, J., M. Torres, A. Almendros, R. Carmona, M. C. Nuñez, A. Rios, and A. Gill. 1994. Effect of dietary nucleotides on small intestinal repair after diarrhoea. Histological and ultrastructural changes. *Gut* 35: 926-933.
- Bueno, L., F. Praddaude, J. Fioramonti, and Y. Ruckebush. 1981. Effect of dietary fiber on gastrointestinal motility and jejunal transit time in dogs. *Gastroenterology*. 8: 701-707.

- Burrin, D. G., R. J. Shulman, M. C. Stern, and P. J. Reeds. 1991. Glutamine or glutamic acid effects on intestinal growth and disaccharidase activity in infant piglets receiving total parenteral nutrition. *JPEN – Parenter Enter.* 15(3): 262-266.
- Burrin, D. G., B. Stoll, R. Jiang, X. Chang, B. Hartmann, J. J. Holst, G. H. Greely, and P. J. Reeds. 2000. Minimal enteral nutrient requirements for intestinal growth in neonatal piglets: how much is enough? *Am. J. Clin. Nutr.* 71: 1603-1610.
- Calder, P. C. 1998. Dietary fatty acids and the immune system. *Nutr. Rev.* 56: S70-S83.
- Carver, J. D., and W. A. Walker. 1995. The role of nucleotides in human nutrition. *Nutr. Biochem.* 6: 58-72.
- Caspary, W. F. 1987. Absorption: general aspects and transport mechanisms in the small intestine. Pages 63-88 in *Structure and Function of the Small Intestine; delaying of absorption as a therapeutic principle; treatment of diabetes mellitus*. W. F. Caspary, ed. Excerpta Medica, Amsterdam, The Netherlands
- Caspary, W. F. 1992. Physiology and pathophysiology of intestinal absorption. *Am. J. Clin. Nutr.* 55: 299S-308S.
- Cera, K. R., D. C. Mahan, R. F. Cross, G. A. Reinhart, and R. E. Whitmoyer. 1988. Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. *J. Anim. Sci.* 66: 574-584.
- Cera, K. R., D. C. Mahan, and G. A. Reinhart. 1990a. Effect of weaning, week postweaning and diet composition on pancreatic and small intestinal luminal lipase response in young swine. *J. Anim. Sci.* 68: 384-391.
- Cera, K. R., D. C. Mahan, and G. A. Reinhart. 1990b. Evaluation of various extracted vegetable oils, roasted soybeans and animal-vegetable fat blend for postweaning swine. *J. Anim. Sci.* 68: 2756-2765.
- Chen, K., T. Okuma, K. Okamura, Y. Torigoe, and Y. Miyauchi. 1994. Glutamine-supplemented parenteral nutrition improves gut mucosa integrity and function in endotoxemic rats. *JPEN – Parenter Enter.* 18: 167-171.
- Cohen, V. L., A. L. Scott, C. A. Dinarello, and R. A. Prendergast. 1991. Interleukin-1 is a mucus secretagogue. *Cell. Immunol.* 136: 425-434.
- Deitch, E. A. 1993. Nutrition and the gut mucosal barrier. *Curr. Opin. General Surg.* 85-91.
- Deprez, P., P. Deroose, C. van den Ende, E. Muylle, and W. Oyaert. 1987. Liquid versus dry feeding in weaned piglets: The influence on small intestinal morphology. *J. Vet. Med. B.* 34: 254-259.
- Dufour, C., G. Dandrifosse, P. Forget, F. Vermesse, N. Romain, and P. Lepoint. 1988. Spermine and spermidine induce intestinal maturation in the rat. *Gastroenterology.* 95: 112-116.
- Dugan, M. E. R., and M. I. McBurney. 1995. Luminal glutamine perfusion alters endotoxin-related changes in ileal permeability of the piglet. *JPEN – Parenter Enter.* 19: 83-87.
- Dunsford, B. R., W. E. Haensly, and D. A. Knabe. 1991. Effects of diet on acidic and neutral goblet cell populations in the small intestine of early weaned pigs. *Am. J. Vet. Res.* 52 (10): 1743-1746.
- Dunsford, B. R., D. A. Knabe, and W. E. Haensly. 1989. Effect of dietary soybean meal on the microscopic anatomy of the small intestine in the early weaned pig. *J. Anim. Sci.* 67: 1855-1863.
- Dyce, K. M., W. O. Sack, and C. J. G. Wensing. 1987. Pages 746-758 in *Textbook of Veterinary Anatomy*. W. B. Saunders Company, Philadelphia, USA.
- Efird, R. C., W. D. Armstrong, and D. L. Herman. 1982. The development of digestive capacity in young pigs: effects of age and weaning system. *J. Anim. Sci.* 55(6): 1380-1387.

- Elsasser, T. H., K. C. Klasing, N. Filipov, and F. Tomphson. 2000. The metabolic consequences of stress: targets for stress and priorities of nutrient use. Pages 77-110 in *The Biology of Animal Stress: basic principles and implications for animal welfare*. G. Moberg, and J. A. Mench, eds. Cabi Publishing, Wallingford, UK.
- Epple, H. J., K. M. Kreusel, C. Hanski., J. D. Schulzke, E. O. Riecken, and M. Fromm. 1997. Differential stimulation of intestinal mucin secretion by cholera toxin and carbachol. *Pflügers Arch. – Eur. J. Physiol.* 433: 638-647.
- Ewtushik, A. L., R. F. P. Bertolo, and R. O. Ball. 2000. Intestinal development of early weaned piglets receiving diets supplemented with selected amino acids or polyamines. *Can. J. Anim. Sci.* 653-662.
- Fan, M. Z., B. Stoll, R. Jiang, and D. G. Burrin. 2001. Enterocyte digestive enzyme activity along the crypt-villus and longitudinal axes in the neonatal pig small intestine. *J. Anim. Sci.* 79: 371-381.
- Fraser, D., B. N. Milligan, E. A. Pajor, P. A. Philips, A. A. Taylor, and D. M. Weary. 1998. Behavioural perspectives on weaning in domestic pigs. Pages 121-138 in *Progress in Pig Science*. J. Wiseman, M. A. Varley, and J. P. Chadwick, eds. Nottingham University Press, Nottingham, UK.
- Forstner, J. F. and G. G. Forstner. 1994. Gastrointestinal mucus. Pages 1255-1283 in *Physiology of the Gastrointestinal Tract*. L. R. Johnson, ed. 3rd ed. Raven Press, New York, USA.
- Gabert, V. M., W. C. Sauer, R. Mosenthin, M. Schmitz, and F. Ahrens. 1995. The effect of oligosaccharides and lactitol on the ileal digestibilities of amino acids, monosaccharides and bacterial populations and metabolites in the small intestine of weanling pigs. *Can. J. Anim. Sci.* 75: 99-107.
- Ganessunker, D., H. R. Gaskins, F. A. Zuckermann, and S. M. Donovan. 1999. Total parenteral nutrition alters molecular and cellular indices of intestinal inflammation in neonatal piglets. *JPEN – Parenter Enter.* 23(6): 337-344.
- Gaskins, H. R. 1997. Immunological aspects of host / microbiota interactions at the intestinal epithelium. Pages 537-587 in *Gastrointestinal Microbiology: Volume 2, Gastrointestinal Microbes and Host Interactions*. R. L. Mackie, B. A. White, and R. E. Isaacson, eds. Chapman & Hall, New York, USA.
- Gaskins, H. R. 2000. Intestinal bacteria and their influence on swine nutrition. In *Swine Nutrition*. A. J. Lewis, and L. L. Southern, eds. 2nd ed. CRC Press, Boca Raton, Florida, USA.
- Gill, B. P., J. Mellange, and J. A. Rooke. 2000. Growth performance and apparent nutrient digestibility in weaned piglets offered wheat-, barley-, or sugar-beet-pulp diets supplemented with food enzymes. *Anim. Sci.* 70: 107-118.
- Goldstein, R. M., T. Hebiguchi, G. D. Luk, F. Taqi, T. R. Guilarte, F. A. Franklin, Jr., P. W. Niemiec, and D. L. Dudgeon. 1985. The effects of total parenteral nutrition on gastrointestinal growth and development. *J. Pediatr. Surg.* 20(6): 785-791.
- Grant, A. L., R. E. Holland, J. W. Thomas, K. J. King, and J. S. Liesman. 1989. Effects of dietary amines on the small intestine in calves fed soybean protein. *J. Nutr.* 1034-1041.
- Grant, A. L., J. W. Thomas, K. J. King, and J. S. Liesman. 1990. Effects of dietary amines on small intestinal variables in neonatal pigs fed soy protein isolate. *J. Anim. Sci.* 68: 363-371.
- Gruys, E., M. J. M. Toussaint, W. J. M. Landman, M. Tivapasi., R. Chamanza, and L. van Veen. 1999. Infection, inflammation and stress inhibit growth. Mechanism and non-specific assessment of the processes by acute phase proteins. Pages 72-84 in *Production Diseases in Farm Animals*. Th. Wensing, ed. Wageningen Pers, Wageningen, The Netherlands.
- Halász, A., and A. Baráth. 1998. Biogenic amines the chemical compounds of special biological activity. Pages 1-7 in *Biogenically Active Amines in Food. Volume II; Metabolic Effects of Biologically Active Amines in*

- Food. S. Bardócz, A. White, and G. Hajós, eds. Office for Official Publications of the European Communities, Luxembourg.
- Hall, G. A., and T. F. Byrne. 1989. Effects of age and diet on small intestinal structure and function in gnotobiotic piglets. *Res. Vet. Sci.* 47: 387-392.
- Hambrecht, E., 1998. Effect of non-starch polysaccharides on performance, incidence of diarrhoea and gut growth in weaned pigs. M.S. Thesis, Institute of Animal Nutrition. Hohenheim Univ., Stuttgart. Germany.
- Hampson, D. J. 1986. Attempts to modify changes in the piglet small intestine after weaning. *Res. Vet. Sci.* 40: 313-317.
- Houdijk, A. P. J., P. A. M. van Leeuwen, M. A. Boermeester, T. van Lambalgen, T. Teerlink, E. L. Flinkerbush, H. P. Sauerwein, and R. I. C. Wesdorp. 1994. Glutamine-enriched enteral diet increases splanchnic blood flow in rat. *Am. J. Phys.* 267 (Gastrointestinal Liver Physiology): G1035-G1040.
- Houdijk, J. 1998. Effects of non-digestible oligosaccharides in young pig diets. Ph.D. Diss., Wageningen Univ., Wageningen, The Netherlands.
- Houle, V. M., E. A. Schroeder, J. Odle, and S. M. Donovan. 1997. Small intestinal disaccharidase activity and ileal villus height are increased in piglets consuming formula containing recombinant human insulin-like growth factor-I. *Pediatr. Res.* 42: 78-86.
- Inamoto, T., and M. Watanabe. 1998. Effects of commercial preparations of lactobacilli and streptococci on the performance of weaned piglets. *Bulletin of the Akita Prefectural College of Agriculture.* 24: 69-72.
- Jaeger, L.A., C. H. Lamar, T. R. Cline, and C. J. Cardona. 1990. Effect of orally administered epidermal growth factor on the jejunal mucosa of weaned pigs. *Am. J. Vet. Res.* 51: 471-474.
- Jensen, P., and B. Recén. 1989. When to wean – observations from free-ranging domestic pigs. *App. Anim. Behav. Sci.* 23: 49-60.
- Jensen, and Mikkelsen. 1998. Feeding liquid diets to pigs. Pages 107-126 in *Recent Advances in Animal Nutrition*. P.C. Gransworthy, and J. Wiseman, eds. Nottingham University Press, Nottingham, UK.
- Jiang, R., X. Chang, B. Stoll, M. Z. Fan, J. Arthington, E. Weaver, J. Campbell, and D.G. Burrin. 2000. Dietary plasma protein reduces small intestinal growth and lamina propria cell density in early weaned pigs. *J. Nutr.* 130: 121-126.
- Jin, L., L. P. Reynolds, D. A. Redmer, J. S. Caton, and J. D. Crenshaw. 1994. Effects of dietary fiber on intestinal growth, cell proliferation and morphology in growing pigs. *J. Anim. Sci.* 72: 2270-2278.
- Johnson, R. W. 1997. Inhibition of growth by pro-inflammatory cytokines: an integrated view. *J. Anim. Sci.* 75: 1244-1255.
- Johnson, L. R., and S. A. McCormack. 1994. Regulation of gastrointestinal mucosal growth. Pages 611-641 in *Physiology of the Gastrointestinal Tract*. L. R. Johnson, ed. Raven Press, New York, USA.
- Jost, M., and A. Bracher-Jakob. 1998. The effect of Sanobiotic RS, a multiactive probiotic growth promoter in rearing piglets. *Agrarforschung.* 5(9): 413.
- Junqueira, L., and J. Carneiro. 1980. Pages 307-357 in *Basic Histology*. 3rd ed., Lange Medical Publication, Los Altos, California, USA.
- Kastel, R., V. Revajova, D. Magic, J. Pistl, M. Levkut, L. Bindas, J. Sajbidor, and M. Horvath. 1999. Effect of oil containing n-3 polyunsaturated fatty acids (PUFA) on the immune response and growth factors in piglets. *Acta Vet. Hung.* 47 (3): 325-334.
- Kelly, D. 1994. Colostrum, growth factors and intestinal development in pigs. Pages 151-166 in *Digestive Physiology in Pigs*. W. B. Souffrant, and H. Hagemeister, eds. EAAP publication no 80, Wageningen Pers, Wageningen, The Netherlands.

- Kelly, D., J. A. Smyth, and K. J. McCracken. 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 weak post-weaning. *Brit. J. Nutr.* 65: 169-180.
- Kelly, D., J. A. Smyth, and K. J. McCracken. 1991b. Digestive development of the early weaned pig; effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. *Brit. J. Nutr.* 65: 181-188.
- Kelly, D., T. P. King, D. S. Brown, and M. McFadyen. 1991c. Polyamine profiles of porcine milk and of intestinal tissue of pigs during suckling. *Reprod. Nutr. Dev.* 31: 73-80.
- Kim, J. D., Y. Hyun, K. S. Sohn, H. J. Woo, T. J. Kim, and In K. Han. 2000. Effects of immunostimulators on growth performance nad immune response in pigs weaned at 21 days of age. *J. Anim. Feed Sci.* 9: 333-346.
- Kitt, S. J., P. S. Miller, A. J. Lewis, and R. L. Fischer. 2001. Effect of diet and crystalline glutamine supplementation on growth performance and small intestine morphology of weanling pigs. *J. Anim. Sci.* 79 (suppl. 1): 148.
- Kudsk, K. A. 1994. Clinical applications of enteral nutrition. *Nutr. Clin. Pract.* 9: 165-171.
- Kyriakis, S. C., V. K. Tsiloyiannis, J. Vlemmas, K. Sarris, A. C. Tsinas, C. Alexopoulos, and L. Jansegers. 1999. The effect of probiotic LSP 122 on the control of post-weaning diarrhoea syndrome of piglets. *Res. Vet. Sci.* 67: 223-228.
- Lacey, J. M., and D. W. Wilmore. 1990. Is glutamine a conditionally essential amino acid? *Nutr. Rev.* 48 (8): 297-309.
- Lackeyram, D., X. Yue, and M. Z. Fan. 2001. Effects of dietary supplementation of crystalline L-glutamine on the gastrointestinal tract and whole body growth in early-weaned piglets fed corn and soybean meal-based diets. *J. Anim. Sci.* 79(suppl. 1): 322.
- Lamont, J. T. 1992. Mucus: the front line of intestinal mucosal defence. *Ann. NY. Acad. Sci.* 190-201
- Le Dividich, J., and P. Herpin. 1994. Effects of climatic conditions on the performance, metabolism and health status of weaned piglets: a review. *Liv. Prod. Sci.* 38: 79-90.
- Leibbrandt, V. D., R. C. Ewan, V. C. Speer, and D. R. Zimmerman. 1975. Effect of weaning and age at weaning on baby pig performance. *J. Anim. Sci.* 40(6): 1077-1080.
- Leibholz, J. 1981. Digestion in the pig between 7 and 35 d of age. 6. The digestion of hydrolysed milk and soya-bean proteins. *Br. J. Nutr.* 46: 59-69.
- LeLeiko N.S., and M. J. Walsh. 1996. The role of glutamine, short-chain fatty acids, and nucleotides in intestinal adaptation to gastrointestinal disease. *Pediatric Gastroenterology II.* 43 (2): 451 - 469.
- Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, R. D. Klemm, D. W. Giesting, J. D. Hancock, G. L. Allee, and R. D. Goodband. 1991. Measuring suitability of soybean products for early-weaned pigs with immunological criteria. *J. Anim. Sci.* 69: 3299-3307.
- Li, D. F., R. C. Thaler, J. L. Nelssen, D. L. Harmon, G. L. Allee, and T. L. Weeden. 1990. Effect of fat sources and combinations on starter pig performance, nutrient digestibility and intestinal morphology. *J. Anim. Sci.* 68: 3694-3704.
- Lizardo, R. J. Peiniau, and A. Aumaitre. 1997. Inclusion of sugar-beet pulp and change of protein source in the diet of the weaned piglet and their effects on digestive performance and enzymatic activities. *Anim. Feed Sci. Techn.* 66: 1-14.
- Longland, A. C. J. Carruthers, and A. G. Low. 1994. The ability of piglets 4 to 8 weeks old to digest and perform on diets containing two contrasting sources of non-starch polysaccharide. *Anim. Prod.* 58: 405-410.



- Lopez-Pedrosa, J. M., M. Ramirez, M. I. Torres, and A. Gill. 1999. Dietary phospholipids rich in long-chain polyunsaturated fatty acids improve the repair of small intestine in previously malnourished piglets. *J. Nutr.* 129: 1149-1155.
- Lopez-Pedrosa, J. M., M. I. Torres, M. I. Fernandez, A. Rios, and A. Gill. 1998. Severe malnutrition alters lipid composition and fatty acid profile of the small intestine in newborn piglets. *J. Nutr.* 128: 224-233.
- Madara, J. L. 1989. Loosening tight junctions; Lessons from the intestine. *J. Clin. Invest.* 83: 1089-1094.
- Madara, J. L., S. Nash, R. Moore, and K. Atisook. 1990. Structure and function of the intestinal epithelial barrier in health and disease. Pages 306-324 in *Gastrointestinal Pathology*. H. Goldman, H. D. Appelman, and N. Kaufman, eds. Williams & Wilkins, Baltimore, USA.
- Mahan, D. C. 1991. Efficacy of initial postweaning diet and supplemental coconut oil or soybean oil for weanling swine. *J. Anim. Sci.* 69: 1397-1402.
- Makinde, M. O., E. Umaphy, B. T. Akingbemi, K. T. Mandisodza, and E. Skadhauge. 1996. Effects of dietary soybean and cowpea on gut morphology and faecal composition in creep and noncreep-fed pigs. *J. Vet. Med. A.* 43: 75-85.
- Makkink, C. A. 1993. Of piglets, dietary proteins and pancreatic proteases. Ph.D. Diss., Wageningen Univ., Wageningen, The Netherlands.
- Mantle, M., and A. Allen. 1989. Gastrointestinal mucus. Pages 202-229 in *Gastrointestinal Secretion*. J. S. Davison, ed. Wright, London, UK.
- Mathew, A. G., C. M. Robbins, S. E. Chattin, and J. D. Quigley. 1997. Influence of galactosyl lactose on energy and protein digestibility, enteric microflora and performance of weanling pigs. *J. Anim. Sci.* 75: 1009-1016.
- McCauley, R. D., K. A. Heel, K. J. Christiansen, and J. C. Hall. 1996. The effect of minimum luminal nutrition on bacterial translocation and atrophy of the jejunum during parenteral nutrition. *J. Gasteren. Hepatol.* 11: 65-70.
- McCormack, S. A., and L. R. Johnson. 1991. Role of polyamines in gastrointestinal mucosal growth. *Am. J. Phys.* 260 (Gastrointestinal Liver Physiology 23): G795-G806.
- McCracken, B. A., H. R. Gaskins, P. J. Ruwe-Kaiser, K. C. Klasing, and D. E. Jewell. 1995. Diet-dependent and diet-independent metabolic responses underlie growth stasis of pigs at weaning. *J. Nutr.* 125: 2838-2845.
- McCracken, B. A., M. E. Spurlock, M. A. Roos, F. A. Zuckermann, and H. R. Gaskins. 1999. Weaning anorexia may contribute to local inflammation in the piglet small intestine. *J. Nutr.* 129: 613-619.
- McCracken, B. A., R. T. Zijlstra, S. M. Donovan, J. Odle, E. L. Lien, and H. R. Gaskins. 1998. Neither intact nor hydrolysed soy proteins elicit intestinal inflammation in neonatal piglets. *JPEN – Parenter Enter.* 22: 91-97.
- Miller, B. G., P. S. James, M. W. Smith, and F. J. Bourne. 1986. Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. *J. Agric. Sci. Camb.* 107: 579-589.
- Moon, H. W. 1971. Epithelial cell migration in the alimentary mucosa of the suckling pig. *Proc. Soc. Exp. Biol. Med.* 137: 151-154.
- Moore, R. J., E. T. Kornegay, R. L. Grayson, and M.D. Lindemann. 1988. Growth, nutrient utilization and intestinal morphology of pigs fed high fiber diets. *J. Anim. Sci.* 66: 1570-1579.
- Mosenthin, R., and E. Hambrecht. 1998. Physiology of dietary fibers in monogastric animals. Pages 78-91 in *Proc. Symposium Series 1 of the 8<sup>th</sup> World Conference of Animal Production*. Seoul National Univ., Seoul, South Korea.
- Mouwen, J. M. V. M. 1972. White scours in piglets at three weeks of age. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.

- Nabuurs, M. J. A., 1991. Etiologic and pathogenic studies on postweaning diarrhea. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- Nabuurs, M. J. A., A. Hoogendoorn, E. J. van der Molen, and A. L. M. van Osta. 1993. Villus height and crypt dept in weaned and unweaned pigs, reared under various circumstances in the Netherlands. *Res. Vet. Sci.* 55: 78-84.
- Nagafuchi, S., T. Katayanagi, E. Nakagawa, T. Takahashi, T. Yaijma, A. Yonekubu, and T. Kuwata. 1997. Effects of dietary nucleotides on serum antibody and splenic cytokine production in mice. *Nutr. Res.* 17: 1163-1174.
- National Research Council (NRC), 1998. Page 190 in *Nutrient Requirements of Swine*. 10th edition. National Academy Press, Washington DC, USA.
- Newport, M. J., and H. D. Keal. 1983. Artificial rearing of pigs 13. Effect of replacement of dried skim-milk by a functional fish protein concentrate on the performance of the pigs and digestion of protein. *Br. J. Nutr.* 49: 43-50.
- Noy, Y., and D. Sklan. 1999. Different types of early feeding and performance in chicks and poult.s. *J. Appl. Poultry Res.* 8: 16-24.
- Núñez, M. C., J. D. Bueno, M. V. Ayudarte, A. Almendros, A. Rios, M. D. Suarez, and A. Gil. 1996. Dietary restriction induces biochemical and morphometric changes in the small intestine of nursing pigs. *J. Nutr.* 126: 933-944.
- Odle, J., R. T. Zijlstra, and S. M. Donovan. 1996. Intestinal effects of milkborne growth factors in neonates of agricultural importance. *J. Anim. Sci.* 74: 2509-2522.
- Okai, D. B., F. X. Aherne, and R. T. Hardin. 1976. Effects of creep and starter composition on feed intake and performance of young pigs. *Can. J. Anim. Sci.* 56: 573-586.
- Opleta-Madsen, K. J. Hardin, and D. G. Gall. 1991. Epidermal growth factor upregulates intestinal electrolyte and nutrient transport. *Am. J. Physiol.* 260 (Gastrointest. Liver Physiol. 23): G807-G814.
- Orban, J. I., J. A. Patterson, O. Adeola, A. L. Sutton, and G. N. Richards. 1996. Growth performance and intestinal microbial populations of growing pigs fed diets containing sucrose thermal oligosaccharide caramel. *J. Anim. Sci.* 74: 170-175.
- Osman, N. E., B. Weström, Q. Wang, L. Persson, and B. Karlsson. 1998. Spermine affects intestinal in vitro permeability to different-sized molecules in rats. *Comp. Biochem. Phys. C.* 120: 211-216.
- Owsley, W. F., D. E. Orr, and L. F. Tribble. 1986. Effects of age and diet on the development of the pancreas and the synthesis and secretion of pancreatic enzymes in the young pig. *J. Anim. Sci.* 63: 497-504.
- Park, Y. K., M. M. Monaco, and S. M. Donovan. 1998. Delivery of total parenteral nutrition (TPN) via umbilical catheterization: development of a piglet model to investigate therapies to improve gastrointestinal structure and enzyme activity during TPN. *Biol. Neonate.* 73: 295-305.
- Pekas, J. C. 1991. Digestion and absorption capacity and their development. Pages 37-73 in *Swine Nutrition*. E. R. Miller, and D. E. Ullrey, eds. Butterworth – Heinemann, Boston, USA.
- Pell, J. D., J. M. Gee, G. M. Wortly, and I. T. Johnson. 1992. Dietary corn oil and guar gum stimulate intestinal crypt cell proliferation in rats by independent but potentially synergistic mechanisms. *J. Nutr.* 122: 2447-2456.
- Pinchasov, Y., and Y. Noy. 1993. Comparison of post-hatch holding time and subsequent early performance of broiler chicks and turkey poult.s. *Brit. Poultry Sci.* 34: 111-120.

- Plaisancié, P., A. Barcelo, F. Moro, J. Claustre, J. A. Chayvialle, and J. C. Cuber. 1998. Effects of neurotransmitters, gut hormones and inflammatory mediators on mucus discharge in rat colon. *Am. J. Phys.* 275 (Gastrointest. Liver Physiol. 38): G1073-G1084.
- Playford, R. J., A. C. Woodman, P. Clark, P. Watanapa, D. Vesey, P. H. Deprez, R. C. N. Williamson, and J. Calam. 1993. Effect of luminal growth factor preservation on intestinal growth. *Lancet*. 341: 843-848.
- Pluske, J. R., D. J. Hampson, and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Liv. Prod. Sci.* 51: 215-236.
- Pluske, J. R., G. N. Power, P. D. Cranwell, S. G. Pierzynowski, R. G. Campbell, D. J. Kerton, R. H. King, and F. R. Dunshea. 1997. Sex and age at weaning affect small intestinal histology and enzymatic capacity. Page 67 in *Manipulating Pig Production VI.*, P. D. Cranwell, ed. Australasian Pig Science Association, Werribee, Australia.
- Pluske, J. R., I. H. Williams, and F. X. Aherne. 1995. Nutrition of the neonatal pig. Pages 187-235 in *The Neonatal Pig: Development and Survival*. M. A. Varley, ed. CAB International, Wallingford, UK.
- Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996a. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. *Anim. Sci.* 62: 131-144.
- Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996b. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. *Anim. Sci.* 62: 145-158.
- Poullain, M. G., J. P. Cezard, L. Roger, and F. Mendy. 1989. Effect of whey proteins, their oligopeptide hydrolysates and free amino acid mixtures on growth and nitrogen retention in fed and starved rats. *JPEN – Parenter Enter.* 13 (4): 382-386.
- Reeds, P. J., D. G. Burrin, T. A. Davis, B. Stoll, L. J. Wijkes, D. Wray-Cahen, F. Jahoor, M. A. Dudley, M. L. Fiorotto, and N. J. Gannon. 1997. A review – Growth, development and nutrient metabolism in piglets and infants. Pages 1-32 in *Manipulating Pig Production VI.*, P. D. Cranwell, ed. Australian Pig Science Association. Werribee, Australia.
- Roberfroid, M. 1993. Dietary fiber, inulin and oligofructose: a review comparing their physiological effects. *Crit. Rev. Food Sci.* 33 (2): 103-148.
- Roediger, W. E. W. 1982. Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* 83: 424-429.
- Rooke, J. A., M. Slessor, H. Fraser, and J. R. Thomson. 1998. Growth performance and gut function of piglets weaned at four weeks of age and fed protease-treated soya-bean meal. *Anim. Feed. Sci. Techn.* 70: 175-190.
- Roomi, N., M. Laburthe, N. Fleming, R. Crowther, and J. Forstner. 1984. Cholera-induced mucin secretion from rat intestine: lack of effect cAMP, cycloheximide, VIP, and colchicine. *Am. J. Phys.* 247 (Gastrointest. Liver Physiol. 10): G140-G148.
- Russell, P. J., T. M. Geary, P. H. Brooks, and A. Campbell. 1996. Performance, water use and effluent output of weaner pigs fed ad libitum with either dry pellets or liquid feed and the role of microbial activity in the liquid feed. *J. Sci. Food Agric.* 72: 8-16.
- Roth, F. X., M. Kirchgessner, and U. Eidelsburger. 1993. Zur Nutritiven Wirksamkeit von Milchsäure in der Ferkelaufzucht. *Agribiol. Res.* 46: 229-239.
- Sakata, T. 1987. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. *Br. J. Nutr.* 58: 95-103.
- Salway, J. G. 1995. Page 97 in *Metabolism at a Glance*. Blackwell Science, Oxford, UK.

- Schellingerhout, A. B., A. J. van Dijk, H. Everts, R. Hovenier, A. G. Lemmens, J. Van der Kuilen, G. Hemke, and A. C. Beynen. 2002a. Effect of increasing intakes of linseed oil on growth performance, fatty acid status and plasma lipids in weanling piglets. Submitted for publication.
- Schellingerhout, A. B., G. W. Jimmink, H. Everts, and A. C. Beynen. 2002b. High water content of feed raises dry matter intake by weanling piglets. Submitted for publication.
- Scheppach, W., W. Burghardt, P. Bartram, and H. Kasper. 1990. Addition of dietary fiber to liquid formula diets: the pros and cons. *JPEN- Parenter Enter.* 14: 204-209.
- Scholten, R. 2001. Fermentation of liquid diets for piglets. Ph.D. Diss., Wageningen Univ., Wageningen, The Netherlands.
- Shanahan, F., 1994. Pages 643-684 in *Physiology of the Gastrointestinal Tract*. L. R. Johnson, ed. 3rd ed., Raven Press, New York, USA.
- Silk, D. B. A. 1989. Fibre and enteral nutrition. *Gut*. 30: 246-264.
- Smith, S. D., M. A. Cardona, S. A. Wishnev, A. G. Kurkchubasche, and M. I. Rowe. 1992. Unique characteristics of the neonatal intestinal mucosal barrier. *J. Pediatr. Surg.* 27(3): 333-338.
- Smith, M. W., B. G. Miller, P. S. James, and F. J. Bourne. 1985. Effect of weaning on the structure and function of piglet small intestine. Pages 75-78 in *Digestive Physiology in the Pig*. A. Just, H. Jorgensen, and J. A. Fernandez, eds. National Institute of Animal Science, Report no. 580, Denmark.
- Smolders, M. A. H. H., M. M. Krimpen, R. H. J. van Scholten, and D. J. P. H. Loo. 2000. De invloed van melkzuur op de technische en financiële resultaten en gezondheid van gespeende biggen. *Praktijkonderzoek Varkenshouderij*, Proefverslag No. P 1.246, Rosmalen, The Netherlands.
- Souba, W. W. 1993. Intestinal glutamine metabolism and nutrition. *J. Nutr. Biochem.* 4: 2-9.
- Spaeth, G., R. D. Berg, R. D. Specian, and E. A. Deitch. 1990. Food without fiber promotes bacterial translocation from the gut. *Surgery*. 108: 240-247.
- Specian, R. D., and M. R. Neutra. 1982. Regulation of intestinal goblet cell secretion. I. Role of parasympathic stimulation. *Am. J. Phys.* 242 (Gastrointest. Liver Physiol 5): G370-G379.
- Stokes, C., and J. F. Bourne. 1989. Pages 164-191 in *Veterinary Clinical Immunology*. R. E. W. Halliwell, ed. W. B. Saunders Co., Philadelphia, USA.
- Stoll, B., J. Henry, P. J. Reeds, H. Yu, F. Jahoor, and D. G. Burrin. 1998. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. *J. Nutr.* 128: 606-614.
- Trier J. S., and J. L. Madara, 1981. Functional morphology of the mucosa of the small intestine. Pages 925-961 in *Physiology of the Gastrointestinal Tract*. L.R. Johnson, ed. Raven Press, New York, USA.
- Touchette, K. J., G. L. Allee, K. Watanabe, Y. Toride, I. Shinzato, and J. L. Usry. 2000. The effect of arginine and glutamine on postweaning performance and intestinal morphology of pigs. *J. Anim. Sci.* 83(suppl. 1): 182.
- Uil, J. J., R. M. van Elburg, F. M. van Overbeek, and C. J. J. Mulder. 1997. Clinical implications of the sugar absorption test: intestinal permeability test to assess mucosal barrier function. *Scand. J. Gastroenterol.* 32 Suppl. 223: 70-78.
- Uni, Z., S. Ganot, and D. Sklan. 1998. Posthatch development of mucosal function in the broiler small intestine. *Poultry Sci.* 77: 75-82.
- Van Beers-Schreurs, H. 1996. The changes in the function of the large intestine of weaned pigs. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- Van Dijk, A. J., P. M. M. van Enthoven, S. G. C. van den Hoven, M. M. M. H. van Laarhoven, T. A. Niewold, M. J. A. Nabuurs, and A. C. Beynen. 2002. The effect of dietary spray-dried porcine plasma on clinical response in weaned piglets challenged with pathogenic *Escherichia coli*. *Vet. Micr.* 84: 207-218.

- Van Dijk, A. J., H. Everts, M. J. A. Nabuurs, R. J. C. F. Margry, and A. C. Beynen. 2001. Growth performance of weanling pigs fed spray-dried animal plasma: a review. *Liv. Prod. Sci.* 68: 263-274.
- Van Dijk, A. J., T. A. Niewold, R. J. C. F. Margry, S. G. C. van den Hoven, M. J. A. Nabuurs, N. Stockhofe-Zurwieden, and A. C. Beynen. 2001. Small intestinal morphology in weaned piglets fed a diet containing spray-dried porcine plasma. *Res. Vet. Sci.* 71: 17-22.
- Van Weerden, E. J., and J. Huisman, eds. 1989. Antinutritional factors (ANF) in the nutrition of monogastric farm animals. PUDOC, Wageningen, The Netherlands.
- Vellenga, L., 1989. Intestinal permeability in pigs and rats. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- Verdonk, J. M. A. J., M. A. M. Spreeuwenberg, G. C. M. Bakker, and M. W. A. Verstegen. 2001. Nutrient intake level affects histology and permeability of the small intestine in newly weaned piglets. Pages 332-334 in *Digestive Physiology of Pigs*. J. E. Lindberg, and B. Ogle, eds. CABI Publishing, Wallingford, UK.
- Walker, R. I., and R. L. Owen. 1990. Intestinal barriers to bacteria and their toxins. *Ann. Rev. Med.* 41: 393-400.
- Wang, Q. 1995. Pathologically and experimentally induced intestinal barrier changes evaluated by permeability measurements. Ph.D. Diss., Department of Animal Physiology, Lund Univ., Sweden.
- Windmueller, H. G. 1982. Glutamine utilization by the small intestine. *Adv. Enzym.* 53: 201-237.
- Wirén, M., J. D. Söderholm, J. Lindgren, G. Olaison, J. Permert, H. Yang, and J. Larsson. 1999. Effects of starvation and bowel resection on paracellular permeability in rat small-bowel mucosa in vitro. *Scand. J. Gastroentero.* 34: 156-162.
- Worsaae, H., and M. Schmidt, 1980. Plasma cortisol and behaviour in early weaned piglets. *Acta Vet. Scan.* 21: 640-657.
- Wu, G. 1998. Intestinal mucosal amino acid catabolism. *J. Nutr.* 128: 1249-1252.
- Wu, G., N. E. Flynn, D. A. Knabe, and L. A. Jaeger. 2000a. A cortisol surge mediates the enhanced polyamine synthesis in porcine enterocytes during weaning. *Am. J. Physiol. Regulatory Integrative Comp Physiol.* 279: R554-R559.
- Wu, G., and D. A. Knabe. 1994. Free and protein bound amino acids in sow's colostrum and milk. *J. Nutr.* 124: 415-424.
- Wu, G., S. A. Meijer, and D. A. Knabe. 1996. Dietary glutamine supplementation prevents jejunal atrophy in weaned pigs. *J. Nutr.* 126: 2578-2584.
- Wu, G., C. J. Meininger, K. Kelly, M. Watford and S. M. Morris, Jr. 2000b. A cortisol surge mediates the enhanced expression of pig intestinal pyrroline-5-carboxylate synthase during weaning. *J. Nutr.* 130: 1914-1919.
- Wu G., and S. M. Morris Jr. 1998. Arginine metabolism: nitric oxide and beyond. *Biochem. J.* 336: 1-17.
- Xu, R. J. 1996. Development of the newborn GI tract and its relation to colostrum / milk intake: a review. *Reprod. Fertil. Dev.* 8: 35-48.
- Yang, H., J. Söderholm, J. Larsson, J. Permert, G. Olaison, J. Lindgren, and M. Wirén. 1999. Glutamine effects on permeability and ATP content of jejunal mucosa in starved rats. *Clin. Nutr.* 18(5): 301-306.
- Yoo, S. S., C. J. Field, and M. I. McBurney. 1997. Glutamine supplementation maintains intramuscular glutamine concentration and normalizes lymphocyte function in infected early weaned pigs. *J. Nutr.* 127: 2253-2259.
- Zarkadas, L. N., and J. Wiseman. 2000a. Inclusion of differently processed full fat soya bean in diets for piglets I. Performance. Page 45 in *The Weaner Pig*. M. Varley, and J. Wiseman, eds. BSAS Occasional Meeting. British society of animal science. Penicuik, Midlothian, UK.

- Zarkadas, L. N., and J. Wiseman. 2000b. Inclusion of differently processed full fat soya bean in diets for piglets II. Digestibility and intestinal morphology. Page 46 in *The Weaner Pig*. M. Varley, and J. Wiseman, eds. BSAS Occasional Meeting. British society of animal science. Penicuik, Midlothian, UK.
- Zarrinkalam, M.R., J. Le Dividich, F. Strullu, and D. R. Tivey. 1999. Insulin like growth factor-I (IGF-I) alters the morphology of epithelial tight junctions in the duodenum of 36-hour old piglets. Page 124 in *Manipulating Pig Production VII.*, P. D. Cranwell, ed. Australian Pig Science Association. Werribee, Australia.
- Zijlstra, R. T., J. Odle, W. F. Hall, B. W. Petschow, H. B. Gelberg, and R. E. Litov. 1994. Effect of orally administered epidermal growth factor on intestinal recovery of neonatal pigs infected with rotavirus. *J. Pediatr. Gastr. Nutr.* 19: 382-390.

---

## CHAPTER 3

---

### **Small Intestine Epithelial Barrier Function is compromised in Pigs with Low Feed Intake at Weaning**

M. A. M. Spreeuwenberg <sup>1</sup>, J. M. A. J. Verdonk <sup>2</sup>, H. R. Gaskins <sup>3</sup>, and  
M. W. A. Verstegen <sup>4</sup>.

<sup>1</sup> Nutreco Swine Research Centre, Boxmeer, The Netherlands; <sup>2</sup> ID TNO Animal Nutrition, Lelystad, The Netherlands; <sup>3</sup> Departments of Animal Sciences and Veterinary Pathobiology, University of Illinois at Urbana-Champaign, IL, USA; <sup>4</sup> Division of Animal Nutrition, Department of Animal Sciences, University of Wageningen, The Netherlands.

Journal of Nutrition 131: 1520-1527, 2001.

Reproduced with permission of the American Society of Nutritional Sciences

## ABSTRACT

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

## INTRODUCTION

Pigs are confronted by multiple stressors at weaning. Under commercial conditions, weaning may involve complex social changes, including separation from the sow, a new housing system, separation from littermates and exposure to unfamiliar pigs (Fraser et al., 1998). Diet composition also changes at weaning; the liquid milk from the sow is replaced by pelleted dry feed with carbohydrates instead of fat as the main energy source.

Abrupt weaning is typically accompanied by low feed intake, which seems to be the main reason for the growth stasis after weaning (Leibrandt et al., 1975). Weaning also causes morphologic and histologic changes of the small intestine of pigs (Miller et al., 1986; Cera et al., 1988; Dunsford et al., 1989; Hall and Byrne, 1989; Kelly et al., 1991; Nabuurs et al., 1993; McCracken et al., 1995; 1999; Pluske et al., 1996a; 1996b). These changes include reduction in villus height and an increased crypt depth. The magnitude of the intestinal responses seems to be related to feed intake of the piglets (Kelly et al., 1991; Pluske et al., 1996b), independent of diet composition (McCracken et al., 1995; 1999). Beers-Schreurs (1996) found that the weaning transition itself explained part of the reduction in villus height and increased crypt depth. Villus height decreased and crypt depth increased in weaned



piglets compared with unweaned piglets given sow's milk at a high energy level after weaning. The reduction in villus height was even more pronounced when the piglets were fed a weanling diet or sow's milk at a comparable low energy level (Beers-Schreurs, 1996). Starvation itself decreased jejunal villus height and increased paracellular permeability in the ileum and jejunum of adult rats (Wirén et al., 1999). An inverse relationship was found between ATP concentrations in jejunal mucosa and permeability (Yang et al., 1999), indicating that at a low energy level, permeability is increased.

The relationship between epithelial barrier function and villus atrophy at weaning is not understood. A compromise in epithelial barrier function possibly increases paracellular permeability. With increased paracellular permeability, toxins, allergenic compounds or bacteria may enter systemic tissues, resulting in inflammatory or immunologic responses (Deitch, 1993; Wang, 1995).

Providing piglets sow's milk after weaning resulted in less villus atrophy compared with a weanling diet (Beers-Schreurs, 1996); thus milk components seem to be favorable. Sow's milk is composed mainly of fat ( $40.6 \text{ g} \cdot (100 \text{ g milk})^{-1}$ ), protein ( $29.4 \text{ g} \cdot (100 \text{ g milk})^{-1}$ ) and lactose ( $28.3 \text{ g} \cdot (100 \text{ g milk})^{-1}$ ) (Darragh and Moughan, 1998). Lactose is converted by lactase to galactose and glucose; glucose can be an energy source for epithelial cells (Wu et al., 1995). Lactose seems, therefore, a key energy source for intestinal epithelial cells in young piglets. Some amino acids in the milk protein can be used as an energy source for epithelial cells (e.g., glutamine), as well as contribute to protein synthesis.

This experiment investigated mucosal variables over time in response to low energy intake and compared the effectiveness of lactose vs. protein in preserving mucosal integrity during the weaning transition. We postulated that the energy supply is more limiting than the protein supply for epithelial cells in contributing to mucosal integrity, i.e., a diet with a high lactose/protein ratio would better preserve mucosal integrity. T-lymphocyte cellularity was measured as an indicator of inflammation. Transepithelial permeability was measured as a functional indicator of mucosal integrity.

## **MATERIALS AND METHODS**

### **Animals and weaning**

Barrows (n = 66) procured from a commercial maternal line herd [Great York X (Dutch Landrace x Finnish Landrace)) were used. The piglets were weaned at 25.9 (SD: 2.01) days of age. Creep feed was not provided during the suckling period to avoid adaptation to experimental diets and to make the piglets' treatment uniform. At weaning, pigs were removed from the sow and transported 10 km to the TNO Nutrition research facility in Wageningen (The Netherlands). Upon arrival from the source farm, pigs were weighed and

housed individually in 50 x 90 cm<sup>2</sup> floor pens. The walls of the pens were transparent plastic, enabling visual contact among the piglets. Each pen was equipped with a plastic trough. Water was supplied via the liquid milk replacer diets. Environmental temperature was maintained at 24 °C. Lights were on continuously. The experimental protocol was approved by the Animal Care and Ethics Committee of the research institute TNO.

### **Feeds, feeding and experimental design**

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

Piglets were fed at a relatively low energy level; the digestible energy (DE) offered was one third of the calculated energy intake according to equation 1. This equation describes the voluntary DE intake of weaned piglets from 5 to 15 kg based on BW (Beers-Schreurs, 1996; National Research Council, 1998):

$$DE = ((455.5 \times BW) - (9.46 \times BW^2) - 1531) \times 4.181 \quad [1]$$

where DE is the digestible energy intake (kJ · day<sup>-1</sup>) and BW is body weight (kg). The amount of milk replacer offered to the piglets was calculated daily. BW was calculated on the basis of BW upon arrival and the expected growth of 60 g · day<sup>-1</sup> [based on Pluske et al. (1996b)]. The milk replacer was fed at a concentration of 62 g · (l water)<sup>-1</sup>. The pigs were fed 4 times per day at 0900, 1230, 1700 and 2130 h. Feed refusals were collected, weighed and subtracted from the amount of milk offered to calculate actual daily feed intake.

### **Growth and health**

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

Table 1 Diet composition of milk replacers that differ in the lactose and protein ratio: low lactose/high protein (LL/HP), control (C), or high lactose/low protein (HL/LP).

Item	LL/HP		C		HL/LP	
Ingredient (g · kg <sup>-1</sup> )						
Casein	265.0		175.0		85.0	
Whey protein concentrate	265.0		175.0		85.0	
Dry Fat Concentrate <sup>1</sup>	340.0		340.0		340.0	
Lactose	75.0		240.5		405.5	
Vegetable oils	5.0		13.0		21.0	
Premix	10.0		10.0		10.0	
Minerals	35.0		41.5		48.5	
Salt	5.0		5.0		5.0	
Digestible Energy (MJ · kg <sup>-1</sup> )	24.0		23.6		23.1	
Nutrients (g · kg <sup>-1</sup> )	Calculated	Analysed	Calculated	Analysed	Calculated	Analysed
Dry matter	968.8		978.2		987.6	
Crude protein	448.4	441	299.0	300	149.6	153
Crude fat	299.5	275	299.8	287 <sup>2</sup>	300.1	270
Ash	56.5	61	55.2	63	54.5	61
Carbohydrates <sup>3</sup>	164.4		324.2		483.4	

<sup>1</sup> Based on butter oil

<sup>2</sup> Fatty acid composition (in %): C6:0 = 2.0; C6:1 = 2.0; C8:0 = 1.3; C10:0 = 2.6; C12:0 = 3.8; C14:0 = 10.5; C14:1 = 1.0; C15:0 ISO = 0.3; C15:0 ANTE ISO = 0.5; C15:1 = 0.9; C15:2 = 0.2; C16:0 = 28.9; C16:1 = 1.8; C17:0 ISO = 0.5; C17:0 ANTE ISO = 0.4; C17:1 = 0.7; C17:2 = 0.3; C18:0 = 9.7; C18:1 = 24.1; C18:2 = 7.7; C18:3 = 1.4; C18:4 = 0.6; C20:0 = 0.2; C20:1 = 0.2; C20:3 < 0.1; C20:4 < 0.1; C20:5 = 0.1; C22:0 = 0.1

<sup>3</sup> Carbohydrates = dry matter - crude protein- crude fat – ash – crude fiber (=0)

## Sampling of gut for histology and permeability

At day 0, 1, 2 and 4 postweaning, piglets to be killed were weighed and anesthetized by inhalation of a mixture of N<sub>2</sub>O/O<sub>2</sub> (ratio 2/1) and isoflurane. The concentration of isoflurane was adjusted to the depth of the narcosis (Guedel, stadium III, phase 2). A midline laparotomy was performed. At three different segments of the small intestine, tissue samples were taken as follows: 0.5 m distal of the ligament of Treitz (proximal small intestine), 3.5 m distal of the ligament of Treitz (mid small intestine) and 0.5 m proximal to the ileocecal ligament (distal small intestine). For the villus height, crypt depth and villus/crypt ratio, the mean value of the three sampled segments was calculated. After samples were taken, piglets were killed by an intracardiac injection (2 mL) of T61 (a watery solution containing a combination of embutramide, mebezoniumiodide and tetracaine hydrochloride; Hoechst Holland, Amsterdam, The Netherlands).

For histologic analysis, tissue samples of the proximal, mid, and distal small intestine were cut open longitudinally at the antimesenteric attachment, prepared on dental wax with the villi on the upper side and fixed in 0.1 mol per L phosphate buffered formalin solution ( $40 \text{ ml} \cdot \text{l}^{-1}$ ). 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 ( $5 \text{ }\mu\text{m}$ ) were cut and stained with either the periodic acid/Schiff procedure (PA/S) or a combination of the basophilic dyes, high iron diamine (HID) and alcian blue (AB). From the PA/S-stained sections, crypt depth ( $\mu\text{m}$ ), villus height ( $\mu\text{m}$ ) and the number of goblet cells (per  $100 \text{ }\mu\text{m}$  crypt) were determined. From the HID/AB-stained sections, goblet cells of 5 crypts were classified as either sialomucin-containing (blue) or sulfomucin-containing (brown) to investigate the chemical nature of the mucins in the goblet cells. The percentage of sulfomucin-containing cells was calculated. The percentage of sialomucin-containing cells was 100 minus the percentage of sulfomucin-containing cells (data not shown).

To measure the number of  $\text{CD4}^+$  and  $\text{CD8}^+$  cells, mid-small intestinal tissues (3 cm) were deep frozen in liquid nitrogen for  $\sim 30$  min, stored frozen at  $-80^\circ\text{C}$  until cryosectioning at  $5 \text{ }\mu\text{m}$  thickness and fixed in acetone for 7 min at room temperature (CD or cell differentiation molecules are cell surface markers of various leukocyte subsets). Cell labeling was performed by incubating the preparations overnight with murine antibodies directed against either porcine CD4 (clone number MIL-17, # MCA 1749, Serotec, Oxford, UK) or CD8 surface antigens (clone number MIL-12, # MCA 1223, Serotec). Subsequently, the samples were incubated with horse anti-mouse antibodies for 30 min followed by Universal peroxidase AEC (3- amino-9-ethyl carbazole substrate solution) for 25 min. Isotonic PBS was used to repeatedly wash the preparations. The tissue sections were counterstained using hematoxylin, washed with tap water and mounted. The number of  $\text{CD4}^+$  and  $\text{CD8}^+$  cells was determined per  $\mu\text{m}^2$  in the lamina propria of the crypts using light microscopy.

To measure transepithelial transport, mid-small intestinal tissue samples (5 cm) were taken. Transepithelial transport of two compounds was measured in TNO transport chambers, i.e., [ $^{14}\text{C}$ ] GlySar (Cambridge Research Biochemicals, Northwich, UK) and [ $2\text{-}^3\text{H}$ ] mannitol (ICN Biomedicals, Zoetermeer, NL). GlySar is a small hydrophilic compound with a molecular weight of 146 Da. It is transported mainly via a transcellular route with a  $\text{H}^+$ -coupled di/tripeptide carrier (Duizer, 1999). Mannitol has a molecular weight of 182 Da and is transported mainly via a paracellular route (Duizer, 1999). Intestinal tissues were rinsed with an ice-cold buffer solution of HEPES-buffered phenol red-free Dulbecco's modified Eagles medium (DMEM) and cut open longitudinally. The tissue was placed with the mucosa on the upper side on a flat underground; with a blunt razor blade, the mucosal layer was carefully stripped off the muscle layer to preserve mucosal integrity. Samples of the mucosal layer were taken using a 9-mm steel punch. Flat sheets, in which isolated intestinal segments ( $0.2 \text{ cm}^2$ )

separate a 1.5 mL mucosal and a 1.5 mL serosal compartment, were placed in the Ussing chambers. The effective exposed area in the Ussing chamber was 0.196 cm<sup>2</sup>. The radiolabeled GlySar and mannitol were mixed with unlabeled compounds to yield final concentrations of 10 µmol · l<sup>-1</sup>. The donor compartment (mucosal side) was filled with 1.25 mL HEPES DMEM medium containing radiolabeled GlySar (10 µmol · l<sup>-1</sup>) and mannitol (10 µmol · l<sup>-1</sup>). The receptor compartment (serosal side) was filled with 1.25 mL HEPES DMEM medium. Both compartments were aerated (O<sub>2</sub>/CO<sub>2</sub>, 95/5) at a temperature of 37°C and stirred by gas lift. At indicated time points (15, 30, 45, 75 and 105 min), 0.5-mL samples were taken from the serosal side and the volume was reconstituted with DMEM without phenol red. <sup>3</sup>H and <sup>14</sup>C radioactivity was determined in the samples and the tissue (at the end of the experiment) by liquid scintillation counting with the Digital Overlay Technique using the Spectrum Library and the External Standard Spectrum for quench correction. Permeability coefficients (P<sub>ms</sub>) were determined on the basis of the appearance of the probe at the serosal side according to the following equation:

$$P_{ms} = R / (A \cdot C_o) \quad [2]$$

where P<sub>ms</sub> = permeability coefficient from mucosal to serosal side (cm · s<sup>-1</sup>); R = permeability rate (mol · s<sup>-1</sup>); A = exposed intestinal area (cm<sup>2</sup>); C<sub>o</sub> = initial mucosal concentration of the test substance (mol · ml<sup>-1</sup>).

### Statistical analysis

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

$$y_{ijk} = \mu + B_i + S_j + (B \times S)_{ij} + e_{ijk} \quad [3]$$

where y<sub>ijk</sub> = dependent variable; µ = overall mean; B<sub>i</sub> = fixed effect of batch (i = 1, 2); S<sub>j</sub> = fixed effect of day postweaning (j = 1, 2, 3 and 4); (B × S)<sub>ij</sub> = interaction between batch (B) and day postweaning (S); e<sub>ijkl</sub> = error term.

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

$$y_{ijkl} = \mu + B_i + S_j + D_k + e_{ijkl} \quad [4]$$

where  $y_{ijkl}$  = dependent variable;  $\mu$  = overall mean;  $B_i$  = fixed effect of batch, ( $i = 1, 2$ );  $S_j$  = fixed effect of day postweaning ( $j = 1, 2, 3$ );  $D_k$  = fixed effect of diet composition ( $k = 1, 2, 3$ );  $e_{ijkl}$  = error term.

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

## RESULTS

### General

BW at weaning was 7.8 kg (SD: 0.13). Daily weight loss ( $\text{g} \cdot \text{day}^{-1} \cdot \text{pig}^{-1}$ ) through the 4-day treatment period was 97.2 (SD: 128.59) for LL/HP, 65.3 (SD: 127.23) for C, and 69.4 (SD: 146.17) for HL/LP. None of the piglets developed watery faeces during the experimental period (score 3). Two had thick liquid faeces (score 2); of these, 1 piglet received the C treatment and 1 the HL/LP treatment. Eight piglets had shapeless faeces (score 1). Of these, 2 piglets received the C treatment, 1 piglet received LL/HP and 5 received HL/LP. The diarrhoea scores were not significantly different among groups ( $P > 0.10$ ). Inclusion of an independent binomial variable in the statistical model indicating the occurrence/absence of diarrhoea, or exclusion of the piglets with diarrhoea from the data did not affect the results and conclusions; therefore, the piglets with a diarrhoea score were left in the database. None of the piglets received medical treatment during the experimental period.

### Energy intake

Figure 1 shows the DE intake of pigs fed the three milk replacers for 4 days postweaning. The number of piglets for the calculation of the mean DE intake decreased from 54 piglets at day 1, to 36 at day 2 and to 18 at day 3 and 4, due to dissection. DE intake did not differ among diet groups on the different sampling days. DE intake ( $\text{kJ} \cdot \text{pig}^{-1}$ ) was 648 (SD: 388.93) on day 1, 1668 (SD: 625.54) on day 2, 1995 (SD: 605.25), on day 3, and 1990 (SD: 670.80) on day 4 postweaning. Independent of diet, the DE intake was lower than the amount offered to the piglets. The percentage of actual energy intake compared with the total amount offered was 43 % at day 1, 81 % at day 2, 96 % at day 3 and 94 % at day 4. Over time, intake increased ( $P < 0.01$ ) for pigs fed each of the three diets.

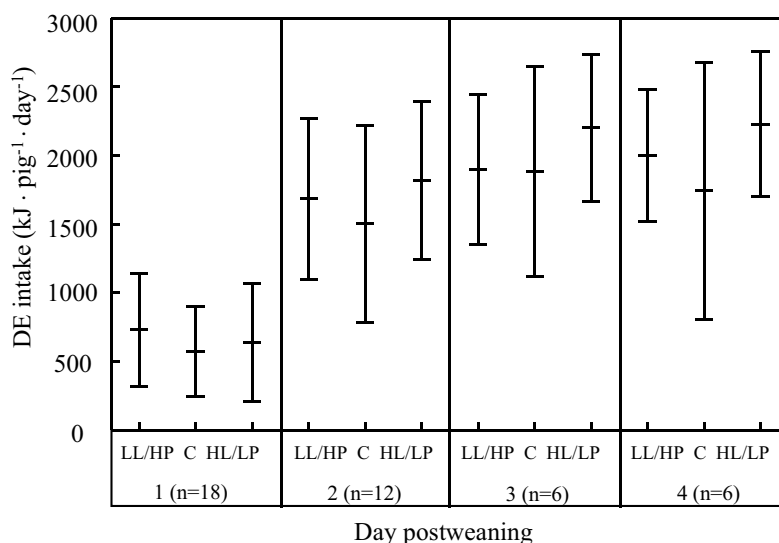


Figure 1 Digestible energy (DE) intake of piglets fed a low lactose/high protein (LL/HP), control (C) or high lactose/low protein (HL/LP) milk replacer for the first 4 days postweaning. Values are means  $\pm$  SD.

### Villus height, crypt depth and small intestinal weight

Histologic parameters and weight of the small intestine per kg BW or per cm length of the small intestine at day 0, 1, 2 and day 4 postweaning are shown in Table 2. Decreased villus height, shallower crypt depths and decreased villus/crypt ratios were most pronounced at the proximal and mid-small intestine. At the distal small intestine, no differences were observed. Villus height of the three sampled sites decreased significantly compared with day 0 ( $P < 0.01$ ) with the shortest villi at day 2. Villus heights at the three sampled segments were 369  $\mu\text{m}$  on day 0, 349  $\mu\text{m}$  on day 1, 258  $\mu\text{m}$  on day 2 and 317  $\mu\text{m}$  on day 4 (SEM, 12.8). The same mean decrease in villus height over time postweaning could be seen at the proximal and mid-small intestine.

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

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

Table 2 Least square means ( $\pm$  SEM) of histological parameters and weight of small intestine per kg of body weight (BW) or per cm length of the small intestine of piglets fed a liquid milk replacer at 0, 1, 2, or 4 days post weaning. The histological parameters - villus height, crypt depth and ratio between villus height and crypt depth - were determined at proximal (prox.), mid or distal part of the small intestine.

	Villus height (μm)				Crypt depth (μm)				Villus/Crypt ratio				Weight of small intestine	
	prox. <sup>1</sup>	mid	distal	mean <sup>2</sup>	prox.	mid	distal	mean	prox.	mid	distal	mean	per kg body weight (g · (kg BW) <sup>-1</sup> )	per cm of length (g · cm <sup>-1</sup> )
Day post weaning														
0	502 <sup>a</sup>	351 <sup>ab</sup>	255	369 <sup>a</sup>	178 <sup>ab</sup>	176 <sup>ab</sup>	158	170 <sup>ab</sup>	2.8 <sup>a</sup>	2.0 <sup>b</sup>	1.7	2.2 <sup>a</sup>	30.4 <sup>a</sup>	7.5
1	433 <sup>c</sup>	376 <sup>a</sup>	231	349 <sup>a</sup>	168 <sup>b</sup>	163 <sup>b</sup>	143	157 <sup>b</sup>	2.6 <sup>a</sup>	2.4 <sup>a</sup>	1.7	2.3 <sup>a</sup>	24.1 <sup>bc</sup>	7.6
2	317 <sup>b</sup>	253 <sup>c</sup>	214	258 <sup>c</sup>	166 <sup>b</sup>	162 <sup>b</sup>	145	157 <sup>b</sup>	2.0 <sup>b</sup>	1.6 <sup>c</sup>	1.5	1.7 <sup>b</sup>	23.6 <sup>c</sup>	7.8
4	388 <sup>b</sup>	318 <sup>b</sup>	244	317 <sup>b</sup>	187 <sup>a</sup>	183 <sup>a</sup>	153	175 <sup>a</sup>	2.1 <sup>b</sup>	1.8 <sup>bc</sup>	1.7	1.9 <sup>b</sup>	26.4 <sup>b</sup>	7.9
SEM	23.4	19.4	15.8	12.8	6.5	5.8	8.1	5.4	0.17	0.13	0.17	0.11	1.02	0.24
P-value <sup>3</sup>														
Day	**	**	ns	**	t	*	ns	*	**	**	ns	**	**	ns

<sup>1</sup> Different letters within a column are significantly different; the level of significance is identified by the P-value.

<sup>2</sup> Mean value of 3 segments.

<sup>3</sup> P-value of the model: \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; t,  $P < 0.10$ ; ns, not significant.



The weight of the small intestine per kg BW decreased significantly over time postweaning with the lowest weight at day 2 ( $23.6 \text{ g} \cdot (\text{kg BW})^{-1}$ ) (Table 2). The weight (g) per cm of the small intestine did not change during time postweaning and was, on average,  $7.7 \text{ g} \cdot \text{cm}^{-1}$  (SD: 1.08).

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

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

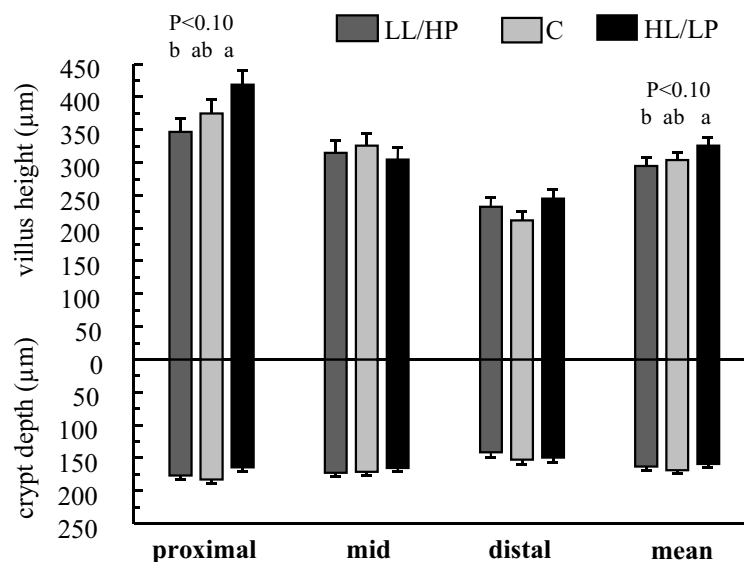


Figure 2 Villus height and crypt depth at the proximal, mid and distal small intestine and the mean value of the three segments of piglets fed low lactose/high protein (LL/HP), control (C) or high lactose/low protein (HL/LP) milk replacer. Values are means  $\pm$  SEM,  $n=18$ .

## Crypt goblet cells

Overall, the number of goblet cell per 100  $\mu\text{m}$  of crypt was not different over time postweaning or across dietary treatments (data not shown). The number of crypt goblet cells was 5.5 (SD: 1.39) at the proximal, 5.6 (SD: 1.46) at the mid-, and 7.8 (SD: 1.81) at the distal small intestine (data not shown). Furthermore, the percentage of sulfomucin-containing cells in intestinal crypts was not different over time postweaning or across dietary treatments (data not shown). The percentage of crypt sulfomucin-containing cells was 35.4 % (SD: 24.73) at the proximal, 27.2 % (SD: 25.56) at the mid-, and 32.8 % (SD: 25.04) at the distal small intestine (data not shown).

Table 3 Least square means ( $\pm$  SEM) of transcellular (GlySar) and paracellular (mannitol) transport and CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets of the mid small intestine of piglets fed a liquid milk replacer at 0, 1, 2, or 4 days post weaning.

	Transepithelial transport ( $\times 10^{-6} \text{ cm} \cdot \text{s}^{-1}$ )		number of positive T-cell lymphocytes (per $10^6 \mu\text{m}^2$ crypt)		
	GlySar	mannitol <sup>1</sup>	CD4 <sup>+</sup>	CD8 <sup>+</sup>	CD4 <sup>+</sup> /CD8 <sup>+</sup>
Days post weaning					
0	16.6	6.6 <sup>a</sup>	216 <sup>a</sup>	117	2.2 <sup>a</sup>
1	15.6	8.1 <sup>a</sup>	125 <sup>b</sup>	116	1.1 <sup>c</sup>
2	16.8	12.2 <sup>b</sup>	195 <sup>a</sup>	168	1.4 <sup>bc</sup>
4	19.8	11.9 <sup>b</sup>	226 <sup>a</sup>	167	2.0 <sup>ab</sup>
SEM	1.52	0.88	30.7	28.9	0.26
P-value <sup>2</sup>					
Day	ns	**	t	ns	*

<sup>1</sup> Different letters within a column are significantly different; the level of significance is identified by the P-value

<sup>2</sup> P-value of the model: \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; t,  $P < 0.10$ ; ns, not significant.

## T-lymphocytes

The numbers of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells (per  $10^6 \mu\text{m}^2$  crypt) at the mid-small intestine on day 0, 1, 2 or 4 postweaning are shown in Table 3. The number of CD4<sup>+</sup> T-cells tended to be lower at day 1 compared with day 0 and 4 ( $P < 0.10$ ). The number of CD8<sup>+</sup> T-cells at 0 or 1 postweaning was numerically lower than at day 2 and 4 postweaning, but this difference was not significant. The CD4<sup>+</sup>/CD8<sup>+</sup> ratio was significantly lower on day 1 and 2 compared with day 0 ( $P < 0.05$ ), with the lowest ratio on day 1. The ratio of CD4<sup>+</sup>/CD8<sup>+</sup> T-cell lymphocytes had increased significantly by day 4 compared with day 1 postweaning. Diet composition did not affect the number of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells or the CD4<sup>+</sup>/CD8<sup>+</sup> ratio (data not shown). A positive correlation was found between the number of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells (Table 4;  $R = 0.49$ ,  $P < 0.01$ ). The number of CD4<sup>+</sup> T-cells tended to be negatively correlated with

villus height ( $R = -0.23$ ,  $P < 0.10$ ) and the villus/crypt ratio ( $R = -0.22$ ,  $P < 0.10$ ) at the mid small intestine. The number of  $CD8^+$  T-cells was negatively correlated with villus height ( $R = -0.27$ ,  $P < 0.05$ ) and the villus/crypt ratio ( $R = -0.25$ ,  $P < 0.05$ ) at the mid-small intestine. The mean DE intake tended to be positively correlated with  $CD4^+$  T-cells ( $R = 0.25$ ,  $P < 0.10$ ) and the  $CD4^+/CD8^+$  ratio ( $R = 0.22$ ,  $P < 0.10$ ).

## Permeability

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

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

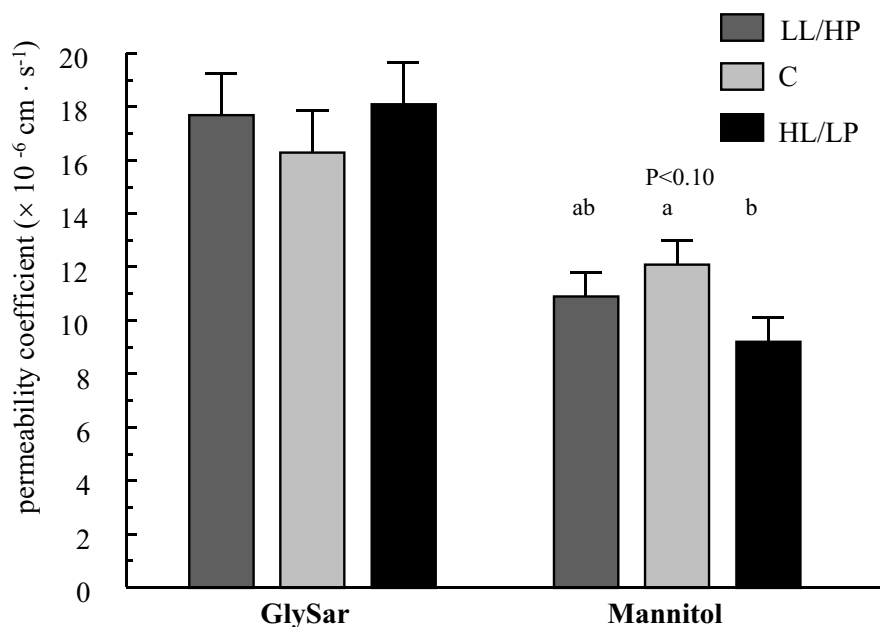


Figure 3 Transcellular (GlySar) and paracellular (mannitol) transport ( $10^{-6} \text{ cm} \cdot \text{s}^{-1}$ ) of the mid small intestine of piglets fed low lactose/high protein (LL/HP), control (C) or high lactose/low protein (HL/LP) milk replacer. Values are means  $\pm$  SEM,  $n = 12$ .

Table 4 Pearson correlation coefficients between the histological parameters, T-cell subsets, transcellular transport, and digestible energy (DE) intake at the mid small intestine of piglets fed a liquid milk replacer at 0, 1, 2, or 4 days post weaning.

	Glysar <sup>1</sup>	CD4 <sup>+</sup> T-cells <sup>2</sup>	CD8 <sup>+</sup> T-cells <sup>2</sup>	CD4 <sup>+</sup> /CD8 <sup>+</sup> T-cell ratio	Villus height <sup>3</sup>	Crypt depth <sup>3</sup>	Villus/Crypt ratio	DE intake <sup>4</sup>
Mannitol <sup>1</sup>	0.32 * <sup>5</sup>	ns	0.42 **	ns	ns	ns	ns	ns
Glysar		ns	0.32 *	ns	ns	ns	ns	ns
CD4 <sup>+</sup>			0.49 **	0.46 **	-0.23 t	ns	-0.22 t	0.25 t
CD8 <sup>+</sup>				-0.33 **	-0.27 *	ns	-0.25 *	ns
CD4 <sup>+</sup> /CD8 <sup>+</sup>					ns	ns	ns	0.22 t
Villus height						ns	0.88 **	ns
Crypt depth							-0.44 **	ns
Villus/Crypt ratio								ns

<sup>1</sup>  $\times 10^{-6} \text{ cm} \cdot \text{s}^{-1}$

<sup>2</sup> per  $10^6 \mu\text{m}^2$  crypt

<sup>3</sup>  $\mu\text{m}$

<sup>4</sup>  $\text{kJ} \cdot \text{day}^{-1} \cdot \text{piglet}^{-1}$

<sup>5</sup> P-value of the model: \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; t,  $P < 0.10$ ; ns, not significant.

## DISCUSSION

These data demonstrate an acute and sequential decline of mucosal barrier function in the pig small intestine during the first 4 days postweaning. The piglets were weaned abruptly at 26 days of age and fed one of three liquid milk replacers. For each of the three diets, the piglets consumed only  $648 \text{ kJ} \cdot \text{pig}^{-1}$  on day 1 postweaning; this corresponded to 43 % of the amount offered. Voluntary milk consumption before weaning was not measured, but averages  $5 \text{ MJ ME} \cdot \text{pig}^{-1} \cdot \text{day}^{-1}$  according to Harrell and colleagues (1993). Thus, the small intestine was subject to a brief but substantial decrease in enteral stimulation at weaning. The importance of enteral stimulation for mucosal homeostasis is well documented (Kelly et al., 1991; McCracken et al., 1995; 1999; Pluske et al., 1997; Park et al., 1998; Ganessunker et al., 1999), although the functional consequences of diminished enteral stimulation for the gut wall during the weaning transition in pigs are not clear. These data demonstrate a temporal relationship between low feed intake, increased paracellular transport, decreased ratio of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets and compromised epithelial architecture.

Stress and starvation both precede an acute temporal increase in paracellular transport and thereby affect mucosal integrity (Wirén et al., 1999; Yang et al., 1999; Spitz et al., 1996; Kiliaan et al., 1998). Weaning may be regarded as a stressor as indicated by an increase of plasma cortisol concentration and certain behavioral modifications (Worsaae and Schmidt, 1980). Plasma cortisol concentrations were 258 % greater in weanling pigs on day 2 postweaning compared with unweaned pigs (Wu et al., 2000). Kiliaan and coworkers (1998) demonstrated that macromolecular protein uptake (horseradish peroxidase) increased in rats after exposure to restraint stress at 8°C, via both the transcellular and paracellular pathways. They found that acetylcholine release during the stress response was critical in the enhanced uptake of the macromolecules across the epithelium. Starvation also increases paracellular transport across intestinal epithelium (Wirén et al., 1999; Yang et al., 1999). Moreover, Spitz and others (1996) demonstrated that the combination of starvation and stress (by glucocorticosteroid injection) resulted in a larger decrease in transepithelial resistance, indicating decreased tight junction resistance, compared with animals either starved or stressed. An increase in intestinal permeability can occur quickly. For example, within 12 h after administration of nonsteroidal anti-inflammatory drugs (NSAID), intestinal permeability to <sup>51</sup>Cr-EOT A was increased (Bjarnason, 1994).

By increased paracellular permeability, luminal antigens rather than bacteria may enter the lamina propria, resulting in inflammation. This is suggested by the fact that starvation alone does not appear sufficient for bacterial translocation, but after endotoxin challenge, starvation predisposes to bacterial translocation (Van Leeuwen et al., 1994; Deitch, 1994; Katayama et al., 1997). Locally increased intestinal permeability leads to an imbalance in normal interactions between luminal aggressive factors (in the small intestine, mainly bile, pancreas secretion, bacteria and their degradation products) and intestinal mucosa, resulting in low grade inflammation perhaps similar to that observed with NSAID-induced enteropathy (Bjarnason, 1994). Although a significant difference in paracellular transport was not observed between day 0 and 1 in this experiment, a numeric increase was noted ( $P = 0.24$ ). The positive correlation, however, between either para- and transcellular transport and the CD8<sup>+</sup> T-cell subset predicts the direct involvement of acute inflammation in small intestinal permeability. We postulate that initial translocation of luminal antigens due to increased paracellular transport might have contributed to the alteration in CD4<sup>+</sup> and CD8<sup>+</sup> T-cell populations, which might have led to a further increase in paracellular transport during the following days.

These data demonstrate a brief decline in the number of CD4<sup>+</sup> T-cells at day 1, followed by an expansion of CD8<sup>+</sup> T-cells at day 2 and 4 postweaning. The changes in T-cell subsets resulted in a significant decrease in the ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T-cells at day 1 and 2 compared with day 0. The ratio of the number of CD4<sup>+</sup> to CD8<sup>+</sup> T-cells seems critical. The number of crypt

goblets in cells was not affected by time postweaning or diet composition in this trial and was similar to that observed in an earlier piglet study (Van Leeuwen et al., 1995). Dunsford and co-workers (1989) showed incidentally a decrease in the number of goblet cells in the crypts after weaning. The results, however, were inconsistent across the small intestinal sites or across diets. In piglets administered total parenteral nutrition (TPN), the number of goblet cells increased in the villi but did not change in the crypts compared with baseline and orally fed piglets. The chemical composition of mucins was also altered in piglets administered TPN compared with baseline and orally fed piglets (Ganessunker et al., 1999). A possibly adaptive response of goblet cells in the crypts to compromised integrity of the mucosal barrier at low feed intake level was not observed in the present study, although villus goblet cells were not evaluated.

Cytokine profiles were not measured here. In a study of De Winter and colleagues (1999), however, downregulation of CD4<sup>+</sup> T-cells altered interleukin 10 and transforming growth factor  $\beta$ . Regulatory CD4<sup>+</sup> T-cells normally antagonize the expansion, localization, differentiation or effector function of T-cells involved in inflammatory responses (De Winter et al., 1999). Expansion of CD8<sup>+</sup> cells likely results in the secretion of proinflammatory cytokines (e.g., tumor necrosis factor- $\alpha$  and interferon- $\gamma$ ), which further compromises barrier function (Madara, 1989, Taylor et al., 1997). A systemic increase of proinflammatory cytokines decreases feed intake, resulting in starvation (Johnson, 1995). The T-cell alterations affected the villi more than the crypts, indicated by the negative correlation between the number of CD8<sup>+</sup> T-cells and villus height. The relationship between DE intake and the ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T-cell numbers tended to be positive, indicating that after weaning, DE intake might be important. The CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets did not differ among dietary treatments. This is in agreement with the results of McCracken (1999), who also showed that a low feed intake rather than diet composition contributes to local inflammation and affects the mucosal architecture after weaning.

The data demonstrate the onset of repair at day 4 postweaning for villus height, crypt depth, CD4<sup>+</sup> T-cells and the ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T-cell subsets. McCracken and co-workers (1995) reported the lowest villus/crypt ratio at day 5 instead of day 2, in comparing the sequential effect of the villus/crypt ratio of a liquid milk replacer on day 0, 1, 2, 5 and 7 postweaning. The resolution of inflammation is dependent on full restoration of epithelial barrier function, and the data indicate that paracellular transport remains elevated at day 4 postweaning. Plasma cortisol returned to preweaning levels on day 8 postweaning, comparing preweaned piglets and piglets at day 2 and 8 postweaning (Wu et al., 2000). Cessation of the stress likely corresponds with the observation that repair has begun at day 4.

Interestingly, despite the wide range of protein and lactose contents, diet effects were generally less pronounced than the sequential effects of low feed intake at weaning. A high

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

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

## **LITERATURE CITED**

- Beers-Schreurs, H. M. G. 1996. The changes in the function of the large intestine of weaned pigs. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- Bjarnason, I. 1994. Intestinal permeability. *Gut* 35 (suppl. 1): S18-S22.
- Cera, K. R., D. C. Mahan, R. F. Cross G. A. Reinhart, and R. E. Whitmoyer. 1988. Effect of age, weaning and postweaning diet on small intestinal growth and small intestinal morphology in young swine. *J. Anim. Sci.* 66: 574-584.
- Darragh, A. J., and P. J. Moughan. 1998. The composition of colostrum and milk. Pages 3-21 in *The Lactating Sow*. M. W. A. Verstegen, P. J. Moughan, and J. W. Schrama, eds. Wageningen Pers, Wageningen, The Netherlands.
- De Winter, H., H. Cheroute, and M. Kronenberg. 1999. Mucosal immunity and inflammation II. The yin and yang of T cells in intestinal inflammation: pathogenic and protective roles in a mouse colitis model. *Am. J. Physiol.* 276: G1317-G1321.
- Deitch, E. A. 1993. Nutrition and the gut mucosal barrier. *Curr. Opin. Gen. Surg.* 85-91.
- Deitch, E. A. 1994. Bacterial translocation: the influence of dietary variables. *Gut* 35 (suppl. 1): S23-S27.
- Duizer, E. 1999. Permeability and modulation of the intestinal epithelial barrier in vitro. Ph.D. Diss., Wageningen Univ., Wageningen, The Netherlands.
- Dunsford, B. R., W. E. Haensly, and D. A. Knabe. 1989. Effects of diet on acidic and neutral goblet cell populations in the small intestine of early weaned pigs. *Am. J. Vet. Res.* 52: 1743-1746.
- Dunsford, B. R., D. A. Knabe, and W. E. Haensly. 1989. Effect of dietary soybean meal on the microscopic anatomy of the small intestine in the early weaned pig. *J. Anim. Sci.* 67: 1855-1863.
- Fraser, D., B. N. Milligan, E. A. Pajor, P. A. Philips, A. A. Taylor, and D. M. Weary. 1998. Behavioural perspectives on weaning in domestic pigs. Pages 121-138 in *Progress in Pig Science*. J. M. Wiseman, A. Varley, and J. P. Chadwick, eds. Nottingham University Press, Nottingham, UK.
- Ganessunker, D., H. R. Gaskins, F. A. Zuckermann, and S. M. Donovan. 1999. Total parenteral nutrition alters molecular and cellular indices of intestinal inflammation in neonatal piglets. *JPEN Parent. Enter.* 23: 337-344.

- Hall, G. A., and T. F. Byrne. 1989. Effects of age and diet on small intestinal structure and function in gnotobiotic piglets. *Res. Vet. Sci.* 47: 387-392.
- Harrell, R. J., M. J. Thomas, and R. D. Boyd. 1993. Limitations of sow milk yield on baby pig growth. Pages 156-164 in *Proc. of the 1993 Cornell Nutrition Conference for Feed Manufacturers*. Cornell Univ., Ithaca, New York, USA.
- Johnson, R. W. 1995. Inhibition of growth by pro-inflammatory cytokines: an integrated view. *J. Anim. Sci.* 75: 1244-1255.
- Katayama, M., D. Xu., R. D. Specian, and E. A. Deitch. 1997. Role of bacterial adherence and the mucus barrier on bacterial translocation; effects of protein malnutrition and endotoxin in rats. *Ann. Surg.* 225: 317-326.
- Kelly, D., J. A. Smyth, K. J. McCracken. 1991. 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. *Br. J. Nutr.* 65: 169-180.
- Kiliaan, A. J., P. R. Saunders, P. B. Bijlsma, M. C. Berin, J. A. Taminiau, J. A. Groot, and M. H. Perdue. 1998. Stress stimulates transepithelial macromolecular uptake in rat jejunum. *Am. J. Physiol.* 275: G1037-G1044.
- Leibrandt, V. D., R. C. Ewan, V. C. Speer, and D. R. Zimmerman. 1975. Effect of weaning and age at weaning on baby pig performance. *J. Anim. Sci.* 40: 1077-1080.
- Madara, J. L. 1989. Loosening tight junctions; lessons from the intestine. *J. Clin. Invest.* 83: 1089-1094.
- McCracken, B. A., H. R. Gaskins, P. J. Ruwe-Kaiser, K. C. Klasing, and D. E. Jewell. 1995. Diet-dependent and diet-independent metabolic responses underlie growth stasis of pigs at weaning. *J. Nutr.* 125: 2838-2845.
- McCracken, B. A., M. E. Spurlock, M. A. Roos, F. A. Zuckermann, and H. R. Gaskins. 1999. Weaning anorexia may contribute to local inflammation in the piglet small intestine. *J. Nutr.* 129: 613-619.
- Miller, B. G., P. S. James, M. W. Smith, and F. J. Bourne. 1986. Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. *J. Agric. Sci. (Cambridge)* 107: 579-589.
- Nabuurs, M.J.A., A. Hoogendoorn, E. J. van der Molen, and A. L. M. van Osta. 1993. Villus height and crypt depth in weaned and unweaned pigs, reared under various circumstances in the Netherlands. *Res. Vet. Sci.* 55: 78-84.
- National Research Council (NRC). 1998. *Nutrient Requirements of Swine*, 10th ed. National Academy Press, Washington, DC.
- Park, Y. K., M. M. Monaco, and S. M. Donovan. 1998. Delivery of total parenteral nutrition (TPN) via umbilical catheterization: development of a piglet model to investigate therapies to improve gastrointestinal structure and enzyme activity during TPN. *Biol. Neonate* 73: 295-305.
- Pluske, J. R., D. J. Hampson, and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livest. Prod. Sci.* 51: 215-236.
- Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. *Anim. Sci.* 62: 131-144.
- Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996. Villous height and crypt depth in piglets in response to increases in the intake of cow's milk after weaning. *Anim. Sci.* 62: 145-158.
- Spitz, J. C., S. Ghandi, M. Taveras, E. Aoys, J. Alverdy, and C. John. 1996. Characteristics of the intestinal epithelial barrier during dietary manipulation and glucocorticoid stress. *Crit. Care Med.* 24: 635-641.
- Taylor, C. T., A. Murphy, D. Kelleher, and A. W. Baird. 1997. Changes in barrier function of a model intestinal epithelium by intraepithelial lymphocytes require new protein synthesis by epithelial cells. *Gut* 40: 634-640.



- van Leeuwen, P., A. J. M. Jansman, J. Wiebenga, J. F. J. Koninkx, and J. M. V. M. Mouwen. 1995. Dietary effects of faba-bean (*Vicia faba* L.) tannins on the morphology and function of the small-intestinal mucosa of weaned pigs. *Br. J. Nutr.* 73: 31-39.
- van Leeuwen, P.A.M., M. A. Boermeester, A. P. J. Houdijk, Ch. C. Ferwerda, M. A. Cuesta, S. Meyer, and R. I. C. Wesdorp. 1994. Clinical significance of translocation. *Gut* 35 (suppl. 1): S28-834.
- Wang, Q. 1995. Pathologically and Experimentally Induced Intestinal Barrier Changes Evaluated by Permeability Measurements. Doctoral thesis, Lund Univ., Lund, Sweden.
- Wirén, M., J. D. Söderholm, J. Lindgren, G. Olaison, J. Permert, H. Yang, and J. Larsson. 1999. Effects of starvation and bowel resection on paracellular permeability in rat small-bowel mucosa in vitro. *Scand. J. Gastroenterol.* 34: 156-162.
- Worsaae, H., and M. Schmidt. 1980. Plasma cortisol and behaviour in early weaned piglets. *Acta Vet. Scand.* 21: 640-657.
- Wu, G., D. A. Knabe, W. Yan, and N. E. Flynn. 1995. Glutamine and glucose metabolism in enterocytes of the neonatal pig. *Am. J. Physiol.* 268: R334-R342.
- Wu, G., C. J. Meininger, K. Kelly, and M. Watford, and S. M. Morris. 2000. A cortisol surge mediates the enhanced expression of pig intestinal pyrroline-5-carboxylate synthase during weaning. *J. Nutr.* 130: 1914-1919.
- Yang, H., J. Söderholm, J. Larsson, J. Permert, G. Olaison, J. Lindgren and M. Wirén. 1999. Glutamine effects on permeability and ATP content of jejunal mucosa in starved rats. *Clin. Nutr.* 18: 301-306.



---

## CHAPTER 4

---

### **Effect of Dietary Protein Source on Feed Intake and Small Intestinal Morphology in Newly Weaned Piglets**

M.A.M. Spreeuwenberg <sup>1</sup>, J. M. A. J. Verdonk <sup>2</sup>, G. C. M. Bakker <sup>2</sup>, A. C. Beynen <sup>3</sup>, and M. W. A. Verstegen <sup>4</sup>.

<sup>1</sup> Swine Research Centre, Nutreco, Boxmeer, The Netherlands; <sup>2</sup> ID TNO Animal Nutrition, Lelystad, The Netherlands; <sup>3</sup> Department of Nutrition, Faculty of Veterinary Medicine, University of Utrecht, Utrecht, The Netherlands; <sup>4</sup> Division of Animal Nutrition, Department of Animal Sciences, University of Wageningen, The Netherlands.

## ABSTRACT

An experiment was designed to study the potential effect of dietary protein source on feed intake and on small intestinal morphology in newly weaned piglets. In total, 108 piglets were used, without access to creep feed during suckling period. Piglets were weaned at 27 days of age. Piglets were fed *ad libitum* one of two experimental diets containing either skim milk powder (SMP) or hydrolysed feather meal (FM), the latter component having low ileal protein digestibility. Diets contained equal amounts of indispensable amino acids. On day 4 postweaning, 18 piglets with a similar high feed intake were selected within each dietary treatment and sampled for small intestinal morphology on days 4, 7, or 14 postweaning. The dietary protein source did not affect feed intake during the first three days after weaning. From day 0 (day of weaning) to 2, the mean feed intake increased from 28.9 (SD: 45.2) to 202.1 (SD: 129.9) g · day<sup>-1</sup> · piglet<sup>-1</sup>. In the second week, the feed intake of the selected piglets receiving the SMP diet was higher ( $P < 0.05$ ) than that of the piglets receiving the FM diet. Villus height and crypt depth were significantly higher for the selected piglets fed the SMP diet when compared to those fed the FM diet. It is concluded that SMP had a positive effect on villus height, this effect being mediated through its high degree of ileal protein digestibility rather than through its stimulatory effect on feed intake.

## INTRODUCTION

At weaning, the digestive system of piglets has to adapt to a dry pelleted diet instead of liquid sow milk. As a consequence, the piglets often show depressed feed intake and growth (Leibbrandt et al., 1975; Okai et al., 1976). After weaning, villus height is generally reduced and crypt depth increased (Miller et al., 1986; Cera et al., 1988; Pluske et al., 1996), which may explain the increased occurrence of diarrhoea (Nabuurs, 1991). The changes in gut morphology are primarily related to the low feed intake immediately after weaning (Kelly et al., 1991; Pluske et al., 1996). Villus height is lowest at 3-4 days after weaning (Hampson, 1986; Nabuurs et al., 1993) and reaches pre-weaning values again between 11 and 14 days postweaning (Nabuurs et al., 1993). This study addressed two different questions. First, we wanted to know whether the protein source in the weaner diets affects feed intake during the first three days after weaning. Secondly, we addressed the question whether the source of dietary protein, differing in the degree of ileal digestibility (CVB, 2000), influences the recovery of villus height. To enhance the contrast in protein source, we compared highly digestible skim milk powder (SMP) and poorly digestible hydrolyzed feather meal (FM) as main dietary protein sources. Piglets were given free access to the diets containing either SMP or FM and their feed intake was measured during the first three days after weaning. Then,

piglets fed either the diet containing SMP or FM were matched on the basis of their feed intake on day 3 after weaning and their small intestinal morphology was assessed on days 4, 7, and 14. The piglets selected had high feed intake because it was assumed that in these animals the recovery process would be most active. It was hypothesized that highly digestible SMP would stimulate recovery of small intestinal morphology when compared with poorly digestible FM. There is evidence that the availability of amino acids in the digesta determines proliferation of enterocytes (Wu, 1998).

## **MATERIALS AND METHODS**

### **Piglets and Weaning**

The 108 newly weaned castrated males used in this experiment were procured from a commercial maternal line herd (Great York × (Dutch Landrace × Finnish Landrace)). The piglets were weaned at 27 d of age. Piglets did not receive creep feed during the suckling period. At weaning, pigs were removed from the sow and transported over 10 km to the former ILOB Animal Nutrition research facility in Wageningen. Upon arrival at the experimental facility, pigs were weighed, and housed individually in 50 × 90 cm-floor pens. The pens had transparent plastic partitionings that enabled visual contact between the piglets. Each pen was equipped with a manual feeder and a nipple waterer. Water was available ad libitum. The environmental temperature was maintained at 24 °C. Lights were on from 06.00 to 24.00 h. The experimental protocol was approved by the Animal Care and Ethics Committee of the TNO research institute.

### **Feeds and Feeding**

The two experimental diets differed in protein source. The diet composition of the feed is shown in Table 1. The diets were formulated to contain 80 g CP · kg<sup>-1</sup> from the variable sources, SMP or FM. The protein digestibility at the terminal ileum of SMP and FM was estimated to be 88 % and 65 %, respectively (CVB, 2000). SMP or FM accounted for 41 % of the CP in the diet, the other nutrients in both diets being similar (Table 2). The diets were balanced for ileal digestible, indispensable AA and lactose content. Before pelleting, the feed was milled at 4 mm. The piglets were fed ad libitum. Feed intake was measured daily.

### **Experimental Design**

The experiment was carried out in 2 consecutive batches. On day 0 (= day of weaning), the experiment started with 27 piglets per dietary treatment per batch. Piglets were assigned to the 2 experimental diets based on body weight (BW). Littermates were equally divided across dietary treatments. This study addressed two different questions. First, we wanted to know

Table 1 Diet composition (as fed basis) of the diets containing skimmed milk powder (SMP) or feather meal (FM) as main protein source.

Item (g · (kg feed) <sup>-1</sup> )	SMP	FM
Feather meal	-	100.0
Skimmed milk powder	230.0	-
Maize, pre-gelatinized	332.1	305.5
Barley	350.0	350.0
Lactose	-	117.5
Potato protein	30.0	30.0
Fish meal (70 % crude protein)	30.0	30.0
Soybean oil	3.0	9.0
Premix <sup>1</sup>	10.0	10.0
Calcium carbonate	7.5	9.5
Monocalcium phosphate	3.0	14.0
Sodium chloride	2.0	4.0
Potassium carbonate	2.0	10.5
Sodium carbonate	-	2.0
L-lysine. HCl	-	6.5
DL-methionine	0.2	0.2
L-threonine	-	0.4
L-tryptophan	0.2	0.9

<sup>1</sup> Lactose based premix supplied (mg · kg<sup>-1</sup> dry feed): retinol acetate, 6.9 (i.e. 20000 IU); cholecalciferol, 0.1 (i.e. 4000 IU); tocopherol, 50; thiamin, 6; riboflavin, 10; pyridoxine, 4; cyanocobalamin, 0.25; d-pantothenic acid, 25; niacin amide, 40; l-ascorbic acid, 80; menadione, 4; folic acid, 1; biotin, 0.5; choline chloride, 1000; zinc oxide, 100; potassium iodate, 0.65; di-sodium selenium oxide pentahydrate, 0.5; copper sulfate pentahydrate, 80; ferrous sulfate heptahydrate, 400; manganous sulfate tetrahydrate, 60; cobalt sulfate heptahydrate, 10; magnesium oxide, 1,000

whether the protein source in the weaner diets affects feed intake during the first three days after weaning. Secondly, we addressed the question whether the source of dietary protein influences the recovery of villus height. To address the first question, feed intake of each piglet was measured for days 0, 1 and 2. To address the second question, 9 piglets per dietary treatment per batch were selected on day 3 as based on their high feed intake. The mean feed intake, on day 3, of the selected piglets was 98 g higher (267 g · day<sup>-1</sup> · piglet<sup>-1</sup>) than that of the remaining piglets (169 g · day<sup>-1</sup> · piglet<sup>-1</sup>). The selected piglets had similar body weights. On days 4, 7, and 14, 3 piglets per treatment per batch were dissected and tissue samples of the small intestine were taken for histological analysis. On day 0, 3 piglets per batch had been randomly chosen and were subjected to dissection to obtain reference data. The piglets were weighed upon arrival and on the day of dissection to determine individual growth rates. Any abnormal faeces consistency and health problems were noted.

Table 2 Calculated and analysed nutrient composition (as fed basis) of the diets containing skimmed milk powder (SMP) or feather meal (FM) as main protein source.

Nutrient content (g · (kg feed) <sup>-1</sup> )	SMP		FM	
	Calculated	Analysed	Calculated	Analysed
DM	892.7	888	899.0	900
CP	192.4	193	199.4	201
Ash	42.2		37.9	
Fat	32.7		40.0	
CF	23.0		22.5	
GE pigs (MJ · kg <sup>-1</sup> )	16.3		16.5	
DE pigs (MJ · kg <sup>-1</sup> )	14.8		14.6	
ME pigs (MJ · kg <sup>-1</sup> )	14.2		14.0	
NE pigs (MJ · kg <sup>-1</sup> )	10.0		10.0	
Ca	7.4		7.4	
P	5.9		6.2	
Total essential amino acids	87.7		88.4	
Total non-essential amino acids	104.3		107.3	
Ileal digestible Lys	10.4		10.4	
Ileal digestible Met	4.3		2.5	
Ileal digestible Met + Cys	6.3		6.5	
Ileal digestible Thr	6.5		6.2	
Ileal digestible Trp	2.0		2.0	
Ileal digestible Ile	7.3		6.6	

### Sampling of Small Intestine for Histology

On days 0, 4, 7 and 14, the piglets to be euthanized were weighed and anaesthetized by inhalation of a mixture of N<sub>2</sub>O/O<sub>2</sub> (ratio 2/1) and isoflurane. The concentration of isoflurane was adjusted to the depth of the narcosis (Guedel, stadium III, phase 2). A midline laparotomy was performed. At 3 different locations of the small intestine, tissue samples were taken: 0.5 m distal of the ligament of Treitz (proximal small intestine), 3.5 m distal of the ligament of Treitz (mid small intestine), and 0.5 m proximal to the ileo-caecal ligament (distal small intestine). After sampling, piglets were euthanized by an intra-cardiac injection (2 cc) of T61 (a watery solution containing a combination of embutramide, mebezoniumiodide and tetracaine hydrochloride; Hoechst Holland N.V., Amsterdam, The Netherlands).

For histological analysis, tissue samples of the proximal, mid, and distal small intestine were cut open longitudinally at the anti-mesenteric attachment, prepared on dental wax with the villi on the upper side, and fixed in 0.1 M phosphate-buffered formalin solution (40 ml formalin · (l buffer)<sup>-1</sup>). A three-mm wide zone from the mesenteric site was cut at right angles to the surface of the mucosa and was embedded in paraffin wax. Sections were cut (5 µm) and stained with the periodic acid Schiff method (PAS staining). From the stained sections, crypt depth (µm) and villus height (µm) were determined.

## Statistical Analysis

A GLM procedure (SAS version 6.12) was used to estimate the least square means for the treatments. The effect of dietary treatment on growth rate and feed efficiency (FE) was evaluated per period until the day of dissection. Batch and protein source were the independent variables in the model to evaluate feed intake during the first three days post weaning, growth and feed efficiency. The interaction of batch  $\times$  protein source was not significant and therefore was excluded from the final model. The final model was:

$$y_{ijk} = \mu + B_i + P_j + e_{ijk} \quad [1]$$

where  $y_{ijk}$  = dependent variable;  $\mu$  = overall mean;  $B_i$  = fixed effect of batch ( $i = 1, 2$ );  $P_j$  = fixed effect of protein source ( $j = 1, 2$ );  $e_{ijk}$  = error term.

The effect of protein source and feed intake on villus height, crypt depth and villus/crypt ratio was examined with batch, day of dissection, protein source, and mean feed intake (co-variable) as independent variables. The two-way interactions were not significant, and therefore excluded from the final model. Histology was analysed per location (proximal, mid and distal small intestine). The final model was:

$$y_{ijkm} = \mu + B_i + S_j + P_k + b \times fi + e_{ijkm} \quad [2]$$

where  $y_{ijkm}$  = dependent variable;  $\mu$  = overall mean;  $B_i$  = fixed effect of batch ( $i = 1, 2$ );  $S_j$  = fixed effect of day of dissection ( $j = 1, 2, 3$ );  $P_k$  = fixed effect of protein source ( $k = 1, 2$ );  $fi$  = effect of mean feed intake from weaning until dissection (co-variable);  $e_{ijkm}$  = error term.

To compare the morphology values of piglets fed either the SMP or FM diet on days 4, 7 and 14 with those at weaning, the following model was used with batch and treatment as independent variables:

$$y_{ijk} = \mu + B_i + T_j + e_{ijk} \quad [3]$$

where  $y_{ijk}$  = dependent variable;  $\mu$  = overall mean;  $B_i$  = fixed effect of batch ( $i = 1, 2$ );  $T_j$  = fixed effect of treatment (including day of dissection and diet) ( $j = 1, 2, \dots, 7$ );  $e_{ijk}$  = error term.

The recovery of villus height was assessed as the difference between group-mean villus height on day 14 minus that on day 4. The standard deviation (SD) of the difference was calculated as squareroot of the sum of the variances for days 4 and 14. The dietary protein effect (SMP vs. FM) on villus height recovery was evaluated with Student's t-test with 10 degrees of freedom. A similar procedure was followed to determine any diet effects on the change in crypt depth.

Chi-square analysis was used to analyse the effect of dietary treatment on diarrhoea, which was expressed as either absence or occurrence. For evaluating the predictive value of the feed intake on a certain day for subsequent feed intake, Pearson correlation coefficients were



calculated. Throughout, significance was assigned at  $P < 0.05$ ; tendencies were assigned at  $0.05 < P < 0.10$ .

## **RESULTS**

### **General**

In general, the experiment went according to the design. The 108 piglets weighed 7.4 kg (SD: 0.81) at weaning. One animal died on the day of arrival, without known cause. Eleven piglets showed inconsistent faeces during the experiment. On average in the diarrhoea-positive piglets, diarrhoea occurred on day 7 (SD: 2.5) after weaning. Piglets receiving the FM diet showed less consistent faeces ( $P < 0.01$ ) when compared with those fed the SMP diet. None of the piglets received any medical treatment during the trial.

### **Feed Intake for days 0 - 2**

Histograms in Figure 1 show the distribution of the voluntary daily feed intake, on day 0, 1 and 2, of 54 weaned piglets receiving either the SMP or FM diet. The mean values for feed intake, SEM, and the median for both diets on the first three days postweaning are also shown in Figure 1. The distribution illustrates the large difference in daily feed intake between individual piglets. On day 0, intake of the SMP diet varied from 0 to 239 g, and for the FM diet it varied from 1 to 290 g. The overall mean feed intake during the first day was only 29 g (SD: 45.2), with 50 % of the piglets eating 10 g of feed or less (data not shown). Figure 1 shows that during the first three days postweaning the feed intake increased rapidly. When compared to day 0, the mean voluntary feed intake on day 1 had increased by 373 and 271 % for the SMP and FM diet respectively. From days 1 to 2 the feed intake increased by 60 and 73 % for the SMP and FM diet. During the first 3 days the feed intake was not significantly affected by protein source in the diet ( $P > 0.10$ ).

For the 108 piglets, the Pearson correlation coefficient (R) between the feed intake on day 0 and that on 1 was 0.48, between days 0 and 2 it was 0.34, and between day 1 and 2 was 0.74. All three correlations were statistically significant ( $P < 0.01$ ). The body weight at weaning was not correlated with the mean feed intake during the first three days postweaning ( $P > 0.10$ ).

### **Feed Intake, growth and feed efficiency for the selected piglets**

Feed intake for days 3 to 14 in the selected piglets is shown in Figure 2. On all days subsequent to day 3, the mean feed intake for the piglets receiving the SMP diet was higher than that for their counterparts consuming the FM diet. The difference showed a tendency towards significance on day 13 ( $P < 0.10$ ) and was significant on days 5 and 11 ( $P < 0.05$ ).

The mean feed intake during the first week was not different between the dietary groups, but during the second week it was higher for the piglets given the SMP diet ( $P < 0.05$ ).

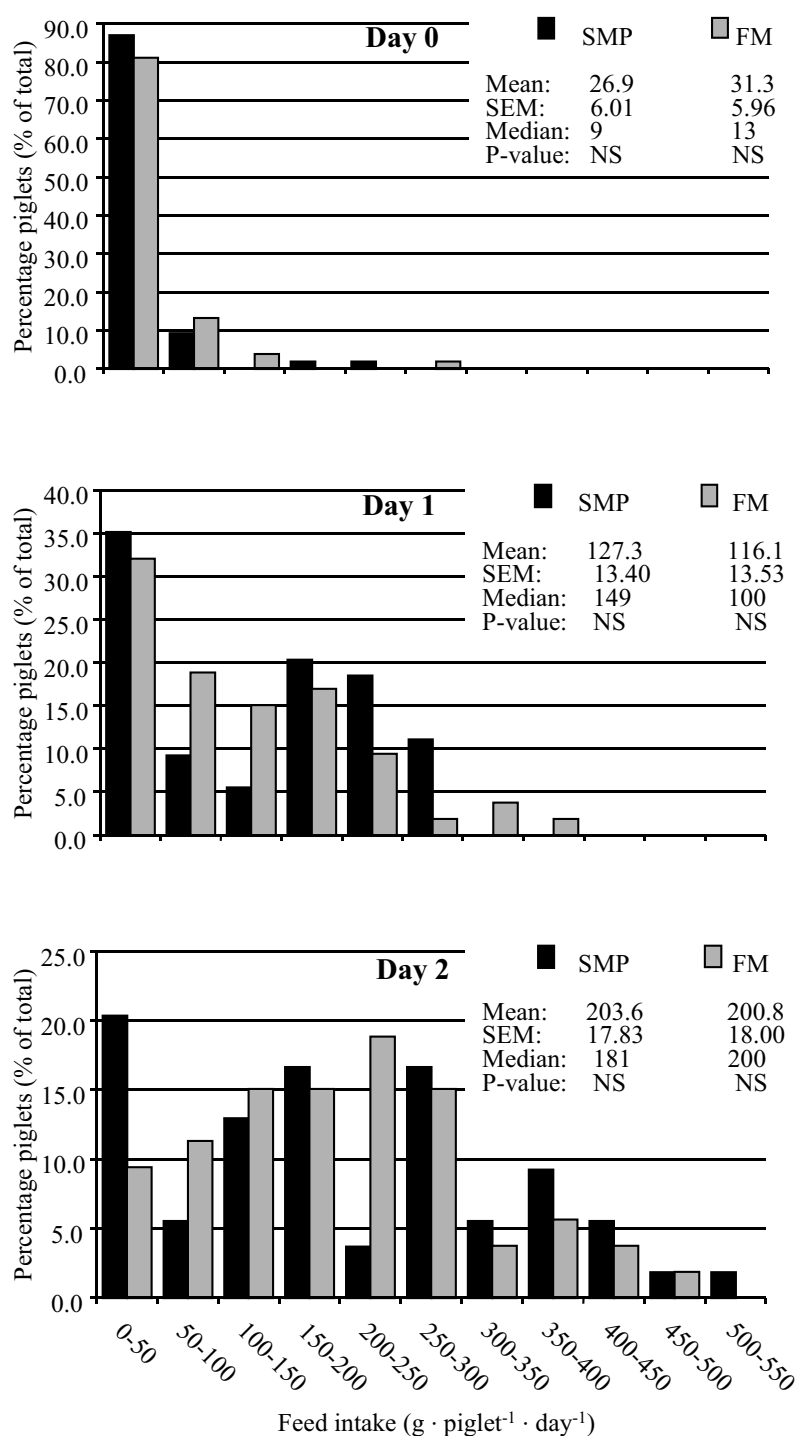


Figure 1 Histograms of the voluntary feed intake with mean value, SEM, and median for the diets containing either skimmed milk powder (SMP;  $n=54$ ) or feather meal (FM;  $n=53$ ) on days 0, 1, and 2 postweaning. NS: no significant difference between dietary treatments.

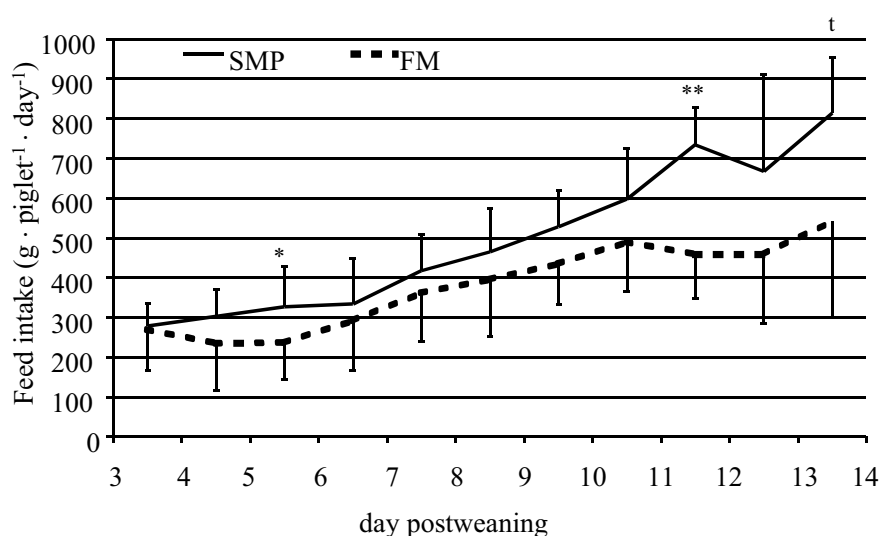


Figure 2 The voluntary daily feed intake (with SD) from days 3 to d 14 post weaning in piglets fed the diets containing either skimmed milk powder (SMP) or feather meal (FM). The number of piglets per treatment decreased with time: days 4-7, n=12; days 7-14, n=6 (P-value of effect of diet composition: \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; t,  $P < 0.10$ ).

For the 36 selected piglets, the feed intake of subsequent days was significantly correlated. During days 0 to 3 the Pearson correlation coefficients for feed intakes on subsequent days were rather low ( $R < 0.5$ ), but from day 4 onwards the correlation was higher ( $R > 0.5$ ). The feed intake during days 0 to 3 had no predictive value for that during days 7 to 14. The feed intake for each set of two days in the period of days 9 to 13 was correlated ( $P < 0.05$ ). The predictive value of the feed intake on a certain day for subsequent days increased with the number of days postweaning.

Table 3 shows the growth rate and feed to gain ratio (feed efficiency, FE) for the selected piglets receiving either the SMP or FM diet. Protein source and the mean feed intake ( $P < 0.01$ ) affected growth and FE during both the first week and the first two weeks postweaning. From days 0-7 and 0-14, the growth rate and FE of the piglets was higher for the SMP-fed piglets when compared to the piglets fed the FM diet.

### Small intestinal histology at weaning and in the selected piglets

Figure 3 shows villus height and crypt depth (with SEM) of the selected piglets fed either the SMP or FM diet. At the proximal and mid small intestine, piglets fed either diet showed lower ( $P < 0.05$ ) villi on days 4 and 7 when compared to the values on day 0. On day 14, the villus

Table 3 Least square means ( $\pm$  pooled SEM;  $n=6$ ) for growth rate and feed efficiency from weaning to dissection in the selected piglets fed ad libitum diets based on either skim milk powder (SMP) or feather meal (FM).

	SMP	FM	SEM	P-value <sup>1</sup>
Growth ( $\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \cdot \text{piglet}^{-1}$ )				
days 0 – 4	150	100	26.7	ns
days 0 – 7	233	64	36.4	**
days 0 – 14	424	201	22.8	**
Feed efficiency ( $\text{g gain} \cdot \text{g feed}^{-1}$ )				
days 0 – 4	0.91	0.50	0.187	ns
days 0 – 7	0.95	0.27	0.086	**
days 0 – 14	0.98	0.59	0.037	**

<sup>1</sup> P-value of the model: \*\*,  $P < 0.01$ ; ns, not significant.

height reached weaning levels again for the proximal intestine, but was even higher than that on day 0 for the mid and distal small intestine. Crypt depth had increased at all three sites ( $P < 0.05$ ) during the first 2 weeks postweaning, when compared to day 0. As mentioned, villus height was lowest on day 4 and then rose towards or even above weaning values. The rate of recovery was assessed as the difference in mean villus height between days 14 and 4. For the SMP-fed piglets, recovery was 125, 200 and 113  $\mu\text{m}$  at the proximal, mid and distal small intestine, and for the FM-fed piglets it was 180, 148 and 150  $\mu\text{m}$ , respectively. Likewise, for the SMP-fed piglets, the increase in crypt depth was 101, 72 and 3  $\mu\text{m}$  at the proximal, mid and distal small intestine and for the FM-fed piglets this was 91, 85 and 39  $\mu\text{m}$ , respectively. There was no significant diet effect on the recovery of villus height, but there were tendencies ( $P < 0.10$ ) for the recovery being greater in the proximal small intestine for the FM-fed piglets and in the mid small intestine for the SMP-fed piglets. Supplying either the SMP or FM diet did not affect the change in crypt depth over the period days 4 to 14 postweaning in the proximal and mid small intestine. In the distal small intestine, the FM-fed piglets showed a larger increase in crypt depth ( $P < 0.05$ ) when compared to the SMP-fed piglets.

Table 4 shows the results on the small intestinal histology in relation to type of diet and small intestinal site. The data for days 4, 7, and 14 postweaning were pooled. In the SMP-fed piglets, the villus height was higher ( $P < 0.05$ ) at the mid small intestine and tended to be higher at the distal small intestine ( $P < 0.10$ ), when compared to the piglets receiving the FM diet. Crypt depth was higher at the proximal small intestine ( $P < 0.05$ ) and tended to be higher at the mid small intestine ( $P < 0.10$ ) in the piglets receiving the SMP diet. Villus/crypt ratio was not affected by protein source. The co-variable feed intake did not significantly affect the small intestinal architecture ( $P > 0.05$ ).

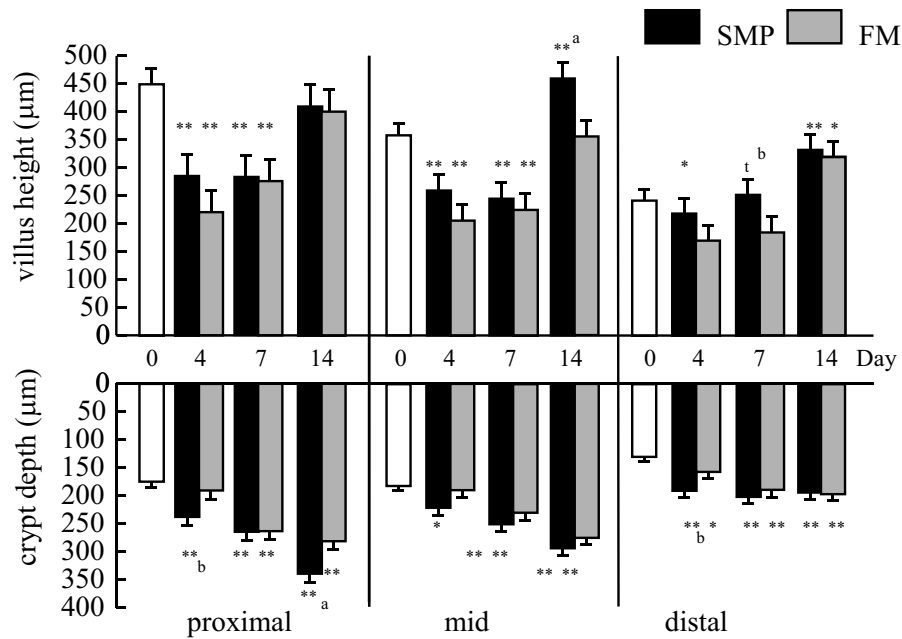


Figure 3 The villus height and crypt depth (with SEM; n=18) of piglets fed the diets containing either skimmed milk powder (SMP) or feather meal (FM). Statistical notes refer to differences versus the day of weaning (0) (\*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; t,  $P < 0.10$ ) and differences between diets within days (a,  $P < 0.05$ ; b,  $P < 0.10$ ).

Table 4 Least square means ( $\pm$  pooled SEM; n=6) for villus height and crypt depth in the selected piglets fed ad libitum diets based on either skim milk powder (SMP) or feather meal (FM) for 4, 7, or 14 days.

Small intestinal site		SMP diet	FM diet	SEM	P-value <sup>1</sup>	Feed intake <sup>2</sup> P-value
Villus height (μm)	proximal	320	304	17.6	ns	ns
	mid	314	266	15.1	*	ns
	distal	265	225	14.3	t	ns
	mean <sup>3</sup>	300	265	11.7	t	ns
Crypt depth (μm)	proximal	276	250	9.6	*	t
	mid	254	231	7.9	t	ns
	distal	196	179	7.2	ns	ns
	mean	242	220	5.9	*	ns
Villus/Crypt ratio	proximal	1.2	1.2	0.08	ns	ns
	mid	1.2	1.2	0.06	ns	ns
	distal	1.4	1.3	0.09	ns	ns
	mean	1.3	1.2	0.06	ns	ns

<sup>1</sup> P-value of the model: \*,  $P < 0.05$ ; t,  $P < 0.10$ ; ns, not significant.

<sup>2</sup> covariable of statistical model

<sup>3</sup> mean value for the 3 small intestinal sites

## DISCUSSION

A piglet with a BW of 7.4 kg needs about 2.1 MJ DE per day for maintenance (NRC, 1998), which is equivalent to about 143 g of the feed used in this study. On day 3 postweaning the average voluntary feed intake supplied sufficient energy for maintenance, but 36 % of the piglets did not eat enough to meet maintenance requirements. The mean energy intake on day 0 was 19 % of that required for maintenance; 3 % of the piglets ate above maintenance. On days 1 and 2 mean energy intake was 89 % and 142 % of maintenance with 44 % and 64 %, of the piglets eating above maintenance, respectively. Le Dividich and Herpin (1994) summarized several data sets and concluded that on average the metabolisable energy (ME) requirement for maintenance was not met until the fifth day after weaning. The optimal feed intake required for piglets, weaned between 28 and 35 days of age, in order to meet the protein requirement is  $25 \text{ g feed} \cdot (\text{kg BW})^{-1}$  (Aumaitre et al., 1995). For the pigs in our experiment the feed intake for optimal protein provision would be 185 g. This level was reached on the third day postweaning. To realise pre-weaning growth rates between 200 and  $280 \text{ g} \cdot \text{day}^{-1}$ , the piglets would need to consume between 320 and  $475 \text{ g feed} \cdot \text{day}^{-1}$  (Fowler and Gill, 1989; Pluske et al. 1995). The feeding level of 320 g was attained on day 5 when the SMP diet was fed and on day 7 when the FM diet was fed. These calculations highlight the importance of attempts to raise feed intake after weaning. The first objective of this study was to investigate whether the dietary protein source affects feed intake during the first three days after weaning. The results showed that the initial drop in feed intake after weaning was not influenced by the feeding of the diets containing either SMP or FM. This result was not anticipated because SMP has been regarded as highly palatable for piglets. FM on the hand would be expected not to be palatable. Possibly, the inclusion levels of the two protein sources were too low for a diet effect on palatability to become apparent and/or any effect of SMP versus FM was masked by the other components of the diets. In any event, it is clear that in this study the use of SMP or FM as protein source did not influence feed intake during the first three days after weaning.

The second objective of this study was to examine whether protein source, differing in the ileal digestibility of dietary protein, influences the recovery of villus architecture in newly weaned piglets. FM was assumed to have a lower ileal protein digestibility than SMP (CVB, 2000), but the amounts of ileal digestible Lys, Met + Cys, Thr and Trp in the diet were balanced by the addition of crystalline amino acids (AA). Both villus height and crypt depth were affected by the protein source in the diet. The selected piglets receiving the SMP diet generally had longer villi and deeper crypts than the piglets receiving the FM diet. Specific dispensable AA are metabolized in enterocytes for energy generation (Glu, Gln, Asp), proliferation (Arg, Gln, Pro) and protection (Arg, Cys, Glu, Gly) of the small intestinal mucosa (Wu, 1998) and thereby may affect villus length. In practical piglet diet formulation,

only the first limiting indispensable AA for protein deposition are taken into account; i.e. Lys, Met, Cys, Thr and Trp. The AA needs are estimated on the basis of data for growing pigs of 60 kg. The AA needs of growing pigs might differ from those of newly weaned piglets, which could require specific dispensable AA to support the morphology and the functions of the small intestinal mucosa. A lower content of dispensable AA in the small intestinal chyme of the piglets receiving the FM diet could have caused the observed shorter villi.

In the literature, only a few protein sources have been described regarding small intestinal integrity. The presence of anti-nutritional factors (ANF) has received considerable attention and may explain the contradictory results obtained with soybean meal. Several workers noted a decrease in villus height in weanling piglets fed a diet with a high level of soybean meal instead of SMP (Dunsford et al., 1989; Li et al., 1991; Makinde et al. 1996), but other reports do not confirm this (Makkink, 1993; McCracken et al., 1999, Jiang et al., 2000). Recently, Zarkadas and Wiseman (2000a; 2000b) demonstrated a negative correlation between the trypsin inhibitor level in soybean meal and villus height or growth in weaned piglets. Makkink (1993) showed that gut wall morphology was not affected by protein source in piglets receiving dry diets with either 25.4 % soy protein concentrate, 34.4 % soybean meal, 47.0 % SMP or 21.3 % fish meal. Jiang and colleagues (2000) weaned piglets at 14 days of age and fed them a dry diet based on either 15 % soybean meal or 10 % porcine spray dried plasma. Protein source did not affect villus height, crypt depth or cell proliferation as measured by 5-bromo-2-deoxyuridine (BrDU) incorporation (Jiang et al., 2000). The ileal protein digestibility of the above mentioned protein sources is in decreasing order: SMP, 88 % > spray dried animal plasma, 87 % > soybean meal, 84 % > fish meal, 83 % > FM, 65 % (CVB, 2000). SMP, spray dried animal plasma and fish meal had similar protein digestibilities and, according to the studies mentioned above feeding of these sources to piglets resulted in similar villus heights. Only FM has a substantial lower ileal protein digestibility and the results of the present study showed a negative effect on villus architecture. Thus, it seems that ileal protein digestibility affects intestinal morphology. It should be stressed that FM versus SMP lowered feed intake, which is an important determinant of villus height (Kelly et al., 1991; Pluske et al., 1996; Verdonk et al., 2001). However, feed intake was not a significant covariable in the influence of dietary protein source on villus height and crypt depth. Therefore, it seems that the decrease in villus height in the FM-fed piglets when compared to the SMP-fed piglets was caused by the lower ileal protein digestibility.

In conclusion, SMP versus FM in the diet had no effect on feed intake during the first 3 days after weaning. From day 4 postweaning onwards, the dietary protein source did affect the feed intake in selected piglets with high feed intake. Postweaning feed intake was too low to meet the maintenance requirements for energy during the first three days and was not sufficient to

reach pre-weaning growth rates until the end of the first week postweaning. In the selected piglets, SMP versus FM in the diet positively affected small intestinal morphology.

## LITERATURE CITED

- Aumaitre, A., J. Peiniau, and F. Madec. 1995. Digestive adaptation after weaning and nutritional consequences in the piglet. *Pig News Info*. 16: 73N–79N.
- Cera, K. R., D. C. Mahan, R. F. Cross, G. A. Reinhart, and R. E. Whitmoyer. 1988. Effect of age, weaning and postweaning diet on small intestinal growth and small intestinal morphology in young swine. *J. Anim. Sci.* 66: 574-584.
- Centraal Veevoederbureau (CVB). 2000. Chemical composition, digestibility, and feeding value of feedstuffs. *Veevoedertabel 2000*, Centraal Veevoederbureau, Lelystad, The Netherlands (in Dutch).
- Dunsford, B. R., D. A. Knabe, and W. E. Haensly. 1989. Effect of dietary soybeanmeal on the microscopic anatomy of the small intestine in the early weaned pig. *J. Anim. Sci.* 67: 1855-1863.
- Fowler V. R., and B. P. Gill. 1989. Voluntary food intake in the young pig. Pages 51–60 in *The Voluntary Food Intake of Pigs*. J. M. Forbes, M. A. Varley and T. L. J. Lawrence, eds. Occasional Publication No. 13, British Society of Animal Production, Edinburgh, UK.
- Hampson, D. J. 1986. Alterations in piglet small intestinal structure at weaning. *Res. Vet. Sci.* 40: 32-40.
- Jiang, R., X. Chang, B. Stoll, M. Z. Fan, J. Arthington, E. Weaver, J. Campbell, and D.G. Burrin. 2000. Dietary plasma protein reduces small intestinal growth and lamina propria cell density in early weaned pigs. *J. Nutr.* 130: 121-126.
- Kelly, D., J. A. Smyth, and K. J. McCracken. 1991. Digestive development of the early weaned pig; effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. *Brit. J. Nutr.* 65: 181-188.
- Le Dividich, J., and P. Herpin. 1994. Effects of climatic conditions on the performance, metabolism and health status of weaned piglets: a review. *Livest. Prod. Sci.* 38: 79-90.
- Leibbrandt, V. D., R. C. Ewan, V. C. Speer, and D. R. Zimmerman. 1975. Effect of weaning and age at weaning on baby pig performance. *J. Anim. Sci.* 40:1077-1080.
- Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, R. D. Klemm, D. W. Giesting, J. D. Hancock, G. L. Allee, and R. D. Goodband. 1991. Measuring suitability of soybean products for early-weaned pigs with immunological criteria. *J. Anim. Sci.* 69: 3299-3307.
- Makinde, M. O., E. Umaphy, B. T. Akingbemi, K. T. Mandisodza, and E. Skadhauge. 1996. Effects of dietary soybean and cowpea on gut morphology and faecal composition in creep and noncreep-fed pigs. *J. Vet. Med. A.* 43: 75-85.
- Makkink, C. A. 1993. Of piglets, dietary proteins and pancreatic proteases. Ph.D. Diss., Wageningen Univ., Wageningen, The Netherlands.
- McCracken, B. A., M. E. Spurlock, M. A. Roos, F. A. Zuckermann, and H. R. Gaskins. 1999. Weaning anorexia may contribute to local inflammation in the piglet small intestine. *J. Nutr.* 129:613-619.
- Miller, B. G., P. S. James, M. W. Smith, and F. J. Bourne. 1986. Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. *J. Agric. Sci. Camb.* 107: 579-589.
- Nabuurs, M. J. A., 1991. Etiologic and pathogenic studies on postweaning diarrhea. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.



- Nabuurs, M. J. A., A. Hoogendoorn, E. J. van der Molen, and A. L. M. van Osta. 1993. Villus height and crypt depth in weaned and unweaned pigs, reared under various circumstances in the Netherlands. *Res. Vet. Sci.* 55: 78-84.
- National Research Council (NRC). 1998. Page 190 in *Nutrient Requirements of Swine*. 10th ed., National Academy Press, Washington DC, U.S.A.
- Okai, D. B., F. X. Aherne, and R. T. Hardin. 1976. Effects of creep and starter composition on feed intake and performance of young pigs. *Can. J. Anim. Sci.* 56: 573-586.
- Pluske, J. R., I. H. Williams, and F. X. Aherne. 1995. Nutrition of the neonatal pig. Pages 187-235 in *The Neonatal Pig; Development and survival*. M. A. Varley, ed. CAB International, Wallingford, UK.
- Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. *Anim. Sci.* 62: 145-158.
- Verdonk, J. M. A. J., M. A. M. Spreeuwenberg, G. C. M. Bakker, and M. W. A. Verstegen. 2001. Nutrient intake level affects histology and permeability of the small intestine in newly weaned piglets. Pages 332-334 in *Digestive Physiology of Pigs*. J. E. Lindberg, and B. Ogle, eds. Cabi Publishing, Wallingford, UK.
- Wu, G., 1998. Intestinal mucosal amino acid catabolism. *J. Nutr.* 128 1249-1252.
- Zarkadas, L. N., and J. Wiseman. 2000a. Inclusion of differently processed full fat soya bean in diets for piglets I. Performance. Page 45 in *The Weaner Pig*. M. Varley, and J. Wiseman, eds. BSAS Occasional Meeting. British Society of Animal Science. Penicuik, Midlothian, UK.
- Zarkadas, L. N., and J. Wiseman, 2000b. Inclusion of differently processed full fat soya bean in diets for piglets II. Digestibility and intestinal morphology. Page 46 in *The Weaner Pig*. M. Varley, and J. Wiseman, eds. BSAS Occasional Meeting. British Society of Animal Science. Penicuik, Midlothian, UK.



---

## CHAPTER 5

---

### **Dietary Protein Hydrolysates versus the Intact Proteins do not enhance Mucosal Integrity and Growth Performance in Weaned Piglets**

M. A. M. Spreeuwenberg <sup>1</sup>, J. M. A. J. Verdonk <sup>2</sup>, J. F. J. G. Koninkx <sup>3</sup>, A. C. Beynen <sup>4</sup>, and M. W. A. Verstegen <sup>5</sup>.

<sup>1</sup> Swine Research Centre, Nutreco, Boxmeer, The Netherlands, <sup>2</sup> ID TNO Animal Nutrition, Lelystad, The Netherlands, <sup>3</sup> Department of Veterinary Pathology and <sup>4</sup> Department of Nutrition, Faculty of Veterinary Medicine, University of Utrecht, Utrecht, The Netherlands, <sup>5</sup> Division of Animal Nutrition, Department of Animal Sciences, University of Wageningen, Wageningen, The Netherlands.

## ABSTRACT

Two separate experiments, but using the same diets, were designed to study whether the addition to the diet of protein hydrolysates or crystalline glutamine (gln) affect small intestinal integrity and growth performance. It was hypothesized that dietary supplementation of hydrolysed proteins would increase the availability of amino acids for the gut wall and therefore result in an improved small intestinal integrity and growth performance of piglets after weaning. The five isonitrogenous diets differed in their protein composition: soybean meal and wheat gluten (SBM+WG), SBM and hydrolysed wheat gluten (SBM+HWG), hydrolysed soybean meal and WG (HSBM+WG), SBM and potato protein (SBM+PP), 2 % of gln added to SBM and PP (SBM+PP+gln). In experiment 1, 88 piglets of 8.4 kg (SD: 0.82) were weaned at 26 d of age (day 0). Piglets were dissected and sampled on days 0, 3, or 7 postweaning. Results showed that the protein hydrolysates did not increase villus height, did not reduce crypt depth and did not raise brush-border aminopeptidase and isomaltase-sucrase activity when compared to the diets containing the native proteins. In experiment 2, 165 piglets of 8.5 kg (SD: 1.33) were weaned at 26 d of age. Feed intake and weight gain were not enhanced by the protein hydrolysates. The addition to the diet of crystalline gln resulted in improved average daily gain and feed efficiency by 22 % and 17 %, respectively ( $P < 0.05$ ) when compared to the other diets and measured during the first 2 weeks postweaning. Gln did not influence growth performance during days 0-7 postweaning. The feeding of protein hydrolysates led to more non-consistent faeces when compared to the SBM+WG diet ( $P < 0.05$ ). In conclusion, dietary supplementation of protein hydrolysates did not ameliorate the weaning-induced compromise of small intestinal integrity and did not enhance growth performance. Dietary supplementation of gln did not affect villus architecture during the first week postweaning, but it enhanced growth performance during the second week.

## INTRODUCTION

The weaning transition of piglets is accompanied by low feed intake, causing growth stasis (Leibbrandt et al., 1975) and decreased villus height and brush border enzyme activity, and raised paracellular permeability of the small intestine (Kelly et al., 1991b; McCracken et al. 1999; also described in Chapter 3). Recovery of the intestinal mucosa with compromised integrity and low digestive capacity might be enhanced by the availability of amino acids or peptides that can be absorbed rapidly (Wu, 1998). It was thus hypothesized that dietary supplementation of peptides, in the form of protein hydrolysates, would result in an improved small intestinal integrity and growth performance of newly weaned piglets. To test our hypothesis, the feeding of either hydrolysed wheat gluten or soybean meal was compared to

that of the native proteins. We used villus height, crypt depth and brush border activities of isomaltase-sucrase and aminopeptidase as indicators of mucosal integrity. As to mucosal integrity, glutamine (gln) is thought to play a specific role in that it is a preferred energy source for enterocytes (Souba, 1993; Wu, 1998). However, gln supplementation studies on mucosal integrity in weanling piglets showed conflicting results (Ayonrinde et al., 1995; Wu et al., 1996; Kitt et al., 2001). In the present study, the possible effect on mucosal integrity and growth of weanling piglets of dietary gln was evaluated, either supplied via wheat gluten, which is rich in gln, or in crystalline form.

## **MATERIALS AND METHODS**

Two experiments, in the form of two consecutive batches each, were performed simultaneously at the Swine Research Centre (SRC) of Nutreco (Boxmeer, The Netherlands). Experiment 1 investigated the mucosal integrity of the small intestine. Experiment 2 was a growth performance trial. A total of 253 weaned piglets were used [(Duroc × Yorkshire synthetic) × (Yorkshire × Dutch landrace synthetic)]. Creep feed was not provided during the suckling period so as to enhance the differential response, if any, to the experimental diets and to prevent the induction of inter-individual variability by variable, pre-weaning ingestion of solid feed (Bruininx et al., 2002). The experimental protocol was approved by the Animal Care and Ethics Committee of the University of Nijmegen (The Netherlands).

### **Experiment 1: Mucosal integrity of small intestine**

#### ***Piglets and weaning***

Barrows (n=44 per batch) were weaned at 26 days (SD: 1.4) of age; they had an average body weight of 8.4 kg (SD: 0.82). At weaning (day 0), pigs were removed from the sow, weighed and housed individually in pens (77×76 cm<sup>2</sup>). Each pen was equipped with a trough and a water nipple. Environmental temperature was maintained at 27 °C. Lights were on from 06.00 to 22.00 h.

#### ***Feeds, feeding and experimental design***

On the day before weaning (day -1), piglets were blocked by body weight (BW) and randomly allocated to one of 10 groups (batch 1) consisting of 4 piglets each or 11 groups (batch 2) consisting of 4 piglets for 12 groups and 8 piglets for 1 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 one group of 8 piglets in batch 2. The remaining 10 groups in batch 2 and the 10 groups in batch 1 were dissected on day 3 or 7 postweaning and received one of 5 experimental diets (Table 1).

Table 1 Ingredient composition of the experimental diets.

Diet code <sup>1</sup>	SBM WG	SBM HWG	HSBM WG	SBM PP	SBM PP
Ingredients (g · (kg feed) <sup>-1</sup> )					gln
Wheat gluten (CP <sup>2</sup> , 81.5 %)	100.0	-	100.0	-	-
Hydrolysed wheat gluten <sup>3</sup>	-	98.8	-	-	-
Soybean meal (CP, 51.2 %)	160.0	160.0		160.0	160.0
Hydrolysed soybean meal <sup>4</sup>	-	-	162.2	-	-
Potato protein (CP, 79.2 %)	-	-	-	102.9	102.9
Pre-gelatinised corn starch	192.9	190.8	199.0	195.6	174.3
Limestone	10.8	10.6	11.1	11.2	11.2
Mono calcium phosphate	16.3	16.5	15.8	15.6	15.6
Salt	9.4	8.1	5.2	9.5	9.5
Soya oil	43.0	45.5	39.0	45.0	46.3
Lysine	5.6	6.0	5.2	-	-
Methionine	-	1.0	0.1	0.4	0.4
Threonine	2.2	2.4	2.1	-	-
Tryptophan	0.1	0.6	0.6	-	-
L-Glutamine <sup>5</sup>	-	-	-	-	20.0
Constant components <sup>6</sup>	459.8	459.8	459.8	459.8	459.8

<sup>1</sup> Abbreviations: gln = glutamine; HSBM = hydrolysed soybean meal; HWG = hydrolysed wheat gluten; PP = potato protein; SBM = soybean meal; WG = wheat gluten.

<sup>2</sup> CP = crude protein

<sup>3</sup> DMV International, Veghel, The Netherlands: CP, 82.5 %; average molecular weight (MW), 800 D; degree of hydrolysis (DH) is the number of peptide bonds broken upon hydrolysis as % of the total number of peptide bonds present in the intact protein, 9 %; free amino acids (FAA), 2 %

<sup>4</sup> DMV International, Veghel, The Netherlands: CP, 50.5 %; MW, 320 D; DH, 27 %; FAA, 7 %

<sup>5</sup> Purity ≥ 99 %

<sup>6</sup> The constant components consisted of (g per kg feed): corn, 250; barley, 150; lactose, 50; choline chloride (purity 50 %), 2.8; titanium oxide, 5; vitamin and trace element premix, 2. The vitamin and trace element inclusion supplied (per kg feed): vitamin A, 10000 IE; vitamin D3, 2000 IE; vitamin E, 65000 IE; vitamin K3, 2 mg; vitamin B1, 1 mg; vitamin B2, 3 mg; panthotenic acid, 10 mg; niacin, 20 mg; biotin, 30 µg; vitamin B12, 20 µg; folic acid, 0.2 mg; vitamin B6, 4 mg; Fe, 160 mg; Cu, 160 mg; Zn, 100 mg; Mn, 30 mg; I, 10 mg; Se, 0.2 mg; antioxidants (E130, E320, E321), 60 mg

The diets were pelleted and then crumbled prior to feeding. The experimental diets differed with respect to protein composition. Wheat gluten (WG), soybean meal (SBM) and potato protein (PP) were fed as native proteins. One diet contained a combination of SBM and WG. To formulate another diet, WG was replaced on a protein basis ( $N \times 6.25$ ) by hydrolysed wheat gluten (HWG); the diet code is SBM+HWG. SBM was exchanged on a protein basis by hydrolysed soybean meal (HSBM) in the diet with code HSBM+WG. To formulate the low-glutamine diet, WG was exchanged by PP, which is low in gln; the diet code is SBM+PP. Two percent of gln ( $20 \text{ g} \cdot \text{kg}^{-1}$ ) was added to the PP+SBM diet to make the diet with code SBM+PP+gln. The amount of supplementary gln was based on research by Ayonrinde et al.

(1995) and Wu et al. (1996), showing that the addition of gln improved mucosal integrity of weanling piglets. The effect of protein hydrolysates on mucosal integrity was evaluated by comparing piglets fed the diet SBM+WG with either the diets HSBM + WG or SBM + HWG. The effect of gln could be ascertained by contrasting the piglets fed the diet SBM + PP + gln with those receiving the diet PP + SBM. Table 2 shows the calculated nutrient composition of the diets. Analysed macronutrient composition is shown in Table 3. The calculated and analysed compositions agreed well.

Table 2 Calculated nutrient composition of the experimental diets.

Diet code <sup>1</sup>	SBM WG	SBM HWG	HSBM WG	SBM PP	SBM PP gln
Nutrients (g · (kg feed) <sup>-1</sup> )					
Macronutrients <sup>2</sup>					
Moisture	88	87	82	92	91
Crude protein	208	209	208	201	221
Crude fat	64	60	61	63	64
Crude fibre	24	24	24	24	24
Ash	54	52	51	53	53
Starch	426	424	431	421	402
Sugar	22	22	23	22	22
NE (MJ) <sup>3</sup>	10.7	10.7	10.7	10.7	10.7
Ileal digestible amino acids <sup>3</sup>					
Lysine	11.0	11.0	11.0	11.0	11.0
Methione	2.8	3.3	2.9	3.7	3.7
Cystine	3.3	2.9	3.3	2.5	2.5
Methionine + Cystine	6.2	6.2	6.2	6.2	6.2
Threonine	7.6	7.6	7.6	7.6	7.6
Tryptophan	2.0	2.0	2.0	2.0	2.0
Glutamate + Glutamine	45.4	51.2	45.1	25.5	45.5

<sup>1</sup> Abbreviations: gln = glutamine; HSBM = hydrolysed soybean meal; HWG = hydrolysed wheat gluten; PP = potato protein; SBM = soybean meal; WG = wheat gluten.

<sup>2</sup> Calculated amounts of vitamin E and selected minerals and trace elements were as follows (per kg feed): Ca, 7.5 g; P, 6.1 g; Na, 3.7 g; K, 5.3 g; Cl, 6.4 g; Cu, 162.1 mg; Fe, 240.6 mg; Zn, 117.2 mg; Mn, 39.4 mg; Vit E, 40.0 IE.

<sup>3</sup> Calculated with the use of the Dutch feed tables (CVB, 2000).

After weaning (days 0 to 7) the piglets were given access to a maximum of dietary energy. Formula 1 describes the maintenance requirement for net energy (NE<sub>m</sub>) of the piglets according to their metabolic weight on the day of weaning (NRC, 1998):

$$NE_m (\text{kJ} \cdot \text{day}^{-1}) = 326.4 \times BW_0^{0.75} \quad [1]$$

where NE<sub>m</sub> is the net energy intake at maintenance level (kJ · day<sup>-1</sup>) and BW<sub>0</sub> is BW on day 0 (kg). The piglets received 0.25 × NE<sub>m</sub> on day 0, 0.5 × NE<sub>m</sub> on day 1, 0.75 × NE<sub>m</sub> on day 2,

$1 \times \text{NE}_m$  on day 3,  $1.5 \times \text{NE}_m$  on day 4,  $2 \times \text{NE}_m$  on day 5,  $2.5 \times \text{NE}_m$  on days 6 and 7. Piglets were fed equal portions of feed 4 times per day at 0900, 1130, 1400 and 1700 h. Feed refusals were collected, weighed and subtracted from the amount of feed offered to calculate actual daily feed intake which was expressed as  $\text{g} \cdot (\text{kg BW}_0^{0.75})^{-1}$ .

Table 3 Analysed nutrient composition of the experimental diets.

Diet code <sup>1</sup> :	SBM WG	SBM HWG	HSBM WG	SBM PP	SBM PP gln
Macronutrients ( $\text{g} \cdot \text{kg}^{-1}$ feed)					
Moisture	98	92	86	98	99
Crude protein	199	209	203	199	223
Crude fat	59	54	55	56	59
Crude fibre	19	18	19	21	25
Ash	50	52	52	51	49
Starch	453	428	446	452	428

<sup>1</sup> Abbreviations: gln = glutamine; HSBM = hydrolysed soybean meal; HWG = hydrolysed wheat gluten; PP = potato protein; SBM = soybean meal; WG = wheat gluten.

### ***Growth and faeces consistency***

Piglets were weighed on days –1, 0, 3 and 7 postweaning. 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 (watery diarrhoea). Scoring was done by experienced care takers who were blinded to treatment modality.

### ***Sampling of small intestine***

On days 0, 3 and 7 postweaning, piglets to be killed were weighed and euthanized with a 5-ml intra-cardiac injection of Euthestate® (pentobarbital sodium,  $200 \text{ mg} \cdot \text{ml}^{-1}$ ; Ceva Sante Animale B.V. Maasluis, The Netherlands). The piglets were killed between 0800 and 1600 h on days 3 and 7, the order being stratified according to the type of diet fed. At 30 min before killing on days 3 and 7, each piglet was given access to its pre-set amount of feed. A midline 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 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 small intestinal tract was collected to determine its dry matter content. Empty body weight (EBW) was determined as animal weight without the gastrointestinal tract, liver and spleen.



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 0.1 mol per l phosphate buffered formalin (40 ml formalin · l<sup>-1</sup>). A 3-mm wide zone from the mesenteric site was cut at a right angle 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 5 villi and crypts.

To measure the specific activity of brush-border-membrane-associated isomaltase-sucrase and aminopeptidase, a proximal small intestinal tissue sample (15 cm) was taken and rinsed with phosphate-buffered saline (PBS), pH 7.2 (0.01 M NaH<sub>2</sub>PO<sub>4</sub>, 0.01 M Na<sub>2</sub>HPO<sub>4</sub>, 0.9 % (w/v) NaCl). The mucosal layer was carefully scraped off from the muscle layer, quickly frozen in liquid nitrogen and stored at -80 °C 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 0 °C to give a final concentration of 5 % (v/w). Subsequently, the homogenates were sonicated twice at 0 °C for 15 s, separated by an interval of 30 s, at an amplitude of 24 µm with an 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 · ml<sup>-1</sup> and used to measure enzyme activities. The activities were tested under conditions of linearity with 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 · min<sup>-1</sup>), 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 · min<sup>-1</sup>) and expressed as enzyme units · g protein<sup>-1</sup>.

### ***Statistical analysis***

A GLM procedure (SAS version 6.12, SAS Institute, Cary, NC) was used to estimate the least-square means (LSMeans) for the different treatments. The effect of diet composition was analysed for each day of sampling separately. The two-way interaction of batch × diet composition was not significant and therefore not included in the final model:

$$y_{ijk} = \mu + B_i + D_j + b \times FI + e_{ijk} \quad [2]$$

where  $y_{ijk}$  = dependent variable;  $\mu$  = overall mean;  $B_i$  = fixed effect of batch ( $i = 1, 2$ );  $D_j$  = fixed effect of diet composition ( $j = 1, 2, 3, 4, 5$ ); FI = effect of average feed intake (co-variable), during days 1-3 for piglets dissected on day 3, or during days 3-7 for piglets

dissected on day 7 and expressed as  $g \cdot (kg BW^{0.75})^{-1}$ ;  $e_{ijk}$  = error term. Postweaning feed intake is an important determinant of mucosal integrity and growth performance of piglets (Kelly et al., 1991b; McCracken et al., 1995; Van Beers-Schreurs 1996). To assess the effect of diet composition rather than that of diet-induced feed intake, it was decided to incorporate feed intake as a co-variable into the statistical model. An additional reason was the observed, large inter-individual variation of postweaning feed intake. The values for faeces inconsistency were not normally distributed. Therefore the effect of dietary treatment was analysed with the  $\chi^2$  test of the Cadmod procedure. For the feed intakes of all dietary treatments combined a box-whisker plot as function of postweaning days was constructed.

## **Experiment 2: Growth trial**

### ***Piglets and weaning***

To evaluate the effect of the experimental diets on growth performance during the first two weeks after weaning, a growth trial was done. After piglets had been assigned to Experiment I, remaining barrows (n=30) and gilts (n=135) with an average BW of 8.5 kg (SD: 1.33) were used in two identical growth trials. At weaning, pigs were removed from the sow, weighed and housed with 3 piglets per pen ( $150 \times 100 \text{ cm}^2$ ). Each pen was equipped with a trough and a water nipple. Environmental temperature was maintained at 27 °C during the first week and at 25 °C during the second week postweaning. Lights were on from 06.00 to 22.00 h.

### ***Feeds, feeding and experimental design***

On day -1, all piglets were weighed, blocked by sexe and BW and subsequently randomly allocated to 5 experimental groups within each batch. Littermates were evenly distributed among groups. During the first 2 weeks postweaning, piglets received one of five experimental diets as described above (Tables 1-3). Water and feed were available ad libitum.

### ***Growth and faeces consistency***

Piglets were weighed on days -1, 0, 7 and 14 postweaning. Feed intake was measured for the periods: days 0-7 and days 7-14 after weaning. Faeces consistency and shape was scored twice a day per pen as described above.

### ***Statistical analysis***

The GLM procedure was used to calculate the LSMeans for each treatment. The experimental unit was a pen with 3 piglets. The effect of diet composition on the technical results was evaluated with batch, stable, diet composition and BW at weaning (co-variable) as independent variables. BW at weaning was used as co-variable because there was considerable variation and because it is negatively related with initial feed intake after

weaning (Bruininx et al., 2001). The two-way interactions of batch  $\times$  diet composition and stable  $\times$  diet composition were not significant and were not included in the final model:

$$y_{ijkl} = \mu + B_i + S(B)_j + D_k + b \times BW_0 + e_{ijkl} \quad [3]$$

where  $y_{ijkl}$  = dependent variable;  $\mu$  = overall mean;  $B_i$  = fixed effect of batch ( $i = 1, 2$ );  $S(B)_j$  = fixed effect of stable, nested within batch ( $j = 1, 2$ );  $BW_0$  = effect of weight at weaning (co-variable);  $D_k$  = fixed effect of diet composition ( $k = 1, 2, 3, 4, 5$ );  $e_{ijkl}$  = error term. The data on faeces inconsistency were analysed as described above.

## RESULTS

### Experiment 1: mucosal integrity of small intestine

#### *Performance*

Feed intake did not differ significantly between dietary treatments. Group mean feed intake during days 3-7 was lowest for the groups fed the diets SBM+HWG and HSBM+WG (Table 4). Daily feed intake ( $\text{g} \cdot (\text{kg BW}^{0.75})^{-1} \cdot \text{day}^{-1}$ ) for all dietary treatments combined are shown in Figure 1 as a box-whisker plot and as means and SD. The increase in the amount of feed offered was associated with an increase in both average daily feed intake and SD. Based on the energy content of the feeds (Table 2) and assumed maintenance requirement (NRC, 1998), the feed intake to meet the net energy requirement for maintenance was calculated to be about  $31 \text{ g} \cdot (\text{kg BW}^{0.75})^{-1} \cdot \text{day}^{-1}$ . On day 7, 27.5 % of the piglets ate below their maintenance requirement. Overall, average daily feed intake during the first week was  $119 \text{ g} \cdot \text{day}^{-1} \cdot \text{pig}^{-1}$  (SD: 45.1).

BW at weaning was 8.4 kg (SD: 0.72;  $n=88$ ). ADG did not differ between dietary treatments. Overall ADG was 273 g (SD: 138.7) from days -1 to 0 ( $n=80$ ), -28 g (SD: 74.9) from days 0 to 3 ( $n=80$ ), 120 g (SD: 117.6) from days 3 to 7 ( $n=40$ ) and 82 g (SD: 60.8) from days 0 to 7 ( $n=40$ ). EBW did not differ between dietary treatments (Table 4). EBW decreased from 7.7 kg (SD: 0.81) on day 0, to 7.3 kg (SD: 0.69) on day 3 and 7.3 kg (SD: 0.79) on day 7. On day 7, feed intake as co-variable was positively correlated with EBW.

#### *Faeces consistency*

None of the piglets received medical treatment during the experimental period. Only 1 piglet showed signs of illness. The average incidence of faeces inconsistency (% of days with faeces score  $\geq 1$ ) was 11 % (SD: 24.3) for the piglets dissected on day 3 and 34 % (SD: 25.8) for the piglets dissected on day 7. The incidence of faeces inconsistency for the piglets dissected on day 7 was significantly lower ( $P < 0.05$ ) when they had received the SBM+PP diet (18 %, SD: 23.8) instead of the other 4 experimental diets (38 %, SD: 25.1). Inclusion of an independent

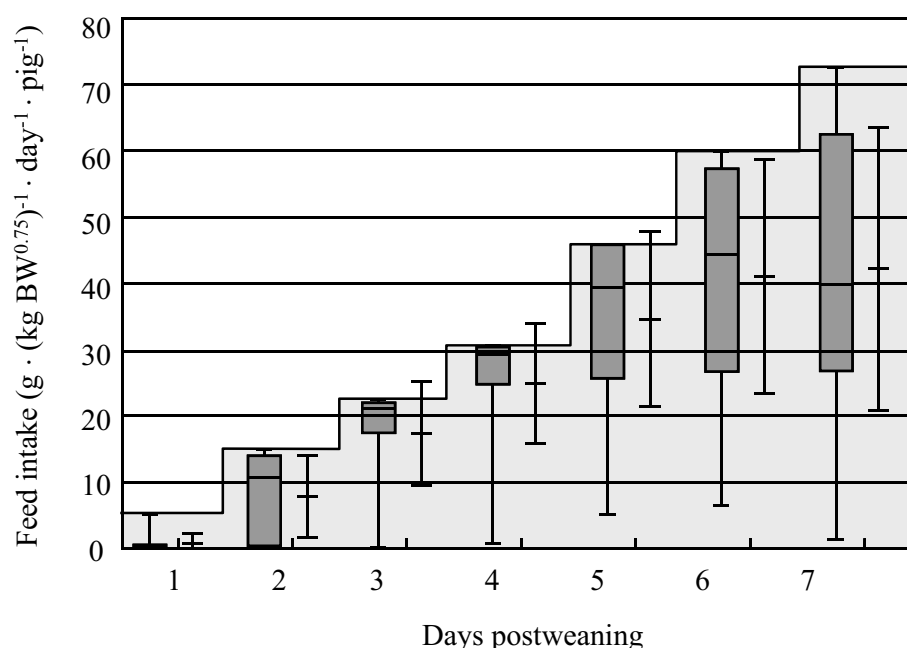


Figure 1 Box-Whisker graph of feed intake expressed as g per kg metabolic weight by piglets for the first 7 days after weaning (days 1-3,  $n=80$ ; days 4-7,  $n=40$ ). The graph shows the means  $\pm$  SD and the median within the boxes and the range as adjacent bars. The upper and lower closures of the boxes indicate the quartiles. The gray area represents the amount of feed offered. The amount of feed needed for maintenance per pig is  $30.6 \text{ g} \cdot (\text{kg BW}^{0.75})^{-1} \cdot \text{day}^{-1}$ .

binomial variable in the statistical model for piglets with a faeces score of  $\geq 2$  at two consecutive days did not affect the outcome and the data of these piglets were not excluded from the statistical analysis. The dry matter content of the chyme in the last 2 m of the small intestine was not affected by diet composition (Table 5). Feed intake was positively associated with the dry matter content of chyme.

### **Organ weights**

Table 4 shows the effect of diet composition and feed intake on organ weights expressed as  $\text{g} \cdot (\text{kg EBW})^{-1}$ . Diet composition did not affect weight of the gastrointestinal tract on day 3. On day 7, diet composition tended to affect liver and pancreas when corrected for EBW, but not as absolute values (data not shown). The addition of crystalline gln to the diet tended to increase the liver weight. Diets containing PP tended to induce a heavier pancreas weight when compared to diet SBM+HWG. Hydrolysis of WG or SBM neither affected liver nor pancreas weight on day 7. Feed intake during days 1-3 was positively correlated with the weight of small intestine, large intestine and liver on day 3. Feed intake during days 3-7 was positively correlated with EBW and the weight of the small intestine and pancreas on day 7, but was negatively correlated with stomach weight on that day.

Table 4 The effect of diet composition and feed intake as co-variable on empty-body weight and organ weights of newly weaned piglets dissected on day 3 or day 7 after weaning (Experiment 1)<sup>1, 2, 3</sup>.

	Diet codes							Feed intake as co-variable <sup>4</sup>	
	SBM	SBM	HSBM	SBM	SBM	P-value <sub>5</sub>	b <sup>6</sup>	P-value	
	WG	HWG	WG	PP	PP				
			gln	SEM					
Day 3									
Feed intake, d 1 – 3 (g · (kg <sup>0.75</sup> BW <sub>0</sub> ) <sup>-1</sup> )	7.4	9.6	8.6	6.6	9.2	1.46	ns		
EBW (kg)	7.3	7.2	7.3	7.3	7.2	0.26	ns	0.01	ns
Organ weights (g · (kg EBW) <sup>-1</sup> )									
- Stomach	6.8	6.6	5.9	6.2	6.1	0.43	ns	-0.001	ns
- Small intestine	26.4	26.3	26.8	28.4	27.1	1.67	ns	0.54	**
- Large intestine	13.4	14.3	13.7	14.8	14.4	0.84	ns	0.30	**
- Liver	28.1	30.4	30.0	29.2	29.5	1.06	ns	0.33	*
- Pancreas	1.1	1.4	1.5	1.3	1.3	0.13	ns	0.02	ns
Day 7									
Feed intake, d 3 – 7 (g · (kg BW <sub>0</sub> <sup>0.75</sup> ) <sup>-1</sup> )	40.1	30.2	31.3	38.5	38.3	4.71	ns		
EBW (kg)	7.2	7.3	7.4	7.3	7.1	0.25	ns	0.04	**
Organ weights (g · (kg EBW) <sup>-1</sup> )									
- Stomach	7.6	7.5	7.9	8.8	8.3	0.40	ns	-0.06	**
- Small intestine	38.3	39.2	39.8	38.0	42.9	1.86	ns	0.11	t
- Large intestine	19.0	19.7	20.6	21.1	20.6	0.96	ns	0.02	ns
- Liver <sup>7</sup>	31.6 <sup>ab</sup>	29.2 <sup>bc</sup>	30.3 <sup>bc</sup>	28.8 <sup>c</sup>	32.9 <sup>a</sup>	1.05	t	-0.06	ns
- Pancreas <sup>7</sup>	2.2 <sup>ab</sup>	2.0 <sup>b</sup>	2.2 <sup>ab</sup>	2.4 <sup>a</sup>	2.5 <sup>a</sup>	0.12	t	0.01	*

<sup>1</sup> Abbreviations: BW<sub>0</sub> = body weight at weaning; EBW = empty-body weight; gln = glutamine; HSBM = hydrolysed soybean meal; HWG = hydrolysed wheat gluten; PP = potato protein; SBM = soybean meal; WG = wheat gluten

<sup>2</sup> Data in the table are presented as least-square means (LSMeans) and standard error of the mean (SEM) for 8 piglets per dietary group

<sup>3</sup> Baseline values (means and SD; n=8) for piglets killed at weaning (day 0) were as follows: EBW, 7.7 kg (SD: 0.81); stomach, 5.3  $\text{g} \cdot (\text{kg EBW})^{-1}$  (SD: 1.24); small intestine, 32.7  $\text{g} \cdot (\text{kg EBW})^{-1}$  (SD: 5.32); large intestine, 12.7  $\text{g} \cdot (\text{kg EBW})^{-1}$  (SD: 2.91); liver, 29.4  $\text{g} \cdot (\text{kg EBW})^{-1}$  (SD: 3.55); pancreas, 0.8  $\text{g} \cdot (\text{kg EBW})^{-1}$  (SD: 0.29).

<sup>4</sup> Feed intake ( $\text{g} \cdot (\text{kg BW}_0^{0.75})^{-1}$ ) is the average for day 1-3 in piglets dissected on day 3 and for days 3-7 in piglets dissected on day 7. The effect of feed intake was statistically analysed by treating it as co-variable.

<sup>5</sup> P-value of the model: \*\*, P < 0.01; \*, P < 0.05; t, P < 0.10; ns, not significant.

<sup>6</sup> Slope for the influence of feed intake as co-variable.

<sup>7</sup> LSMeans in a row without a common superscript letter differ significantly (P < 0.10).

Table 5 The effect of diet composition and feed intake as co-variable on small intestinal (SI) characteristics of newly weaned piglets dissected on day 3 or day 7 after weaning (Experiment 1)<sup>1, 2, 3</sup>.

	Diet codes							Feed intake as co-variable	
	SBM WG	SBM HWG	HSBM WG	SBM PP	SBM PP gln	SEM	P-value <sup>4</sup>	b <sup>5</sup>	P-value
<b>Day 3</b>									
SI length / kg EBW (cm · kg <sup>-1</sup> )	100.3	101.3	98.5	110.1	106.4	5.29	ns	1.17	t
SI weight / length (g · cm <sup>-1</sup> )	0.27	0.26	0.27	0.26	0.26	0.012	ns	0.002	ns
Protein content SI mucosa (g · g <sup>-1</sup> )	0.46	0.46	0.55	0.56	0.56	0.048	ns	0.001	ns
Dry matter in SI chymus (%)	8.0	7.7	11.3	5.6	7.0	1.47	ns	0.49	**
Specific enzyme activity (units · (g protein) <sup>-1</sup> )									
- aminopeptidase	338.2	415.7	404.9	339.3	292.5	55.75	ns	10.22	ns
- isomaltase-sucrase	23.7	19.0	16.5	15.0	13.2	2.71	ns	0.90	ns
<b>Day 7</b>									
SI length / kg EBW (cm · kg <sup>-1</sup> )	113.7	111.0	110.8	111.0	116.1	4.61	ns	-0.26	ns
SI weight / length (g · cm <sup>-1</sup> )	0.34	0.35	0.36	0.34	0.37	0.011	ns	0.002	**
Protein content SI mucosa (g · g <sup>-1</sup> )	0.65	0.68	0.65	0.68	0.63	0.031	ns	0.005	**
Dry matter in SI chymus (%)	5.4	6.5	4.9	6.0	6.0	0.81	ns	0.13	**
Specific enzyme activity (units · (g protein) <sup>-1</sup> )									
aminopeptidase	365.0	330.7	289.8	367.2	293.5	30.71	ns	2.76	*
isomaltase-sucrase <sup>6</sup>	17.4 <sup>b</sup>	13.3 <sup>b</sup>	14.3 <sup>b</sup>	24.4 <sup>a</sup>	12.9 <sup>b</sup>	2.87	*	0.12	ns

<sup>1</sup> Abbreviations used: BW<sub>0</sub> = body weight at weaning; EBW = empty-body weight; gln = glutamine; HSBM = hydrolysed soybean meal; HWG = hydrolysed wheat gluten; PP = potato protein; SBM = soybean meal; WG = wheat gluten

<sup>2</sup> Data in the table are presented as least-square means (LSMeans) and standard error of the mean (SEM) for 8 piglets per dietary group

<sup>3</sup> Baseline values (means and SD; n=8) for piglets killed at weaning (day 0) were as follows: SI length, 99.2 cm · (kg EBW)<sup>-1</sup> (SD: 13.73); SI weight, 0.3 g · cm<sup>-1</sup> (SD: 0.03), protein content SI mucosa 0.8 g · g<sup>-1</sup> (SD: 0.07); aminopeptidase specific activity 538.2 units · (g protein)<sup>-1</sup> (SD: 135.62); isomaltase-sucrase specific activity 59.8 units · (g protein)<sup>-1</sup> (SD: 20.33).

<sup>4</sup> P-value of the model: \*\*, P < 0.01; \*, P < 0.05; t, P < 0.10; ns, not significant.

<sup>5</sup> Slope for the influence of feed intake as co-variable.

<sup>6</sup> LSMs in a row without a common superscript letter differ significantly (P < 0.05).

### Enzyme activity

The activity of brush-border aminopeptidase and isomaltase-sucrase was expressed as units per g mucosa protein. The protein content of the small intestinal mucosa was not affected by diet composition and was on average 0.52 g · (g mucosa)<sup>-1</sup> (SD: 0.148) on day 3 and 0.66 g · (g mucosa)<sup>-1</sup> (SD: 0.110) on day 7 (Table 5). On day 7, feed intake was positively correlated with the protein content of the mucosa. Diet composition did not affect the

aminopeptidase activity. Overall aminopeptidase activity was 538.2 (SD: 135.62) on day 0, 358.1 (SD: 167.09) on day 3 and 329.2 (SD: 99.07) on day 7. Overall isomaltase-sucrase activity decreased from 59.8 (SD: 20.33) on day 0, to 17.4 (SD: 9.17) on day 3 and 16.5 (SD: 10.12) on day 7. Diet composition did not affect isomaltase-sucrase on day 3, but on day 7 isomaltase-sucrase activity was higher in piglets fed the diet SBM+PP when compared to piglets fed the other diets.

### ***Morphology***

Villus height and crypt depth at the proximal and mid small intestine on days 3 and 7 postweaning is shown in Figure 2. Diet composition did not affect villus height at the proximal and mid small intestine. When all data were combined, villus height ( $\mu\text{m}$ ) at the proximal small intestine decreased from 525 (SD: 94.9) on day 0, to 285 (SD: 113.6) on day 3 and 320 (SD: 98.6) on day 7. Villus height ( $\mu\text{m}$ ) at the mid small intestine decreased from 487 (SD: 94.9) on day 0 to 312 (SD: 93.3) on day 3 and 263 (SD: 70.8) on day 7. Crypt depth at the proximal and mid small intestine on day 3 or at the mid small intestine on day 7 was not affected by diet composition. Crypt depth at the proximal and mid small intestine increased with postweaning time. On day 7, crypt depth at the proximal small intestine was significantly deeper for piglets fed the diets containing hydrolysed protein sources when compared to their counterparts fed the native protein sources. On day 7, feed intake for all treatments combined was positively correlated with villus height at the proximal and mid small intestine ( $P < 0.01$ ), the explained variance ( $R$ ) being 71 and 45 %, respectively. On day 3, feed intake was positively correlated with villus height at the proximal small intestine ( $R = 53 \%$ ,  $P < 0.01$ ), but was not correlated with the villus height at the mid small intestine. Crypt depth at the proximal small intestine was not correlated with feed intake, neither on day 3 nor on day 7. However, crypt depth at the mid small intestine was correlated with feed intake on both days 3 and 7 ( $P < 0.05$ ).

### **Experiment 2: Growth trial**

Average BW at weaning was 8.5 kg (SD: 1.33). Feed intake did not differ between dietary treatments (Table 6). During the first week postweaning, growth and feed efficiency were not significantly different between dietary treatments, but during the second week, piglets receiving the diet supplemented with crystalline gln had higher ADG and feed efficiency than the piglets fed the other diets. A similar effect was seen when the data for the first two weeks were combined. In the second week postweaning, feeding the diet HSBM+WG depressed growth and feed efficiency.

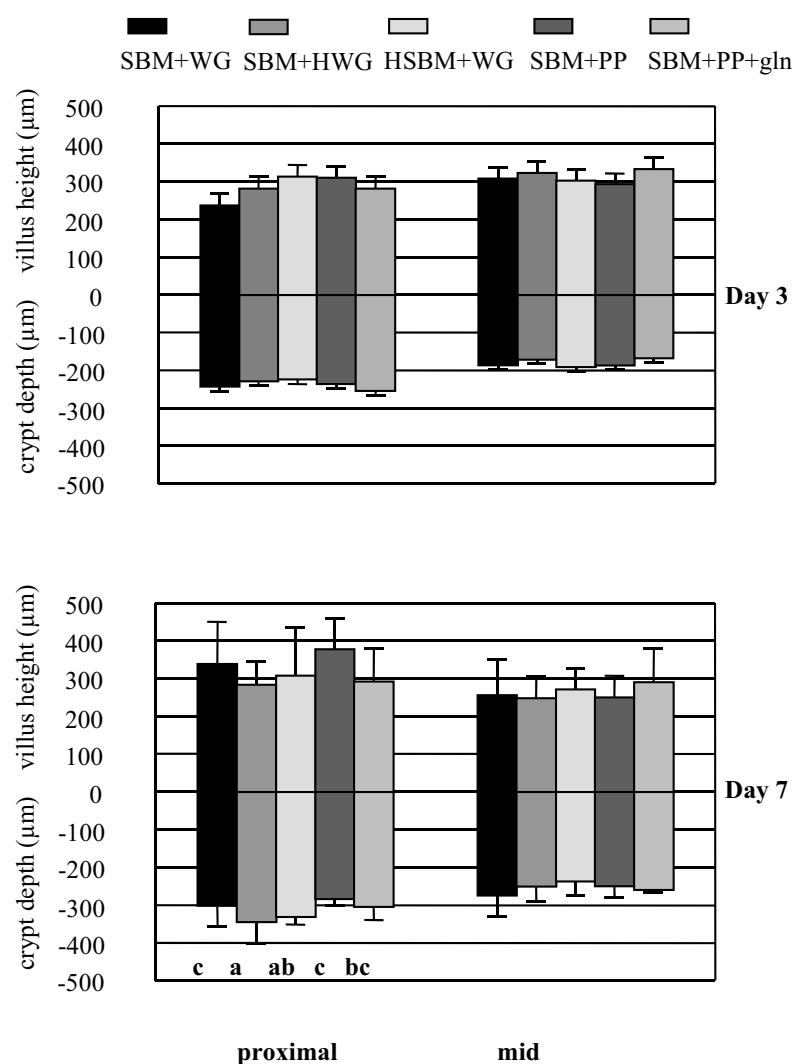


Figure 2 Effect of diet composition on villus height and crypt depth at the proximal and mid small intestine of piglets dissected on day 3 (upper graph) or day 7 (lower graph) after weaning. Diets codes are as follows: SBM = soybean meal; WG = wheat gluten; HWG = hydrolysed wheat gluten; HSBM = hydrolysed soybean meal; PP = potato protein; gln = glutamine. Results are presented as means  $\pm$  SD for 8 piglets per diet per postweaning day. Means with different letters are significantly different ( $P < 0.05$ ).

Four piglets showed vomiting and two piglets showed signs of lameness. During on average 43 % (SD: 15.6) of the days in the trial, the faeces was considered not to be consistent. During the first week after weaning, the diet SBM+WG had reduced incidence of faeces inconsistency (Table 7). During the second week and during the first two weeks combined, piglets receiving the diet SMB+WG also showed more consistent faeces when compared to piglets fed the other diets.



Table 6 Effect of diet composition on growth performance of piglets during 2 weeks postweaning (Experiment 2) <sup>1</sup>

Diet code <sup>2</sup> :	SBM <sup>1</sup> WG	SBM HWG	HSBM WG	SBM PP	SBM PP gln	SEM	P-value <sup>2</sup>
Average daily gain (g · day <sup>-1</sup> · pig <sup>-1</sup> )							
week 0-1	93	79	86	92	95	13.0	ns
week 1-2 <sup>3</sup>	216 <sup>b</sup>	211 <sup>bc</sup>	184 <sup>c</sup>	233 <sup>b</sup>	271 <sup>a</sup>	13.3	**
week 0-2	155 <sup>bc</sup>	145 <sup>bc</sup>	135 <sup>c</sup>	163 <sup>a</sup>	183 <sup>ab</sup>	10.0	*
Average daily feed intake (g · day <sup>-1</sup> · pig <sup>-1</sup> )							
week 0-1	174	152	168	172	167	10.4	ns
week 1-2	310	286	289	317	325	13.4	ns
week 0-2	242	219	228	245	246	10.3	ns
Feed efficiency (g gain · (g feed) <sup>-1</sup> )							
week 0-1	0.51	0.52	0.50	0.52	0.55	0.054	ns
week 1-2	0.70 <sup>bc</sup>	0.73 <sup>b</sup>	0.63 <sup>c</sup>	0.74 <sup>b</sup>	0.84 <sup>a</sup>	0.033	**
week 0-2	0.64 <sup>bc</sup>	0.66 <sup>b</sup>	0.59 <sup>c</sup>	0.66 <sup>b</sup>	0.74 <sup>a</sup>	0.028	**

<sup>1</sup> Results are expressed as least-square means (LSMeans) for 11 pens per dietary treatment, each pen containing three piglets.

<sup>2</sup> gln = glutamine; HSBM = hydrolysed soybean meal; HWG = hydrolysed wheat gluten; PP = potato protein; SBM = soybean meal; WG = wheat gluten.

<sup>3</sup> P-value of the model: \*\*, P < 0.01; \*, P < 0.05; ns, not significant.

<sup>4</sup> LSMs in a row without a common character in the superscript differ significantly.

## DISCUSSION

Weaning of piglets is known to compromise their mucosal integrity as indicated by a fall of the activity of the brush-border enzymes isomaltase-sucrase and aminopeptidase (Hampson, 1986; Miller et al., 1986; Kelly et al., 1991a) and a decrease in villus height and an increase in crypt depth (Hampson, 1986; Miller et al., 1986; Cera et al., 1988; Dunsfort et al., 1989; Hall and Byrne, 1989; Kelly et al., 1991a; Nabuurs 1991; Pluske et al., 1996; also described in Chapter 3). The well-known effects of weaning on mucosal integrity were also found in this study. When the data of all treatment groups were combined, villus height was positively correlated with feed intake. This correlation has been reported earlier (Kelly et al. 1991b; McCracken et al., 1995; Van Beers-Schreurs, 1996; Pluske et al., 1996; Verdonk et al., 2001).

The weaning-induced reduction in villus height and aminopeptidase activity lowers the capacity of the small intestine to digest and absorb dietary proteins. Makkink (1993) found an increased ratio of precipitable protein to total crude protein in the jejunal digesta on 3 and 6 days after weaning, indicating that protein digestion was impaired. Caine (1997) showed that

Table 7 Effect of diet composition on incidence and severity of faeces inconsistency of piglets during 2 weeks postweaning (Experiment 2)<sup>1</sup>.

Diet code <sup>2</sup> :	SBM WG		SBM HWG		HSBM WG		SBM PP		SBM PP gln	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Incidence of faeces inconsistency (%) <sup>3</sup>										
week 0-1	16.9 <sup>a</sup>	16.68	37.7 <sup>b</sup>	19.45	35.1 <sup>b</sup>	17.34	28.6 <sup>b</sup>	16.90	37.7 <sup>b</sup>	18.37
week 1-2	54.5 <sup>a</sup>	34.89	83.1 <sup>c</sup>	21.01	85.7 <sup>c</sup>	21.19	70.1 <sup>b</sup>	28.89	67.5 <sup>b</sup>	26.41
week 0-2	35.7 <sup>a</sup>	24.12	60.4 <sup>c</sup>	17.89	60.4 <sup>c</sup>	13.31	49.4 <sup>b</sup>	16.43	52.6 <sup>bc</sup>	17.86
Severity of faeces inconsistency <sup>4</sup>										
week 0-1	1.2	0.99	1.9	0.69	1.7	0.79	1.7	0.74	2.0	0.36
week 1-2	1.5	0.57	1.8	0.35	2.0	0.36	1.7	0.33	1.8	0.39
week 0-2	1.5	0.58	1.9	0.36	2.0	0.31	1.8	0.31	1.8	0.32

<sup>1</sup> Results are given for 11 pens per dietary treatment, each pen containing three piglets. The data were not normally distributed but are presented as means  $\pm$  SD to enhance interpretation.

<sup>2</sup> gln = glutamine; HSBM = hydrolysed soybean meal; HWG = hydrolysed wheat gluten; PP = potato protein; SBM = soybean meal; WG = wheat gluten.

<sup>3</sup> Data presented as means  $\pm$  SD and expressed as % of days within the period shown with faecal consistency score of 1, 2 or 3, i.e. inconsistent faeces. Means in a row without a common superscript letter differ significantly,  $P < 0.05$  as analyzed with  $\chi^2$  test

<sup>4</sup> Data presented as means  $\pm$  SD and expressed as the average faecal score when piglets show inconsistent faeces within the period shown.

the apparent ileal protein digestibility was low 7 days after weaning. Thus, it was hypothesised that hydrolysis of dietary proteins before ingestion would increase the availability of amino acids for the gut wall and therefore counteracts the decrease in the small intestinal integrity after weaning. Evaluation of the present data leads to rejection of the hypothesis. The incorporation into the diet of either HWG or HSBM instead of the native proteins did not increase villus height and did not raise brush border activities of aminopeptidase and isomaltase-sucrase. In addition, the hydrolysed versus intact proteins did neither increase organ weights nor feed intake, or growth in the current study. However, on day 7 after weaning, the protein hydrolysates had induced deeper crypts at the proximal small intestine. Similarly to our study, other investigators showed that villus height was similar for hydrolysed versus intact SBM (McCracken et al., 1998; Rooke et al., 1998). However, when hydrolysed casein was fed, villus height was decreased at the proximal small intestine (Hampson, 1986). Rooke et al. (1998) showed that piglets fed protease-treated SBM showed similar aminopeptidase, lactase and maltase activities, but had increased sucrase activity when compared to piglets fed untreated SBM. Hampson (1986) showed an increase in lactase and sucrase activity in the mid small intestine, but not in the proximal and distal small intestine, when piglets were fed hydrolysed instead of untreated casein. However, isomaltase-sucrase

activity was not affected by hydrolysis in the current study. Other piglet trials comparing the feeding of native proteins with hydrolysed proteins showed either similar (Leibholz, 1981; Richert et al., 1994; Caine, 1997) or increased (McCracken et al., 1998; Rooke et al., 1998) ADG.

Faeces consistency of piglets fed the protein hydrolysates when compared with those fed the untreated protein was generally decreased. In line with the tendency towards diarrhoea in the piglets fed the protein hydrolysates is the observed, significantly lower weight gain in piglets fed the diet HSBM+WG. In contrast, McCracken and colleagues (1998) showed less diarrhoea when diets with hydrolysed soy protein isolate instead of either intact soy protein isolate or milk protein were fed to piglets that were weaned 2 days postpartum. Hampson (1986) showed no effect of hydrolysis of casein on the number of weanling pigs with diarrhoea.

The inclusion of 2 % crystalline gln in the diet did not affect villus architecture in the current study. Some trials with weaned piglets showed no effect on villus height with either 1 % (Kitt et al., 2001) or 1.2 % gln in the diet (Touchette et al., 2000). Other trials showed that 1 % gln (Wu et al., 1996) or 6.5 % glutamate (glu) (Ewtushik et al., 2000) had a positive effect on one site of the proximal small intestine, but not further along the intestine. Ayonrinde and colleagues (1995) showed that 4 % gln increased villus height in both the duodenum and ileum. In contrast to the results of Ewtushik and colleagues (2000), liver weight expressed per kg of EBW tended to be higher upon the addition of gln to the diet in the current study. Surprisingly, the diet with extra gln caused a decrease in isomaltase-sucrase activity on day 7 after weaning. However, in earlier work the addition of 6.5 % glu did not affect total and specific enzyme activity of lactase, sucrase and maltase (Ewtushik et al., 2000). Even though there was a decrease in isomaltase-sucrase enzyme activity and no effect on gut morphoplogy, growth and feed efficiency during the second week postweaning were increased by the addition of gln to the diet. Wu and colleagues (1996) showed improved feed efficiency but similar growth during the second week postweaning when extra gln was fed. In other studies, growth was either similar (Ewtushik et al., 2000) or increased by the addition of gln to the diet (Kitt et al., 2001). Lackeyram and colleagues (2001) noted increased growth with 0.8 % gln, but no effect with either 1.6 % or 2.4 % gln. It may be concluded that the effects of gln supplementation are equivocal, possibly due to interactions with the background composition of the diet and/or other experimental conditions such as infectious pressure. WG contains a high and PP a low content of gln. The diets containing either WG, HWG or gln contained a similar amount of calculated gln plus glu in the diet, i.e. about 5 %. However, when compared to the PP+SBM diet, which contained 2.5 % of gln plus glu, the diets containing either WG or HWG did not improve growth performance unlike the diet containing crystalline gln. This observation could also point at the gln effect being subject to nutrient interactions.

## IMPLICATIONS

The present study shows that the feeding of diets containing hydrolysates of either soybean meal or wheat gluten or added crystalline glutamine did not ameliorate the weaning-induced decrease in growth, villus height and specific enzyme activity as measured during the first week postweaning. This information is relevant for the formulation of diets for weanling piglets as the hydrolysates and gln are relatively expensive. When the data of all treatment groups were combined, feed intake was positively correlated with organ weight, villus architecture and brush-border enzyme activity. This indicates that, at least under the conditions of this study, small intestinal development in weaned piglets depended on feed intake rather than on diet composition.

## LITERATURE CITED

- Ayonrinde, A. I., I. H. Williams, R. McCauley, and B. P. Mullan. 1995. Glutamine stimulates intestinal hyperplasia in weaned piglets. Page 180 in *Manipulating Pig Production V.*, D. P. Hennessy and P. D. Cranwell, eds. Australasian Pig Science Association, Werribee, Australia.
- Bruininx, E. M. A. M. 2002. Individually measured feed intake characteristics in group-housed weanling pigs. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- Bruininx, E. M. A. M., C. M. C. van der Peet-Schwering, J. W. Schrama, P. F. G. Vereijken, P. C. Vesseur, H. Everts, L. A. Den Hartog, and A. C. Beynen. 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. *J. Anim. Sci.* 79: 301-308.
- Caine, W. R. 1997. Ileal recovery of endogenous amino acids in pigs. Ph.D. Diss., Wageningen Univ., Wageningen, The Netherlands.
- Cera, K. R., D. C. Mahan, R. F. Cross, G. A. Reinhart, and R. E. Whitmoyer. 1988. Effect of age, weaning and postweaning diet on small intestinal growth and small intestinal morphology in young swine. *J. Anim. Sci.* 66: 574-584.
- Centraal Veevoederbureau (C.V.B). 2000 Chemical composition, digestibility and feeding value of feedstuffs. Veevoedertabel, Centraal Veevoederbureau, Lelystad, The Netherlands (in Dutch).
- Dunsford, B. R., D. A. Knabe, and W. E. Haensly. 1989. Effect of dietary soybeanmeal on the microscopic anatomy of the small intestine in the early weaned pig. *J. Anim. Sci.* 67: 1855-1863.
- Ewtushik, A. L., R. F. P. Bertolo, and R. O. Ball. 2000. Intestinal development of early-weaned piglets receiving diets supplemented with selected amino acids or polyamines. *Can. J. Anim. Sci.* 80: 653-662.
- Hall, G. A., and T. F. Byrne. 1989. Effects of age and diet on small intestinal structure and function in gnotobiotic piglets. *Res. Vet. Sci.* 47: 387-392.
- Hampson, D. J. 1986. Attempts to modify changes in the piglet small intestine after weaning. *Res. Vet. Sci.* 40: 313-317.
- Kelly, D., J. A. Smyth, and K. J. McCracken. 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. *Brit. J. Nutr.* 65, 169-180.

- Kelly, D., J. A. Smyth, and K. J. McCracken. 1991b. Digestive development of the early weaned pig; effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. *Brit. J. Nutr.* 65: 181-188.
- Kitt, S. J., P. S. Miller, A. J. Lewis, and R. L. Fischer. 2001. Effects of diet and crystalline glutamine supplementation on growth performance and small intestine morphology of weanling pigs. *J. Anim. Sci.* 79 (suppl. 1): 148.
- Lackeyram, D., X. Yue, and M. Z. Fan. 2001. Effects of dietary supplementation of crystalline L-glutamine on the gastrointestinal tract and whole body growth in early-weaned piglets fed corn and soybean meal-based diets. *J. Anim. Sci* (suppl. 1): 322.
- Le Dividich, J., and P. Herpin. 1994. Effects of climatic conditions on the performance, metabolism and health status of weaned piglets: a review. *Liv. Prod. Sci.* 38:79-90.
- Le Huërou-Luron, I., J. Peiniau, P. Guilloteau, and A. Aumaître. 2001. Are the activities of intestinal peptidases age- and diet-dependent in piglets. Pages 20-22 in *Digestive Physiology of Pigs*. J. E. Lindberg, and B. Ogle, eds. Cabi Publishing, Wallingford, UK.
- Leibbrandt, V. D., R. C. Ewan, V. C. Speer, and D. R. Zimmerman. 1975. Effect of weaning and age at weaning on baby pig performance. *J. Anim. Sci.* 40: 1077-1080.
- Leibholz, J. 1981. Digestion in the pig between 7 and 35 d of age. 6. The digestion of hydrolysed milk and soya-bean proteins. *Br. J. Nutr.* 46: 59-69.
- Makkink, C. A. 1993. Of piglets, dietary proteins and pancreatic proteases. Ph.D. Diss., Wageningen Univ., Wageningen, The Netherlands.
- Marouz, S., D. Louvard, and J. Barratti. 1973 The aminopeptidase of pig intestinal brush border. *Biochem. Biophys. Acta* 321: 282-295.
- McCracken, B. A., H. R. Gaskins, P. J. Ruwe-Kaiser, K. C. Klasing, and D. E. Jewell. 1995. Diet-dependent and diet-independent metabolic responses underlie growth stasis of pigs at weaning. *J. Nutr.* 125: 2838-2845.
- McCracken, B. A., M. E. Spurlock, M. A. Roos, F. A. Zuckermann, and H. R. Gaskins. 1999. Weaning anorexia may contribute to local inflammation in the piglet small intestine. *J. Nutr.* 129: 613-619.
- McCracken, B. A., R. T. Zijlstra, S. M. Donovan, J. Odle, E. L. Lien, and H. R. Gaskins. 1998. Neither intact nor hydrolysed soy proteins elicit intestinal inflammation in neonatal piglets. *JPEN – Parenter. Enter.* 22: 91-97.
- Messer, M., and A. Dahlqvist. 1966 A one step ultramicromethod for the assay of intestinal disaccharidases. *Anal. Biochem.* 14: 376-392.
- Miller, B. G., P. S. James, M. W. Smith, and F. J. Bourne. 1986. Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. *J. Agric. Sci. Camb.* 107: 579-589.
- Nabuurs, M. J. A. 1991. Etiologic and pathogenic studies on postweaning diarrhea. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- National Research Council (NRC). 1998. Page 190 in *Nutrient Requirements of Swine*. 10th ed., National Academy Press, Washington DC, USA.
- Pusztai, A., J. Koninkx, H. Hendriks, W. Kok, S. Hulshcer, E. J. M. Van Damme, W. J. Puemans, G. Grant, and S. Bardocz. 1996. Effect of the insecticidal *Galanthus nivalis* agglutinin on metabolism and the activities of brush border enzymes in the rat small intestine. *Nutr. Biochem.* 7: 677-682.
- Pluske, J.R., I. H. Williams, and F. X. Aherne. 1996. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. *Anim. Sci.* 62: 145-158.
- Richert, B. T., J. D. Hancock, and J. L. Morrill. 1994. Effects of replacing milk and soybean products with wheat glutens on digestibility of nutrients and growth performance in nursery pigs. *J. Anim. Sci.* 72: 151-159.

- Rooke, J. A., M. Slessor, H. Fraser, and J. R. Thomson. 1998. Growth performance and gut function of piglets weaned at four weeks of age and fed protease-treated soya-bean meal. *Anim. Feed. Sci. Techn.* 70: 175-190.
- Smith P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk. 1985. Measurements of protein using bicinchoninic acid. *Anal. Biochem.* 150: 76-85
- Souba, W. W., 1993. Intestinal glutamine metabolism and nutrition. *J. Nutr. Biochem.* 4: 2-9.
- Touchette, K. J., G. L. Allee, K. Watanabe, Y. Toride, I. Shinzato, and J. L. Usry. 2000. The effect of arginine and glutamine on post-weaning performance and intestinal morphology of pigs. *J. Anim. Sci.* 78 (suppl. 1): 182-183.
- Van Beers-Schreurs, H., 1996. The changes in the function of the large intestine of weaned pigs. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- Verdonk, J. M. A. J., M. A. M. Spreeuwenberg, G. C. M. Bakker, and M. W. A. Verstegen. 2001. Nutrient intake level affects histology and permeability of the small intestine in newly weaned piglets. Page 332-334 in *Digestive Physiology of Pigs*. J. E. Lindberg, and B. Ogle, eds. Cabi Publishing, Wallingford, UK.
- Wu, G. 1998. Intestinal mucosal amino acid catabolism. *J. Nutr.* 128: 1249-1252.
- Wu, G., S. A. Meijer, and D. A. Knabe. 1996. Dietary glutamine supplementation prevents jejunal atrophy in weaned pigs. *J. Nutr.* 126: 2578-2584.

---

## CHAPTER 6

---

### **Villus Height and Gut Development in Weaned Piglets receiving Diets containing either Glucose, Lactose or Starch**

M. A. M. Spreeuwenberg <sup>1</sup>, J. M. A. J. Verdonk <sup>2</sup>, M. W. A. Verstegen <sup>3</sup>, and A. C. Beynen <sup>4</sup>.

<sup>1</sup> Swine Research Center, Nutreco, Boxmeer, The Netherlands; <sup>2</sup> ID TNO Animal Nutrition, Lelystad, The Netherlands; <sup>3</sup> Division of Animal Nutrition, Department of Animal Sciences, University of Wageningen, Wageningen, The Netherlands; <sup>4</sup> Department of Nutrition, Faculty of Veterinary Medicine, University of Utrecht, Utrecht, The Netherlands.

## ABSTRACT

This study was designed to evaluate differential effects of dietary glucose, lactose and starch on small intestinal morphology, organ weights, pH of chyme and haptoglobin levels in blood plasma of weaned piglets. It was hypothesised that lactose consumption would ameliorate the weaning-induced decrease in gut integrity. A total of 42 barrows was used. Piglets were weaned at 26 days (SD: 0.8) of age and weighed 7.8 kg (SD 1.0). On the day before weaning (day -1) all pigs were blocked according to body weight and randomly assigned to 7 groups (n=6 per group). The groups differed in diet and day of dissection. On the day of weaning (day 0), dissection was performed on one group of six piglets. The remaining groups were fed one of three experimental diets in which glucose, lactose or starch had been iso-energetically exchanged, supplying 24 % of the dietary energy. Piglets received a liquid diet (air-dry meal: water = 1:2, w:w). The piglets were given access to a maximum of dietary energy in order to prevent entanglement between feed intake and villus architecture. On days 0, 3 and 10 postweaning, pigs were weighed and euthanized. The results show that the carbohydrate source did not affect growth performance, organ weights, villus architecture, pH of chyme and plasma haptoglobin level. The weaning transition resulted in decreased villus height and increased haptoglobin levels. In the contents of the caecum and large intestine, the pH decreased after weaning. It is concluded that at least under conditions of unaltered feed intake and low infectious pressure, dietary lactose does not ameliorate the weaning induced compromise of small intestinal integrity when compared to either glucose or starch.

## INTRODUCTION

At weaning, the diet composition of piglets changes drastically. The liquid sow milk is replaced by pelleted dry feed with carbohydrates, instead of fat, as the main energy source. In addition, lactose, the main carbohydrate in milk, is replaced by starch. The weaning transition is accompanied by low feed intake (Leibbrandt et al., 1975; Okai et al., 1976), which causes a reduction in villus height (Kelly et al., 1991; Pluske et al., 1996; Verdonk et al., 2001a).

We have shown that increasing amounts of lactose in the weaner diet at the expense of protein were associated with higher group-mean villus height in the proximal small intestine of piglets (described in Chapter 3), and we hypothesized that lactose has specific properties contributing to mucosal integrity in newly weaned piglets. Epithelial cells need energy to maintain gut integrity. By providing lactose as a preferred energy source for the epithelial cells, the effect of postweaning low feed intake on small intestinal architecture might be ameliorated. To test the specificity of lactose, three experimental weaner diets were formulated containing 24 % of total net energy in the form of either glucose, lactose or starch.



The diets were supplied to weanling piglets and their villus height and crypt depth were measured on 3 and 10 days postweaning.

## **MATERIALS AND METHODS**

### **Piglets and weaning**

Barrows (n = 42) used were from the Swine Research Centre of Nutreco [(Duroc × Yorkshire synthetic) × (Yorkshire × Dutch Landrace synthetic)]. The piglets were weaned at 27 days (SD:0.8) of age (= day 0) and weighed 8.0 kg (SD: 0.53). Creep feed was not provided during the suckling period so as to enhance the differential response, if any, to the experimental diets and to prevent the induction of inter-individual variability by variable, pre-weaning ingestion of solid feed (Bruininx, 2002). At weaning, pigs were removed from the sow, weighed and housed individually in pens (80×100 cm<sup>2</sup>). Each pen was equipped with two feed throughs and a water nipple. Environmental temperature was maintained at 27 °C. During the day of weaning, lights were on continuously. From day 1 onwards lights were on from 0600 till 2200 h. The experimental protocol was approved by the Animal Care and Ethics Committee of the University of Nijmegen (The Netherlands).

### **Feeds, feeding and experimental design**

On the day before weaning (day –1), piglets were blocked on the basis of body weight (BW) and randomly allocated to one of seven groups. Littermates were evenly distributed among the groups. The groups differed as to diet and/or day of dissection. On day 0, dissection was performed on a group of 6 piglets. The remaining 6 groups were dissected on day 3 or 10 postweaning and received one of the 3 experimental diets in the form of a slurry. The water to air-dry feed ratio was 2:1 (w:w).

A mixture of constant components was formulated (Table 1). The experimental diets differed in their carbohydrate composition. Glucose, lactose and wheat starch were iso-energetically exchanged and supplied 24 % of total energy of the diet. Wheat starch is regarded rapidly digestible as based on its in-vitro, fractional digestion rate and has a total potential digestibility of 99.9 % (SD: 0.93) (Weurding et al., 2001). The calculated nutrient composition of the experimental diets is shown in Table 2.

After weaning (days 0 to 10), the piglets were given access to a maximum amount of dietary energy. The following formula describes the amount of net energy for maintenance (NE<sub>m</sub>) of the piglets according to their metabolic weight on the day of weaning (NRC, 1998):

$$\text{NE}_m (\text{kJ} \cdot \text{day}^{-1}) = 326.4 \times \text{BW}_0^{0.75} \quad [1]$$

where  $NE_m$  is the net energy intake at maintenance level ( $\text{kJ} \cdot \text{day}^{-1}$ ) and  $BW_0$  is BW on day 0 (kg). The piglets were offered  $0.5 \times NE_m$  on day 0,  $1.0 \times NE_m$  on day 1,  $1.5 \times NE_m$  on day 2 and  $2.0 \times NE_m$  from day 3 onwards. Piglets were fed equal portions of feed 3 times per day from day 0 to 3 (at 1000, 1300 and 1600 h) and 2 times per day from day 4 onwards (at 1000 and 1600 h). Feed refusals were collected, weighed and dried during overnight stay at 100 °C. Actual daily dry matter intake (g) and net energy intake per kg of metabolic weight were calculated ( $\text{kJ} \cdot (\text{kg } BW_0^{0.75})^{-1}$ ).

Table 1 Ingredient composition of the experimental diets.

Dietary variable:	Glucose	Lactose	Starch
Constant components <sup>1</sup> (g)	800.0	800.0	800.0
Glucose <sup>2</sup> (g)	213.8	-	-
Lactose <sup>3</sup> (g)	-	200.0	-
Native wheat starch <sup>4</sup> (g)	-	-	203.9
Total (g)	1013.8	1000.0	1003.9

<sup>1</sup> The constant components consisted of (g per 800 g feed): wheat, 464.8; wheat bran, 120.0; wheat gluten, 24.8; soybean concentrate, 80.0; potato protein, 24.0; fishmeal, 40.0; soya oil, 20.1; limestone, 9.1; mono calcium phosphate, 3.3; fytase liquid, 0.08 ; choline chloride (purity 50 %), 0.64 ; salt, 5.4; methionine, 1.2; lysine, 3.3; thryptophan, 0.64; threonine, 1.2; vitamin and trace element premix, 1.6.

The vitamin and trace element inclusion supplied (per 800 g constant components): vitamin A, 8000 IE; vitamin D3, 1600 IE; vitamin E, 52000 IE; vitamin K3, 1.6 mg; vitamin B1, 0.8 mg; vitamin B2, 2.4 mg; panthotenic acid, 8.0 mg; niacin, 16.0 mg; biotin, 24.0 µg; vitamin B12, 16.0 µg; folic acid, 0.16 mg; vitamin B6, 3.2 mg; Fe, 128.0 mg; Cu, 128.0 mg; Zn, 80.0 mg; Mn, 24.0 mg; I, 8.0 mg; Se, 0.16 mg; antioxidants (E130, E320, E321), 48.0 mg

<sup>2</sup> C-Dex (Cerestar, Sas van Gent, The Netherlands); dry matter content, 91.4 %; dextrose, 92.29 %.

<sup>3</sup> Lactopure (Borculo Domo Ingredients, Zwolle, The Netherlands); dry matter content, 99.9 %; lactose, 94.2 %

<sup>4</sup> Cerestar PT 20002 (Cerestar, Sas van Gent, The Netherlands); dry matter content, 88.3 %; starch, 86.50 %.

## Growth performance and faeces consistency

Piglets were weighed on days -1, 0, 3 and 10 postweaning. Average daily gain (ADG) was calculated for the periods -1 to 0, 0 to 3 and 3 to 10 days. Faecal consistency was monitored twice a day and quantified using a score on a scale from 0 to 3 with 0 = normally shaped faeces, 1 = shapeless (loose) faeces, 2 = thick, liquid (soft) faeces, and 3 = thin, liquid faeces (watery diarrhoea). Scoring was done by experienced care takers who were blinded to treatment modality.

Table 2 Calculated nutrient composition of experimental diets <sup>1, 2</sup>

	Glucose	Lactose	Starch
Dry matter (g · kg <sup>-1</sup> )	894	910	887
Crude protein (g · kg <sup>-1</sup> )	194	197	196
Fat (g · kg <sup>-1</sup> )	36	37	36
Crude fibre (g · kg <sup>-1</sup> )	24	25	25
Ash (g · kg <sup>-1</sup> )	42	42	43
Total carbohydrates (g · kg <sup>-1</sup> ) <sup>3</sup>	598	609	587
Total Sugars	215	20	20
Lactose	0	188	0
Starch	311	315	490
NE (MJ · kg <sup>-1</sup> ) <sup>4</sup>	10.21	10.07	10.17

<sup>1</sup> Minerals and vitamins (per kg of feed): Ca, 5.9 g; P, 5.2 g; Na, 2.9 g; K, 6.3 g; Cl, 5.7 g; Cu, 131.2 mg; vitamin E, 51.7 IE.

<sup>2</sup> Apparently ileal digestible amino acids (per kg of feed): lysine, 10.7 g; methionine, 4.2; methionine + cystine, 6.7; threonine, 6.8 g; tryptophan, 2.5 g

<sup>3</sup> Calculated as: dry matter - (crude protein + fat + crude fibre + ash)

<sup>4</sup> Calculated with the use of the Dutch feed tables (CVB, 2000)

## Sampling

On days 0, 3 and 10 postweaning, piglets to be killed were weighed and euthanised with a 5-ml intra-cardiac injection of Euthestate<sup>®</sup> (pentobarbital sodium 200 mg · ml<sup>-1</sup>; Ceva Sante Animale B.V. Maasluis, The Netherlands). A midline laparotomy was performed. From the vena cava caudalis a blood sample was taken into a 9-ml tube with heparin (Lithium-Heparin-Monovette<sup>®</sup>, Sarstedt, Nümbrecht, Germany). After mixing carefully, the tubes were immediately put on ice and then centrifuged (10 min, 2500 rpm). Plasma was stored in the freezer (-20 °C) until analysis. A jejunal segment was taken at 0.5 m distal to the ligament of Treitz (proximal jejunum) and a second segment at 3.5 m distal of this ligament (mid jejunum). Surface area and weight of the mucosal layer was determined for a 10-cm sample from the proximal jejunum. Liver and pancreas were removed and weighed. The small intestine was divided into 3 parts: the first 2 m distal of stomach (proximal small intestine), the last 2 m proximal of the caecal valve (distal small intestine) and the middle, remaining part (mid small intestine). Chyme was collected and mixed, pH was measured and empty weight was determined of the stomach, proximal, mid and distal small intestine, caecum and large intestine. Empty body weight (EBW) was determined as animal weight without the gastrointestinal tract, liver and spleen.

For histological analysis, tissue samples (2 cm) of the proximal and mid jejunum were cut open longitudinally at the anti-mesenteric attachment, fixed onto dental wax with the villi on the upper side and put in 0.1 mol · l<sup>-1</sup> phosphate buffered formalin solution (40 ml formalin · l<sup>-1</sup>). 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 5 villi and crypts.

Total antibody titers to haptoglobin (Hp) in plasma were determined by ELISA (Biofocus GmbH, Recklinghausen, Germany) as described (His, 2001; His et al., 2001). Briefly, biotinylated porcine Hp was used as tracer and was incubated together with either Hp standard or plasma in microtiter plates coated with sheep anti-rabbit crystalline-fragment immunoglobulins. After adding the specific rabbit antiserum, plates were incubated for 1 h, washed and evaluated via a streptavidin peroxidase system with tetramethylbenzidine as substrate. Haptoglobin levels are expressed as  $\text{mg} \cdot (\text{ml plasma})^{-1}$ .

### Statistical analysis

A GLM procedure (SAS version 6.12, SAS Institute, Cary, NC) was used to estimate the least-square means (LSMeans) of the different variables. The effect of diet composition was evaluated within the  $3 \times 2$  experimental design with 3 experimental diets and days 3 and 10 as dissection days:

$$y_{ijk} = \mu + D_i + C_j + (D \times C)_{ij} + e_{ijk} \quad [2]$$

where  $y_{ijk}$  = dependent variable;  $\mu$  = overall mean;  $D_i$  = fixed effect of day of dissection ( $i = 1, 2$ );  $C_j$  = fixed effect of diet composition ( $j = 1, 2, 3$ );  $(D \times C)_{ij}$  = interaction between day of dissection and diet composition;  $e_{ijk}$  = error term.

The effect of day postweaning was evaluated across diets with day postweaning as the only independent variable:

$$y_{ij} = \mu + D_i + e_{ij} \quad [3]$$

where  $y_{ij}$  = dependent variable;  $\mu$  = overall mean;  $D_i$  = fixed effect of day of dissection ( $i = 1, 2, 3$ );  $e_{ij}$  = error term.

To compare the effect of a specific diet on day 3 or 10 with the day of weaning, the 7 groups were regarded as different treatments:

$$y_{ij} = \mu + T_i + e_{ij} \quad [4]$$

where  $y_{ij}$  = dependent variable;  $\mu$  = overall mean;  $T_i$  = fixed effect of treatment ( $i = 1, 2, \dots, 7$ ). The experimental groups differing in diet and day of dissection were regarded as different treatments;  $e_{ij}$  = error term. Only pre-planned comparisons were made, i.e. between diets within either day 3 or 10 and between days (day 0, 3 and 10) for the same diet.

The repeated measures option of the GLM procedure was used to analyse differences between pH in the different parts of the gastrointestinal tract. The incidence of faeces inconsistency

was not distributed normally. Therefore, the effect of dietary treatment on faeces inconsistency was analysed by  $\chi^2$  analysis of the Cadmod procedure. Pearson correlation analysis was performed to evaluate selected correlations. For all data combined, feed intake as a function of days postweaning was plotted in the form of a Box-Whisker graph and as means and SD. Significance was assigned at  $P < 0.05$ ; tendencies were assigned at  $P < 0.10$ .

## RESULTS

None of the piglets showed signs of illness. Energy intake and average daily gain (ADG) did not differ between dietary treatments. Daily feed intake ( $\text{kJ} \cdot (\text{kg BW}^{0.75})^{-1} \cdot \text{pig}^{-1}$ ) across dietary treatments is shown in Figure 1 as a Box-Whisker plot. There was substantial inter-individual variation in feed intake. On average, the energy intake required for maintenance was reached on day 4 postweaning. For all piglets combined, average daily dry matter intake during the first week was  $122 \text{ g} \cdot \text{day}^{-1} \cdot \text{pig}^{-1}$  (SD: 49.2) and during the entire 10-day period it was  $163 \text{ g} \cdot \text{day}^{-1} \cdot \text{pig}^{-1}$  (SD: 39.9). ADG was 281 g (SD: 145.2) from days -1 to 0 ( $n=42$ ), -40 g (SD: 96.2) from days 0 to 3 ( $n=36$ ), 202 g (SD: 58.9) from days 3 to 10 and 128 g (SD: 61.6) from days 0 to 10. Feed intake and growth were positively correlated ( $P < 0.01$ ).

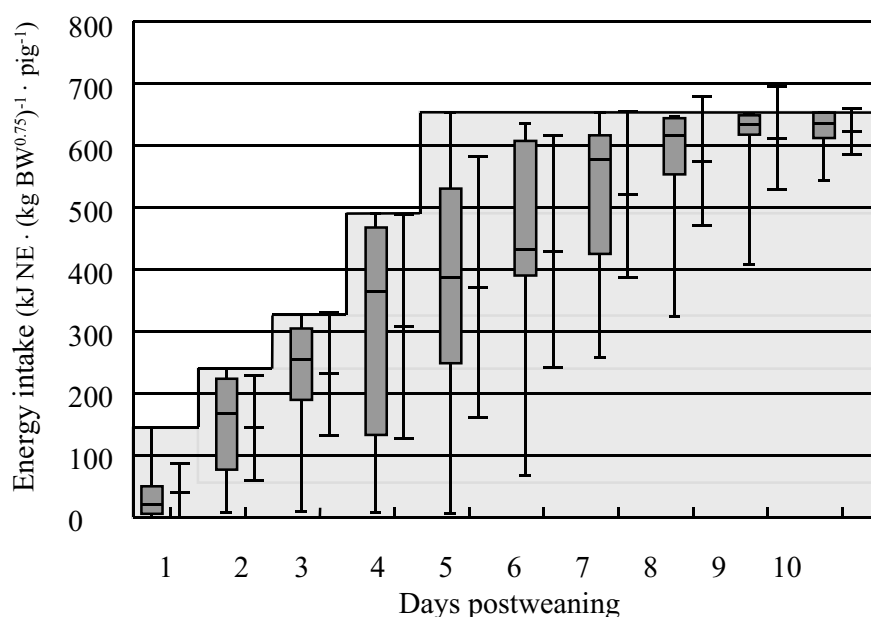


Figure 1 Box-Whisker graph of feed intake by piglets, expressed as g per kg metabolic weight on the day of weaning, for the period of first 10 days after weaning (days 1-3,  $n=36$ ; days 4-10,  $n=18$ ). The graph shows the means  $\pm$  SD and the median within the boxes and the range as adjacent bars. The upper and lower closures of the boxes indicate the quartiles. The continuous line represents the amount of feed offered. The amount of feed needed for maintenance per pig is  $326.4 \text{ kJ NE} \cdot (\text{kg BW}^{0.75})^{-1} \cdot \text{day}^{-1}$ .

The percentage of days that a piglet had non-consistent faeces (score either 1, 2, or 3) did not differ between dietary treatments and for all piglets combined was 8 % (SD: 18.5) from days 0 to 3 and 16 % (SD: 15.8) from days 0 to 10.

Dietary treatment did neither affect the EBW nor organ weights, small intestinal length or mucosal weight (data not shown). For all piglets combined, Table 3 shows the effect of postweaning time on organ weights and various small intestinal characteristics. Organ weights are expressed per kg of EBW. When compared with day 0, EBW was decreased on day 3, but the pre-weaning level was reached again on day 10 postweaning ( $P < 0.01$ ). Specific weight of the stomach increased from days 0 to 3 and then to day 10 ( $P < 0.01$ ). Specific weights of liver, pancreas, small intestine, caecum and large intestine were higher on day 10 than on days 0 and 3 ( $P < 0.01$ ). Length of the small intestine and the weight of the small intestinal mucosa were also higher on day 10 when compared to days 0 and 3. However, the small intestinal or mucosal weight expressed per cm was not affected by postweaning day.

Table 3 Pooled data for relative organ weights and small intestinal morphology of piglets in relation to postweaning days.

Day post weaning:	0	3	10	RSD <sup>1</sup>	P-value <sup>2</sup>
n:	6	18	18		
Empty body weight (EBW) (kg)	7.3 <sup>a</sup>	6.9 <sup>b</sup>	7.5 <sup>a</sup>	7.95	**
Liver (g · (kg EBW) <sup>-1</sup> )	29.0 <sup>b</sup>	28.4 <sup>b</sup>	32.8 <sup>a</sup>	3.15	**
Pancreas (g · (kg EBW) <sup>-1</sup> )	1.5 <sup>b</sup>	1.7 <sup>b</sup>	2.8 <sup>a</sup>	0.50	**
Stomach (g · (kg EBW) <sup>-1</sup> )	5.0 <sup>c</sup>	6.4 <sup>b</sup>	10.5 <sup>a</sup>	1.27	
Small intestine (g · (kg EBW) <sup>-1</sup> )	31.2 <sup>b</sup>	31.2 <sup>b</sup>	47.2 <sup>a</sup>	6.25	**
length (cm)	736 <sup>b</sup>	759 <sup>b</sup>	847 <sup>a</sup>	59.95	**
weight / length (g · cm <sup>-1</sup> )	0.3	0.3	0.4	0.04	ns
mucosa (g)	1.4 <sup>b</sup>	1.5 <sup>b</sup>	1.8 <sup>a</sup>	0.34	**
mucosa weight / surface (g · cm <sup>-1</sup> )	0.05	0.06	0.06	0.016	ns
villus length, proximal (µm)	394 <sup>a</sup>	275 <sup>c</sup>	324 <sup>b</sup>	77.5	**
villus length, mid (µm)	337 <sup>a</sup>	229 <sup>b</sup>	303 <sup>a</sup>	76.7	**
crypt depth, proximal (µm)	166 <sup>a</sup>	183 <sup>a</sup>	289 <sup>b</sup>	34.1	**
crypt depth, mid (µm)	157 <sup>a</sup>	181 <sup>a</sup>	254 <sup>b</sup>	36.8	**
Caecum (g · (kg EBW) <sup>-1</sup> )	1.5 <sup>b</sup>	1.7 <sup>b</sup>	2.0 <sup>a</sup>	0.41	**
Large intestine (g · (kg EBW) <sup>-1</sup> )	11.5 <sup>b</sup>	12.8 <sup>b</sup>	19.1 <sup>a</sup>	3.05	**

<sup>1</sup> RSD, residual standard deviation

<sup>2</sup> LSMeans with different superscript letters in a row are significantly different ( $P < 0.01$ )

<sup>3</sup> P-value of day postweaning: \*\*,  $P < 0.01$ ; ns, not significant.

Villus length and crypt depth were not differently affected by dietary carbohydrate source (Figure 2). Irrespective of the type of diet, villus height decreased from day 0 to 3 and increased again between day 3 and 10 ( $P < 0.01$ ). Between days 3 and 10 postweaning, the group-mean increase in villus height for both the proximal and mid small intestine was greater in piglets fed the diet with lactose than in those fed the other diets. In general, villus height on

day 10 was intermediate between that on day 0 and 3. Crypt depth was deeper on day 10 compared to that on days 0 and 3 ( $P < 0.01$ ), both at the proximal and mid jejunum. Pearson correlation analysis indicated that the values of the proximal and mid small intestine for either villus height or crypt depth were positively correlated ( $P < 0.01$ ). Villus height was neither correlated with crypt depth, nor with feed intake or growth. However, crypt depth at the mid jejunum ( $P < 0.01$ ), but not at the proximal jejunum, was positively correlated with both feed intake and growth between days 3 and 10 and between days 0 and 10. Crypt depths at the proximal and mid jejunum were positively correlated with the specific weight of the proximal, mid and distal small intestine ( $P < 0.01$ ). Villus height was not correlated with the specific weights of the small intestine.

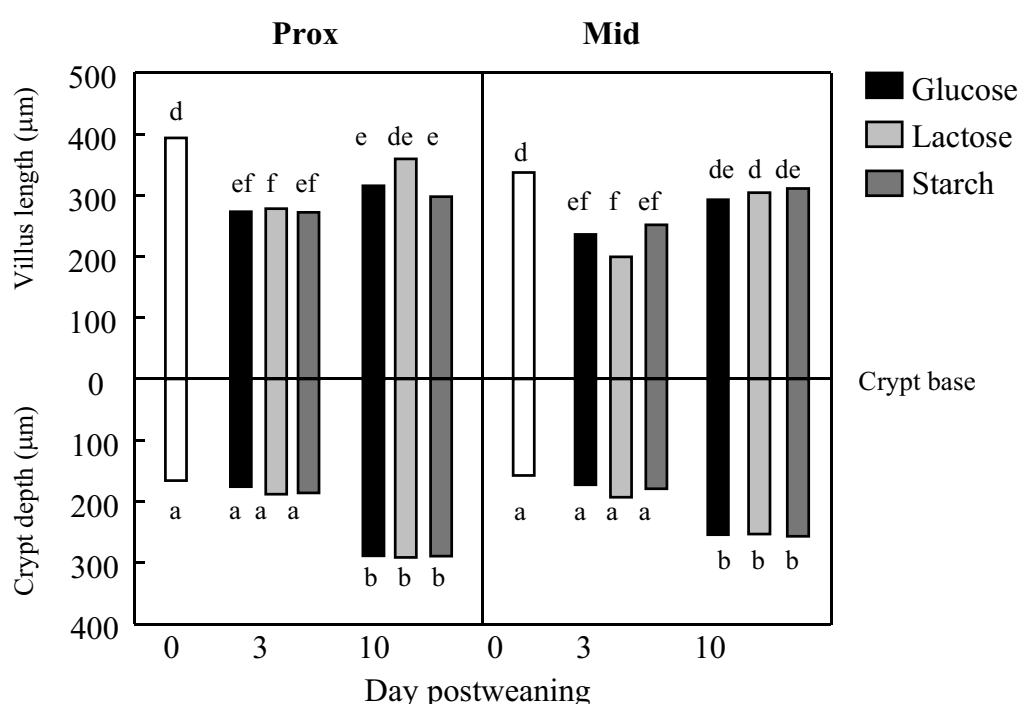


Figure 2 Villus height and crypt depth at the proximal (prox) and mid small intestine of piglets fed either the glucose, lactose or starch diet. Data are given for 0, 3 and 10 days postweaning. Values are LSMeans; values for residual standard deviation (RSD) are: prox villus height, 79.7; mid villus height, 79.3; prox crypt depth, 35.8; mid crypt depth, 38.3 ( $n = 6$ ). Per site for both villus length and crypt depth, statistical comparisons were made between diets within days and between days for the same diet. There were no effects of the type of dietary carbohydrate within day 3 or 10. Postweaning day had significant effects: abc,  $P < 0.05$ ; def,  $0.10 > P > 0.05$ .

Table 4 Effect of diet composition and day postweaning on pH of the chyme at different sites in the gastrointestinal tract <sup>1</sup>.

n:	Dietary variable				Day postweaning				
	Glucose	Lactose	Starch	RSD	0	3	10	RSD	P-value <sup>3</sup>
	12	12	12		6	18	18		
Stomach	4.5	4.2	4.4	0.76	4.7	4.2	4.6	0.80	ns
Proximal SI	5.7	6.0	5.8	0.44	5.9	6.0	5.7	0.42	ns
Mid SI <sup>2</sup>	6.4	6.4	6.3	0.33	6.5 <sup>ab</sup>	6.5 <sup>a</sup>	6.3 <sup>b</sup>	0.32	t
Distal SI	6.7	6.8	6.8	0.57	7.2 <sup>a</sup>	7.0 <sup>a</sup>	6.5 <sup>b</sup>	0.51	**
Caecum	5.9	5.9	6.2	0.49	6.6 <sup>a</sup>	6.2 <sup>b</sup>	5.8 <sup>c</sup>	0.46	**
Large intestine	6.4	6.5	6.6	0.42	6.8 <sup>a</sup>	6.7 <sup>a</sup>	6.3 <sup>b</sup>	0.42	**

<sup>1</sup> Abbreviations used: RSD, residual standard deviation; SI, small intestine

<sup>2</sup> LSMeans with different superscript letters in a row are significantly different.

<sup>3</sup> P-value of day postweaning: \*\*,  $P < 0.01$ ; t,  $P < 0.10$ ; ns, not significant. Diet composition did not significantly affect pH of chyme at different sites. Repeated measures procedure of GLM indicated an effect of site ( $P < 0.01$ ) and an interaction between site and day of dissection ( $P < 0.01$ ).

Table 4 shows the pH of the chyme at different sites of the gastrointestinal tract. Diet composition did not affect the pH. In the stomach and proximal small intestine, pH of the contents was not affected by day postweaning. However, further along the gastrointestinal tract pH was decreased on day 10 compared with days 0 and 3. The pH was not correlated to feed intake, growth and villus length. For the mid and distal small intestine, caecum and large intestine, the pH of the contents was negatively correlated with crypt depth and specific weight of small intestine.

Haptoglobin levels in plasma were not affected by diet composition (Figure 3). On day 10 postweaning, haptoglobin levels were increased ( $P < 0.05$ ) when compared with those on either day 0 or 3. Haptoglobin levels were not correlated with feed intake, growth and villus height ( $P > 0.10$ ), but were positively correlated with crypt depth ( $P < 0.05$ ).

## DISCUSSION

It has been shown that feed intake is positively correlated to villus height (Pluske et al., 1996; Kelly et al., 1991; Verdonk et al., 2001a). To study the effect of carbohydrate source on small intestinal architecture independently of feed intake, the piglets were offered a pre-determined maximum amount of feed. Consequently, feed intake did not differ between dietary treatments. Likewise, ADG and feed efficiency were not affected by carbohydrate source in the diet. Earlier growth performance trials (Jin et al., 1998; Lee et al., 2000; Mavromichalis et al. 2001) with piglets weaned at 3 weeks of age and fed ad libitum showed that dextrin, molasses and mono- and disaccharides were utilised equally efficiently. However, these



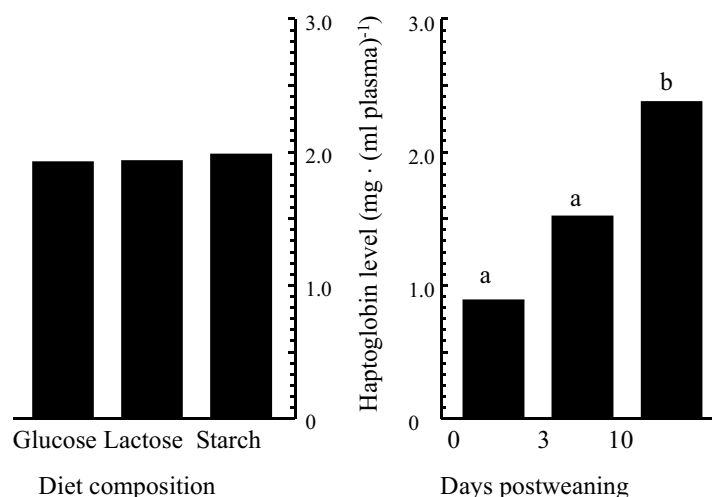


Figure 3 Haptoglobin levels on days 0, 3 and 10 postweaning in plasma of piglets fed either the glucose, lactose or starch diet. Left part of graph: residual standard deviation (RSD) = 1.2;  $n=12$  for days 3 and 10 postweaning combined. Right part of graph: RSD = 1.1;  $n = 6$  on day 0,  $n = 18$  on days 3 and 10. Values are LSMeans. Values with different letters differ significantly ( $P < 0.05$ ).

carbohydrates induced higher feed intake and better growth performance than did starch. Dry matter digestibility was either not affected by carbohydrate source (Lee et al., 2000; Mavromichalis et al. 2001) nor was decreased by the use of starch (Jin et al., 1998). Veum and Mateo (1986) found similar growth performance for piglets weaned at 1 day of age and fed a liquid diet containing either 53 % glucose, lactose, sucrose or cornstarch.

The observed decrease in villus length after weaning followed by partial recovery within 10 days postweaning is in agreement with results of others (Nabuurs et al., 1993; Van Beers-Schreurs, 1996; Van Dijk, 2001; Verdonk et al. 2001b). It was hypothesized that lactose in the weaner diet would preserve villus length. However, the results show that villus length was not affected by carbohydrate source. Villus length at the proximal small intestine of piglets receiving the diet with lactose seemed to recover somewhat faster than that of piglets receiving the diets containing either glucose or starch. However, the apparent lactose effect was mainly due to 1 piglet with a villus length of 535  $\mu\text{m}$  on day 10 postweaning, while villus height of the other 5 piglets of that experimental group ranged between 236 and 365  $\mu\text{m}$ . Therefore, it may be concluded that lactose has no specific effect on villus architecture.

Easily fermentable dietary substrates like lactose and sucrose, but not starch, are thought to induce a favourable pH for digestion (Ewing and Cole, 1994). However, pH in the contents of the gastrointestinal tract at the various sites was not affected by the carbohydrate source in the diet, which agrees with work of Ly (1992). The optimal pH for pepsin action is 2 and for

trypsin and chymotrypsin it is 8 (Whitaker, 1994). However, most piglets did not reach these pH values in the stomach and proximal small intestine, respectively. It seems that the pH values did not allow optimum digestion. Values found in the current study agree with data of Makkink (1993). However, the pH values were measured in the total, mixed chyme of each segment. Kamphues (1987) reported that the pH of digesta close to the gastric wall or at the pyloric site is higher than in other parts of the stomach. Therefore, the above-mentioned conclusion as to discrepancies between measured and optimum values requires caution.

The pH in the caecum and large intestine decreased with time postweaning. Van Beers-Schreurs (1996) showed that volatile fatty acid production in the large intestine, including that of butyric acid, increased during the first week postweaning. With increased production of volatile fatty acids, pH decreases. In ileally fistulated rats, the infusion of propionic, butyric and acetic acid at physiological doses into the fistula was found to increase crypt cell production rate of both small and large intestine in a dose-dependent manner (Sakata, 1987). Thus, a decrease in pH might be due to volatile fatty acids which also increase proliferation of crypt cells and thereby increase mucosal weight. This reasoning may explain the observed negative correlation between pH and either crypt depth and mucosal weight.

The acute-phase response to infection, inflammation or trauma is mediated by a combination of cytokines and is associated with increased concentrations of plasma proteins produced by the liver, i.e. the acute-phase proteins (Gruys et al., 1999). Haptoglobin is a major acute-phase protein in the pig (Eckersall et al., 1996). Haptoglobin levels in the blood were not affected by diet composition. Likewise, Hiss (2001) found no effect of diet composition on haptoglobin levels after a lipopolysaccharide injection; different levels of yeast beta-glucans did not ameliorate the inflammatory response. It has been suggested that the level of haptoglobin in the blood might be used as a tool to evaluate the general health status and consequently the growth performance on a farm (Knura et al., 2000). We found that the weaning transition increased the level of haptoglobin in the blood.

In conclusion, the present experiment rejects our hypothesis that dietary lactose, when compared to glucose and starch, is beneficial for the weaning-induced compromise in small intestinal integrity. It should be noted that the hypothesis was tested under conditions of unaltered feed intake and that the piglets used were kept under low infection pressure.

## **IMPLICATIONS**

The formulation of diets for weanling piglets aims at reducing the weaning-induced decrease in gut integrity. There was suggestive evidence that lactose could have a positive effect on villus height and crypt depth. However, this study shows that lactose, glucose and starch had no differential effect on villus architecture. It should be noted that a specific feeding regimen

was used so that the experimental diets would not induce differences in feed intake. For all piglets combined, feed intake and growth were positively correlated. This study corroborates earlier work in that feed intake rather than feed composition determines postweaning growth performance and mucosal integrity in piglets.

## LITERATURE CITED

- Bruininx, E. M. A. M. 2002. Individually measured feed intake characteristics in group-housed weanling pigs. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands
- Centraal Veevoederbureau (CVB). 2000. Chemical composition, digestibility and feeding value of feedstuffs. Veevoedertabel, Centraal Veevoederbureau, Lelystad, The Netherlands (in Dutch).
- Eckersall, P. D., P. K. Saini, and C. McComb. 1996. The acute phase response of acid soluble glycoprotein,  $\alpha_1$ -acid glucoprotein, ceruloplasmin, haptoglobin and C-reactive protein, in the pig. *Vet. Immunol. Immunop.* 51: 377-385.
- Ewing, W. N., and D. J. A. Cole. 1994. Page 220 in *The Living Gut. Context*, Dungannon, Ireland.
- Gruys, E., M. J. M. Toussaint, W. J. M. Landman, M. Tivapasi, R. Chamanza, and L. van Veen. 1999. Infection, inflammation and stress inhibit growth. Mechanisms and non-specific assessment of the processes by acute phase proteins. Pages 72-84 in *Production Diseases in Farm Animals*. 10th Int. Conf. Th. Wensing, ed. Wageningen Pers, Wageningen, The Netherlands.
- Hiss, S. 2001. Entwicklung und Validierung von enzyimmunologischen Messverfahren zur Bestimmung von Haptoglobin bei verschiedenen Haustierspezies und erste Anwendungen in Pilotstudien. Ph.D. Diss., Hannover, Germany.
- Hiss, S., M. Hennies, S. Gymnich, B. Petersen, and H. Sauerwein. 2001. Haptoglobin in pigs: development and validation of an enzyme immunoassay for various body fluids and establishment of physiological reference levels. Page 61 in *Proc. 11th Int. Conf. on Production Diseases in Farm Animals*. Frederiksberg, Denmark.
- Jin, C. F., J. H. Kim, H. K. Moon, W. T. Cho, Y. K. Han, and I. K. Han. 1998. Effects of various carbohydrate sources on the growth performance and nutrient utilization in pigs weaned at 21 days of age. *Asian Aus. J. Anim. Sci.* 11: 285-292.
- Kamphues, J. 1987. Untersuchungen zu Verdauungsvorgängen bei Absetzferkeln in Abhängigkeit von Futtermenge und -zubereitung sowie van Futterzusätzen. Ph.D. Diss., Tierärztliche Hochschule Hannover, Hannover, Germany.
- Kelly, D., J. A. Smyth, and K. J. McCracken. 1991. Digestive development of the early weaned pig; effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. *Brit. J. Nutr.* 65: 181-188.
- Knura, S., C. Lipperheide, B. Petersen, and M. Wendt. 2000. Impact of hygienic environment on haptoglobin concentration in pigs. Pages 537-541 in *Proc. 10th Int. Cong. of Animal Hygiene*. Maastricht, The Netherlands.
- Lee, C. H., Y. K. Han, K. U. Lee, J. D. Kim, W. T. Cho, T. G. Ko, and I. K. Han. 2000. Study on the nutritive value of dextrin as a carbohydrate source for pigs weaned at 21 days of age. *J. Anim. Feed Sci.* 9: 647-663.
- Leibbrandt, V. D., R. C. Ewan, V. C. Speer, and D. R. Zimmerman. 1975. Effect of weaning and age at weaning on baby pig performance. *J. Anim. Sci.* 40: 1077-1080.

- Ly, J. 1992. Studies of the digestibility of pigs fed dietary sucrose, fructose or glucose. *Arch. Anim. Nutr.* 42: 1-9.
- Makkink, C. A. 1993. Of piglets, dietary proteins and pancreatic proteases. Ph.D. Diss., Wageningen Univ., Wageningen, The Netherlands.
- Mavromichalis, I., J. D. Hancock, R. H. Hines, B. W. Senne, and H. Cao. 2001. Lactose, sucrose, and molasses in simple and complex diets for nursery pigs. *Anim. Feed Sci. Techn.* 93: 127-135.
- Nabuurs, M. J. A., A. Hoogendoorn, E. J. van der Molen, and A. L. M. van Osta. 1993. Villus height and crypt dept in weaned and unweaned pigs, reared under various circumstances in the Netherlands. *Res. Vet. Sci.* 55: 78-84.
- National Research Council (NRC). 1998. *Nutrient Requirements of Swine*. 10th ed. National Academy Press, Washington DC, USA.
- Okai, D. B., F. X. Aherne, and R. T. Hardin. 1976. Effects of creep and starter composition on feed intake and performance of young pigs. *Can. J. Anim. Sci.* 56: 573-586
- Pluske, J. R., I. H. Williams and F. X. Aherne. 1996. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. *Anim. Sci.* 62: 145-158.
- Sakata, T. 1987. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. *Brit. J. Nutr.* 58: 95-103.
- Van Beers-Schreurs, H. 1996. The changes in the function of the large intestine of weaned pigs. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands
- Van Dijk, A. 2001. Spray-dried animal plasma in the diet of weanling piglets: influence on growth performance and underlying mechanisms. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- Verdonk, J. M. A. J., M. A. M. Spreeuwenberg, G. C. M. Bakker and M. W. A. Verstegen. 2001a. Nutrient intake level affects histology and permeability of the small intestine in newly weaned piglets. Page 332-334 in *Digestive Physiology of Pigs*. J. E. Lindberg, and B. Ogle eds. Cabi Publishing, Wallingford, UK.
- Verdonk, J. M. A. J., M. A. M. Spreeuwenberg, G. C. M. Bakker, and M. W. A. Verstegen. 2001b. Effect of protein source and feed intake level on the small intestine in newly weaned piglets. Page 347-349 in *Digestive Physiology of Pigs*. J. E. Lindberg, and B. Ogle eds. Cabi Publishing, Wallingford, UK.
- Veum, T. L., and J. P. Mateo. 1986. A review of the utilization of lactose, glucose, sucrose and cornstarch by neonatal piglets reared artificially. Page 735-743 in *Swine in Biomedical Research*. M. E. Tumbleson, ed. Plenum Press, New York, USA.
- Weurding, R. E., A. Veldman, W. A. G. Veen, P.J. van der Aar, and M. W. A. Verstegen. 2001. In vitro starch digestion correlates well with rate and extent of starch digestion in broiler chickens. *J. Nutr.* 131: 2336-2342.
- Whitaker, J. R. 1994. Pages 273-300 in *Principles of Enzymology for the Food Sciences*. 2nd ed. Marcel Dekker inc., New York, USA.

---

## CHAPTER 7

---

### **Interrelationship between Gut Morphology and Faeces Consistency in Newly Weaned Piglets**

M. A. M. Spreeuwenberg <sup>1</sup>, J. M. A. J. Verdonk <sup>2</sup>, A. C. Beynen <sup>3</sup>, and M. W. A. Verstegen <sup>4</sup>.

<sup>1</sup> Swine Research Centre, Nutreco, Boxmeer, The Netherlands, <sup>2</sup> ID TNO Animal Nutrition, Lelystad, The Netherlands, <sup>3</sup> Department of Nutrition, Faculty of Veterinary Medicine, University of Utrecht, Utrecht, The Netherlands, <sup>4</sup> Division of Animal Nutrition, Department of Animal Sciences, University of Wageningen, Wageningen, The Netherlands

## 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 days (SD: 1.4) of age and weighed 8.4 kg (SD: 0.70). On the day of weaning (day 0), dissection was performed on one group of eight piglets. The remaining piglets were given access to a maximum amount of dietary energy in the form of diets with different protein sources. On days 3 and 7 postweaning pigs were weighed and euthanized. Diet composition did not effect small intestinal integrity and the data were pooled for further analysis. The weight of the stomach, large intestine and pancreas increased with time postweaning ( $P < 0.001$ ). Small intestinal weight decreased from day 0 to 3 and was increased again on day 7, exceeding the preweaning value ( $P < 0.001$ ). Isomaltase-sucrase and aminopeptidase activity were decreased on days 3 and 7 when compared to day 0. Villus height was decreased after weaning followed by an increase on day 7 postweaning at the proximal small intestine which was accompanied by a further decrease at the mid small intestine ( $P < 0.001$ ). Crypt depth was increased after weaning ( $P < 0.001$ ). Faeces were scored twice a day on a scale from 0 to 3 with increasing faeces inconsistency. The average percentage of days during which piglets had inconsistent faeces was 26 %. During the first week postweaning, 73 % of the piglets showed a faeces score of 2 during at least 1 day. Villus height was positively correlated to feed intake level, brush-border enzyme activity and dry matter content of the chyme. Villus height was negatively correlated to the degree of faeces inconsistency. Crypt depth was positively associated with the weight of various parts of the gastrointestinal tract. This study supports the concept that feed 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.

## INTRODUCTION

Abrupt weaning of piglets around four 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 feed intake after weaning is highly variable between piglets and the latency time to the first solid-feed intake can take up to 3 days (Bruininx et al., 2001). The low feed 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 small

intestinal morphology and function may impair the ability to digest and absorb nutrients and to predispose the weanling piglet to development of malabsorption and diarrhoea. Indeed, in practice, postweaning 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 small intestinal integrity or growth performance on the other hand. In the experiment described here, the weanling piglets had inconsistent faeces during on average one quarter of the 7 postweaning days. On day 7 after weaning, 75 % of the piglets produced inconsistent faeces. Due to the relative high incidence of inconsistent faeces and considerable variation between piglets, the data were considered suitable to assess the associations between faeces consistency, small intestinal morphology, enzyme activity, feed intake and growth during the first week postweaning. It was anticipated that the information thus obtained would provide insight into the determinants of postweaning diarrhoea and provide clues as to the prevention of diarrhoea given the current management of weanling piglets.

## **MATERIALS 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 days (SD: 1.4) of age; they had an average body weight of 8.4 kg (SD: 0.70). At weaning (day 0), piglets were removed from the sow, weighed and housed individually in pens (77×76 cm<sup>2</sup>). Each pen was equipped with a through and a water nipple. Environmental temperature was maintained at 27 °C. Lights were on from 0600 to 2200 h. Creep feed 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).

### **Feeds, 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 4 piglets each or to 13 groups (batch 2) consisting of 4 piglets each for 12 groups and having 8 piglets for 1 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 8 piglets in batch 2. The 12 groups with 4 piglets each per batch were dissected on day 3 or 7 postweaning and received one of 6 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, soybean meal and potato protein. One diet contained extra glutamine and another arginine. The composition of the diets have been described elsewhere (Chapter 5), except for the arginine-rich diet. The ingredient composition of the arginine-rich diet was as follows ( $\text{g} \cdot \text{kg feed}^{-1}$ ): corn, 250.0; pre-gelatinised corn starch, 181.9; soybean meal, 160.0; barley, 150.0; wheat gluten, 100.0; lactose, 50.0; soya oil, 44.0; mono calcium phosphate, 16.3; limestone, 10.8; salt, 9.4; choline chloride (purity 50 %), 2.8; titanium oxide, 5.0; vitamin and trace element premix, 2.0; lysine, 5.6; threonine, 2.2, tryptophan, 0.1; arginine, 10.0. Calculated net energy (NE) content of all diets was  $10.7 \text{ MJ NE} \cdot (\text{kg feed})^{-1}$ . The calculated and analysed nutrient compositions agreed well. Diet composition after weaning did not differentially affect small intestinal integrity as measured for 3 and 7 days postweaning (Chapter 5). Therefore, the data for the various diets were pooled and selected correlations calculated.

After weaning (days 0 to 7) the piglets were given access to a maximum of dietary energy. The following formula describes the amount of net energy requirement for maintenance ( $\text{NE}_m$ ) of the piglets according to their metabolic weight on the day of weaning (NRC, 1998):

$$\text{NE}_m (\text{kJ} \cdot \text{day}^{-1}) = 326.4 \times \text{BW}^{0.75} \quad [1]$$

where  $\text{NE}_m$  is the net energy requirement at maintenance level ( $\text{kJ} \cdot \text{day}^{-1}$ ) and  $\text{BW}_0$  is BW at day 0 (kg). The piglets received  $0.25 \times \text{NE}_m$  on day 0,  $0.5 \times \text{NE}_m$  on day 1,  $0.75 \times \text{NE}_m$  on day 2,  $1 \times \text{NE}_m$  on day 3,  $1.5 \times \text{NE}_m$  on day 4,  $2 \times \text{NE}_m$  on day 5,  $2.5 \times \text{NE}_m$  on days 6 and 7. Piglets were given equal portions of their allowance 4 times per day at 09.00, 11.30, 14.00 and 17.00 h. Feed refusals were collected, weighed and subtracted from the amount of feed offered to calculate actual daily feed intake which was expressed as  $\text{g} \cdot (\text{kg BW}_0^{0.75})^{-1}$ .

## Growth and faeces consistency

Piglets were weighed on days -1, 0, 3 and 7 postweaning. 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 care takers who were blinded to treatment modality.

## Sampling of small intestine

On days 0, 3 and 7 postweaning, piglets to be killed were weighed and euthanized with a 5-ml intra-cardiac injection of Euthestate<sup>®</sup> (pentobarbital sodium,  $200 \text{ mg} \cdot \text{ml}^{-1}$ ; Ceva Sante Animale B.V. Maasluis, The Netherlands). The piglets were killed between 0800 and 1600 h on days 3 and 7, the order being stratified according to the type of diet fed. At 30 min before killing on days 3 and 7, each piglet was given access to its feed. A midline 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 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 small intestinal tract was collected to determine its dry matter content. Empty body weight (EBW) was determined as animal weight without the gastrointestinal tract, liver and spleen.

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)<sup>-1</sup>) formalin (40 ml formalin · (l buffer)<sup>-1</sup>). 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 5 villi and crypts.

To measure the specific activity of brush-border-membrane associated activity of isomaltase-sucrase and aminopeptidase, a proximal small intestinal tissue sample (approximately 15 cm) was taken and rinsed with phosphate-buffered saline (PBS), pH 7.2 (0.01 M NaH<sub>2</sub>PO<sub>4</sub>, 0.01 M Na<sub>2</sub>HPO<sub>4</sub>, 0.9 % (w/v) NaCl). The mucosal layer was carefully scraped off from the muscle layer, quickly frozen in liquid nitrogen and stored at -80°C 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 0 °C to give a final concentration of 5 % (v/w). Subsequently, the homogenates were sonicated twice at 0 °C for 15 seconds, separated by an interval of 30 sec, 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 · ml<sup>-1</sup> 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/min), and the activity of aminopeptidase (EC 3.4.11.2) using L-alanine-p-nitroanilide (Marouze et al, 1973) as substrate (1 unit = 1 nmol substrate hydrolysed/min) and expressed as enzyme units/g protein.

## Statistical 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 postweaning and batch were the independent variables in the final model:

$$y_{ijk} = \mu + B_i + D_j + e_{ijk} \quad [2]$$

where  $y_{ijk}$  = dependent variable;  $\mu$  = 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 magnitude of eating and severity of diarrhoea was evaluated for the piglets dissected on day 7 with the following model:

$$y_{ijk} = \mu + E_i + F_j + (E \times F)_{ij} + e_{ijk} \quad [3]$$

where  $y_{ijk}$  = dependent variable;  $\mu$  = overall mean;  $E_i$  = fixed effect of eating ( $i=1, 2$ ), a piglet was regarded as eater if the average daily net energy intake for the 3-day period just before dissection (days 5-7) was above the energy level for maintenance (NEm);  $F_j$  = 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 5-7);  $(E \times F)_{ij}$  = interaction between eating and diarrhoea;  $e_{ijk}$  = error term.

A multivariate analysis (Simca-P version 3.01, Umetri AB & 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. Bilinear projections are made between the X and Y variables. The relation between the X and Y weight vectors (shown as w and c, respectively) in the first ( $w \cdot c[1]$ ) and second ( $w \cdot c[2]$ ) dimension were plotted. Variables with numerically large w-values are important for modelling Y. Variables with similar profiles of w-values provide common information (Eriksson et al., 1997). PLS analysis provides inside in which X variables contribute to predict the independent Y variables. A correlation matrix on the other hand shows only the explained variation between two variables. Three PLS analyses were performed. In total, 24 variables were taken into account: weight at weaning; feed intake per kg metabolic weight from days 1 to 3 (MFI<sub>13</sub>) and from days 3 to 7 (MFI<sub>37</sub>); organ weights, expressed as g per kg empty body weight (EBW), i.e. stomach, small intestine (SI), large intestine (LI); total weight of gastrointestinal tract (GIT); weight of small intestine per cm (SI g/cm); day of dissection i.e. day 0, 3 or 7; occurrence of inconsistent 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 analysis, respectively, villus height, crypt depth and enzyme activity were used as Y variable and the remaining variables as X variables.

## RESULTS

Piglets weighed on average 8.4 kg (SD: 0.70) at weaning ( $BW_0$ ). Daily feed intake ( $\text{g} \cdot (\text{kg } BW_0^{0.75})^{-1}$ ) increased from 1 (SD: 1.4,  $n = 96$ ) on day 1, to 7 (SD: 6.2,  $n = 96$ ) on day 2, 16 (SD: 8.3,  $n = 96$ ) on day 3, 24 (SD: 9.1,  $n = 48$ ) on day 4, 35 (SD: 12.7,  $n = 48$ ) on day 5, 41 (SD: 16.8,  $n = 48$ ) on day 6 and 40 (SD: 20.7,  $n = 48$ ) on day 7. Based on the energy content of the feeds ( $10.7 \text{ MJ} \cdot (\text{kg feed})^{-1}$ ) and assumed maintenance requirement (NRC, 1998), the feed intake to meet the net energy requirement for maintenance was estimated to be about  $31 \text{ g} \cdot (\text{kg } BW_0^{0.75})^{-1} \cdot \text{day}^{-1}$ . Of the piglets dissected on day 7, 31 % ate below their maintenance requirements during the 3-day period before dissection. Average daily gain ( $\text{g} \cdot \text{day}^{-1} \cdot \text{piglet}^{-1}$ ) was 280 (SD: 145.7) from days -1 to 0 ( $n=104$ ), - 39 (SD: 84.0) from days 0 to 3 ( $n = 96$ ) and 119 (SD: 109.4) from days 3 to 7. Feed intake and average daily gain within the periods of days 1 to 3 and days 3 to 7 were positively correlated ( $P < 0.001$ ) and explained 59 % ( $n = 96$ ) and 66 % ( $n = 48$ ) of the variation ( $R^2$ ), respectively. Average feed intake during days 1 to 3 explained 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 inconsistent faeces, expressed as % of days with a faecal consistency score of either 1, 2, or 3, was 10 % (SD: 24.0) for the piglets dissected on day 3 and 33 % (SD: 26.7) for the piglets dissected on day 7. Figure 1 shows the distribution of faeces scores per day. During the first week postweaning, 73 % of the piglets showed a faeces score of 2 during at least 1 day.

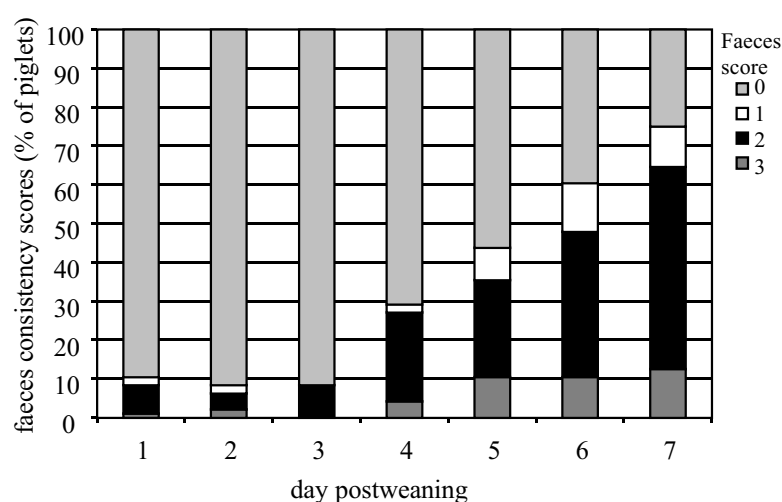


Figure 1 The distribution of faeces score of piglets per day during the first week postweaning (days 1-3,  $n = 96$ ; days 4-7,  $n = 48$ ).

The time course of body weight, organ weights and small intestinal characteristics in weaned the piglets is shown in Table 1. Body weight tended to be higher on day 7 postweaning than on day 3 ( $P < 0.10$ ). Organ weights are expressed per kg of EBW. Relative stomach, large

intestinal and pancreatic weight increased with time postweaning ( $P < 0.001$ ). The small intestinal weight was decreased on day 3 when compared to either day 0 or 7. On day 7, the weight of the small intestine per cm exceeded preweaning 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 preweaning 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 villus height on day 7 was lower than on day 3 ( $P < 0.001$ ). Crypt depth increased with time postweaning, both at the proximal and mid small intestine ( $P < 0.001$ ). Villus/crypt ratio decreased with time postweaning ( $P < 0.001$ ). Specific isomaltase-sucrase and aminopeptidase activity was lower on days 3 and 7 when compared to day 0 ( $P < 0.001$ ). Group mean activity of the two enzymes was lower on day 7 than on day 3.

Table 1 Body weights, organ weights and small intestinal characteristics in weaned piglets in relation to day postweaning <sup>1</sup>.

Day postweaning: n:	0 8	3 48	7 48	RSD <sup>2</sup>	P-value <sup>3</sup>
<u>General</u>					
Weight (kg)	8.5 <sup>ab</sup>	8.3 <sup>b</sup>	8.7 <sup>a</sup>	0.80	t
Empty body weight (EBW; kg)	7.6	7.3	7.3	0.72	ns
Organ weights (g · (kg EBW) <sup>-1</sup> )					
- Stomach	5.3 <sup>c</sup>	6.2 <sup>b</sup>	8.0 <sup>a</sup>	1.24	***
- Small intestine	32.2 <sup>b</sup>	27.0 <sup>c</sup>	39.0 <sup>a</sup>	5.00	***
- Large intestine	11.6 <sup>c</sup>	13.9 <sup>b</sup>	20.2 <sup>a</sup>	2.53	***
- Liver	29.8	29.5	30.4	3.15	ns
- Pancreas	0.9 <sup>c</sup>	1.3 <sup>b</sup>	2.2 <sup>a</sup>	0.38	***
<u>Small intestinal (SI) characteristics</u>					
Length / kg EBW (cm · kg <sup>-1</sup> )	96.6 <sup>b</sup>	102.8 <sup>b</sup>	111.7 <sup>a</sup>	13.75	**
Weight / length (g · cm <sup>-1</sup> )	0.3 <sup>a</sup>	0.3 <sup>b</sup>	0.4 <sup>a</sup>	0.03	***
Protein content mucosa (g · g <sup>-1</sup> )	0.7 <sup>a</sup>	0.5 <sup>b</sup>	0.7 <sup>a</sup>	0.11	***
Villus height (µm)					
proximal SI	560 <sup>a</sup>	280 <sup>c</sup>	324 <sup>b</sup>	101.8	***
mid SI	522 <sup>a</sup>	313 <sup>b</sup>	259 <sup>c</sup>	77.4	***
Crypt depth (µm)					
proximal SI	220 <sup>b</sup>	240 <sup>b</sup>	313 <sup>a</sup>	42.2	***
mid SI	166 <sup>b</sup>	180 <sup>b</sup>	251 <sup>a</sup>	33.9	***
Villus/crypt ratio					
proximal SI	2.7 <sup>a</sup>	1.2 <sup>b</sup>	1.1 <sup>b</sup>	0.41	***
mid SI	3.2 <sup>a</sup>	1.8 <sup>b</sup>	1.0 <sup>c</sup>	0.45	***
Isomaltase-sucrase (units · (g cp) <sup>-1</sup> ) <sup>4</sup>	63.9 <sup>a</sup>	17.2 <sup>b</sup>	16.2 <sup>b</sup>	9.58	***
Aminopeptidase (units · (g cp) <sup>-1</sup> )	587 <sup>a</sup>	359 <sup>b</sup>	326 <sup>b</sup>	132.5	***

<sup>1</sup> Data in the table are presented as least-square means (LSMeans). LSMean within a row with different superscript letters are significantly different.

<sup>2</sup> RSD is residual standard deviation

<sup>3</sup> P-value of day post weaning: \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; t,  $P < 0.10$ ; ns, not significant.

<sup>4</sup> cp = crude protein

Table 2 Eating <sup>1</sup> and diarrhoea <sup>2</sup> in relation to either gut morphology at the proximal and mid small intestine or specific enzyme activities at the proximal small intestine in piglets <sup>3</sup>.

Eating:		Eating		Non-eater		RSD <sup>4</sup>	P-value <sup>5</sup>		
Diarrhoea :		No	Yes	No	Yes		E	F	E × F
n:		16	19	4	9				
Villus height (µm)	proximal	386	321	287	236	80.6	**	*	ns
	mid	303	259	241	190	58.4	**	*	ns
Crypt depth (µm)	proximal	332	315	312	277	40.7	*	t	ns
	mid	260	254	240	234	35.5	ns	ns	ns
Villus crypt ratio	proximal	1.2	1.0	1.0	0.9	0.32	t	ns	ns
	mid	1.2	1.0	1.0	0.8	0.26	t	t	ns
Isomaltase-sucrase (units · (g cp) <sup>-1</sup> ) <sup>6</sup>		19.8	15.3	18.8	10.5	9.09	ns	*	ns
Aminopeptidase (units · (g cp) <sup>-1</sup> )		377	321	311	252	88.0	*	t	ns

<sup>1</sup> A piglet was regarded as eater if the average net energy intake during the 3-day period just before dissection (days 5-7) was above the energy level for maintenance (NEm), i.e.  $NEm (kJ) > 326.4 \times kg BW_0^{0.75}$ , where  $BW_0$  is body weight at weaning (kg).

<sup>2</sup> Diarrhoea was defined as inconsistent 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 5-7).

<sup>3</sup> Data in the table are presented as least-square means (LSMeans) and refer to day 7 postweaning.

<sup>4</sup> RSD is residual standard deviation

<sup>5</sup> Influence of eating (E) and diarrhoea (F) and the interaction  $E \times F$ : \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; t,  $P < 0.10$ ; ns, not significant.

<sup>6</sup> cp = crude protein

Table 2 shows villus architecture and enzyme activity in piglets at 7 days post weaning in relation to eating and diarrhoea. A piglet was regarded as an eater when the average net energy intake during the 3-day period before dissection (days 5 to 7) was above the energy level for maintenance. Diarrhoea was defined as inconsistent faeces (score 1, 2, or 3) during 2 days out of the 3-day period before dissection. 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 labeled non-eaters with diarrhoea had 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 ( $P < 0.05$ ). 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 higher activity than those with diarrhoea ( $P < 0.05$ ). Aminopeptidase activity was higher in eaters than in non-eaters ( $P < 0.05$ ) and tended to be higher in piglets without diarrhoea when compared to those with diarrhoea ( $P < 0.10$ ). On day 7 postweaning, the

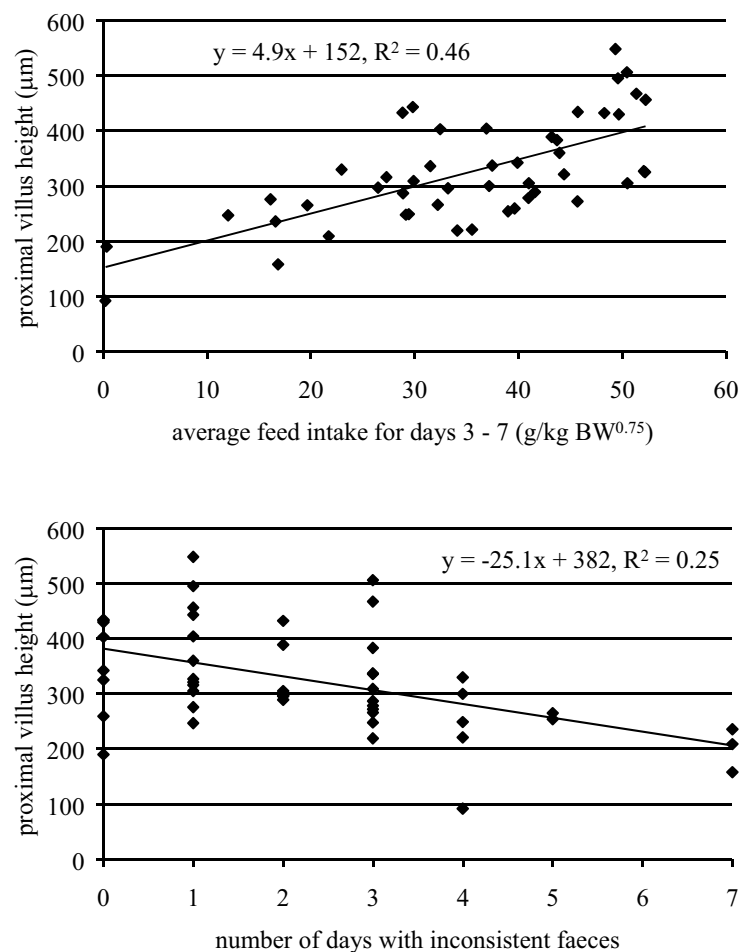


Figure 2 Relation between either average feed intake for days 3 to 7 (upper graph), and the the number of days with inconsistent faeces during the period of days 0-7 (lower graph) with villus height on day 7 postweaning in the proximal small intestine of individual piglets.

average feed intake from day 3 to 7 and the number of days with inconsistent faeces, respectively, explained 46 % and 25 % of the variation ( $R^2$ ) in villus height in the proximal small intestine (Figure 2). Feed intake and villus height were positively correlated, whereas the occurrence of inconsistent faeces on days 4 to 6 and villus height were negatively correlated. The average feed intake and the number of days with inconsistent faeces explained 20 % and 22 %, respectively, of the variation ( $R^2$ ) in villus height, in the mid small intestine (data not shown).

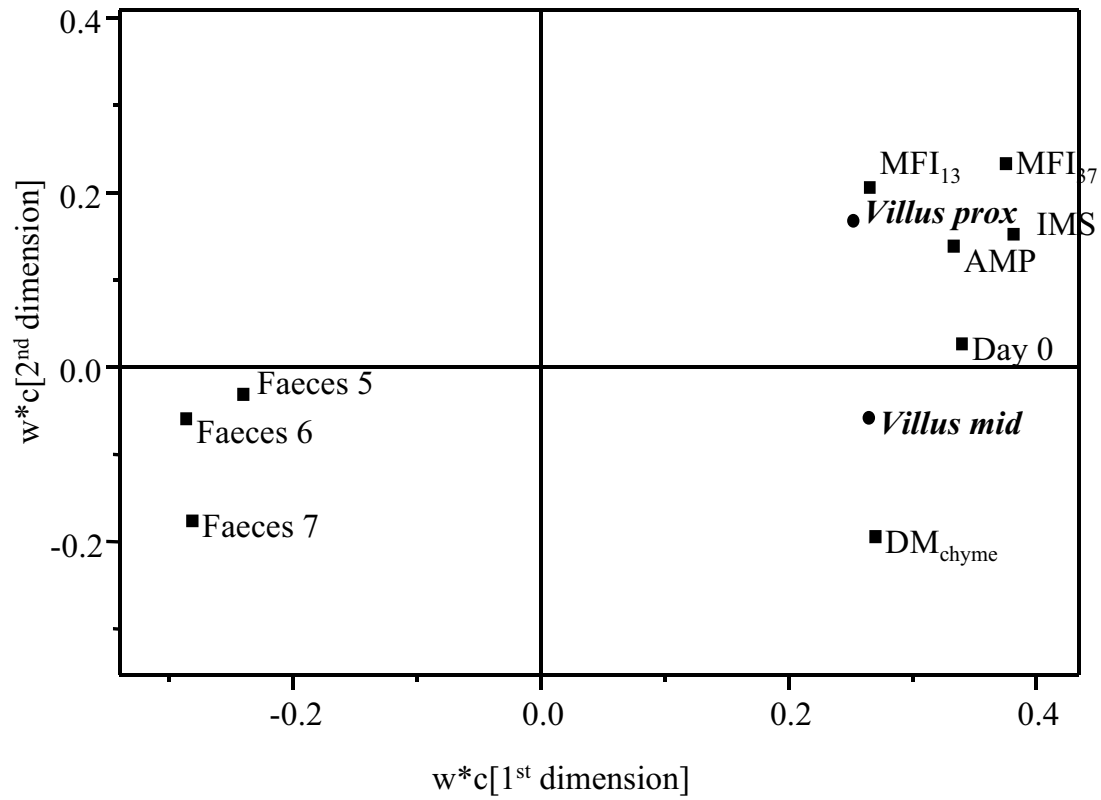
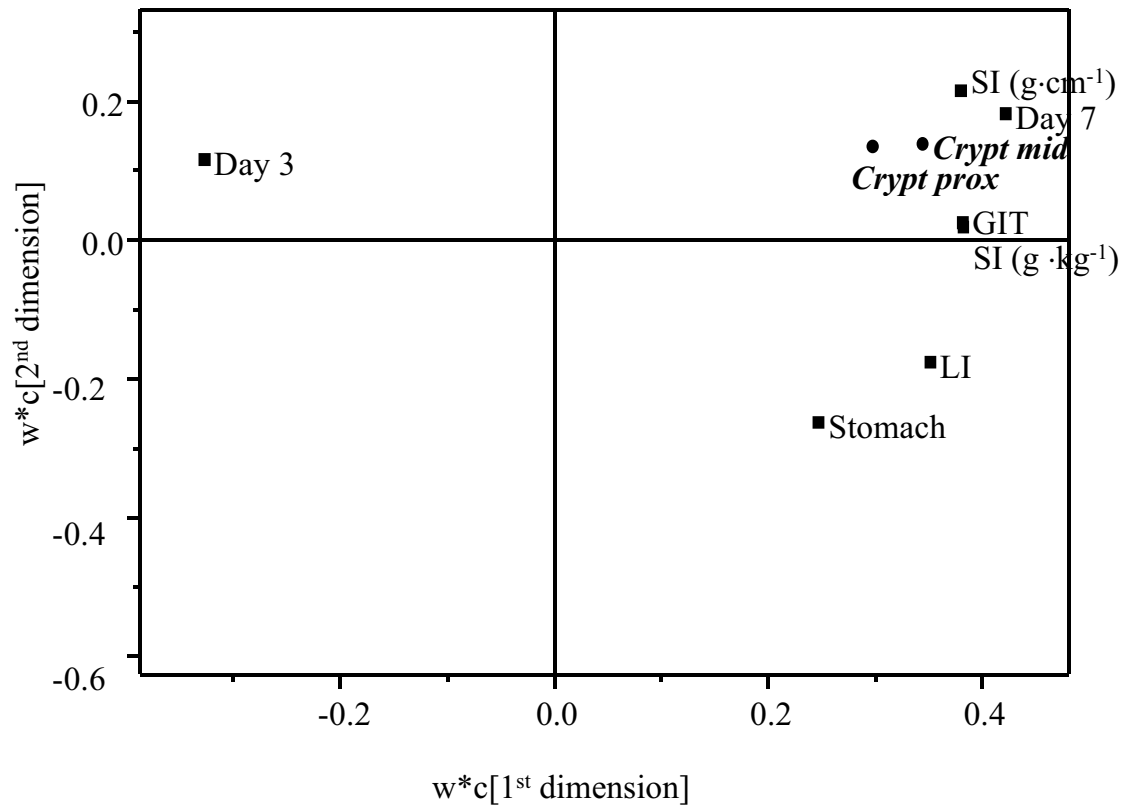
Figure 3 shows the association between the variables as based on multivariate analysis. Only those variables that predict villus height (1A), crypt depth (1B) or specific enzyme activity (1C) are shown. The variables shown on the right side of the vertical zero line are positively associated and those 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 feed intake, enzyme activities and dry matter content of the chyme and negatively associated with faeces consistency 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 feed intake and dry matter content of the chyme.

## DISCUSSION

This study provides new information as to the associations between faeces consistency, small intestinal integrity, energy intake and organ weights. Weaning usually is associated with a dramatic reduction in feed intake, resulting in growth stasis and altered structure of the small intestine. Indeed, the piglets showed weight loss during the first three days postweaning. In agreement with previous work, the weaning transition also was 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; also described in Chapter 3) and a decrease in isomaltase-sucrase and aminopeptidase activity (Hampson, 1986a; Miller et al., 1986; Kelly et al., 1991a). Feed 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., 1991; Pluske et al., 1996; Lopez-Pedrosa et al., 1998; Verdonk et al., 2001).

Regarding the temporal changes in small intestinal 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 ( $\text{g} \cdot \text{kg EBW}^{-1}$ ), the segmental weight of the small intestine ( $\text{g} \cdot \text{cm}^{-1}$ ) and the protein content of the mucosa ( $\text{g} \cdot \text{g}^{-1}$ ) were decreased on day 3, followed by an increase on day 7 postweaning. On day 7, the weight of the small intestine exceeded the preweaning value. Although small intestinal weight had fully recovered on day 7 postweaning, the morphology of the gut wall and the enzyme activity of the brush border had not reached preweaning levels. Crypt depth was greater on day 7 when compared to both days 3 and 0, pointing at 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 gastrointestinal 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

**A: Villus height****B: Crypt depth**



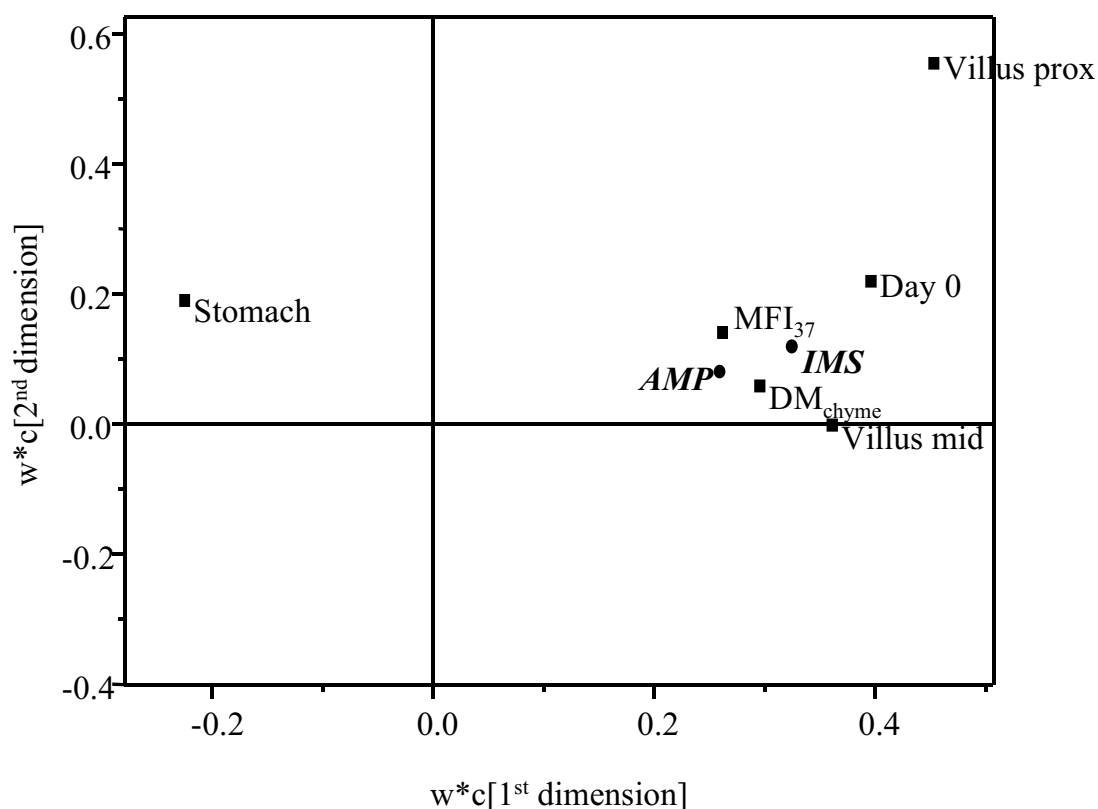
**C: Enzyme activity**

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 (bold, italic) differ per 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 contribute to predict the Y-variables are shown, where MFI<sub>13</sub>, MFI<sub>37</sub> = feed intake per kg metabolic weight from days 1 to 3 and days 3 to 7; organ weights expressed per kg empty body weight, i.e. stomach ( $\text{g} \cdot \text{kg}^{-1}$ ); SI ( $\text{g} \cdot \text{kg}^{-1}$ ) = small intestinal weight, LI ( $\text{g} \cdot \text{kg}^{-1}$ ) = large intestinal weight; GIT = total weight of gastrointestinal tract ( $\text{g}$ ); SI ( $\text{g} \cdot \text{cm}^{-1}$ ) = weight of small intestine per cm ; day 0, 3 or 7 = dissection on day 0, 3, 7; faeces 4, 5 or 6 = occurrence of inconsistent faeces (faeces score either 1, 2, or 3) on day 4, 5, or 6.

(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 ( $P < 0.001$ ). 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 and colleagues (2002) found in piglets weaned at 7 days of age that villus height was numerically lower on day 3 postweaning 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 a 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 postweaning when the proximal and mid small intestine are compared.

During the first week postweaning, 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 postweaning. Nabuurs (1991) reported an incidence of diarrhoea of 40 % during the first, 69 % during the second and 50 % during the third week postweaning 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 inconsistent faeces had shorter villi, which may reflect that shorter villi result in faeces inconsistency. Nabuurs and colleagues (1993) showed on a herd level that mean villus height in diarrhoeic herds was relatively lower than in specific pathogen free (SPF) herds. Low feed intake may lead to shorter villi which in turn causes diarrhoea through maldigestion, malabsorption and increased diarrhoea. Indeed, the variation in villus height ( $R_{\text{Pearson}} = -0.38$ ,  $P < 0.01$ ) explained more variation in faeces consistency than did the variation in feed intake ( $R_{\text{Pearson}} = -0.14$ ,  $P > 0.10$ ). Crypt depth was not associated with faeces consistency. Villus height and crypt depth may influence the pathogenesis of postweaning diarrhoea, as suggested by Nabuurs and colleagues (1993), through the absorptive and secretive properties of small intestinal enterocytes (Powell, 1987). Other factors also play a role in the onset of diarrhoea. The pathogens *E. coli* and rotavirus are frequently detected in piglets with postweaning diarrhoea (Hampson, 1986a; Van Beers-Schreurs et al., 1998; Nabuurs et al., 1993).

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

## LITERATURE CITED

- Ball, R. O., and F. X. Aherne. 1982. Effect of diet complexity and feed restriction on the incidence and severity of diarrhea in early-weaned pigs. *Can. J. Anim. Sci.* 62: 907-913.
- Bruininx, E. M. A. M., C. M. C. van der Peet-Schwering, J. W. Schrama, P. F. G. Vereijken, P. C. Vesseur, H. Everts, L. A. Den Hartog, and A. C. Beynen. 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. *J. Anim. Sci.* 79: 301-308.
- Cera, K. R., D. C. Mahan, R. F. Cross, G. A. Reinhart, and R. E. Whitmoyer. 1988. Effect of age, weaning and postweaning diet on small intestinal growth and small intestinal morphology in young swine. *J. Anim. Sci.* 66: 574-584.
- Dunsford, B. R., D. A. Knabe, and W. E. Haensly. 1989. Effect of dietary soybeanmeal on the microscopic anatomy of the small intestine in the early weaned pig. *J. Anim. Sci.* 67: 1855-1863.
- Efird, R. C., W. D. Armstrong, and D. L. Herman. 1982. The development of digestive capacity in young pigs: effects of age and weaning system. *J. Anim. Sci.* 55, 1380-1387.
- Eriksson, L., J. L. M. Hermens, E. Johansson, H. J. M. Verhaar, and S. Wold. 1995. Multivariate analysis of aquatic toxicity data with PLS. *Aquat. Sci.* 57: 217-241.
- Fan, M. Z., B. Stoll, R. Jiang, and D. G. Burrin. 2001. Enterocyte digestive enzyme activity along the crypt-villus and longitudinal axes in the neonatal pig small intestine. *J. Anim. Sci.* 79, 371-381.
- Fraser, D., B. N. Milligan, E. A. Pajor, P. A. Philips, A. A. Taylor and D. M. Weary. 1998. Behavioural perspectives on weaning in domestic pigs. Pages 121-138 in *Progress in Pig Science*. J. Wiseman, M. A. Varley, and J. P. Chadwick, eds. Nottingham University Press, Nottingham, UK.
- Hall, G. A., and T. F. Byrne. 1989. Effects of age and diet on small intestinal structure and function in gnotobiotic piglets. *Res. Vet. Sci.* 47: 387-392.
- Hampson, D. J. 1986a. Attempts to modify changes in the piglet small intestine after weaning. *Res. Vet. Sci.* 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. *Res. Vet. Sci.* 41: 63-69.
- Kelly, D., J. A. Smyth, and K. J. McCracken. 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 weak post-weaning. *Brit. J. Nutr.* 65: 169-180.
- Kelly, D., J. A. Smyth, and K. J. McCracken. 1991b. Digestive development of the early weaned pig; effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. *Brit. J. Nutr.* 65: 181-188.

- Le Dividich, J., and P. Herpin. 1994. Effects of climatic conditions on the performance, metabolism and health status of weaned piglets: a review. *Liv. Prod. Sci.* 38: 79-90.
- Leibbrandt, V. D., R. C. Ewan, V. C. Speer, and D. R. Zimmerman. 1975. Effect of weaning and age at weaning on baby pig performance. *J. Anim. Sci.* 40, 1077-1080.
- Lopez-Pedrosa, J. M., M. I. Torres, M. I. Fernandez, A. Rios, and A. Gill. 1998. Severe malnutrition alters lipid composition and fatty acid profile of the small intestine in newborn piglets. *J. Nutr.* 128: 224-233.
- Marion, J., M. Biernat, G. Savaray, F. Thomas, R. Zabielski, I. Le Huërou-Luron, and J. Le Dividich. 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.* 34: 89-95.
- Marouz, S., D. Louvard, and J. Barratti. 1973 The aminopeptidase of pig intestinal brush border. *Biochem. Biophys. Acta* 321: 282-295.
- Messer, M., and A. Dahlqvist. 1966. A one step ultramicromethod for the assay of intestinal disaccharidases. *Anal. Biochem* 14:376-392.
- Miller, B. G., P. S. James, M. W. Smith, and F. J. Bourne. 1986. Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. *J. Agric. Sci. Camb.* 107: 579-589.
- Nabuurs, M., 1991. Etiologic and pathogenic studies on postweaning diarrhea. Ph.D. Diss., Univ. Utrecht, The Netherlands.
- Nabuurs, M. J. A., A. Hoogendoorn, E. J. van der Molen, and A. L. M. van Osta. 1993. Villus height and crypt dept in weaned and unweaned pigs, reared under various circumstances in the Netherlands. *Res. Vet. Sci.* 55: 78-84.
- National Research Council (NRC). 1998. Nutrient Requirements of Swine. 10th ed., National Academy Press, Washington DC, USA.
- Núñez, M. C., J. D. Bueno, M. V. Ayudarte, A. Almendros, A. Rios, M. D. Suarez, and A. Gil. 1996. Dietary restriction induces biochemical and morphometric changes in the small intestine of nursing pigs. *J. Nutr.* 126: 933-944.
- Pluske, J. R., D. J. Hampson and I. H Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livest. Prod. Sci.* 51: 215-236.
- Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. *Anim. Sci.* 62: 145-158.
- Powell, D. W., 1987. Intestinal water and electrolyte transport. Pages 1267-1305 in *Physiology of the Gastrointestinal Tract*. L. R. Johnson, ed., 2nd ed. Raven Press, New York, USA.
- Pusztai, A., J. Koninkx, H. Hendriks, W. Kok, S. Hulshcer, E. J. M. Van Damme, W. J. Puemans, G. Grant, and S. Bardocz. 1996. Effect of the insecticidal *GaIanthus nivalis* agglutinin on metabolism and the activities of brush border enzymes in the rat small intestine. *Nutr. Biochem.* 7: 677-682.
- Smith P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk. 1985. Measurements of protein using bicinchoninic acid. *Analytical Biochemistry* 150: 76-85.
- Van Beers-Schreurs, H. M. G., M. J. A. Nabuurs, L. Vellenga, Th. Wensing, H. J. Breukink. 1998. Role of the large intestine in the pathogenesis of diarrhoea in weaned pigs. *J. Nutr.* 59: 696-703.
- Verdonk, J. M. A. J., M. A. M. Spreeuwenberg, G. C. M. Bakker, and M. W. A. Verstegen. 2001. Effect of protein source and feed intake level on the small intestine in newly weaned piglets. Pages 347-349 in *Digestive Physiology of Pigs*. J. E. Lindberg, and B. Ogle, eds. Cabi Publishing, Wallingford, UK.

---

## **CHAPTER 8**

---

### **General Discussion**

## General

The weaning transition of piglets at 4 weeks of age is associated with low feed intake, growth stasis, a decrease in small intestinal integrity, and diarrhoea. However, the generally assumed negative association between small intestinal integrity on one hand and digestive disorders on the other hand is not clear. The aim of the experiments described in this thesis was to investigate the postweaning changes of weaning on small intestinal integrity and whether diet composition can ameliorate the weaning-induced decrease in small intestinal integrity or enhance its recovery also in relation to faeces consistency. Figure 1 in Chapter 2 shows an integrated concept of the pathogenesis of the post-weaning syndrome. This concept is used as the basis for testing various interventions with diet composition. Briefly, weaning results in social and diet changes for piglets. These changes result in low feed intake and lack of enteral nutrition for the small intestinal enterocytes, which is followed by an impairment of mucosal function. This in turn may lead to maldigestion/malabsorption or to local inflammation due to the uptake of antigens, toxins or translocation of bacteria. Both conditions will contribute to poor performance, the occurrence of diarrhoea and infection. In this chapter the main findings of this thesis are discussed in relation to nutrition, small intestinal integrity, diarrhoea and growth performance.

Table 1 summarises the experimental designs of the experiments described in this thesis. In all trials, feed intake was measured daily and at dissection the intestine was sampled to measure intestinal morphology. Other parameters, which can describe intestinal integrity, were only measured in a single experiment. The day of dissection varied between experiments, due to differences in focus. In experiment II, focus was on the first 4 days after weaning in order to investigate how rapid small intestinal integrity deteriorates. In experiments I and IV the onset of recovery was studied.

## Feed intake

Feed intake is an important determinant of performance and the structure and function of the gastrointestinal tract. Individual pigs respond differently to weaning stress with regard to food intake during the first days after weaning, as suggested by Makkink (1993). Some pigs respond initially by refraining from eating followed by a rapid increase in food intake. Other animals may start eating immediately after weaning followed by only a gradual increase in food intake. As hypothesised by Makkink (1993) a period of underfeeding will result in a damaged gut architecture while a rapid increase in food intake following starvation may cause overloading of the digestive and absorptive capacity. The overload of undigested nutrients may trigger undesirable microbial activity (e.g. fermentation of proteins) in the gastrointestinal tract, which eventually might lead to diarrhoea (Kamphues 1987).

Table 1 Summary of experimental designs of the trials described in this thesis.

Experiment, Chapter	I, Chapter 4	II, Chapter 3	III, Chapter 5 and 7	IV, Chapter 6
Weaning characteristics	weight, 7.2 ± 1.01; n, 48; weaned in fourth week of age	weight, 7.8 ± 1.02; n, 66; weaned at 26 ± 2.0 days of age	weight, 8.4 ± 0.70; n, 104; weaned at 26 ± 1.4 days of age	weight, 8.0 ± 0.53; n, 42; weaned at 27 ± 0.8 days of age
Feeding form and level	pellets fed ad libitum; piglets with high feed intake selected on day 3	liquid milk replacer fed restrictedly	crushed pellets fed at pre-set maximum	liquid feed fed at pre-set maximum
Research question:	effect of protein digestibility	importance of energy vs. protein	effect of protein predigestion	effectiveness of carbohydrates
Diet composition (values expressed as % in the feed)	protein sources exchanged on protein basis:	ratio carbohydrates (± lactose) / protein:	protein sources exchanged on protein basis:	carbohydrates exchanged on net energy basis:
Diet 1	23 % skimmed milk powder (well digestible)	lactose/protein (resembles sow milk): 32 %/ 30 %	10 % wheat gluten + 16 % soybean meal	21 % glucose
Diet 2	10 % feather meal (poorly digestible)	low lactose/high protein: 16 %/ 45 %	10 % hydrolysed wheat gluten + 16 % soybean meal	20 % lactose
Diet 3	-	high lactose/low protein : 48 %/ 15 %	10 % wheat gluten + 16 % hydrolysed soybean meal	20 % wheat starch
Diet 4	-	-	10 % potato protein + 16 % soybean meal	-
Diet 5	-	-	10 % potato protein + 16 % soybean meal + 2 % glutamine	-
Diet 6	-	-	10 % wheat gluten + 16 % soybean meal + 1 % arginine	-
Dissection days	0, 4, 7, 14	0, 1, 2, 4	0, 3, 7	0, 3, 10
Performance indicators	body weight: days 0, 4, 7, 14; feed intake and faeces score: daily	body weight: days 0, 1, 2, 4; feed intake and faeces score: daily	body weight: days 0, 3, 7; feed intake and faeces scores: daily	body weight: days 0, 3, 10; feed intake and faeces scores: daily
Histology	at dissection	at dissection	at dissection	at dissection
Permeability	-	at dissection	-	-
Enzyme activity	-	-	at dissection	-
T-lymphocyte cellularity	-	at dissection	-	-
Acute phase protein	-	-	-	at dissection
Small intestinal length	-	at dissection	at dissection	at dissection
Organ weights	-	-	at dissection	at dissection
pH chyme	-	-	-	at dissection

Table 2 Means, standard deviation (SD) and covariance (CV) of daily postweaning net energy intake (kJ) of piglets.

[illegible]



On the other hand it is hypothesised that piglets that start eating immediately after weaning in combination with a gradual increase in food intake are considered to be at a lower risk to digestive and absorptive problems (Makkink, 1993). Bruininx (2002) tested the hypothesis in group-housed piglets by measuring individual feed intake characteristics and indicators of gut physiology like gut morphology, disaccharidase activity, number of goblet cells, and index of mitosis. It was concluded that within the range of feed intake as occurred, the physiology and function of the gut was neither affected by the time between weaning and the onset of eating nor by the subsequent increase in daily feed intake.

The unfamiliarity with dry feed may contribute to a low feed intake after weaning. Creep feeds in practice are made available to suckling piglets from the 2nd week of life in order to encourage dry feed consumption after weaning. Pajor and colleagues (1991) showed that creep feed consumption varied greatly between individual piglets, both between litters and between littermates. Creep feed consumption showed either no clear effect (Hampson, 1986b; Barnett et al., 1989; Kelly et al., 1990; Appleby et al., 1992; Pajor et al., 1994) or a positive effect (Bruininx et al., 2002) on postweaning feed intake and growth. The lack of response to creep feeding in most studies, seems due to the variation in creep feed consumption between individual piglets (Barnett et al., 1989) or due to the complexity of the creep feed (Fraser et al., 1994; Bruininx, 2002). Bruininx (2002) showed that the provision of a highly digestible and complex creep feed resulted in a higher mean creep feed consumption than a creep feed that is more typical for the first two weeks after weaning. This was partially explained by more piglets within a litter that actually consumed the creep feed. It should be pointed out that in the experiments described in this thesis, creep feed was not provided during the suckling period in order to prevent the induction of extra inter-individual variability by variable, pre-weaning ingestion of solid feed.

The intestinal changes after weaning are strongly related to feed intake of the piglets during the first days postweaning (Kelly et al., 1991; Van Beers-Schreurs et al., 1998; Pluske et al., 1996), which is confirmed by own research (Verdonk et al., 2001a; 2001b). A gradual or fast increase in feed intake seems less important than the actual level of feed intake (Bruininx, 2002). Thus, in the current experiments, special attention was given to the amount of the feed intake during the first days postweaning. Piglets were housed individually in order to measure individual feed intakes of piglets. Feeding strategy, i.e. the amount of feed offered and feed form, differed across trials in an attempt to decrease the inter-piglet variation in feed and consequently energy intake in order to enhance the effect of diet composition.

Depending on the objective of the trial, feed form and daily feed allowance varied between experiments. In experiment I, piglets with a high feed intake on day 3 postweaning were selected for further study. Piglets with low feed intake are vulnerable for a compromised small intestinal integrity and therefore piglets were fed a predetermined maximum amount of

feed in experiments II to IV. In experiment II, of the piglets dissected at day 4, 88 % ate the amount of feed offered and therefore the feeding level is considered to be restricted. However in experiments III and IV, only during 33 % of the experimental days the piglets had consumed more than 90 % of the amount of feed offered. Therefore the feeding level in those experiments is considered as “approaching ad libitum”, because feed was available ad libitum for most piglets.

Feed was supplied in a different form in the consecutive experiments. In the first experiment, the feed was supplied as pellets. In the second experiment, a liquid milk replacer was provided. However, because providing a liquid milk replacer is not common in practice, the piglets were fed crumbs in the third experiment. Feeding a liquid diet instead of pellets is known to stimulate feed intake as reviewed by Jensen and Mikkelsen (1998). Therefore, in the last experiment the feed was fed as gruel.

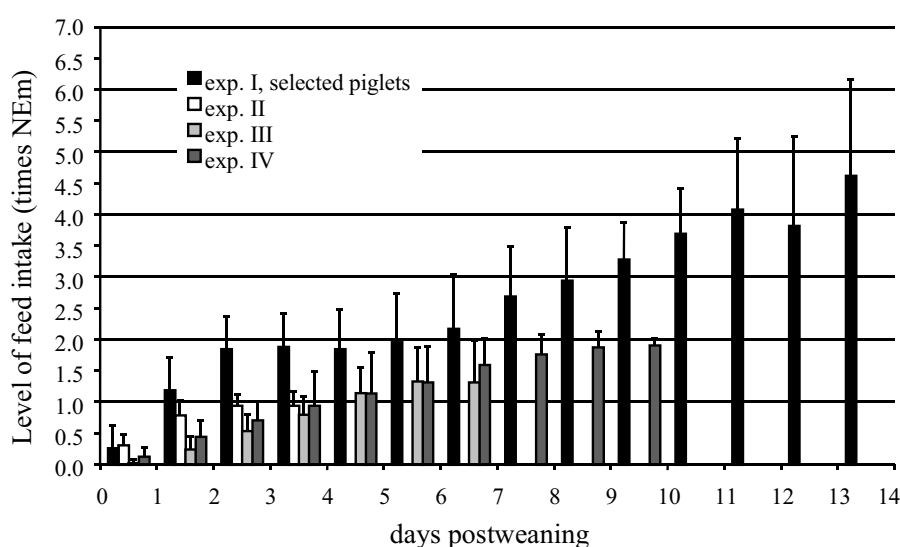


Figure 1 Daily net energy (NE) intake per experiment expressed on a scale of the daily maintenance requirements for net energy (NEm) during the first two weeks after weaning.  $NEm (kJ \cdot kg^{0.75}) = 326.4 \times BW^{0.75}$ , where BW is body weight at weaning (NRC, 1998).

The development of the mean feed intake as based on the piglets pooled per experiment, expressed as units of daily maintenance requirements for net energy, is shown in Figure 1. In experiment I, mean energy intake for maintenance requirement was met on the second day postweaning in the case of piglets selected for a high feed intake and on the third day by the non-selected and selected piglets together. In experiments III and IV, mean energy intake for maintenance requirements was met on fifth day postweaning, which is in agreement with LeDividich and Herpin (1994) and Bruininx (2002). Bark and colleagues (1986) found that

the basal energy requirement was not met until day 4. Therefore, energy intakes found in the current experiments are in line with findings of others.

The increase in feed intake and the decrease in inter-individual variation with day postweaning indicate the adaptation to the weaning process, as shown in Table 2. Although an attempt was made to standardise feed intake, feed intake during the first week was still very variable as shown by the high coefficient of variation. Inter-individual variation in energy intake for piglets receiving the diets in different forms was in increasing order for piglets fed: liquid milk replacer (experiment II) < selected for high feed intake (experiment I) < liquid feed (experiment IV) < crumbled pellets (experiment III). The daily feed intakes differed per experiment and therefore the data of all experiments were not pooled for further analysis.

### **Intestinal variables**

Various variables describing the morphology, functionality and the inflammatory status of the small intestine were investigated. Morphology was described by villus height, crypt depth, number of Goblet cells and mucin type in goblet cells. Functionality was described by permeability across the gut wall and brush-border enzyme activity, i.e. aminopeptidase and isomaltase-sucrase activity. The inflammatory status was described by cell-differentiation molecules of T-cell lymphocytes, i. e. CD4<sup>+</sup> and CD8<sup>+</sup> T-cell lymphocytes, and haptoglobin levels in blood plasma. Villus height and crypt depth was investigated in all experiments, but the other intestinal parameters were only measured in a single experiment and are discussed in the specific chapters above. Table 3 shows villus height and crypt depth on different days postweaning at the different intestinal sites in each experiment, but expressed as percentage of the villus height or crypt depth on the day of weaning. The absolute values on the day of weaning are also given.

Crypt depth generally became deeper after weaning, which is in agreement with work of others (Hampson, 1986a; Miller et al., 1986; Nabuurs et al., 1993; Pluske et al., 1996; Van Beers-Schreurs et al., 1998). Villus height generally decreased after weaning. The decrease in villus height after weaning in experiment I to III was most pronounced at the proximal small intestine, both based on absolute values and relative to the day of weaning. This is in agreement with observations of others (Hampson, 1986a; Nabuurs et al., 1993; Pluske et al., 1996). In experiment IV, the decrease in villus height compared to the day of weaning was 119 and 108 µm in the proximal and mid small intestine, respectively, but there was no clear difference in the relative decrease of villus height. However, Miller and colleagues (1986) found no difference in decrease in small intestinal villus height for piglets sampled at 25, 50 and 75 % along the small intestinal. In the mid small intestine, villus height on either day 10 or 14 postweaning seemed to have recovered faster than in the proximal small intestine, which is in agreement with data of Nabuurs and colleagues (1993). In the experiment of those

authors, piglets were weaned between 30 and 32 days of age, whereas others were left with the sow. On days 11 and 14 after weaning, villus height was higher at the mid small intestine and similar at the proximal small intestine when comparing the weaned and unweaned littermates of the same age (Nabuurs et al., 1993). Therefore, the weaning process seems to have more effect on the gut morphology in the proximal part of the small intestine than on that in the mid and distal parts.

**Table 3** The effect of day postweaning on mean villus height and crypt depth expressed as percentage of the value on the day of weaning at the proximal (prox), mid and distal small intestinal site in each experiment.

Experiment	I			II			III		IV	
Intestinal site	prox	mid	distal	prox	mid	distal	prox	mid	prox	mid
Day postweaning										
Villus height										
0 (µm)	449	357	241	502	351	255	525	487	394	337
(%)	100	100	100	100	100	100	100	100	100	100
1				-13.3	7.0	-9.6				
2				-36.9	-27.9	-16.6				
3							-46.8	-35.7	-30.3	-32.1
4	-43.8	-35.2	-19.6	-22.8	-9.4	-4.2				
7	-38.0	-34.4	-9.8				-38.3	-46.9		
10									-17.7	-10.3
14	-9.9	13.5	34.8							
Crypt depth										
0 (µm)	174	181	129	178	176	158	210	161	166	157
(%)	100	100	100	100	100	100	100	100	100	100
1				-5.4	-7.6	-9.0				
2				-6.5	-7.8	-8.2				
3							14.3	11.3	10.3	15.3
4	24.1	13.1	34.7	5.3	4.1	-2.6				
7	51.1	32.3	50.7				49.5	55.6		
10									74.6	61.8
14	77.9	56.6	50.7							

## Diet composition

An attempt was made to modify small intestinal integrity by the macronutrients protein and carbohydrates. However, in contrast to feed intake level (Verdonk et al., 2001a; 2001b), dietary constituents studied only had marginal effects on small intestinal integrity in the weaned piglet (table 4). Feed intake did not differ between experimental diets in experiments II to IV. Therefore the effect of diet composition could be investigated without entanglement with the actual level of feed intake.

Table 4 Effect of diet composition on mean villus height at the proximal and mid small intestine per experiment <sup>1</sup>.

Diet composition	Villus height <sup>2</sup>			
	proximal %	P-value	mid %	P-value
Effect of protein versus carbohydrates (Experiment II)				
Medium lactose/protein ratio (control) versus low lactose/protein ratio	8	ns	3	ns
High lactose/protein ratio versus control	9	ns	-6	ns
High lactose/protein ratio versus low lactose/protein ratio	18	t	-3	ns
Effect of protein digestibility (Experiment I)				
Skimmed milk powder versus feather meal	9	ns	23	*
Effect of protein predigestion (Experiment III)				
Wheat gluten versus hydrolysed wheat gluten	5	ns	4	ns
Soybean meal versus hydrolysed soybean meal	12	ns	3	ns
Addition of 2 % glutamine	-11	ns	18	ns
Addition of 1 % arginine	7	ns	0	ns
Effect of carbohydrate source (Experiment IV)				
Lactose versus glucose	-5	ns	8	ns
Lactose versus starch	-10	ns	12	ns
Glucose versus starch	-6	ns	4	ns

<sup>1</sup> Data were pooled for day postweaning

<sup>2</sup> P-value of effect of diet composition on villus height: \*,  $P < 0.05$ ; t,  $P < 0.10$ ; ns, not significant.

Diets in the experiments described were formulated to increase the availability of nutrients and energy for the small intestinal mucosa. Table 4 summarises the studied effects of diet composition on villus height. The effectiveness of protein versus lactose was investigated by changing the ratio of protein to lactose in the diet (Experiment II; Chapter 3). The major components of sow's milk roughly are protein: carbohydrates: fat = 30: 30: 40 (Darragh and Moughan, 1998). Fat can vary much more than the other components (Darragh and Moughan, 1998). The lactose/protein ratio of the control milk replacer resembled sow milk. In the high protein/low lactose diet, half of the lactose was replaced by protein, in the low protein/high lactose diet, half of the protein was replaced by lactose. Results indicated that a high lactose/protein ratio in the diet tended to result in greater villus length in the proximal small intestine and less paracellular transport, suggesting that lactose had specific properties for the mucosal integrity. The specificity of lactose in preserving mucosal integrity was tested in experiment IV (Chapter 6). Piglets were fed a diet consisting of either glucose, lactose or starch. Results indicated that different carbohydrate sources did not affect mucosal integrity differently. Furthermore, it was hypothesised that increasing the digestibility or availability of the protein would increase the digestive/absorptive capacity of the small intestine and consequently would positively influence the small intestinal integrity. The effect of protein digestibility was investigated by supplying either poorly or highly digestible protein (Experiment I, Chapter 4). The piglets fed the diet with highly digestible skimmed milk

powder had higher villi than those fed the diet with poorly digestible feather meal. The effect of predigestion of protein was investigated by using protein hydrolysates or by adding the single amino acids glutamine or arginine to the diet (Experiment III, Chapter 5). However, hydrolysed protein, glutamine or arginine did not affect villus height or brush-border enzyme activity in newly weaned piglets differently.

Other potential functional feed ingredients are reviewed in Chapter 2. It was concluded that only limited studies on functional ingredients have been conducted with piglets weaned at 3 or 4 weeks of age. Most studies have been conducted with rodents or neonatal piglets. Functional feed ingredients should be selected to stimulate epithelial cell proliferation and differentiation, to enhance the immune function, to promote the growth of beneficial bacteria and to prevent the proliferation of pathogens. These properties of feed ingredients should receive more attention in the future. Combinations of functional feed ingredients may be more successful than the use of single ingredients. The cost-efficiency of the ingredients will determine their application in practice.

### **Faeces consistency**

A visible indicator of piglet health is diarrhoea. Faeces inconsistency is an indicator for diarrhoea. Figure 2 shows the incidence of faeces inconsistency across experiments. In experiments II to IV, experienced caretakers, who were blinded to treatment modality, scored faeces twice a day on a scale from 0 to 3. Score 0 was given for normally shaped faeces, 1 for shapeless (loose) faeces, 2 for thick, liquid (soft) faeces, and 3 for thin, liquid faeces. Faeces were considered to be inconsistent on a certain day when faecal score was  $\geq 1$ . In experiment I, only those piglets showing signs of diarrhoea were noted and faeces were not scored twice daily as in experiments II to IV. In experiment III the piglets showed more inconsistent faeces than in the other trials. In experiments III and IV, piglets originated from the same sow herd, but with different sows and the diet was fed as crumbs and gruel, respectively. Thus, the higher incidence of inconsistent faeces in experiment III cannot be explained.

A piglet with a faeces score of 2 is not equivalent to overt diarrhoea. Across trials, 39 % of the piglets showed inconsistent faeces during at least 1 day. However, there were no clinical signs of illness and medical treatments were not performed. If diarrhoea was defined as a faecal score of  $\geq 1$  for at least two consecutive days, then 29 % and 22 % of the piglets in experiment III and IV, respectively, would be considered to have diarrhoea. Reported incidences of diarrhoea are 22 % from weaning to slaughter (Hampson, 1986a), 32 % (Ball and Aherne, 1982) and 39 % (Hampson, 1986b) from weaning to 14 days postweaning and 40 % during the first, 69 % during the second and 50 % during the third week postweaning of piglets reared under commercial conditions (Nabuurs, 1991). The percentage of piglets, pooled for experiments, with inconsistent faeces gradually increased from day 0 to 4. On day

4, 29 % of the piglets showed inconsistent faeces. Most piglets, i.e. 52 %, showed inconsistent faeces on day 6 and this percentage decreased thereafter. It should be noted that the number of piglets decreased with time, which may influence the accuracy of calculating the percentage of piglets with inconsistent faeces.

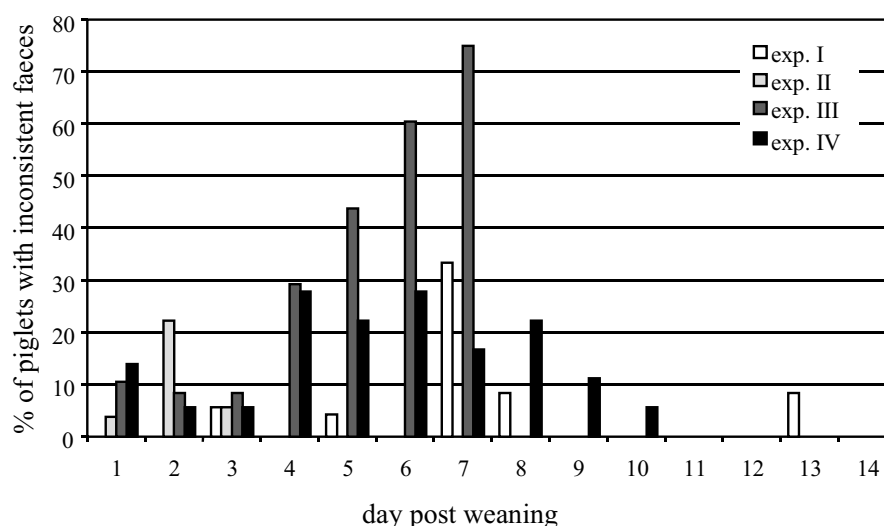


Figure 2 Percentage of piglets with inconsistent faeces on each postweaning day in the 4 experiments. Piglets were considered to have inconsistent faeces if the faecal score was 1, 2, or 3.

### Association between performance, small intestinal parameters and faeces consistency

Generally, small intestinal integrity decreases after weaning, which is also clear from the current studies. Faeces consistency can be regarded as a visible health parameter. Table 5 shows the partial correlation coefficients between the feed intake, growth, faeces inconsistency, and villus height. Feed intake and growth are positively correlated. The positive correlation between feed intake and villus height is also shown by others (e.g. Kelly et al., 1991; Pluske et al., 1996; Bruininx, 2002). Villus height and growth were also positively correlated. For piglets showing a high incidence of inconsistent faeces, there was a negative correlation between villus length and the occurrence of inconsistent faeces (experiment III, Chapter 7). However for piglets showing a lower incidence of inconsistent faeces, the decrease in villus length occurred irrespectively of the occurrence of less consistent faeces. Furthermore, the association between villus height and diarrhoea was positive on day 3 postweaning in experiment IV. Overall, it can be concluded that villus height is a poor predictor of faeces inconsistency, because the association depends on the overall occurrence of diarrhoea.

Table 5 Partial correlation coefficients between cumulative feed intake, growth performance, faeces inconsistency, and villus height of weanling pigs from weaning until day of dissection <sup>1</sup>.

day post weaning:		3		4		7		10	14	Overall impression <sup>2</sup>
n:		48	18	12	18	12	48	18	12	
experiment:		III	IV	I	II	I	III	IV	I	
cum. feed intake <sup>3</sup>	growth	0.71 ***	0.79 ***	0.45 -	0.66 **	0.68 *	0.90 ***	0.88 ***	0.81 **	++
cum. feed intake	villus height prox	0.54 ***	0.25 -	-0.04 -	0.26 -	0.25 -	0.65 ***	-0.20 -	0.48 -	+
cum. feed intake	villus height mid	0.22 -	0.39 -	-0.16 -	0.38 -	0.43 -	0.44 **	-0.30 -	0.52 t	+
villus height prox	growth	0.63 ***	0.23 -	0.21 -	0.32 -	0.26 -	0.60 ***	-0.01 -	0.13 -	+
villus height mid	growth	0.41 **	0.41 t	0.28 -	0.42 t	0.49 -	0.41 **	0.01 -	0.66 *	++
cum. feed intake	faeces inconsistency <sup>4</sup>	-0.20 -	0.06 -	0.03 -	0.71 **	-0.19 -	-0.11 -	0.06 -	-0.22 -	0
cum. feed intake	diarrhoea <sup>5</sup>	0.02 -	0.32 -	na -	0.39 -	na -	-0.14 -	-0.46 t	na -	0
faeces inconsistency	growth	-0.31 *	-0.11 -	na -	0.38 -	-0.63 *	-0.02 -	-0.02 -	-0.59 *	--
diarrhoea	growth	-0.12 -	0.36 -	na -	0.06 -	na -	-0.23 -	-0.44 t	na -	0
villus height prox	faeces inconsistency	-0.02 -	0.05 -	-0.25 -	0.17 -	0.12 -	-0.13 -	0.19 -	0.32 -	0
villus height prox	diarrhoea	0.20 -	0.53 *	na -	0.38 -	na -	-0.38 **	0.00 -	na -	0
villus height mid	faeces inconsistency	-0.13 -	-0.20 -	-0.29 -	-0.05 -	-0.34 -	-0.17 -	-0.09 -	-0.38 -	0
villus height mid	diarrhoea	-0.06 -	0.24 -	na -	0.22 -	na -	-0.39 **	0.20 -	na -	0

<sup>1</sup> P-value of correlation: \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; t,  $P < 0.10$ ; -, not significant; na, not applicable because none of the piglets showed inconsistent faeces

<sup>2</sup> Overall impression: ++ strong positive, + moderate positive, 0 no effect, - moderate negative, -- strong negative

<sup>3</sup> Cumulative feed intake from weaning until dissection

<sup>4</sup> Piglets were considered to have inconsistent faeces if the faecal score was 1, 2, or 3 for  $\geq 1$  day.

<sup>5</sup> Piglets were considered to have diarrhoea if the faecal score was  $\geq 1$  during at least 2 days out of the 3-day period before dissection

Faeces inconsistency and growth are negatively correlated. Figure 3 shows the average daily gain of piglets with either consistent or inconsistent faeces on at least 1 day of the experimental period. As shown with the regression analysis, the daily increase in growth is lower when piglets show inconsistent faeces. Including a binomial variable for faeces



consistency, and a nominal variable for day postweaning in a linear regression analysis, results in the following formula for average daily gain as dependent variable:

$$y_{\text{growth}} = -121.3 + 32.8 \times \text{day} - 56.8 \times \text{faeces consistency}, R^2 = 50.8 \%. \quad [1]$$

This indicates that average daily gain was decreased with  $56.8 \text{ g} \cdot \text{day}^{-1}$  lower when inconsistent faeces occurred during the experimental period.

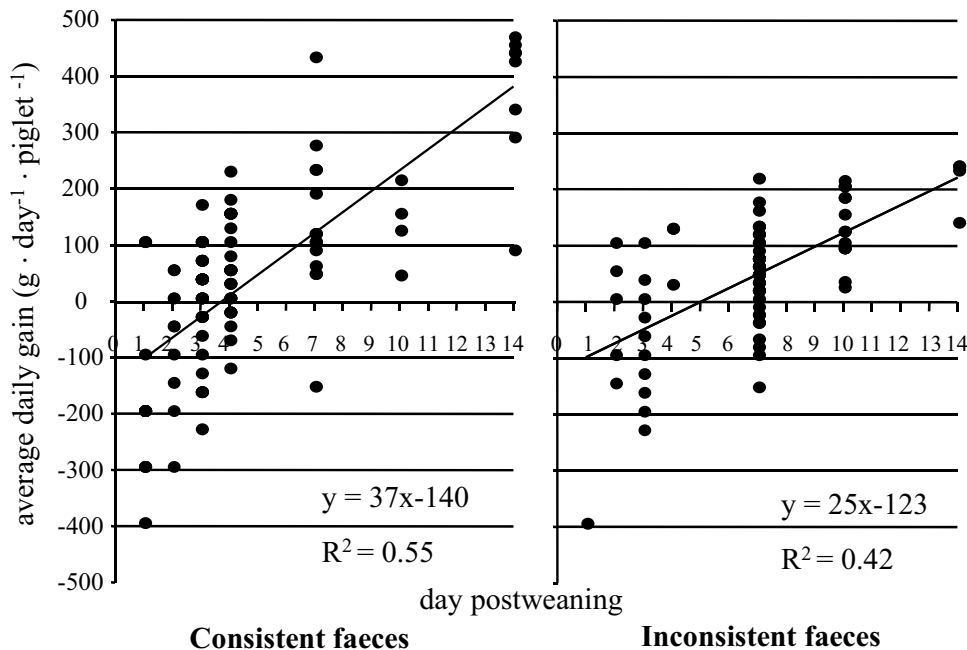


Figure 3 Association between faeces consistency and average daily gain from weaning until dissection. In the left graph, piglets showed consistent faeces ( $n_{\text{consistent faeces}} = 136$ ), in the right graph piglets showed inconsistent faeces ( $n_{\text{inconsistent faeces}} = 86$ ). Piglets were considered to have inconsistent faeces if the faecal score was 1, 2, or 3 for  $\geq 1$  day. Data are derived from all 4 experiments.

The associations found in the current experiments are visualised in figure 4. Feed intake was positively correlated with growth and villus height, but not with faeces consistency. Growth is positively correlated with villus height and negatively with faeces inconsistency. Only with a high incidence of inconsistent faeces, faeces inconsistency and villus height were correlated. Thus, a decrease in villus height and consequently a decrease in absorptive capacity does not automatically lead to diarrhoea. Therefore, it seems questionable whether small intestinal integrity as measured with villus height, is an appropriate indicator for the risk of diarrhoea in weaned piglets.

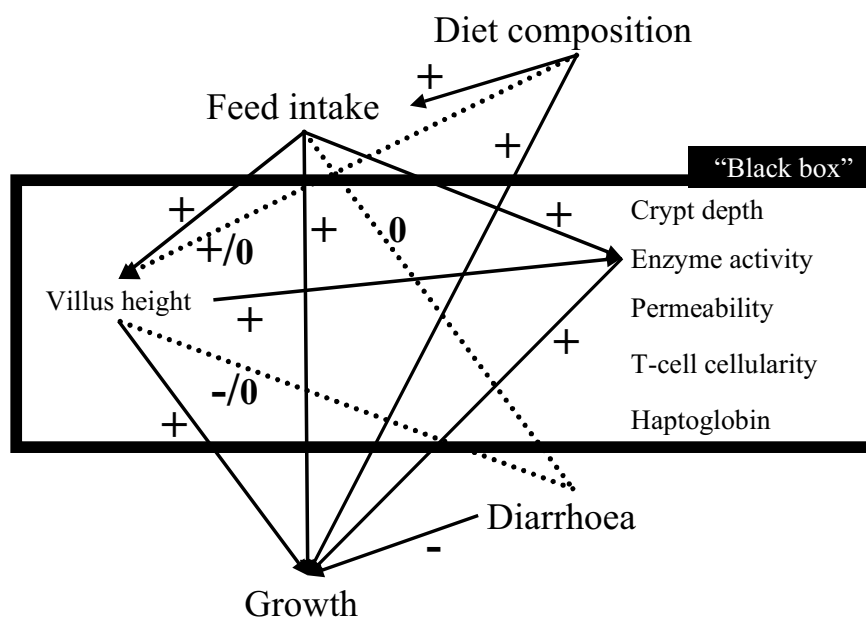


Figure 4 Associations between input (feed intake), output (growth and diarrhoea) and small intestinal characteristics of the weaned piglets, where a drawn line with + indicates a positive association, a drawn line with - indicates a negative association, a dotted line indicates no unequivocal association.

## General conclusions

Based on the general discussion, the following conclusions can be drawn:

- Small intestinal integrity was diminished after the first day postweaning
- The postweaning degeneration of the small intestinal integrity seemed most pronounced in the proximal small intestine; regeneration seemed to occur faster in the mid small intestine.
- The predictability of diarrhoea with measurements on small intestinal morphology was poor.
- Feed intake during the first three days postweaning was low and variable, independent of diet composition.
- Equalising voluntary feed intake of individual piglets during first week postweaning is difficult.
- The effect of feed intake level on small intestinal integrity is more profound than the effect of diet composition.

## Practical implications

The problems associated with weaning are mainly a consequence of the commercial prerequisites: weaning of piglets at an age as young as possible to increase the number of piglets per sow per year. According to the current European legislation, it is not allowed to

wean piglets before 3 weeks of age, while the natural age of weaning is between 12-16 weeks of age (Jensen and Recén, 1989; Fraser et al., 1998).

For early-weaned piglets, postweaning feed intake is the most important determinant regarding the weaning induced decrease in small intestinal integrity and the dietary components tested only showed marginal effects. The variables used to measure small intestinal integrity were histology, permeability, enzyme activity, T-lymphocyte cellularity, acute phase proteins, pH of intestinal chyme, small intestinal length and organ weights. However, those variables did not accurately predict the occurrence of diarrhoea and consequently the health status of weanling piglets. Additionally, most variables are rather expensive and interpretation of the results of those variables often leads to difficulties. It seems that due to accuracy, price and interpretability, the current variables describing small intestinal integrity are poorly applicable variables for investigating the effect of diet composition.

There is an urgent need for new sensitive bio-markers to predict the health status of a piglet in vivo, both as a tool for research and for farm advisement. For research these bio-markers can be used to investigate new feed ingredients in relation to the health status of a piglet. At a farm level, bio-markers can be incorporated into checklists, which can be used by consultants to determine the origin of occurring problems and support their advice.

Regarding the diet of weanling piglets, practical diet formulation should focus on critical determinants of feed intake immediately after weaning. For practical diet formulation it means that more “palatable” feed ingredients should be used. Therefore, more insight into preferences of piglets should be obtained. Furthermore, the postweaning degeneration of the small intestinal integrity seemed most pronounced in the proximal part of the small intestine. Therefore, the ideal weaner-diet stimulates feed intake with highly digestible, rapidly available nutrients in order to prevent indigestible materials, which can cause proliferation of pathogens. However, the cost-efficiency of the ingredients will determine their application in practice.

The occurrence of diarrhoea is determined by many factors, including stress, management, hygiene, occurrence of pathogens, climate, changes in diet. Diet composition is only one of the factors. New innovations are needed combining handling of weanling piglets, housing and nutrition in order to increase postweaning performance and health. During those developments, the weanling piglet rather than production should be placed central. In conclusion a multifactorial approach is needed in order to control postweaning diarrhoea.

## LITERATURE CITED

- Appleby, M. C., E. A. Pajor, and D. Fraser. 1992. Individual variation in feeding and growth of piglets: effects of increased access to creep food. *Anim. Prod.* 55: 147-152.
- Ball, R. O., and F. X. Aherne. 1982. Effect of diet complexity and feed restriction on the incidence and severity of diarrhea in early-weaned pigs. *Can. J. Anim. Sci.* 62: 907-913.
- Bark, L. J., T. D. Crenshaw, and V. D. Leibbrandt. 1986. The effect of meal intervals and weaning on feed intake of early weaned pigs. *J. Anim. Sci.* 62: 1233-1239.
- Barnett, K. L., E. T. Kornegay, C. R. Risely, M. D. Lindemann, and G. G. Schurig. 1989. Characterization of creep feed consumption and its subsequent effects on immune response, scouring index and performance of weanling pigs. *J. Anim. Sci.* 67: 2698-2708.
- Bruininx, E. M. A. M. 2002. Individually measured feed intake characteristics in group-housed weanling pigs. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- Bruininx, E. M. A. M., G. P. Binnendijk, C. M. C. van der Peet-Schwering, J. W. Schrama, L. A. den Hartog, H. Everts, and A. C. Beynen. 2002. Effect of creep feed consumption on individual feed intake characteristics and performance of group-housed weanling pigs. *J. Anim. Sci.* 80(6): 1413-1418.
- Darragh, A. D., and P. J. Moughan. 1998. The composition of colostrum and milk. Pages 3-21 in *The Lactating Sow*. M. W. A. Verstegen, P. J. Moughan, and J. W. Schrama, eds. Wageningen Pers, Wageningen, The Netherlands.
- Fraser, D., J. J. R. Feddes, and E. A. Pajor. 1994. The relationship between creep feeding behaviour of piglets and adaptation to weaning: effect of diet quality. *Can. J. Anim. Sci.* 74: 1-6.
- Fraser, D., B. N. Milligan, E. A. Pajor, P. A. Philips, A. A. Taylor, and D. M. Weary. 1998. Behavioural perspectives on weaning in domestic pigs. Pages 121-138 in *Progress in Pig Science*. J. Wiseman, M. A. Varley, and J. P. Chadwick, eds. Nottingham University Press, Nottingham, UK.
- Hampson, D. J. 1986a. Attempts to modify changes in the piglet small intestine after weaning. *Res. Vet. Sci.* 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. *Res. Vet. Sci.* 41: 63-69.
- Jensen, and Mikkelsen. 1998. Feeding liquid diets to pigs. Pages 107-126 in *Recent Advances in Animal Nutrition*. P.C. Gransworthy, and J. Wiseman, eds. Nottingham University Press, Nottingham, UK.
- Jensen, P., and B. Recén. 1989. When to wean – observations from free-ranging domestic pigs. *App. Anim. Behav. Sci.* 23: 49-60.
- Kamphues, J. 1987. Untersuchungen zu Verdauungsvorgängen bei Absetzferkeln in Abhängigkeit von Futtermenge und –zubereitung sowie von Futterzusätzen. Habilitationsschrift zur Erlangung der Venia Legendi. Tierärztliche Hochschule Hannover, Hannover, Deutschland.
- Kelly, D. J. 1990. Effect of creep feeding on the incidence duration and severity of post-weaning diarrhoea in pigs. *Res. Vet. Sci.* 49: 223-228.
- Kelly, D., J. A. Smyth, and K. J. McCracken. 1991. Digestive development of the early weaned pig; effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. *Brit. J. Nutr.* 65: 181-188.
- Le Dividich, J., and P. Herpin. 1994. Effects of climatic conditions on the performance, metabolism and health status of weaned piglets: a review. *Liv. Prod. Sci.* 38: 79-90.
- Makkink, C. A. 1993. Of piglets, dietary proteins and pancreatic proteases. Ph.D. Diss., Wageningen Univ., Wageningen, The Netherlands.

- Miller, B. G., P. S. James, M. W. Smith, and F. J. Bourne. 1986. Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. *J. Agric. Sci. Camb.* 107: 579-589.
- Nabuurs, M., 1991. Etiologic and pathogenic studies on postweaning diarrhea. Ph.D. Diss., Univ. Utrecht, Utrecht, The Netherlands.
- Nabuurs, M. J. A., A. Hoogendoorn, E. J. van der Molen, and A. L. M. van Osta. 1993. Villus height and crypt depth in weaned and unweaned pigs reared under various circumstances in the Netherlands. *Res. Vet. Sci.* 55: 78-84.
- Pajor, E. A., D. Fraser, and D. L. Kramer. 1991. Consumption of solid food by suckling pigs: individual variation and relation to weight gain. *Appl. Anim. Behav. Sci.* 32: 139-155.
- Pluske, J.R., I. H. Williams, and F. X. Aherne. 1996. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. *Anim. Sci.* 62: 145-158.
- Van Beers-Schreurs, H. M. G., M. J. A. Nabuurs, L. Vellenga, H. j. Kalsbeek-van der Valk, T. Wensing, and H. J. Breukink. 1998. Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. *J. Nutr.* 128: 947-953.
- Verdonk, J. M. A. J., M. A. M. Spreeuwenberg, G. C. M. Bakker, and M. W. A. Verstegen. 2001. Nutrient intake level affects histology and permeability of the small intestine in newly weaned piglets. Pages 332-334 in *Digestive Physiology of Pigs*. J. E. Lindberg, and B. Ogle, eds., CABI Publishing, Wallingford, UK.
- Verdonk, J. M. A. J., M. A. M. Spreeuwenberg, G. C. M. Bakker, and M. W. A. Verstegen. 2001. Effect of protein source and feed intake level on histology of the small intestine in newly weaned piglets. Pages 347-349 in *Digestive Physiology of Pigs*. J. E. Lindberg, and B. Ogle, eds. CABI Publishing, Wallingford, UK.



---

## SUMMARY

---

In the Netherlands, piglets are weaned at 3 to 4 weeks of age. During the weaning transition, piglets are faced with multiple changes. These changes include social, environmental and nutritional changes and generally result in low voluntary feed intake and decreased growth performance. In practice, diarrhoea frequently occurs. The occurrence of diarrhoea is determined by many factors including stress, management, hygiene, occurrence of pathogens, climate, changes in diet. The small intestine is thought to play an important role in the etiology of diarrhoea. The small intestine has two main functions. Firstly, to digest and absorb nutrients. Secondly, to exclude pathogens, toxins and allergic compounds that otherwise may reach the systemic organs and tissues causing an immunologic response. The concerted actions of the small intestine regarding absorption and exclusion of pathogenic compounds are addressed as small intestinal integrity. The generally assumed negative association between small intestinal integrity on one hand and digestive disorders on the other hand is not well understood. Increasing the knowledge regarding the relation between small intestinal integrity and digestive disorders may lead to solutions for the weaning induced growth stasis and diarrhoea.

The aim of the experiments described in this thesis was to investigate the effect of diet composition on small intestinal integrity and on digestive disorders in weaned piglets. The chosen approach to improve small intestinal integrity was either to ameliorate the loss of small intestinal integrity or to enhance its recovery. During the weaning transition of piglets, two successive phases in the small intestinal integrity can be distinguished: a de- and regenerative phase. It was assumed that increasing the availability of nutrients for the mucosa would support the intestinal integrity in both the de- and regenerative phase. The effect of the macronutrients protein and carbohydrates on small intestinal integrity were investigated. The importance of protein versus lactose was investigated by changing the ratio of protein to lactose in the diet (Chapter 3). The specificity of lactose as carbohydrate source was investigated by feeding the piglets either glucose, lactose or native starch (Chapter 6). The effect of protein digestibility was investigated by supplying either poorly or highly digestible protein (Chapter 4). The effect of predigestion of protein was investigated by using protein hydrolysates or by adding the single amino acid glutamine to the diet (Chapter 5).

Small intestinal integrity is assessed on the basis of indicators regarding morphology (villus length, crypt depth), functionality (enzyme secretion and permeability across the gut wall) and inflammation (cell differentiation molecules on T-cell lymphocytes, i. e. CD4<sup>+</sup> and CD8<sup>+</sup> T-cell lymphocytes and haptoglobin levels in blood plasma). The day of weaning is addressed to as day 0.

A literature review was conducted (Chapter 2). The first part of the review describes the small intestinal integrity and methods to assess it. Additionally, an integrated concept of the pathogenesis of the post-weaning syndrome is shown. Briefly, the low feed intake after weaning results in a lack of enteral nutrition for the small intestinal enterocytes, followed by an impairment of mucosal function. This results either in maldigestion/malabsorption or in inflammation due to exposure to antigens/pathogens and subsequently in poor growth performance possibly accompanied by diarrhoea. The second part of the review focussed on the weaning-induced impairment of small intestinal integrity and the specific role of the feed intake. It was concluded that the temporal low feed intake immediately after weaning is the main cause of the decrease in small intestinal integrity. Thus, the lower the energy intake immediately after weaning, the more impaired the small intestinal integrity after weaning is. In the third part, the effect of dietary components on small intestinal integrity was discussed. Studies reviewed were those dealing with potential functional feed ingredients, including protein source, specific amino acids, fatty acids, fibres including non-digestible oligosaccharides, growth factors, polyamines and nucleotides. It was concluded that the individual feed constituents have only marginal effects on small intestinal integrity of the weaned pig. Combinations of functional feed ingredients may be more successful than the use of single ingredients. The cost-efficiency of the ingredients will determine their application in practice.

The contribution of energy versus protein to mucosal integrity in weaned piglets was investigated using three experimental diets with varying lactose to protein ratios (Chapter 3). Piglets were sampled on days 0, 1, 2 and 4 postweaning and were offered feed at a low energy intake level. The effect of diet composition within the degenerative phase of small intestinal integrity was studied as measured with morphology, T-lymphocyte cellularity and transepithelial permeability. The ratio of CD4<sup>+</sup>/CD8<sup>+</sup> T-lymphocytes decreased during the first day postweaning. More paracellular transport, higher villi, shallower crypts and lower villus/crypt ratio was observed on day 2 compared to day 0 postweaning. Piglets consuming the high lactose/low protein diet tended to exhibit lower paracellular transport and higher villi. This might indicate that energy from lactose is more limiting than protein for epithelial cells in contributing to mucosal integrity during the first days after weaning. The effect of diet composition was not as important as the sequential effects of low feed intake during the first 4 days postweaning. Already on day 1 postweaning, intestinal integrity was affected.



The effect of dietary protein source on feed intake and on small intestinal morphology was studied in newly weaned piglets (Chapter 4). This study addressed two different questions. First we wanted to know whether the protein source in the weaner diets affects feed intake during the first three days after weaning. Secondly, we addressed the question whether the dietary protein source, including the degree of ileal digestibility, influences the recovery of villus height. Increasing the protein digestibility might increase the availability of amino acids in the digesta resulting in increased proliferation of enterocytes and therefore the rate of recovery of the villi. Two iso-nitrogenous and iso-energetic diets were formulated based on equal calculated amounts of ileal indispensable amino acids, but derived from protein sources that extremely differ in total ileal protein digestibility. It was hypothesised that highly digestible skimmed milk powder would stimulate recovery of small intestinal morphology when compared to poorly digestible feather meal. Results showed that the protein source did not affect the feed intake during the first three days after weaning. On day 4 postweaning, 18 piglets with a similar high feed intake were selected within each dietary treatment and sampled for small intestinal morphology on days 0, 4, 7 or 14. Piglets were selected for high feed intake because it was assumed that in these piglets the recovery process would be most active. In the second week, the feed intake, growth and feed efficiency of the selected piglets receiving the skim milk powder diet was higher than that of the piglet receiving the feather meal diet. Villus height and crypt depth were significantly higher for the selected piglets fed the skim milk powder diet when compared to those fed the feather meal diet. It was concluded that skim milk powder had a positive effect on villus height, this effect being mediated through its high degree of ileal digestibility rather than through its stimulatory effect on feed intake.

The effect of predigestion of protein was investigated (Chapter 5). It was hypothesised that dietary supplementation of hydrolysed proteins when compared to their native proteins or that the addition of the single amino acid glutamine would increase the availability of nutrients for the gut wall and therefore result in an improved small intestinal integrity and growth performance of piglets after weaning (Chapter 5). Two separate experiments were performed, but using the same diets. Piglets were sampled for small intestinal integrity in experiment I on days 0, 3, or 7 postweaning. Piglets were fed a predetermined maximum amount of feed in order to decrease differences in feed intake. Results showed that the protein hydrolysates did not increase villus height, did not reduce crypt depth and did not raise brush-border aminopeptidase and isomaltase-sucrase activity. In experiment II, growth performance, feed intake and feed efficiency were measured after 7 and 14 days postweaning. Both feed intake and weight gain were not enhanced by the protein hydrolysates. The addition to the diet of crystalline gln resulted in improved average daily gain and feed efficiency by 22 % and 17 %, respectively when compared to the other diets and measured during the first 2 weeks postweaning. In conclusion, dietary supplementation of protein hydrolysates did not

ameliorate the weaning-induced compromise of small intestinal integrity and did not enhance growth performance. Dietary supplementation of gln did not affect villus architecture but enhanced growth performance.

Increasing amounts of lactose in the weaner diet at the expense of protein were associated with longer villi in the proximal small intestine and decreased paracellular transport as shown in Chapter 3. Therefore we hypothesised that lactose might have specific properties contributing to mucosal integrity in newly weaned piglets. To test the specificity of lactose, three experimental weaner diets were formulated containing 24 % of total energy in the form of either glucose, lactose or wheat starch (Chapter 6). The effects of dietary glucose, lactose and starch were evaluated on small intestinal morphology, organ weights, pH of chyme and haptoglobin levels in blood plasma of piglets dissected on days 0, 3 and 10 postweaning. Piglets received a liquid diet (air-dry meal: water = 1:2, w:w). The piglets were given access to a maximum of dietary energy in order to prevent entanglement between feed intake and villus architecture. The results show that the carbohydrate source did not affect growth performance, organ weights, villus architecture, pH of chyme and plasma haptoglobin level. The weaning transition resulted in decreased villus height and increased haptoglobin levels. In the contents of the caecum and large intestine, the pH decreased after weaning. It is concluded that under conditions of unaltered feed and low infectious pressure, dietary lactose does not ameliorate the weaning induced compromise of small intestinal integrity when compared to glucose or wheat starch.

The weanling piglets from the study described in Chapter 5 had inconsistent faeces during on average 2 of the 7 postweaning days, using a faecal consistency score ranging from 0 to 3. On day 7 after weaning, 75 % of the piglets produced inconsistent faeces. Due to the relatively high incidence of inconsistent faeces, the dataset was considered suitable to assess the association between feed intake, faeces consistency and mucosal integrity, as measured with specific aminopeptidase and isomaltase-sucrase activity, villus height and crypt depth. The piglets were sampled on days 0, 3 and 7 postweaning. Across diets, the weight of the stomach, large intestine and pancreas increased with time postweaning. Small intestinal weight decreased from day 0 to 3 and was increased again on day 7, exceeding preweaning levels. Isomaltase-sucrase and aminopeptidase activity decreased on day 3 and 7 compared to day 0. Villus height decreased after weaning followed by an increase on day 7 postweaning at the proximal small intestine, but a further decrease at the mid small intestine. Crypt depth was increased after weaning. Villus height was positively correlated with feed intake level and dry matter content of the chyme, but was negatively correlated with faeces consistency. Crypt depth was positively correlated with the weight of the different parts of the gastrointestinal tract. Brush-border enzyme activity was positively associated with feed intake level, villus height and dry matter content of the chyme. This study supports the concept that feed 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.

It was concluded that small intestinal integrity is already diminished after one day postweaning. The postweaning degeneration of the small intestinal integrity seemed more pronounced in the proximal small intestine; regeneration seemed to occur faster in the mid small intestine. The correlation between measurements on faeces consistency and on small intestinal morphology was poor. Feed intake during the first three days postweaning was low and variable, independent of diet composition. Equalising voluntary feed intake of individual piglets during first week postweaning in order to prevent entanglement of the effect of feed intake and diet composition on small intestinal integrity is difficult. Overall it was concluded that the effect of effect of feed intake level on small intestinal integrity is more profound than the effect of diet composition. A multifactorial approach combining handling, housing and nutrition is needed in order to control postweaning diarrhoea.



---

## SAMENVATTING

---

In Nederland worden biggen gespeend op een leeftijd van 3 tot 4 weken. Dit gaat gepaard met meerdere veranderingen. Zo worden biggen van verschillende tomen gemengd en eventueel getransporteerd naar een andere stal met een hogere omgevingstemperatuur. Vervolgens verandert de voeding drastisch van vloeibare, warme zeugenmelk naar droge, gepelleteerde brokjes met koolhydraten in plaats van vet als belangrijkste energiebron. Al deze veranderingen tijdens het speenproces leiden tot een lage voeropname en verminderde groei. In de praktijk treedt veel diarree op na het spenen. Het optreden van diarree wordt veroorzaakt door vele factoren waaronder stress, management, hygiëne, vóórkomen van pathogene bacteriën, klimaat en voer veranderingen. Er wordt over het algemeen aangenomen dat de dunne darm een belangrijke rol speelt in de etiologie van diarree. De dunne darm heeft twee belangrijke functies. Ten eerste, het verteren en absorberen van nutriënten. Ten tweede het buitensluiten van onder andere pathogenen en toxines. Dit om te voorkomen dat lichaamsvreemde stoffen in de bloedbaan en de organen terechtkomen en een immunologische reactie veroorzaken. Beide functies samen worden “dunne darm integriteit” genoemd. Er is niet veel bekend over de negatieve relatie tussen dunne darm integriteit aan de ene kant en verteringsproblemen/diarree aan de andere kant. Meer kennis over deze relatie kan leiden tot het vinden van een oplossing voor de speenproblematiek.

Het doel van de experimenten die in dit proefschrift beschreven zijn was om het effect van voersamenstelling op de dunne darm integriteit en op verteringsstoornissen in gespeende biggen te onderzoeken. De gekozen aanpak om dunne darm integriteit te verbeteren is het verminderen van de afname in dunne darm integriteit en het versnellen van het herstel. In de opeenvolgende veranderingen die in de dunne darm integriteit optreden, kunnen twee fases onderscheiden worden: een de- en een regeneratieve fase. Er is aangenomen dat door een toename van nutriënt-beschikbaarheid voor de dunne darm cellen, de dunne darm integriteit behouden blijft in zowel de de- als regeneratieve fase. De effecten van eiwitten en koolhydraten op de dunne darm integriteit zijn onderzocht. Het belang van eiwit ten opzichte van lactose voor de dunne darm integriteit is onderzocht door de ratio eiwit/lactose in het voer te veranderen (Hoofdstuk 3). De specificiteit van lactose voor biggen als koolhydraatbron is onderzocht met een voer op basis van glucose, lactose of natief zetmeel (Hoofdstuk 6). Het effect van eiwitverteerbaarheid is onderzocht door zowel slecht als goed verteerbaar eiwit te

verstrekken (Hoofdstuk 4). Het effect van voorvertering van eiwit was onderzocht door eiwit hydrolysaten te voeren of door het aminozuur glutamine toe te voegen aan het voer (Hoofdstuk 5).

Dunne darm integriteit, in de experimenten beschreven in dit proefschrift, is gemeten door indicatoren aangaande darm morfologie (darmvlok lengte, crypte diepte), functionaliteit (enzym secretie door de borstelzoom van de dunne darm, permeabiliteit over de darmwand) en inflammatie (cel differentiatie moleculen op T-cellen, d.w.z.  $CD4^+$  en  $CD8^+$  T-lymfocyten en haptoglobine concentratie in het bloed). De dag van spenen wordt aangegeven met dag 0.

Een overzicht van de literatuur is gegeven in Hoofdstuk 2. In het eerste deel van het overzicht wordt de dunne darm integriteit beschreven en hoe je deze kan meten. Ook is een geïntegreerd concept beschreven over de problemen die optreden na het spenen van biggen op 4 weken leeftijd. In het kort, de lage voeropname na spenen zorgt voor een tekort aan nutriënten voor de dunne darm cellen. Dit veroorzaakt een verslechtering van de dunne darm integriteit, wat kan leiden tot maldigestie/malabsorptie en tot inflammatie door de blootstelling aan lichaamsvreemde stoffen. Beiden resulteren in verminderde groei, mogelijk gepaard gaande met diarree. Het tweede deel van het overzicht focust op de specifieke rol van de voeropname als veroorzaker van een verminderde dunne darm integriteit. Geconcludeerd werd dat de tijdelijke lage energie opname direct na spenen de belangrijkste oorzaak is van de verminderde dunne darm integriteit. Dus, hoe meer energie opname, hoe beter de dunne darm integriteit. In het derde deel wordt het effect van specifieke voedingscomponenten op de dunne darm bediscussieerd. De besproken potentiële functionele voedingscomponenten zijn: verschillende eiwitbronnen, specifieke aminozuren, vetzuren, vezels waaronder niet verteerbare oligosacchariden, groeifactoren, polyaminen en nucleotiden. Geconcludeerd werd dat de individuele voedingscomponenten slechts een marginaal effect hebben op de dunne darm integriteit van de pasgespeende big. Combinaties van de ingrediënten hebben waarschijnlijk meer effect dan de enkelvoudige ingrediënten zelf. De kosten/baten verhouding van deze ingrediënten zal bepalen of ze in de toekomst ook gebruikt gaan worden in de mengvoeder- en levensmiddelenindustrie.

De bijdrage van energie ten opzichte van eiwit aan de dunne darm integriteit van de pasgespeende big was onderzocht met behulp van drie experimentele voeders die verschilden in de ratio lactose/eiwit (Hoofdstuk 3). Biggen zijn bemonsterd op 0, 1, 2 en 4 dagen na spenen en zijn gevoerd op een laag energie aanbod niveau. Het effect van voersamenstelling binnen de degeneratieve fase op de dunne darm integriteit was onderzocht met behulp van morfologie, T-lymfociet cellulariteit en transepitheliale permeabiliteit. De ratio  $CD4^+/CD8^+$  T-lymfocieten was al na 1 dag verminderd. Op dag 2 na spenen ten opzichte van de dag van spenen zelf, was er meer paracellulair transport (transport tussen de darmcellen door), langere darmvlokken en diepere crypten. Biggen die het voer met de hoog lactose/laag eiwit ratio

kregen, leken minder paracellulair transport te hebben en langere darmvlokken. Dit kan een indicatie zijn dat energie uit lactose eerder limiterend is voor de epitheelcellen dan eiwit en dat lactose daardoor een bijdrage levert aan een verbeterde dunne darm integriteit tijdens de eerste dagen na spenen. Het effect van voersamenstelling was niet zo groot als de tijdsgerelateerde effecten van een lage voeropname tijdens de eerste 4 dagen na spenen. Al op 1 dag na spenen was de dunne darm integriteit aangetast.

Het effect van verschillende eiwitbronnen in het voer op de voeropname en op de dunne darm morfologie was onderzocht (Hoofdstuk 4). Dit experiment behandelde 2 verschillende vragen. Ten eerste wilden we weten of de eiwitbron in het speenvoer de voeropname gedurende de eerste drie dagen na spenen beïnvloedt. Ten tweede wilden we weten of de dunne darm verteerbaarheid van een eiwitbron, het herstel van de lengte van de darmvlokken beïnvloedt. Door een toename in eiwit verteerbaarheid zou de beschikbaarheid van aminozuren in de digesta toenemen en daardoor ook de snelheid van herstel van de darmvlokken. Twee voeders zijn geformuleerd met een gelijke hoeveelheid aan eiwit, dunne darm verteerbare aminozuren en energie. Echter, de gebruikte eiwitbronnen verschilden in totale dunne darm eiwit verteerbaarheid. Het goed verteerbare magere melkpoeder was verondersteld het herstel van de dunne darm morfologie te versnellen ten opzichte van het slecht verteerbare verenmeel. De resultaten toonden aan dat de eiwitbron de voeropname gedurende de eerste drie dagen na spenen niet beïnvloedt. Op dag 4 na spenen zijn 18 biggen met een vergelijkbare hoge voeropname geselecteerd binnen elke voerbehandeling. De biggen zijn bemonsterd voor analyse van de dunne darm morfologie op dag 0, 4, 7 of 14 na spenen. De biggen zijn voor een hoge voeropname geselecteerd omdat er aangenomen was dat juist in deze biggen de herstelfase zou zijn begonnen. De geselecteerde biggen die het magere melkpoeder rantsoen kregen hadden langere darmvlokken dan de biggen die het verenmeel rantsoen kregen. In de tweede week na spenen was de voeropname, groei en voerefficiëntie hoger voor de geselecteerde biggen die het magere melkpoeder rantsoen kregen dan voor de biggen die het verenmeel rantsoen kregen. Geconcludeerd werd dat magere melkpoeder een positief effect heeft op de vlok lengte, dit effect werd met name veroorzaakt door de hoge mate van eiwitverteerbaarheid en niet door de stimulerende werking op de voeropname.

Het effect van voorvertering van eiwit was onderzocht (Hoofdstuk 5). De hypothese was dat de beschikbaarheid van nutriënten voor de darmwand toeneemt door het gebruik van gehydrolyseerde eiwitten ten opzichte van de natieve eiwitten in het voer of door toevoeging van het aminozuur glutamine aan het voer. Door de toename van nutriënt beschikbaarheid, zou de dunne darm integriteit en de groei van biggen na spenen moeten toenemen. Twee afzonderlijke experimenten zijn uitgevoerd, maar met gebruik van dezelfde voeders. In experiment I zijn de biggen bemonsterd voor dunne darm integriteit op dag 0, 3 en 7 na spenen. Biggen kregen een vooraf bepaalde, maximale hoeveelheid voer verstrekt om de

verschillen in voeropname tussen biggen te verminderen. Resultaten toonden aan dat het verstrekken van eiwithydrolysaten aan biggen geen effect had op de lengten van darmvlokken, de diepten van crypten en de activiteit van borstelzoom enzymen. In experiment II zijn de groei, voeropname en voerefficiëntie gemeten na 7 en 14 dagen na spenen. Het gebruik van eiwithydrolysaten had geen effect op de voeropname en groei. De toevoeging van kristallijne glutamine aan het voer ten opzichte van de andere voeders verhoogde de groei en voerefficiëntie tijdens de tweede week na spenen met 22 % en 17 %, respectievelijk. In conclusie, toevoeging van eiwithydrolysaten aan het voer had geen effect op de dunne darm integriteit en groei van pasgespeende biggen. Toevoeging van glutamine aan het voer had geen effect op de vloklengthe, maar het verbeterde de groei in de tweede week.

Zoals beschreven in hoofdstuk 3 leidde het verhogen van de hoeveelheid lactose in het voer, ten koste van eiwit, tot langere darmvlokken in het voorste gedeelte van de dunne darm en tot een verminderd transport tussen de cellen. Om de specificiteit van lactose te onderzoeken zijn 3 experimentele speenvoeders geformuleerd die elk 24 % van de totale hoeveelheid energie in de vorm van glucose, lactose of tarwezetmeel bevatten (Hoofdstuk 6). De biggen zijn bemonsterd op 0, 3 en 10 dagen na spenen om dunne darm morfologie, orgaan gewichten, pH van de chymus en haptoglobine concentraties in het bloed te bepalen. De biggen kregen brijvoer. Biggen kregen een vooraf bepaalde, maximale hoeveelheid voer verstrekt om de verschillen in voeropname tussen biggen te verminderen. De groei, orgaan gewichten, darmmorfologie, pH van de chymus en plasma haptoglobine concentraties werden niet beïnvloed door de koolhydraatbron. Het speenproces zelf zorgde voor kortere darmvlokken en een verhoogde concentratie haptoglobine in het bloed. De pH in de chymus van de blinde en dikke darm werd lager na spenen. Geconcludeerd werd dat bij vergelijkbare voeropnames, lactose ten opzichte van glucose of zetmeel in het voer de afname in darmintegriteit niet verminderde.

De gespeende biggen uit Hoofdstuk 5 hadden gemiddeld gedurende 2 van de 7 dagen na spenen inconsistente mest. Mestconsistentie werd tweemaal daags gescoord, met een score die varieerde van 0 tot 3. Op dag 7 na spenen had 75 % van de biggen inconsistente mest. Omdat er relatief veel biggen inconsistente mest hadden, was de dataset erg geschikt om de correlatie tussen voeropname, mestconsistentie en dunne darm integriteit te berekenen. Darmintegriteit is gemeten aan de hand van aminopeptidase en isomaltase-sucrase activiteit, lengten van darmvlokken en diepten van crypten. De biggen zijn bemonsterd op dag 0, 3 en 7 na spenen. Data zijn gepoold voor voersamenstelling. Over dagen heen nam het gewicht van de maag, dikke darm en pancreas toe. Het gewicht van de dunne darm nam af van dag 0 tot 3 en nam weer toe op dag 7. Op dag 7 was het gewicht van de dunne darm hoger dan op de dag van spenen. Aminopeptidase en isomaltase-sucrase activiteit was lager op dag 3 en 7 dan op dag



0. De lengte van de darmvlokken nam ook af na spenen, gevolgd door een toename op dag 7 na spenen in het voorste gedeelte van de dunne darm, maar een verdere afname in het middelste gedeelte. Crypten werden dieper na spenen. De lengte van de darmvlokken was positief gecorreleerd met voeropname, borstelzoom enzymen en droge stof gehalte van de chymus, maar was negatief gecorreleerd met de mestconsistentie. Crypte diepte was positief gecorreleerd met het gewicht van verschillende delen van het maagdarmkanaal. Deze studie bevestigt dat de voeropname bij gespeende biggen de lengte van de darmvlokken en de productie van borstelzoom enzymen beïnvloedt. Vervolgens bepaalt de lengte van de darmvlokken en de productie van borstelzoom enzymen de kans op de ontwikkeling van diarree.

Op basis van de in dit proefschrift beschreven onderzoeksresultaten wordt geconcludeerd dat de dunne darm integriteit al na een dag na spenen verminderd is. De degeneratie van de dunne darm integriteit lijkt sneller op te treden in het voorste gedeelte van de dunne darm. Het herstel lijkt sneller op te treden in het middelste gedeelte van de dunne darm. De correlatie tussen de mestconsistentie en de lengte van darmvlokken is laag. De voeropname gedurende de eerste drie dagen na spenen is laag en variabel, onafhankelijk van de voersamenstelling. Het gelijkstellen van de vrijwillige voeropname gedurende de eerste week na spenen, om verstrengeling van het effect van voer opname en samenstelling op dunne darm integriteit te voorkomen, is moeilijk. Over het algemeen werd geconcludeerd dat het effect van voeropname op de dunne darm integriteit groter is dan het effect van voer samenstelling. Er is een multifactoriële aanpak nodig, die management van biggen, huisvesting en voeding combineert, om de speenproblematiek aan te pakken.



---

## LIST OF PUBLICATIONS

---

- Goelema, J. O., **M. A. M. Spreeuwenberg**, G. Hof, A. F. B. van der Poel, and S. Tamminga. 1998. Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. *Anim. Feed. Sci. Techn.* 76: 35-50.
- Huirne, R. B. M., **M. A. M. Spreeuwenberg**, S. C. Scheer, A. A. Dijkhuizen, and S. B. Harsh. 1996. Consistency in goals, information needs and risk management of Dutch dairy farmers. Pages 14-25 in *Farmers in small-scale and large-scale farming in a new perspective. Objectives, decision making and information requirements*. G. Beers et al., eds. LEI-DLO Publ. No. 143. Den Haag, The Netherlands.
- Spreeuwenberg, M. A. M.** and A. C. Beynen. Diet-mediated modulation of small intestinal integrity in weaned piglets. In *The Weaner pig: Concepts and Consequences*. J. Pluske, J. Le Dividich and M. W. A. Verstegen, eds. Wageningen pers, Wageningen, The Netherlands. Accepted for publication.
- Spreeuwenberg, M. A. M.**, R. B. M. Huirne, A. A. Dijkhuizen, and J. B. Wilmink. 1995. Stierkeuze en risicohouding. Hoe veranderen ze in de tijd? (Dutch). *Veeteelt*. 1190-1192.
- Spreeuwenberg, M. A. M.**, J. M. A. J. Verdonk, G. C. M. Bakker, and M. W. A. Verstegen. Effect of dietary protein source on feed intake and small intestinal morphology in newly weaned piglets. Submitted
- Spreeuwenberg, M. A. M.**, J. M. A. J. Verdonk, A. C. Beynen, and M. W. A. Verstegen. 2002. The effect of carbohydrate source on intestinal morphology of weaned pigs. *J. Anim. Sci.* 80 (Suppl. 1): 152 (Abstr).
- Spreeuwenberg, M. A. M.**, J. M. A. J. Verdonk, A. C. Beynen, and M. W. A. Verstegen. Villus height and gut development in weaned piglets receiving diets containing either glucose, lactose or starch. Submitted.
- Spreeuwenberg, M. A. M.**, J. M. A. J. Verdonk, A. C. Beynen, and M. W. A. Verstegen. Interrelationship between gut morphology and faeces consistency in newly weaned piglets. Submitted.
- Spreeuwenberg, M. A. M.**, J. M. A. J. Verdonk, H. R. Gaskins, and M. W. A. Verstegen. 2001. Small intestine epithelial barrier function is compromised in pigs with low feed intake at weaning. *J. Nutr.* 131: 1520-1527.
- Spreeuwenberg, M. A. M.**, J. M. A. J. Verdonk, J. F. J. G. Koninkx, A. C. Beynen, and M. W. A. Verstegen. 2002. Dietary protein hydrolysates versus the intact proteins do not enhance mucosal integrity and growth performance in weaned piglets. Submitted.
- Spreeuwenberg, M. A. M.**, J. M. A. J. Verdonk, and M. W. A. Verstegen. 2000. The effect of composition of liquid milk replacer at a low energy level on the small intestinal permeability of piglets after weaning. *J. Anim. Sci.* 78 (Suppl. 1): 137 (Abstr.).
- Verdonk, J. M. A. J., **M. A. M. Spreeuwenberg**, G. C. M. Bakker, and M. W. A. Verstegen. 2001. Nutrient intake level affects histology and permeability of the small intestine in newly weaned piglets. Pages 332-334 in *Digestive Physiology of Pigs*. J. E. Lindberg and B. Ogle, eds. Cabi Publishing, Wallingford, UK.
- Verdonk, J. M. A. J., **M. A. M. Spreeuwenberg**, G. C. M. Bakker, and M. W. A. Verstegen. 2001. Effect of protein source and feed intake level on histology of the small intestine in newly weaned piglets. Pages 347-349 in *Digestive Physiology of Pigs*. J. E. Lindberg and B. Ogle, eds. Cabi Publishing, Wallingford, UK.

- Verdonk .J. M. A. J., **M. A. M. Spreeuwenberg**, G. C. M. Bakker, Z. Mroz, and M. W. A. Verstegen. Gut integrity of piglets fed a diet in liquid and dry form. J. Anim. Sci. 80 (Suppl. 1): 197 (Abstr.).
- Verdonk, J. M. A. J., **M. A. M. Spreeuwenberg**, R. J. T. Rodenburg, R. C. A. Onderwater, and M. W. A. Verstegen. 2002. Nutrient intake affects in vivo interleukin 1 $\beta$  expression of the small intestine in pigs. Page 66 in Proc. 1st Nutrigenomics Conf. Noordwijk aan Zee, The Netherlands.

---

# NAWOORD

---

Klaar!!! Maar niet zonder een aantal mensen te bedanken voor de hulp én discussies én steun én gezelligheid. Zoals in menig dankwoord te lezen is sta je er niet alleen voor, maar je moet de kar wel trekken en vooral stug doorgaan.

Gedurende de afgelopen jaren is de begeleidingscommissie van samenstelling veranderd. Martin Verstegen, Anton Beynen, Coen Smits, Manfred Hessing, Han Verdonk, Gertruud Bakker en Joop Huisman bedankt voor jullie bijdrage.

Martin, gedurende de hele periode was je een rots in de branding. Ik mocht altijd binnen lopen, zelfs op je eigen schoenen! Je optimisme is aanstekelijk. Bedankt voor je enthousiasme en ik hoop dat we elkaar nog regelmatig zullen zien.

Anton, je was met name bij het tweede gedeelte van mijn promotie betrokken. We hebben intensief samengewerkt aan het afronden van de manuscripten en het bediscussiëren van proefopzetten. Het is plezierig om met je samen te werken. Bij het afronden van manuscripten wist je de zaken net wat krachtiger en beknopter te omschrijven. Met weinig woorden veel zeggen is een vak apart.

Manfred, je hebt dit onderzoeksproject opgezet en mij aangenomen om het uit te voeren. Later heeft Coen de directe begeleiding overgenomen. Coen, bedankt voor de discussies en voor de vele pagina's die je hebt doorgelezen en voorzien van opmerkingen. Ik wil jullie bedanken voor de mogelijkheid om tijd aan dit project te besteden. Nu heb ik alle tijd om me op andere onderzoeksprojecten te storten en daar heb ik ook erg veel zin in.

Han, als medepromovendus vanuit ID TNO Diervoeding was jij binnen dit project vaak mijn eerste aanspreekpunt. Om je nog extra voor de prettige samenwerking te bedanken heb ik je gevraagd om paranimf te worden.

I also would like to thank Rex Gaskins of the University of Illinois. We had fruitful discussions and we collaborated in an experiment. During my visit at the University of Illinois, you helped me writing my first paper. Finishing the first paper gave me a lot of energy.

En dan zijn er nog véééééle anderen die ik graag wil bedanken, zoals mijn collega's op het SRC. Jan, we hebben vaak een boom opgezet over hoe je gezondheid "eenvoudig" kan meten. Volgens mij zijn we er nog niet volledig uit, dus er is nog voldoende discussiestof. Tien, Jos(sen), Ben, Martien en Harry, bedankt voor de hulp tijdens de experimenten. Ellen bedankt voor de gezelligheid als kamergenote. Mijn vrienden en familie wil ik ook graag bedanken voor de betrokkenheid. Elke, wauw, ik vind de voorkant die jij samen met Bas met veel enthousiasme hebt gemaakt erg mooi. Je geeft daarmee de inhoud van het proefschrift een gezicht, bedankt daarvoor. Pap, je bent zelf jaren actief geweest in de mengvoederindustrie, daarom en omdat ik aan jou en mama veel te danken heb, ben ik trots dat je mijn paranimf bent. Als laatste natuurlijk Johan, lest best ...

---

## CURRICULUM VITAE

---

Maria Antoinette Martina Spreeuwenberg, Mirjam, is op 20 april 1973 geboren te Vierlingsbeek. Zij groeide op in Veghel. Aldaar behaalde zij aan het Zwijsen College in 1991 het VWO diploma. In 1991 begon zij met de studie Zoötechniek – specialisatie veevoeding – aan de Universiteit van Wageningen. In 1995 liep zij 4 maanden stage op de afdeling internationale marketing van Elanco Animal Health in Indianapolis, Amerika. In 1996 studeerde zij af in de Zoötechniek met als hoofdvakken Veevoeding, Agrarische Bedrijfseconomie en Toegepaste Wiskunde. Meteen na het beeindigen van haar studie begon ze als product manager bij Franklin Products International in Raamsdonksveer. Vanaf maart 1998 is zij werkzaam als onderzoeker biggenvoeding bij Nutreco te Boxmeer. In een samenwerkingsverband met ID TNO Diervoeding, Wageningen Universiteit en de Universiteit van Utrecht werden de proeven voor haar promotieonderzoek uitgevoerd. Zij zal haar werkzaamheden op het Swine Research Centre van Nutreco in Boxmeer voortzetten.

Mirjam Spreeuwenberg (Christian name: Maria Antoinette Martina Spreeuwenberg) was born on the 20<sup>th</sup> of April in 1973 in Vierlingsbeek, The Netherlands. She grew up in Veghel. In 1991 she graduated from secondary education. In the same year she started the study Animal Science at Wageningen University. In 1995 she went for 4 months to the U.S.A. for her practical training at the department of international marketing of Elanco Animal Health in Indianapolis. In 1996 she graduated for her masters after MSc research in Animal Nutrition, Agricultural Economy and Applied Mathematics. Immediately after her study she was appointed as product manager for Franklin Products International b.v. in Raamsdonksveer. In March 1998 she started as researcher for piglet nutrition at Nutreco, Boxmeer. In collaboration with ID TNO Animal Nutrition, Wageningen University and the University of Utrecht, the experiments for her Ph.D. were performed. She will continue her work as researcher at the Swine Research Centre of Nutreco in Boxmeer, The Netherlands.

Omslag: Elke van den Berg & Bas Kools  
Druk: Grafisch Bedrijf Ponsen & Looijen BV, Wageningen

**Abstract.** This thesis deals with the effects of dietary modulation of protein and carbohydrates under controlled energy intake on small intestinal integrity in weaned piglets. Small intestinal integrity is assessed on the basis of indicators regarding morphology (villus length, crypt depth), functionality (enzyme secretion and permeability across the gut wall) and inflammation (cell differentiation molecules on T-cell lymphocytes and haptoglobin levels in blood plasma). The piglets were offered a predetermined maximum amount of feed in order to prevent entanglement between the effect of feed intake level and the effect of diet composition itself on small intestinal integrity. The effectiveness of protein versus lactose was investigated by changing the ratio of protein to lactose in the diet. Results indicated that lactose seemed more limiting than protein for epithelial cells in contributing to mucosal integrity during the first days after weaning. Therefore the specificity of lactose as carbohydrate source was investigated by feeding the piglets either glucose, lactose or native starch. However, those carbohydrate sources did not differentially affect mucosal integrity. Furthermore, changing protein digestibility or predigestion of protein did not affect mucosal integrity. Across diets, degeneration of the small intestine occurred already after one day postweaning and seemed more pronounced in the proximal small intestine, regeneration during the second week postweaning seemed more pronounced in the mid small intestine. The association between measurements regarding faeces consistency and small intestinal morphology was poor. It was concluded that the effect of the weaning induced low energy intake is in general more pronounced than the effect of diet composition on small intestinal integrity.