Nutrition driven small-intestinal development and performance of weaned pigs and young broilers

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Thesis

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Abstract

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The relative importance of animal husbandry and nutrition during the first weeks after weaning in pigs and after hatch in broilers has increased considerably over the past 50 years as a result of the tremendous improvement in daily body weight (BW) gain. Substantial changes in weight, architecture, and physiology of the small intestine occur early in the life of these animals. The optimal function of the small intestines is fundamental for nutrient absorption from the diet and for health. Nutrient requirement studies conducted on these animals have largely overlooked the very young animal. It is therefore logical that there are still gaps in our knowledge of the nutrition of these animals during this particular stage of life. The objective of this thesis was to improve small-intestinal development and performance of pigs after weaning and young broilers by ways of an optimal nutrient composition of the diet. In experiments with broilers, it was shown that enhanced dietary ideal protein (IP) concentrations in the starter diet increased BW gain in the starter phase and in the subsequent grower phase. Moreover, the effects of enhanced IP concentrations in the starter diet on BW gain are more marked than the effects in the grower and finisher diets. However, BW gain hardly improved in response to dietary IP increment during the first 3 d after hatch, whereas in the consecutive 3 d, BW gain improved substantially with enhanced dietary IP concentrations. This suggests that the first 3 d after hatch, from a nutritional point of view, are substantially different from the next consecutive days in the life of broiler chicks. Moreover, a 30% increase in dietary IP increased the duodenum weight between 6 and 9 d of age. Thus, in young broilers, a greater relative small-intestinal weight is associated with a greater BW gain. However, this thesis did not make a clear determination of the functional changes of the small intestine after hatch in broilers. A review of the literature showed that after weaning in pigs, the barrier function of the tight junctions of the small intestine is disturbed, and transcellular barrier function seems to improve after weaning. In the first study with pigs, the data here showed that paracellular barrier functions, as measured with orally administered lactulose, did not correlate with bacterial translocation or transcellular barrier function, as measured with horseradish peroxidase in Ussing chambers. Therefore, it was concluded that lactulose recovery in the urine of pigs after weaning is not associated with risk factors for infection. The last study with pigs showed that dietary protein with dextrose stimulates mucosal weight after weaning. However, the combination of protein with dextrose had no substantial effect on small-intestinal barrier function, whereas dietary starch with dextrose improved small-intestinal barrier function. In conclusion, optimising protein nutrition in broilers after hatch has a great potential to further improve overall broiler performance. In particular, knowledge regarding optimal nutrition during the first 3 d after hatch is still lacking. Furthermore, dietary protein is a potent stimulator for growth of the proximal small intestine in broilers and of the small-intestinal mucosa in pigs. However, mucosal mass and luminal protein are of minor importance for small-intestinal barrier function in pigs after weaning. In contrast, the luminal carbohydrate supply or energy level is important for maintaining small-intestinal barrier function.

Voorwoord

Zoals voor bijna iedereen was ook voor mij het uitvoeren van een promotieonderzoek een loodzware taak met heel veel opofferingen en teleurstellingen maar ook (schaarse) momenten van euforie. Het uiteindelijke resultaat geeft een gevoel van voldoening en gepaste trots. Het voltooien van dit promotieonderzoek is vooral een eenzame weg geweest maar toch is voorafgaand aan dit proces en tijdens het onderzoek de hulp van anderen essentieel geweest om het uiteindelijk succesvol te kunnen afsluiten. Allereerst wil ik Henry en Pim bedanken omdat vooral zij mij gevormd hebben tot de onderzoeker die ik nu ben. Tijdens mijn eerste afstudeervak in Wageningen werd ik begeleid door Henry, wiens enthousiasme mede heeft bijgedragen aan het besluit om in het onderzoek verder te gaan. Gedurende mijn eerste jaren bij Provimi heeft Pim mij de fijne kneepjes bijgebracht van het voedingsonderzoek bij varkens en kippen. De grootste dank verdient echter Hink omdat hij mij heeft overgehaald om aan dit hele proces te beginnen, maar vooral ook omdat hij de mogelijkheid heeft gecreëerd om binnen Provimi dit promotieonderzoek te kunnen doen. Martin wil ik vooral bedanken voor de mentale ondersteuning op de momenten dat ik mogelijk wat te kritisch was op het geleverde resultaat en voor zijn onuitputtelijke correcties van de aangeleverde artikelen. Wouter wil ik bedanken voor de gedetailleerde aanwijzingen om de laatste puntjes op de i te zetten in de eindfase. Uiteraard verdienen ook alle coauteurs een woord van dank voor de aanwijzingen bij het wegwerken van de scherpe kantjes aan de artikelen. Het belangrijkste bij het uitvoeren van een promotieonderzoek is uiteraard het genereren van goede proefgegevens. Daarom wil ik iedereen die betrokken is geweest bij de uitvoering van de dierproeven bedanken. Extra aandacht hiervoor verdienen John en Esther voor hun inzet en toewijding bij het uitvoeren van respectievelijk de laatste biggen- en kuikenproef van dit onderzoek die wel heel erg intensief waren. Johan, jij bent denk ik de enige die bij de uitvoering van alle proeven van mijn promotieonderzoek betrokken is geweest. Bedankt voor je toewijding! Onder jouw leiding is iedere proef in goede handen. Jaap en Evelien jullie zijn fantastische collega's met een enorm enthousiasme voor het onderzoek. Bedankt voor de steun bij het opzetten, organiseren en uitvoeren van de proeven. Ik voel mij vereerd dat jullie mijn paranimfen willen zijn! Als laatste en belangrijkste wil ik een woord richten tot het thuisfront. Zonder jullie was ik zeker verstrikt geraakt in het web van het promotieonderzoek. Stan en Timo, jullie hebben zelfs je eigen stelling gekregen omdat jullie zorgden voor de broodnodige ontspanning. Tanja, voor jou is de opoffering voor mijn promotieonderzoek misschien nog wel groter geweest dan die van mij. Het was voor jou niet je eigen keuze en met twee jonge kinderen moest je meestal alles alleen regelen als ik weer "gezellig" op zolder zat. Bedankt dat je ondanks dat al die tijd een luisterend oor had zodat ik mijn hart kon luchten om vervolgens weer een stapje dichter bij het eindresultaat te komen. We hebben nog heel wat verloren tijd in te halen samen!

Table of Contents

General Introduction

At the end of the 19th century, similar breeds of domestic fowl were used for both meat and egg production. Over the first half of the 20th century, a few synthetic breeds of fowl were developed and separate strains were gradually selected for meat production and for egg laying[1]. During the same period, the central organisation of pig breeding was being developed: however, it remained largely focused on selection within a single breed. Consequently, breeding efforts to improve the performance of pigs had limited effects[2]. The second half of the 20th century saw a tremendous increase in the performance level of meat-type animals, including broilers and pigs, especially due to improvements in their genetic backgrounds. For broilers, the time from hatching to a body weight (BW) of 1815 g decreased from 101 d in 1957 to 32 d in 2001[3]. Likewise, but to a lesser extent, the average daily weight gain in pigs nearly doubled between the beginning of the 20th and 21st centuries (Table 1) with the majority of improvements occurring after 1950[2]. Consequently, for pigs, the length of time from birth to a BW of 120 kg decreased from about 340 d in 1900 to 170 d in the year 2000. These findings demonstrate that the relative importance of the first weeks of life has tripled for broilers and has doubled for pigs over a period of 44 and 100 years, respectively. As such, the relative importance of animal husbandry and nutrition during the first few weeks subsequent to weaning or hatching has increased considerably over the past 50 years. This process is likely to continue over the next several decades.

Table 1. Development of animal growth in the 20th century

Decade	Average daily gain, g	
	Broilers (hatch–1815 g)[3]	Pigs (20–120 kg)[2]
1900s	–	500
1950s	18	600
2000s	55	950

Coinciding with improved performance, production-related diseases and disorders have increased significantly, particularly in broilers[4]. For instance, broilers have become more susceptible both to ascites as well as leg-problems[5-7]. Baghbanzadeh and Decuypere[8] have stressed that controlling excessive growth of broilers during early stages of development is paramount to reducing the incidence of metabolic disorders. From their work it can be hypothesised that the basis for metabolic disorders seen in broilers may lie early in development when their relative growth rate is at its maximum. It has been suggested that early embryonic[9] or post-hatching[10] organ development are important for subsequent growth and organ function. Therefore, inadequate organ development during early growth stages may directly relate to the occurrence of metabolic diseases later in life and as such the first weeks after hatch seem to be critical for health of the broilers up to market age.

In pigs, the most severe health problems that arise early during development are related to the process of weaning. In nature, piglets gradually learn to eat solid food, and weaning is a gradual process that is completed at an age of about 10 wk[11]. In most pig-producing countries, the weaning age has decreased from about 8 wk in the 1950s to around 3 to 4 wk today[12]. Weaning pigs at such a young age is associated with abrupt social, environmental, and dietary changes, resulting in high levels of stress[13]. Studies show that concentrations of cortisol and corticotrophin-releasing factor in the blood of pigs increase markedly after weaning[14,15]. Moreover, about ten percent of pigs do not ingest any feed during the first 48 h after weaning[16], and most other pigs have low intake of feed. Weaning is associated with

a variety of health problems, and after weaning, pigs are highly susceptible to enteric diseases and infections[13,17]. In general, the detrimental effects of weaning on the health and performance of pigs are more severe when pigs are weaned at 3 wk or earlier than if the pig is 4 wk or older. This is best illustrated with data from studies using Ussing chambers, which measured transepithelial electrical resistance (TEER) and mannitol flux over the small intestine. There is an increase in mannitol flux and a concurrent decrease in TEER when the paracellular barrier function of the small intestine is disturbed. Moeser *et al.*[18] showed that mannitol flux and TEER over the mid-jejunum were similar at 1 d after weaning compared with unweaned controls in pigs weaned at 4 wk of age. However, in pigs weaned at 3 wk of age, Moeser *et al.*[14,18] and Boudry *et al.*[19] reported that the mannitol flux over the proximal or mid-jejunum was significantly increased and that TEER was significantly decreased at 1 and 2 d after weaning compared to weaning or compared to unweaned controls. In line with the parracellular barrier function, active nutrient absorption is also more affected after weaning when pigs are weaned at 3 wk compared to 4 wk of age. In the study by Boudry *et al.*[19], pigs were weaned at 3 wk of age. These authors showed that Na^+-dependent glucose absorption increased transiently in the proximal jejunum at 2 d after weaning and decreased at 15 d after weaning compared to pre-weaning absorption. Moreover, in the same study, ileal glucose absorption decreased at 2, 5, and 15 d after weaning. In line with this, Smith[20] found that the Na^+-dependent alanine uptake by enterocytes of the mid-small intestine (measured with a rapid-uptake-apparatus using radiolabelled tracer amino acids) decreased considerably at 5 d after weaning at 2 or 3 wk of age. Furthermore, in 4-wk-old pigs, Na^+-dependent alanine uptake by enterocytes of the mid-small intestine was lower for weaned pigs (5 d after weaning, thus weaning at 23 d) than for unweaned pigs[21]. However, the alanine uptake of 6-wk-old pigs (both weaned and unweaned) was similar to the alanine uptake of 4-wk-old weaned pigs[21]. This study suggests that weaning before 4 wk of age and ageing appear to decrease the number of enterocytes that are involved in active alanine uptake[21]. The studies described above show that active small-intestinal absorption of nutrients decreases after weaning when pigs are weaned between 14 and 23 d of age. However, when pigs are weaned after 4 wk of age, active absorption is not affected by the weaning process. Miller *et al.*[21] observed that the decreased absorption rates after weaning also occur in unweaned pigs, but over a much longer time course. This suggests that the decrease in active absorption is part of a maturation process that is enhanced by the process of weaning. The small intestine of pigs is still maturing at 3 wk of age, and weaning at a young age may aggravate the negative effects observed at weaning at a later age. Therefore, the conditions (environmental, nutritional, and social) during the first week after weaning are very important for the health of pigs, and in this regard the weaning age is an important factor.

The most obvious anatomical changes during the first week after hatch in broilers and during the first week after weaning in pigs occur in the small intestine. In broilers, the relative weight of the small intestine increases from about 2% at hatch to about 8% at 8 d after hatch[22]. Subsequently, the relative weight of the small intestine decreases again. Other digestive organs do not show similar increases in relative size during the first week after hatch. Weaning results in villous atrophy and a reduced crypt-cell production rate[23]. The lowest villous height and deepest crypts are generally found at 3–5 d after weaning, and the effect on crypt dept is slower than on villous height. At about 11 d after weaning, these parameters are largely restored to pre-weaning values. In the gastrointestinal tract, and especially in the small intestine, feed constituents are hydrolysed and degraded into simple

molecules or compounds (i.e. small peptides, amino acids (AA), free fatty acids, and monosaccharides). Subsequently, these molecules are absorbed and transported predominantly through the blood circulation, but may also be used by the gut wall. At the same time, the small intestine has to function as a barrier to resist harmful bacteria, toxins, and allergic compounds from entering the body[24]. This barrier is a combination of a chemical/physical defence (i.e. gastric acid, mucus and epithelium with tight junctions between cells) and immunological defence (i.e. secretory IgA and several types of leucocytes)[24,25]. As discussed above, the paracellular barrier function of the jejunum may be compromised during the process of weaning in pigs[14,19,26]. Moreover, oedema disease, caused by the shiga-like II variant toxin from some *Escherichia coli* strains, is associated with the process of weaning and requires a disrupted intestinal barrier function in order for the large toxin molecules to pass through the intestinal epithelium[27]. Thus, the small intestine undergoes large changes after hatch and weaning, and has a key function in both the supply of nutrients to the body and in maintaining health. In consideration of these points, it is inevitable that many health problems occur in these animals or have their origin during this phase of life when the small intestine is rapidly developing or degenerating. Moreover, in early life, pigs and broilers are subjected to abrupt changes in the macronutrient composition of their "diets". Early in life, these animals use the fatty acids and AA in egg yolk and milk as the main energy yielding substrates. In addition, the lactose in milk is one of the primary energy sources for piglets. In comparison, glucose from complex carbohydrates is the main energy yielding substrate in the diet of chicks and pigs after this period. Thus, in addition to the structural and functional changes of the small intestine, the small intestine has to adapt to substantial changes in nutrient supply.

Starvation reduces villous height and villous surface area in young broilers[28,29]. Geyra *et al.*[29] showed that the effects of starvation on villous height were more pronounced during the first days after hatch than from 6 to 8 d after hatch. In addition, it has been determined that the decrease in the total small intestine weight, mucosal weight, and villous height after weaning is more severe and the recovery is slower when very low levels of feed intake occur after weaning[26,30-32]. The intestinal barrier function and development of the intestinal absorptive capacity depends on a sufficient supply of nutrients from the diet[33,34]. This is also observed in pigs after weaning. Verdonk[26] measured the mannitol flux in the mid-jejunum of pigs with a low vs. high intake level of milk replacer after weaning. The mannitol flux was not affected in pigs with a high feed intake level at 1, 2, and 4 d after weaning. However, mannitol flux was increased in pigs with low feed intake levels at 2 and 4 d after weaning compared to pre-weaning. On average over d 1, 2, and 4, this resulted in a higher mannitol flux for the low intake pigs compared to the high intake pigs (at least 2.5 times energy maintenance). In line with this, a 2 d fast of 23-d-old pigs increased transepithelial electrical conductance (the opposite of TEER)[35]. Thus, sufficient feed intake after weaning prevents loss of barrier function of the tight junctions after weaning. From the above findings, some scientists have concluded that at low feed intake levels, it is specifically the low energy intake that causes the compromised small-intestinal barrier function and architecture. However, protein is the main energy source and AA are the main building blocks for the small intestine. Therefore, one can hypothesise that specifically a shortage of dietary protein, but not energy, may be the major cause of the reduced mucosal integrity and barrier function of the small intestine. This hypothesis is supported by some data in the literature. Enhanced lysine concentrations in the starter diet of broilers increased duodenal villous height at 21 d of age[28]. Moreover, the addition of glutamine (1 or 4%) in the diet of

broilers increased small-intestinal villous height at 7, 14, and 21 d of age[36,37]. Reduction of protein concentrations in broiler starter diets from 23% (maize-soybean meal diet) to 14% (maize-starch-crystalline AA diet) reduced jejunal villous height and small-intestinal weight at 7 d of age[38]. These authors reported no differences in small-intestinal weight at 21 d of age, whereas villous height remained lower for the 14% protein diet at 21 d of age compared to the 23% protein diet. In agreement with these findings, Swatson *et al.*[39] showed that differences in dietary protein concentrations or an AA imbalance had no consistent effect on small-intestinal weight at 24 d of age. However, the AA imbalance reduced the villous height. Reduction of protein concentrations in pig diets after weaning (23% vs. 17% protein in the diet) reduced duodenal and jejunal villous height at 2 wk after weaning[40]. Supplementation of glutamine to weaner diets increased villous height in the duodenum and increased mucosal weight in the proximal jejunum at 14 d after weaning[41], and increased ileal villous height at 4 wk after weaning[42,43]. Moreover, Wu *et al.*[44] reported that dietary glutamine supplementation increased jejunal villous height at 7 d after weaning, whereas villous height at 14 d after weaning was only numerically increased. In addition, duodenal villous height at 7 and 14 d after weaning was increased after dietary glutamine supplementation, whereas jejunal and ileal villous height was only numerically increased[45]. Finally, glutamine and glutamate increased villous height in the proximal, mid, and distal small intestine at 7 d after weaning, and to a lesser extent at 14 d after weaning[46]. Supplementing the diet of weaned pigs with tryptophan tended to increase villous height in the distal small intestine[47]. Threonine deficient diets reduced villous height in the ileum but not in the jejunum of young pigs after weaning[48,49]. Moreover, in the latter study[49], threonine deficiency increased the ileal permeability for fluorescein isothiocyanate dextran and also tended to decrease TEER, showing that the paracellular barrier function was disturbed. The above studies indicate that small-intestinal weight and architecture are sensitive to dietary AA supply, especially during the first 2 wk after hatch or weaning. Moreover, one study with pigs indicates that dietary AA may also be important for small-intestinal barrier function[49].

There have been few reported studies that have investigated the nutrient requirements of very young animals (first weeks after hatch or weaning). This may be related to the feed costs during this phase, which are relatively low compared to the total feed costs from the time of hatch or birth to slaughter. In addition, in pigs, feed intake stimulation and health improvement after weaning are without a doubt the most important aspects of nutrition after weaning. Therefore, studies have mainly focused on ingredient composition to improve feed intake and health, with less attention focused on the nutrient composition of the diet. The importance of the small intestine for animal performance and health was emphasised above, and particularly in young animals, this organ is subjected to many changes. Therefore, nutrient requirement studies during these phases in broilers and pigs should also focus on the development of the different organs and their functionality, with special interest on the small intestine.

The objective of this thesis is to improve intestinal development and performance of pigs after weaning and young broilers by optimising the nutrient composition of the diet. The first aim is to gain more insight into the anatomical and functional changes of the small intestine after hatch in broilers and after weaning in pigs. The second aim is to investigate the effect of the level of feed intake and the effect of protein nutrition on performance, small-intestinal development, and health in broilers after hatch and pigs after weaning. For broilers, the studies reported in this thesis concentrate on the further improvement of

performance and suppression of metabolic disorders in high performing birds. For pigs after weaning, the focus is on the optimal nutritional support for intestinal barrier and absorption functions of pigs at low levels of feed intake.

Table 2. Most important parameters of the different animal studies of this thesis

Chapter (specie)	Primary parameters			
	Performance	Mortality	Mucosa or small intestine weight	Intestinal barrier and absorptive function
2 (broilers)	X			
3 (broilers)	X	X	X	
5 (pigs)				X
6 (pigs)			X	X

Chapter 1 provides a review of the normal growth of intestinal villous in chicks and pigs and the development of the small intestine in broilers after hatch and in pigs around weaning. In addition, it describes various nutritional factors that can affect the development of the small intestine during these critical phases. The latter may contribute to the development of strategies that optimise small-intestinal development and function for these animals. **Chapter 2** reports the effect of dietary AA concentrations on performance of broilers during the starter, grower, and finisher phase (Table 2), and further develops our understanding of the relative importance of the starter phase with respect to AA nutrition. Subsequently, in **Chapter 3**, the effect of AA nutrition in broiler starter diets on performance is studied in more detail. Moreover, the effect of AA nutrition and feed intake restriction on small-intestinal weight and mortality is also reported, and further insight regarding the importance of the small-intestinal weight with respect to broiler performance and health is provided. **Chapter 4** provides a review of the effect of weaning and dietary treatments after weaning on intestinal barrier function and absorption, which allows the development of nutritional strategies to aid weaning disorders. **Chapter 5** reports the change in small intestine barrier function over time, as measured by lactulose, and the relationship with putative causes of infection and disease. The lactulose recovery in urine is compared with bacterial translocation as well as with macromolecular transport of horseradish peroxidase in Ussing chambers. This study further develops our understanding of health disorders after weaning in relation to small-intestinal barrier function. In **Chapter 6**, the effect of non-protein energy or protein intake immediately after weaning on the intestinal mucosal mass and permeability in the first week after weaning is reported. This study clarifies the role of dietary protein supply in small-intestinal atrophy and barrier function in pigs after weaning. Finally, in the **General Discussion**, the conclusions from Chapters 1–6 are aligned and discussed in the context of optimal early life nutrition, and the practical implications and suggestions for further research are provided.

Chapter 1

Intestinal development in chicks after hatch and in pigs around the time of weaning: a review

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Submitted

Abstract

This review describes small-intestinal development after hatch in broilers and around the time of weaning in pigs. In broilers, the relative small-intestinal weight and villous height increase rapidly during the first week after hatch. After the first week, the relative weight decreases gradually, but the villous height continues to increase. At 4 d after weaning, villous height in pigs decreases to about 60% of the pre-weaning height. Two weeks after weaning, this recovers to similar values as in unweaned control animals independent of weaning age. Small-intestinal development after weaning and after hatch consistently deteriorates at low feed intake levels. Suboptimal protein nutrition is the predominant factor that depresses small-intestinal development. This is more pronounced during the first 2 wk after hatch or weaning than later in life. These findings stress the importance of applying an optimal nutritional strategy in these phases of life to reach optimal small-intestinal development.

Keywords: *feed intake, intestinal weight, protein nutrition, villous height.*

Introduction

In the gastrointestinal tract, and especially in the small intestine, feed constituents are hydrolysed and degraded into simple molecules (i.e. free small peptides, amino acids (AA), free fatty acids, and monosaccharides). Subsequently, these molecules are absorbed and may be used by the gut wall, but are mainly transported via blood circulation. At the same time, the small intestine has to function as a barrier to resist harmful bacteria, toxins, and allergic compounds[(24)]. This barrier is a combination of chemical/physical defence (i.e. gastric acid, mucus, and epithelium with tight junctions between cells) and immunological defence (i.e. secretory IgA and several types of leucocytes)[(24,25)]. The small intestine is the largest immunological organ in the body[(50)]. Thus, the small intestine has a key function both in the supply of nutrients to the body and in disease prevention. Early in life, chickens and piglets use fatty acids and AA as the main energy yielding substrates in egg yolk and milk. Moreover, lactose in milk is also available as an energy source for piglets. Thereafter, glucose from complex carbohydrates is the main energy yielding substrate in the diet of chicks and pigs. In addition, chicks and piglets are subjected to several other stressors (a.o. intensive handling, transportation, and separation from the mother) that coincide with these nutritional changes. Because of the dietary changes and the other stressors occurring

in this phase of life, it is a critical period for development and for adaptation of the small intestine to the new environment. The intestinal barrier function and development of the intestinal absorptive capacity depends on a sufficient supply of nutrients from the diet[33,34]. Moreover, intestinal barrier function may be further compromised in response to the different stressors. Hence, an adequate development of the intestines can be at risk in chicks after hatch and in pigs after weaning. An inadequate intestinal development will result in negative effects on body development and health. With the increasing growth rates for pigs and broilers, the time from birth or from hatch to slaughter has decreased considerably and this will most likely continue over the next several decades. Because of this, the relative effect of growth shortly after weaning or shortly after hatch is becoming increasingly important in respect to the economical performance of pigs and broilers.

This review aims to describe the normal growth of intestinal villi in chicks and pigs and the development of the size of the small intestine in broilers after hatch and in pigs around weaning. Villi height and small intestine weight are relatively easy to measure, and therefore there is ample information on this topic in the literature. These parameters can be used as rough indicators of the digestive and absorptive capacity of the small intestine. In addition, this review describes various nutritional factors that can affect the development of the small intestine in these critical phases. This may be useful to develop strategies that optimise small-intestinal development and function in this critical phase.

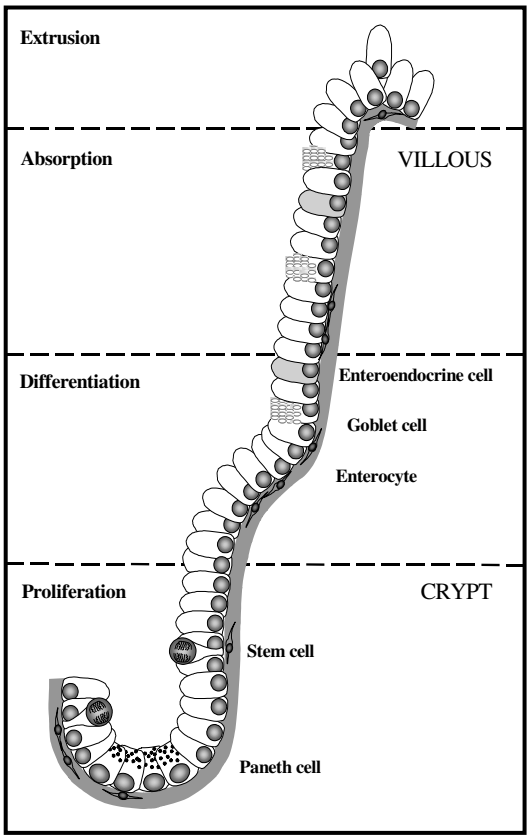

Figure 1. Architecture of a villous and crypt, from Van 't Land[51].

Villous growth: specie differences and effect of nutrition on growth rate

A single layer of epithelial cells covers the small-intestinal villous and provides the interface between the gut wall and intra-luminal chime constituents (Figure 1). This layer is covered with mucus and has to sustain the balance between (1) absorption of nutrients and (2) prevent harmful substances from entering the body. Stem cells that divide (proliferate) and generate new cells are located in the crypts. After division, the cells either migrate to the bottom of the crypt or to the top of the villous. The cells that migrate to the crypt are differentiated into Paneth cells (cells secreting antimicrobial polypeptides) or into enteroendocrine cells (cells producing serotonin, enteroglucagon, or gastrin). The cells that migrate to the villous differentiate into enteroendocrine cells, goblet cells (mucus producing cells), or absorptive enterocytes. During the migration of the enterocytes to the top of the villous (2–10 d), microvilli develop. Transport proteins that are essential for the active absorption of nutrients are synthesised and inserted into the luminal cell membrane. Moreover, at the top of the villi, digestive enzymes are produced. Immature cells have no absorptive or digestive abilities, and

therefore most absorption of nutrients occurs at the tips of the villi. The migrating cells push older cells to the villous tip where they are extruded into the lumen[51-53].

Under normal conditions, proliferation of cells is restricted to the crypt base. In neonatal mammals and in birds shortly after hatching, however, all epithelial cells, including villous cells will proliferate[54,55]. Moreover, as a reaction to refeeding, the majority of jejunal crypt and villous cells can proliferate after a period of fasting, which allows for quick villous recovery[29,56,57]. The renewal of the entire small-intestinal epithelium takes 7–10 d in 1 d old pigs[58], about 8 d in 14 d old pigs[59], 2–4 d in 3 wk old pigs[58], and 2–3 d in 7 wk old pigs[60]. In 1–2 d old chicks, the renewal of the small-intestinal epithelium takes 2–5 d[55,61-63]. In contrast to pigs, the lifespan of enterocytes in chickens increases as they age[55,61,63]. As an example, only 75% and 50% of the small-intestinal epithelial cells were renewed over a period of 5 d in 3 wk and 6 mo old chickens, respectively[63]. It is striking that the enterocyte lifespan in broilers seems to increase with age, whereas the enterocyte lifespan in pigs seems to decrease with age. This is in agreement with findings that villous height increases with age in broilers and decreases with age in pigs (see also sections below).

Enterocyte lifespan in rats was not affected by dietary protein concentration (50 vs. 200 g protein/kg). Moreover, enterocyte lifespan in pigs was not affected by a 50% reduction in dietary energy content[60]. Starvation for 2 or more days decreases proliferation and migration rate of enterocytes in both rats and chickens[29,34,57]. In line with this, total parenteral nutrition (no luminal feed supply) in pigs decreases enterocyte proliferation and migration rates[64]. Refeeding after a period of starvation or malnutrition increases enterocyte proliferation and migration rates[29,65]. In addition, a period of starvation up to 24 h has no effect on enterocyte proliferation or migration rate[66]. These findings show that nutritional status itself can affect villous growth in respect to cell proliferation and migration rate and most likely does not affect enterocyte lifespan very much. However, the latter has been tested with relatively smaller contrasts between nutritional treatments.

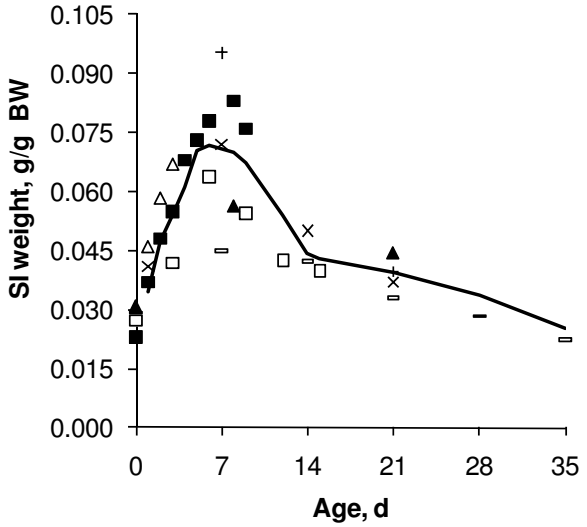

Figure 2. Small-intestinal (SI) weight relative to body weight (BW) after hatch based on data of 7 different literature sources: □[67], ▲[68], ■[69], ×[61], +[38], Δ[70], and –[71].

Intestinal development of chicks after hatch

Small intestine weight

The relative weight of the small intestine increases from about 2.5% of BW at hatch to about 7% at 1 wk after hatch (Figure 2). Other digestive organs do not show similar increases in relative size during the first week after hatch[22]. A higher relative small intestine weight of broilers is associated with higher growth, indicating that the small intestine facilitates rapid growth[72]. After the first week of life, the relative weight of the small intestine decreases rapidly to about 4.5% of BW at 2 wk of age and decreases gradually thereafter to 3% at 5 wk of age. The pattern of development of the small intestine given in various studies reported in the literature is rather similar. However, the relative

weight at 1 wk of age varies largely between different studies reported in the literature, with the extremes at 4.5%[71] and 10%[38]. These differences in reported relative weights may be related to the strain of chickens used and to environmental differences (i.e. diet composition, diet form, feeding strategy, ambient temperature, and microbial challenge) and to the methodology of the determination of the empty intestine weight. The latter is often not well described. Literature regarding the separate description of weight development over time of the duodenum, jejunum, and ileum is scarce. Data on poults showed that the development of the duodenum and the jejunum was similar between the time of hatch and 12 d of age, and also showed that the weight increase of the ileum was less pronounced within that same time frame compared to that of the duodenum and jejunum[73].

In conclusion, the chick gives a high priority to the development of the small intestine during the first week of life. This conclusion underscores the importance of this organ to the newly hatched chick.

Villous development

Several studies have shown that villous height and villous volume increase rapidly during the first week after hatch. After the first week, villi height continue to increase with age[63,74]. This increase in villi height is most marked in the jejunum and ileum. The age at which the villi height stops increasing has not be studied in detail, however it seems to continue to increase to at least the age at which birds are slaughtered (5–6 wk)[74]. These studies show that both the height and volume of the villi increase rapidly after hatch. In the duodenum, the villous volume has increased 2-fold at 7 d of age compared with hatch, and stabilises thereafter[75,76]. The villous volume in the jejunum and in the ileum increases 3-fold from hatch up to at least 10 d of age[75,76]. Others have shown that villous height continues to increase after 10 d of age[63,74]. These authors found that villous height in the duodenum increased by 30% between 7 and 21 d of age[74] and again by 30% between 21 d and 6 mo of age[63]. In the jejunum, the villous height increases by 75% between 21 d and 6 mo of age[63]. Furthermore, the villous height increases by 55% between 7 and 21 d of age in the ileum, by 37% between 21 and 42 d of age[74], and by 72% between 21 d and 6 mo of age[63]. In line with these findings, the villi height at 21 d of age in the duodenum, jejunum, and ileum had somewhat higher values than the height at 14 d of age[61].

In conclusion, villous height in chicks continues to increase for a much longer period than the relative small intestine weight. This most likely enables maturing birds to efficiently absorb nutrients while the relative small intestine weight and length decrease.

Effect of nutritional status on small-intestinal development

There are a limited number of studies in the literature that have investigated the effect of diet composition on small-intestinal development of broilers within a short time after hatch. A few studies have focused on micro-ingredients in the diet, such as enzymes and minerals. Dietary xylanase supplementation had no effect on duodenal and jejunal villous height in 7 and 21 d old broiler chicks[77,78]. However, dietary xylanase supplementation reduced the relative weight and length of the duodenum, jejunum, and ileum at 21 d of age[77]. This reduction in weight may be the adaptive response of the small intestine that is related to an improvement in digestion of the diet due to the enzyme supplementation. Consequently, the bird can change its digestive capacity. Moreover, part of the effect of xylanase is

believed to be related to a reduction in the microbial activity in the small intestine of the bird[79]. The bird may respond to this by reducing the activity of the local immunological function, which can result in a lower weight of the mucosa and submucosa. Supplementation of chelated-Se to breeder hen and broiler diets was shown to increase villous height in the duodenum, jejunum, and ileum of 21 d old broilers[80]. These authors hypothesised that the chelated-Se reduced oxidative stress, and as a result, the lifespan of enterocytes was increased, which resulted in increased villous height. Most studies reported in the literature have focused on the effects of macro-nutrients, such as protein, fat, and glucose, or the effect of feed restriction. Starvation depresses proliferation and migration of epithelium cells (see above) and reduces villous height and surface area in young birds[28,29]. Moreover, Geyra *et al.*[29] showed that the effects of starvation on villi heights were more pronounced during the first days after hatch than from 6–8 d after hatch. In line with this, jejunal villous height tended to decrease with a 25% feed intake restriction in 24-d-old broilers[39]. In contrast, the jejunal villous height increased in 6-wk-old broilers after 24 h of starvation[66]. These authors hypothesised that broilers increased the villous height to maximise later potential absorption capacity. Enhanced lysine concentrations in the starter diet increased duodenal villous height at 21 d of age[28]. Noy and Sklan[81] varied dietary protein concentrations (18, 23, and 28%) but maintained indispensable AA concentrations (lysine, methionine + cystine, and threonine) at similar concentrations in all diets. They reported no difference in relative small-intestinal weight at 7 d of age. This may indicate that with respect to small-intestinal development one of the indispensable AA is limiting. The dietary addition of glutamine (1 or 4%) increased small-intestinal villi height at 7, 14, and 21 d of age[36,37]. Reduction in dietary protein concentration in the starter diet from 23% (maize-soybean meal diet) to 14% (maize-starch-crystalline AA diet) reduced jejunal villous height and small-intestinal weight at 7 d of age[38]. These authors reported no differences in small-intestinal weight at 21 d of age, whereas villous height remained lower with the 14% protein diet at 21 d of age compared to the 23% protein diet. In agreement with these findings, Swatson *et al.*[39] showed that differences in dietary protein concentrations or an AA imbalance had no consistent effect on small-intestinal weight at 24 d of age. However, the AA imbalance had reduced the villous height. Thus, these studies[38,39] indicate that at 1 wk of age, the small intestine is more sensitive to differences in dietary protein concentrations than at 3 wk of age, and that villous height is more sensitive than small-intestinal weight. Maneewan and Yamauchi[82] showed that dietary protein is more important than fat or fibres for villi recovery after a 3 d period of feed withdrawal. However, in another study, both low protein (12.6 vs. 23.1%) and low fat (4.3 vs. 11.0%) depressed relative small-intestinal weight at 5 d of age[83]. The dietary addition of egg powder (15%) or glucose syrup (20%), for 1 or 2 d showed no consistent effects on duodenal, jejunal, and ileal villi heights at 7 and 21 d of age[84]. Moreover, intubation of starch, casein, or soybean oil within a very short time after hatch with the subsequent *ad libitum* supply of feed from 24 h onwards showed no differences at 3 and 21 d of age for the relative duodenum, jejunum, and ileum weights[85]. The lack of an effect in the latter 2 studies[84,85] may be related to 3 different factors. First, the application period of the treatment (24 or 48 h) may have been too short. Second, the tested ingredients may not have had a clear effect on small-intestinal development. Finally, the period between treatment application and intestinal measurements may have been too long.

In conclusion, malnutrition or suboptimal nutrition deteriorates small-intestinal development, especially in young birds. This appears to be particularly true in the case of

malnutrition of AA. However, studies with nutrients other than AA are very limited. Thus, firm conclusions on the effects of these other nutrients on small-intestinal development cannot be drawn. With respect to AA, glutamine and indispensable AA seem to be especially important for small-intestinal development.

Intestinal development of pigs around the time of weaning

Small-intestinal development before weaning

The relative weight of the small intestine and the small-intestinal mucosa decreases between a few days after birth until about 21 d of age (Figure 3a and 4a). Villous height continues to decrease until 28 d of age (Figure 5a). Cera *et al*.[86] showed that after 21 d of age, the relative weight of the small intestine increases again in suckling pigs, but not in piglets that are weaned. The piglets in that trial had access to creep feed before weaning. In general, piglets start to eat considerable amounts of creep feed after 3 wk of age. The increased small-intestinal weight after 3 wk of age in the trial of Cera *et al*.[86] may therefore be related to an increased intake of creep feed. Creep feed is less digestible than sow milk and may therefore require a higher intestinal motility that stimulates muscular development. In addition, feed intake and the associated increased microbial challenge can stimulate the development of immunological function and the digestive glands and tissues that are located in the mucosa and submucosa layers. Therefore, the weight of these layers may be increased as well. In conclusion, small-intestinal villous height and mucosa weight have been shown to already decrease before weaning, indicating that this process is part of the natural development of the small intestine.

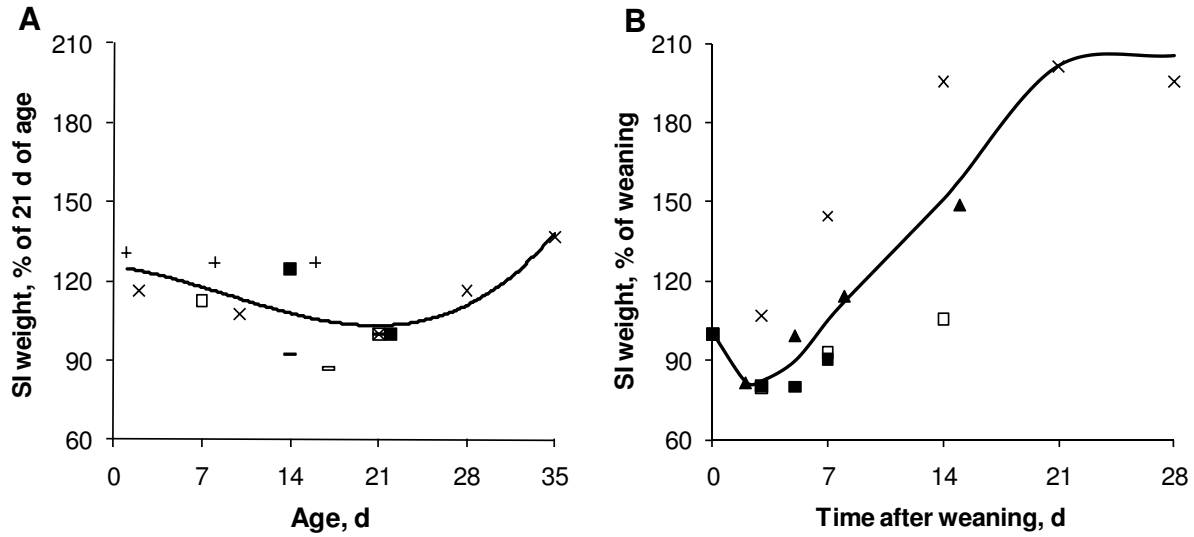

Figure 3. Small intestine (SI) weight relative to body weight before weaning (relative to 21 d of age, panel A) and after weaning (relative to weaning age, panel B) based on data of 5 different literature sources: + & –[87], ×[86], ■[88], □[31], and ▲[89].

Small-intestinal development after weaning

The relative small-intestinal weight decreases to about 80% of the pre-weaning weight at 2–3 d after weaning (Figure 3b). Subsequently, the small intestine weight recovers rapidly to pre-weaning level 1 wk after weaning and continues to increase to about 200% of the pre-

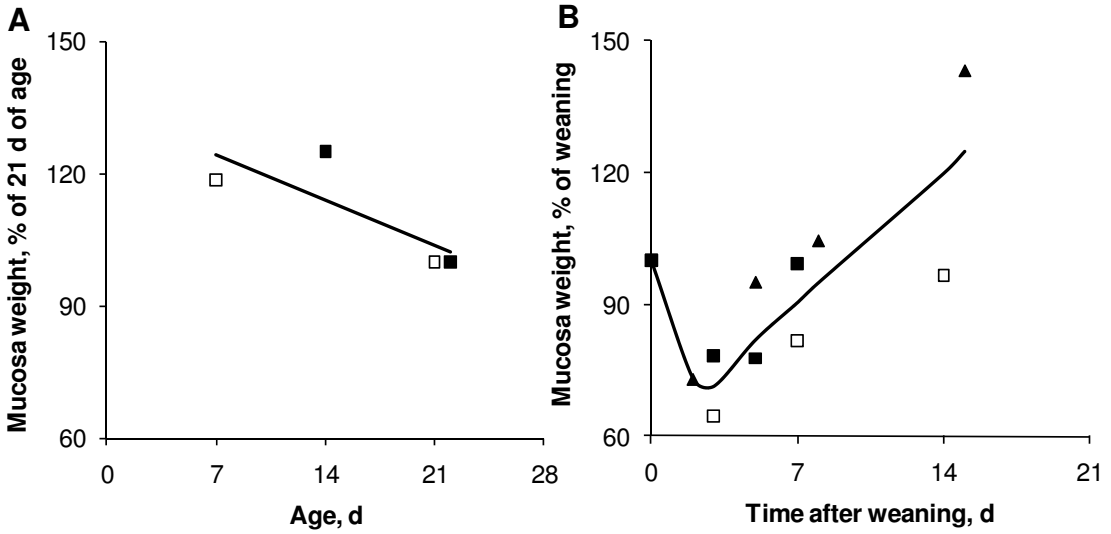

Figure 4. Small-intestinal mucosa weight relative to body weight before weaning (relative to 21 d of age, panel A) and after weaning (relative to weaning age, panel B) based on data of 3 different literature sources: ■[88], □[31], and ▲[89].

weaning level at 3 wk after weaning. In agreement with the hypothesis before weaning, this rapid increase of the small intestine weight is probably related to the consumption of solid feed. The consumption of solid feed may increase the muscularis, submucosa, and mucosa weight of the intestine. The villous height in the mid-small intestine drops rapidly after weaning to about 60% of the pre-weaning level between 3 and 5 d after weaning (Figure 5a). When pigs are weaned at 1 wk of age, the villous height has been shown to decrease to 60% of the pre-weaning level 3 d after weaning, and remains at this level for up to 2 wk after weaning[31]. When the pigs are weaned at 3 wk of age, the small-intestinal weight had recovered to about 80% of the pre-weaning level 2–3 wk after weaning[86,88]. When pigs are weaned at 4 wk of age, villous height recovers to pre-weaning values about 2 wk after

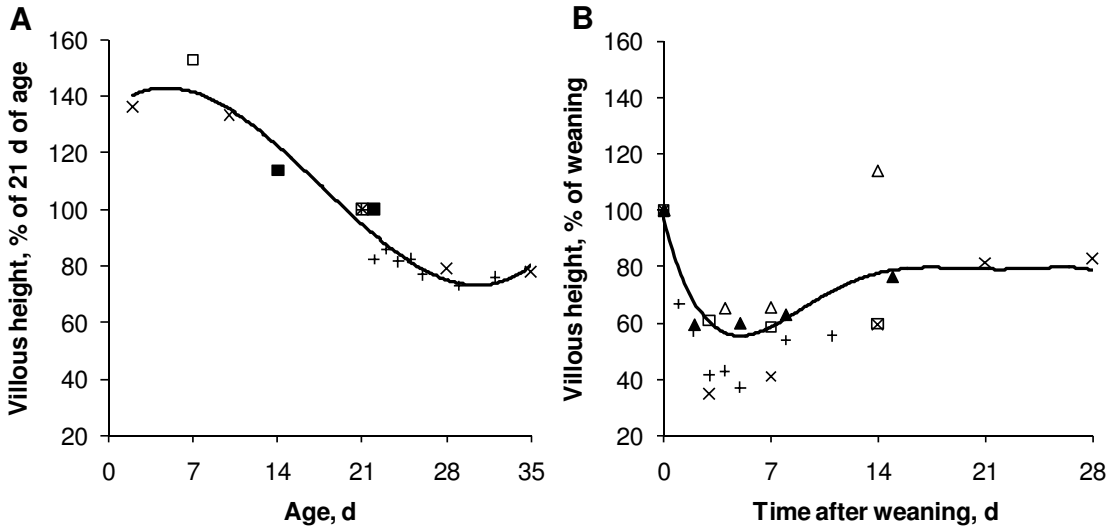

Figure 5. Villous height in the small intestine of unweaned pigs (relative to 21 d of age, panel A) and after weaning (relative to weaning age, panel B) based on data of 5 different literature sources in the mid-small intestines: +[90] ×[86], ■ [88], □[31], Δ[26], and one source in the proximal small intestine: ▲[89].

weaning (Figure 5b)[26]. Therefore, this suggests that that at all weaning ages (1, 3, or 4 wk), the villous height at about 2 wk after weaning is similar to the villous height of unweaned counterparts of the same age. Thus, it appears that pigs weaned before 4 wk of age do not completely recover from villous atrophy due to the process of weaning, whereas pigs do recover from villous atrophy when weaned at 4 wk or thereafter. However, this could be misleading, because this is most likely due to a reduction of villous height, which is part of a natural intestinal maturation process that also occurs in unweaned pigs up to 4 wk of age. In conclusion, the severity of villous atrophy after weaning is similar for pigs weaned between 1 and 4 wk of age, taking into account the natural decrease in villous height that occurs at this age.

Effect of nutritional status on small-intestinal development

It has been determined that the decrease in the total small intestine weight, mucosal weight, and villous height after weaning is more severe and the recovery is slower with very low levels of feed intake after weaning[26,30-32]. Starvation has similar effects on villous height in both rats and chickens[29,56]. The fast decrease of mucosa weight and villous height after weaning is therefore most likely due to starvation or low feed intake. Verdonk[26] showed that the mean jejunal permeability for mannitol at 1, 2, and 4 d after weaning was higher in pigs fed a low level of milk replacer compared to pigs fed a high level of milk replacer. In contrast, this author and co-workers found that jejunal permeability for mannitol was not affected by the feed intake level of a pelleted diet[91]. This shows that although feed intake level after weaning consistently affects small-intestinal architecture, the effect on intestinal barrier function is less consistent. Page *et al.*[34], hypothesised that additional stress is needed in addition to starvation (as a food stressor) to compromise intestinal barrier function. Several authors have reported that the reduction of villous height due to low feed intake after weaning is more pronounced in the proximal than in the distal small intestine[31,89]. Stoll *et al.*[92] reported that protein synthesis in the proximal small intestine is more dependent on luminal nutrient supply than protein synthesis occurring in the distal small intestine. This may explain the more pronounced reduction of villous height in the proximal compared to the distal small intestine with very low levels of feed intake. Vente-Spreeuwenberg and Beynen[93] concluded from their review that soybean meal vs. milk protein, skimmed milk powder, fish meal, or hydrolysed soybean meal reduced villous height or had no effect on villous height. Moreover, dietary feather meal reduced villous height when compared with skimmed milk powder[94]. Thus, these findings show that some raw materials that are generally believed to have a negative effect on the performance and health of pigs after weaning can reduce intestinal villous height. The addition of spray dried plasma, dietary fibre, calcium formate, and sodium butyrate to the diet after weaning had no consistent effects on villous height after weaning[93,95]. All of these components are in general believed to be positive for the health and performance after weaning but have no clear effect on villous recovery or prevention of villous atrophy. Reduction in dietary protein concentration after weaning (23 vs. 17% protein in the diet) reduced duodenal and jejunal villous height at 2 wk after weaning[40]. However, other studies that applied a similar change in dietary protein concentration in the weaning diet showed no clear effect on small-intestinal villous height at 3 wk after weaning[96,97]. Supplementation of extra glutamine to weaner diets increased villous height in the duodenum and increased mucosal weight in the proximal jejunum at 14 d after weaning[41] and increased ileal villous height at 4 wk after weaning[42,43]. Moreover, Wu *et al.*[44] reported that dietary glutamine supplementation

increased jejunal villous height at 7 d after weaning, whereas villous height at 14 d after weaning was only numerically increased. In addition, duodenal villous height at 7 and 14 d after weaning increased after dietary glutamine supplementation, whereas jejunal and ileal villous height was only numerically increased[45]. Finally, glutamine and glutamate increased villous height in the proximal, mid, and distal small intestine at 7 d after weaning and to a lesser extent at 14 d after weaning[46]. The supplementation of tryptophan to the diet of weaned pigs tended to increase villous height in the distal small intestine[47]. Moreover, threonine deficient diets reduced villous height in the ileum but not in the jejunum of young pigs after weaning[48,49].

In conclusion, small-intestinal mucosal atrophy is strongly related to the feed intake level after weaning. The data on the effects of different raw materials and bioactive dietary ingredients on mucosal architecture are limited and inconsistent. In contrast, dietary protein concentrations and specific AA have a significant and consistent effect on mucosal architecture after weaning. These effects are most pronounced in the proximal small intestine and seem to be most marked in the first week after weaning.

Villous height differences over time for broilers vs. pigs

In the previous sections, studies were discussed that showed that the villous height of broilers increases with age and the villous height of pigs decreases with age. This contradiction most likely relates to differences in enterocyte lifespan that increase in broilers and decreases in pigs over time (Table 1). It seems that broilers experience a rapid increase in the small-intestinal mass at young age in order to support the rapid increase in BW. However, when broilers get older, the small-intestinal mass decreases, and concomitantly small-intestinal absorptive capacity is refined with a higher absorptive capacity per mass unit, as indicated by the higher villi. This enables the birds to obtain a lower weight compared to mammals, which is especially important for birds during flight. In addition, the retention time of the digesta in the small intestine is shorter, the pH of the stomach (proventriculus and gizzard) is higher, and there is more reflux of the digesta in the small intestine of broilers compared to pigs. These conditions in the small intestine of broilers may be less favourable for pathogenic bacteria to adhere to the lumen wall to colonise. Consequently, this may reduce the risk of infections and subsequent inflammations in broilers compared to pigs.

Table 1. Enterocyte lifespan of broilers and pigs at different ages

	Enterocyte lifespan, d	
Age	Broilers[55,61-63]	Pigs[58-60]
1–2 d	2–5	7–10
2 wk	–	8
3 wk	7	2–4
7 wk	–	2–3
6 mo	10	–

Local or systemic inflammations have consistently been shown to be related to a reduction in small-intestinal villous height[98-100]. Moreover, the type of bacteria in broiler and pig small intestines are probably different. Different bacteria may have a different direct stimulating or suppressing effect on villous growth. For example, it has been shown that dietary probiotics can affect small-intestinal villous heights[99,101]. Thus, in addition to a different evolutionary background, villi may grow higher in broilers than in pigs due to differences in

bacterial challenges or differences in bacterial stimulation of villous growth between broilers and pigs.

Conclusions

The relative small-intestinal weights and villous heights increase rapidly in broilers during the first week after hatch. After this week, the relative weights decrease gradually, but the villous heights continue to increase. Thus, the chick gives a high priority to the development of the small intestine during the first week of life. This underscores the importance of this organ to the newly hatched chick, and if this development can be improved, it may be beneficial for its performance and health. After weaning, the villous heights in pigs decrease to about 60% of the pre-weaning level 4 d after weaning. Two weeks after weaning, the villous height recovers to a similar value as in unweaned control animals, independent of the weaning age. Thus, the intestinal architecture is largely affected by weaning, but the severity of this effect seems to be unrelated to the weaning age. Small-intestinal villous heights increase in broilers and decrease in pigs with age. This contradiction may relate to the need of flying birds to have highly efficient nutrient absorption at a low intestinal mass. Moreover, it may also relate to differences in bacterial and immunological stimulation or suppression of villous growth between broilers and pigs. Small-intestinal development after weaning and after hatch consistently deteriorates at low feed intake levels. Moreover, suboptimal protein nutrition depresses small-intestinal development, which is more pronounced during the first 2 wk after hatch or weaning than later in life. Thus, small-intestinal weights and villous heights are sensitive to nutritional differences in recently hatched birds and in recently weaned pigs. This underscores the importance of an optimal nutrient supply in these periods of life for optimal small-intestinal development. The importance of protein in this regard should be especially studied in detail. Explicit information with regard to the optimal protein intake and optimal AA composition of the protein is missing with respect to intestinal development. Better insight into the relationship between AA nutrition and small-intestinal development should lead to more balanced nutrition in young farm animals.

Chapter 2

Effects of different dietary ideal protein concentrations on male and female broiler performance during different phases of life: single phase effects, carryover effects, and interaction between phases

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Abstract

Several experiments in which the dietary ideal protein (IP) concentrations were increased indicate that with current IP recommendations the maximum performance of broilers will not be achieved. However, available data of this IP-increment approach is scarce and, for the starter phase, entirely lacking. The objective of the present study, therefore, was to generate data regarding the effects in the starter phase and to test the impact of adequate vs. high IP concentrations in preceding phases on the response to IP increment in the phase under study. To evaluate this, an IP dose response in the starter phase and factorial arrangements combining adequate or high IP concentrations in starter and grower diets with low, adequate, or high IP concentrations in finisher diets were carried out with male and female broilers. Enhanced dietary IP concentrations in the starter diet increased BW gain in the starter phase and in the consecutive grower phase. Moreover, it was shown that a delay in BW gain due to suboptimal IP concentrations in the starter diet could only be partly compensated for in later phases of life. These results demonstrate a need for a reevaluation of IP concentrations used in practical starter diets. Gain to feed ratio and BW gain responses to increased IP concentrations in the grower and finisher diets were less pronounced when high compared with adequate IP concentrations were fed in the preceding phase. This difference in response could not be detected statistically but was consistent between experiments and phases. Therefore, this phenomenon should not simply be neglected.

Keywords: age, broiler, ideal protein, sex, age, phase.

Introduction

Besides energy, amino acids (AA) are the most critical dietary factors determining feed costs and performance in the broiler industry. Therefore, it is of considerable financial importance to continuously increase our knowledge of broiler requirements for AA.

In the 1950's, Almquist[(102)] established that growth rate depends on the intake of the first limiting (indispensable) AA. Animal performance, furthermore, improves in response to supplementation of that AA in the diet up to the concentration that the second

indispensable AA becomes limiting. This theory has been fully accepted and is used in feed formulation practice.

It is understood that requirements of poultry for AA are influenced by dietary, environmental, and genetic factors. The ideal protein (IP) concept has been developed, in which AA requirements are often expressed as ratio to Lys, leading to an ideal AA profile in which all indispensable AA are equally limiting. This approach was first implemented in pigs[103,104]. Until now, the optimum concentration of this profile in the diet has been established mainly based on studies designed according to the graded supplementation technique in which Lys was increased in diets deficient in Lys[105-109]. Recent studies[110-112] showed, however, that increasing IP concentrations in male broiler grower and finisher diets improves broiler performances up to higher Lys concentrations than was expected based on studies using the graded supplementation technique. Furthermore, Eits *et al.*[110] showed that gain to feed ratio (G:F) and BW gain responses to increased dietary IP concentrations are similar for male and female broilers during the grower phase (11–26 d), whereas, during the finisher phase (26–41 d) responses were of a higher magnitude for male broilers.

These data from experiments using the IP-increment approach strongly indicate that with current IP recommendations, the maximum performance of broilers will not be achieved. Compared to the tremendous amount of data available regarding the graded supplementation technique, however, data with the IP-increment approach is scarce, and data for the starter phase is even entirely lacking. Hence, an effort has to be made to generate a substantial amount of data to estimate the full potential of this approach.

The impact of protein concentrations [either a specific AA, crude protein (CP), or IP] in preceding phases, on the response to protein increment in the phase under study is generally neglected in requirement studies. It has been reported, however, that low protein concentrations in preceding phases enhance the response to protein in the phase studied[110,113,114]. In general, however, these studies are designed with deficient vs. high protein concentrations in preceding phases and the effect of adequate vs. high protein concentrations remains unknown.

The objective of the present study was to generate more data with the IP-increment approach regarding the effects in the starter phase and to test the impact of adequate vs. high IP concentrations in preceding phases, on the response to IP increments in the phase under study. To evaluate this, an IP dose response in the starter phase and factorial arrangements combining adequate[109] or high IP concentrations in starter and grower diets with low, adequate, or high IP concentrations in finisher diets were carried out with male and female broilers

Materials and methods

Birds and Housing

Two consecutive experiments were performed in the same broiler unit. The unit consisted of 2 rooms with 36 cages each. Birds were housed in battery cages (1.1 m^2) with raised wire floors. Feed and water were provided *ad libitum*. Temperature, relative humidity, and ventilation were computer controlled. Temperature decreased by 2.5^oC per week, from 34^oC at the day of arrival (1-d-old chicks) to a final temperature of 21^oC at 37 d of age during the trial. Lights were on 23 h/d. Birds were spray-vaccinated against Newcastle disease immediately after hatching and at 20 d of age.

Nine hundred fifty 1-d-old male and 950 female chicks (for experiment 1) and 1800 male

chicks (for experiment 2) of a commercial broiler strain (Ross 308) were purchased and weighed individually. Chicks weighing between 38 and 48 g for experiment 1 and between 35 and 45 g for experiment 2 were randomly assigned to a cage; chicks outside these ranges were not used in the trials. The experiments commenced with 20 birds per cage with similar average initial weights of 43 g/chick in experiment 1 and 40 g/chick in experiment 2. In experiment 1, birds were housed with 1 sex per cage and sexes were evenly divided over both rooms.

Experimental Design and Diets

In both experiments, the effects of different dietary IP concentrations were studied. Experiments were divided into starter (0–14 d of age), grower (14–30 d of age), and finisher phases (30–37 d of age).

In experiment 1 (Table 1), in the starter and grower phase, adequate[109] or high (20% above recommendation) IP concentrations were fed according to a 2 × 2 factorial arrangement. In addition, 2 treatment groups were fed 10 to 30% above recommendation in the starter diet and adequate IP concentrations in the grower diet. All birds were fed an adequate IP diet in the finisher phase. Treatments receiving an adequate IP concentration in the grower phase, after graded dietary IP concentrations in the starter phase comprised experiment 1A, whereas the treatments of the 2 × 2 factorial arrangement comprised experiment 1B. Consequently, the treatment receiving adequate IP concentrations in the starter and grower phase and the treatment receiving a high IP concentration in the starter and an adequate IP concentration in the grower phase were integrated in both experiments.

In experiment 2 (Table 1) adequate or high (20% above recommendation) IP concentrations in the starter and grower phase and low (10% below recommendation), adequate (recommended), or high (10% above recommendation) IP concentrations in the finisher phase were fed according to a 2 × 2 × 3 factorial arrangement.

Table 1. Experimental design of experiment 1 and experiment 2

	Dietary ideal protein (percentage of recommendations[1])			
Treatment	Starter	Grower	Finisher	Experiment
Experiment 1				
1	100	100	100	1B/1A
2	100	120	100	1B
3	120	100	100	1B/1A
4	120	120	100	1B
5	110	100	100	1A
6	130	100	100	1A
Experiment 2				
1	100	100	90	2
2	100	100	100	2
3	100	100	110	2
4	100	120	90	2
5	100	120	100	2
6	100	120	110	2
7	120	100	90	2
8	120	100	100	2
9	120	100	110	2
10	120	120	90	2
11	120	120	100	2
12	120	120	110	2

[1]Recommendations of Schutte[109].

Table 2. Ingredient composition (g/kg) of the experimental diets

	Experiment 1					Experiment 2					
	Starter		Grower		Finisher	Starter		Grower		Finisher	
AFD[1] Lys, g/kg	10.5	13.7	10.2	12.2	9.9	10.5	12.6	10.2	12.2	8.9	10.9
Maize	309.5	405.4	292.5	353.0	362.4	375.2	452.2	307.1	370.5	326.3	401.0
Soybean meal	215.0	279.5	246.3	295.6	259.8	232.0	278.4	246.9	296.3	247.3	302.3
Soy isolate	34.3	44.5	19.6	23.5	10.2	3.3	3.9	10.2	12.2		
Wheat	76.9	100.0	125.0	150.0	130.0	83.3	100.0	125.0	150.0	122.7	150.0
Potato protein	26.9	35.0	25.0	30.0	30.0	29.2	35.0	25.0	30.0	18.3	22.4
Fish meal	30.8	40.0	16.7	20.0		33.3	40.0	16.7	20.0		
Soybean oil	33.0	33.0	38.4	38.4	35.0	14.7	14.7	30.2	30.2	30.6	30.6
Animal fat	10.0	10.0	40.0	40.0	40.0	20.0	20.0	40.0	40.0	40.0	40.0
Monodicalcium phosphate	12.4	10.4	11.5	10.4	12.0	11.7	10.3	11.3	10.2	12.5	11.7
Ground limestone	17.2	16.6	12.5	12.1	12.5	16.8	16.2	12.3	11.9	12.5	12.3
Sodium bicarbonate	0.6	0.2	1.3	1.2	2.0	2.4	2.5	1.9	1.9	2.7	2.9
Potassium bicarbonate	1.3										
L-Lysine-HCL (80%)	0.7	1.0	1.1	1.3	1.3	1.6	1.9	1.6	2.0	1.8	2.2
DL-Methionine	2.3	3.0	2.3	2.7	2.1	2.3	2.8	2.4	2.8	2.0	2.5
L-Threonine			0.1	0.1							0.1
L-Arginine	0.3	0.4	0.3	0.4	0.3	1.2	1.4	0.9	1.1	0.6	0.8
Sodium chloride	1.5	1.0	1.3	1.1	1.7	1.0	0.6	1.2	0.9	1.6	1.4
Maize starch	183.7	5.0	127.5	5.0	74.0	138.5	5.0	131.3	5.0	142.7	5.0
Cellulose (Arbocel B 800)	16.8	2.5	14.2	2.5	8.4	11.8	2.5	13.1	2.5	14.2	2.5
Diamol GM	16.8	2.5	14.2	2.5	8.4	11.8	2.5	13.1	2.5	14.2	2.5
Vitamin-mineral premix[2]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0

[1]AFD = apparent faecal digestible.

[2]Contributed per kg diet: riboflavin, 5 mg; niacinamide, 40 mg; d-pantothenic acid, 12 mg; choline chloride, 500 mg; cyanocobalamin, 15 µg; vitamin E (dl-α-tocopheryl acetate), 15 IU; menadione, 5 mg; vitamin A (retinyl-acetate), 10,000 IU; cholecalciferol, 50 µg; biotin, 0.1 mg; folic acid, 0.75 mg; $FeSO_4.H_2O$, 200 mg; MnO_2, 100 mg; $CuSO_4.5H_2O$, 60 mg; $ZnSo_4.H_2O$, 150 mg; Se (organic), 0.15 mg; KI, 1 mg; $CoSO_4.7H_2O$, 1 mg; antioxydant (ethoxyquin), 100 mg.

Table 3. Nutritional composition of the experimental diets

	Experiment 1					Experiment 2					
	Starter		Grower		Finisher	Starter		Grower		Finisher	
AFD[1] Lys, g/kg	10.5	13.7	10.2	12.2	9.9	10.5	12.6	10.2	12.2	8.9	10.9
Calculated composition, g/kg[2]											
$AME_{n\ (broilers)}$, MJ/kg	11.92	11.92	12.55	12.55	12.55	11.92	11.92	12.55	12.55	12.55	12.55
P (available)	4.5	4.5	4.0	4.0	3.8	4.5	4.5	4.0	4.0	3.8	3.8
K	7.3	8.9	7.5	9.0	7.9	7.4	8.9	7.5	9.0	7.4	9.0
Na	1.7	1.7	1.5	1.5	1.5	1.7	1.7	1.5	1.5	1.5	1.5
Cl	1.8	1.8	1.6	1.6	1.6	1.8	1.8	1.6	1.6	1.6	1.6
AFD amino acids											
Lys	10.5	13.7	10.2	12.2	9.9	10.5	12.6	10.2	12.2	8.9	10.9
Met + Cys	7.9	10.2	7.7	9.2	7.4	7.9	9.5	7.6	9.2	6.7	8.2
Thr	6.6	8.6	6.4	7.7	6.3	6.6	8.0	6.4	7.7	5.6	6.9
Trp	2.2	2.9	2.2	2.6	2.2	2.2	2.6	2.2	2.6	1.9	2.3
Ile	8.0	10.4	7.7	9.2	7.6	7.5	9.0	7.3	8.8	6.4	7.9
Val	8.9	11.5	8.5	10.2	8.5	8.5	10.2	8.3	9.9	7.2	8.8
Arg	11.8	15.3	11.4	13.7	11.1	11.8	14.1	11.4	13.7	10.0	12.2
Gly + Ser	15.5	20.2	14.9	17.9	14.6	15.6	18.7	15.2	18.3	13.3	16.3
Analysed composition, g/kg											
CP	208	272	204	245	202	208	245	204	242	180	218
Crude fat	64	72	101	104	98	61	63	93	98	98	94
Ca	10.5	10.0	7.9	7.9	7.6	10.9	10.8	9.1	8.9	8.6	8.4
P	6.3	7.0	6.2	6.6	6.3	6.5	6.9	6.3	6.6	6.0	6.5
Lys	12.5	16.2	12.1	14.8	12.1	12.9	15.4	12.7	15.1	11.1	13.2
Met + Cys	8.7	11.5	8.9	10.5	8.8	8.5	10.3	8.7	10.2	7.5	8.7
Thr	8.3	10.8	8.3	9.9	8.0	7.5	8.8	7.2	8.7	6.4	7.6
Trp	2.6	3.3	2.6	3.0	2.5	2.4	2.8	2.4	2.8	2.1	2.6
Ile	8.9	11.5	9.0	10.4	8.6	8.4	10.2	8.5	10.1	7.3	8.7
Val	10.3	13.1	10.2	12.0	9.9	9.4	11.4	9.5	11.4	8.2	9.7
Arg	13.8	17.9	13.7	16.5	13.3	13.9	16.7	14.0	16.7	12.2	14.5
Gly + Ser	19.0	24.6	18.7	22.3	18.1	17.9	21.1	17.4	21.1	15.5	18.5

[1]AFD = apparent faecal digestible.

[2]Based on amino acid, N and dry matter analyses of raw materials and chemical composition, digestibility and energy value for broilers of the CVB Livestock Feed Table[(115)]. $AME_{n\ (broiler)}$ content of maize starch was estimated at 13.37 MJ/kg and zero for cellulose and diamol.

In both experiments, adequate IP concentrations met the IP recommendations of Schutte[109], being 10.5 g of apparent faecal digestible (AFD) Lys/kg in the starter, 10.2 g of AFD Lys/kg in the grower and 9.9 g of AFD Lys/kg in the finisher diets. This represents the recommendations of the Central Bureau for Livestock Feeding (CVB)[115]. The recommendations are widely used in commercial operations in the Netherlands and other West European countries and they are approximately 10% higher than NRC[116] recommendations.

For each experiment, battery cages were assigned to the treatments according to a randomised block design (block = room), with 6 cages of male broilers and 6 cages of female broilers per treatment in experiment 1, and 6 cages with male broilers per treatment in experiment 2.

For each experiment, batches of maize, wheat, soybean meal, soy isolate, fish meal, and potato protein were reserved and analysed for AA[117], N (Dumas)[118], and dry matter content. Based on these analyses and on digestibility and nutrient data provided by CVB[115], the starter, grower, and finisher diets containing the highest IP concentrations (Table 2) were formulated first for each experiment. The diets with the lowest IP concentrations (Table 2) were formulated by diluting the high IP diets with a protein-free mixture. This mixture contained maize starch, inert materials (cellulose: Arbocel B 800, J.Rettenmaier & Sohne GmbH + Co, Rosenberg, Germany and silica: Diamol GM: Franz Bertram GmbH, Hamburg, Germany), soybean oil, animal fat, and all necessary minerals and vitamins. All diets were calculated to be isocaloric within each phase and adequate in all nutrients, except CP and AA (Table 3). The ratios between digestible Lys and all other indispensable digestible AA met or exceeded the recommendations of Mack *et al.*[108] for Met + Cys (0.75), Thr (0.63), Trp (0.19), Ile (0.71), Val (0.81), and Arg (1.12) and the recommendations of Schutte[109] for Met (0.38) and Gly + Ser (1.43). The diets with intermediate IP concentrations for the starter phase of experiment 1 (11.55 and 12.6 g AFD Lys/kg) and the finisher phase of experiment 2 (9.9 g AFD Lys/kg) were prepared by blending the highest and lowest IP diet for the specific phase and experiment. After mixing, the diets were pelleted (2.5 mm) with steam addition. All diets were analysed for AA[117], N (Dumas)[118], crude fat (6-h extraction with petroleum-ether), ash (incineration at 550^{o}C for 4 h), Ca (ISO 6490.2), and P (ISO 6491) content.

Data Collection

In both experiments, for each cage all birds were weighed together at the start and were weighed individually at 14, 30, and 37 d of age, and BW gain for each phase was calculated. In addition, feed consumption for each cage was recorded during each phase. Feed spillage at the research accommodation was extremely low because birds could only access the feeding troughs with their heads. Based on gain and feed consumption, G:F was calculated as grams of live weight gain per gram of feed consumed.

At 37 d of age, 5 birds per cage (birds being nearest to the average cage weight) were deprived of feed (for approximately 10 h) and slaughtered the next day. The birds were successively weighed, euthanised by cervical dislocation (method approved by the Ethical Committee of the Erasmus University, Rotterdam, The Netherlands), and bled, and the feathers were removed. After removal of the feathers, the birds were excised. The weight of the carcass (whole bird without feathers, blood, organs, intestines, head, and legs below the hock) was determined and carcass yield was calculated as percentage of the feed-deprived bird weight (whole bird weight before slaughtering). In addition, the breast weight

(pectoralis major, pectoralis minor, sternum, and clavicle), and the abdominal fat pad weight (included fat surrounding the gizzard) were determined and their yields were calculated as percentage of the carcass weight.

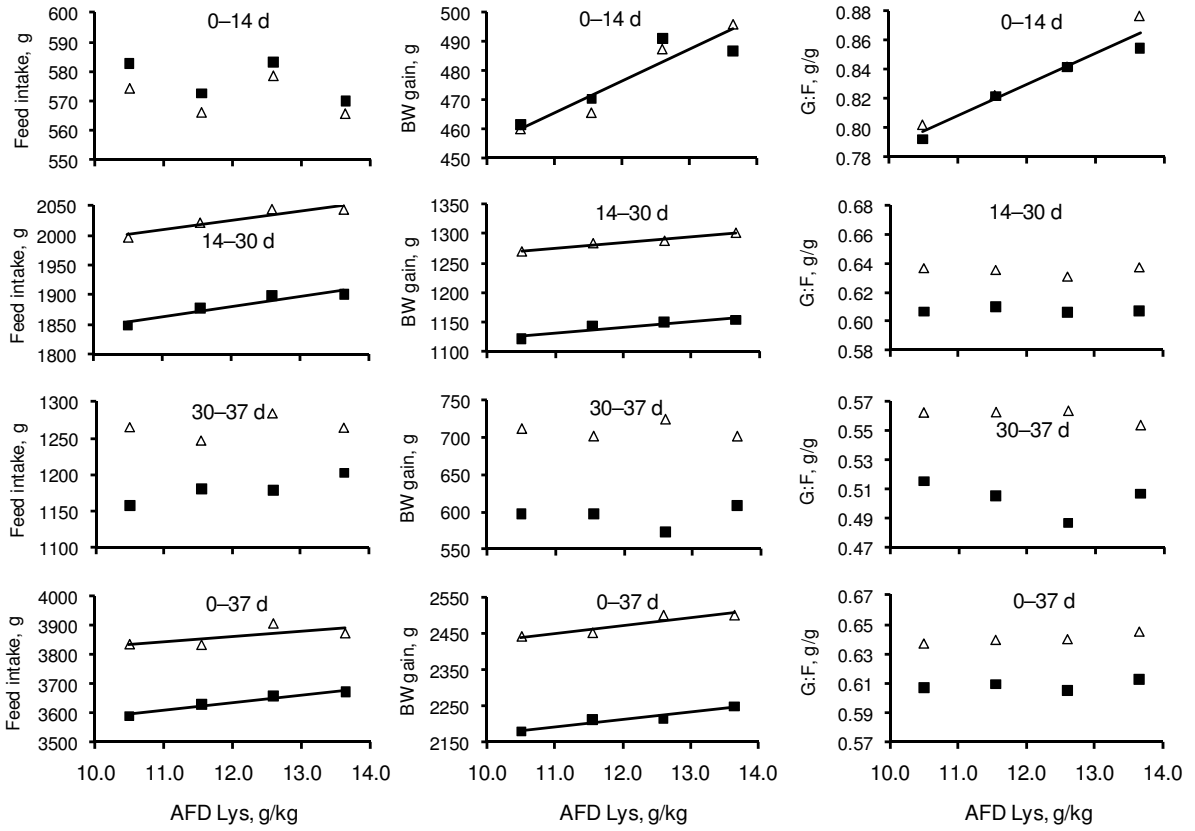

Figure 1. Feed intake, BW gain, and gain to feed ratio (G:F) of male (Δ) and female (■) broilers as a function of ideal protein concentrations [expressed as apparent faecal digestible (AFD) Lys, g/kg] in the starter diet (0–14 d). Regression lines are displayed when significant ($P \leq 0.05$, Table 4), experiment 1A.

Statistical Analyses

A linear model was fitted to treatment means for all data of experiment 1A. The model included AFD Lys concentration (g/kg) in the starter diet, sex effect, and the interaction between AFD Lys and sex. However, the interaction for none of the parameters was significant and therefore omitted from the model. In addition, the sex effect for BW gain, feed intake, and G:F of the starter period were not significant and were omitted from the model. All data from experiment 1B and experiment 2 were subjected to ANOVA according to the following statistical models:

$$\textit{(experiment 1B)}\ Y_{ijklm} = \mu + R_i + S_j + G_k + SX_l + S{\times}G_{jk} + S{\times}SX_{jl} + G{\times}SX_{kl} + e_{ijklm},$$

$$\text{(experiment 2)}\ Y_{ijklm} = \mu + R_i + S_j + G_k + F_l + S{\times}G_{jk} + S{\times}F_{jl} + G{\times}F_{kl} + e_{ijklm},$$

where Y = variance associated with a parameter; μ = overall mean; R = room; S = IP concentration starter diet; G = IP concentration grower diet; F = IP concentration finisher diet; SX = sex; S×G, S×SX, G×SX, S×F, and G×F are the interactions between the different factors; and e = the residual error term. Differences between the 3 IP concentrations of the finisher phase of experiment 2 were analysed for significance ($P \leq 0.05$) using the Student-Newman-Keuls test[119]. In both experiments, cages were treated as experimental units. Effects were considered significant for $P \leq 0.05$. The sixth edition of Genstat for Windows[120], was used to analyse all data.

Table 4. Intercept and slope (SE in parentheses) estimates for the linear responses[1] of BW gain, feed intake, gain to feed ratio (G:F), and slaughter yields of male and female broilers[2] to dietary ideal protein concentrations (expressed as apparent faecal digestible Lys, g/kg) in the starter diet (0–14 d), experiment 1A

		Intercept (a)				
Dependent variable (y)		Female	Male	Slope (b)	R^2	P^3
BW gain, g	0–14 d	348	348	10.8 (1.7)	0.85	***
	14–30 d	1025	1169	9.7 (1.5)	0.99	***
	30–37 d	591	708	0.3 (4.3)	0.95	NS
	0–37 d	1964	2224	20.7 (3.3)	0.99	***
Feed intake, g	0–14 d	598	598	-2.0 (2.1)	-	NS
	14–30 d	1685	1829	16.3 (2.6)	0.99	***
	30–37 d	1084	1169	7.9 (4.3)	0.91	NS
	0–37 d	3370	3593	22.3 (6.5)	0.97	*
G:F, g/g	0–14 d	0.573	0.573	0.0214 (0.0022)	0.93	***
	14–30 d	0.610	0.638	-0.0003 (0.0008)	0.97	NS
	30–37 d	0.544	0.601	-0.0033 (0.0026)	0.93	NS
	0–37 d	0.587	0.619	0.0018 (0.0008)	0.98	†
Carcass yield, % of BW		72.7	71.5	-0.06 (0.11)	0.76	NS
Breast yield, % of carcass		29.2	29.2	0.17 (0.08)	0.32	†
Abdominal fat pad yield[4]		3.41	2.83	-0.065 (0.016)	0.97	**

[1]The model used was: y = a + bx where y = dependent variable; a = intercept; b = slope; and x = apparent faecal digestible Lys (g/kg) in the starter diet. Parallel lines were fitted for male and female broilers because slopes between sexes did not differ ($P > 0.1$). Separate intercepts were estimated per sex.

[2] One intercept was estimated for male and female broilers for all data from d 0–14 and for breast yield because sex effects were not present ($P > 0.1$) and therefore omitted from the model.

[3] Probabilty of the Lys effect in the model, †$P \leq 0.1$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; NS = $P > 0.1$.

[4] As percentage of the carcass weight.

Results

Starter Diet Effects, Experiment 1A

Increasing IP concentrations in the starter diet (Figure 1, Table 4), linearly improved ($P < 0.05$) BW gain (11 g per gram AFD Lys) and G:F (0.021 g/g per gram AFD Lys). During the grower phase, as a carry-over-effect, BW gain (10 g per gram AFD Lys) and feed intake (16 g per gram AFD Lys) improved linearly ($P < 0.05$). Over the entire growth period, the former effects resulted in a linear increase ($P < 0.05$) in BW gain (21 g per gram AFD Lys) and feed intake (22 g per gram AFD Lys). Breast yield showed a nonsignificant increase and abdominal fat pad yield (0.07% per gram AFD Lys) decreased linearly (Figure 2, Table 4). Ideal protein responses for performance during the starter, grower, and finisher phase and for slaughter yields were similar for male and female broilers.

Phase Effects and Phase Interactions, Experiment 1B and 2

Increasing IP concentrations in the starter diet by 20% significantly improved BW gain and G:F in the starter phase in experiment 1B (Tables 5, 6) and experiment 2 (Tables 7, 8). In the consecutive grower phase, BW gain increased significantly only in experiment 2, whereas feed intake increased and G:F decreased ($P < 0.05$) in both experiments. In experiment 1B, the decreased G:F was more pronounced in birds fed high IP concentrations in the grower phase compared with birds fed adequate IP concentrations in the grower phase. Over the entire growth period, BW gain improved ($P < 0.05$) by 43 g/bird (mean of experiments 1B

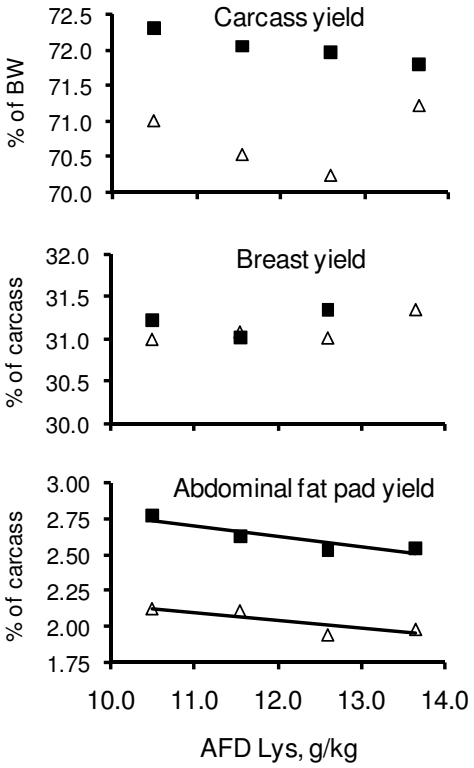

Figure 2. Slaughter yields of male (Δ) and female (■) broilers as a function of ideal protein concentrations [expressed as apparent faecal digestible (AFD) Lys, g/kg] in the starter diet (0–14 d). Regression lines are displayed when significant ($P \leq 0.05$, Table 4), experiment 1A.

and 2) and in experiment 2, feed intake increased ($P < 0.05$) by 55 g/bird, because of the increased IP concentration in the starter diet.

Increasing IP concentrations in the grower diet by 20% significantly improved BW gain and G:F in the grower phase in experiment 1B (Tables 5, 6) and experiment 2 (Tables 7, 8). In experiment 1B, the improved G:F was of a higher magnitude in males than in females, resulting in a significant grower IP × sex interaction. In the consecutive finisher phase, G:F decreased ($P < 0.05$) because of increased IP concentrations in the grower diet. The increased BW gain obtained in the grower phase was partly compensated in the finisher phase and therefore was nonsignificant over the entire growth period. Gain to feed ratio improved ($P < 0.05$) over the entire growth period, because of the increased IP concentrations in the grower diet. Breast yield increased ($P < 0.05$) in both experiments and abdominal fat pad yield decreased ($P < 0.05$) in experiment 2 because of high dietary IP concentrations in the grower diet.

Increasing IP concentrations in the finisher diet (experiment 2) significantly improved BW gain in the finisher phase, and G:F in the finisher phase and the entire growth period (Tables 7, 8). In addition, breast yield ($P < 0.05$) improved because of increased dietary IP concentrations in the finisher diet. In general, BW gain and G:F responses to increased dietary IP concentrations were of a lower magnitude in treatments that received high IP diets in the phase preceding the test phase compared with treatments that received adequate IP concentrations in the phase preceding the test phase. However, in none of the cases did this lead to a significant interaction between starter and grower IP concentrations.

Discussion

In the present study, breast yield, abdominal fat pad yield, BW gain, and G:F (Tables 4–8, Figures 1–3) responded to higher dietary IP concentrations than those recommended in the literature for starter, grower, and finisher diets[109,121]. For the grower and finisher diets, these results confirm previous findings with the IP-increment approach[110-112]. However, compared with Wijtten *et al.*[112], BW gain responses during the grower and in particularly during the finisher phase, were of a lower magnitude. The lower magnitude of these responses might be related to the difference in absolute performance level between both studies. Daily feed intake during the grower phase of the present study was higher than that observed by Wijtten *et al.*[112]. Consequently, at similar dietary IP concentrations, daily IP intake in the present study was higher. D'Mello[122] showed that AA responses are better predicted by the absolute daily intake rather than by the dietary AA concentration.

Table 5. Effect of different ideal protein concentrations in the starter (0–14 d), and grower (14–30 d) diet on BW gain and feed intake of male and female broilers, experiment 1B

	AFD[1] Lys, g/kg		BW gain, g				Feed intake, g			
Sex	Starter	Grower[2]	0–14 d	14–30 d	30–37 d	0–37 d	0–14 d	14–30 d	30–37 d	0–37 d
Male	10.50	10.20	460	1270	713	2443	574	1996	1265	3835
		12.24	446	1326	683	2455	559	1963	1263	3784
	12.60	10.20	487	1289	725	2501	579	2043	1285	3907
		12.24	495	1339	701	2534	580	2027	1284	3891
Female	10.50	10.20	462	1121	597	2180	583	1849	1158	3589
		12.24	462	1174	588	2224	575	1851	1183	3609
	12.60	10.20	491	1151	574	2215	583	1898	1178	3660
		12.24	479	1146	586	2211	566	1845	1180	3591
Pooled SEM			8.8	14.5	14.5	28.0	11.1	22.5	17.4	45.2
Factorial analysis[3]										
Starter	10.50		457	1223	645	2326	573	1914	1217	3704
	12.60		488	1231	646	2365	577	1953	1232	3762
Probability			***	NS	NS	*	NS	*	NS	†
Grower		10.20	–	1208	652	2335	–	1946	1222	3748
		12.24	–	1246	640	2356	–	1921	1227	3719
Probability			–	***	NS	NS	–	NS	NS	NS
df			43	39	39	39	43	39	39	39

[1]Ideal protein concentrations expressed as apparent faecal digestible (AFD) Lys, g/kg.
[2]Means of 6 cages (each containing 20 birds) per treatment.
[3] Probabilities for the interactions are not shown, but none were significant ($P > 0.1$).
†$P \leq 0.1$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; NS = $P > 0.1$.

Table 6. Effect of different ideal protein concentrations in the starter (0–14 d), and grower (14–30 d) diet on gain to feed ratio (G:F) and slaughter yields of male and female broilers, experiment 1B

	AFD[1] Lys, g/kg		G:F, g/g				Slaughter yields[3]		
Sex	Starter	Grower[2]	0–14 d	14–30 d	30–37 d	0–37 d	Carcass	Breast	Abdominal fat pad
Male	10.50	10.20	0.802	0.637	0.562	0.637	71.0	31.0	2.12
		12.24	0.799	0.675	0.540	0.649	70.6	31.8	1.74
	12.60	10.20	0.842	0.631	0.564	0.640	70.2	31.0	1.95
		12.24	0.853	0.660	0.545	0.651	70.9	31.7	1.79
Female	10.50	10.20	0.792	0.607	0.515	0.607	72.3	31.2	2.77
		12.24	0.804	0.634	0.497	0.616	71.6	32.1	2.65
	12.60	10.20	0.842	0.606	0.487	0.605	72.0	31.3	2.53
		12.24	0.846	0.621	0.497	0.616	72.0	31.6	2.61
Pooled SEM			0.0079	0.0043	0.0073	0.0026	0.28	0.29	0.11
Factorial analysis[4]									
Starter	10.50		0.799	0.638	0.529	0.627	71.4	31.6	2.32
	12.60		0.846	0.630	0.523	0.628	71.3	31.4	2.22
Probability			***	**	NS	NS	NS	NS	NS
Grower		10.20	–	0.620	0.532	0.622	71.4	31.1	2.34
		12.24	–	0.648	0.520	0.633	71.3	31.8	2.20
Probability			–	***	*	***	NS	**	†
df			43	39	39	39	39	39	39

[1]Ideal protein concentrations expressed as apparent faecal digestible (AFD) Lys, g/kg.
[2]Means of 6 cages (each containing 20 birds, 5 birds per cage for slaughter yields) per treatment.
[3]As percentage of the feed-deprived BW for carcass yield and as percentage of the carcass for breast and abdominal fat pad yield.
[4]Starter × grower interactions were significant for G:F 14–30 d ($P \leq 0.1$) and for carcass yield ($P \leq 0.05$); starter × sex interaction was significant for G:F 30–37 d ($P \leq 0.1$); and grower × sex interaction was significant for G:F 14–30 d ($P > 0.05$). Other interactions were not significant.
†$P \leq 0.1$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; NS = $P > 0.1$.

Table 7. Effect of different ideal protein concentrations in the starter (0–14 d), grower (14–30 d), and finisher (30–37 d) diet on BW gain and feed intake of male broilers, experiment 2

AFD[1] Lys, g/kg			BW gain, g				Feed intake, g			
Starter	Grower	Finisher[2]	0–14 d	14–30d	30–37d	0–37 d	0–14 d	14–30d	30–37d	0–37 d
10.50	10.20	8.91	439	1213	576	2228	563	1856	1143	3562
		9.90	444	1209	579	2232	548	1861	1094	3503
		10.89	435	1225	612	2272	547	1852	1136	3535
	12.24	8.91	444	1262	566	2272	550	1861	1119	3531
		9.90	437	1260	567	2264	537	1853	1132	3521
		10.89	442	1265	603	2310	549	1878	1145	3572
12.60	10.20	8.91	473	1266	568	2307	543	1945	1143	3632
		9.90	470	1256	588	2314	535	1946	1136	3617
		10.89	467	1222	605	2293	548	1899	1119	3566
	12.24	8.91	470	1276	564	2309	539	1900	1144	3583
		9.90	466	1261	580	2307	545	1889	1147	3581
		10.89	473	1280	575	2327	543	1902	1131	3576
Pooled SEM			6.1	16.1	15.5	23.4	8.8	24.8	18.3	41.5
Factorial analysis[3]										
Starter										
10.50			440	1239	584	2263	549	1860	1128	3537
12.60			470	1260	580	2310	542	1914	1137	3592
Probability			***	*	NS	***	NS	***	NS	*
Grower	10.20		–	1232	588	2274	–	1893	1129	3569
	12.24		–	1267	576	2298	–	1881	1136	3561
Probability			–	***	NS	†	–	NS	NS	NS
Finisher		8.91	–	–	568[b]	2279	–	–	1137	3577
		9.90	–	–	579[ab]	2279	–	–	1127	3556
		10.89	–	–	599[a]	2301	–	–	1133	3562
Probability			–	–	*	NS	–	–	NS	NS
df			69	67	59	59	69	67	59	59

[a,b]Means within a column (for finisher effect) with no common superscript differ significantly ($P < 0.05$).
[1]Ideal protein concentrations expressed as apparent faecal digestible (AFD) Lys, g / kg.
[2]Means of 6 cages (each containing 20 birds) per treatment.
[3]Probabilities for the interactions are not shown, but none were significant ($P > 0.1$).
†$P \leq 0.1$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; NS = $P > 0.1$.

This may explain the differences in G:F and BW gain responses between the 2 studies. The initial weight at the start of the finisher phase in the present experiment (30 d) was approximately 450 g above that of the birds (28 d) of Wijtten *et al*.[112]. Pesti and Fletcher[114] and Eits *et al*.[110] showed that BW responses to dietary CP or IP concentrations were more pronounced in birds restricted in weight (by protein restriction in their case) in the preceding phase. In the context of compensatory growth, it has been hypothesised that birds have a set point for BW for a specific age, and when behind their scheduled growth curve (for whatever reason), they try to reach the set point weight in the shortest possible time[123,124]. Compensatory growth can partly be explained by a better G:F because of decreased fat content in the gain[114], or by a higher water content in the carcass as was hypothesised by Eits *et al*.[110]. These findings and theories might explain the higher responses in the finisher phase found by Wijtten *et al*.[112] compared with the present study.

Starter Diet Effects, Experiment 1A

The BW gain and G:F responses (Figure 1, Table 4) due to increased dietary IP concentrations in the starter phase reported herein, agreed well with findings of Morris and Njuru[125], Morris and Abebe[126] and Sklan and Noy[127]. Those studies varied dietary CP

Table 8. Effect of different ideal protein concentrations in the starter (0–14 d), grower (14–30 d), and finisher (30–37 d) diet on gain to feed ratio (G:F) and slaughter yields of male broilers, experiment 2

AFD[1] Lys, g/kg			G:F, g/g				Slaughter yields[3]		
Starter	Grower	Finisher[2]	0–14 d	14–30 d	30–37 d	0–37 d	Carcass	Breast	Abdominal fat pad
10.50	10.20	8.91	0.781	0.654	0.504	0.626	71.4	29.5	1.72
		9.90	0.809	0.650	0.529	0.637	71.9	29.8	1.67
		10.89	0.798	0.663	0.538	0.643	71.8	30.1	1.93
	12.24	8.91	0.807	0.678	0.505	0.644	71.3	30.3	1.69
		9.90	0.814	0.680	0.501	0.643	71.5	29.9	1.41
		10.89	0.805	0.673	0.527	0.647	71.7	31.0	1.50
12.60	10.20	8.91	0.871	0.651	0.496	0.635	72.0	30.1	1.79
		9.90	0.879	0.645	0.517	0.639	71.7	30.0	1.75
		10.89	0.853	0.643	0.540	0.643	71.3	30.4	1.59
	12.24	8.91	0.872	0.672	0.492	0.644	71.5	30.7	1.52
		9.90	0.857	0.667	0.506	0.644	71.7	30.4	1.59
		10.89	0.870	0.673	0.508	0.651	71.7	30.7	1.35
Pooled SEM			0.0115	0.0062	0.0095	0.0044	0.28	0.29	0.10
Factorial analysis[4]									
Starter									
10.50			0.802	0.666	0.518	0.640	71.6	30.1	1.65
12.60			0.867	0.658	0.510	0.643	71.6	30.4	1.60
Probability			***	*	NS	NS	NS	NS	NS
Grower	10.20		-	0.651	0.521	0.637	71.7	30.0	1.74
	12.24		-	0.674	0.506	0.645	71.5	30.5	1.51
Probability			-	***	**	**	NS	**	***
Finisher		8.91	-	-	0.500[c]	0.637[b]	71.5	30.2[ab]	1.68
		9.90	-	-	0.513[b]	0.641[ab]	71.7	30.0[b]	1.61
		10.89	-	-	0.528[a]	0.646[a]	71.6	30.6[a]	1.59
Probability			-	-	***	*	NS	*	NS
df			69	67	59	59	59	59	59

[a,b,c]Means within a column (for finisher effect) with no common superscript differ significantly ($P < 0.05$).

[1]Ideal protein concentrations expressed as apparent faecal digestible (AFD) Lys, g/kg.

[2]Means of 6 cages (each containing 20 birds, 5 birds per cage for slaughter yields) per treatment.

[3]As percentage of the feed-deprived BW for carcass yield and as percentage of the carcass for breast and abdominal fat pad yield.

[4]Starter × finisher interaction was significant for abdominal fat pad yield ($P \leq 0.05$). Other interactions were not significant; †$P \leq 0.1$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; NS = $P > 0.1$.

concentrations in broilers up to 3 wk of age between 170 and 250 g/kg[(125)], 180 and 300 g/kg[(126)], or 160 and 280 g/kg[(127)]. Morris and Njuru[(125)] and Sklan and Noy[(127)], however, did not balance the AA composition of the diets when increasing the CP content, and observed that feed intake increased along with increased dietary CP concentrations. This effect may have been related to an inadequate AA intake in the low CP diets or might have been caused by an effect on the palatability of the diets due to the shifts in ingredients. Hence, their response on BW gain could also have been influenced by a higher intake level of other nutrients and most likely were not solely an AA intake effect. The data of Noy and Sklan[(81)] and MacLeod[(128)] support this theory because they increased an unbalanced CP in the diets of male broilers during the first week of life and did not observe an effect on feed intake and BW gain[(128)], or BW gain was even depressed[(81)]. Morris and Abebe[(126)], on the contrary, balanced the AA composition of their diets, did not find an effect on feed intake, and reported comparable responses to those found in the present experiment. This supports the theory that a balanced IP is necessary to achieve the effects on BW gain as reported herein and as shown before[(110-112)].

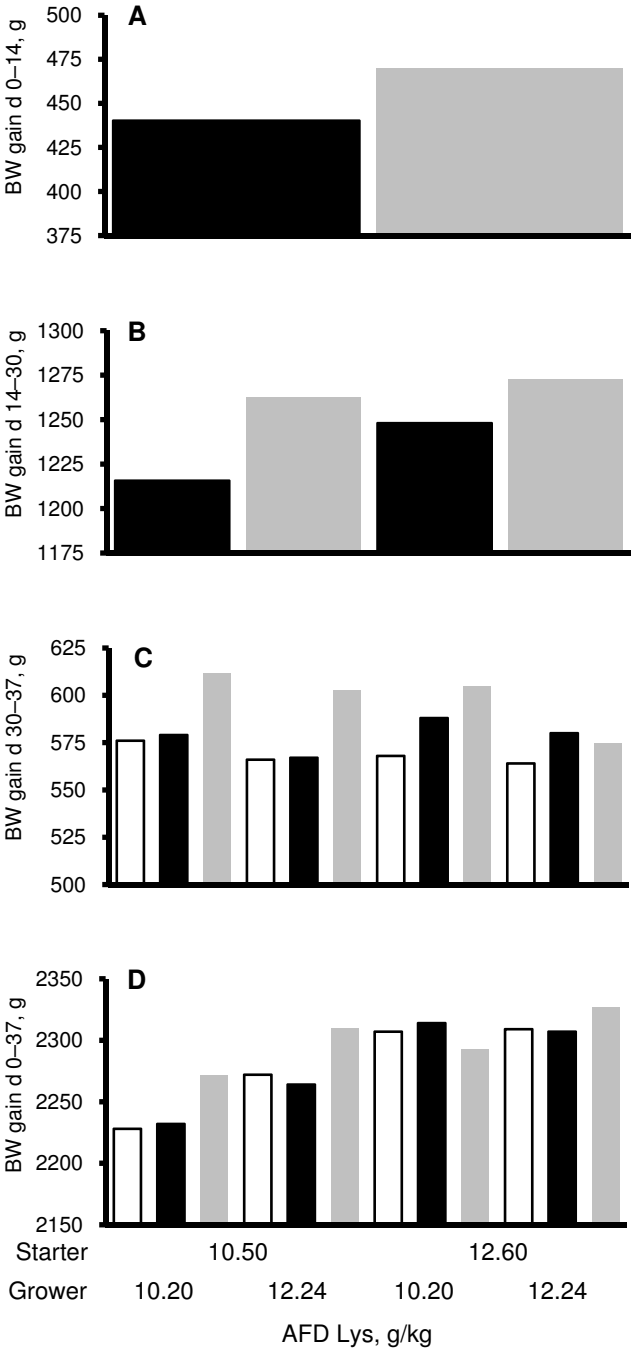

Figure 3. Effect of different ideal protein (IP) concentrations (expressed as apparent faecal digestible (AFD) Lys, g/kg) in the starter (0–14 d), grower (14–30 d), and finisher (30–37 d) diet on BW gain of male broilers, experiment 2. Bars represent Low (□), adequate (■), or high (■) IP concentrations fed in the particular phase (Panel A, B, and C); and represent the respective concentrations fed in the finisher phase for panel D (entire experimental period).

The present study showed that increased IP concentrations in the starter diets improve feed intake and BW gain in the consecutive grower phase (Figure 1, Table 4). The physiological background of this might be related to a better development of the gastrointestinal tract after hatch. This better developed intestinal tract has been reported to affect the capacity and efficiency of digestion and subsequently can improve broiler performance up to market age [129,130]. In addition, muscle cell maturation appears to depend on nutrition soon after hatch[131]. Delayed muscle cell maturation cannot be fully compensated in later phases of life and will result in decreased muscle growth[131,132]. In contrast with our observation, Pesti and Fletcher[114] and Eits *et al*.[110] showed that differences in BW at the end of the grower period, caused by different CP or IP concentrations in the grower phase, were compensated in the consecutive phase. The observation that delayed muscle cell maturation in early life cannot be fully compensated[131,132] might explain the discrepancy between our findings in the starter phase and findings of Pesti and Fletcher[114] and Eits *et al*.[110] in the grower phase. However, Pesti and Fletcher[114] and Eits *et al*.[110] also showed that compensation in BW gain was more pronounced for treatments receiving diets with high CP or IP concentrations in the consecutive phase. This finding might indicate that with a higher IP concentration in the diet of the consecutive grower phase our birds would have shown some compensatory growth. This aspect will be further discussed in the next section.

Finally, abdominal fat yield in the present experiment (Figure 2, Table 4) was significantly decreased as a result of the

increased IP concentration in the starter diet, showing that nutrition in early phases of life is capable of influencing the body composition at slaughtering.

Phase Effects and Phase Interactions, Experiments 1B and 2

The effects due to increased IP concentrations in the starter diets of experiments 1B and 2 (Tables 5–8, Figure 3) were generally in line with the effects of experiment 1A as discussed above. However, 2 additional findings can be added to the previous discussion. First, in contrast with experiment 1A, G:F was significantly decreased in the consecutive grower phase of experiment 1B and 2 as a result of increased IP concentrations in the starter diet. In experiment 1B this effect was almost entirely attributed to the treatment groups receiving high IP concentrations in the grower diet (treatments not included in experiment 1A) and hence this effect was not observed in experiment 1A. Second, BW gain and G:F responses to increased dietary IP concentrations in the grower phase were less pronounced when high IP concentrations were fed in the preceding starter phase. Although BW responses due to increased IP concentrations in the grower diet were less pronounced when high IP concentrations were fed in the preceding starter phase, birds fed adequate IP starter diets and high IP grower diets, compared with those fed high IP starter diets and high IP grower diets, were still not able to compensate for the lower BW gain during the starter phase. This clearly shows the importance of sufficient IP concentrations in the starter phase to obtain optimum broiler performance.

Feed conversion efficiency was significantly lower in the finisher phase (experiment 2) for birds fed high IP grower diets (Table 8). This phenomenon has been reported in literature[110,114,133]. It might be caused by a higher fat deposition rate in the finisher phase[133] as compensation for the lower fat gain in the grower phase, or due to a higher maintenance requirement[134] because of a heavier weight at the end of the grower period.

In agreement with findings in the grower phase, BW gain and G:F responses to increased IP concentrations in the finisher diets were less pronounced when high IP concentrations were fed in the preceding grower phase (Tables 7 and 8, Figure 3). These effects could not be detected statistically. However, the consistency of this observation between experiment 1B and experiment 2 and between the grower and finisher phases indicates the existence of this effect at adequate vs. high IP concentrations in preceding phases. Moreover, it is in agreement with the comparison of deficient vs. high CP or IP concentrations in the preceding phases of Pesti and Fletcher[114] and Eits *et al*.[110].

During the grower phase of experiment 1B, G:F responses of females to the increased IP concentration were significantly lower compared with those of males. This is not surprising because AA requirements have been reported to be higher for male than for female broilers[121]. In addition, Wijtten *et al*.[135] showed that in the grower phase (14–35 d), responses of female broilers to increased dietary IP concentrations were of a lower magnitude than those of male broilers.

Conclusions

The results of the present study showed that a delay in BW gain due to suboptimal IP concentrations in the starter diet could only partly be compensated for in later phases of life, and then at the expense of high IP concentrations in the grower and finisher diets. High IP concentrations in the starter phase can be financially attractive due to the relatively low contribution of the starter diet to the total feed costs of a broiler up to market age. For the

same reason, the effect of high IP concentrations on nitrogen excretion in the starter phase would have a minor effect on total nitrogen excretion. The results of the present study, therefore, demonstrate a need for a reevaluation of IP concentrations used in practical starter diets.

This study and literature data indicate that BW gain and G:F responses to increased dietary IP concentrations in the phase under study depend on the IP concentrations fed in preceding phases. For accurate requirement studies and for implication of practical recommendations, it is important to know if this effect truly exists. Therefore, this possible effect should not simply be neglected. Hence, this phenomenon should be further studied in a well-designed experiment that can detect and accurately quantify such an effect, if present.

The current study demonstrated that G:F responses due to IP increment of female broilers, in the grower phase, are of a lower magnitude than those of male broilers. This finding shows that additional research is required to establish the differences in male and female responses to dietary IP concentrations in the grower and finisher phase.

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Chapter 3

Dietary amino acid concentrations and feed restriction affect small-intestinal development, mortality, and weight gain of male broilers

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Abstract

This study investigated the effect of 2 different dietary amino acid (AA) treatments and feed restriction in early life vs. a control treatment on development of the small intestine segments (weights), mortality, and broiler performance. Each treatment was applied to 6 cages with Ross 308 male broilers and to 6 cages with Cobb 500 male broilers with 24 birds per cage. A control treatment [100% ideal protein (IP)] was compared with a treatment with 30% extra IP, a treatment with daily adjustment of the dietary AA concentration and profile, and a feed restriction treatment. The protein treatments were applied from 0–14 d of age. The feed restriction was applied from 4–21 d of age. Restriction was 15% from d 4–14 of age and diminished with equal daily steps thereafter to 5% at 21 d of age. Birds were weighed and dissected for evaluation of small intestine weights at 6, 9, 14, and 36 d of age. Feed intake restriction reduced leg problems in Ross and Cobb broilers. Extra dietary protein reduced leg problems in Ross broilers only. The present experiment does not show that small-intestinal weight development is related to mortality. Thirty percent extra dietary ideal protein increased duodenum weight between 6 and 9 d of age. This was not further increased by the daily optimisation of the dietary AA concentration and profile. The increased duodenum weights coincided with an improved BW gain. This indicates that duodenum weight may be important in facilitating BW gain in young broilers. Thus, it may be worthwhile to pay more attention to the relation between nutrition and duodenum weight and duodenum function in further studies.

Key words: *amino acid, broiler, feed restriction, mortality, small intestine.*

Introduction

The growth rate of broilers has increased considerably over the last decades. As a side effect, broilers have become more susceptible to metabolic disorders than in the past[(5-7)]. Baghbanzadeh and Decuypere[(8)] stressed that particularly growth reduction of broilers in early life is very effective in reducing metabolic disorders. It has been suggested that early embryonic[(9)] or post-hatch[(10)] organ development is important for growth and for organ functioning. From this, we hypothesised that the basis for metabolic disorders lies in early life when relative growth rate is at its maximum. The development of the small intestines in

birds occurs rapidly. It increases from 2 to 8% of the BW from 0–8 d of age[22]. It is known that organs develop in concert with the need of the body. The increase at d 8 indicates a high metabolic activity of the small intestine and this is indicative of the high importance of this organ for young broilers. Amino acids (AA) are both energy sources and building blocks for the small intestine. They are very important for optimal development of the small intestine. There are very few studies on the AA requirement of very young broilers, especially with regard to intestinal development. It has been shown that dietary protein quality (AA profile) and its concentration can affect gene expression of transporter proteins in the small intestines[136,137]. As a consequence, suboptimal dietary concentrations may suppress gut function. We hypothesised that optimal AA nutrition in the starter diet will improve intestinal development and its capacity in digestion and absorption of nutrients from feed.

Our previous studies demonstrated that increased dietary AA concentrations in early life also improve BW gain in consecutive phases[138] and that early life feed intake restriction decreases mortality rate[139]. The present study was specifically designed to investigate the effect of these treatments on small intestine and heart weight development in addition to the effects on performance and mortality. When these treatments affect organ weights, this may be used as an indication that organ function also is affected. Subsequently, more sophisticated studies can be designed to evaluate the effects on organ functionality. In previous studies, it was shown that performance responses to dietary AA treatments[140] and also mortality[139,141] can differ largely between Ross and Cobb broilers. Therefore, in the present study we included both breeds.

Materials and methods

Birds and housing

The experiment was performed in a broiler unit that consisted of 2 rooms with 24 cages each. Birds were housed in battery cages, which were 1.1 m^2 each and had a raised wire floor. Water was provided *ad libitum*, whereas feed was either provided restricted or *ad libitum* according to treatments as shown in Table 1. Temperature, relative humidity, and ventilation were automatically controlled. Temperature decreased by 2.5°C per wk, from 34°C at the day of arrival (1-d-old chicks) to a final temperature of 21.5°C at 36 d of age. Lights were on during 23 h/d. Birds were spray-vaccinated against Newcastle disease at 9 d of age. The experimental methods were approved by the Ethical Committee of the Animal Science Group of Wageningen University and Research Centre, Lelystad, the Netherlands.

Eight hundred 1-d-old Ross 308 and 800 Cobb 500 male chicks were purchased from a commercial hatchery and weighed individually. Ross 308 chicks weighing between 38 and 46 g and Cobb 500 chicks weighing between 40 and 48 g were each randomly assigned to a

Table 1. Experimental design[1]

		Experimental phase		
Treatment		0–4 d	4–14 d	14–21 d[2]
1	Control (CONTR)	100% IP (AL)	100% IP (AL)	100% IP (AL)
2	130% IP (130IP)	130% IP (AL)	130% IP (AL)	100% IP (AL)
3	Optimised (OPT)	Variable AA (AL)	Variable AA (AL)	100% IP (AL)
4	Restricted (RES)[3]	100% IP (AL)	100% IP (15% RES)	100% IP (15 to 5% RES)

[1]AA = amino acid; IP = ideal protein; AL = *ad libitum*.
[2]All birds had free access to a 100% IP diet from d 21 until the end of the experiment (d 36).
[3]Feed restriction was 15% from 4–14 d and diminished with equal daily steps thereafter to 5% at 21 d of age.

Table 2. Feed intake, body weight (BW), and empty BW of Ross 308 male broilers in the preliminary experiment[1]

Day	Feed intake, g/d	BW, g	Empty BW[2], % of BW
0		47 ± 0.1	91.6 ± 1.2
1	8 ± 0.5	57 ± 2.2	84.5 ± 1.8
2	12 ± 0.7		
3	17 ± 1.0	88 ± 3.7	87.2 ± 1.2
4	20 ± 1.1		
5	25 ± 0.9	130 ± 2.2	88.3 ± 1.5
6	30 ± 1.8		
7	34 ± 1.1	184 ± 5.7	90.8 ± 0.6
8	40 ± 1.9		
9	46 ± 1.8		
10	54 ± 1.8	299 ± 10	92.4 ± 1.0
11	58 ± 3.1		
12	68 ± 3.5		
13	71 ± 2.4		
14	76 ± 2.3	508 ± 20	94.7 ± 1.0
14–21[3]	94 ± 3.0	974 ± 29	95.5 ± 1.4
21–29[3]	145 ± 2.7	1742 ± 36	95.7 ± 0.6
29–35[3]	180 ± 2.8	2403 ± 40	96.6 ± 0.6

[1]For the experiment, 6 cages with broilers (63 broilers at d 0) were fed a diet with a similar nutritional composition as the control treatments in the present experiment. Data represents the mean of 6 cages ± SD.
[2] At each dissection day, 5 broilers per cage were dissected to determine the empty BW (BW minus yolk and gut content); [3] Body weight and empty BW at d 21, 29 and 35, respectively.

cage. Chicks outside these weight ranges were not used in the trial. Breeds were equally divided over the 2 rooms with 3 cages per breed by treatment combination in each room. The experiment commenced with 24 chicks per cage. The average initial weight in each cage was 43 g/chick for Ross 308 and 44 g/chick for Cobb 500.

Experimental design and diets

In this experiment, the effects of 4 dietary treatments were studied each in 2 broiler breeds (Ross 308 and Cobb 500) according to a 4 × 2 factorial arrangement. The 4 dietary treatments were applied in the starter phase and partly in the first week of the grower phase (Table 1). The experiment was divided in a starter phase (0–14 d of age), grower phase (14–30 d of age), and a finisher phase (30–36 d of age). The control (CONTR) treatment (100% ideal protein, IP) met the Lys recommendations according to Schutte[(109)]. These recommendations are 10.5, 10.2, and 9.9 g of apparent faecal digestible Lys/kg in the starter, grower, and finisher diets, respectively. This corresponds, respectively, to 11.0, 10.7, and 10.4 g true faecal digestible Lys if it is taken into account that endogenous Lys losses are 0.5 g/kg feed[(142)]. The profile of Mack *et al*.[(108)] was applied for the other AA, being a ratio to Lys of 0.75 for TSAA, 0.63 for Thr, 0.19 for Trp, 0.71 for Ile, 0.81 for Val, and 1.12 for Arg. In the second treatment, the dietary IP concentration of the starter diet was increased by 30% (130IP, 13.7 g apparent faecal digestible Lys/kg) compared with the CONTR treatment and remaining similar ratios among all AA. The IP was increased by increasing the content of all high-protein raw materials (soybean meal, fish meal, soybean isolate, maize gluten meal, and potato protein) at the expense of maize. This ensured that Trp, Ile, Val, and Arg met or exceeded 130% of the recommendations. Subsequently, Lys, TSAA, and Thr were set at 130% of the recommendations with synthetic AA. This 130IP treatment showed significantly more

Table 3. Approach used for amino acid (AA) requirement calculations of the optimised diets

Item	Approach
1	Based on our previous studies[138], we assumed that the dietary crude protein (CP) and indispensable AA content for maximal growth is well established in 28-d-old broilers. Based on this, the assumption was that at this age, the dietary Lys recommendations of Schutte[109]+10% are sufficient to maximise growth.
2	In the above trial (item 1), the AA profile of Mack *et al.*[108] was applied and all diets exceeded the recommendations for His, Leu, and Phe + Tyr of Han and Baker[105].
3	We predicted daily empty gain (gain minus yolk and gut fill) and daily feed intake based on a preliminary experiment with male Ross 308 broilers in our own research facilities (Table 2). In that experiment, feed intake was determined daily until 14 d of age and weekly afterward.
4	Daily CP gain was estimated based on the daily empty BW gain of the preliminary study (item 3) and the CP content of carcasses calculated based on equations from Sklan and Noy[143].
5	Requirements for indispensable AA and CP intake at 28 d of age were based on the estimated feed intake at that day in the preliminary study (item 3) and the estimated optimum dietary CP and AA compositions based on item 1 and 2.
6	To estimate the indispensable AA and CP requirement for CP gain, the maintenance requirements for CP and indispensable AA based on literature [Leveille and Fisher[144-146], Leveille *et al.*[147], Baker *et al.*[148], Edwards III *et al.*[149,150] Edwards III and Baker[151], Samadi and Liebert[152] and Kebreab *et al.*[153]] were subtracted from the total AA and CP requirements (item 5).
7	Based on item 5 and 6, the CP and indispensable AA requirements per gram CP gain and for maintenance are available and daily requirements were calculated based on CP gain (item 4).
8	Amino acid digestibility is lower during the first 10 d posthatch compared with older broilers[154,155].
9	Based on daily indispensable AA and CP requirements (item 7) and predicted daily feed intake (item 3), the dietary AA and CP content were calculated.

weight gain in previous experiments compared with the 100% IP treatment[138]. For the third treatment, the dietary crude protein (CP) concentration and AA profile were optimised (OPT) based on data of a preliminary study (Table 2), our own calculations, and data from literature. This is described in more detail in Table 3. The calculations resulted in daily CP and AA formulation targets for d 0–14 (Table 4). Changes in dietary CP and AA composition for the OPT treatment were realised in the same manner as for the 130IP diet. For the fourth treatment, feed intake of the birds was restricted (RES, Table 1) based on a previously tested program[139]. This showed that 15% feed restriction from 4–14 d of age

Table 4. Formulation targets for the diets of the optimised treatment

	Trial day[1]			
Nutrient	1	2	3	11–14
AFD[2] amino acids, g/kg				
Lys	11.7	14.5	14.1	11.3
TSAA	8.9	10.9	10.5	8.4
Thr	7.6	9.1	8.8	7.0
Trp	2.3	2.7	2.6	2.1
Ile	8.5	10.3	9.9	8.0
Arg	9.6	11.7	11.4	9.1
Val	13.5	16.2	15.6	12.5
His	3.5	4.5	4.4	3.5
Leu	13.4	16.0	15.5	12.4
Phe + Tyr	12.3	15.2	14.8	11.9
Crude protein	241	282	270	217

[1]Formulation targets for d 4–10 were intermediate targets of the d 3 and d 11–14 targets and can be calculated by applying the following ratios for the d 3 and d 11–14 targets: d 4, 0.800:0.200; d 5, 0.642:0.358; d 6, 0.509:0.491; d 7, 0.381:0.619; d 8, 0.255:0.745; d 9, 0.144:0.856; d 10, 0.063:0.937.
[2]AFD = apparent faecal digestible.

and thereafter gradually diminishing this restriction to 5% at 21 d of age, reduced mortality markedly. Feed intake restriction was based on the feed intake of the CONTR groups (per breed) on the previous day and an estimated increase in daily feed intake based on our preliminary study (Table 2). The feed of the RES birds (same diets as the CONTR treatment) was provided once per day between 0800 and 0900 h. This resulted in a period of 4–6 h/d without feed during the first week of the restriction that decreased to no or almost no time without feed at the end of the restriction phase. After the starter diets (fed until 14 d of age), the birds of all treatments switched to the grower diet (from 14–30 d of age) and to the finisher diet (from 30–36 d of age).

Before the preparation of the experimental diets, batches of soybean meal, soybean isolate, maize gluten meal, fish meal, and potato protein were analysed for AA content[156-158] In addition, these ingredients and the maize and wheat were analysed for N (Dumas)[118] and DM content (103^{o}C for 4 h). Based on the analysed values and digestibility figures and nutrient contents derived from CVB[142], the different experimental diets were formulated (Table 5). All diets were composed to be isocaloric with regard to AME_n within each phase and adequate in all nutrients. For the CONTR diets and the 130IP starter diet, the ratios between digestible Lys and all other indispensable digestible AA met or exceeded the recommendations of Mack *et al*.[108]. Diets for d 4–10 of the OPT treatment were intermediate diets of the d 3 and d 11–14 diets and were blended at the production plant at different ratios before pelleting (Table 5). All diets were pelleted (2.5 mm) with steam addition. All diets were analysed for N (Dumas)[118], crude fat (AOCS Am 5-04), Ca, and P (both inductive coupled plasma-atomic emission spectrometry, ICP-AES) content. The AA composition of the diets was calculated from analysed composition of the ingredients before composing the diets and was not analysed after preparation of the diets. The diets were prepared in a plant specialised in the production of experimental diets (Research Diet Services, Wijk bij Duurstede, the Netherlands), and the quality standards at this plant ensured that the diet composition was as formulated.

Data Collection

For each cage, all birds were weighed individually at 0, 3, 6, 9, 11, 14, 21, 30, and 36 d of age and weight gain for each phase was calculated per cage. In addition, feed consumption for each cage was determined at the time of weighing. Based on gain and feed consumption, gain to feed ratio (G:F) was calculated as grams of live weight gain per gram of feed consumed. Every dead or culled bird during the experiment was dissected to determine the reason for mortality or disease.

Two birds per cage (chosen at random) were dissected to determine the weights of various organs at 6, 9, 14, and 36 d of age. The birds were weighed and euthanised (by decapitation) without previous feed deprivation (fed birds). Subsequently, they were dissected and the empty weights of the duodenum (duodenal loop), jejunum (end duodenum to the Meckel's diverticulum), ileum (Meckel's diverticulum to the ceca), and the heart weight were measured. The intestines were emptied by gently squeezing out the content. The organ weights were calculated as percentage of the fed metabolic BW ($BW^{0.75}$). Five additional birds per cage (chosen at random) were deprived of feed (for 8–12 h) at 36 d of age and were slaughtered the next day. The birds were successively weighed, euthanised, bled, and feathers were removed. After removal of the feathers, the birds were excised. The weight of the carcass (whole bird without feathers, blood, organs, intestines, head, and legs below the hock) was measured. Carcass yield was determined and calculated as percentage

Table 5. Ingredient and nutritional composition of the experimental diets

	Starter diets						Grower diet	Finisher diet
	Tr. 1 & 4	Tr. 2	Treatment (Tr.) 3[1]				All Tr.	All Tr.
Item	d 1–14	d 1–14	d 1	d 2	d 3	d 11–14	d 14–30	d 30–36
Ingredient composition, g/kg								
Maize	575.2	426.8	468.8	360.7	392.3	537.0	430.6	461.2
Wheat	100.0	100.0	100.0	100.0	100.0	100.0	150.0	150.0
Soybean meal	199.2	294.7	269.3	338.2	317.5	224.0	306.8	287.4
Fish meal	20.3	30.0	27.4	34.4	32.3	22.8		
Soybean isolate	14.3	21.2	19.4	24.4	22.9	16.1		
Maize gluten meal	13.5	20.0	18.3	23.0	21.6	15.2		
Potato protein	13.5	20.0	18.3	23.0	21.6	15.2		
L-Lysine-HCl	1.9	1.4	0.1	0.4	0.9	1.8	1.4	1.6
DL-Methionine	2.1	3.1	2.1	3.1	3.0	2.3	2.4	2.2
L-Threonine	0.2	0.1				0.1	0.2	0.2
Soybean oil	17.1	43.0	36.0	54.7	49.1	23.8	34.8	31.1
Animal fat							34.8	31.1
Ground limestone	16.8	16.0	16.2	15.6	15.8	16.6	13.7	12.0
$CaHPO_4$,$Ca(H_2PO_4)_2.H_2O$	11.9	10.5	10.9	9.8	10.1	11.6	11.3	9.1
Sodium chloride	1.2	1.3	1.7	1.5	1.4	1.2	1.5	1.4
Sodium bicarbonate	2.6	1.9	1.5	1.3	1.6	2.5	2.6	2.8
Vitamin-mineral premix[2]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Calculated composition[3], g/kg								
$AME_{n\ (Broiler)}$, MJ/kg	11.92	11.92	11.92	11.92	11.92	11.92	12.55	12.55
P (available)	4.3	4.3	4.3	4.3	4.3	4.3	3.8	3.3
K	7.2	9.0	8.6	9.9	9.5	7.7	9.0	8.7
Na	1.6	1.6	1.6	1.6	1.6	1.6	1.4	1.4
Cl	1.8	1.8	1.8	1.8	1.8	1.8	1.6	1.6
AFD[4] amino acids								
Lys	10.5	13.7	11.7	14.5	14.1	11.3	10.2	9.9
Met	5.2	7.0	5.8	7.3	7.1	5.6	5.0	4.8
TSAA	7.9	10.2	8.9	10.9	10.5	8.4	7.7	7.4
Thr	6.6	8.6	8.0	9.4	9.0	7.0	6.4	6.2
Trp	2.0	2.7	2.5	3.0	2.9	2.2	2.2	2.1
Ile	7.6	10.0	9.4	11.2	10.6	8.2	7.6	7.3
Arg	11.8	15.8	14.7	17.6	16.8	12.8	12.6	12.1
Val	8.5	11.1	10.4	12.2	11.7	9.2	8.3	8.0
Analysed composition, g/kg								
Crude protein	205	263	249	292	284	220	211	201
Crude fat	45	67	60	78	74	49	91	86
Ca	10.1	9.9	10.0	9.6	9.5	9.4	8.2	6.5
P	6.0	6.3	6.4	6.3	6.2	5.9	6.0	5.2

[1]Diets for d 4–10 of treatment 3 were intermediate diets of the d 3 and d 11–14 diets and, respectively, blended at the production plant before pelleting in the following ratios: d 4, 0.800:0.200; d 5, 0.642:0.358; d 6, 0.509:0.491; d 7, 0.381:0.619; d 8, 0.255:0.745; d 9, 0.144:0.856; d 10, 0.063:0.937.

[2]Contributed per kg diet: riboflavin, 5 mg; niacinamide, 40 mg; D-pantothenic acid, 12 mg; choline chloride, 500 mg; cyanocobalamin, 15 µg; vitamin E (DL-α-tocopheryl acetate), 15 IU; menadione, 5 mg; vitamin A (retinyl-acetate), 10,000 IU; cholecalciferol, 50 µg; biotin, 0.1 mg; folic acid, 0.75 mg; $FeSO_4.H_2O$, 200 mg; MnO_2, 100 mg; $CuSO_4.5H_2O$, 60 mg; $ZnSo_4.H_2O$, 150 mg; Se (organic), 0.15 mg; KI, 1 mg; $CoSO_4.7H_2O$, 1 mg; antioxydant (ethoxyquin), 100 mg.

[3]Based on amino acid, N, and dry matter analyses of raw materials and chemical composition, digestibility, and energy value for broilers of the CVB Livestock Feed Table[(142)].

[4]AFD = apparent faecal digestible.

of the feed-deprived bird weight (whole bird weight before slaughtering). In addition, the breast weight (pectoralis major, pectoralis minor, sternum, and clavicle) and the abdominal fat pad weight (including fat surrounding the gizzard) were measured and their yields were calculated as percentage of the carcass weight.

Statistical Analyses

All data (except mortality) were subjected to ANOVA according to the following statistical model:

$$Y_{ijkl} = \mu + R_i + D_j + B_k + D \times B_{jk} + e_{ijkl},$$

where Y = variable; μ = overall mean; R = room (i = 1,2); D = Dietary treatment (j = 1,4); B = Breed (k = 1,2); D × B is the interaction between D and B; and e = the residual error term. For all parameters, except for carcass, breast and abdominal fat yield, room effects were significant for at least 1 time point. However, only for duodenum weight at d 36, room × dietary treatment interaction was significant. For the other parameters, therefore, the above model was used, in which the room × dietary treatment interaction was omitted. Contrasts were calculated for the 130IP (CONTR vs. 130IP), OPT (CONTR vs. OPT), RES (CONTR vs. RES), and protein (130IP vs. OPT) effects and for the breed × contrast interactions. The data from each cage were the experimental unit. Mortality data were analysed by comparing individual treatments as binomial proportions calculating the same contrasts as mentioned above. Effects were considered significant for $P < 0.05$. The 11th edition of Genstat for Windows[159] was used to analyse all data.

Results

Performance

Feed intake from d 0–6 of the 130IP birds was lower ($P < 0.05$) than of the CONTR birds (Table 6). Feed intake from d 0–3 of the OPT birds was lower ($P < 0.01$) than of the CONTR birds. From d 6–14, feed intake of the OPT birds was higher ($P < 0.001$) than of the CONTR birds. From d 6–14, breed × contrast interactions for feed intake were significant ($P < 0.05$) for the CONTR vs. 130IP birds and for CONTR vs. OPT birds. These contrasts were due to a more marked increase in feed intake due to the 130IP and OPT treatments in Ross broilers compared with Cobb broilers. Feed intake of the 130IP and OPT birds was higher ($P < 0.001$) from d 14–21 compared with CONTR birds. On average, feed intake of RES birds from d 3–14 was 16% lower ($P < 0.001$) than feed intake of CONTR birds. Feed intake of RES birds from d 14–21 was lower (−10%, $P < 0.001$) than of CONTR birds. From d 21–36, feed intake was similar for all treatments.

Body weight (BW) gain from d 3–4 was higher ($P < 0.001$) for the 130IP (+13%) and the OPT birds (+10%) than for the CONTR birds (Table 7). From d 9–14, BW gain was lower ($P < 0.05$) for the OPT birds (−5%) compared with the 130IP birds but was still higher ($P < 0.001$) for the OPT birds (+7%) compared with the CONTR birds. The RES treatment reduced ($P < 0.001$) BW gain (−16%) from d 3–14 compared with the CONTR treatment. Body weight gain was similar for all treatments from 14–36 d of age.

Body weight gain relative to metabolic BW from d 3–9 was higher ($P < 0.05$) for the 130IP birds (+7%) and higher ($P < 0.05$) for the OPT birds (+9%) than for the CONTR birds (Figure 1). From d 30–36, relative BW gain for the 130IP birds (−7%) was lower ($P < 0.05$)

Table 6. Effect of different amino acid treatments and feed restriction on feed intake of male broilers[1]

		Feed intake, g/d								Feed intake, g		
Item[2]		0–3 d	3–6 d	6–9 d	9–11 d	11–14 d	14–21 d	21–30 d	30–36 d	0–14 d	0–30 d	0–36 d
Ross 308	CONTR	11.8	27.0	41.4	53.4	70.4	95.9	143	169	559	2515	3532
	130IP	11.5	26.6	42.2	57.1	73.5	107.4	147	169	576	2646	3659
	OPT	11.2	27.3	45.0	60.8	76.5	103.8	141	169	602	2595	3608
	RES	11.8	21.4	35.8	45.0	60.1	85.9	145	176	477	2382	3435
Cobb 500	CONTR	11.8	27.4	42.6	55.9	69.8	92.2	133	163	566	2412	3387
	130IP	10.5	25.7	40.6	54.3	69.4	96.8	138	161	547	2467	3434
	OPT	10.6	26.1	44.0	57.3	72.8	96.8	138	160	575	2492	3452
	RES	10.6	20.7	35.5	45.6	60.0	82.9	137	165	472	2285	3273
Pooled SEM (n = 6)		0.3	0.4	0.5	0.6	0.8	1.8	2.3	3.7	4.8	31	46
Main effects												
Breed												
Ross 308		11.6	25.6	41.1	54.1	70.1	98.2	144	171	553	2535	3559
Cobb 500		10.9	25.0	40.6	53.3	68.0	92.2	137	162	540	2414	3387
Pooled SEM (n = 24)		0.2	0.2	0.2	0.3	0.4	0.9	1.2	1.9	2.4	15	23
Starter diet												
	CONTR	11.8	27.2	42	55	70	94	138	166	563	2463	3459
	130IP	11.0	26.2	41	56	71	102	142	165	561	2557	3547
	OPT	10.9	26.7	45	59	75	100	139	164	588	2543	3530
	RES	11.2	21.1	36	45	60	84	141	170	474	2333	3354
Pooled SEM (n = 12)		0.2	0.3	0.4	0.4	0.6	1.3	1.7	2.6	3.4	22	33
P-value of factorial analysis												
Breed		0.005	0.037	0.183	0.069	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001
Feed treatment		0.037	<0.001	<0.001	<0.001	<0.001	<0.001	0.286	0.492	<0.001	<0.001	<0.001
Breed × Feed treatment		0.345	0.183	0.046	<0.001	0.043	0.145	0.546	0.953	0.001	0.492	0.819
P-value of contrasts												
CONTR vs. 130IP		0.017	0.010	0.225	0.105	0.113	<0.001	0.074	0.784	0.784	0.004	0.067
CONTR vs. OPT		0.009	0.225	<0.001	<0.001	<0.001	0.001	0.625	0.682	<0.001	0.013	0.134
CONTR vs. RES		0.068	<0.001	<0.001	<0.001	<0.001	<0.001	0.227	0.277	<0.001	<0.001	0.028
130IP vs. OPT		0.798	0.152	<0.001	<0.001	<0.001	0.332	0.187	0.891	<0.001	0.665	0.723

[1]Each cage contained 24 male broilers at the start of the experiment.

[2]CONTR = the control 100% ideal protein starter diet; 130IP = 130% ideal protein starter diet; OPT = daily optimised ideal protein composition of the starter diet; and RES = feed intake restriction from d 4–21.

Table 7. Effect of different dietary amino acid treatments and feed restriction on BW gain of male broilers[1]

		BW gain[3], g/d								BW gain, g		
Breed	Feed treatment[2]	0–3 d	3–6 d	6–9 d	9–11 d	11–14 d	14–21 d	21–30 d	30–36 d	0–14 d	0–30 d	0–36 d
Ross 308	CONTR	14.3	23.0	33.2	41	53	67	91	97	453	1743	2324
	130IP	15.2	28.1	37.3	48	60	71	92	95	515	1842	2410
	OPT	14.5	28.1	37.4	46	57	70	88	96	504	1787	2365
	RES	14.7	18.8	29.1	36	45	62	92	103	395	1664	2284
Cobb 500	CONTR	15.2	24.2	33.4	43	51	61	81	97	459	1614	2196
	130IP	14.4	26.6	36.5	47	57	63	83	91	500	1687	2234
	OPT	13.9	26.3	36.4	45	55	63	84	92	483	1685	2234
	RES	13.6	17.9	28.4	36	45	60	86	98	385	1577	2164
Pooled SEM (n = 6)		0.4	0.6	0.7	0.9	0.9	1.8	2.2	3.6	4.7	27	38
Main effects												
Breed												
Ross 308		14.7	24.5	34.2	43	54	68	91	98	467	1759	2346
Cobb 500		14.3	23.7	33.7	43	52	62	83	94	457	1641	2207
Pooled SEM (n = 24)		0.2	0.3	0.3	0.5	0.5	0.9	1.1	1.8	2.3	13	19
Starter diet												
	CONTR	14.7	23.6	33.3	42	52	64	86	97	456	1679	2260
	130IP	14.8	27.4	36.9	48	58	67	87	93	507	1764	2322
	OPT	14.2	27.2	36.9	46	56	67	86	94	494	1736	2300
	RES	14.2	18.3	28.7	36	45	61	89	101	390	1621	2224
Pooled SEM (n = 12)		0.3	0.4	0.5	0.6	0.6	1.2	1.5	2.6	3.3	19	27
P-value of factorial analysis												
Breed		0.231	0.068	0.248	0.741	0.009	<0.001	<0.001	0.185	0.004	<0.001	<0.001
Feed treatment		0.288	<0.001	<0.001	<0.001	<0.001	0.005	0.470	0.173	<0.001	<0.001	0.063
Breed × Feed treatment		0.105	0.059	0.829	0.172	0.654	0.468	0.379	0.866	0.044	0.594	0.874
P-value of contrasts												
CONTR vs. 130IP		0.843	<0.001	<0.001	<0.001	<0.001	0.071	0.623	0.295	<0.001	0.003	0.108
CONTR vs. OPT		0.220	<0.001	<0.001	<0.001	<0.001	0.114	0.968	0.436	<0.001	0.040	0.299
CONTR vs. RES		0.191	<0.001	<0.001	<0.001	<0.001	0.126	0.179	0.312	<0.001	0.035	0.347
130IP vs. OPT		0.156	0.740	0.976	0.012	0.009	0.813	0.594	0.786	0.006	0.295	0.557

[1]Each cage contained 24 male broilers at the start of the experiment.

[2]CONTR = the control 100% ideal protein starter diet; 130IP = 130% ideal protein starter diet; OPT = daily optimised ideal protein composition of the starter diet; RES = feed intake restriction from d 4–21. [3]For d 3–6 and d 6–9, the results of 1 pen of the Ross 130IP treatment was excluded from the calculations. The residual variance of this pen was higher than 4 times SE, most probably due to an inaccurate weighing of the birds of that pen at d 6.

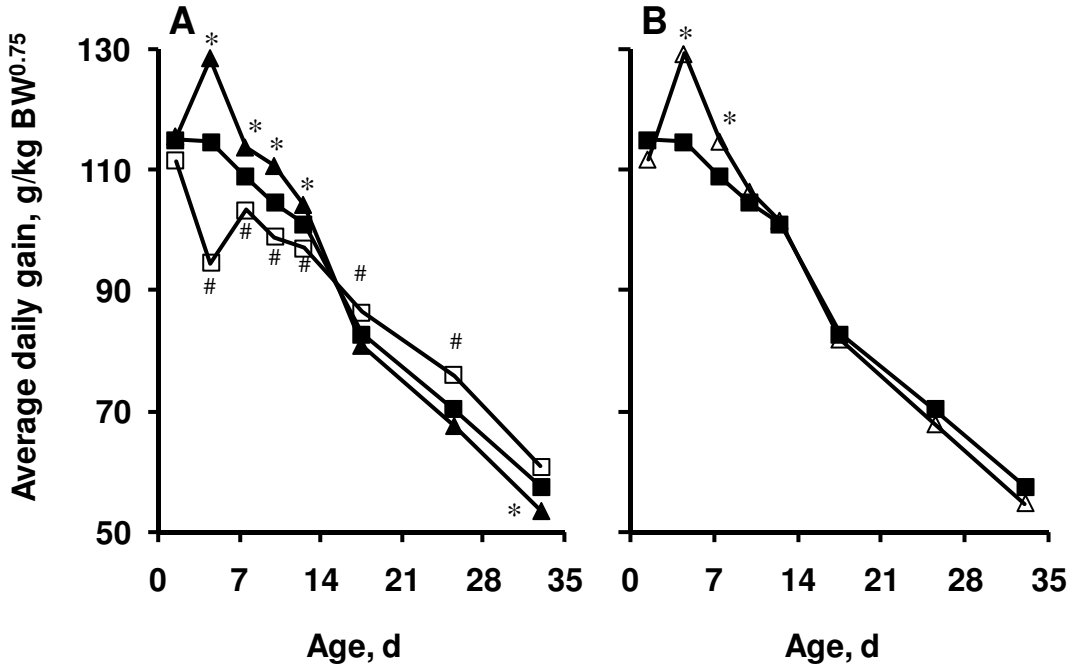

Figure 1. Combined results of the Ross and Cobb broilers of the average daily gain expressed relative to the metabolic BW ($BW^{0.75}$) of the control (■, panel A and B), the 130% ideal protein (▲, panel A), the restriction (□, Panel A), and the optimised (Δ, panel B) treatments. *Differences ($P < 0.05$) for the 130% ideal protein or optimised treatment compared with the control treatment. # Differences ($P < 0.05$) for the restriction treatment compared with the control treatment. The Pooled SEM were 1.9 (d 0–3), 1.7 (d 3–6), 1.5 (d 6–9), 1.6 (d 9–11), 1.1 (d 11–14), 1.1 (d 14–21), 1.0 (d 21–30) and 1.3 (d 30–36).

than for the CONTR birds. The RES treatment reduced (−9%, $P < 0.001$) relative BW gain from d 3–14 compared with the CONTR treatment. Relative BW gain was higher ($P < 0.05$) from d 14–21 (+4%) and higher ($P < 0.05$) from d 21–30 (+8%) for the RES birds than for the CONTR birds.

Gain to feed ratio from d 0–14 was higher ($P < 0.001$) for the 130IP birds compared with the CONTR birds (Table 8). G:F ratio from d 0–9 was higher ($P < 0.05$) for the OPT birds compared with the CONTR birds. From d 0–3 and from d 6–14, G:F was higher ($P < 0.05$) for the 130IP birds compared with the OPT birds. G:F ratio from d 14–21 was lower ($P < 0.05$) for the 130IP birds than for CONTR birds and was higher ($P < 0.001$) for the RES birds compared with the CONTR birds.

Breast weight relative to carcass weight was lower ($P < 0.001$) and abdominal fat yield relative to carcass weight was higher ($P < 0.01$) at d 37 for RES birds compared with CONTR birds (Table 9).

Small Intestines

Relative duodenum weight (Table 10) was higher ($P < 0.05$) for the 130IP birds compared with the CONTR birds at d 6 (+11%) and at d 9 (+15%). Relative duodenum weight at d 6 (+11%) and relative jejunum weights at d 6 (+11%) and at d 9 (+9%) of the OPT birds were higher ($P < 0.05$) than those of the CONTR birds. At d 9, the relative duodenum weight was higher ($P < 0.05$) in the 130IP birds than in the OPT birds. The relative duodenum weight for RES birds at d 6 (−24%) was lower ($P < 0.001$) than for CONTR birds. The relative jejunum weight at d 6 (−18%) and d 14 (−17%) was lower ($P < 0.001$) for RES birds than for CONTR birds and tended ($P = 0.076$) to be lower at d 9 (−6%). The relative ileum weight at d 6

Table 8. Effect of different dietary amino acid treatments and feed restriction on gain to feed ratio (G:F) of male broilers[1]

		G:F[3], g/g										
Item[2]		0–3d	3–6d	6–9d	9–11d	11–14d	14–21d	21–30d	30–36d	0–14 d	0–30 d	0–36 d
Ross 308	CONTR	1.204	0.855	0.802	0.770	0.756	0.696	0.640	0.570	0.811	0.693	0.658
	130IP	1.325	1.051	0.886	0.847	0.811	0.661	0.627	0.561	0.893	0.696	0.659
	OPT	1.293	1.027	0.831	0.767	0.748	0.677	0.624	0.570	0.839	0.689	0.655
	RES	1.247	0.879	0.813	0.797	0.751	0.726	0.638	0.587	0.828	0.698	0.665
Cobb 500	CONTR	1.292	0.882	0.784	0.772	0.735	0.663	0.605	0.595	0.810	0.669	0.648
	130IP	1.371	1.038	0.901	0.875	0.825	0.653	0.598	0.565	0.913	0.683	0.650
	OPT	1.341	1.006	0.829	0.777	0.750	0.652	0.613	0.570	0.839	0.676	0.647
	RES	1.280	0.864	0.800	0.784	0.745	0.723	0.626	0.593	0.817	0.690	0.661
Pooled SEM (n = 6)		0.013	0.017	0.015	0.014	0.007	0.010	0.008	0.011	0.004	0.005	0.004
Main effects												
Breed												
Ross 308		1.267	0.953	0.833	0.795	0.767	0.690	0.632	0.572	0.843	0.694	0.659
Cobb 500		1.321	0.947	0.828	0.802	0.764	0.673	0.611	0.581	0.845	0.680	0.652
Pooled SEM (n = 24)		0.006	0.009	0.007	0.007	0.004	0.005	0.004	0.006	0.002	0.002	0.002
Starter diet												
	CONTR	1.248	0.868	0.793	0.771	0.745	0.679	0.623	0.583	0.810	0.681	0.653
	130IP	1.348	1.044	0.893	0.861	0.818	0.657	0.612	0.563	0.903	0.690	0.654
	OPT	1.317	1.017	0.830	0.772	0.749	0.664	0.618	0.570	0.839	0.682	0.651
	RES	1.263	0.872	0.806	0.790	0.748	0.725	0.632	0.590	0.822	0.694	0.663
Pooled SEM (n = 12)		0.009	0.012	0.010	0.010	0.005	0.007	0.006	0.008	0.003	0.003	0.003
P-value of factorial analysis												
Breed		<0.001	0.653	0.670	0.486	0.609	0.019	<0.001	0.279	0.477	<0.001	0.018
Feed treatment		< 0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.140	0.080	<0.001	0.035	0.053
Breed × Feed treatment		0.165	0.501	0.685	0.522	0.107	0.409	0.412	0.685	0.009	0.443	0.889
P-value of contrasts												
CONTR vs. 130IP		< 0.001	<0.001	<0.001	<0.001	<0.001	0.035	0.226	0.079	<0.001	0.095	0.749
CONTR vs. OPT		< 0.001	<0.001	0.016	0.936	0.622	0.150	0.579	0.248	<0.001	0.822	0.670
CONTR vs. RES		0.240	0.842	0.365	0.162	0.686	<0.001	0.280	0.536	0.007	0.012	0.031
130IP vs. OPT		0.019	0.123	<0.001	<0.001	<0.001	0.481	0.506	0.531	<0.001	0.146	0.457

[1]Each cage contained 24 male broilers at the start of the experiment.

[2]CONTR = the control 100% ideal protein starter diet; 130IP = 130% ideal protein starter diet; OPT = daily optimised ideal protein composition of the starter diet; RES = feed intake restriction from d 4–21. [3]For d 3–6 and d 6–9 the results of 1 pen of the Ross 130% IP treatment was excluded from the calculations. The residual variance of this pen was higher than 4 times SE, most probably due to an inaccurate weighing of the birds of that pen at d 6.

Table 9. Effect of different dietary amino acid treatments and feed restriction on slaughter characteristics at 37 d of age[1]

Item[2]		Carcass, % of BW	Breast, % of carcass	Abdominal fat, % of carcass
Ross 308	CONTR	72.6	34.5	1.55
	130IP	71.5	35.1	1.44
	OPT	72.0	34.6	1.37
	RES	71.6	32.6	1.79
Cobb 500	CONTR	71.7	34.3	1.40
	130IP	71.3	33.8	1.36
	OPT	72.2	34.6	1.46
	RES	71.4	32.7	1.71
Pooled SEM (n = 6)		0.5	0.6	0.12
Main effects				
Breed				
Ross 308		71.9	34.2	1.54
Cobb 500		71.6	33.9	1.48
Pooled SEM (n = 24)		0.3	0.3	0.06
Starter diet				
	CONTR	72.1	34.4	1.48
	130IP	71.4	34.4	1.40
	OPT	72.1	34.6	1.41
	RES	71.5	32.7	1.75
Pooled SEM (n = 12)		0.4	0.4	0.09
P-value of factorial analysis				
Breed		0.207	0.235	0.376
Feed treatment		0.069	<0.001	<0.001
Breed × Feed treatment		0.581	0.320	0.558
P-value of contrasts				
CONTR vs. 130IP		0.038	0.877	0.402
CONTR vs. OPT		0.920	0.571	0.477
CONTR vs. RES		0.072	<0.001	0.004
130IP vs. OPT		0.048	0.679	0.897

[1]Five birds were slaughtered per cage.

[2]CONTR = the control 100% ideal protein starter diet; 130IP = 130% ideal protein starter diet; OPT = daily optimised ideal protein composition of the starter diet; RES = feed intake restriction from d 4–21.

(−23%), d 9 (−8%), and d 14 (−17%) was lower ($P < 0.05$) for RES birds than for CONTR birds. Relative small intestine weights at d 36 were similar for all treatments.

Mortality and Heart Weight

For RES birds, the relative heart weight (Table 11) was lower ($P < 0.05$) than for the CONTR birds at d 6 (−12%), d 9 (−11%), and d 14 (−8%). Mortality due to leg problems was lower ($P < 0.01$) for RES Ross and Cobb broilers compared with CONTR birds (Table 12). Mortality due to leg problems was lower ($P < 0.05$) for 130IP and OPT Ross birds compared with Ross CONTR birds and was not different from CONTR birds for Cobb.

Discussion

Performance

In the current experiment, higher dietary protein concentrations (130IP and OPT) initially decreased feed intake. This is in line with the general opinion that high dietary protein suppresses feed intake[(160)]. However, after 6 d on a high protein diet, feed intake equalled

Table 10. Effect of different dietary amino acid treatments and feed restriction on relative weight of the duodenum, jejunum, and ileum of male broilers[1]

		Duodenum[3], g/kg $BW^{0.75}$				Jejunum, g/kg $BW^{0.75}$				Ileum, g/kg $BW^{0.75}$			
Item[2]		d 6	d 9	d 14	d 36	d 6	d 9	d 14	d 36	d 6	d 9	d 14	d 36
Ross 308	CONTR	30.2	34.3	34.5	26.4	45.2	53.3	53.9	55.6	32.2	35.5	35.1	29.7
	130IP	35.9	39.5	37.5	24.0	45.0	56.2	53.6	45.1	31.0	36.4	37.0	26.8
	OPT	32.7	36.2	35.9	25.3	43.2	59.2	51.7	48.9	30.7	37.3	36.4	27.5
	RES	22.5	30.8	30.0	25.0	36.0	49.9	42.4	54.1	23.8	30.7	32.5	29.4
Cobb 500	CONTR	30.6	32.8	35.7	25.5	39.7	53.2	51.2	48.7	29.8	34.1	36.4	30.0
	130IP	31.3	37.8	38.1	25.4	44.7	55.0	51.4	48.1	32.5	37.7	36.1	29.3
	OPT	34.9	34.1	35.3	27.0	50.9	57.1	55.4	48.1	34.9	35.2	36.6	28.7
	RES	23.6	34.5	34.4	28.0	33.5	50.5	45.1	48.4	24.0	33.1	31.4	29.6
Pooled SEM (n = 6)		1.3	1.7	1.9	1.9	1.8	1.7	2.3	2.3	1.1	1.2	1.4	1.6
Main effects													
Breed													
Ross 308		30.3	35.2	34.5	25.2	42.3	54.6	50.4	50.9	29.4	35.0	35.3	28.4
Cobb 500		30.1	34.8	35.9	26.5	42.2	53.9	50.8	48.3	30.3	35.0	35.1	29.4
Pooled SEM (n = 24)		0.6	0.8	1.0	0.9	0.9	0.8	1.2	1.1	0.6	0.6	0.7	0.8
Starter diet													
	CONTR	30.4	33.5	35.1	26.0	42.5	53.2	52.6	52.2	31.0	34.8	35.7	29.8
	130IP	33.6	38.7	37.8	24.7	44.9	55.6	52.5	46.6	31.7	37.0	36.6	28.0
	OPT	33.8	35.1	35.6	26.2	47.0	58.2	53.6	48.5	32.8	36.3	36.5	28.1
	RES	23.1	32.7	32.2	26.5	34.7	50.2	43.7	51.2	23.9	31.9	31.9	29.5
Pooled SEM (n = 12)		0.9	1.2	1.4	1.3	1.2	1.2	1.6	1.6	0.8	0.8	1.0	1.1
P-value of factorial analysis													
Breed		0.820	0.752	0.303	0.337	0.923	0.559	0.812	0.113	0.289	0.967	0.890	0.359
Feed treatment		<0.001	0.006	0.053	0.797	<0.001	<0.001	<0.001	0.074	<0.001	<0.001	0.007	0.560
Breed × Feed treatment		0.056	0.279	0.626	0.774	0.005	0.860	0.390	0.124	0.040	0.198	0.835	0.870
P-value of contrasts													
CONTR vs. 130IP		0.015	0.004	0.177	0.507	0.182	0.162	0.990	0.019	0.514	0.065	0.579	0.260
CONTR vs. OPT		0.010	0.345	0.813	0.914	0.013	0.005	0.665	0.119	0.120	0.211	0.602	0.274
CONTR vs. RES		<0.001	0.609	0.143	0.792	<0.001	0.076	<0.001	0.691	<0.001	0.018	0.012	0.813
130IP vs. OPT		0.861	0.046	0.262	0.441	0.221	0.126	0.656	0.404	0.357	0.533	0.973	0.973

[1]Per dissection day, 2 birds were dissected per cage. $BW^{0.75}$ = metabolic BW.

[2]CONTR = the control 100% ideal protein starter diet; 130IP = 130% ideal protein starter diet; OPT = daily optimised ideal protein composition of the starter diet; RES = feed intake restriction from d 4–21. [3]Room × dietary treatment interaction was significant (*P* = 0.047) for relative duodenum weight at d 36. This was reflected in a clear difference between the relative duodenum weights for the CONTR treatment between rooms (28.5 vs. 23.4) and hardly any difference between rooms for the 130IP (23.5 vs. 25.9), OPT (30.0 vs. 28.4) and the RES treatments (27.5 vs. 25.5).

Table 11. Effect of different dietary amino acid treatments and feed restriction on relative heart weight of male broilers[1]

		Heart, g/kg $BW^{0.75}$			
Item[2]		d 6	d 9	d 14	d 36
Ross 308	CONTR	17.4	19.2	17.1	23.2
	130IP	17.1	18.5	17.6	22.7
	OPT	16.6	18.7	17.1	22.2
	RES	15.2	16.5	15.6	22.7
Cobb 500	CONTR	16.6	17.6	16.6	20.9
	130IP	16.5	18.2	16.4	20.9
	OPT	15.6	17.7	17.1	20.7
	RES	14.8	16.1	15.4	21.4
Pooled SEM (n = 6)		0.4	0.6	0.6	0.7
Main effects					
Breed					
Ross 308		16.6	18.2	16.8	22.8
Cobb 500		15.9	17.4	16.4	21.3
Pooled SEM (n = 24)		0.2	0.3	0.3	0.4
Starter diet					
	CONTR	17.0	18.4	16.8	22.0
	130IP	16.8	18.4	17.0	21.8
	OPT	16.1	18.2	17.1	21.4
	RES	15.0	16.3	15.5	22.1
Pooled SEM (n = 12)		0.3	0.4	0.4	0.5
P-value of factorial analysis					
Breed		0.023	0.047	0.282	0.002
Feed treatment		<0.001	0.002	0.032	0.780
Breed × Feed treatment		0.944	0.698	0.770	0.919
P-value of contrasts					
CONTR vs. 130IP		0.644	0.982	0.800	0.717
CONTR vs. OPT		0.052	0.752	0.661	0.386
CONTR vs. RES		<0.001	<0.001	0.027	0.965
130IP vs. OPT		0.131	0.735	0.853	0.612

[1]Per dissection day, 2 birds were dissected per cage. $BW^{0.75}$ = metabolic body weight.
[2]CONTR = the control 100% ideal protein starter diet; 130IP = 130% ideal protein starter diet; OPT = daily optimised ideal protein composition of the starter diet; RES = feed intake restriction from d 4–21.

(Cobb broilers) or exceeded (Ross broilers) feed intake of CONTR birds. This higher feed intake with high-protein diets is probably related with the higher BW of birds on a high-protein diet because when expressed as percentage of the BW, the feed intake of the birds on a high-protein diet remains lower than that of the CONTR birds (data not given). When the birds (130IP and OPT) at d 14 were switched to the same diet as the CONTR treatment, the feed intake clearly exceeded the feed intake of the CONTR birds. However, when expressed again relative to BW, feed intake after d 14 was similar to that of CONTR birds (data not given). The increased dietary AA concentrations (130IP and OPT treatments) in the starter diet improved BW gain during the starter period. These differences in BW gain remained significant until 30 d of age. This effect on BW gain in the starter phase agrees with results of Morris and Abebe[126] and of Wijtten *et al*.[138]. In the current experiment, between d 3 and 6, relative BW gain of the 130IP (+12%) and OPT (+13%) birds was higher than that of the CONTR birds (Figure 1). The stimulation of BW gain due to high dietary protein in this phase may have been crucial in stimulation of feed intake and BW gain in subsequent phases. Between d 9 and 14, BW gain of the OPT birds was significantly lower (−5%) than of the 130IP birds. This indicates that dietary AA requirements for maximal BW gain in this phase are probably higher than those calculated for our OPT diets. Thus, dietary

Table 12. Effect of different dietary amino acid treatments and feed restriction on mortality due to ascites or leg problems up to d 36 of male broilers[1]

		Mortality, %	
Item[2]		Ascites	Leg problems
Ross 308	CONTR	1.9	4.6
	130IP	0.0	0.0
	OPT	0.9	0.0
	RES	0.9	0.0
Cobb 500	CONTR	2.8	4.6
	130IP	2.8	4.6
	OPT	0.9	7.4
	RES	0.0	0.9
Starter diet			
	CONTR	2.3	4.6
	130IP	1.4	2.3
	OPT	0.9	3.7
	RES	0.5	0.5
P-value of contrasts			
CONTR vs. 130IP		0.475	0.189
CONTR vs. OPT		0.253	0.630
CONTR vs. RES		0.100	0.006
130IP vs. OPT		0.653	0.398

[1]Mortality percentage is based on 18 broilers per pen (24 broilers at the start minus 6 broilers dissected at d 6, 9 or 14).
[2]CONTR = the control 100% ideal protein starter diet; 130IP = 130% ideal protein starter diet; OPT = daily optimised ideal protein composition of the starter diet; RES = feed intake restriction from d 4–21.

AA concentrations should remain at a high concentration for a longer period, as was done with the 130IP treatment.

The RES treatment reduced BW gain during the restriction phase, as was expected. After the restriction phase, this BW difference became gradually smaller with age until the end of the experiment. This was not yet completely compensated at d 36. For the Ross broilers, this is in line with our previous experiments[(139)]. For the Cobb broilers, however, this was in contrast with our previous experiments, in which Cobb broilers did fully compensate their BW at 37 d of age. The lower breast yield and higher abdominal fat yield due to feed restriction were also observed in our previous experiment with the same restriction program. Thus, with this restriction program in young broilers, final weights can be similar to CONTR birds. However, carcass quality is consistently compromised. In our previous experiment[(139)] the abdominal fat yield was similar to the CONTR treatment when a more severe restriction program or an abrupt change from restriction to *ad libitum* feeding was applied. Plavnik and Hurwitz[(161)] showed that with a severe restriction program, abdominal fat yield was lower in RES-fed birds compared with CONTR birds. The differences in effect of feed restriction on abdominal fat yield among studies may relate to differences in the restriction programs and changes in broiler genetics over time.

Small Intestines

In the present experiment, increased dietary AA concentrations (130IP and OPT treatments) in the starter diet increased duodenum weights at d 6 and 9. Jejunum weights were only increased with the OPT treatment at d 6 and 9. The ileum weights were not affected by the 130IP and OPT treatments. Thus, effects were most pronounced in the duodenum, decreased in the jejunum, and nearly disappeared in the ileum. It has been shown in pigs

that the proximal small intestine is more dependent on luminal nutrient supply for intestinal protein synthesis than the distal small intestine[162]. This may explain why the effect of dietary protein on small-intestinal weight in the current study was more pronounced in the duodenum than in the jejunum and ileum. Moreover, this indicates that the luminal supply of AA is important for duodenum weight. Batal and Parsons[38] investigated the small-intestinal weight of broilers either fed a maize-soybean meal-based diet with 23% CP or a crystalline AA-based diet with 14% CP. In line with our study, they showed that relative weight of the jejunum and ileum at 7 d of age was higher when fed the 23% CP diet compared with the 14% CP diet. At 21 d of age, Batal and Parsons[38] found no effect of the different diets on ileum weight and they found a lower jejunum weight for the 23% CP diet compared with the 14% CP diet. Lemme *et al*.[163] found that the relative weight of the entire small intestine was increased at 7 d of age with high dietary IP concentrations, but this effect had disappeared at 14 d of age. Finally, Swatson *et al*.[39] found no consistent effects of different dietary protein concentrations (20–40% with balanced or unbalanced AA fed from 10–24 d of age) on relative small intestine weight. In our experiment and in those of Batal and Parsons[38] and Lemme *et al*.[163], the most pronounced effects were found between 6 and 9 d of age and hardly any effects were seen in birds of 14 d and older. This indicates that suboptimal protein nutrition will suppress small-intestinal weight in birds between 6 and 9 d of age but may not affect small-intestinal weight in birds of 2 wk and older. In the present experiment, weights of the different small-intestinal segments were similar for the 130IP and the OPT treatments at most days. This means that a daily optimisation of the AA concentration and profile in the starter diet does not further increase small-intestinal weight compared with a treatment with a constantly high dietary IP concentration (130IP). To the contrary, duodenum weight at d 9 was lower for the OPT birds than for the 130IP birds, which is probably related to the suboptimal dietary AA concentrations at that day in the OPT diets.

Feed intake restriction reduced the duodenum, jejunum, and ileum weight at d 6 in the present experiment. At d 9 and 14, feed intake restriction reduced jejunum and ileum weights. In line with this, Palo *et al*.[164] also reported a decrease of the relative duodenum weight and jejunum weight at the end of a 7-d feed restriction period. In contrast to this, others reported no difference in relative small intestine weights due to feed restriction or an increase in relative small intestine weights due to feed restriction[39,165]. The above shows that feed intake restriction gives differential effects on relative small intestine weights. These discrepancies are most probably related to differences in the severity of the restriction between studies. Moreover, there were differences among studies in the time between the last meal and dissection in RES birds and in fed birds. These differences make it difficult to draw firm conclusions regarding the relation between feed intake restriction and small-intestinal weight.

The small intestine is an important organ for the digestion and absorption of nutrients. Therefore, an adequate development of this organ is essential for digestion and absorption. In this respect, a study of Maisonnier *et al*.[166] with broilers showed that the weight:length ratio of the duodenum of individual birds was positively related with the digestibility percentage of nutrients. To a lesser extend, this relation was also found for duodenum weight relative to BW. For the jejunum and ileum, the relation between weight:length ratio and nutrient digestion was less pronounced or did not exist in the experiment of Maisonnier *et al*.[166]. To the contrary, the addition of enzymes or antibiotics to the diet, which have shown to improve BW gain and feed efficiency, are often reported to reduce the length and

weight of the small intestines[167,168]. Marquardt[169] hypothesised that a reduced intestine size after enzyme supplementation is related to a more efficient nutrient digestion, which reduces the need for an enlarged intestine. On the other hand, a thinner intestinal wall due to a lower bacterial challenge after antibiotic feeding is hypothesised to be one of the mechanisms that improves feed efficiency due to antibiotics[167]. Thus, an increased intestinal weight can be related to an improved as well as a deteriorated animal performance. The above indicates that when an improved performance is associated with an increased intestinal size, this may be related to an enhanced digestive and absorptive capacity. Moreover, when a deteriorated performance is associated with an increased intestinal size, this may be related with an enhanced defence mechanism (e.g., immunity and physical barrier function) of the intestines. This may occur as a result of a bacterial challenge. In the present study, the increased duodenum weights, due to enhanced dietary AA concentrations, coincided with an improved BW gain. Moreover, Maisonnier *et al*.[166] showed that the weight:length ratio of the duodenum, and to a lesser extend the relative duodenum weight, was positively related with digestion. Based on this, we hypothesise that especially the increased duodenum weight may have been important in facilitating the increased BW gain with the enhanced AA treatments in the present experiment. However, this remains speculative and the mechanism should be revealed in further studies.

Mortality

Feed restriction reduced the incidence of ascites numerically and reduced the incidence of leg problems significantly in the present experiment. This is in line with findings from Robinson *et al*.[170] and Demir *et al*.[171]. The relative heart weight of the restriction treatment, in the present experiment, was lower on d 6, 9, and 14 than the weights of the heart of the CONTR treatment. In literature, a lower right heart ventricle weight has been associated with a low susceptibility for ascites[172]. It is well known that feed restriction can reduce the incidence of ascites[173]. Thus, the positive effect of feed restriction on mortality may be related to a lower oxygen demand as a consequence of a lower metabolic rate of the birds during the first weeks of life. However, the 130IP treatment in the present experiment increased relative BW gain during the first 2 wk of life, which coincided with a lower mortality due to leg problems in Ross broilers. The absence of increased mortality in these faster growing 130IP birds weakens the direct relation between early life BW gain and mortality in latter life. This contributes to the idea that the effect of early life feed restriction on early life organ development may also be a factor that is critical for ascites and leg problems. In the current experiment, feed intake restriction decreased small-intestinal weight and the 130IP and OPT treatments increased small-intestinal weights. Feed intake restriction reduced mortality due to leg problems in Ross and Cobb broilers. Extra dietary protein decreased mortality due to leg problems in Ross broilers and exhibited no effect in Cobb broilers. Thus, small intestine weights and leg problems are not consistently related between treatments and breeds in the present experiment. This indicates that weight development of the small intestines is not related to leg problems. The effect of feed intake restriction on leg problems is consistently shown in literature and in the present experiment. However, evidence for the positive effect of enhanced protein concentrations in the starter diet on leg problems in the present experiment is only observed in Ross broilers and not in Cobb broilers and should be repeated to be convincing.

Conclusions and Implication

Feed intake restriction reduces mortality due to leg problems in Ross and Cobb broilers and extra dietary protein decreases mortality due to leg problems in Ross broilers but not in Cobb broilers. The present experiment does not show that small-intestinal weight development is related to mortality. Enhanced dietary AA concentrations increase duodenum weight in birds between 6 and 9 d of age and hardly affect small-intestinal weight in 14-d-old or older birds. The increased duodenum weights coincide with an improved BW gain. This indicates that duodenum weight may be important in facilitating BW gain in young broilers. Thus, it may be worthwhile to pay more attention to the relation between nutrition and duodenum weight and duodenum function in further studies. The daily optimisation of the AA concentration and profile in the starter diet (OPT) does not further increase BW gain or duodenum weight compared with a treatment with a constant high dietary IP concentration (130IP).

Chapter 4

Intestinal barrier function and absorption in pigs after weaning: a review

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Abstract

Under commercial conditions, weaning of piglets is associated with social, environmental and dietary stress. Consequently, small-intestinal barrier and absorptive functions are deteriorated within a short time after weaning. Most studies that have assessed small-intestinal permeability in pigs after weaning either used Ussing chambers or orally administered marker probes. Paracellular barrier function and active absorption decrease when pigs are weaned at 3 wk of age or earlier. However, when weaned at 4 wk of age or later, the barrier function is less affected, and active absorption is not affected or is increased. Weaning stress is a critical factor in relation to the compromised paracellular barrier function after weaning. Adequate feed intake levels after weaning prevent the loss of intestinal barrier function. Transcellular transport of macromolecules and passive transcellular absorption decrease after weaning. This may reflect a natural intestinal maturation process that is enhanced by the weaning process and prevents the pig from an antigen overload. It seems that passive and active absorption after weaning adapt accurately to the new environment after weaning when pigs are weaned after 3 wk of age. However, when weaned at 3 wk of age or earlier, the decrease in active absorption indicates that pigs are unable to sufficiently adapt to the new environment. To improve weaning strategies, future studies should distinguish whether the effect of feed intake on barrier function can be directed to a lack of a specific nutrient, i.e. energy or protein.

Keywords: pigs, weaning, intestinal barrier, intestinal absorption.

Introduction

The small intestinal epithelium has 3 major functions; (1) the digestion and absorption of nutrients; (2) the secretion and absorption of water and electrolytes to maintain a proper viscosity of the luminal content and to flush out noxious components; (3) serving as a barrier against noxious antigens and pathogens. Impaired intestinal barrier function or an increased intestinal permeability may promote the translocation of bacteria and the entering of allergenic compounds from the gut into the body. This results in immunological responses and an increased susceptibility for infections[(174,175)]. Weaning of pigs is associated with social, environmental and dietary stress[(13)], and in rats and humans various stressors will

deteriorate small-intestinal barrier function[176,177]. Evidence for weaning stress in pigs is that cortisol and corticotrophin-releasing factor concentrations in the blood plasma are increased after weaning[14,15]. Moreover, about 10% of the pigs do not ingest any feed during the first 48 h after weaning[16], and most other pigs have a low feed intake. In addition, low feed intake after weaning is consistently associated with villous atrophy in the small intestine[13,23,32]. After weaning, pigs are especially susceptible for infections[13]. Oedema disease, caused by the Shiga-like toxin type II variant from some *Escherichia coli* strains, is associated with the process of weaning but requires a disturbed intestinal barrier function in order to enable large toxin molecules to pass the intestinal epithelium[27]. Thus, the effect of weaning on intestinal morphology, susceptibility for infections and occurrence of oedema disease indicates that intestinal barrier function is disturbed after weaning. Moreover, a reduced villous surface after weaning implicates a reduction in intestinal absorptive capacity as well. The present review discusses the effect of weaning and dietary treatments after weaning on intestinal barrier function and absorption. The study opens with some background information regarding epithelial transport in the small intestine. Subsequently, the most commonly used techniques, Ussing chambers and orally administered marker probes, to asses intestinal barrier function and intestinal absorption are discussed. Eventually, the review concentrates on the effects of weaning and treatments after weaning on intestinal barrier function and absorption in pigs.

Background of epithelial transport

Transport across the small-intestinal epithelium can be separated into paracellular and transcellular pathways. Paracellular transport represents diffusion between epithelial cells. Networks of proteins called tight junctions connect the epithelial cells and 'seal' the space between the cells. The tight junctions are selectively permeable for ions, small molecules and water[178]. Transcellular transport represents either the uptake of small molecules (e.g. nutrients) by carrier-mediated (active) or carrier-unmediated (passive) transport through absorptive small-intestinal cells (enterocytes) or the uptake of macromolecules by endocytosis. Endocytosis is specifically important with respect to maternal Ig uptake after birth and for antigen uptake. In healthy animals, antigen uptake is precisely regulated to train the immune system. Antigen uptake by endocytosis is more common in the follicle-associated epithelium, covering the Peyer's patches, than in enterocytes[179]. In the present review, we distinguish between small-intestinal barrier function and small-intestinal absorption. For this distinction, we associate a disturbed barrier function with increased paracellular transport and transepithelial transport (paracellular and transcellular) of macromolecules into the body.

Techniques to measure epithelial transport

Ussing chamber

Intestinal barrier function and absorption in pigs after weaning has mainly been assessed in *ex vivo* studies with Ussing chambers. In Ussing chambers a section of intestinal mucosa is mounted between 2 chambers. Marker probes are added to the solution in the chamber at the mucosal site. The appearance of these marker probes in the chamber at the serosal site represents the permeability for these probes. Table 1 gives an overview of probes that have been used in studies with pigs after weaning. In addition, Table 1 gives 3 electrophysical

Table 1. Marker probe characteristics and electrophysical parameters

Probe	Molecule size, Da	Transepithelial routes / description
Probes used in Ussing chambers		
Ovalbumin	45000	Transcellular by endocytosis[180]
Horseradish peroxidase	40000	Transcellular by endocytosis[181,182] as well as paracellular through tight junctions[183]
Na-Flu	376	Passive transcellularly[15,180,184]
Mannitol	182	Mainly via a paracellular route[185,186], passive transcellular routes cannot be excluded[187].
D-glucose[1]	180	Carrier-mediated transcellular transport[19]
Gln[1]	146	Carrier-mediated transcellular transport[19]
Glycylsarcosine	146	Via a H^+ carrier-mediated transcellular route[188]
Electrophysical parameters used in Ussing chambers		
TEER	-	Reflects mainly paracellular transport[189]
Conductance	-	Inverse of TEER[189]
Short-circuit current	-	Measures active ion transport and gives indication of water movement and electrolyte dependent glucose and amino acid absorption[189]
Probes used in vivo		
Bovine serum albumin	66000	Transcellular by endocytosis[180]
Lactulose	342	Paracellular[190]
Mannitol	182	Mainly via a paracellular route[185,186], passive transcellular routes cannot be excluded[187].
L-rhamnose	164	Transcellular (aqueous pores) or paracellular[185]
D-xylose	150	Passive transcellular, paracellular[191] or carrier-mediated transcellular transport[185]

Na-Flu, sodium-fluorescein isothiocyanate; TEER, transepithelial electrical resistance.
[1]Determined by measuring the change in short-circuit current after the addition of glucose or Gln to the mucosal site of the chamber.

parameters that can be determined in Ussing chambers. First, the transepithelial electrical resistance (TEER) of the mounted intestinal mucosa can be determined. This is considered to reflect the opening of the tight junctions between epithelial cells, i.e. the paracellular permeability of the intestinal mucosa[189]. An increased TEER reflects decreased paracellular permeability, and a decreased TEER reflects increased paracellular permeability. Second, the transepithelial electrical conductance can be determined. This is the inverse of the TEER[189]. Finally, the short-circuit current (Isc) over the mucosa can be measured. This is a measurement of active electrogenic ion transport across the epithelium. Increased Isc either reflects increased electrogenic anion secretion (e.g. Cl^-, HCO_3^-) or increased electrogenic cation absorption (Na^+). *In vivo,* the water flow over the intestinal epithelium follows the osmotic gradient induced by actively transported electrolytes. Therefore, Isc can be used to indicate water movement over the epithelium[189]. Moreover, the change in Isc after the addition of specific nutrients to the mucosal solution (e.g. glucose, Gln) is an indirect measure of Na-dependent nutrient absorption.

Orally administered marker probes

In vivo permeability tests have been used to assess intestinal permeability in human and medical research for many decades[192]. The principle of the test is that orally administered test substances (probes) that are not metabolised in the body pass through the intestinal epithelium and are excreted in the urine within a short time after administering. Most frequently used probes are those monosaccharides and disaccharides that are not degraded

by digestive enzymes, that are hardly metabolised in the body but that are fermented by 'colonic' bacteria[185]. Because of these 3 characteristics, the sugars are almost exclusively absorbed by the small intestine, and the influences of digestion and metabolism are minimised. Therefore, depending on their permeation route, they can be used as specific markers for small-intestinal permeability function, absorption function or both[185].

In vivo permeability tests have been used in pigs around weaning. The most frequently used test in this respect is the D-xylose absorption test[23,193-197]. In studies with pigs, D-xylose is exclusively analysed in blood 1 h after an oral dose. In humans both blood and urine are used for the D-xylose test. In animals, it is easier to sample blood than to perform a quantitative urine collection. However, a single blood measurement is affected by many factors, for instance, rate of gastric emptying, intestinal absorption rate and clearance rate from the blood[198]. Therefore, in general, a correct quantitative urine collection is better than a single blood sampling in order to get an accurate permeability or absorption estimate[198]. Even though, absorption and permeability tests based on quantitative urine collection can also be disturbed by several factors. Most important in this respect are a marked renal dysfunction, an incomplete urine collection and an increased luminal clearance of the marker probes by bacterial overgrowth[198,199].

It is evident that knowledge about the permeation routes of the used marker probes is required in order to draw conclusions with physiologic relevance. Of the marker probes that are most commonly used with this technique, we have gathered the most relevant information in Table 1. The test results with this technique can, however, be influenced by many premucosal factors (gastric emptying, intestinal transit time and bacterial degradation) and postmucosal factors (metabolism, endogenous production, completeness of urinary collection and renal function)[185]. To reduce the effects of those premucosal and postmucosal factors, the theory of differential urinary excretion of marker probes has been introduced[185,200]. In this approach, it is common practice to use both a disaccharide and a monosaccharide, which are both transported over the epithelium by unmediated diffusion. The transport of monosaccharides used for this test occurs either through tight junctions between the epithelial cells or through aqueous pores in the cell. For disaccharides, the transport occurs through the tight junctions of the crypts[175,185]. The ratio of the urinary recovery of the 2 sugars provides information about the intestinal barrier function. The assumption is that both probes are affected by the premucosal and postmucosal factors to a similar extent, and their ratio is not disturbed by those factors[175]. In this so-called 'dual sugar test', most often, lactulose is used as a disaccharide to assess paracellular permeability and L-rhamnose or mannitol (a sugar alcohol) is used as a monosaccharide. As an example, an increase in the lactulose:L-rhamnose ratio indicates a decrease in the intestinal barrier function, whereas a decrease in the lactulose:L-rhamnose ratio indicates an improved intestinal barrier function. The use of the dual sugar test in pigs is to our knowledge limited to 4 studies. In 2 studies, the effect of parenteral vs. enteral nutrition in neonatal piglets[201,202] has been addressed with lactulose and mannitol as marker probes. In another study, the effect of an lipopolysaccharide challenge in 20 kg gilts has been evaluated with lactulose and L-rhamnose as marker probes[203]. In the fourth study, enhanced dietary Zn concentrations in the weaner diet decreased the lactulose:mannitol ratio in pigs at 2 wk after weaning[204].

Thus, studies in pigs shortly after weaning with orally dosed marker probes so far have been limited to a few that have assessed the absorptive small-intestinal function with D-

xylose. Up to now, the use of the dual sugar tests to address small-intestinal barrier function in pigs shortly after weaning is limited to 1 study.

Comparison of techniques

With orally administered marker probes, intestinal barrier function and absorption itself can be measured without killing the pig. This enables repeated measurements on the same pig over time, which allows correlating permeability and absorption parameters with performance and health parameters over time. This is a clear advantage above the Ussing chamber technique for which the pigs need to be euthanised. However, Ussing chamber measurements enable the assessment of the permeability at a specific intestinal site. Thus both techniques are complementary.

Intestinal barrier function after weaning

The effects of weaning and weaning conditions on the intestinal barrier function in pigs have been assessed in a small number of studies using Ussing chambers. Mannitol and TEER have been used to assess the barrier function related to paracellular transport, and horseradish peroxidase (HRP) has been used to assess the barrier function related to endocytosis. Mannitol is a sugar alcohol with a molecular mass of 182 Da. It is thought to cross the mucosa mainly via a paracellular route[185,186], but also transcellular routes cannot be excluded[187]. Horseradish peroxidase is a 40 kDa protein with enzymatic activity. The enzymatic activity makes it possible to detect low concentrations of HRP. Therefore, HRP is a very sensitive marker to measure transport of low amounts of macromolecules over the epithelium. The basal flux of intact HRP across the intestinal epithelium occurs mainly through transcelluar transport via endocytosis[182,183]. A compromised barrier function may increase trancellular endocytosis of HRP[182] as well as paracellular transport of HRP through large pores in the tight junctions[183]. Therefore, HRP is primarily used as a marker of antigen uptake through endocytosis[205].

Transcellular transport (endocytosis)

In 2 studies with pigs, it has been revealed that in the proximal jejunum, the HRP flux was decreased at 2, 5 and 15 d after weaning[19] and at 4 and 7 d after weaning[91] compared with pre-weaning levels (Table 2). We suggest that after weaning, the natural maturation process, enhanced by weaning may reduce the permeability for macromolecules by a reduction in endocytosis rate. This is further supported by a 90% lower HRP flux at 35 d after weaning compared with 15 d after weaning, as has been established by Boudry *et al.*[19]. This suggested that the maturation process may be a beneficial mechanism that prevents the animal suffering from an antigen overload. Such an antigen overload may result in an excessive activation of the immune system when the pigs are subjected to their new environment after weaning. In a study of van der Meulen *et al.*[15], in the mid- jejunum, the HRP flux increased at 4 and 7 d after weaning compared with 1 d after weaning. In that study, a pre-weaning measurement was not done (first measurement 1 d after weaning) and the pigs were transported and separated from the other pigs and fasted overnight before Ussing chamber measurements were performed. The handling stress in this experiment the day before measurements can explain the contradiction with the results of the 2 studies that measured HRP flux in the proximal jejunum[19,91]. Stress has been shown

Table 2. Small-intestinal barrier function in pigs after weaning as measured by horseradish peroxidase flux, mannitol flux and transepithelial electrical resistance in Ussing chambers

References	Intestinal segment	Weaning age, d	Feed intake after weaning (fraction of energy requirement for maintenance)	Change after weaning, %	Treatment description (first mentioned = control if unclear)	Change compared with control, %
Horseradish peroxidase						
Boudry *et al.*[19]	Proximal jejunum	21	d2: 0 d5: 1 d8: 2 d15: 4	d2: –80* d5: –64* d8: –27 d15: –63* d35: –96*		
	Ileum			d2: 25 d5: –53 d8: –39 d15: 29		
Verdonk *et al.*[91]	Proximal jejunum	26	Low: d1: 0 d2: 0 d3: 1 d4: 1.5 d5: 1.5 d6: 1.5 d7: 1.5	d4: –77* d7: –51*	Low vs. high feed intake	d4: 130 d7: 10
			High d1: 0.5 d2: 1 d3: 1.5 d4: 2 d5: 2.5 d6: 2 d7: 2	d4: –47 d7: –47		
Van der Meulen *et al.*[15]	Mid-jejunum	28	Control: d1: 1 d2: 2 d3: 2 d4: 2 d5: 2 d6: 2 d7: 2	–	No creep feed or creep feed	Average d 1, 4 & 7: 17
			Creep feed: d1: 1 d2: 2 d3: 2 d4: 2 d5: 2 d6: 2 d7: 2			
		49	Control: d1: 2 d2: 2 d3: 2 d4: 3 d5: 3 d6: 3 d7: 4	–	Weaning at 28 or 49 d of age	Average d 1, 4 & 7: 9
			Creep feed: d1: 3 d2: 3 d3: 3 d4: 4 d5: 4 d6: 4 d7: 4			
Boudry *et al.*[206]	Proximal jejunum	28	d28 to 25kg: 4.5 (milk diet). Diet switch at 25 kg, thereafter: d1: 0 d2: 1.5 d3: 3 d4: 4.5	–	Switch to barley diet at 25 kg	d4: 20
					Switch to wheat diet at 25 kg	d4: 33
Koopmans *et al.*[47]	Mid-jejunum	25	Average of d 0–d 10: 1.5 (first days low intake)	–	5 g/kg Trp in the weaner diet	d4: –53 d5: –42 d6: 31
Egberts *et al.*[207]	Proximal jejunum	21†	–	–	Enterotoxigenic *Escherichia coli* infection	d2 Not affected
Mannitol						
Verdonk *et al.*[91]	Proximal jejunum	26	Low: d1: 0 d2: 0 d3: 1 d4: 1.5 d5: 1.5 d6: 1.5 d7: 1.5	d4: 12 d7: –22	Low vs. high feed intake	d4: 16 d7: –37
			High d1: 0.5 d2: 1.0 d3: 1.5 d4: 2 d5: 2.5 d6: 2 d7: 2	d4: 29 d7: –51		
Verdonk[26]	Proximal jejunum	26	Dry: d1: 0 d2: 0 d3: 0.5 d4: 1.5 d5: 1.5 d6: 1.5	d2: 216* d6: 51	Dry vs. wet feed	Average d2 & 6: 11
			Wet: d1: 0.5 d2: 0.5 d3: 1 d4: 1 d5: 1.5 d6: 1.5	d2: 180* d6: 159*		
	Mid-jejunum	26	Low: d1: 0.5 d2: 1 d3: 1 d4: 1	d1: 53 d2: 82* d4: 111*	Low vs. high feed intake	Average d1, 2 & 4: –32*
			High: d1: 2.5 d2: 3.5 d3: 3.5 d4: 3.5	d1: 4 d2: 35 d4: 31		
Spreeuwenberg *et al.*[188]	Mid-jejunum	26	d1: 0.5 d2: 1 d3: 1 d4: 1	d1: 23 d2: 85* d4: 80*	24% lactose and 30% dietary protein vs. 41% lactose and 15% dietary protein	Average d1, 2 & 4: –25
					24% lactose and 30% dietary protein vs. 8% lactose and 45% dietary protein	Average d1, 2 & 4: –10

Table 2. *Continued*

References	Intestinal segment	Weaning age, d	Feed intake after weaning (fraction of energy requirement for maintenance)	Change after weaning, %	Treatment description (first mentioned = control if unclear)	Change compared with control, %
Moeser *et al.*[18]	Mid-jejunum	19	*Ad libitum* available, intake not given	d1: 151* (compared with unweaned)	Injection with mast cell-stabilising drug cromolyn before and after weaning	d1: –38*
		28	*Ad libitum* available, intake not given	d1: –4 (compared with unweaned)		
Moeser *et al.*[14]	Mid-jejunum	19	*Ad libitum* available, intake not given	d1: 148* (compared with unweaned)	Injection with corticotrophin-releasing factor antagonist α-helical before and after weaning	d1: –29*
Lodemann *et al.*[208]	Mid-jejunum	28	Not given	d7: –44* d28: 27*	Probiotic (*Enterococcus faecium* SF68) added to the sow and piglet diets	d0: –31*, d7: –7 & d28: –2
Lodemann *et al.*[209]	Mid-jejunum	28	Not given	Control: d7: –31 d28: –7	Probiotic (*Bacillus cereus var. toyoi*) added to the sow and piglet diets	Average d0, 7 & 28: –3
				Probiotic: d7: –25 d28: –5		
Transepithelial electrical resistance						
Boudry *et al.*[19]	Proximal jejunum	21	d2: 0 d5: 1 d8: 2 d15: 4	d2: –67* d5: 21 d8: –6 d15: 3		
	Ileum			d2: –18 d5: 116* d8: 64 d15: 91*		
Boudry *et al.*[206]	Proximal jejunum	28	d28-25kg: 4.5 (milk diet). Diet switch at 25 kg, thereafter: d1: 0 d2: 1.5 d3: 3 d4: 4.5	–	Switch to barley diet at 25 kg	d4: –14
					Switch to wheat diet at 25 kg	d4: –15
Moeser *et al.*[18]	Mid-jejunum	19	*Ad libitum* available, intake not given	d1: –40* (compared with unweaned)		
		28	*Ad libitum* available, intake not given	d1: 9 (compared with unweaned)		
Moeser *et al.*[14]	Mid-jejunum	19	*Ad libitum* available, intake not given	d1: –74* d2: –48* d7: –18* (compared with unweaned)	Injection with corticotrophin-releasing factor antagonist α-helical before and after weaning	d1: 52*
Lodemann *et al.*[209]	Mid-jejunum	28	Not given	Control: d7: –11 d28: 8	Probiotic (*Bacillus cereus var. toyoi*) added to the sow and piglet diets	Average d0, 7 & 28: 2
				Probiotic: d7: –2 d28: 11		
Carlson *et al.*[210]	Ileum	28	Not given but probably already nearly at maintenance level the second day after weaning	d1–2: 12 d5–6: 32* d14–15: 27*	(100 mg Zn, 20 mg Cu/kg) vs. (100 mg Zn, 175 mg Cu/kg)	d5–7: –10
					(100 mg Zn, 20 mg Cu/kg) vs. (2500 mg Zn, 20 mg Cu/kg)	d5–7: –10
					(100 mg Zn, 20 mg Cu/kg) vs. (2500 mg Zn, 175 mg Cu/kg)	d5–7: 0
Carlson *et al.*[211]	Mid-jejunum	28	d0–d6: 1.5 (100 mg Zn) & 1 (2500 mg Zn)	–	100 mg Zn/kg vs. 2500 mg Zn/kg	d5–6: 0
Hamard *et al.*[49]	Ileum	7	d1: 1 d2: 1 d 3–14: 2	–	Adequate (9.3 g/kg) vs. deficient (6.5 g/kg) dietary Thr	d14: –29

Values were significantly different from those at weaning or from the control treatment: *$P < 0.05$.
†Piglets were delivered by caesarian section and subsequently housed in isolators and fed *ad libitum* condensed cows' milk until the treatment started at 3 wk of age.

to increase small-intestinal HRP flux in rats[205]. The handling stress the day before Ussing chamber measurements on top of the effect of weaning may have generated the increased HRP flux over time in the experiment of van der Meulen *et al.*[15]. Unlike in the jejunum, the HRP flux in the ileum was not affected after weaning in the study of Boudry *et al.*[19]. These differences of the ileum compared with the jejunum may relate to the fact that the basal HRP flux at weaning in the ileum was only 35% of that in the jejunum. This indicates that at weaning, antigen sampling is already at a lower level in the ileum, and a further reduction (maturation) in this respect may not be beneficial.

The HRP flux in the proximal jejunum was not affected by feed intake level after weaning[91], and in the mid-jejunum, it was not affected by feed intake level before weaning and by weaning age (4 vs. 7 wk)[15,91]. Boudry *et al.*[206] changed the diet of pigs of 25 kg (4–6 wk after weaning) from a milk replacer to a barley- or wheat-based diet. At 4 d after this dietary change, the HRP flux in the proximal jejunum of the barley- and wheat-fed pigs was not different from that of control pigs fed the milk replacer. Also, extra Trp in the diet after weaning (5 g/kg diet) did not affect HRP flux in the mid-small intestine at 4, 5 or 6 d after weaning of pigs at 25 d of age[47]. Egberts *et al.*[207] found no effect of enterotoxigenic *E. coli* infection on proximal jejunal permeability for HRP (measured *in vivo*) 48 h after the infection in 3-wk-old pigs. In suckling piglets, Boudry *et al.*[212] reported that the effect of mast cell degranulation (this is a stressor that increases permeability) on ileal permeability to HRP decreased with age. This effect of age occurred earlier in piglets of sows fed *n*-3 fatty acids (minimum at 7 d of age) than in piglets of sows fed the control diet (minimum at 28 d of age). In line with this, Rådberg *et al.*[180] have shown that oral administration of red kidney bean lectins in 2-wk-old suckling piglets reduced the small-intestinal permeability for large molecules (bovine serum albumin, 67 kDa; ovalbumin, 45 kDa). Thus, dietary treatments were not able to affect HRP fluxes after weaning, but specific dietary treatments before weaning could reduce small-intestinal permeability for macromolecules. This indicates that the level of antigen uptake is higher before weaning than after weaning, creating a window that enables dietary treatments to have an effect. This further supports the hypothesis that antigen uptake decreases over time after weaning as a result of a maturation process.

Paracellular transport

Moeser *et al.*[18] have shown that mannitol flux and TEER over the mid-jejunum were not different at 1 d after weaning compared with unweaned controls for pigs weaned at 28 d of age. However, for pigs weaned at 3 wk of age, Moeser *et al.*[14,18] and Boudry *et al.*[19] have reported that the mannitol flux over the proximal or mid-jejunum was significantly increased, and TEER was significantly decreased at 1 and 2 d after weaning compared with weaning or compared with unweaned controls (Table 2). This shows that weaning pigs at a higher age can prevent the loss of paracellular barrier function after weaning. In addition, Moeser *et al.*[14,18] have shown that in 3-wk-old pigs, TEER and mannitol flux were not affected by the weaning process when stress pathways were blocked with a corticotrophin-releasing factor receptor antagonists or with a mast cell-stabilising drug. This shows that stress is a major factor with respect to the disturbed intestinal barrier function after weaning. Moreover, it shows that the immune system through mast cell activation has a critical role in the loss of intestinal barrier function after weaning. Several other studies have shown that intestinal barrier function was compromised in pigs weaned at an age of 26 d[26,89,91,188]. Verdonk[26] measured the mannitol flux in the mid-jejunum of pigs with a low vs. a high intake level of milk replacer after weaning (Table 2). The mannitol flux was not

affected in pigs with a high feed intake level at 1, 2 and 4 d after weaning. However, mannitol flux was increased for the pigs with low feed intake levels at 2 and 4 d after weaning compared with pre-weaning. As an average over d 1, 2 and 4, this resulted in a higher mannitol flux for pigs with low feed intake compared with pigs with high feed intake (at least 2.5 times energy maintenance). In line with this, a 2 d fast of 23-d-old pigs increased transepithelial electrical conductance (the opposite of TEER)[35]. Thus, a sufficient feed intake after weaning prevents loss of barrier function of the tight junctions after weaning. This indicates the importance of a sufficient luminal nutrient supply to maintain barrier function. Sufficient feed intake may be especially important for the proximal small intestine because this part depends more on luminal nutrient supply than the distal small intestine[92]. In the study of Verdonk[26], villous height in the proximal small intestine decreased after weaning for pigs at the low intake level and was hardly affected after weaning in pigs at the high intake level. This illustrates that luminal nutrient supply was adequate in the high-intake group and inadequate in the low-intake group. In line with the study of Verdonk[26] described earlier, Spreeuwenberg *et al.*[188] have shown that the mannitol flux in the mid-jejunum increased at 2 and 4 d after weaning compared with pre-weaning. Moreover, Verdonk *et al.*[26,91] published 2 other experiments in which the mannitol flux was measured over the epithelium of the proximal instead of the mid-jejunum in pigs after weaning (Table 2). In the first experiment, the mannitol flux was increased at 2 and 6 d after weaning compared with the pre-weaning flux. However, in the second experiment, the mannitol flux at 4 and 7 d after weaning was not different from pre-weaning fluxes and was not affected by feed intake level after weaning. Thus, the last study, in contrast with the first 2 studies of Verdonk[26,91] and the study of Spreeuwenberg *et al.*[188], has shown no increase in mannitol flux after weaning, although feed intake levels were reasonably low. This discrepancy is probably related to handling of the pigs before the start of the experiment. In the first 2 studies of Verdonk and in the study of Spreeuwenberg *et al.*[188] pigs were transported from another location before the start of the experiment. However, in the last experiment of Verdonk[26], piglets were weaned and kept at the same location at which the experiment was conducted. Thus, in this last experiment, the weaning process was probably less stressful because pigs were not transported in a trailer. Because stress is an important factor with respect to the intestinal barrier function (see before) this may explain the differences between the studies. Lodemann *et al.*[208,209] found that at 7 d after weaning, the mannitol flux in the mid-jejunum was either significantly or numerically lower from before weaning, and TEER was not different than before weaning. In these experiments, the timing of permeability measurements may have been too late to detect a disturbed barrier function or, again, the stress level around weaning may have been lower than in the other experiments. In the ileum, TEER was increased at 5 and 15 d after weaning compared with pre-weaning levels in a study of Boudry *et al.* [19]. Carlson *et al.*[210] have shown similar results in the ileum with pigs weaned at 4 wk of age. In their study, the transepithelial electrical conductance (the opposite of TEER) was not different from weaning at 1–2 d after weaning and was lower at 5–6 and 14–15 d after weaning compared with weaning. Thus, in the ileum, in contrast to the proximal and mid-jejunum, intestinal barrier function is not compromised due to the weaning process. Finally, Berkeveld *et al.*[197] administered pigs an oral mannitol dose before and 0.5, 2, 4 and 7 d after weaning and measured plasma mannitol concentrations 1 h after each dose. They observed that the plasma mannitol concentrations decreased gradually after weaning, being significantly different from pre-weaning concentration at 4 d after weaning. This contradiction between

the results of Berkeveld *et al.*[197] (orally administered mannitol) with the other studies (Ussing chambers) may relate to the different techniques used to determine intestinal mannitol transport. The study of Berkeveld *et al.*[197] may reflect mannitol permeability in the whole small intestine, and in this study, mannitol was measured in blood instead of in the urine, which is a complicating factor in evaluating the results (see above).

In general, dietary treatments after weaning showed only minor effects on paracellular intestinal permeability (Table 2). Supplementing different probiotics to the diets of the sow and piglets had no effect on mid-jejunal mannitol flux and TEER of piglets after weaning[208,209]. In 2 studies of Carlson *et al.*[210,211], it was revealed that dietary Cu and Zn concentrations had no effect on the transepithelial electrical conductance of the jejunum and ileum at 5–7 d after weaning. However, in a study of Zhang and Guo[204], urinary latulose:mannitol ratios were decreased after feeding enhanced dietary Zn concentrations for a periode of 2 wk after weaning. The discrepancy between the studies of Carlson *et al.* and the study of Zhang and Guo may again relate to the stress level during the study. In the study of Zhang and Guo, pigs were separated from the other pigs, individually housed and fasted overnight before the permeability test. Boudry *et al.*[206] changed 25 kg pigs from a milk replacer diet to a barley- or wheat-based diet on an equal intake level. The jejunal TEER was not different from the milk-fed pigs 4 d after the dietary change. Finally, the combination of an increased dietary lactose concentration (41 vs. 24%) and a decreased dietary protein concentration (15 vs. 30%) tended to decrease mannitol flux in the mid-small intestine[188]. Thus, no distinction could be made between a possible (positive) effect of lactose and a possible (negative) effect of protein on mannitol flux. In the same study, the combination of a decreased dietary lactose concentration (8 vs. 24%) and an increased dietary protein concentration (45 vs. 30%) had no effect on mannitol flux. In contrast to the studies described earlier, Hamard *et al.*[49] weaned pigs at 7 d of age and showed that dietary Thr deficiency, for a period of 2 wk after weaning, increased the ileal permeability for fluorescein isothiocyanate dextran (4 kDa) and also tended to decrease TEER. The aforementioned studies indicate that diet composition in general has not a major effect on paracellular permeability. However, when diets deficient in nutrients (e.g. Thr) are fed, paracellular permeability deteriorates. In line with this, intestinal barrier function is compromised at low feed intake levels, and, in this respect a low feed intake level is similar to a diet being deficient in all nutrients. In addition, diet composition may affect paracellular permeability when permeability measurements are accompanied with additional stressors (i.e. fasting and individual housing).

Conclusions regarding barrier function

The current review clarifies that small intestinal barrier function in pigs is affected by the process of weaning. In the literature, 4 factors (i.e. weaning age, weaning stress, feed intake and diet composition) have been identified that can have a major effect on the barrier function after weaning. In addition, barrier function is differently affected after weaning in the proximal and mid-jejunum than in the ileum. The relationships between these different aspects of intestinal barrier function after weaning are illustrated in Figure 1. The stress that is associated with weaning manipulates the immune system, resulting in mast cell activation that has a critical role in the loss of the barrier function of the tight junctions in the small intestine. In addition, the loss of paracellular barrier function is prevented when feed intake after weaning is at an adequate level such that the loss of the villous height is prevented. This shows that low feed intake is another factor that seems to have a critical role in the

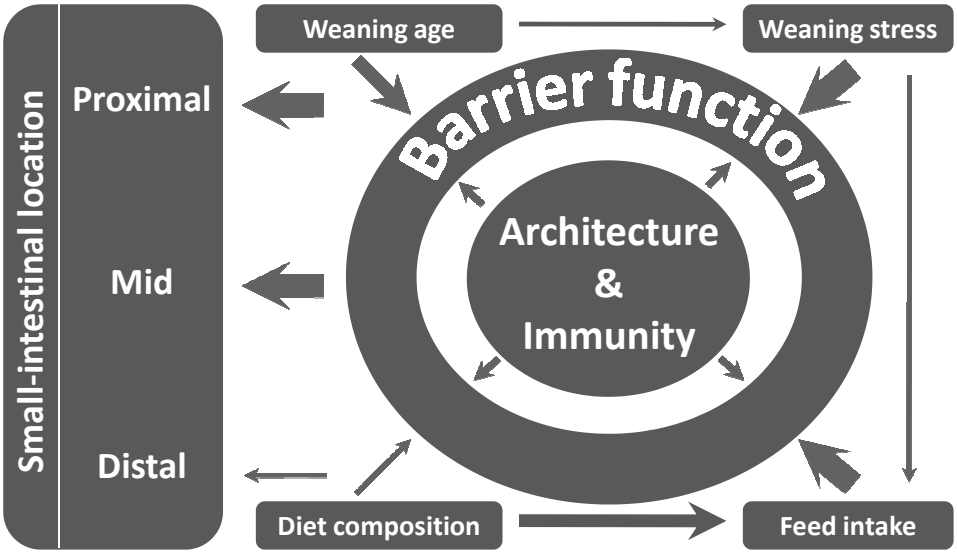

Figure 1. Scheme representing the relationship between small-intestinal barrier function, small-intestinal location and factors (age, stress, feed intake or diet composition) that affect the barrier function. The thickness of the arrows indicates the significance of the relation. Barrier function is less affected at high than at low weaning age, which relates probably to intestinal maturation rate. Weaning stress compromises the paracellular barrier function indirectly through mast cell activation (immunity). Adequate feed intake levels after weaning prevent the loss of the barrier function probably indirectly through preservation of intestinal architecture. The direct effect of diet composition on intestinal barrier function seems to be limited unless diets are deficient in specific nutrients. Barrier function is most affected in the proximal and mid-small intestine and hardly in the distal small intestine.

compromised barrier function after weaning. In contrast to the proximal and the distal jejunum, paracelular barrier function after weaning is not compromised in the ileum. Because luminal nutrient supply is most critical in the proximal small intestine, this indicates that the loss of the barrier function due to low feed intake is due to a shortage of luminal nutrient supply. In line with this, it was shown that barrier function was compromised in pigs after feeding a Thr-deficient diet for a period of 2 wk. This effect of nutrient supply on barrier function may be a secondary effect as a result of a compromised intestinal architecture. The loss of the paracellular barrier function is almost exclusively found in the first week after weaning and returns to pre-weaning level at 2 wk after weaning. Pigs are less susceptible for a compromised barrier function when weaned at an older age and also on the long term have a better barrier function[18,213]. This is probably related to the earlier stage of maturation of the small intestine when pigs are weaned at a young age. Although paracellular barrier function is consistently compromised after weaning, one can argue whether this is a direct risk for the health of the pig. Moreover, transcellular barrier function for macromolecules through endocytosis improves after weaning. We suggest that this maturation process enhanced by weaning may prevent the animal suffering from an antigen overload. Such an antigen overload may result in an excessive activation of the immune system when pigs are subjected to their new environment after weaning. We hypothesise that the increased paracellular permeability also indicates that the intestine is extra susceptible for the disturbance of the transcelluar barrier function when several stressors occur simultaneously.

Based on the present review, 3 different approaches can be followed to improve intestinal barrier function after weaning by ways of dietary composition: first, the old-fashioned approach to improve palatability of the diet to increase feed intake after weaning.

This has only been partially successful up to now; second, to identify crucial nutrients (e.g. protein or specific amino acids) that may be supplied to pigs with low feed intake in a concentrated form or through the drinking-water in order to prevent loss of intestinal function; third, to add specific biologically active components to the diet to modulate the stress response or the subsequent immune response, to prevent the loss of the barrier function. With this last approach, it is essential that the diet should be eaten, otherwise the active component needs to be supplied through the drinking water.

Intestinal absorption after weaning

Small-intestinal transcellular absorption can be divided into active and passive absorption. Active absorption takes place in specific transporters that transport nutrients such as glucose or amino acids (AA) over the intestinal epithelium, which coincides with Na^+ transport. This Na^+ transport enables us to estimate the active transport over the intestinal epithelium in Ussing chambers. In pigs after weaning with this technique, the Na^+-dependent glucose and Gln absorption have been investigated (Table 1). The change in Isc after the addition of glucose or Gln to the mucosal site estimates the transport of glucose or Gln to the serosal site of the chamber[189]. In addition, after weaning, glycylsarcosine (GlySar) has been used to assess active transport in Ussing chambers. GlySar is a dipeptide (146 Da, Table 1), which is believed to pass through the intestinal epithelium via a H^+carrier-mediated transcellular route[188]. As addressed earlier, active absorption coincides with electrolyte transport (i.e. Na^+ and H^+) over the epithelium. The transport of electrolytes, but also the transport of nutrients, coincides with water movement through the tight junctions because water follows the osmotic gradient. Thus, active transport of electrolytes is also important with respect to fluid absorption. It should be noted that electrolytes are not only absorbed by the small intestine but also secreted into the lumen. Carvey *et al.*[35] have shown that the net Na^+ and Cl^- movement in fasted pigs was only 25% of the total mucosal to serosal movement. In addition, Miller and Skadhauge[214] have shown that weaning reduced Na^+ absorption but had hardly any effect on Na^+ secretion. These studies have shown that in addition to a net movement of charge, as measured by Isc, unidirectional electrolyte movements occur in the small intestines.

Sodium-fluorescein isothiocyanate (Na-Flu) and D-xylose have been used to study passive absorption in pigs after weaning. Na-Flu is a small (376 Da) fluorescent-labelled molecule with a 50:50 lipid–water solubility (Table 1)[15,184]. It has been used to study intestinal absorption with Ussing chambers. D-xylose has been used to test the absorptive intestinal function before and after weaning *in vivo* in several studies. In these studies, D-xylose was orally administered, and plasma D-xylose concentrations were determined after 1 h. D-xylose is a monosaccharide (150 Da, Table 1), which besides a passive transcellular route is also thought to pass through the intestinal mucosa by a carrier-mediated route or by paracellular diffusion[175,185,191].

Active absorption

In a study of Boudry *et al.*[19], pigs were weaned at 21 d of age. In that study, the Na^+-dependent glucose absorption increased in the proximal jejunum at 2 d after weaning and decreased at 15 d after weaning compared with pre-weaning absorption (Table 3). Moreover, in the same study, ileal glucose absorption decreased at 2, 5 and 15 d after weaning. In line with this, Smith[20] has found that the Na^+-dependent Ala uptake by

Table 3. Small-intestinal molecular absorption in pigs after weaning as measured for Na^+-dependent glucose, Na^+-dependent Gln, glycylsarcosine and sodium-fluorescein isothiocyanate absorption

References	Intestinal segment	Weaning age, d	Feed intake after weaning (fraction of energy requirement for maintenance)	Change after weaning, %	Treatment description (first mentioned = control if unclear)	Change compared with control, %
Na^+-dependent glucose						
Boudry *et al.*[19]	Proximal jejunum	21	d2: 0 d5: 1 d8: 2 d15: 4	d2: 78* d5: –32 d8: –43 d15: –83*		
	Ileum			d2: –62* d5: –74* d8: –13 d15: –54*		
Boudry *et al.*[206]	Proximal jejunum	28	d28 to 25kg: 4.5 (milk diet). Diet switch at 25 kg, thereafter: d1: 0 d2: 1.5 d3: 3 d4: 4.5	–	Switch to barley diet at 25 kg	d4: 76*
					Switch to wheat diet at 25 kg	d4: 74*
Lodemann *et al.*[208]	Mid-jejunum	28	Not given	d7: 6 d28: –12	Probiotic (*Enterococcus faecium* SF68) added to the sow and piglet diets	d0: 27, d7: 24 & d28: –16
Lodemann *et al.*[209]	Mid-jejunum	28	Not given	Control: d7: –14 d28: –28	Probiotic (*Bacillus cereus var. toyoi*) added to the sow and piglet diet	Average d0, 7 & 28: –7
				Probiotic: d7: –2 d28: –9		
Carlson *et al.*[210]	Ileum	28	Not given but probably already at maintenance at 2 d after weaning	d1–2: 111* d5–6: 112* d14–15: 130*	(100 mg Zn, 20 mg Cu/kg) vs. (100 mg Zn, 175 mg Cu/kg)	d5–7: 12
					(100 mg Zn, 20 mg Cu/kg) vs. (2500 mg Zn, 20 mg Cu/kg)	d5–7: 3
					(100 mg Zn, 20 mg Cu/kg) vs. (2500 mg Zn, 175 mg Cu/kg)	d5–7: –14
Gabler *et al.*[215]	Proximal jejunum	14–17	d1: 0	–	Sow gestation and lactation diets supplemented with either fish oil, DHA or coconut fat	Measurement at d 1: Fish oil: 355* DHA: 510* Cocconut fat: 190
Gabler *et al.*[216]	Proximal jejunum	15–19	d1: 0	–	Sow gestation/lactation diets supplemented or not with fish oil resulting in 4 treatments: control/control, control/fish, fish/control, fish/fish	Measurement at d 1: Control/fish: 189, Fish/control: 316*, Fish/fish: 389*
Hamard *et al.*[49]	Ileum	7	d1: 1 d2: 1 d 3–14: 2	–	Adequate (9.3 g/kg) vs. deficient (6.5 g/kg) dietary Thr	d14: 81
Glycylsarcosine						
Verdonk *et al.*[91]	Proximal jejunum	26	Low: d1: 0 d2: 0 d3: 1 d4: 1.5 d5: 1.5 d6: 1.5 d7: 1.5	d4: 29 d7: 33	Low vs. high feed intake	d4: 18 d7: –15
			High d1: 0.5 d2: 1 d3: 1.5 d4: 2 d5: 2.5 d6: 2 d7: 2	d4: 53 d7: 14		
Verdonk[26]	Proximal jejunum	26	Dry: d1: 0 d2: 0 d3: 0.5 d4: 1.5 d5: 1.5 d6: 1.5	d2: 169* d6: 209*	Dry vs. wet feed	Average d2 & 6: –28*
			Wet: d1: 0.5 d2: 0.5 d3: 1 d4: 1 d5: 1.5 d6: 1.5	d2: 109* d6: 118*		
	Mid-jejunum	26	Low: d1: 0.5 d2: 1 d3: 1 d4: 1	d1: –13 d2: –2 d4: 9	Low vs. high feed intake	Average d1, 2 & 4: –20
			High: d1: 2.5 d2: 3.5 d3: 3.5 d4: 3.5	d1: –35* d2: –1 d4: –29		

Table 3. *Continued*

References	Intestinal segment	Weaning age, d	Feed intake after weaning (fraction of energy requirement for maintenance)	Change after weaning, %	Treatment description (first mentioned = control if unclear)	Change compared with control, %
Spreeuwenberg *et al.*[188]	Mid-jejunum	26	d1: 0.5 d2: 1 d3: 1 d4: 1	d1: –6 d2: 1 d4: 19	24% lactose and 30% dietary protein vs. 41% lactose and 15% dietary protein	Average d1, 2 & 4: 10
					24% lactose and 30% dietary protein vs. 8% lactose and 45% dietary protein	Average d1, 2 & 4: 8
Na^+-dependent Gln						
Lodemann *et al.*[208]	Mid-jejunum	28	Not given	d7: 17 d28: –44	Probiotic (*Enterococcus faecium* SF68) added to the sow and piglet diets	d0: 40, d7: 19 & d28: 11
Lodemann *et al.*[209]	Mid-jejunum	28	Not given	Control: d7: –17 d28: 12	Probiotic (*Bacillus cereus var. toyoi*) added to the sow and piglet diets	Average d0, 7 & 28: 19
				Probiotic: d7: 1 d28: –12		
Gabler *et al.*[215]	Proximal jejunum	14–17	d1: 0	–	Gestation and lactation diets supplemented with either fish oil, DHA or with coconut fat	Measurement at d 1: Fish oil: 400 DHA: 2425* Cocconut fat: 1625
Sodium-fluorescein isothiocyanate						
Verdonk *et al.*[91]	Proximal jejunum	26	Low: d1: 0 d2: 0 d3: 1 d4: 1.5 d5: 1.5 d6: 1.5 d7: 1.5	d4: –48* d7: –42*	Low vs. high feed intake	d4: 6 d7: 21
			High d1: 0.5 d2: 1 d3: 1.5 d4: 2 d5: 2.5 d6: 2 d7: 2	d4: –45* d7: –30		
Van der Meulen *et al.*[15]	Mid-jejunum	28	Control: d1: 1 d2: 2 d3: 2 d4: 2 d5: 2 d6: 2 d7: 2	–	No creep feed or creep feed	Average d 1, 4 & 7: –10
			Creep feed: d1: 1 d2: 2 d3: 2 d4: 2 d5: 2 d6: 2 d7: 2			
		49	Control: d1: 2 d2: 2 d3: 2 d4: 3 d5: 3 d6: 3 d7: 4	–	Weaning at 28 or 49 d of age	Average d 1, 4 & 7: –18
			Creep feed: d1: 3 d2: 3 d3: 3 d4: 4 d5: 4 d6: 4 d7: 4			
Koopmans *et al.*[47]	Mid-jejunum	25	Average of d 0–10: 1.5 (first days low intake)	–	5 g/kg Trp in the weaner diet	d4: 11 d5: 38 d6: –1

Values were significantly different from those at weaning or from the control treatment: *$P < 0.05$

enterocytes of the mid-small intestine (measured with a rapid uptake apparatus, using radiolabelled tracer AA) decreased considerably at 5 d after weaning at 2 or 3 wk of age. Furthermore, in 4-wk-old pigs, Na^+-dependent Ala uptake by enterocytes of the mid-small intestine was lower for weaned pigs (5 d after weaning, thus weaning at 23 d) than for unweaned pigs[21]. However, the Ala uptake of 6-wk-old pigs (both weaned and unweaned) was similar to the Ala uptake of 4-wk-old weaned pigs[21]. This study suggests that weaning (before 4 wk of age) and ageing appear to decrease the number of enterocytes that are involved in active Ala uptake[21]. The aforementioned studies have shown that active small-intestinal absorption decreases after weaning when pigs are weaned between 14 and 23 d of age. However, when weaned after 4 wk of age, active absorption is not affected by the weaning process. Miller *et al.*[21] have observed that the decreased absorption rates after weaning also occur in unweaned pigs but over a much longer time course. This suggests that this decrease in active absorption is part of a maturation process that is enhanced by the process of weaning. This may relate to a decrease in the relative demand for nutrients when pigs get older because weight gain expressed relative to body weight (BW) decreases when pigs get older. Several studies have investigated the active absorption of glucose, Gln or GlySar in the proximal, mid and distal small intestine for pigs that were weaned at 26 or 28 d of age[26,91,188,208-210]. In all these studies, active absorption between 1 and 15 d after weaning was either similar to or higher than absorption before weaning. This confirms that active small-intestinal absorption after weaning is only suppressed when pigs are weaned before 4 wk of age. The only contradiction to this is the study of Buddington *et al.*[217]. They measured carrier-mediated Asp, Leu, Lys, Met and Pro absorption (per unit of wet mass) in the mid-small intestine for pigs weaned between 32 and 35 d of age. They showed that for all AA, active absorption was lower after weaning (42 d of age) than before weaning (at 28 d of age). The discrepancies of the study of Buddington *et al.*[217] with the others studies may relate to the fact that they did not use Ussing chambers or a rapid uptake apparatus.

In a study of Verdonk[26], energy intake during the first 2 d after weaning was either low (close to 0 for pigs on a dry diet) or moderate (0.5 times maintenance for pigs fed a wet diet). In this study, the average GlySar absorption at d 2 and 6 was higher for pigs with low feed intake than for pigs with moderate feed intake. In rats, starvation increased the expression of mRNA of peptide transporter 1, and along with the up-regulation of this transporter protein, the activity of GlySar uptake was enhanced[218]. This may explain why GlySar absorption increased in pigs with low feed intake levels after weaning. In another study of Verdonk[26], pigs were fed milk replacers, and the energy intake during the first 2 d after weaning was either moderate (0.5 times maintenance) or high (3.0 times maintenance). GlySar absorption in the mid-jejunum at 1, 2 and 4 d after weaning was not different from pre-weaning for treatments with the moderate energy intake. However, GlySar absorption at d 1 was lower compared with pre-weaning in pigs with high energy intake. Thus, low feed intake stimulates active absorption. In agreement with this, active glucose absorption in the proximal jejunum was stimulated after pigs were fasted for a period of 2 d[35]. Moreover, Boudry *et al.*[19] have shown that active glucose absorption in the proximal jejunum increased when pigs were fasted for a period of 2 d after weaning. In contrast, in the same study, ileal glucose absorption decreased after weaning. This difference in glucose absorption between the proximal and distal small intestine is probably because the proximal region depends more on luminal nutrient supply than the distal region[92]. Boudry *et al.*[206] changed pigs of 25 kg (4–6 wk after weaning) from a milk replacer to a barley- or wheat-based diet. It was observed that 4 d after this dietary change,

the Na^+-dependent glucose absorption in the proximal jejunum of the barley- or wheat-fed pigs was higher than that of the control pigs fed the milk replacer. This indicates that after weaning, the shift from a milk- to a cereal-based diet increases active small-intestinal absorption. In conclusion, the short-term increase in active absorption in the proximal small intestine after weaning is due to the low feed intake after weaning. The long-term increase is related to the shift from a milk- to a cereal-based diet. The addition of fish oil or DHA to gestation diets of the sow increased Na^+-dependent glucose and Gln absorption in the proximal jejunum at 24 h after weaning in 15- to 20-d-old pigs[215,216]. Dietary Thr deficiency tended to increase ileal Na^+-dependent glucose absorption at 14 d after weaning[49]. Studies with differences in dietary minerals (Cu and Zn), lactose and protein concentration and dietary probiotic addition have found no effect on active small-intestinal glucose, Gln or GlySar absorption after weaning[188,208-211]. Thus, the shift from a milk- to a cereal-based diet and dietary fatty acid composition have a significant effect on active small-intestinal absorption, whereas some other dietary changes have no effect on absorption.

Passive absorption

Results of 3 studies[193,195,197] revealed that D-xylose absorption in piglets before weaning is hardly affected over time after 3 wk of age. Results of 5 studies with pigs after weaning showed that absorption of D-xylose [23,193-196] decreased gradually to about 50% of the pre-weaning level at 7 d after weaning. In line with this, the absorption of Na-Flu in the proximal jejunum decreased at 4 and 7 d after weaning compared with pre-weaning levels in pigs weaned at 26 d of age[26]. Berkeveld *et al.*[197], however, observed that plasma D-xylose concentrations were significantly higher at 2 and 7 d after weaning than pre-weaning and were not different between pre-weaning and 0.5 and 4 d after weaning. Apart from the results of the study of Berkeveld *et al.*[197], passive absorption seems to decrease consistently after weaning. This seems to be a permanent effect because even at 14 d after weaning D-xylose absorption was only at 65% of the absorption level measured before weaning[193]. We hypothesise that the reduced passive transcellular absorption after weaning is a defence mechanism that prevents uncontrolled transport of potential harmful agents to enter the body. This is more or less in line with what was described before with respect to transcellular transport of macromolecules after weaning. In all these studies, weaning age, varying from 14 to 29 d of age, does not seem to have a major effect on the response after weaning. Creep feed intake of piglets before weaning had no effect on D-xylose absorption from 1 to 14 d after weaning[193,195,196]. Moreover, Na-Flu absorption was not affected by creep feed intake before weaning or by weaning age (4 or 7 wk of age) at 1, 4 and 7 d after weaning[15].Kelly *et al.*[30] have found no difference in D-xylose absorption at 5 d after weaning for tube-fed pigs fed at a low (0, 0.25, 0.5, 0.75, 1.0 times energy maintenance on d 1–5, respectively) or at a high (1.5, 1.75, 2.0, 2.25, 2.5 times energy maintenance on d 1–5, respectively) intake level of the diet. In addition, Pluske *et al.*[23] have shown that D-xylose absorption after weaning was not affected by the intake level of cows' milk after weaning (1.0, 2.5 or 4.0 times energy maintenance) and was not different for pigs fed cows' milk vs. pigs fed a dry weaner diet. Thus, passive absorption is not affected by feed intake level before or after weaning. Finally, the addition of extra Trp (5 g/kg diet) after weaning had no effect on Na-Flu flux in the mid-small intestines at 4, 5 or 6 d after weaning of pigs weaned at 25 d of age[47].

Conclusions regarding absorption

The present review shows that active and passive absorption are differently affected after weaning. Active absorption after weaning is influenced by 3 important factors (weaning age, feed intake level and feed composition). However, passive absorption decreases after weaning irrespective of these 3 factors. This reduced passive transcellular absorption after weaning may be a defence mechanism that prevents uncontrolled transport of potential harmful agents to enter the body. This is more or less in line with what was previously described with respect to the transcellular transport of macromolecules after weaning. The decreased passive absorption may reflect a natural maturation process of the intestines that occurs rapidly after weaning, as hypothesised earlier by other authors[20,21]. In general, active small-intestinal absorption decreases after weaning when pigs are weaned at 3 wk of age or at a lower age. In line with the decrease in passive absorption, this decrease in active absorption may be part of a maturation process that is enhanced by the process of weaning. This may relate to a decrease in the relative demand for nutrients when pigs get older because of a decrease in weight gain relative to BW. However, when weaned at an age of 4 wk or later in life, active absorption is not affected by weaning or stimulated by the weaning process. This may indicate that with respect to active absorption, the small intestine is mature at 4 wk of age. The shift from a milk- to a cereal-based diet and addition of fish oil or DHA to the diet increase active absorption. Thus, diet composition after weaning can have a significant effect on active small-intestinal absorption. Moreover, this indicates that the shift from a milk- to a cereal based-diet may be responsible for the long-term increase in active absorption after weaning. It seems that passive and active absorption after weaning adapt accurately to the changed environment after weaning with respect to feeding status when weaned after 3 wk of age. Only when weaned at 3 wk of age or earlier, the decrease in active absorption indicates an insufficient adaptation to the new environment that may result in an insufficient absorptive capacity. A diet that stimulates active absorption, for instance DHA, may help to overcome or prevent this sudden decrease in absorption.

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Chapter 5

Lactulose as a marker of intestinal barrier function in pigs after weaning

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Abstract

Intestinal barrier function in pigs after weaning is almost exclusively determined in terminal experiments with Ussing chambers. Alternatively, the recovery in urine of orally administered lactulose can be used to assess intestinal permeability in living animals. This experiment was designed to study the barrier function of the small intestine of pigs over time after weaning. The aim was to relate paracellular barrier function (measured by lactulose recovery in the urine) with macromolecular transport [measured by horseradish peroxidase (HRP) using Ussing chambers] and bacterial translocation to assess whether lactulose recovery is related to possible causes of infection and disease. Forty gonadectomised male pigs (6.7 ± 0.6 kg) were weaned (d 0) at a mean age of 19 d, fitted with urine collection bags, and individually housed. Pigs were dosed by oral gavage with a marker solution containing lactulose (disaccharide) and the monosaccharides L-rhamnose, 3-*O*-methylglucose, and D-xylose at 2 h and at 4, 8, and 12 d after weaning. The recovery of sugars in the urine was determined over 18 h after each oral gavage. The day after each permeability test, the intestines of 10 pigs were dissected to determine bacterial translocation to the mesenteric lymph nodes (MLN) and jejunal permeability for HRP in Ussing chambers. Recovery of L-rhamnose in urine was affected by feed intake and by the time after weaning ($P \leq 0.05$). Recovery of lactulose from the urine was greater ($P \leq 0.05$) at 4, 8, and 12 d after weaning compared with the first day after weaning and was negatively correlated with feed intake (r = –0.63, $P \leq 0.001$). The mean translocation of aerobic bacteria to the mesenteric lymph nodes was greater at 5 and 13 d after weaning compared with d 1 ($P \leq 0.05$). Lactulose recovery showed no correlation with permeability for HRP nor with bacterial translocation ($P > 0.05$). Although both lactulose recovery and bacterial translocation increased over time after weaning, lactulose recovery did not correlate with the permeability for HRP nor bacterial translocation within a pig ($P > 0.05$). Therefore, we conclude that lactulose recovery in the urine of pigs after weaning is not associated with risk factors for infections. However, it appears to be possible to measure paracellular barrier function with orally administered lactulose in pigs shortly after weaning. Further studies will

reveal whether this variable is relevant for the long-term performance or health of pigs after weaning.

Key words: bacterial translocation, horseradish peroxidase, barrier function, lactulose, pig, weaning.

Introduction

Decreased feed intake after weaning results in atrophy of the villi of the small intestine within a few days after weaning[13,23,32]. Additionally, weaning increases the paracellular permeability of the jejunum[14,19,26]. To date, the intestinal permeability in pigs after weaning has been measured by terminal experiments with Ussing chambers[219]. Orally administered marker probes have yet to be used in pigs to assess intestinal permeability. After administration, the recovery of the marker probe in urine is used to measure barrier and absorption functions of the small intestine. This technique has been successfully used for decades in studies with human patients and in medical research with animals. Traditionally, monosaccharides and lactulose have been used to assess trancellular absorption and paracellular barrier function, respectively[185]. Orally administered marker probes in pigs are more advantageous than Ussing chambers because multiple measurements can be obtained from the same animal over time. This technique also allows one to determine when barrier function is least effective and, therefore, determine the time at which each pig is most vulnerable. Moreover, a correlation of intestinal function with health and performance variables later in life can be determined. We sought to study the changes in small intestine barrier function over time as measured with lactulose and to test its relationship with putative causes of infection and disease. Lactulose recovery in urine was compared with bacterial translocation, as well as with macromolecular transport of horseradish peroxidase (HRP) in Ussing chambers. This study aimed to improve our understanding of health disorders after weaning and may aid in the development of strategies that prevent or cure postweaning disorders.

Materials and methods

The study was approved by the Ethical Committee of the Animal Science Group of Wageningen University and Research Centre, Lelystad, The Netherlands.

Animals and Housing

Forty Piétrain × (Large White × Dutch Landrace) gonadectomised male pigs were selected from 18 litters of our institutional herd (Provimi Research Centre 'De Viersprong', Velddriel, The Netherlands). The pigs were assigned to 4 groups of 10 pigs each with a similar mean body weight (BW; 6.7 ± 0.6 kg). Animals had free access to creep feed before weaning from 4 d of age onwards. The pigs were weaned at a mean age of 19.3 ± 0.5 d and subsequently housed individually. All pigs had free access to water through nipple drinkers, but had limited access to a standard nursery diet during the first 4 d after weaning (5, 30, 75, and 115 g/pig for each of the 4 subsequent days, respectively). The feed supply corresponded to typical intakes of pen-housed pigs in the same facility on the same diet. After 4 d of dietary restriction, pigs had free access to the diet. Feed intake was determined daily, and pigs were

weighed (without feed and water restriction) at weaning and subsequently at 4, 8, and 12 d after weaning.

Experimental Procedures

Permeability of the small intestines of pigs was assessed *in vivo* at d 0, 4, 8, and 12 after weaning (Figure 1). Pigs were initially fasted for 2 h and then each pig was subsequently dosed by oral gavage (gastro-duodenal feeding tube, Levin type, Vygon Nederland B.V., Valkenswaard, The Netherlands) with a marker solution containing 2.83 g lactulose, 0.19 g L-rhamnose, 0.04 g 3-*O*-methylglucose (3OMG), and 0.09 g D-xylose (Sigma, Zwijndrecht, The Netherlands) dissolved in deionised water for a total dose of 15 g of sugar mixture per pig. The amount of L-rhamnose, 3OMG, and D-xylose was derived from a human study[220] and adapted for pigs based on metabolic BW. The dose of lactulose used was similar to the dose used in a previous study with neonatal piglets[201]. Total voided urine was collected in a pouch glued and taped to the belly of each pig. At 2, 8, and 18 h after marker administration, urine from the pouch was collected and refrigerated at 6°C in a jar containing 100 µL of a thimerosal solution (100 g/L; Sigma) for preservation. For pigs that did not urinate between 2 and 18 h after marker administration, the urine collection period was prolonged until urine was voided, or urine was directly collected from the bladder if pigs were dissected the next day (see below). At d 0, 4, 8, and 12 after weaning, 25, 57, 20, and 10% of the pigs were subjected to prolonged urine collection, respectively.

Dissection

Two hours after marker administration (after 4 h of feed deprivation), access to feed was restored. From about 18 through 22 h after marker administration, 10 previously assigned pigs were dissected as described below. The experimental design allowed for repeated measurements over time of the recovery of permeability markers. However, the number of measurements per pig ranged from 1 for the pigs dissected at d 1, to 4 for the pigs dissected

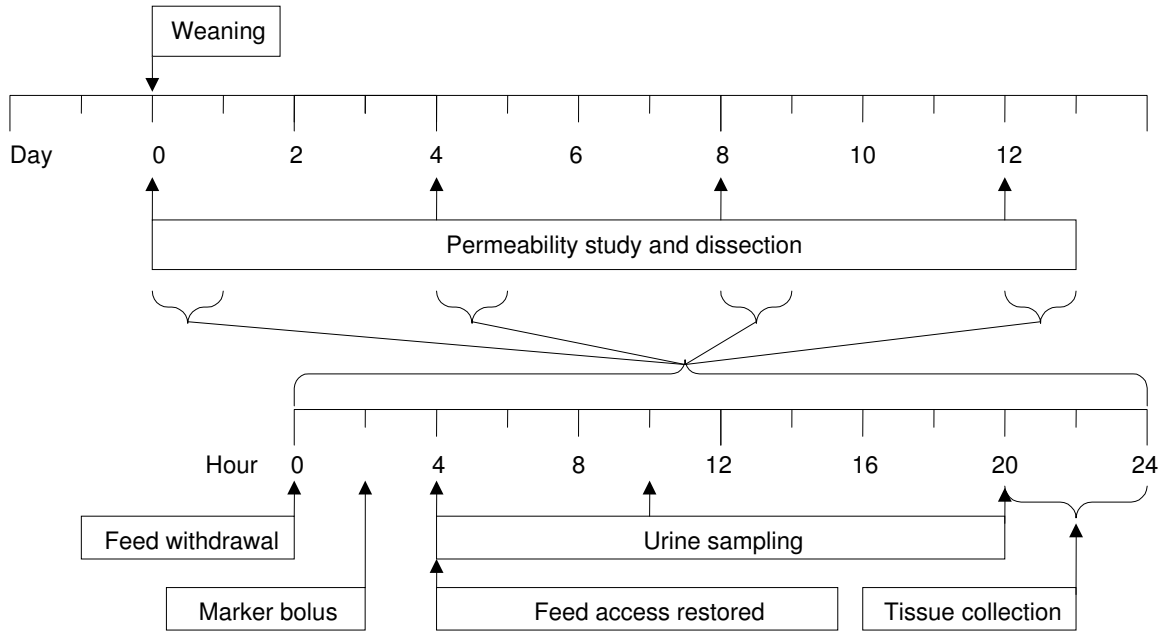

Figure 1. Schematic diagram of the experimental design.

at d 13. Pigs were anesthetised with 24 mg sodium pentobarbital/kg BW (Euthasol, ASTfarma, Oudewater, The Netherlands). The abdominal cavity was opened under sterile conditions, and samples of the mesenteric lymph nodes (MLN) of the proximal and distal small intestines and the liver were isolated to determine bacterial counts. Twenty centimeter of the small intestine (between 100 and 150 cm distal from the stomach) was then removed to measure the permeability to HRP using Ussing chambers. The total urine in the bladder was also collected. The urine was used to analyse marker sugars if the pigs did not urinate from 2 h after marker administration to dissection. Pigs were subsequently euthanised by exsanguination.

Sample Handling and Analyses

Aliquots of urine were stored at –20^{o}C before the analyses for marker sugars. For these analyses, urine samples were diluted 10-fold using deionised water. Chloride was subsequently precipitated by adding silver nitrate. After centrifugation (13000 × *g*, 10 min at 21^{o}C) the supernatant was separated on an OnGuard Ba and H column (Dionex, Amsterdam, The Netherlands) followed by analysis using high-performance anion-exchange chromatography with a pulsed amperometric detector (Dionex, Amsterdam, The Netherlands). The method was performed according to the manufacturer's instructions[221,222]. The limits of detection for lactulose and the monosaccharides were 100 µmol/L and 60 µmol/L, respectively. Sugars that were recovered below the limit of detection were assumed to be 50% of the detection limit. In those cases, the estimated amounts were applied in the statistical analyses.

Liver and MLN samples for bacterial counts were stored in transport medium [buffered peptone water supplemented with 0.5% Cysteine-HCl (Oxoid Ltd., Cambridge, UK)]. Before analysis, the samples were homogenised using an UltraTorax in an anaerobic cabinet (Bactron X, Shellab, Cornelius, OR). Ten-fold dilutions of the homogenised samples were made in reduced peptone physiological salt solution (1 g of peptone and 8.5 g of NaCl per liter). One hundred microliters of each solution was plated in duplicate on either brain heart infusion agar plates or blood-reinforced clostridial agar plates. The brain heart infusion plates were incubated for 24 h at 37^{o}C in normal air to determine total aerobic counts. The blood-reinforced clostridial agar plates were incubated for 3 d under anaerobic conditions to determine total anaerobic counts.

The jejunal segment of the intestine was analysed using an Ussing chamber. The tissue was placed in Ringer buffer containing 25 mmol/L $NaHCO_3$, 117.5 mmol/L NaCl, 2.5 mmol/L $CaCl_2$, 5.7 mmol/L KCl, 1.2 mmol/L NaH_2PO_4, 1.2 mmol/L $MgSO_4$, and 27.8 mmol/L D-glucose at room temperature that had previously been oxygenated with 95% O_2/5% CO_2. Smooth muscle layers were stripped off and 3 adjacent sections per segment, which were free of Peyer's patches, were mounted in Ussing chambers (TNO, Zeist, The Netherlands). Tissues were bathed on their serosal and mucosal sides with 1.5 ml Ringer's solution, oxygenated, and maintained at 37°C. After equilibration for 30 min the permeability was assessed by placing 10^{-5} mol/L HRP (Sigma) on the mucosal side of the Ussing chamber. Serosal samples (20 µL) were taken at 30, 60, 90, and 120 min and replaced with 20 µL fresh Ringer's solution to keep the volume constant. Enzymatic activity of HRP was analysed using 3,3,5,5-tetramethylbenzydine substrate (Pierce, Rockford, IL) and 10% H_2SO_4 for the stop reagent as published by Gallati and Pracht[223]. The reaction was assessed by measuring optical density at 450nm.

Table 1. Best fit covariance structure for each variable and corresponding *P*-value and degrees of freedom for the different variance sources

			Variance source					
		BW d 0	Group[1]		Day		Group[1] × Day	
Variable	Covariance structure	*P*	*P*	df	*P*	df	*P*	df
Repeated measurements over days								
Lactulose (L)	Compound symmetry	0.41	0.05	27	<0.001	49	0.29	49
L-rhamnose (R)	Unstructured[2]	0.80	0.74	24.5	0.03	13.4	0.03	13.4
L:R ratio	Unstructured[2]	0.14	0.02	20.4	<0.001	18.3	<0.01	18.4
3OMG[3]	Compound symmetry	0.40	0.36	27	0.90	49	0.10	49
D-xylose	Compound symmetry	0.30	0.45	27	0.85	49	0.91	49
Body weight (BW)	1st order autoregressive	<0.001	<0.001	27	<0.001	54	<0.001	54
No repeated measurements[4]								
HRP	–	0.99	–	–	0.73	19	–	–
Aerobic liver	–	0.04	–	–	0.13	35	–	–
Aerobic prox. MLN[5]	–	0.32	–	–	0.03	34	–	–
Aerobic dist. MLN[5]	–	0.05	–	–	0.02	34	–	–
Anaerobic liver	–	0.55	–	–	0.15	35	–	–
Anaerobic prox. MLN[5]	–	0.41	–	–	0.10	35	–	–
Anaerobic dist. MLN[5]	–	0.19	–	–	0.15	34	–	–

[1]Group = eater (pigs that had eaten at least 30 g/d during 1 of the first 5 d after weaning) or noneater (all other pigs).

[2]Degrees of freedom were estimated with the Kenward-Roger method with an unstructured covariance structure.

[3]3OMG = 3-*O*-methylglucose.

[4]HRP = horseradish peroxidase; MLN = mesenteric lymph nodes (of the small intestine); prox. = proximal; dist. = distal.

Statistical Analyses

Feed intake after weaning has been shown to affect intestinal barrier function[(26)]. To account for substantial differences in level of feed intake, the animals were classified as eaters or noneaters. Pigs were defined as eaters if they had eaten at least 30 g/d during 1 of the first 5 d after weaning. Marker recovery, the lactulose: L-rhamnose ratio (L:R ratio), and BW were analysed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) to allow for correlations between repeated observations over time using pig as the experimental unit. Mixed linear models were fitted with fixed effects for group (eaters or noneaters), day, and their interaction, and BW at weaning as the covariate. Different correlation structures were compared for the repeated measurements over days, applying a compound symmetric, first-order autoregressive, and an unstructured covariance structure[(224)], and then subsequently selecting the model with the least Akaike's information criterion (Table 1). Horseradish peroxidase flux and bacterial counts (in the proximal and distal MLN and in the liver) were analysed using day as the only factor and BW as a covariate in the model. In addition, HRP flux and bacterial counts were analysed using group (eaters or noneaters) and group × day interactions in the model, but without the inclusion of data obtained from d 1 (grouping was not yet possible as these pigs were dissected at d 1). Due to difficulties in marker administration for 2 pigs on d 0 and 4, and for 1 pig on d 8, these were considered outliers and were omitted from the analysis of intestinal permeability for that day. Moreover, the data for 3 samples of bacterial counts were also omitted from statistical analyses due to incorrect sample handling. Residuals were plotted to evaluate normality of distribution and homogeneity of variance. Based on these data, some variables were log-transformed (lactulose recovery, L:R ratio and HRP flux using $\log_e$ and by convention, bacterial counts using $\log_{10}$) and monosaccharide recoveries were square root-transformed before the final statistical analyses. All results were presented as least square means, and if transformed,

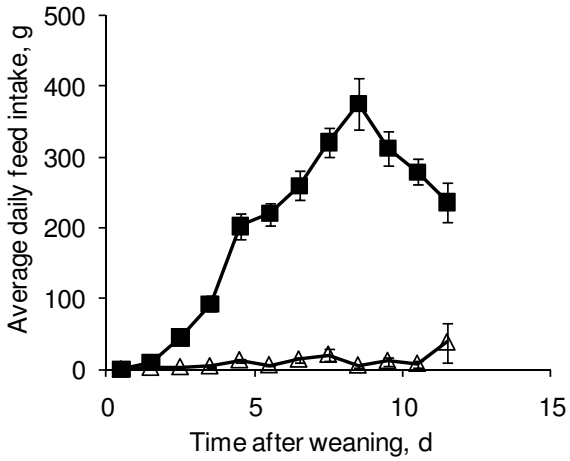

Figure 2. Average daily feed intake of eaters (■) and noneaters (Δ). The number of pigs for eaters was 19, 19, 13, and 7 on d 0, 4, 8, and 12, respectively, and for non-eaters was 11, 11, 7, and 3, respectively. Error bars represent SE of the mean.

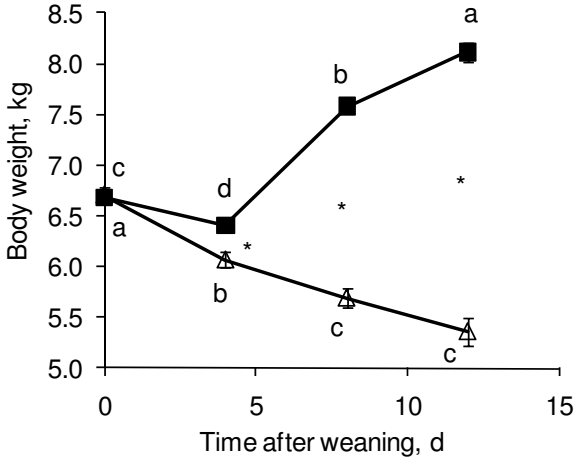

Figure 3. Body weight of eaters (■) and noneaters (Δ). Different letters indicate differences between days ($P \leq 0.05$) and * indicates differences within days between groups ($P \leq 0.05$). The number of pigs for eaters was 19, 19, 13, and 7 on d 0, 4, 8, and 12, respectively, and for noneaters was 11, 11, 7, and 3, respectively. Error bars represent SE of the least square mean.

the means of the data before transformation were also presented (with the exception of bacterial counts). Differences between day × group combinations or only differences between days were analysed for significance ($P \leq 0.05$) using the Tukey procedure to account for multiple comparisons. Pearson correlation coefficients were calculated between HRP flux, bacterial counts, marker recoveries, L:R, and level of feed intake the day before dissection using the CORR procedure of SAS. Partial correlations were calculated between the residuals of the variables after correction of the variables for the day by ANOVA using the GLM procedure of SAS.

Results

The noneaters ate almost nothing for the first 5 d after weaning and ate only minor amounts thereafter (Figure 2). The mean BW decreased gradually after weaning for the noneaters but increased ($P < 0.001$) for the eaters (Figure 3). The noneaters and eaters were evenly distributed over the day of sample collection.

Lactulose recovery in the urine was greater at d 4, 8, and 12 after weaning compared with the first day after weaning (Figure 4; $P \leq 0.05$). Moreover, lactulose recovery after weaning was greater for the noneaters than for the eaters (Table 1; $P \leq 0.05$). Although no significant association between the group and day was found ($P = 0.29$), the overall difference between the groups seemed to be due to the difference at d 4 and 8 (Table 1 and Figure 4). For L-rhamnose recovery in the urine, the feed intake group was associated with the test day (Table 1; $P = 0.03$). This association was the consequence of a constant L-rhamnose recovery over time for the noneaters and a decrease in recovery over time for the eaters (Figure 4). Recovery of 3OMG and D-xylose in the urine did not differ over time (Figure 5; $P = 0.90$ and $P = 0.85$, respectively). Feed intake differences between groups (eaters or noneaters) had no effect on small intestinal HRP flux ($P = 0.80$). Therefore, the HRP flux was not presented separately for eaters and noneaters (Figure 6). In addition, the

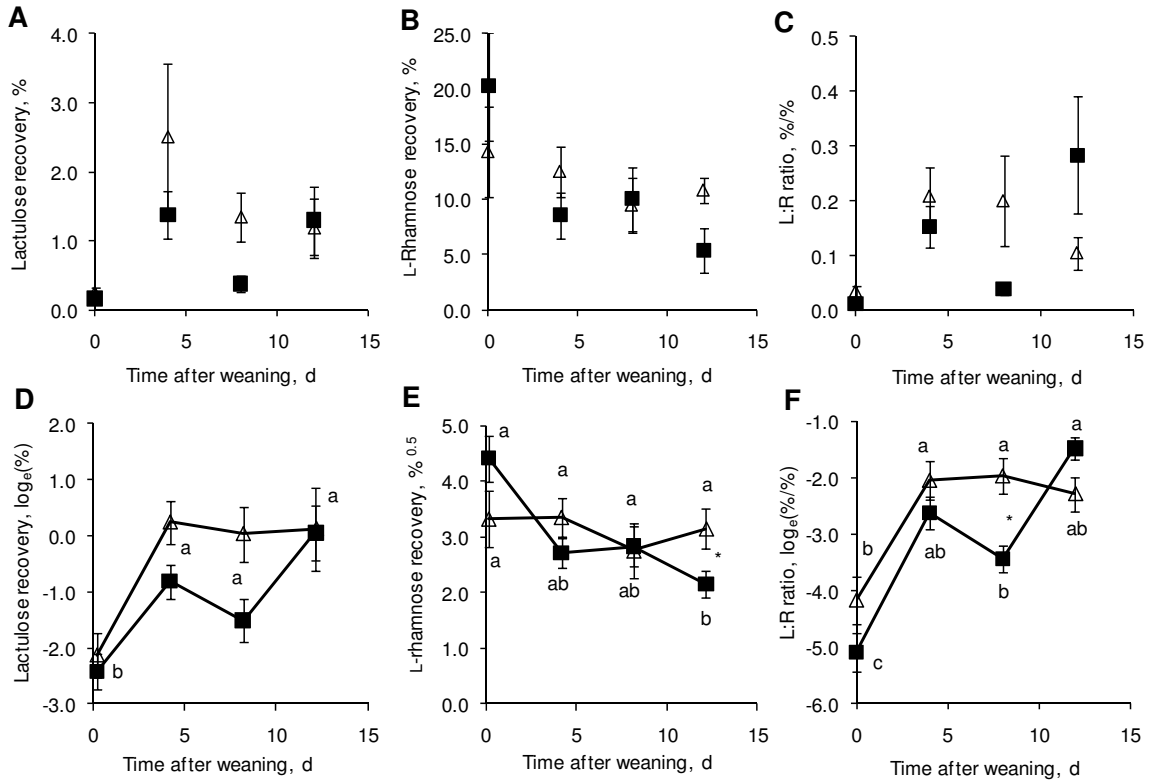

Figure 4. Recovery of lactulose and L-rhamnose from urine and lactulose: L-rhamnose ratio (L:R ratio) of eaters (■) and noneaters (Δ). Panel A to C represent untransformed data, and panel D to F represent $\log_e$ (lactulose and L:R ratio) or square root (L-rhamnose) transformed data. $^{a-c}$Different letters indicate differences between days ($P \leq 0.05$) and * indicates differences within days between groups ($P \leq 0.05$). The number of pigs for eaters was 17, 17, 12, and 7 on d 0, 4, 8, and 12, respectively, and for noneaters was 11, 11, 7, and 3, respectively. Error bars represent SE of the mean (panel A to C) or of the least square mean (panel D to F).

HRP flux was not affected over time (Figure 6; $P = 0.73$). Feed intake differences between groups (eaters or noneaters) had no effect on the bacterial counts in the liver or in the proximal and distal MLN ($P \geq 0.24$). Therefore, the bacterial counts from the 3 sample locations were not separately presented for eaters and noneaters (Figure 7). The anaerobic bacteria counts were not different over time for any of the sample locations (Figure 7; $P \geq 0.10$). The aerobic bacteria counts in the proximal MLN at d 5 and in the proximal and distal MLN at d 13 were increased compared with the counts at d 1 after weaning ($P = 0.04$, $P = 0.04$, and $P = 0.03$, respectively).

The lactulose recovery in the urine was negatively correlated with feed intake (Table 2; $r = -0.63$, $P \leq 0.001$). In contrast, bacterial translocation to the MLN and liver and HRP flux in the small intestine were not correlated with feed intake (Table 2; $P \geq 0.31$). Moreover, lactulose recovery was not correlated with HRP flux or with bacterial translocation ($P \geq 0.05$). Lactulose recovery was negatively correlated with the translocation of aerobic bacteria to the MLN of the distal small intestine ($P = 0.04$).

Discussion

Animals were divided into groups of eaters and noneaters, because feed intake after weaning has been shown to be related to intestinal barrier function [26]. The non-eaters ate almost nothing for the first 5 d after weaning and ate only minor amounts thereafter. This as also reflected in the change in BW, which decreased gradually after weaning for the

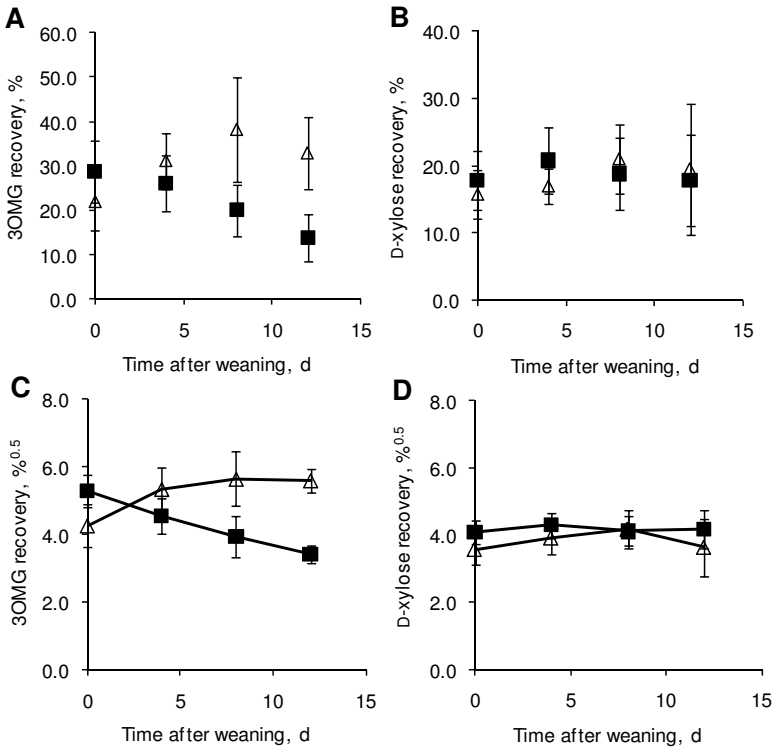

Figure 5. 3-*O*-methylglucose (3OMG) and D-xylose recovery in the urine of eaters (■) and noneaters (Δ). Panel A and B represent untransformed data, and panel C and D represent square root-transformed data. The number of pigs for eaters was 17, 17, 12, and 7 on d 0, 4, 8, and 12, respectively, and for noneaters was 11, 11, 7, and 3, respectively. Error bars represent SE of the mean (panel A and B) or of the least square mean (panel C and D).

noneaters but increased for the eaters. The noneaters and eaters were evenly distributed over the day of sample collection. The total number of noneaters (11 out of 30) was large. It has been reported that about 90% of pigs start eating during the first 48 h after weaning [16].The large number of noneaters observed in the present study may be related to the individual housing of the pigs, as well as to the experimental protocol that was used. In other experiments in this facility, we have also observed a large number of noneaters, although this is highly variable among experiments. The stresses that were associated with the intensive handling of the pigs during this study (oral gavage, connection of the pouch,

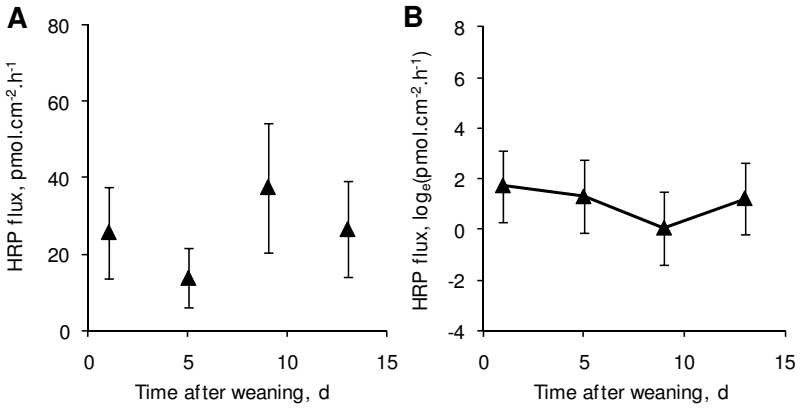

Figure 6. Horseradish peroxidase flux of the proximal small intestine measured in Ussing chambers over a period of 90 min. Panel A represents untransformed data, and panel B represents $\log_e$ transformed data. All data points represent the data of 6 pigs. Error bars represent SE of the mean (panel A) or of the least square mean (panel B).

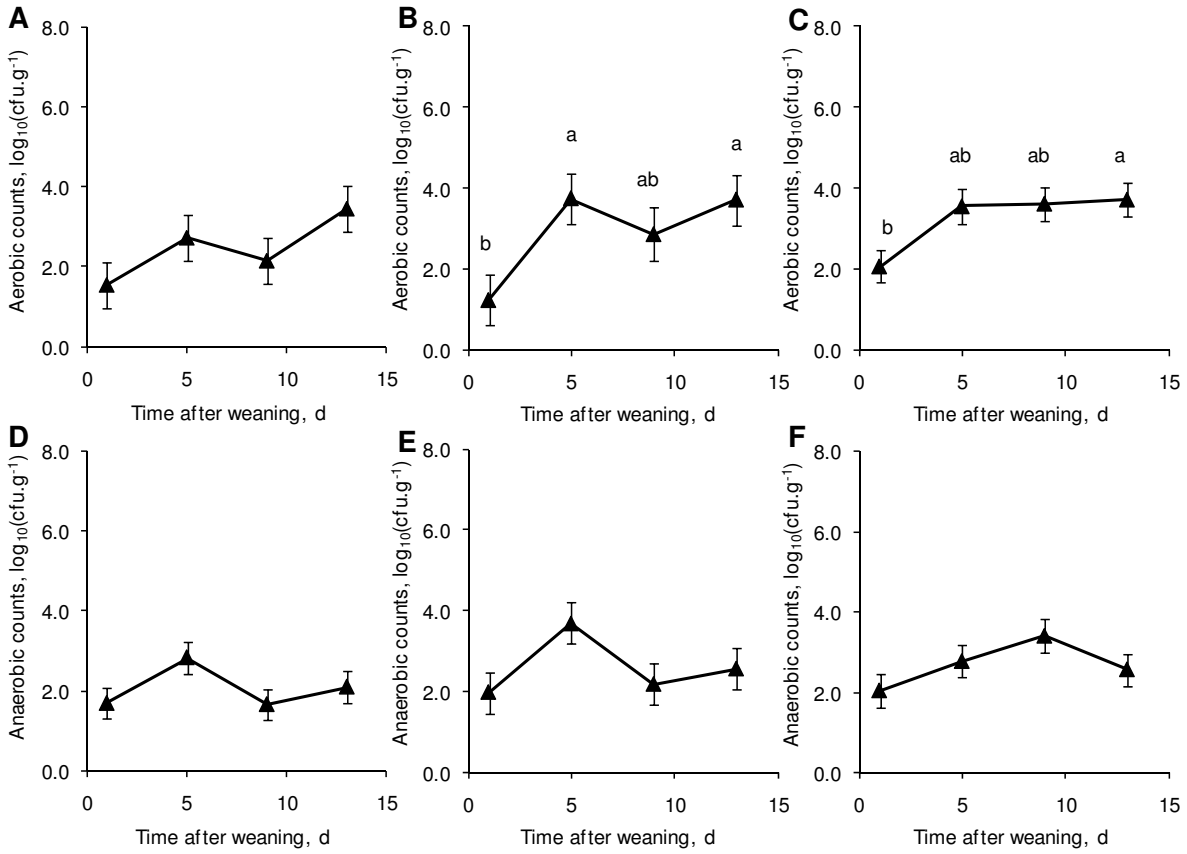

Figure 7. Mean results of aerobic bacteria counts of the liver (panels A and D) and the mesenteric lymph nodes (MLN) of the proximal (panels B and E) and distal (panels C and F) small intestine. [a,b]Different letters indicate significant differences between days ($P \leq 0.05$). All days represent the data of 10 pigs, with the exception of d 5 (distal MLN, n = 9 pigs) and d 9 (proximal MLN, n = 9 pigs). Error bars represent SE of the least squares mean.

and urine collection) may also have been contributing factors that increased the number of noneaters. It is not likely that the oral marker probes themselves were the cause of the high number of noneaters because the total sugar dose per oral gavage was relatively small (about 3 g) compared with the normal levels of feed intake of pigs.

In this discussion section, we distinguish between the paracellular barrier function and absorption function of the small intestine. The paracellular barrier function was measured by lactulose recovery in the urine, and the absorption function was measured by monosaccharide recovery in the urine.

Paracellular Barrier Function

Lactulose is a marker that can be used as an orally administered probe to assess paracellular barrier function of the small intestine by measuring the recovery of lactulose in the urine[(185)]. However, the test results with this technique can be influenced by many premucosal (e.g. bacterial degradation) and postmucosal (e.g. completeness of urine collection) factors[(185)]. To reduce the effects of those premucosal and postmucosal factors, the dual sugar test was introduced by Menzies[(192)]. The principle of the test is based in the fact that orally administered disaccharides (e.g. lactulose) will pass the intestinal epithelium through the paracellular route when the barrier function is compromised. In addition, a monosaccharide, such as L-rhamnose, is also administered, which passes the intestinal epithelium by unmediated diffusion either through paracellular or transcellular routes, and therefore

Table 2. Pearson correlation coefficients between variables of intestinal permeability, intestinal absorption, and feed intake[1]

							Aerobic bacteria[5]			Anaerobic bacteria[5]		
	Lactulose	rhamnose	L:R[2]	3OMG[3]	D-xylose	HRP[4]	Liver	Prox. MLN[5]	Dist. MLN	Liver	Prox. MLN	Dist. MLN
Feed intake	–0.63 ***	ns[6]	–0.44 *	–0.38 *	ns	ns	ns	ns	ns	ns	ns	ns
Lactulose		0.37 *	0.80 ***	0.38 *	ns	ns	ns	ns	–0.34 *	ns	ns	ns
L-rhamnose			ns	0.84 ***	0.64 ***	ns	ns	ns	ns	ns	ns	ns
L:R				ns	ns	ns	ns	ns	ns	ns	ns	ns
3OMG					0.77 ***	ns	ns	ns	ns	ns	ns	ns
D-xylose						ns	ns	ns	ns	ns	ns	ns
HRP							ns	ns	ns	ns	ns	ns

[1]Pearson correlations were calculated for the residuals from ANOVA analyses with only the day in the model. Correlations were calculated between the dissection variables and the marker recoveries in the urine and feed intake of the pigs the day before the dissection.
[2]L:R = L-rhamnose:lactulose ratio; [3]3OMG = 3-*O*-methylglucose; [4]HRP = horseradish peroxidase.
[5]MLN = mesenteric lymph nodes; Prox. = proximal; Dist. = distal; [6]ns = non significant; **P* < 0.05; ****P* < 0.001.

provides a measure of the absorptive surface. After absorption, the sugars are not metabolised and the majority of the absorbed sugar is excreted in the urine. The assumption of this test is that both probes are affected by the premucosal and postmucosal factors to a similar degree, and therefore their ratio is not disturbed by those factors[(175)]. The ratio between the lactulose and L-rhamnose excreted in the urine provides information regarding the intestinal barrier function[(185)]. For example, an increase in the L:R ratio indicates a decrease of the intestinal paracellular barrier function, whereas a decrease in the L:R ratio indicates improved intestinal paracellular barrier function.

In the current study, L-rhamnose recovery decreased after weaning in the eaters group and was not affected over time in the noneaters group. This unexpected result makes it difficult to interpret the L:R ratio for evaluating intestinal barrier function after weaning because an increase in the ratio may reflect a decreased L-rhamnose recovery, as well as an increased lactulose recovery. Therefore, in the present study with pigs, it was better to assess paracellular barrier function solely based on lactulose instead of the L:R. Lactulose recovery in the urine was greater at d 4, 8, and 12 after weaning compared with the first day after weaning. Moreover, lactulose recovery after weaning was greater for the noneaters than the eaters. It cannot be excluded that the differences in lactulose recovery in the urine over time or between eater groups were influenced by differences in bacterial degradation. However, this possibility was not likely for 2 reasons. First, the total number of bacteria per gram of digesta in the lumen of the small intestine has been shown to decrease during the first 2 d after weaning and were similar or increased during the second week after weaning compared with the numbers before weaning[(89,225)]. Therefore, if lactulose recovery over time was markedly influenced by bacterial degradation in the current study, we would expect a decrease in recovery over time instead of an increase, which was not observed. Second, the recovery of L-rhamnose, D-xylose, and 3OMG in the urine was either constant or decreased over time in the current experiment, which is opposite to the recovery of lactulose over time. Therefore, if the monosaccharides and lactulose were markedly influenced by bacterial degradation over time, we would expect a similar change over time for all saccharides. We cannot exclude the possibility that changes in specific bacterial

species over time or between eaters and noneaters influenced the recovery of individual sugars in the urine differently. However, this would contradict the assumption that all sugars are influenced by premucosal and postmucosal factors to a similar degree[175]. In the literature, mannitol, which is a sugar alcohol, has also been used to assess paracellular permeability of the pig intestine after weaning in Ussing chambers. Although mannitol has a similar molecular size as monosaccharides, and therefore is one-half the size of lactulose, it is believed to pass the intestinal epithelium predominantly through a paracellular route when barrier function is compromised, similar to lactulose[185]. In agreement with our results using lactulose, the Ussing chamber studies revealed that jejunal mannitol fluxes between 1 and 6 d after weaning increase compared to preweaning fluxes[14,18,26,188]. Moreover, Verdonk *et al*.[26] showed that mannitol fluxes at 2 and 4 d after weaning compared with preweaning were not affected in pigs with greater feed intake, whereas flux was increased when compared with preweaning for pigs with decreased feed intake. In agreement with our study, Verdonk *et al*.[26] showed that feed intake after weaning is important for intestinal barrier function. Therefore, the lactulose data in the current study are in agreement with the Ussing chamber data using mannitol in pigs with respect to the effects after weaning and the relationship to feed intake. Finally, another study has shown that the L:R in the urine of dogs increased after weaning in gluten-sensitive dogs but was not affected over time in healthy dogs[226]. Therefore, the increase in recovery of lactulose in urine over time after weaning in the current study is in agreement with the previous study using gluten-sensitive dogs.

Absorption Function

Recovery of L-rhamnose in the urine was constant over time for the noneaters and decreased over time for the eaters. To our knowledge, no other study has described the intestinal absorption of L-rhamnose in pigs over time after weaning. However, Verdonk *et al*.[91] measured Na-fluorescein isothiocyanate absorption in pigs after weaning in Ussing chambers. Similar to L-rhamnose, Na-fluorescein isothiocyanate is absorbed through a trancellular passive process[184,185]. Verdonk *et al*.[91] showed that Na-fluorescein isothiocyanate absorption decreased after weaning, which is in agreement with the eaters group in our study. However, in contrast to our study, Verdonk *et al*.[91] found no relationship between feed intake after weaning and passive transcellular absorption. This difference may be related to the fact that the feed intake differences were more pronounced in our study than in the study by Verdonk *et al*.[91]. Recovery of 3OMG, which is a glucose analogue that is absorbed through an active process, was not affected over time in the current study. We previously concluded that active absorption decreases after weaning when pigs are weaned at 3 wk of age[219]. Reduced feed intake stimulates active absorption[26]. Therefore, the discrepancy between our study and the literature with regards to active absorption may relate to the large number of noneaters in our current study. Recovery of D-xylose in the urine did not differ over time in the present study. However, in contrast to this finding, several other studies have shown that at 1 h after oral administration, the serum D-xylose concentrations were less during the first week after weaning compared to preweaning[23,193-196]. In our study, the D-xylose recovery was measured in quantitatively collected urine, whereas in all other studies, the D-xylose concentration was measured in blood serum. The discrepancy of our study with other studies may relate to this difference in technique. Moreover, the largest decrease in D-xylose absorption was shown to occur during the first day after weaning[193]. Because we did

not measure D-xylose absorption before weaning, this may also explain why we did not find differences in D-xylose absorption over time. In agreement with our study, Kelly *et al*.[30] found no difference in D-xylose absorption at 5 d after weaning in pigs at an increased or decreased feed intake. Therefore, D-xylose absorption after weaning seems to be less sensitive to differences in feed intake after weaning than L-rhamnose and 3OMG absorption.

HRP Flux and Bacterial Translocation

In the current study, the HRP flux was not affected by time. In contrast, the HRP flux in the proximal jejunum significantly decreased at different days between 2 and 15 d after weaning compared with preweaning stage[19,91]. We did not measure the HRP flux before weaning in the current study, which may be why a decrease over time was not observed. Horseradish peroxidase is used as a marker of antigen uptake through endocytosis[205]. Therefore, a reduced HRP flux is an indication of improved intestinal barrier function. However, it is generally believed that the intestinal barrier function is disturbed after weaning. This is supported by the greater susceptibility of pigs to infections and oedema disease after weaning than before weaning[13,27]. Moreover, it has been shown that the transepithelial electrical resistance (TEER) of the proximal jejunum decreased after weaning, which indicates a disturbed barrier function[19]. In that study, the TEER responded in the same direction as HRP flux after weaning, whereas these variables are supposed to respond in an opposite manner. One possible explanation for this observation is that in general, only the paracellular barrier function, which is represented by TEER, is compromised after weaning. The barrier function for HRP is mainly related to endocytosis and represents transcellular barrier function[182,183]. This may only be compromised after the occurrence of additional stressors, such as transport stress or a bacterial challenge, as we have previously hypothesised[219].

In the current experiment, the aerobic bacteria counts in the proximal MLN at d 5 and in the proximal and distal MLN at d 13 were increased compared to d 1 after weaning. To our knowledge, there is no comparable study that has reported changes over time of bacterial translocation shortly after weaning in pigs. One study has assessed bacterial translocation 6 d after weaning in pigs that were weaned at 23 d of age[227]. However, in contrast to the current study, the pigs in that study did not have access to creep feed before weaning, were housed in groups in fully slatted pig units, and received a weaner diet that had a relatively large fishmeal concentration (12.5%) compared with our study (3%). The average bacterial count in the distal MLN at 5, 9, and 13 d after weaning for aerobes (3.6 $\log_{10}$ cfu/g) and anaerobes (2.9 $\log_{10}$ cfu/g) in our study was also less than that reported by Broom *et al*.[227]. At 6 d after weaning, they found an average of 4.8 $\log_{10}$ cfu/g for aerobes and 5.5 $\log_{10}$ cfu/g for anaerobes. It has been shown that weaning deteriorates the paracellular barrier function of the small intestine[14,18,26,91,188]. We previously hypothesised that the deteriorated paracellular barrier function may be an indication for an increased risk of bacterial translocation[219]. Therefore, the increase over time of aerobic bacteria counts in the MLN after weaning is in agreement with this hypothesis.

Partial Correlations Between Variables

In the present study, lactulose recovery in the urine was negatively correlated with feed intake. This is in agreement with the previous observation that lactulose recovery is greater in noneaters than in eaters and shows that the intestinal permeability for lactulose is

associated with feed intake at the day of the permeability test. In contrast, bacterial translocation to the MLN and liver, as well as HRP flux in the small intestine, did not correlate with feed intake in the current study. Moreover, in the current study, lactulose recovery did not positively correlate with HRP flux or with bacterial translocation. In agreement with these findings, Verdonk[26] found no correlation between small intestinal HRP flux and mannitol flux in Ussing chambers for pigs at 4 and 7 d after weaning. In the present study, lactulose recovery was even negatively correlated with bacterial translocation to the MLN of the distal small intestine. In addition to the intestinal barrier function, the bacterial concentration in the intestinal lumen is also an important factor with respect to bacterial translocation[174]. We hypothesise that greater bacterial concentrations in the intestinal lumen of specific pigs may have increased bacterial translocation. In addition, we hypothesise that in the same pigs, the greater bacterial concentration may have increased the intestinal fermentation of lactulose, which consequently may have decreased the recovery of lactulose in the urine. This may explain the negative correlation between lactulose recovery and bacterial translocation to the MLN of the distal small intestine. In previous studies with rats, an increased intestinal permeability for lactulose was associated with increased bacterial translocation[228,229]. In agreement with these findings, we found that the mean translocation of aerobic bacteria to the MLN and the recovery of lactulose in the urine showed a similar increase over time after weaning. However, we hypothesise that the Pearson's correlation test in the current study may not have shown a relationship between the recovery of lactulose in the urine and translocation of aerobic bacteria to the MLN because of the variation in concentration of bacteria in the lumen between individual pigs.

Conclusions

Recovery of lactulose in the urine of pigs increased after weaning and was higher in noneaters than in eaters. These findings indicate that based on lactulose, the weaning process disturbs paracellular barrier function of the small intestine, which is more prominent in pigs with reduced feed intake. In contrast, HRP flux was not different between days after weaning or between eaters and noneaters, and showed no correlation with lactulose recovery. Bacterial translocation of aerobic bacteria to the different tissues was higher at 5, 8 and 13 d after weaning than at 1 d after weaning, which was in agreement with the lactulose data. However, bacterial translocation to the different tissues was not different for eaters and noneaters, and showed no relationship with lactulose recovery in a Pearson correlation test. Although both lactulose recovery and bacterial translocation increased over time after weaning, lactulose recovery did not correlate with permeability for HRP nor bacterial translocation. We conclude that lactulose recovery in the urine of pigs after weaning is not associated with risk factors for infections. However, it appears to be possible to measure paracellular barrier function with orally administered lactulose in pigs shortly after weaning. Further studies will reveal whether this variable is relevant for the long-term performance or health of pigs after weaning.

Chapter 6

A diet of starch and dextrose improves small-intestinal barrier function after weaning compared with feed-deprived pigs; however, a diet of protein and dextrose shows no substantial effect on barrier function

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Submitted

Abstract

Low feed intake in pigs after weaning has been associated with villous atrophy and with loss of small-intestinal barrier function. It is assumed that the effects of low feed intake on small-intestinal integrity are due to the low energy intake of the pigs. However, there are no studies that distinguish between the effects of non-protein energy and protein on small-intestinal architecture and barrier function. In this study, the effects of feed deprivation (FD) for 4 d after weaning were compared with feeding either starch and dextrose (StD) or protein and dextrose (PrD) with 18 barrows per treatment. Both diets were fed as liquids with dextrose (44 g/kg) and either pregelatinised rice starch (StD, 112 g/kg) or hydrolysed wheat protein (PrD, 103 g/kg). Small-intestinal mucosal weight was determined at 4 d after weaning and small-intestinal barrier function was measured by urinary lactulose recovery after an intragastrical dose at 3 d after weaning. Mucosa weights were higher for the PrD pigs compared to the FD pigs ($P < 0.05$), while mucosal weight of the StD pigs was between the mucosal weights of the PrD and FD pigs. Lactulose recovery was lower ($P < 0.05$) for the StD pigs than for the FD pigs and was not significantly different ($P = 0.11$) between PrD and FD pigs. Thus, dietary protein with dextrose stimulates an increase in mucosa weight. However, protein does not have a substantial effect on small-intestinal barrier function, whereas dietary starch improves small-intestinal barrier function. These findings suggest that at low feed intake, dietary protein concentrations after weaning are of minor importance for small-intestinal barrier function.

Keywords: barrier function, pig, protein, small intestine, starch, weaning.

Introduction

After weaning, feed intake of pigs, in general, is very low. Ten percent of pigs do not take in any feed during the first 48 h after weaning[(16)]. This low feed intake is consistently associated with villous atrophy in the small intestine[(13,23,32)], which results in a variety of health problems. From previous studies, it has been concluded that a low energy intake is the cause of the observed reduction in gut integrity. Carbohydrates and protein are the main energy sources[(230-232)], and amino acids (AA) are the main building blocks for the small

intestine. Therefore, one can hypothesise that the specific shortage of dietary protein may be the major cause of the reduced mucosal integrity of the small intestine and not energy. In line with this, others have hypothesised that a sufficient dietary protein supply is important for maintaining gut integrity immediately after weaning[40,89]. Hence, maintaining a sufficient protein intake may maintain gut function and integrity, and one can hypothesise that it will also prevent gut permeability problems that are often seen after weaning[14,188]. Data regarding possible differences between non-protein energy and protein in relation to gut integrity is currently lacking. On the contrary, several recent studies have focused on a reduction of dietary protein in diets of weaned pigs for reducing the amount of protein available for pathogenic bacteria[97,233,234]. These studies showed that low protein diets improve the faecal score of the pigs without any compromise on weight gain. However, none of these studies focused on the effects of low protein diets on gut development or gut integrity in the first week after weaning. Therefore, the possible detrimental effects of low protein diets on the integrity of the intestinal wall may have been overlooked. The present study aimed to investigate the effect of the type of dietary energy used (starch or protein) during the first 4 d after weaning on small-intestinal mucosal mass and barrier function.

Materials and Methods

Animals and housing

Fifty-eight Piétrain × (Large White × Dutch Landrace) barrows were selected from 20 litters of our own farm herd (Provimi Research Centre 'De Viersprong', Velddriel, The Netherlands). Based on weight and ancestry, the pigs were assigned to 3 treatment groups of 18 barrows each and 1 group of 4 pigs to be used for dissection at weaning. The mean body weight (BW) was similar for all groups. Pigs had no access to creep feed diets before weaning. The pigs were weaned at 20.4 ± 0.9 d and had a BW of 6.4 ± 0.9 kg (± SD for age and BW). The pigs from the group of 4 barrows were euthanised and dissected immediately at weaning. The remaining 54 pigs were housed individually (0.5 m^2/pen) in either 1 room with 36 pens or 1 room with 18 pens. The ambient temperature was maintained at 29^oC throughout the experiment. Lights were on 24 h per day. The Ethical Committee of the Animal Science Group of the Wageningen University and Research centre (Lelystad, The Netherlands) approved the experiment.

Feeds, feeding, and experimental design

The 3 groups were subjected to 3 different treatments at the time of weaning. The pigs were either given restricted access to a liquid 'diet' containing only minerals (feed-deprived treatment, FD), a liquid diet containing minerals, starch, and dextrose (starch and dextrose treatment, StD), or a liquid diet containing minerals, protein, and dextrose (protein and dextrose treatment, PrD). The daily supply and the exact composition of these liquid diets are given in Table 1. The mineral composition of the liquid diets was a typical mineral composition for electrolyte mixes commonly used in practice. The liquid diets were given 3 times per day (0800, 1600, and 2300 h) during the first 2 d after weaning and were fed 2 times per day (0800 and 1800 h) during the remainder of the experiment. The daily portion was equally divided over the different feeding times and was freshly prepared before each feeding. Four days after weaning, 6 pigs from each treatment group were euthanised and dissected.

Table 1. Composition of liquid test diets of the feed-deprived (FD), starch with dextrose (StD) and protein with dextrose (PrD) fed pigs

	Diet		
Item	FD	StD	PrD[1]
Ingredients		Allowed intake, *g·pig^{-1}·d^{-1}*	
Dextrose		26.3	26.3
Pregelatinised rice starch[2]		66.8	
Hydrolysed wheat protein[3]			61.1
L-Lysine-HCl (80%)			2.38
L-Threonine			0.73
L-Valine			0.21
L-Tryptophan			0.12
Sodium chloride	1.90	1.90	1.13
Potassium chloride	0.23	0.23	0.08
Sodium bicarbonate	1.41	1.41	2.23
Sweetener[4]	0.03	0.03	0.03
Water	500	500	500
Total	504	597	594
Nutrients			
Carbohydrates	–	86.7	30.5
Crude protein	–	0.6	50.0
Crude fat	–	0.1	0.7
Ash	3.5	4.2	3.9
Sodium	1.15	1.15	1.15
Potassium	0.11	0.11	0.11
Chloride	1.25	1.25	1.25
Metabolisable energy , *kJ·pig^{-1}·d^{-1}*	–	1410	1410

[1]Based on supplier information of the wheat protein and an assumed 99% digestibility of the crystalline amino acids this diet supplied the following ileal digestible amino acids (g·pig^{-1}·d^{-1}): Lys, 2.59; Met, 0.92; Cys, 1.02; Thr, 1.91; Trp, 0.48; Val, 1.99; Ile, 1.63; His, 0.85; Leu, 3.14; Phe, 2.70; Tyr, 1.60; Arg, 1.58; Glu, 17.24; Aln, 1.16; Asp, 1.40; Gly, 1.40; Pro, 5.65; Ser, 2.51.

[2]Remyline AX-FG-P, Beneo-Remy NV, Leuven-Wijgmaal, Belgium. Containing 9 g/kg crude protein and 940 g/kg carbohydrates on an as-fed basis.

[3]Solpro 508, Syral, Aalst, Belgium. Containing 768 g/kg crude protein and 108 g/kg carbohydrates on an as-fed basis.

[4]Containing 950 g/kg sodium saccharin and 50 g/kg neohesperidin dihydrochalcone on an as-fed basis.

Growth and feed intake

All pigs were weighed at weaning and at 4 d after weaning. Feed intake was determined daily.

Permeability measurements

For the permeability test, marker probes with different characteristics were intragastrically dosed in order to measure different absorption and permeability functions of the intestine. At 3 d after weaning, the pigs were deprived of the liquid feed from 0800 to 1200 h. In order to avoid suppression of urine production they were given access to 100 mL of water from a bowl during this time of feed deprivation. At 1000 h, all pigs were intragastrically dosed with a marker solution containing 2.83 g of lactulose, 0.19 g L-rhamnose, 0.04 g 3-*O*-methyl glucose (3OMG), and 0.09 g D-xylose (all obtained from Sigma, Zwijndrecht, The Netherlands) and 0.6 g Co-EDTA (prepared according to Udén *et al.*[(235)]). The markers (3.16 g in total)

were dissolved in 11.84 g deionised water to obtain a solution of 15 g per pig. The L-rhamnose, 3OMG, and D-xylose doses were derived from a study with humans[220] and adapted to pigs based on metabolic BW ($BW^{0.75}$). L-rhamnose is a marker for transcellular (aqueous pores) or paracellular absorption[185], 3OMG is a marker for active absorption, and D-xylose is a marker for passive transcellular, paracellular[191], or carrier-mediated transcellular absorption[185]. The lactulose dose was in line with the dose used in a previous study with neonatal piglets[201]. The dose of Co-EDTA was based on previous work (Wijtten *et al*., unpublished). Lactulose is a marker for paracellular barrier function of the small intestine[190]. Co-EDTA is a molecule with a similar size as lactulose and is also transported over the intestinal epithelium by paracellular transport. However, in contrast to lactulose, it is not degraded by bacterial fermentation and therefore it is a marker for both small-intestinal and large-intestinal (colon) permeability[185]. Urine was quantitatively collected in a pouch glued and taped to the belly of the pig until 1400 h the next day. At 2, 7, 12, 22, and 28 h after marker administration, the urine from the pouch was collected and stored in a refrigerator (6°C) in a separate jar for each pig containing 100 µL of a thimerosal (Sigma, Zwijndrecht, The Netherlands) solution (100 g thimerosal/L) for preservation of the urine. At d 4 (1 d after marker administration), 6 pigs per treatment were euthanised and dissected (see below). The urine collected from the bladder of these pigs was stored in the same jar as which was used for collection of other urine samples from the same pig the day before the dissection.

Dissection

On the day of weaning 4 pigs were dissected immediately after weaning. At 4 d after weaning, 6 pigs per treatment were dissected (18 pigs in total). Pigs were anesthetised with 24 mg Na-pentobarbital per kg BW (Euthasol, ASTfarma, Oudewater, The Netherlands) and euthanised by bleeding. The abdominal cavity was opened and the urine in the bladder was collected quantitatively for marker analyses (see above). The small intestine was separated from the mesentery, measured, and then divided into 4 segments with equal length (0–25, 25–50, 50–75, and 75–100% segment). Each segment was opened lengthwise with scissors, rinsed with ice-cold saline (154 mmol NaCl/L), blotted dry with tissue paper, and weighed again. The mucosa was removed with a glass microscope slide from the middle 50 cm of each segment and the weight of the mucosa and remaining muscularis was determined. From the mucosa:muscularis ratio of this middle 50 cm and the total weight of each segment, the weight of mucosa and muscularis of the total intestinal segment was estimated. Relative mucosa and muscularis weights were calculated as mg/g BW.

Sample handling and analyses

After collection, small aliquots of the urine were stored at –20°C until needed for further analyses. All urine samples were analysed for marker sugars. The urine samples were diluted 10-fold using deionised water. Subsequently, Cl was precipitated by adding $AgNO_3$. After centrifugation (13000 × *g*, 10 min at 21°C), the supernatant was separated on an OnGuard Ba and H column followed by analysis using high-performance anion-exchange chromatography with a pulsed amperometric detector (Dionex Corporation, Salt Lake City, UT). The method was performed according to the manufacturer's instructions[221,222]. The detection limit for lactulose was 100 µmol/L and for the mono-sugars 60 µmol/L. Before determining the Co-EDTA content by Co analysis, 10 g of urine was incubated for 2 h at 95°C

with 20 mL HCl (12 mol/L) and 4 mL HNO_3 (16 mol/L). After cooling, deionised water was added to 50 g and the sample was analysed the next day with inductive coupled plasma-atomic emission spectrometry (ICP-AES, Thermo Iris Intrepid II XSP DUO) at wavelengths of 229 and 231nm. The detection limit for Co was 1 mg/kg. The recovery of sugars below the detection limit was assumed to be 50% of the detection limit. In those cases (13% of the samples), the estimated recovery was applied in the statistical analyses. The Co content of all of the urine samples was above the detection limit.

Statistical analyses

All data were analysed using Proc MIXED of SAS (version 9.1, SAS Inst. Inc., Cary, NC) using pig as the experimental unit. Energy intake was analysed to allow for correlations between repeated observations over time. Fixed effects of treatment, day, and their interaction were included in the model. To compare the effects of relative mucosa and muscularis weight of the FD pigs over time, fixed effects of day, intestinal segment (0–25, 25–50, 50–75, 75–100%, repeated over space), and their interaction were included in the model. Because of differences in daily energy intake between the StD and PrD treatments, energy intake was included in the model as a covariate in all analyses below, which allowed for different slopes for the StD and PrD treatments. If no effects ($P > 0.1$) of energy intake could be found, energy intake was excluded from the model and final analyses were done as described below. For analysing treatment effects on relative mucosa and muscularis weights within days, fixed effects of treatment, intestinal segment (repeated over space), and their interaction were included in the model. For all analyses above, different correlation structures were compared for the repeated measurements over days or over intestinal segments, applying a compound symmetric, first-order autoregressive and an unstructured covariance structure[224]. The model with the lowest Akaike's information criterion was selected. For urinary recovery of intestinal permeability and absorption markers and BW at d 4, mixed linear models were fitted with fixed effects for treatment, BW at weaning as covariate, and mother of the pigs and room as random effects. Degrees of freedom were estimated with the Kenward-Roger method for all analyses. Residuals were plotted to evaluate normality of distribution and homogeneity of variance. Based on this, lactulose and Co-EDTA recovery were $\log_e$ transformed and mono sugar recoveries were square root transformed before the final statistical analyses. All results are presented as least square means (LS-means). In case of data transformation, the LS-means were back-transformed

Table 2. Metabolisable energy (ME) intake, crude protein (CP) intake and body weight (BW) of feed-deprived (FD) barrows or of barrows fed a starch with dextrose (StD) or protein with dextrose (PrD) diet during the first 4 d after weaning[1]

Treatment	ME intake	CP intake	d 4 BW[2]	
	kJ/d	*g/d*	*kg*	*n*
FD	–	–	6.04 ± 0.05[c]	18
StD	1120 ± 73[a]	–	6.21 ± 0.05[b]	18
PrD	715 ± 72[b]	25.8 ± 2.7	6.45 ± 0.07[a]	17
P-values				
Treatment	< 0.0001	–	< 0.0001	
Treatment × Energy[3]	–	–	0.14	

[1]Data are LS-means ± SE. Means in columns without a common letter differ, $P < 0.05$.
[2]Estimated LS-means and treatment probabilities for an energy intake of 1120 kJ/d for the StD and PrD treatments and 0 kJ/d for the FD treatment.
[3]Probability for testing H0 that slopes for the StD and PrD treatment are not different.

after analysis. In case of an energy intake effect, estimated LS-means for a daily energy intake of 1120 kJ/d for the StD and PrD treatment and 0 kJ/D for the FD treatment are presented. The 1120 kJ/d was the intake level of the StD treatment. Differences between treatments or differences between days were analysed for significance ($P < 0.05$) using the Bonferroni procedure to account for multiple comparisons.

Because of difficulties with the marker administration for 1 pig and incomplete urine collection of another pig, these were omitted from the statistics for permeability and absorption parameters. Moreover, 1 pig of the PrD treatment, dissected at d 4, consumed only 11% of the allowed intake of the liquid diet. This pig was also omitted from the statistics for all data.

Table 3. Small-intestinal (SI) muscle and mucosa weights of feed-deprived (FD) barrows or of barrows fed a starch with dextrose (StD) or protein with dextrose (PrD) diet during the first 4 d after weaning [1,2]

Item	SI muscle		SI mucosa	
FD	*mg/g BW*	*n*	*mg/g BW*	*n*
d 0	1.93 ± 0.11[b]	4	5.82 ± 0.36[a]	4
d 4	2.25 ± 0.05[a]	6	3.98 ± 0.22[b]	6
P-value	0.019		< 0.0001	
d 4				
FD	2.42 ± 0.07[a]	6	3.59 ± 0.19[b]	6
StD	2.14 ± 0.07[b]	6	4.17 ± 0.19[ab]	6
PrD	2.13 ± 0.07[b]	5	4.68 ± 0.21[a]	5
P-value				
Treatment	0.007		0.009	
Treatment × Energy[3]	0.99		0.25	

[1]Data are LS-means ± SE, means in columns without a common letter differ, $P < 0.05$.
[2]The SI segment and day or treatment did not interact for muscle and mucosa weight, therefore mean weights of the 4 segments are provided. Thus, the presented means represent one-fourth of the total SI muscle and mucosa weight.
[3]Probability for testing H0 that slopes for the StD and PrD treatment are not different.

Results

Performance

Pigs in the StD and PrD treatment were allowed to eat a maximum of 1410 kJ of metabolisable energy (ME) per day for 4 d after weaning. Mean energy intake was higher ($P < 0.001$) for the StD (80% of allowed intake) than for the PrD pigs (51% of allowed intake, Table 2). The BW at 4 d after weaning was higher for StD and PrD pigs than for FD pigs and was higher for PrD than for StD pigs ($P < 0.05$, Table 2).

Intestinal mass

For both the relative small-intestinal mucosa weight and the small-intestinal muscularis weight, treatment or day effects did not interact ($P > 0.1$) with the small-intestinal segment. Therefore, the average results of the 4 small-intestinal segments are presented in Table 3. The relative small-intestinal mucosa weights of the FD pigs were lower and the muscularis weights were higher at 4 d after weaning compared to the day of weaning ($P < 0.05$, Table 3). The mucosa weight at d 4 was higher for the PrD pigs compared to the FD pigs ($P < 0.05$). The muscularis weight at d 4 was lower for the StD and PrD pigs than the FD pigs ($P < 0.05$). Both mucosa weights and muscularis weights at d 4 were not affected by variations in

energy intake levels within the StD and PrD treatments ($P > 0.1$). Consequently, mucosa and muscularis data were not corrected for differences in energy intake levels between the StD and the PrD treatment.

Intestinal permeability and absorption

Urinary Co-EDTA, L-rhamnose, D-xylose, and 3OMG recovery were similar for all treatment groups ($P > 0.05$, Table 4). Urinary lactulose recovery was lower ($P < 0.05$) for the StD pigs than for the FD pigs. The effect of energy intake level on lactulose recovery was different for the PrD treatment compared to the StD treatment group ($P < 0.05$, Table 4). For the StD treatment group, per unit increase in energy intake lactulose recovery decreased by 0.0022 with 95% confidence limits between 0.0006 and 0.0039, whereas for the PrD treatment the decrease was less: 0.0003 with 95% confidence limits between –0.0009 and 0.0015.

Table 4. Twenty-eight hour urinary recovery of intestinal permeability and absorption markers after an intragastrical marker dose of feed-deprived (FD) barrows or of barrows fed a starch with dextrose (StD) or protein with dextrose (PrD) diet during the first 4 d after weaning [1]

Treatment	Co-EDTA[2]	Lactulose[2]	L-rhamnose[2]	D-xylose	3-*O*-methylglucose
			%		
FD	8.9 (7.4-10.7)	1.5[a] (1.0-2.3)	16.1 (10.3-23.3)	44.4 (38.4-50.7)	72.8 (61.3-85.2)
StD	8.7 (7.1-10.5)	0.7[b] (0.4-1.0)	17.8 (11.5-25.4)	40.6 (34.9-46.6)	69.7 (58.5-81.9)
PrD	7.4 (5.5-10.2)	1.2[ab] (0.6-2.2)	8.4 (2.8-16.9)	45.4 (39.4-51.8)	68.9 (57.8-81.0)
P-values					
Treatment	0.62	0.002	0.09	0.51	0.88
Treatment × Energy[3]	0.09	0.049	0.41	0.78	0.46

[1]Data are LS-means with 95% confidence limits between parentheses, $n = 17$. Means in columns without a common letter differ, $P < 0.05$.
[2]Estimated LS-means for an energy intake of 1120 kJ/d for the StD and PrD treatments and 0 kJ/d for the FD treatment.
[3]Probability for testing H0 that slopes for the StD and PrD treatment are not different.

Discussion

The feed deprivation for 4 d in the FD pigs from the current study mimicked the extreme situation of starvation, which is a condition experienced after weaning by some pigs under current practices. The objective of this study was to determine the effect of luminal protein and dextrose or starch and dextrose supplementation compared to feed deprivation on intestinal function and mucosal weight after weaning. In order to minimise positive effects of systemic energy status on intestinal function, the ME intake of the StD and PrD treatment groups, during the first 4 d after weaning was limited to 75% of the maintenance requirements for a pig weighing 6.5 kg (1410 kJ·pig^{-1}·d^{-1})[(236)]. Research with pigs and heifers suggests that at least 25% of maintenance energy is required to maintain basal intestinal functions[(237,238)]. For the PrD treatment group, crude protein (CP) intake was limited to 50 g·pig^{-1}·d^{-1} during the first 4 d of the experiment, which is a level similar to the CP intake from sow milk just before weaning[(239,240)]. Protein requirements for maintenance is 4 g·pig^{-1}·d^{-1} [(236)] for a pig weighing 6.5 kg. However, more protein may be required for a fully active intestine. Actual energy intake for the first 4 d after weaning was 38% of the maintenance requirements for the PrD pigs and 60% of maintenance for the StD pigs. For the PrD treatment group, CP intake during the first 4 d after weaning was 26 g·pig^{-1}·d^{-1}. Thus, both protein and energy intake for the PrD treatment group were higher than the amounts

hypothesised to be needed to maintain intestinal function and architecture. Rice starch was chosen as the main energy source in the StD treatment group because it is highly digestible in pigs after weaning and does not cause diarrhoea problems[241-245]. Hydrolysed wheat protein was chosen as the protein source because of its high digestibility and its high glutamine content, which can be used as energy by the intestine[232]. The wheat protein was supplemented with crystalline AA to yield a level sufficient for protein maintenance and was sufficient to provide 36 g of protein accretion per day[246]. The crystalline AA was supplemented in order to prevent an AA imbalance and the subsequent negative effects on intake. Body weight at 4 d after weaning was higher for the StD pigs than for the FD pigs, because the FD pigs had to release their entire energy requirement from their body reserves. In addition, when energy intake was corrected to the same level for the StD and the PrD pigs, the BW of the PrD pigs was higher than of the StD pigs. This probably relates to the fact that the PrD treatment enabled some protein deposition.

During the last few decades, several authors have shown that the weaning of pigs is associated with mucosal atrophy, which is most pronounced at low feed intake levels[23,31,32]. In agreement with these findings, the relative mucosa weights of the FD pigs at 4 d after weaning was reduced by 40% compared to the weight at time of weaning. With the StD treatment, the relative mucosa weight at 4 d after weaning was 30% lower than at the time of weaning, but was not significantly different from the FD treatment group. The PrD diet caused a 21% lower mucosa weight at 4 d after weaning than at the time of weaning. In agreement with our hypothesis, although not significant, the loss of mucosal weight after weaning was 10% less when protein was included in the diet instead of starch, regardless of the lower energy intake levels in the PrD treatment group. However, energy intake did not show a significant relationship with mucosa weights within treatment groups. These findings are in contrast with the literature. For example, Berkeveld *et al*. showed a positive correlation between villous height at 2 d after weaning and feed intake level after the time of weaning[247]. In that study, the feed intake levels of pigs after weaning varied roughly between 30 and 300% of energy maintenance requirements. In the present study, energy intake was restricted to a maximum of 75% of energy maintenance. In the study of Berkeveld *et al*.[247], the correlation between villous height and feed intake seemed to be most pronounced at high feed intake levels. Hence, the relationship between villous height and feed intake may not hold at low feed intake levels. This may explain the discrepancy between the present study and the literature. One can hypothesise that at feed intake levels below maintenance requirements, villous growth has a lower priority than other biological processes, and therefore is not stimulated by energy supply. In mice, muscularis weights were lower with parenteral nutrition compared with enteral nutrition[248]. In the current experiment, the muscularis weights at d 4 were lower for the StD and PrD pigs than for the FD pigs. Based on the literature, the opposite was expected, and there is no explanation for this discrepancy.

Previous studies with Ussing chambers showed that intestinal barrier function is compromised in pigs after weaning[14,19,188]. Moreover, Verdonk[26] showed that intestinal barrier function is more effective at higher feed intake levels after weaning. However, in another study, Verdonk *et al.*[91] found no effect of the feed intake level on intestinal barrier function. It was shown previously that the *in vivo* permeability for lactulose (a disaccharide crossing the epithelial barrier by paracellular transport) is increased after weaning[249]. In addition, the permeability for lactulose was negatively correlated with the feed intake level[249]. In humans and rats, lactulose is considered a marker for small-intestinal

permeability because it is fermented in the large intestine[185]. In pigs, it may be mainly a marker for the permeability of the proximal small intestine, because at the distal small intestine, 78% of the ingested lactulose has already been fermented[250]. The present study showed that urinary lactulose recovery at 3 d after weaning was lower for the StD than for the FD pigs. This shows that energy from starch and some dextrose improves intestinal barrier function compared with FD pigs. In contrast to our hypothesis, lactulose recovery of the PrD pigs was not different from that of the FD pigs. Thus, the administration of protein with some dextrose did not improve intestinal barrier function, even though lactulose recovery of the PrD treatment group was corrected to the same energy intake level as the StD treatment group. Lactulose recovery was more effectively reduced by dietary starch intake than by dietary protein intake, as is suggested by the energy intake × treatment interaction for the StD and PrD treatment groups. This may relate to the finding that dietary carbohydrates are more effective stimulators of small-intestinal blood flow than protein[251]. Such an increased blood flow improves nutrient supply, but it especially improves oxygen supply to the small intestine, which may have been an important factor in the improved barrier function with the StD diets. Co-EDTA is a molecule with a similar size as lactulose and is also transported over the intestinal epithelium by paracellular transport. In contrast to lactulose, it is not degraded by bacterial fermentation, and therefore it is a marker for both small-intestinal and large-intestinal (colon) permeability[185]. In contrast to lactulose recovery, Co-EDTA recovery was not affected by the StD or PrD treatments. This suggests that dietary carbohydrate or protein supply at the tested levels has little effect on hind gut barrier function. L-Rhamnose absorption was also not affected by the StD or PrD treatments after weaning. In agreement with this, it was previously concluded that passive absorption is not affected by feed intake level after weaning[219]. The absorption of D-xylose was not different between treatments. In line with this, 2 other studies that assessed D-xylose absorption in pigs showed that it was not affected by feed intake level after weaning[23,30]. In the present study, 3OMG absorption (a glucose analogue) was not affected by the different treatments. This indicates that active absorption was not affected by feed intake level after weaning. Another study has shown that active glycylsarcosine (a dipeptide) absorption increases at low feed intake levels after weaning[26]. The discrepancy between the results of our study and the previous study with respect to active absorption may relate to the fact that electrolytes were supplied through the liquid 'diets' for all treatment groups. Thus, StD and PrD have no effect on active absorption after weaning, but electrolyte intake may be important in this respect.

This study showed that the combination of dietary protein and dextrose significantly stimulates an increase in small-intestinal mucosal weight 4 d after weaning, but this was not found with dietary starch in combination with dextrose. On the other hand, dietary protein has no substantial effect on small-intestinal barrier function, whereas dietary starch improves small-intestinal barrier function. Neither protein nor starch had a substantial effect on active absorption of a glucose analogue. These findings suggest that at low feed intake, dietary protein concentrations after weaning are of minor importance for small-intestinal barrier function.

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General Discussion

Introduction

Meat type pigs and chickens (broilers) are used to produce meat at low costs. Nutrient requirement studies conducted on these animals have largely overlooked the very young animal (i.e. the first weeks after hatch or after weaning). This is most likely due to the fact that the feed costs during this phase of animal growth are relatively low compared to the total feed costs from hatch/weaning to slaughter. It is therefore logical that there are still gaps in our knowledge of the nutrition of these animals during this particular stage of life. Therefore, it is likely that performance of these meat type animals at a very young age can be further improved. Moreover, during this phase in the animal's life, the physiological, nutritional, and environmental changes are associated with stress, which makes the animal vulnerable to diseases. The physiological changes include changes in small-intestinal architecture, weight, and function. The fundamental functions of the small intestine are to ensure nutrient absorption, to provide a barrier function against potential harmful bacteria and toxins, and to contribute to overall health as the biggest immunological organ in the body[24,52]. As such, many health problems can occur and may originate during this phase of life when the small-intestinal function is still developing. Therefore, nutrient requirement studies during these phases in broilers and pigs should not only focus on the aspects of efficient production, but should also pay attention to the development of the different organs and their functionality.

The feed intake of pigs shortly after weaning is highly variable and rather unpredictable. It has been reported that 10% of pigs do not take in any feed during the first 48 h after weaning[16]. The optimal nutrient composition of the diet depends very much on the feed intake level of the animals, due to differences in optimal dietary nutrient composition for maintenance and for gain. Feed for gain requires much higher protein concentrations and a different amino acid (AA) profile than feed for maintenance. Thus, after weaning, the dietary composition for pigs with a low feed intake level should mainly support the maintenance processes, whereas for pigs with high intake levels, this should also support protein accretion. Therefore, during this phase of life, the stimulation of feed intake is more important with respect to performance and uniformity of the animals rather than the precise nutritional composition of the diet. Under both maintenance and high production conditions, the small intestine is one of the most important organs in the body. At high production levels, the small intestine has to facilitate sufficient nutrient transport to the body, and under maintenance conditions, it is the most demanding organ with respect to AA and energy consumption for integrity support. Consequently, the research conducted as part of this thesis focused on the most important nutrients for pigs at a low feed intake level to particularly support the physiological functions of the small intestine.

In the experiments described in this thesis, the effect of protein nutrition and feed intake level on performance, intestinal development, intestinal (dys)function, and health were investigated in both piglets after weaning and in young broilers. In this General Discussion, the conclusions from previous Chapters will be aligned and discussed in the context of optimal early life nutrition. At the end of this Chapter, practical implications and suggestions for further research are presented based on the studies reported in this thesis.

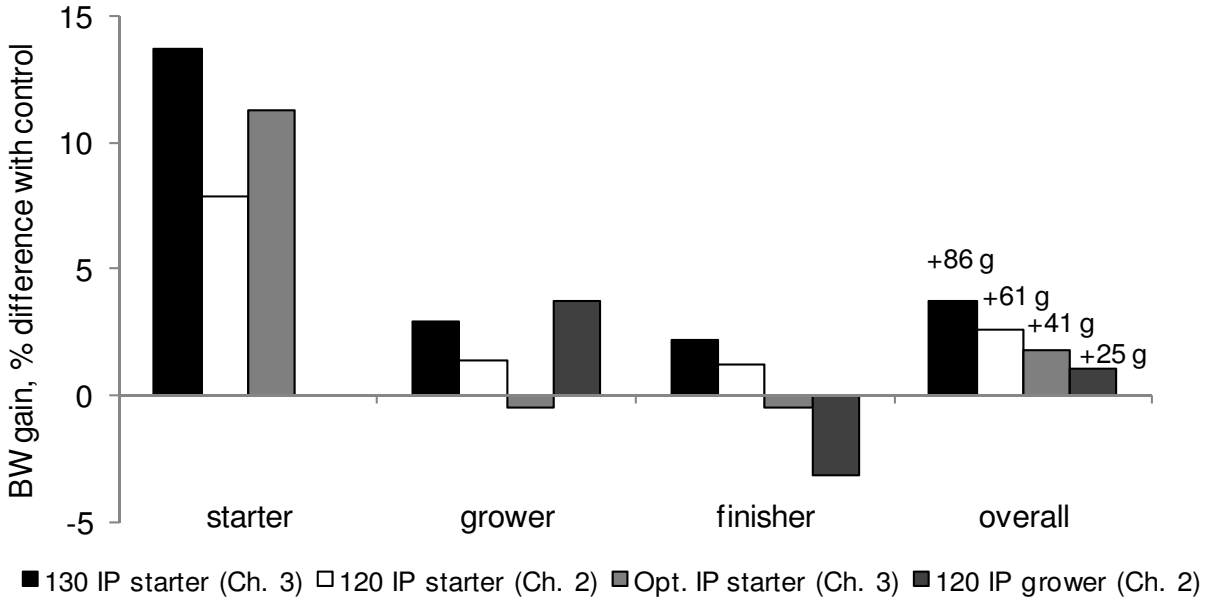

Figure 1. Results of enhanced dietary ideal protein (IP) concentrations in starter and grower diets on BW gain of male Ross 308 broilers from Chapter 2 and 3 are presented in the graph.

Nutrition and performance of broilers

Nutrition is 1 of the major factors that drive the production level of farm animals. For several decades, scientists and nutritionists have conducted studies to determine the most economic concentration of several nutrients in the diet. Energy and AA are the 2 most important components with respect to the costs of the diet. Amino acids are special nutrients because of their important and diverse functions in the animal. First, they are the main building blocks for cells and functional proteins (e.g. hormones, signal molecules) for all types of processes in the body. Second, they are used as an energy source for metabolic processes in the body. In the latter function, AA are the major energy source for enterocytes[230]. Third, AA are precursors for the *de novo* synthesis of special molecules (e.g. nucleotides, creatine, and some vitamins). Today, the requirements for AA by pigs and poultry are studied according to the ideal protein (IP) concept. With this concept, AA requirements are often expressed as a ratio to lysine, which leads to an ideal AA profile where all indispensable AA are equally limiting. This approach was first implemented in pigs[103,104]. In general, protein gain, but also BW gain and gain to feed ratio (G:F), are the main decision parameters for nutritionists to estimate the optimum profile or the optimal dietary IP concentration. In this thesis (**Chapter 2**), the AA requirements to maximise BW gain and G:F in broilers were studied. Instead of varying AA in the diet, an optimum IP concentration in the diet was established. This was done by increasing all AA containing ingredients at the expense of an iso-caloric protein free mixture. Consequently, the ratios among all AA were similar in all diets, and dietary protein concentrations were increased to the same extent as the concentrations of indispensable AA. From the results in **Chapter 2**, it can be concluded that the effects of enhanced IP concentrations in the starter phase are more pronounced than the effects of enhanced IP concentrations in the grower and finisher phase (Figure 1). In addition, enhanced IP concentrations during the starter phase increased BW gain not only during the starter phase, but also during the consecutive grower phase (**Chapter 2 and Chapter 3**). This positive carry-over effect on BW gain found here is not always found in the literature. Some data from previous studies have shown that the

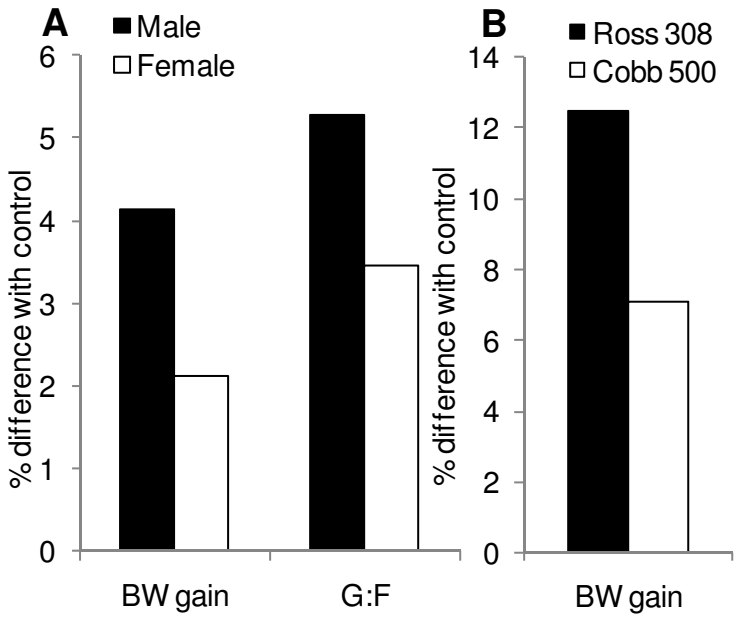

Figure 2. Differences in BW gain and gain to feed ratio (G:F) responses to 120 IP grower diets between male and female broilers in the grower phase are given in panel A (Exp. 1B of Chapter 2). The difference in BW gain response to enhanced ideal protein (IP) concentrations in the starter diet (average of 130 IP and OPT IP treatment, Chapter 3) between Ross 308 and Cobb 500 male broilers in the starter phase is given in Panel B. BW gain and G:F responses to enhanced IP concentrations in the starter phase are similar for male and female broilers (Exp. 1A Chapter 2).

positive effects on BW gain in the starter phase fades in the grower phase[163], indicating that the circumstances (e.g. ambient temperature or bacterial challenge) during the grower phase itself may determine whether the effects on BW gain persist or fade during the grower phase. Nevertheless, high IP concentrations in the diet of broilers during the starter phase can be attractive due to the small share of the starter diet in the total feed costs of a broiler up to slaughter weight. Responses to enhanced dietary IP concentrations in the starter diet and the carry-over effects during the grower phase in male and female broilers were nearly identical. However, G:F and BW gain responses to enhanced dietary IP concentrations in the grower diet of females are less marked than of males (Figure 2). Thus, the results of the experiments reported in **Chapter 2** showed that IP concentrations in starter diets are of particular interest for both males and females from a performance and economic point of view, and need to be evaluated in more detail. Based on these findings, the experiment reported in **Chapter 3** was designed. **Chapter 3** describes a study in which feed intake, BW gain, and G:F in the starter phase were monitored over short intervals in 2 different breeds (Ross 308 and Cobb 500). In addition, for 1 treatment group during the starter phase, the dietary AA composition was adjusted daily using calculated AA requirements. The results showed that the birds hardly responded in terms of BW gain to dietary IP during the first 3 d after hatch. However, over the next 3 consecutive days, BW gain improved substantially with enhanced dietary IP concentrations. In addition, increasing dietary IP concentrations by 30% improved G:F during the first 3 d after hatch by only 8%, whereas over the next 3 consecutive days, G:F improved by 20% with this treatment. Thus, the results of the experiment reported in **Chapter 3** showed that with respect to the fine tuning of the diet, one may consider the first 3 d after hatch as a separate period in order to maximise performance and nutrient efficiency. This may relate to 1) the fact that the yolk-sac is still being utilised during this period, 2) the relative feed intake level during the first 3 d after hatch is lower than in the subsequent 3 d, and 3) birds are still partly poikilotherm[252]

right after hatch, and therefore their physiological state is different than later in life. The protein supply from the yolk-sac during the first 3 d after hatch can be estimated from the weight development of the yolk-sac after hatch in the preliminary study reported in **Chapter 3** and the composition of the yolk-sac at hatch described by Yadgary *et al.*[253]**.** These calculations determined that during these 3 d, a total of 0.8 g protein was supplied by the yolk-sac. This is about 11% of the total dietary protein intake of the control treatment (average of Ross 308 and Cobb 500 birds) during these first 3 d of the trial. Similarly, the total energy supply from the yolk-sac during these 3 d was calculated to be 10% of the dietary energy intake. Thus, the protein to energy ratio in the yolk and in the diet is similar during this time, which indicates that the protein release from the yolk-sac is probably not a large influence with respect to the lower BW gain and G:F responses to enhanced dietary protein concentrations for d 0–3 vs. d 3–6 after hatch. Sakamuro *et al.*[254] estimated that the maintenance energy requirement of broilers was 532 kJ metabolisable energy/$kg^{0.75}$. From d 0–3 after hatch, energy intake from the diet plus energy utilised from the yolk sac was 2.3 times maintenance, and from d 3–6 the energy intake increased to 2.7 times maintenance. The lower energy intake from d 0–3 compared to d 3–6 must have limited the availability of energy for protein deposition. This may be why the broilers did not improve BW gain with enhanced dietary protein concentration. In addition, the lower response for d 0–3 may relate to a different metabolism of the birds right after hatch. For instance, in the first few days after hatch, birds are still in the process of changing from a partly poikilothermic to a homeothermic metabolism. Moreover, it has been shown that the mechanism for protein degradation is different between fish (poikilothermic) and homeothermic mammals[255]. Fish have a low protein turnover (high efficiency of protein deposition), but at the same time have a high rate of protein catabolism, because protein is used to meet their energy requirements[256]. Similarly, it can be hypothesised that shortly after hatch, protein deposition of partly poikilothermic broilers may be more efficient than later in life, and excess dietary protein will be catabolised. Consequently, enhanced dietary protein concentrations show only minor effects on BW gain and G:F shortly after hatch. With an extremely high relative BW gain (12% higher than the control group), the basis for the improved performance with enhanced dietary IP concentrations, is established during 3–6 d of age (Figure 1, **Chapter 3**). However, the experiment reported in **Chapter 3** also showed that higher IP concentrations have to be continued until at least 14 d of age in order to achieve the maximum performance improvement. This was illustrated with the optimised IP treatment, in which the dietary AA concentrations were gradually decreased after 3 d of feeding. The effect on BW gain was clearly less pronounced at the end of the starter phase with this optimised treatment than when enhanced IP concentrations were continued until the end of the starter phase with the 130 IP treatment. In agreement with these findings, Lemme *et al*.[163] showed that 14 d of enhanced dietary protein concentrations clearly improved BW gain. This effect was much less pronounced when feed with increased protein concentrations was only fed until 8 d of age[163]. Finally, **Chapter 3** showed that Cobb 500 broilers responded differently to enhanced IP concentrations than Ross 308 broilers with regard to feed intake, BW gain, and G:F. Thus, this thesis showed that in the starter phase, male and female chicks respond similar to dietary IP concentrations, but there are differences in responses between breeds, which advocates for breed specific recommendations in the starter phase.

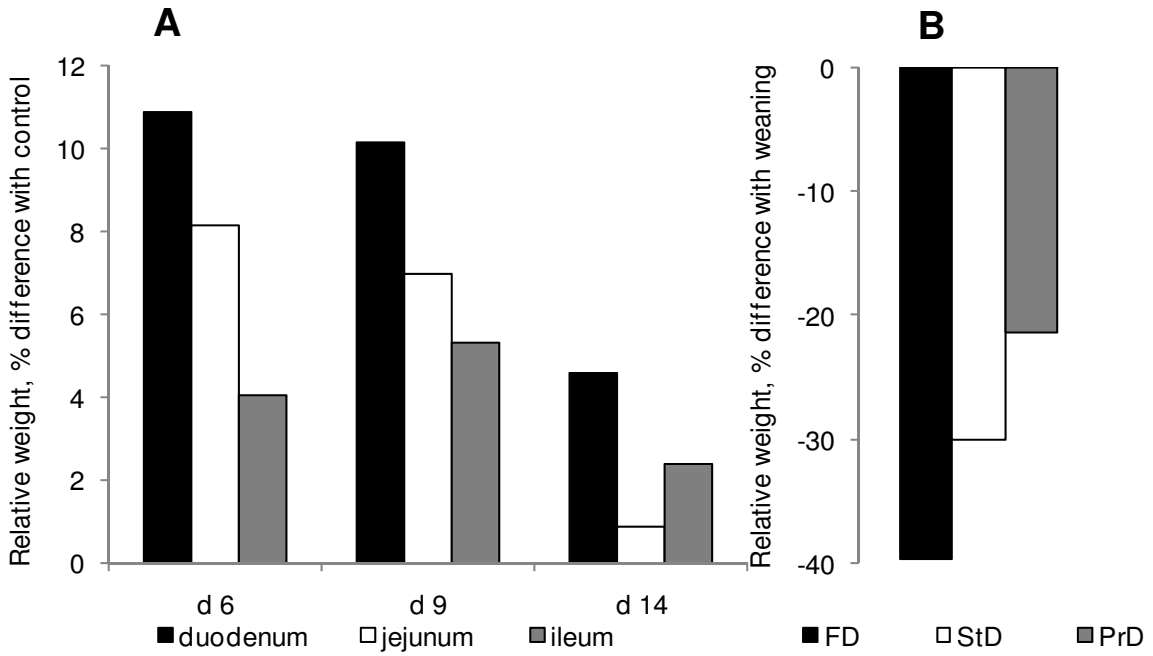

Figure 3. Effects of enhanced dietary ideal protein (IP) concentrations in the starter diet (average of 130 IP and OPT IP treatment, Chapter 3) on relative small-intestinal weights of Cobb 500 and Ross 308 broilers at different days after hatch (Panel A). Effects of feed-deprived (FD) vs. starch with dextrose (StD) or protein with dextrose (PrD) fed weaner pigs on the loss of small-intestinal mucosa weight at 4 d after weaning compared with weaning (Panel B).

Nutrition and the small intestine

In **Chapter 1**, it was shown that the small-intestinal architecture changes considerably after hatch in broilers and around the time of weaning in piglets. In broilers, both the relative small-intestinal weight and villous height increase rapidly during the first week after hatch[38,61,67-71,75,76]. After this week, the relative weight decreases gradually, but the villous height continues to increase[38,61,63,67-71,74]. At 4 d after weaning, villous height in pigs decreases to about 60% of the pre-weaning height[26,31,86,89,90]. Two weeks after weaning, villous height recovers to similar values as in unweaned control animals independent of the weaning age (**Chapter 1**).

In **Chapter 3**, the data showed that enhanced IP concentrations in broiler starter diets (130 IP and OPT IP) increased the relative small-intestinal weight at 6, 9, and 14 d of age (Figure 3A). These effects were more pronounced at 6 and 9 d of age than at 14 d of age. Moreover, these effects were more pronounced in the duodenum than in the jejunum and ileum. These results are in line with the review of the literature (**Chapter 1**), which showed that the small intestine is sensitive to changes in dietary protein concentrations, especially in young broilers. The increased small intestine weights in the birds treated with enhanced dietary IP concentrations coincided with an increased BW. In a Pearson correlation test, empty BW of individual birds at d 6 correlated with relative small-intestinal weight ($r = 0.49$, $P < 0.001$). However, at 9, 14, and 36 d of age, empty BW showed no significant correlation with relative small intestine weight. This discrepancy between d 6 and the other days may relate to the fact that at d 6, the birds are in a developmental stage at which the relative small intestine weight is still increasing. Above 6 d of age, the birds are at a developmental stage where the relative small intestine weight is at the maximum level or is gradually decreasing. Therefore, it can be hypothesised that at d 9, 14, and 36 the heaviest birds are

in a more advanced stage of development compared to the lighter birds at the same age, which coincides with a lower relative small intestine weight. In agreement with these findings, relative small intestine weight correlated with the empty BW at 3 d of age (r = 0.66, $P < 0.001$) in the pilot experiment reported in **Chapter 3**, and did not correlate with empty BW at the other days of dissection. These data underscore the importance of the development of the small intestine in young broilers for maximising BW gain. However, it is not possible to conclude whether the enhanced dietary IP concentrations that were used (**Chapter 3**) stimulated small-intestinal development with a subsequent effect on BW gain, or whether BW gain has stimulated the small-intestinal development. The experiment described in **Chapter 3** lacked parameters that measured small-intestinal function. Further studies should measure the digestibility of the diet and the expression of nutrient transporters after a starter phase with enhanced dietary IP concentrations. These parameters should show whether an enhancement in small intestine weight coincides with improved small intestine digestive function, and will also clarify the role of the small intestine in the improvements in BW gain seen with enhanced dietary IP concentrations.

In **Chapter 6**, studies were conducted in pigs that were either deprived of feed for the first 4 d after weaning or were fed a restricted starch-dextrose (StD) or protein-dextrose (PdD) diet. In the deprived pigs, mucosal weight at 4 d after weaning was reduced by 40% compared to day of weaning, whereas the reduction was less for the StD and PrD fed pigs (30 and 21%, respectively; Figure 3B). These results are in line with previous studies in the literature (**Chapter 1**) that have shown that protein is the most important nutrient for to development of small-intestinal weight and villous height. In the experiment described in **Chapter 6,** mucosal weight was measured instead of villous height. The limitation of this approach is that a higher mucosal weight cannot explicitly be viewed as a positive effect, because aside from increased villous heights, inflammatory responses can also increase the weight of the small-intestinal mucosa. However, in general a decrease in mucosa weight before and shortly after weaning is associated with decreased villous height as well[31,88,89]. The experiment in **Chapter 6** showed that only half of the mucosal loss was prevented by PrD intake, whereas the protein intake was assumed to be sufficient for the prevention of the loss of the entire mucosa. The energy and protein intake of the pigs that received the PrD treatment in **Chapter 6** were only 15% and 40% of a normal pre-weaning intake[239], respectively. This indicates that in addition to luminal nutrient supply the systemic nutrient status is also important for the recovery or prevention of mucosal atrophy. In line with this, it was previously shown that the net protein retention in the portally-drained viscera during 6 h after a single meal was increased in pigs when maltodextrin was fed in addition to a purified protein diet, which resulted in a 4-fold increase in total energy intake[257]. Thus, at a constant protein supply but with a higher (systemic) energy supply in animals, the protein accretion in the small intestine is increased. In a study with laying hens, feed deprivation and parenteral nutrition reduced duodenal villous height to the same extent[258]. This indicates that systemic nutrient status has no effect on mucosal atrophy. However, in 3 wk old pigs, both feed deprivation and parenteral feeding reduced portal blood flow by about 40% compared to enteral feeding[259], and in addition, parenteral nutrition resulted in mucosal atrophy[201,259]. These authors suggested that the parenteral nutrition indirectly induced mucosal atrophy due to the decreased systemic nutrient and oxygen supply to the small intestine as a result of the decreased blood flow[259]. This hypothesis is supported by the finding that during feed deprivation the mucosal microcirculation of blood is reduced[251], indicating that luminal nutrients not only have a direct stimulating effect on small-intestinal

development by luminal nutrient supply, but also an indirect effect through the stimulation of systemic nutrient and oxygen supply to the small intestine. According to De Aguilar-Nascimento, dietary lipids and sugars are effective stimulators of blood flow to the small intestine, whereas most protein and peptides only have a mild stimulating effect on intestinal blood flow[251]. Therefore, the PrD treatment in the experiment reported in **Chapter 6** may have been less effective in stimulating intestinal blood flow than the StD treatment.

In conclusion, based on the experiment detailed in **Chapter 6**, the luminal supply of protein with dextrose seems to only prevent 50% of the total mucosal atrophy that occurs due to feed deprivation after weaning. A luminal supply of carbohydrates only prevented 25% of the mucosal atrophy. This effect of carbohydrates may be mainly related to the stimulating effect that luminal carbohydrates have on intestinal blood flow, and the subsequent systemic nutrient and oxygen supply to the small intestine. Both protein and carbohydrates (or energy) are most likely required in sufficient quantities to fully prevent mucosal atrophy after weaning. The results reported in **Chapter 3** further indicate that small-intestinal weight is important for BW gain. However, this study could not clarify whether an increase in small-intestinal weight could also improve the digestive capacity of the small intestine.

Nutrition, small-intestinal (dys)function and health

The growth rate of broilers has increased considerably over the last few decades. As a side effect, high producing broilers have become more susceptible to metabolic disorders than in the past[5-7]. It has been suggested that early embryonic[9] or post-hatch[10] organ development is important for growth and for organ functioning. In **Chapter 3**, it was hypothesised that this development of organs early in life may be related to metabolic disorders later in life. In this regard the development of the small intestine may be the most critical aspect, because the relative weight of this organ increases from 2 to 8% of the BW from 0–8 d of age[22].The increase in small-intestinal weight is much higher than of any other organ during this phase of life. It was hypothesised that small-intestinal development would be important for broiler survival. However, the experiment shown in **Chapter 3** indicated that there was no relationship between small-intestinal weight development early in life and mortality in broilers. In **Chapter 3**, it was shown that feed intake restriction early in life reduced the mortality due to leg problems later in life (Figure 4). In addition, enhanced dietary IP concentrations decreased mortality due to leg problems in Ross broilers but not in Cobb broilers (Figure 4). In the literature, it has been stressed that the growth reduction of broilers early in life, in particular, is very effective for reducing metabolic disorders[8]. However, in Ross broilers shown in **Chapter 3**, BW gain was increased in the enhanced IP treatments, whereas the mortality due to leg problems decreased. This suggests that the decrease in BW gain does not directly prevent metabolic disorders; however, it may be a physiological side effect of the reduced BW gain or the feed intake restriction that causes the reduction in metabolic disorders. An interesting observation of the study discussed in **Chapter 3** (data not shown) was that feed intake restriction reduced the relative spleen weight at 14 d of age compared to the control treatment (2.1 g/kg $BW^{0.75}$ vs. 1.5 g/kg $BW^{0.75}$; $P < 0.05$). The spleen is an immunologically active organ. The reduced spleen weight with feed intake restriction suggests that feed intake restriction suppresses immune reactivity. In agreement with this, others have shown that feed intake restriction modifies immune parameters in broilers[260-264]. In the human nutrition field, it has been shown that the

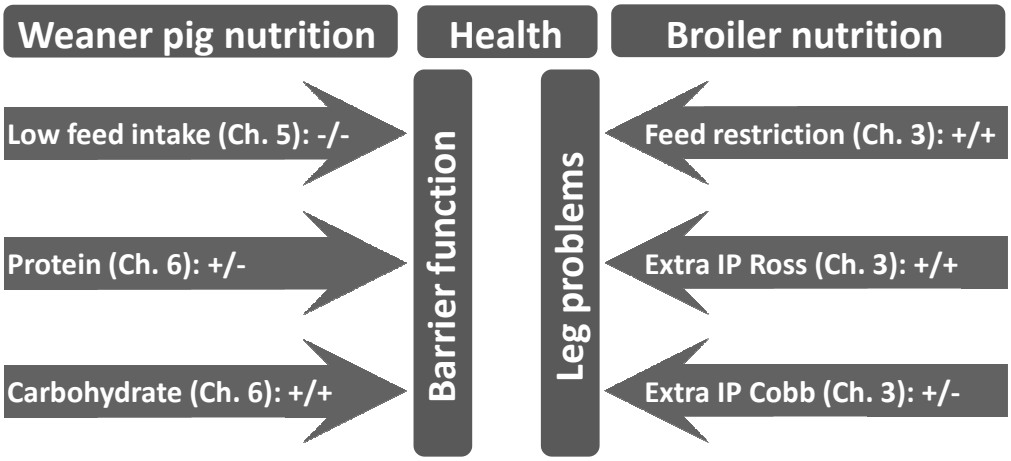

Figure 4. A scheme illustrating the effects of nutrition in Chapters 3, 5, and 6 on small-intestinal paracellular barrier function in pigs after weaning and on leg problems of broilers. The effects represent either positive health effects (+/+), negative health effects (-/-), or no health effects (+/-). In addition, in Chapter 5 it was shown that paracellular barrier function is affected by feed intake level after weaning, but this does not correlate with transcellular barrier function.

immune system and inflammatory responses have an important role in metabolic diseases, such as type 2 diabetes and atherosclerosis[265]. Similarly, it can be hypothesised that in broilers, metabolic disorders, such as ascites and lameness, are related to the negative side effects of inflammatory responses. Thus, the effect of feed restriction on the immune system may be a crucial factor that suppresses mortality due to ascites and leg problems. In addition, metabolites from AA catabolism in mammals have functional properties to control the immune system[266]. This may explain why enhanced dietary IP concentrations, which certainly resulted in higher protein catabolism, were associated with less mortality due to leg problems in Ross broilers (**Chapter 3**). The weak aspect in this suggestion is that the enhanced dietary IP concentrations in **Chapter 3** showed no reduction in the incidence of leg problems in Cobb broilers. Thus, high dietary protein concentrations may be beneficial for leg development. However, further studies with more sophisticated measurements are required to assess whether this can be reproduced and should clarify the role of the immune system in this process.

The results of the experiment reported in **Chapter 5** showed that the paracellular barrier function of the small intestine deteriorates when pigs are weaned at 3 wk of age. These findings are in agreement with the review reported in **Chapter 4**[14,19,267]. However, an adequate feed intake level after weaning prevents the loss of intestinal barrier function[26]. Amino acids, particularly glutamine, are the major energy sources of enterocyte[231,232]. From these studies it can be deduced that the luminal AA supply is especially important for the maintenance of small-intestinal barrier function after weaning. Therefore, it was studied whether luminal protein can prevent the loss of small-intestinal barrier function after weaning (**Chapter 6**). The paracellular barrier function of the small intestine at 3 d after weaning (Figure 4) was similar for both feed deprived and PrD fed pigs. Thus, a luminal supply of protein with dextrose does not protect against the loss of barrier function after weaning. In contrast, StD intake improved paracellular barrier function of the small intestine at 3 d after weaning compared to feed deprived pigs (Figure 4). This indicates that dietary starch improves small-intestinal barrier function after weaning, and that protein has no substantial effect on small-intestinal barrier function. In the previous section it was

mentioned that luminal carbohydrates are a much more potent stimulator of intestinal blood flow than most AA[251]. Moreover, it has been suggested that sufficient intestinal blood flow is essential for mucosal barrier function[251]. Hence, the possible stimulating effect of carbohydrates on intestinal blood flow may be the reason why starch and not protein improved mucosal barrier function after weaning compared to feed deprived pigs in the experiment in **Chapter 6**. Therefore, the level of feed intake after weaning and the type of dietary nutrients are important for maintaining small-intestinal barrier function after weaning. In addition, the review presented in **Chapter 4** showed that weaning stress is a critical factor that is related to the compromised paracellular barrier function after weaning. Moeser *et al*.[18] showed that the detrimental effect of weaning stress on intestinal paracellular barrier function disappeared when mast cells were blocked. This indicates that the loss of intestinal barrier function after weaning is related to detrimental side effects of the immune system. The research reported in this thesis did not specifically focus on immune parameters. However, based on currently available data from the literature and the studies reported here, it can be hypothesised that the negative inflammatory effects of an 'uncontrolled' immune system may be an important factor, which is related to metabolic diseases in broilers and intestinal disorders in pigs. Finally, previous studies discussed in **Chapter 4** indicated that transcellular transport of macromolecules and passive transcellular absorption decreases after weaning, and this effect is not clearly age dependent. This may reflect a natural intestinal maturation process that is enhanced by the weaning process, and may protect the pig from antigen overload. When pigs are weaned at 4 wk of age, the paracellular barrier function and active transcellular absorption are clearly less affected by weaning compared to weaning at 3 wk of age, which indicates a more mature small intestine. This also suggests that these parameters are especially important when studying the effect of weaning on small-intestinal function.

Performance and health in a broader perspective

It is evident from the research reported in this thesis that broiler performance can be improved by increasing dietary AA concentrations to higher concentrations than those currently used in practical starter diets. However, this does not hold true for the first 3 d after hatch. This phase requires more detailed studies to fully understand why optimal dietary AA concentrations seem to be lower in those 3 d than afterward. If this has a physiological mechanism, it is likely that the optimal concentrations of other nutrients (e.g. fat, minerals, and vitamins) would also be affected. This should be studied in more detail during the first days after hatch in order to optimise the performance of broilers. During the last decade, several researchers have investigated the potential for improving broiler performance by early feeding. With early feeding, the chicks are provided access to feed in the hatchery within a few hours after hatch. In addition, a technique for feeding the chick while still in the egg ('*in ovo* feeding') has been developed. Both early feeding and *in ovo* feeding have shown an improvement in BW by 5-10% at market age[268]. Recently, brooding systems have been developed that enable the feeding of broilers immediately or within a very short time after hatch. In addition, the broilers can stay in those systems for several days after hatch. Thus, the development of these systems that advance feeding of broilers at an earlier age is another way to improve overall broiler performance, in addition to diet composition. Moreover, it is important to explore the nutritional requirements of the very young broiler in order to fully utilise the possibilities of these systems.

In general, an increase in BW at 1 or 2 wk of age can be expected to remain stable or even multiply by market age. However, it has been shown that an improvement in BW at the end of the starter phase sometimes fades by market age when these birds are maintained under identical circumstances as control birds after the starter phase. Understanding why an improvement in BW at the end of the starter phase remains or even increases by market age in some cases, but decreases entirely in other cases, would be a tremendous step forward in the improvement of overall broiler performance. In this regard, knowledge can be gained from analysing the factors in the realimentation period that influence compensatory growth after a period of feed or nutrient restriction. Male birds have a more pronounced ability for compensatory growth than female birds, and slow growing birds have a greater ability for compensatory growth after a period of growth restriction than fast growing birds[134]. In addition, the study reported in **Chapter 2** showed that the response of birds to enhanced dietary protein concentrations in the grower phase was more pronounced when birds had been fed low dietary protein concentrations in the preceding starter phase. In agreement with this, AA requirements are increased after feed intake restriction[161]. These 2 findings indicate that compensatory growth after a period of feed restriction or less optimal dietary protein concentrations is more pronounced when this phase is followed by a high protein diet. Other factors that may influence the effect of early life performance improvements on overall performance are the health status, climate (ventilation and temperature), and feed texture. However, there are numerous other factors that could be involved, and factors such as hatching conditions or broiler breeder flock status (age, nutritional status a.o.), for instance, cannot be excluded as variables that may have long-term effects. Future studies should aim to identify the most important factors and subsequently study and optimise them.

The work presented in **Chapter 3** of this thesis and in the literature shows that feed intake restriction early in life is a very effective way of reducing mortality that is related to metabolic disorders in broilers[6,8,269]. Birds have the potential to compensate for the reduction in BW gain, which is mediated by feed intake restriction occurring earlier in life. However, compensatory growth is unpredictable, and birds frequently do not fully recover the 'lost weight gain' by market age[134]. In addition to the potential loss in market weight, from a management point of view, it is difficult to accurately control the feed intake in broiler houses in practice. Thus, metabolic disorders in broilers should ideally be prevented by measures other than feed intake restriction. The data in this thesis show that enhanced dietary protein concentrations in Ross broilers reduce leg problems (**Chapter 3**). In this Chapter, it was previously suggested that this may be related to the possible manipulation of the immune system by metabolites from protein catabolism. Thus, the modulation of the immune system may be a way to prevent metabolic disorders in broilers. Other researchers have shown that the incubation temperature of the eggs influences the incidence of ascites in broilers later in life[270]. In addition, *in ovo* feeding or early feeding systems may potentially reduce long-term mortality and morbidity[268]. However, specific measures used to prevent metabolic disorders may be undone over the long term by changing the genetics of the birds, which can cause slight modifications to the genetic defects that are related to the disorders. Ideally, these metabolic disorders should be prevented by eliminating the genetic defects all together, and other measures should only be used until geneticists can solve these problems.

In pigs after weaning, the initial performance level (predominantly feed intake) and health are closely related. The transition from sow milk to solid feed as well as other stress

factors results in low feed intake levels (below maintenance) for the first day after weaning for nearly all pigs[271]. Thus, after weaning, it is important for both performance and health to stimulate feed intake and to give pigs the best possible nutritional support. From a nutritional point of view, pigs with no or a low feed intake can only be supported by supplementing the water with nutrients. The work reported here has shown that protein is relatively unimportant with respect to supporting the barrier function of the small intestine (**Chapter 6**). In contrast, it seems that sufficient energy intake, specifically through highly digestible carbohydrates, can at least partially prevent the loss of intestinal barrier function. Further studies should define minimum intake levels for this supportive function and should also assess the effects of other nutrients (e.g. minerals, specific AA, or specific bioactive substances). Measures taken around the time of weaning should attempt to smooth the transition from sow milk to solid feed in order to stimulate feed intake after weaning. In addition to the composition and physical form (mash, crumble, micrum, pellet, gruel) of feed, several management factors have a major influence on feed intake after weaning, including weaning age, climate, water supply, housing, light schedule. Weaning at 4 wk instead of 3 wk of age does not only improve small-intestinal barrier function, absorptive function, and architecture after weaning (**Chapters 1 and 4**), but also increases feed intake after weaning. This is nicely illustrated by the data of Brooks *et al.*[16] who showed that piglets weaned at 3 wk of age consumed hardly any feed before weaning, whereas piglets weaned at 4 wk of age consumed in excess of 100 g/d during the last days before weaning. However, increasing the weaning age limits the number of litters that can be produced per year. Combining an older weaning age with intermittent suckling can ensure that pigs have feed intake levels above maintenance immediately after weaning (5 wk of age). In addition, this prevents small-intestinal atrophy after weaning[247]. With intermittent suckling, the sow is separated from the piglets for 10 h/d for a period starting at least 1 wk before weaning. After a week, this procedure induces ovulation in the sow, which consequently reduces the numbers of days lost between farrowings. However, in practice this is a very labour intensive system. Therefore, the current procedure of weaning at 4 wk of age with high quality diets seems to be the most realistic and practical solution. Combining this approach with nutritional support in the drinking water for pigs with a low feed intake may be the best solution to alleviate potential weaning problems. This is the best compromise for a smooth weaning process and an economical method of farming. Weaning pigs at an older age than 4 wk is currently not realistic from an economic point of view, but may be enforced in the future.

Suggestions for further study

Based on the findings of this thesis, 3 areas are suggested for further study. First, it is important to improve our understanding of the physiological nutrient needs in broilers during the first 6 d immediately after hatch. These needs seem to change markedly over a short time frame, and few studies have explored this area. Second, the role of the detrimental effects of an over or incorrectly stimulated immune system on animal health should be investigated in both young broilers and pigs after weaning. Third, the impact of optimal nutritional support through drinking water to pigs after weaning with no or a low feed intake should be investigated in more detail. With this respect, in this thesis research was initiated to explore the role of carbohydrate and protein.

Conclusions

The objective of this thesis was to improve small-intestinal development and performance of pigs after weaning and young broilers by optimising the nutrient composition of the diet.

The first aim was to gain more insight into the anatomical and functional changes of the small intestine after hatch in broilers and after weaning in pigs.

The literature review presented in **Chapter 1** showed that villous heights increase with age in broilers and decrease with age in pigs, indicating a more efficient nutrient absorption over time in broilers. In broilers during the first week after hatch, high duodenum weights coincide with a high BW gain (**Chapter 3**), indicating that the small-intestinal development facilitates a high BW gain. However, from the results in the current thesis, it cannot be concluded whether higher duodenum weights cause higher BW or are the result of a higher BW. Paracellular barrier function deteriorates in pigs after weaning (**Chapter 4** and **Chapter 5**). In line with this, bacterial translocation also increased over time after weaning (**Chapter 5**). However, paracellular barrier function after weaning did not correlate with transcellular barrier function for macromolecules and with bacterial translocation (**Chapter 5**). Transcellular barrier function improves and passive transcellular absorption of the small intestine decreases in pigs after weaning. This suggests that weaning enhances the maturation process of the small intestine (**Chapter 4**). Therefore, in general the disturbance of the barrier function after weaning is restricted to tight junctions, and transcellular barrier function may even be improved after weaning. This thesis provides insight into both the anatomical and functional changes of the small intestine after weaning in pigs. However, this thesis does not provide clear insight into the functional changes of the small intestine after hatch in broilers.

The second aim was to investigate the effect of the level of feed intake and the effect of protein nutrition on performance, small-intestinal development, and health in broilers after hatch and pigs after weaning.

It was shown that the effects of enhanced IP concentrations in the starter diet on BW gain of broilers are more prominent than the effects of enhanced IP concentrations in the grower and finisher diets (**Chapter 2**). Moreover, a more detailed study of the starter phase showed that G:F and BW gain responses to enhanced dietary IP concentrations during the first 3 d after hatch were substantially lower than in the consecutive 3 d (**Chapter 3**). This highlights the importance of studying the physiological requirements during these first 3 d after hatch in more detail in future studies (**Chapter 3**). Mortality in relation to leg problems was markedly decreased due to feed intake restriction early in life (**Chapter 3**). This has been previously shown in the literature, and other researchers have assumed that the reduced mortality relates to a reduction in metabolic rate early in life. However, as shown in the data in **Chapter 3**, enhanced dietary IP concentrations fed to Ross 308 broilers increased BW gain early in life, and thus increased the metabolic rate of the broilers while concomitantly decreasing mortality due to leg problems. This suggested that a physiological mechanism other than metabolic rate is involved in the reduced observed reduction in mortality. The research reported in this thesis shows that dietary protein is a potent stimulator for growth of the proximal small intestine in broilers and of the intestinal mucosa in pigs (**Chapter 3** and **Chapter 6**). Adequate feed intake levels after weaning prevent the loss of paracellular barrier function of the small intestine (**Chapter 4**). Although mucosal mass of the small intestine in pigs was enhanced by dietary protein, it did not support the

maintenance of paracellular barrier function of the small intestine after weaning (**Chapter 6**). This shows that mucosal mass and luminal protein is of minor importance for intestinal barrier function in pigs after weaning (**Chapter 6**). In contrast, luminal carbohydrate supply or energy level is important for maintaining small-intestinal barrier function (**Chapter 6**). Thus, this thesis provides substantial insight into the nutritional requirements needed to optimise performance, small-intestinal function, and health in broilers after hatch and in pigs after weaning. In addition, this thesis has identified areas that will require more detailed study with respect to the nutrition of pigs after weaning and broilers after hatch.

References

1. Arthur JA & Albers GAA (2003) Industrial perspective on problems and issues associated with poultry breeding. In *Poultry Genetics, Breeding and Biotechnology*, pp. 3-12 [WM Muir and SE Aggrey, editors]. Cambridge: CABI Publishing.
2. Merks JWM (2000) One century of genetic changes in pigs and the future needs. In *The Challenge of Genetic Change in Animal Production*, pp. 8-19 [WG Hill, SC Bishop, B McQuirk, JC McKay, G Simm and AJ Webb, editors]. Edinburgh: Br. Soc. Anim. Sc.
3. Havenstein GB, Ferket PR & Qureshi MA (2003) Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. *Poult Sci* **82**, 1500-1508.
4. Jensen P (2010) Domestication, selection, behaviour and welfare of animals - Genetic mechanisms for rapid responses. *Anim Welf* **19**, 7-9.
5. Deeb N, Shlosberg A & Cahaner A (2002) Genotype-by-environment interaction with broiler genotypes differing in growth rate. 4. Association between responses to heat stress and to cold-induced ascites. *Poult Sci* **81**, 1454-1462.
6. Julian RJ (2005) Production and growth related disorders and other metabolic diseases of poultry - A review. *Vet J* **169**, 350-369.
7. Moghadam HK, McMillan I, Chambers JR & Julian RJ (2001) Estimation of genetic parameters for ascites syndrome in broiler chickens. *Poult Sci* **80**, 844-848.
8. Baghbanzadeh A & Decuypere E (2008) Ascites syndrome in broilers: physiological and nutritional perspectives. *Avian Pathol* **37**, 117 - 126.
9. Schmalhausen I (1930) Das Wachstumsgesetz als Gesetz der progressiven Differenzierung. *Wilhelm Roux Arch Entwickl Mech Org* **123**, 153-178.
10. Ricklefs RE (1987) Comparative analysis of avian embryonic growth. *J Exp Zool* **244**, 309-323.
11. Whittemore CT & Green DM (2001) Growth of the young weaned pig. In *The Weaner Pig: Nutrition and Management*, pp. 1-15 [MA Varley and J Wiseman, editors]. Wallingford, UK: CABI Publishing.
12. Pluske JR, Le Dividich J & Verstegen MWA (2003) Introduction. In *Weaning the Pig: Concepts and Consequences*, pp. 15-16 [JR Pluske, J Le Dividich and MWA Verstegen, editors]. Wageningen: Wageningen Academic Publishers.
13. Lallès JP, Boudry G, Favier C, Le Floc'h N, Luron I, Montagne L *et al.* (2004) Gut function and dysfunction in young pigs: physiology. *Anim Res* **53**, 301-316.
14. Moeser AJ, Klok CV, Ryan KA, Wooten JG, Little D, Cook VL *et al.* (2007) Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. *Am J Physiol Gastrointest Liver Physiol* **292**, G173-G181.
15. Van Der Meulen J, Koopmans SJ, Dekker RA & Hoogendoorn A (2010) Increasing weaning age of piglets from 4 to 7 weeks reduces stress, increases post-weaning feed intake but does not improve intestinal functionality. *Animal* **4**, 1653-1661.
16. Brooks PH, Moran CA, Beal JD, Demeckova V & Campbell A (2001) Liquid feeding for the young piglet. In *The Weaner Pig: Nutrition and Management*, pp. 153-178 [MA Varley and J Wiseman, editors]. Wallingford, UK: CABI Publishing.
17. Hopwood DE & Hampson DJ (2003) Interactions between the intestinal microflora, diet and diarrhoea, and their influences on piglet health in the immediate post-weaning period. In *Weaning the Pig: Concepts and Consequences*, pp. 199-218 [JR Pluske, J Le Dividich and MWA Verstegen, editors]. Wageningen: Wageningen Academic Publishers.
18. Moeser AJ, Ryan KA, Nighot PK & Blikslager AT (2007) Gastrointestinal dysfunction induced by early weaning is attenuated by delayed weaning and mast cell blockade in pigs. *Am J Physiol Gastrointest Liver Physiol* **293**, G413-G421.
19. Boudry G, Péron V, Le Huërou-Luron I, Lallès JP & Sève B (2004) Weaning induces both transient and long-lasting modifications of absorptive, secretory, and barrier properties of piglet intestine. *J Nutr* **134**, 2256-2262.
20. Smith MW (1984) Effect of postnatal development and weaning upon the capacity of pig intestinal villi to transport alanine. *J Agric Sci Camb* **102**, 625-633.
21. Miller BG, James PS, Smith MW & Bourne FJ (1986) Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. *J Agric Sci Camb* **107**, 579-589.
22. Sklan D (2001) Development of the digestive tract of poultry. *Worlds Poult Sci J* **57**, 415-428.

23. Pluske JR, Williams IH & Aherne FX (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.
24. Farhadi A, Banan A, Fields J & Keshavarzian A (2003) Intestinal barrier: An interface between health and disease. *J Gastroenterol Hepatol* **18**, 479-497.
25. Cottier H, Hässig A & Stampfli K (1994) Effective and ineffective defence mechanisms in the gastrointestinal tract. In *Effective and Ineffective Defence Mechanisms in the Gastrointestinal Tract*, pp. 1-11 [H Cottier, PJ Heidt, VC Rusch and D Van der Waaij, editors]. Herborn-Dill (Germany): Institute for Microbiology and Biochemistry.
26. Verdonk JMAJ (2006) Nutritional strategy affects gut wall integrity in weaned piglets. PhD Thesis, Wageningen University.
27. Niewold TA, Van Essen GJ, Nabuurs MJA, Stockhofe-Zurwieden N & Van Der Meulen J (2000) A review of porcine pathophysiology: A different approach to disease. *Vet Q* **22**, 209-212.
28. Franco JRG, Murakami AE, Natali MRM, Garcia ERM & Furlan AC (2006) Influence of delayed placement and dietary lysine levels on small intestine morphometrics and performance of broilers. *Rev Bras Cienc Avic* **8**, 233-241.
29. Geyra A, Uni Z & Sklan D (2001) The effect of fasting at different ages on growth and tissue dynamics in the small intestine of the young chick. *Br J Nutr* **86**, 53-61.
30. Kelly D, Smyth JA & McCracken KJ (1991) Digestive development of the early-weaned pig. 2. Effects of level of food intake on digestive enzyme activity during the immediate post-weaning period. *Br J Nutr* **65**, 181-188.
31. Marion J, Biernat M, Thomas F, Savary G, Le Breton Y, Zabielski R *et al.* (2002) Small intestine growth and morphometry in piglets weaned at 7 days of age effects of level of energy intake. *Reprod Nutr Dev* **42**, 339-354.
32. Van Beers-Schreurs HMG, Nabuurs MJA, Vellenga L, Kalsbeek-Van Der Valk HJ, Wensing T & Breukink HJ (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.
33. Bishop WP, Kim SI, Yamazato M, Yoshino H & Kimura K (1992) Mucosal morphology in isolated bowel segments: Importance of exposure to luminal contents. *J Pediatr Surg* **27**, 1061-1065.
34. Page CP (1989) The surgeon and gut maintenance. *Am J Surg* **158**, 485-490.
35. Carey HV, Hayden UL & Tucker KE (1994) Fasting alters basal and stimulated ion transport in piglet jejunum. *Am J Physiol Regul Integr Comp Physiol* **267**, R156-R163.
36. Bartell SM & Batal AB (2007) The effect of supplemental glutamine on growth performance, development of the gastrointestinal tract, and humoral immune response of broilers. *Poult Sci* **86**, 1940-1947.
37. Yi GF, Allee GL, Knight CD & Dibner JJ (2005) Impact of glutamine and oasis hatchling supplement on growth performance, small intestinal morphology, and immune response of broilers vaccinated and challenged with Eimeria maxima. *Poult Sci* **84**, 283-293.
38. Batal AB & Parsons CM (2002) Effects of age on development of digestive organs and performance of chicks fed a corn-soybean meal versus a crystalline amino acid diet. *Poult Sci* **81**, 1338-1341.
39. Swatson HK, Gous R, Iji PA & Zarrinkalam R (2002) Effect of dietary protein level, amino acid balance and feeding level on growth, gastrointestinal tract, and mucosal structure of the small intestine in broiler chickens. *Anim Res* **51**, 501-515.
40. Yue LY & Qiao SY (2008) Effects of low-protein diets supplemented with crystalline amino acids on performance and intestinal development in piglets over the first 2 weeks after weaning. *Livest Sci* **115**, 144-152.
41. Ewtushik AL, Bertolo RFP & Ball RO (2000) Intestinal development of early-weaned piglets receiving diets supplemented with selected amino acids or polyamines. *Can J Anim Sci* **80**, 653-662.
42. Domeneghini C, Di Giancamillo A, Bosi G & Arrighi S (2006) Can nutraceuticals affect the structure of intestinal mucosa? Qualitative and quantitative microanatomy in L-glutamine diet-supplemented weaning piglets. *Vet Res Commun* **30**, 331-342.
43. Domeneghini C, Di Giancamillo A, Savoini G, Paratte R, Bontempo V & Dell'Orto V (2004) Structural patterns of swine ileal mucosa following L-glutamine and nucleotide administration during the weaning period. An histochemical and histometrical study. *Histol Histopathol* **19**, 49-58.
44. Wu G, Meier SA & Knabe DA (1996) Dietary glutamine supplementation prevents jejunal atrophy in weaned pigs. *J Nutr* **126**, 2578-2584.

45. Lee DN, Cheng YH, Wu FY, Sato H, Shinzato I, Chen SP *et al.* (2003) Effect of dietary glutamine supplement on performance and intestinal morphology of weaned pigs. *Asian-Australas J Anim Sci* **16**, 1770-1776.
46. Liu T, Peng J, Xiong Y, Zhou S & Cheng X (2002) Effects of dietary glutamine and glutamate supplementation on small intestinal structure, active absorption and DNA, RNA concentrations in skeletal muscle tissue of weaned piglets during d 28 to 42 of age. *Asian-Australas J Anim Sci* **15**, 238-242.
47. Koopmans SJ, Guzik AC, Van Der Meulen J, Dekker R, Kogut J, Kerr BJ *et al.* (2006) Effects of supplemental L-tryptophan on serotonin, cortisol, intestinal integrity, and behavior in weanling piglets. *J Anim Sci* **84**, 963-971.
48. Hamard A, Sève B & Le Floc'h N (2007) Intestinal development and growth performance of early-weaned piglets fed a low-threonine diet. *Animal* **1**, 1134-1142.
49. Hamard A, Mazurais D, Boudry G, Le Huërou-Luron I, Sève B & Le Floc'h N (2010) A moderate threonine deficiency affects gene expression profile, paracellular permeability and glucose absorption capacity in the ileum of piglets. *J Nutr Biochem* **21**, 914-921.
50. Holmgren J, Czerkinsky C, Lycke N & Svennerholm AM (1992) Mucosal immunity: Implications for vaccine development. *Immunobiology* **184**, 157-179.
51. Van't Land B (2004) Mucosal barrier injury: Regulation and function of small intestinal epithelial cells during methotrexate treatment. PhD Thesis, Catholic University Nijmegen.
52. Moffett DF, Moffett SB & Schauf CL (1993) *Human Physiology: Foundations and Frontiers*, 2nd ed. St. Louis (MO): Mosby.
53. Willemsen LEM (2004) Itestinal barrier function: Regulation of epithelial permeability and mucin experession. PhD Thesis, University of Amsterdam.
54. Hermos JA, Mathan M & Trier JS (1971) DNA synthesis and proliferation by villous epithelial cells in fetal rats. *J Cell Biol* **50**, 255-258.
55. Uni Z, Geyra A, Ben-Hur H & Sklan D (2000) Small intestinal development in the young chick: Crypt formation and enterocyte proliferation and migration. *Br Poult Sci* **41**, 544-551.
56. Hodin RA, Graham JR, Meng S & Upton MP (1994) Temporal pattern of rat small intestinal gene expression with refeeding. *Am J Physiol Gastrointest Liver Physiol* **266**, G83-G89.
57. Holt PR, Wu S & Yeh KY (1986) Ileal hyperplastic response to starvation in the rat. *Am J Physiol Gastrointest Liver Physiol* **251**, G124-G131.
58. Moon HW (1971) Epithelial cell migration in the alimentary mucosa of the suckling pig. *Proc Soc Exp Biol Med* **137**, 151-154.
59. Houle VM, Park YK, Laswell SC, Freund GG, Dudley MA & Donovan SM (2000) Investigation of three doses of oral insulin-like growth factor-I on jejunal lactase phlorizin hydrolase activity and gene expression and enterocyte proliferation and migration in piglets. *Pediatr Res* **48**, 497-503.
60. Smith MW, Paterson JYF & Peacock MA (1984) A comprehensive description of brush border membrane development applying to enterocytes taken from a wide variety of mammalian species. *Comp Biochem Physiol A Physiol* **77**, 655-662.
61. Iji PA, Saki A & Tivey DR (2001) Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. *Br Poult Sci* **42**, 505-513.
62. Imondi AR & Bird FH (1966) The turnover of intestinal epithelium in the chick. *Poult Sci* **45**, 142-147.
63. Moon HW & Skartvedt SM (1975) Effect of age on epithelial cell migration in small intestine of chickens. *Am J Vet Res* **36**, 213-215.
64. Conour JE, Ganessunker D, Tappenden KA, Donovan SM & Gaskins HR (2002) Acidomucin goblet cell expansion induced by parenteral nutrition in the small intestine of piglets. *Am J Physiol Gastrointest Liver Physiol* **283**, G1185-G1196.
65. Butzner JD & Gall DG (1990) Impact of refeeding on intestinal development and function in infant rabbits subjected to protein-energy malnutrition. *Pediatr Res* **27**, 245-251.
66. Thompson KL & Applegate TJ (2006) Feed withdrawal alters small-intestinal morphology and mucus of broilers. *Poult Sci* **85**, 1535-1540.
67. Nitsan Z, Dunnington EA & Siegel PB (1991) Organ growth and digestive enzyme levels to fifteen days of age in lines of chickens differing in body weight. *Poult Sci* **70**, 2040-2048.
68. O'Sullivan NP, Dunnington EA, Larsen AS & Siegel PB (1992) Correlated responses in lines of chickens divergently selected for fifty-six-day body weight. 2. Organ growth, deoxyribonucleic acid, ribonucleic acid, and protein content. *Poult Sci* **71**, 598-609.
69. Noy Y & Sklan D (1999) Energy utilization in newly hatched chicks. *Poult Sci* **78**, 1750-1756.

70. Maiorka A, Santin E, Dahlke F, Boleli IC, Furlan RL & Macari M (2003) Posthatching Water and Feed Deprivation Affect the Gastrointestinal Tract and Intestinal Mucosa Development of Broiler Chicks. *J Appl Poult Res* **12**, 483-492.
71. Ravindran V, Wu YB, Thomas DG & Morel PCH (2006) Influence of whole wheat feeding on the development of gastrointestinal tract and performance of broiler chickens. *Aust J Agric Res* **57**, 21-26.
72. Katanbaf MN, Dunnington EA & Siegel PB (1988) Allomorphic relationships from hatching to 56 days in parental lines and F1 crosses of chickens selected 27 generations for high or low body weight. *Growth Dev Aging* **52**, 11-21.
73. Noy Y & Sklan D (1998) Metabolic responses to early nutrition. *J Appl Poult Res* **7**, 437-451.
74. Miles RD, Butcher GD, Henry PR & Littell RC (2006) Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. *Poult Sci* **85**, 476-485.
75. Uni Z, Ganot S & Sklan D (1998) Posthatch development of mucosal function in the broiler small intestine. *Poult Sci* **77**, 75-82.
76. Uni Z, Noy Y & Sklan D (1995) Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. *Poult Sci* **74**, 1622-1629.
77. Amerah AM, Ravindran V, Lentle RG & Thomas DG (2008) Influence of particle size and xylanase supplementation on the performance, energy utilisation, digestive tract parameters and digesta viscosity of broiler starters. *Br Poult Sci* **49**, 455-462.
78. Yang Y, Iji PA, Kocher A, Mikkelsen LL & Choct M (2008) Effects of xylanase on growth and gut development of broiler chickens given a wheat-based diet. *Asian-Australas J Anim Sci* **21**, 1659-1664.
79. Langhout DJ (1998) The role of the intestinal flora as affected by non-starch polysaccharides in broiler chicks. PhD Thesis, Wageningen University.
80. Read-Snyder J, Edens FW, Cantor AH, Pescatore AJ & Pierce JL (2009) Effect of dietary selenium on small intestine villus integrity in reovirus-challenged broilers. *Int J Poult Sci* **8**, 829-835.
81. Noy Y & Sklan D (2002) Nutrient use in chicks during the first week posthatch. *Poult Sci* **81**, 391-399.
82. Maneewan B & Yamauchi K (2004) Intestinal villus recovery in chickens refed semi-purified protein-, fat-, or fibre-free pellet diets. *Br Poult Sci* **45**, 163-170.
83. Swennen Q, Everaert N, Debonne M, Verbaeys I, Careghi C, Tona K *et al.* (2010) Effect of macronutrient ratio of the pre-starter diet on broiler performance and intermediary metabolism. *J Anim Physiol Anim Nutr* **94**, 375-384.
84. Tabedian SA, Samie A, Pourreza J & Sadeghi G (2010) Effect of fasting or post-hatch diet's type on chick development. *J Anim Vet Adv* **9**, 406-413.
85. Bhanja SK, Anjali Devi C, Panda AK & Shyam Sunder G (2010) Effect of post-hatch nutrient intubation on performance, intestinal growth, meat yield and immune response in broiler chickens. *Asian-Australas J Anim Sci* **23**, 515-520.
86. Cera KR, Mahan DC, Cross RF, Reinhart GA & Whitmoyer RE (1988) Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. *J Anim Sci* **66**, 574-584.
87. Efird RC, Armstrong WD & Herman DL (1982) The development of digestive capacity in young pigs: effects of age and weaning system. *J Anim Sci* **55**, 1380-1387.
88. Kelly D, Smyth JA & McCracken KJ (1991) Digestive development of the early-weaned pig. 1. 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.
89. Montagne L, Boundry G, Favier C, Le Huerou-Luron I, Lallès JP & Sève B (2007) Main intestinal markers associated with the changes in gut architecture and function in piglets after weaning. *Br J Nutr* **97**, 45-57.
90. Hampson DJ (1986) Alterations in piglet small intestinal structure at weaning. *Res Vet Sci* **40**, 32-40.
91. Verdonk JMAJ, Bruininx EMAM, van der Meulen J & Verstegen MWA (2007) Post-weaning feed intake level modulates gut morphology but not gut permeability in weaned piglets. *Livest Sci* **108**, 146-149.
92. Stoll B, Chang X, Fan MZ, Reeds PJ & Burrin DG (2000) Enteral nutrient intake level determines intestinal protein synthesis and accretion rates in neonatal pigs. *Am J Physiol Gastrointest Liver Physiol* **279**, G288-G294.
93. Vente-Spreeuwenberg MAM & Beynen AC (2003) Diet-mediated modulation of small intestinal integrity in weaned piglets. In *Weaning the Pig: Concepts and Consequences*, pp. 145-198 [JR Pluske, J Le Dividich and MWA Verstegen, editors]. Wageningen: Wageningen Academic Publishers.

94. Vente-Spreeuwenberg MAM, Verdonk JMAJ, Bakker GCM, Beynen AC & Verstegen MWA (2004) Effect of dietary protein source on feed intake and small intestinal morphology in newly weaned piglets. *Livest Prod Sci* **86**, 169-177.
95. Lallès JP, Bosi P, Janczyk P, Koopmans SJ & Torrallardona D (2009) Impact of bioactive substances on the gastrointestinal tract and performance of weaned piglets: A review. *Animal* **3**, 1625-1643.
96. Nyachoti CM, Omogbenigun FO, Rademacher M & Blank G (2006) Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. *J Anim Sci* **84**, 125-134.
97. Opapeju FO, Rademacher M, Blank G & Nyachoti CM (2008) Effect of low-protein amino acid-supplemented diets on the growth performance, gut morphology, organ weights and digesta characteristics of weaned pigs. *Animal* **2**, 1457-1464.
98. Peuhkuri K, Vapaatalo H & Korpela R (2010) Even low-grade inflammation impacts on small intestinal function. *World J Gastroenterol* **16**, 1057-1062.
99. Lee KW, Lee SH, Lillehoj HS, Li GX, Jang SI, Babu US *et al.* (2010) Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. *Poult Sci* **89**, 203-216.
100. Jiang ZY, Sun LH, Lin YC, Ma XY, Zheng CT, Zhou GL *et al.* (2009) Effects of dietary glycyl-glutamine on growth performance, small intestinal integrity, and immune responses of weaning piglets challenged with lipopolysaccharide. *J Anim Sci* **87**, 4050-4056.
101. Awad WA, Ghareeb K & Böhm J Effect of addition of a probiotic micro-organism to broiler diet on intestinal mucosal architecture and electrophysiological parameters. *J Anim Physiol Anim Nutr* **94**, 486-494.
102. Almquist HJ (1952) Amino acid requirements of chickens and turkeys - A review. *Poultry Sci* **31**, 966-981.
103. Chung TK & Baker DH (1992) Ideal amino acid pattern for 10-kilogram pigs. *J Anim Sci* **70**, 3102-3111.
104. Wang TC & Fuller MF (1989) The optimum dietary amino acid pattern for growing pigs. 1. Experiments by amino acid deletion. *Br J Nutr* **62**, 77-89.
105. Han Y & Baker DH (1993) Effects of sex, heat stress, body weight, and genetic strain on the dietary lysine requirement of broiler chicks. *Poult Sci* **72**, 701-708.
106. Han Y & Baker DH (1994) Digestible lysine requirement of male and female broiler chicks during the period three to six weeks posthatching. *Poult Sci* **73**, 1739-1745.
107. Han YM & Baker DH (1991) Lysine requirements of fast- and slow-growing broiler chicks. *Poult Sci* **70**, 2108-2114.
108. Mack S, Bercovici D, De Groote G, Leclercq B, Lippens M, Pack M *et al.* (1999) Ideal amino acid profile and dietary lysine specification for broiler chickens of 20 to 40 days of age. *Br Poult Sci* **40**, 257-265.
109. Schutte JB (1996) Aminozuurbehoeften van leghennen en vleeskuikens (Amino acid requirements of laying hens and broilers). Lelystad, The Netherlands: Central Bureau for Livestock Feeding (CVB).
110. Eits RM, Kwakkel RP, Verstegen MWA & Emmans GC (2003) Responses of broiler chickens to dietary protein: Effects of early life protein nutrition on later responses. *Br Poult Sci* **44**, 398-409.
111. Temim S, Chagneau AM, Guillaumin S, Michel J, Peresson R & Tesseraud S (2000) Does excess dietary protein improve growth performance and carcass characteristics in heat-exposed chickens? *Poult Sci* **79**, 312-317.
112. Wijtten PJA, Prak R, Lemme A & Langhout DJ (2004) Effect of different dietary ideal protein concentrations on broiler performance. *Br Poult Sci* **45**, 504-511.
113. Kidd MT, Kerr BJ, Halpin KM, McWard GW & Quarles CL (1998) Lysine levels in starter and grower-finisher diets affect broiler performance and carcass traits. *J Appl Poult Res* **7**, 351-358.
114. Pesti GM & Fletcher DL (1984) The response of male broiler chickens to diets with various protein contents during the growing and finisher phases. *Br Poult Sci* **25**, 415-423.
115. CVB (2001) *Veevoedertabel (Livestock Feed Table)*. Lelystad, The Netherlands: Central Bureau for Livestock Feeding (CVB).
116. NRC (1994) *Nutrient Requirements of Poultry. 9th rev. ed.* Washington, DC: National Academy Press.
117. Llames CR & Fontaine J (1994) Determination of amino acids in feeds: Collaborative study. *J AOAC Int* **77**, 1362-1402.
118. Sweeney RA (1989) Generic combustion method for determination of crude protein in feeds: collaborative study. *J Assoc Off Anal Chem* **72**, 770-774.
119. Bechhofer DH, Santner TJ & Goldsman DM (1995) *Design and Analysis of Experiments for Statistical Selection, Screening, and Multiple Comparisons*. New York, USA: J. Wiley.

120. Baird DB, Harding SA, Lane PW, Murray DA, Payne RW & Soutar DM (2002) *Genstat for Windows (6th Edition) Introduction*. Oxford, United Kingdom: VSN International.
121. Baker DH (1996) Advances in amino acid nutrition and metabolism of swine and poultry. *Nutrient Management of Food Animals to Enhance and Protect the Environment*, 41-53.
122. D'Mello JPF (1994) Responses of growing poultry to amino acids. *Amino Acids in Farm Animal Nutrition*, 205-243.
123. Mosier Jr HD (1986) The control of catch-up growth. *Acta Endocrinologica, Supplement* **113**, 1-7.
124. Wilson PN & Osbourn DF (1960) Compensatory growth after undernutrition in mammals and birds. *Biol Rev* **35**, 324-363.
125. Morris TR & Njuru DM (1990) Protein requirement of fast- and slow-growing chicks. *Br Poult Sci* **31**, 803-810.
126. Morris TR & Abebe S (1990) Effects of arginine and protein on chicks' responses to dietary lysine. *Br Poult Sci* **31**, 261-266.
127. Sklan D & Noy Y (2003) Crude protein and essential amino acid requirements in chicks during the first week posthatch. *Br Poult Sci* **44**, 266-274.
128. MacLeod MG, McNeill L & Kim JH (2003) Food intake, weight gain, food conversion ratio, breast muscle weight and abdominal fat weight in broiler chickens fed on diets of varying protein quality. *Br Poult Sci* **44**.
129. Ravindran V (2002) Early nutrition of chicks - Physiological limitations and potential. *Proceedings of the New Zealand Poultry Industry Conference*, 1-12.
130. Vieira SL & Moran Jr ET (1999) Effects of egg of origin and chick post-hatch nutrition on broiler live performance and meat yields. *Worlds Poult Sci J* **55**, 125-142.
131. Halevy O, Geyra A, Barak M, Uni Z & Sklan D (2000) Early posthatch starvation decreases satellite cell proliferation and skeletal muscle growth in chicks. *J Nutr* **130**, 858-864.
132. Mozdziak PE, Walsh TJ & McCoy DW (2002) The effect of early posthatch nutrition on satellite cell mitotic activity. *Poult Sci* **81**, 1703-1708.
133. Gous RM, Emmans GC & Fisher C (1992) The response of broilers to feeds differing in protein content following a period of fattening. *Poult Sci* **71**, 152.
134. Zubair AK & Leeson S (1996) Compensatory growth in the broiler chicken: A review. *Worlds Poult Sci J* **52**, 197-201.
135. Wijtten PJA, Lemme A & Pack M (2001) The effect of enhanced dietary ideal amino acid levels in maize- and wheat-based diets on performance of male and female broilers. *13th European Symposium on Poultry Nutrition*. Blankenberge, Belgium. pp. 41-42.
136. Chen H, Pan Y, Wong EA & Webb Jr KE (2005) Dietary protein level and stage of development affect expression of an intestinal peptide transporter (cPepT1) in chickens. *J Nutr* **135**, 193-198.
137. Gilbert ER, Li H, Emmerson DA, Webb Jr KE & Wong EA (2008) Dietary protein quality and feed restriction influence abundance of nutrient transporter mRNA in the small intestine of broiler chicks. *J Nutr* **138**, 262-271.
138. Wijtten PJA, Lemme A & Langhout DJ (2004) Effects of different dietary ideal protein levels on male and female broiler performance during different phases of life: Single phase effects, carryover effects, and interactions between phases. *Poult Sci* **83**, 2005-2015.
139. Wijtten PJA, Hangoor E, Perdok HB & Sparla JKWM (2008) The effect of early life feed restriction on performance and mortality of male broilers. *23th World Poultry Congress*. Brisbane, Australia. pp. 234.
140. Sterling KG, Pesti GM & Bakalli RI (2006) Performance of different broiler genotypes fed diets with varying levels of dietary crude protein and lysine. *Poult Sci* **85**, 1045-1054.
141. Berhe ET & Gous RM (2008) Effect of dietary protein content on growth, uniformity and mortality of two commercial broiler strains. *S Afr J Anim Sci* **38**, 293-302.
142. CVB (2005) *Veevoedertabel (Livestock Feed Table)*. Lelystad, The Netherlands: Central Bureau for Livestock Feeding (CVB).
143. Sklan D & Noy Y (2005) Direct determination of optimal amino acid intake for maintenance and growth in broilers. *Poult Sci* **84**, 412-418.
144. Leveille GA & Fisher H (1958) The amino acid requirements for maintenance in the adult rooster. I. Nitrogen and energy requirements in normal and protein-depleted animals receiving whole egg protein and amino acid diets. *J Nutr* **66**, 441-453.
145. Leveille GA & Fisher H (1959) Amino acid requirements for maintenance in the adult rooster. II. The requirements for glutamic acid, histidine, lysine and arginine. *J Nutr* **69**, 289-294.

146. Leveille GA & Fisher H (1960) Amino acid requirements for maintenance in the adult rooster. III. The requirements for leucine, isoleucine, valine and threonine, with reference also to the utilization of the D-isomers of valine, threonine, and isoleucine. *J Nutr* **70**, 135-140.
147. Leveille GA, Shapiro R & Fisher H (1960) Amino acid requirements for maintenance in the adult rooster. IV. The requirements for methionine, cystine, phenylalanine, tyrosine and tryptophan; the adequacy of the determined requirements. *J Nutr* **72**, 8-15.
148. Baker DH, Fernandez SR, Parsons CM, Edwards Iii HM, Emmert JL & Webel DM (1996) Maintenance requirement for valine and efficiency of its use above maintenance for accretion of whole body valine and protein in young chicks. *J Nutr* **126**, 1844-1851.
149. Edwards III HM, Baker DH, Fernandez SR & Parsons CM (1997) Maintenance threonine requirement and efficiency of its use for accretion of whole-body threonine and protein in young chicks. *Br J Nutr* **78**, 111-119.
150. Edwards III HM, Fernandez SR & Baker DH (1999) Maintenance lysine requirement and efficiency of using lysine for accretion of whole-body lysine and protein in young chicks. *Poult Sci* **78**, 1412-1417.
151. Edwards III HM & Baker DH (1999) Maintenance sulfur amino acid requirements of young chicks and efficiency of their use for accretion of whole-body sulfur amino acids and protein. *Poult Sci* **78**, 1418-1423.
152. Samadi & Liebert F (2006) Estimation of nitrogen maintenance requirements and potential for nitrogen deposition in fast-growing chickens depending on age and sex. *Poult Sci* **85**, 1421-1429.
153. Kebreab E, France J, Kuhi HD & Lopez S (2008) A comparative evaluation of functions for partitioning nitrogen and amino acid intake between maintenance and growth in broilers. *J Agric Sci* **146**, 163-170.
154. Batal AB & Parsons CM (2002) Effects of age on nutrient digestibility in chicks fed different diets. *Poult Sci* **81**, 400-407.
155. Noy Y & Sklan D (1995) Digestion and absorption in the young chick. *Poult Sci* **74**, 366-373.
156. Moore S (1963) On the Determination of Cystine as Cysteic Acid. *J Biol Chem* **238**, 235-237.
157. Schram E, Dustin JP, Moore S & Bigwood EJ (1953) Application de la chromatographie sur échangeur d'ions a l'étude de la composition des aliments en acides amines. *Anal Chim Acta* **9**, 149-162.
158. Slump P & Schreuder HAW (1969) Determination of tryptophan in foods. *Anal Biochem* **27**, 182-186.
159. Payne RW, Murray DA, Harding SA, Baird DB & Soutar DM (2008) *Genstat for Windows (11th Edition) Introduction*. Hemel Hempstead, UK: VSN International.
160. Potier M, Darcel N & Tome D (2009) Protein, amino acids and the control of food intake. *Curr Opin Clin Nutr Metab Care* **12**, 54-58.
161. Plavnik I & Hurwitz S (1989) Effect of dietary protein, energy, and feed pelleting on the response of chicks to early feed restriction. *Poult Sci* **68**, 1118-1125.
162. Stoll B, Chang X, Fan MZ, Reeds PJ & Burrin DG (2000) Enteral nutrient intake level determines intestinal protein synthesis and accretion rates in neonatal pigs. *Am J Physiol Gastrointest Liver Physiol* **279**, G288-G294.
163. Lemme A, Janssen MGT, Wijtten PJA, Sparla JKWM & Redshaw M (2007) Increased dietary balanced protein levels at varying length of application in 1-14 days old broilers. *16th European Symposium on Poultry Nutrition*. Strasbourg, France. pp. 193-196.
164. Palo PE, Sell JL, Piquer FJ, Soto-Salanova MF & Vilaseca L (1995) Effect of early nutrient restriction on broiler chickens. 1. Performance and development of the gastrointestinal tract. *Poult Sci* **74**, 88-101.
165. Susbilla JP, Tarvid I, Gow CB & Frankel TL (2003) Quantitative feed restriction or meal-feeding of broiler chicks alter functional development of enzymes for protein digestion. *Br Poult Sci* **44**, 698-709.
166. Maisonnier S, Gomez J, Chagneau AM & Carré B (2001) Analysis of variability in nutrient digestibilities in broiler chickens. *Br Poult Sci* **42**, 70-76.
167. Visek WJ (1978) The mode of growth promotion by antibiotics. *J Anim Sci* **46**, 1447-1469.
168. Wu YB, Ravindran V, Thomas DG, Birtles MJ & Hendriks WH (2004) Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. *Br Poult Sci* **45**, 76-84.
169. Marquardt RR (1997) Enzyme enhancement of the nutritional value of cereals: role of viscous, water-soluble, nonstarch polysaccharides in chick performance. In *Enzyme in Poultry and Swine Nutrition*, pp. 5-17 [RR Marquardt and Z Han, editors]. Ottawa, Canada: International Development Research Centre.
170. Robinson FE, Classen HL, Hanson JA & Onderka DK (1992) Growth performance, feed efficiency and the incidence of skeletal and metabolic disease in full-fed and feed restricted broiler and roaster chickens. *J Appl Poult Res* **1**, 33-41.

171. Demir E, Sarica S, Sekeroglu A, Ozean MA & Seker Y (2004) Effects of early and late feed restriction or feed withdrawal on growth performance, ascites and blood constituents of broiler chickens. *Acta Agric Scand A Anim Sci* **54**, 152-158.
172. Scheele CW, Van Der Klis JD, Kwakernaak C, Buys N & Decuypere E (2003) Haematological characteristics predicting susceptibility for ascites. 2. High haematocrit values in juvenile chickens. *Br Poult Sci* **44**, 484-489.
173. Balog JM (2003) Ascites Syndrome (Pulmonary Hypertension Syndrome) in Broiler Chickens: Are We Seeing the Light at the End of the Tunnel? *Avian Poult Biol Rev* **14**, 99-125.
174. Berg RD (1995) Bacterial translocation from the gastrointestinal tract. *Trends Microbiol* **3**, 149-154.
175. Uil JJ, Van Elburg RM, Van Overbeek FM, Mulder CJJ, Vanberge-Henegouwen GP & Heymans HSA (1997) Clinical implications of the sugar absorption test: Intestinal permeability test to assess mucosal barrier function. *Scand J Gastroenterol* **32**, Suppl. 223, 70-78.
176. Lambert GP (2009) Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. *J Anim Sci* **87**, E101-E108.
177. Santos J, Benjamin M, Yang PC, Prior T & Perdue MH (2000) Chronic stress impairs rat growth and jejunal epithelial barrier function: Role of mast cells. *Am J Physiol Gastrointest Liver Physiol* **278**, G847-G854.
178. Pácha J (2000) Development of intestinal transport function in mammals. *Physiol Rev* **80**, 1633-1667.
179. Keita ÅV & Söderholm JD (2010) The intestinal barrier and its regulation by neuroimmune factors. *Neurogastroenterol Motil* **22**, 718-733.
180. Rådberg K, Biernat M, Linderoth A, Zabielski R, Pierzynowski SG & Weström BR (2001) Enteral exposure to crude red kidney bean lectin induces maturation of the gut in suckling pigs. *J Anim Sci* **79**, 2669-2678.
181. Heyman M, Ducroc R, Desjeux JF & Morgat JL (1982) Horseradish peroxidase transport across adult rabbit jejunum *in vitro*. *Am J Physiol Gastrointest Liver Physiol* **5**, G558-G564.
182. Cameron HL & Perdue MH (2007) Muscarinic acetylcholine receptor activation increases transcellular transport of macromolecules across mouse and human intestinal epithelium *in vitro*. *Neurogastroenterol Motil* **19**, 47-56.
183. Bijlsma PB, Kiliaan AJ, Scholten G, Heyman M, Groot JA & Taminiau JAJM (1996) Carbachol, but not forskolin, increases mucosal-to-serosal transport of intact protein in rat ileum *in vitro*. *Am J Physiol Gastrointest Liver Physiol* **271**, G147-G155.
184. Osman NE, Weström B, Wang Q, Persson L & Karlsson B (1998) Spermine affects intestinal *in vitro* permeability to different-sized molecules in rats. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* **120**, 211-216.
185. Bjarnason I, MacPherson A & Hollander D (1995) Intestinal permeability: An overview. *Gastroenterology* **108**, 1566-1581.
186. Duizer E, Van Der Wulp C, Versantvoort CHM & Groten JP (1998) Absorption enhancement, structural changes in tight junctions and cytotoxicity caused by palmitoyl carnitine in Caco-2 and IEC-18 cells. *J Pharmacol Exp Ther* **287**, 395-402.
187. Johnston SD, Smye M & Watson RGP (2001) Intestinal permeability tests in coeliac disease. *Clin Lab* **47**, 143-150.
188. Spreeuwenberg MAM, Verdonk JMAJ, Gaskins HR & Verstegen MWA (2001) Small intestine epithelial barrier function is compromised in pigs with low feed intake at weaning. *J Nutr* **131**, 1520-1527.
189. Boudry G (2005) The Ussing chamber technique to evaluate alternatives to in-feed antibiotics for young pigs. *Anim Res* **54**, 219-230.
190. Travis S & Menzies I (1992) Intestinal permeability: Functional assessment and significance. *Clin Sci* **82**, 471-488.
191. Craig RM & Ehrenpreis ED (1999) D-xylose testing. *J Clin Gastroenterol* **29**, 143-150.
192. Menzies JS (1974) Absorption of intact oligosaccharide in health and disease. *Biochem Soc Trans* **2**, 1042-1047.
193. Miller BG, Newby TJ, Stokes CR & Bourne FJ (1984) Influence of diet on postweaning malabsorption and diarrhoea in the pig. *Res Vet Sci* **36**, 187-193.
194. Hampson DJ & Kidder DE (1986) Influence of creep feeding and weaning on brush border enzyme activities in the piglet small intestine. *Res Vet Sci* **40**, 24-31.
195. Hampson DJ & Smith WC (1986) Influence of creep feeding and dietary intake after weaning on malabsorption and occurrence of diarrhoea in the newly weaned pig. *Res Vet Sci* **41**, 63-69.
196. Kelly D, Smyth JA & McCracken KJ (1990) Effect of creep feeding on structural and functional changes of the gut of early weaned pigs. *Res Vet Sci* **48**, 350-356.

197. Berkeveld M, Langendijk P, Verheijden JH, Taverne MA, van Nes A, van Haard P *et al.* (2008) Citrulline and intestinal fatty acid-binding protein: longitudinal markers of postweaning small intestinal function in pigs? *J Anim Sci* **86**, 3440-3449.
198. Peled Y, Doron O, Laufer H, Bujanover Y & Gilat T (1991) D-Xylose absorption test. Urine or blood? *Dig Dis Sci* **36**, 188-192.
199. Ehrenpreis ED, Salvino M & Craig RM (2001) Improving the serum D-xylose test for the identification of patients with small intestinal malabsorption. *J Clin Gastroenterol* **33**, 36-40.
200. Menzies IS, Laker MF & Pounder R (1979) Abnormal intestinal permeability to sugars in villous atrophy. *Lancet* **2**, 1107-1109.
201. Kansagra K, Stoll B, Rognerud C, Niinikoski H, Ou CN, Harvey R *et al.* (2003) Total parenteral nutrition adversely affects gut barrier function in neonatal piglets. *Am J Physiol Gastrointest Liver Physiol* **285**, G1162-G1170.
202. Bjornvad CR, Thymann T, Deutz NE, Burrin DG, Jensen SK, Jensen BB *et al.* (2008) Enteral feeding induces diet-dependent mucosal dysfunction, bacterial proliferation, and necrotizing enterocolitis in preterm pigs on parenteral nutrition. *Am J Physiol Gastrointest Liver Physiol* **295**, G1092-G1103.
203. Bruins MJ, Hallemeesch MM, Deutz NEP & Soeters PB (1998) Increase in intestinal permeability after endotoxin challenge is due to fluid load. *Eur J Gastroenterol Hepatol* **10**, A22-A23.
204. Zhang B & Guo Y (2009) Supplemental zinc reduced intestinal permeability by enhancing occludin and zonula occludens protein-1 (ZO-1) expression in weaning piglets. *Br J Nutr* **102**, 687-693.
205. Keita ÅV, Söderholm JD & Ericson AC (2010) Stress-induced barrier disruption of rat follicle-associated epithelium involves corticotropin-releasing hormone, acetylcholine, substance P, and mast cells. *Neurogastroenterol Motil* **22**, 770-778+e221-e222.
206. Boudry G, Lallès JP, Malbert CH, Bobillier E & Sève B (2002) Diet-related adaptation of the small intestine at weaning in pigs is functional rather than structural. *J Pediatr Gastroenterol Nutr* **34**, 180-187.
207. Egberts HJA, de Groot ECBM, Van Dijk JE, Vellenga L & Mouwen JMVM (1993) Tight junctional structure and permeability of porcine jejunum after enterotoxic *Escherichia coli* infection. *Res Vet Sci* **55**, 10-14.
208. Lodemann U, Hübener K, Jansen N & Martens H (2006) Effects of *Enterococcus faecium* NCIMB 10415 as probiotic supplement on intestinal transport and barrier function of piglets. *Arch Anim Nutr* **60**, 35-48.
209. Lodemann U, Lorenz BM, Weyrauch KD & Martens H (2008) Effects of *Bacillus cereus var. toyoi* as probiotic feed supplement on intestinal transport and barrier function in piglets. *Arch Anim Nutr* **62**, 87-106.
210. Carlson D, Poulsen HD & Sehested J (2004) Influence of weaning and effect of post weaning dietary zinc and copper on electrophysiological response to glucose, theophylline and 5-HT in piglet small intestinal mucosa. *Comp Biochem Physiol A Mol Integr Physiol* **137**, 757-765.
211. Carlson D, Sehested J, Feng Z & Poulsen HD (2008) Serosal zinc attenuate serotonin and vasoactive intestinal peptide induced secretion in piglet small intestinal epithelium *in vitro*. *Comp Biochem Physiol A Mol Integr Physiol* **149**, 51-58.
212. Boudry G, Douard V, Mourot J, Lallès JP & Le Huërou-Luron I (2009) Linseed oil in the maternal diet during gestation and lactation modifies fatty acid composition, mucosal architecture, and mast cell regulation of the ileal barrier in piglets. *J Nutr* **139**, 1110-1117.
213. Smith F, Clark JE, Overman BL, Tozel CC, Huang JH, Rivier JEF *et al.* (2010) Early weaning stress impairs development of mucosal barrier function in the porcine intestine. *Am J Physiol Gastrointest Liver Physiol* **298**, G352-G363.
214. Miller BG & Skadhauge E (1997) Effect of weaning in the pig on ileal ion transport measured *in vitro*. *Zentralbl Veterinarmed A* **44**, 289-299.
215. Gabler NK, Spencer JD, Webel DM & Spurlock ME (2007) In utero and postnatal exposure to long chain (n-3) PUFA enhances intestinal glucose absorption and energy stores in weanling pigs. *J Nutr* **137**, 2351-2358.
216. Gabler NK, Radcliffe JS, Spencer JD, Webel DM & Spurlock ME (2009) Feeding long-chain n-3 polyunsaturated fatty acids during gestation increases intestinal glucose absorption potentially via the acute activation of AMPK. *J Nutr Biochem* **20**, 17-25.
217. Buddington RK, Elnif J, Puchal-Gardiner AA & Sangild PT (2001) Intestinal apical amino acid absorption during development of the pig. *Am J Physiol Regul Integr Comp Physiol* **280**, R241-R247.
218. Shimakura J, Terada T, Saito H, Katsura T & Inui KI (2006) Induction of intestinal peptide transporter 1 expression during fasting is mediated via peroxisome proliferator-activated receptor α. *Am J Physiol Gastrointest Liver Physiol* **291**.

219. Wijtten PJA, van der Meulen J & Verstegen MWA (2011) Intestinal barrier function and absorption in pigs after weaning: a review. *Br J Nutr* **105**, 967-981.
220. Blijlevens NMA, Van't Land B, Donnelly JP, M'Rabet L & De Pauw BE (2004) Measuring mucosal damage induced by cytotoxic therapy. *Support Care Cancer* **12**, 227-233.
221. Dionex (2000) Technical note 20: analysis of carbohydrates by high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD).
222. Dionex (2004) Dionex product manual onguard II cartridges document no.031688 revision 06 6 may 2004.
223. Gallati H & Pracht I (1985) Horseradish peroxidase: Kinetic studies and optimization of peroxidase activity determination using the substrate H2O2 and 3,3',5,5' tetramethylbenzidine. *J Clin Chem Clin Biochem* **23**, 435-460.
224. Littell RC, Henry PR & Ammerman CB (1998) Statistical Analysis of Repeated Measures Data Using SAS Procedures. *J Anim Sci* **76**, 1216-1231.
225. Pieper R, Janczyk P, Zeyner A, Smidt H, Guiard V & Souffrant WB (2008) Ecophysiology of the developing total bacterial and Lactobacillus communities in the terminal small intestine of weaning piglets. *Microb Ecol* **56**, 474-483.
226. Garden OA, Manners HK, SÃ¸rensen SH, Rutgers HC, Daniels S, Legrand-Defretin V *et al.* (1998) Intestinal permeability of Irish setter puppies challenged with a controlled oral dose of gluten. *Res Vet Sci* **65**, 23-28.
227. Broom LJ, Miller HM, Kerr KG & Knapp JS (2006) Effects of zinc oxide and Enterococcus faecium SF68 dietary supplementation on the performance, intestinal microbiota and immune status of weaned piglets. *Res Vet Sci* **80**, 45-54.
228. Ding LA, Li JS, Li YS, Zhu NT, Liu FN & Tan L (2004) Intestinal barrier damage caused by trauma and lipopolysaccharide. *World J Gastroenterol* **10**, 2373-2378.
229. Song D, Shi B, Xue H, Li Y, Yang X, Yu B *et al.* (2006) Confirmation and prevention of intestinal barrier dysfunction and bacterial translocation caused by methotrexate. *Dig Dis Sci* **51**, 1549-1556.
230. Burrin DG & Reeds PJ (1997) Alternative fuels in the gastrointestinal tract. *Current Opinion in Gastroenterology* **13**, 165-170.
231. Oba M, Baldwin Vi RL & Bequette BJ (2004) Oxidation of glucose, glutamate, and glutamine by isolated ovine enterocytes in vitro is decreased by the presence of other metabolic fuels. *J Anim Sci* **82**, 479-486.
232. Windmueller HG & Spaeth AE (1980) Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats. Quantitative importance of glutamine, glutamate, and aspartate. *J Biol Chem* **255**, 107-112.
233. Heo JM, Kim JC, Hansen CF, Mullan BP, Hampson DJ & Pluske JR (2008) Effects of feeding low protein diets to piglets on plasma urea nitrogen, faecal ammonia nitrogen, the incidence of diarrhoea and performance after weaning. *Arch Anim Nutr* **62**, 343-358.
234. Htoo JK, Araiza BA, Sauer WC, Rademacher M, Zhang Y, Cervantes M *et al.* (2007) Effect of dietary protein content on ileal amino acid digestibility, growth performance, and formation of microbial metabolites in ileal and cecal digesta of early-weaned pigs. *J Anim Sci* **85**, 3303-3312.
235. Udén P, Colucci PE & Van Soest PJ (1980) Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *J Sci Food Agric* **31**, 625-632.
236. Burrin DG (2001) Nutrient requirements and metabolism. In *Biology of the Domestic Pig*, pp. 309-389 [WG Pond and HJ Mersmann, editors]. Ithaca: Cornell University Press.
237. Yen JT, Nienaber JA, Hill DA & Pond WG (1989) Oxygen consumption by portal vein-drained organs and by whole animal in conscious growing swine. *Proc Soc Exp Biol Med* **190**, 393-398.
238. Reynolds CK, Tyrrell HF & Reynolds PJ (1991) Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers: Whole body energy and nitrogen balance and visceral heat production. *J Nutr* **121**, 994-1003.
239. Csapó J, Martin TG, Csapó-Kiss ZS & Házas Z (1996) Protein, fats, vitamin and mineral concentrations in porcine colostrum and milk from parturition to 60 days. *Int Dairy J* **6**, 881-902.
240. Pluske JR, Fenton TW, Lorschy ML, Pettigrew JE, Sower AF & Aherne FX (1997) A Modification to the Isotope-Dilution Technique for Estimating Milk Intake of Pigs Using Pig Serum. *J Anim Sci* **75**, 1279-1283.
241. Hopwood DE, Pethick DW, Pluske JR & Hampson DJ (2004) Addition of pearl barley to a rice-based diet for newly weaned piglets increases the viscosity of the intestinal contents, reduces starch digestibility and exacerbates post-weaning colibacillosis. *Br J Nutr* **92**, 419-427.

242. Kim JC, Mullan BP, Hampson DJ & Pluske JR (2008) Addition of oat hulls to an extruded rice-based diet for weaner pigs ameliorates the incidence of diarrhoea and reduces indices of protein fermentation in the gastrointestinal tract. *Br J Nutr* **99**, 1217-1225.
243. Li TJ, Huang RL, Wu GY, Lin YC, Jiang ZY, Kong XF *et al.* (2007) Growth performance and nitrogen metabolism in weaned pigs fed diets containing different sources of starch. *Livest Sci* **109**, 73-76.
244. Pluske JR, Montagne L, Cavaney FS, Mullan BP, Pethick DW & Hampson DJ (2007) Feeding different types of cooked white rice to piglets after weaning influences starch digestion, digesta and fermentation characteristics and the faecal shedding of β-haemolytic Escherichia coli. *Br J Nutr* **97**, 298-306.
245. Vicente B, Valencia DG, Pérez-Serrano M, Lázaro R & Mateos GG (2008) The effects of feeding rice in substitution of corn and the degree of starch gelatinization of rice on the digestibility of dietary components and productive performance of young pigs. *J Anim Sci* **86**, 119-126.
246. Fuller MF, McWilliam R, Wang TC & Giles LR (1989) The optimum dietary amino acid pattern for growing pigs. 2 Requirements for maintenance and for tissue protein accretion. *Br J Nutr* **62**, 255-267.
247. Berkeveld M, Langendijk P, Soede NM, Kemp B, Taverne MAM, Verheijden JHM *et al.* (2009) Improving adaptation to weaning: Effect of intermittent suckling regimens on piglet feed intake, growth, and gut characteristics. *J Anim Sci* **87**, 3156-3166.
248. Murali SG, Nelson DW, Draxler AK, Liu X & Ney DM (2005) Insulin-like growth factor-I (IGF-I) attenuates jejunal atrophy in association with increased expression of IGF-I binding protein-5 in parenterally fed mice. *J Nutr* **135**, 2553-2559.
249. Wijtten PJA, Verstijnen JJ, Van Kempen TATG, Perdok HB, Gort G & Verstegen MWA (2011) Lactulose as a marker of intestinal barrier function in pigs after weaning. *J Anim Sci* **89**, 1347-1357.
250. Branner GR, Böhmer BM, Erhardt W, Henke J & Roth-Maier DA (2004) Investigation on the precaecal and faecal digestibility of lactulose and inulin and their influence on nutrient digestibility and microbial characteristics. *Arch Anim Nutr* **58**, 353-366.
251. De Aguilar-Nascimento JE (2005) The role of macronutrients in gastrointestinal blood flow. *Curr Opin Clin Nutr Metab Care* **8**, 552-556.
252. Baarendse PJJ, Debonne M, Decuypere E, Kemp B & Van Den Brand H (2007) Ontogeny of avian thermoregulation from a neural point of view. *Worlds Poult Sci J* **63**, 267-276.
253. Yadgary L, Cahaner A, Kedar O & Uni Z (2010) Yolk sac nutrient composition and fat uptake in late-term embryos in eggs from young and old broiler breeder hens. *Poult Sci* **89**, 2441-2452.
254. Sakomura NK, Longo FA, Oviedo-Rondon EO, Boa-Viagem C & Ferraudo A (2005) Modeling energy utilization and growth parameter description for broiler chickens. *Poult Sci* **84**, 1363-1369.
255. Martin SAM, Blaney S, Bowman AS & Houlihan DF (2002) Ubiquitin-proteasome-dependent proteolysis in rainbow trout (oncorhynchus mykiss): Effect of food deprivation. *Pflug Arch Eur J Physiol* **445**, 257-266.
256. Kaushik SJ & Seiliez I (2010) Protein and amino acid nutrition and metabolism in fish: Current knowledge and future needs. *Aquac Res* **41**, 322-332.
257. Deutz NEP, Ten Have GAM, Soeters PB & Moughan PJ (1995) Increased intestinal amino-acid retention from the addition of carbohydrates to a meal. *Clin Nutr* **14**, 354-364.
258. Tarachai P & Yamauchi K (2000) Effects of luminal nutrient absorption, intraluminal physical stimulation, and intravenous parenteral alimentation on the recovery responses of duodenal villus morphology following feed withdrawal in chickens. *Poult Sci* **79**, 1578-1585.
259. Niinikoski H, Stoll B, Guan X, Kansagra K, Lambert BD, Stephens J *et al.* (2004) Onset of small intestinal atrophy is associated with reduced intestinal blood flow in TPN-fed neonatal piglets. *J Nutr* **134**, 1467-1474.
260. Jang IS, Kang SY, Ko YH, Moon YS & Sohn SH (2009) Effect of qualitative and quantitative feed restriction on growth performance and immune function in broiler chickens. *Asian-Australas J Anim Sci* **22**, 388-395.
261. Hamidi H & Pourreza J (2009) Effects of zinc-methionine and feed restriction on performance, immunocompetence and gut content osmolality of broilers challenged with a mixed coccidial infection. *J Biol Sci* **9**, 669-675.
262. Pan Jq, Tan X, Li Jc, Sun Wd, Huang Gq & Wang Xl (2008) Reduced PKCα expression in pulmonary arterioles of broiler chickens is associated with early feed restriction. *Res Vet Sci* **84**, 434-439.
263. Khajavi M, Rahimi S, Hassan ZM, Kamali MA & Mousavi T (2003) Effect of feed restriction early in life on humoral and cellular immunity of two commercial broiler strains under heat stress conditions. *Br Poult Sci* **44**, 490-497.

264. Pimentel JL, Cook ME & Greger JL (1991) Immune response of chicks fed various levels of zinc. *Poult Sci* **70**, 947-954.
265. Hotamisligil GS & Erbay E (2008) Nutrient sensing and inflammation in metabolic diseases. *Nat Rev Immunol* **8**, 923-934.
266. Grohmann U & Bronte V (2010) Control of immune response by amino acid metabolism. *Immunol Rev* **236**, 243-264.
267. Moeser AJ & Blikslager AT (2007) Mechanisms of porcine diarrheal disease. *J Am Vet Med Assoc* **231**, 56-67.
268. Uni Z & Ferket RP (2004) Methods for early nutrition and their potential. *Worlds Poult Sci J* **60**, 101-111.
269. Julian RJ (1998) Rapid Growth Problems: Ascites and Skeletal Deformities in Broilers. *Poult Sci* **77**, 1773-1780.
270. Molenaar R (2010) Perinatal development and nutrient utilization in chickens: effects of incubation conditions. PhD Thesis, Wageningen University.
271. Bruininx EMAM, Van Der Peet-Schwering CMC, Schrama JW, Vereijken PFG, Vesseur PC, Everts H *et al.* (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.

Summary

The second half of the 20th century saw a tremendous increase in the performance level of meat-type animals, such as broilers and pigs, especially due to genetic improvements. For broilers, the time from hatch to a body weight (BW) of about 1.8 kg decreased from 100 d in the 1950s to 30 d in the 2000s. Similarly, but to a lesser extent, the average daily gain in pigs nearly doubled between the years 1900 and 2000, with the major gains being achieved after 1950. Consequently, for pigs, the time from birth to a BW of 120 kg decreased from about 340 d in the 1900s to 170 d in the 2000s. These data show that the relative importance of the first weeks of life has tripled for broilers over a period of about 50 years and has doubled for pigs over a period of 100 years. As such, the relative importance of animal husbandry and nutrition during the first weeks after weaning and after hatch, with respect to total life time performance, has increased considerably over the past 50 years. This process is likely to continue over the next several decades. In parallel with the improved performance, broilers have also become more susceptible to ascites and leg problems. For pigs, the weaning age has decreased from about 8 wk in the 1950s to 3 to 4 wk today. Weaning pigs at such a young age is associated with abrupt social, environmental and dietary changes, which results in high levels of stress. About ten percent of pigs do not ingest any feed during the first 48 h after weaning, and most others have a low feed intake. As a result, pigs are highly susceptible to enteric diseases and infections after weaning.

Many physiological, nutritional, and environmental changes occur early in life of broilers and pigs. These changes are most marked in the small-intestinal architecture, weight, and function. Previous research that was discussed in Chapter 1 showed that in broilers, the relative small-intestinal weight and villous height increase rapidly during the first week after hatch. After this first week, the relative weight decreases gradually, but the villous height continues to increase. At 4 d after weaning, villous height in pigs decreases to about 60% of the pre-weaning height. Two weeks after weaning, the height recovers to similar values as in unweaned control animals independent of the weaning age. Small-intestinal development after weaning and after hatch consistently deteriorates at low feed intake levels. In addition, small-intestinal weights and villous height are stimulated by dietary protein. This is more pronounced during the first 2 wk after hatch or weaning than it is later in life. The fundamental functions of the small intestine are to ensure nutrient absorption, to provide a barrier against potential harmful bacteria and toxins, and to contribute to overall health as the biggest immunological organ in the body. As such, many health problems can occur or may have their origin early in the life of these animals when the small-intestinal function is still developing. This stresses the importance to applying an optimal nutritional strategy in these phases of life to achieve optimal small-intestinal development.

Nutrient requirement studies conducted on these animals have largely overlooked the very young animal (first weeks after hatch or after weaning). It is therefore logical that there are still gaps in our knowledge of the nutrition of these animals during this particular stage of life. This makes it likely that the performance of these meat type animals can be further improved at a very young age by optimising the nutrition. However, in consideration of the many health problems in these animals and the large changes in small-intestinal architecture and function, nutrient requirement studies in these phases in broilers and pigs should not only focus on the aspects of efficient production, but should also pay attention to health and organ function. The objective of this thesis is to improve intestinal development and performance of pigs after weaning and young broilers by optimising the

nutrient composition of the diet. The first aim is to gain more insight into the anatomical and functional changes of the small intestine after hatch in broilers and after weaning in pigs. The second aim is to investigate the effect of the level of feed intake and the effect of protein nutrition on performance, small-intestinal development, and health in broilers after hatch and pigs after weaning. For broilers, the studies reported in this thesis concentrate on the further improvement of performance and suppression of metabolic disorders in high performing birds. For pigs after weaning, the focus is on the optimal nutritional support for intestinal barrier and absorption functions of pigs at low levels of feed intake.

In the experiments reported in Chapter 2, the amino acid (AA) requirements to maximise BW gain and gain to feed ratio (G:F) in broilers were studied. To evaluate this, an ideal protein (IP) dose response in the starter phase and factorial arrangements combining adequate or high IP concentrations in starter and grower diets with low, adequate, or high IP concentrations in finisher diets were carried out with male and female broilers. Enhanced dietary IP concentrations in the starter diet increased BW gain in the starter phase and in the consecutive grower phase. Moreover, it was shown that the effects of enhanced IP concentrations in the starter diet on BW gain of broilers were more marked than the effects of enhanced IP concentrations in the grower and finisher diets.

An experiment was subsequently designed to study the effects of enhanced dietary IP concentrations in the starter phase and feed intake restriction early in life in more detail (Chapter 3). A control treatment (100% IP) was compared with a treatment with 30% extra IP, a treatment with a daily adjustment of the dietary AA concentration and profile, and a feed restriction treatment. The protein treatments were applied from 0–14 d of age. The feed restriction was applied from 4–21 d of age. The restriction was 15% from d 4–14 of age and diminished with equal daily steps thereafter to 5% at 21 d of age. The birds were weighed and dissected for evaluation of the small intestine weights at 6, 9, 14, and 36 d of age. In agreement with the studies in Chapter 2, BW gain in the starter phase and in the consecutive grower phase was substantially improved by enhanced IP concentrations in the starter diet. Moreover, the results showed that the birds hardly responded in terms of BW gain to dietary IP increment during the first 3 d after hatch. However, in the next consecutive 3 d, BW gain improved substantially with enhanced dietary IP concentrations. In addition, increasing the dietary IP concentrations by 30% improved G:F during the first 3 d after hatch by only 8%, whereas in the next 3 consecutive days, G:F was improved by 20% with this treatment. These results showed that with respect to the fine tuning of the diet, the first 3 d after hatch can be considered as a separate time period in order to maximise performance and nutrient efficiency. This highlights the importance of studying the physiological requirements during these first 3 d after hatch in more detail in future studies. A 30% increase in dietary IP increased the duodenum weight between 6 and 9 d of age. This was not further increased by the daily optimisation of the dietary AA concentration and profile. The increased duodenum weights coincided with an improved BW gain. This indicates that duodenum weight may be important in facilitating BW gain in young broilers. However, it is not possible to conclude whether the enhanced dietary IP concentrations stimulated small-intestinal development with a subsequent effect on BW gain, or whether the enhanced BW gain has stimulated the small-intestinal development.

Mortality in relation to leg problems was markedly decreased due to feed intake restriction early in life (Chapter 3). In addition, enhanced dietary IP concentrations decreased mortality due to leg problems in Ross broilers, but not in Cobb broilers. In the literature, it has been assumed that growth reduction of broilers early in life is particularly

effective in reducing metabolic disorders. However, as shown in Chapter 3, BW gain increased with the enhanced IP treatments for Ross broilers, whereas mortality due to leg problems decreased. This suggests that the decrease in BW gain does not directly prevent metabolic disorders in broilers given restricted feed. However, it may be a physiological side effect of the reduced BW gain or of the feed intake restriction that causes the reduction in metabolic disorders. The feed intake restriction reduced the relative spleen weight, and the spleen is an immunological active organ. Therefore, this finding suggests that feed intake restriction suppresses immune reactivity. In the human nutrition field, the immune system and inflammatory responses have been shown to have an important role in metabolic diseases, such as type 2 diabetes and atherosclerosis. Similarly, it can be hypothesised that metabolic disorders, such as ascites and lameness, are related to the negative side effects of inflammatory responses in broilers.

In Chapter 4, data from the literature with respect to the small-intestinal barrier and absorptive function of pigs after weaning were discussed. Paracellular barrier function (barrier function of the tight junctions) and active absorption decrease when pigs are weaned at 3 wk of age or earlier. However, when weaned at 4 wk of age or later, the barrier function is less affected, and active absorption is not affected or is increased, indicating a more mature small intestine. The transcellular transport of macromolecules as well as passive transcellular absorption decreases after weaning. This may reflect a natural intestinal maturation process that is enhanced by the weaning process, which prevents the pig from an antigen overload. Therefore, in general the disturbance of the barrier function after weaning is restricted to the tight junctions, and transcellular barrier function may even be improved after weaning. In addition, other studies have shown that the detrimental effect of weaning stress on intestinal paracellular barrier function disappeared when mast cells were blocked. This suggests that the immune-system has a negative effect on small-intestinal barrier function after weaning. Moreover, the review presented in Chapter 4 showed that adequate feed intake levels after weaning prevent the loss of the small-intestinal barrier function.

The experiment described in Chapter 5 was designed to study the barrier function of the small intestine of pigs over time after weaning. The aim was to relate paracellular barrier function (measured by lactulose recovery in the urine) with macromolecular transport [measured by horseradish peroxidase (HRP) using Ussing chambers] and bacterial translocation to assess whether lactulose recovery is related to possible causes of infection and disease. Forty barrows were weaned (d 0) at a mean age of 19 d, fitted with urine collection bags, and individually housed. Pigs were dosed by oral gavage with a marker solution containing lactulose (disaccharide) and the monosaccharides L-rhamnose, 3-*O*-methylglucose, and D-xylose at 2 h and at 4, 8, and 12 d after weaning. The day after each permeability test, the intestines of 10 pigs were dissected to determine bacterial translocation to the mesenteric lymph nodes and jejunal permeability for HRP in Ussing chambers. Recovery of lactulose in the urine was greater at 4, 8, and 12 d after weaning compared with the first day after weaning, and was negatively correlated with feed intake. The mean translocation of aerobic bacteria to the mesenteric lymph nodes was greater at 5 and 13 d after weaning compared with d 1. Although both lactulose recovery and bacterial translocation increased over time after weaning, lactulose recovery did not correlate with the permeability for HRP or bacterial translocation within a pig. Therefore, it was concluded that lactulose recovery in the urine of pigs after weaning is not associated with risk factors

for infection. However, it does appear to be possible to measure paracellular barrier function with orally administered lactulose in pigs shortly after weaning.

The results of the study reported in Chapter 5 and other studies in the literature have shown that low feed intake in pigs after weaning is associated with villous atrophy and with the loss of small-intestinal barrier function. It is assumed that these effects of low feed intake on small-intestinal integrity are due to the low energy intake of the pigs. However, there are no studies that have distinguished between the effects of non-protein energy and protein on the small-intestinal architecture and barrier function. In the study presented in Chapter 6, the effects of feed deprivation (FD) for 4 d after weaning was compared with feeding either starch and dextrose (StD) or protein and dextrose (PrD). Small-intestinal mucosal weight was determined at 4 d after weaning, and small-intestinal barrier function was measured by urinary lactulose recovery after an intragastrical dose at 3 d after weaning. Mucosa weights were greater for the PrD pigs compared to the FD pigs, while the mucosal weight of the StD pigs was intermediate. Lactulose recovery was lower for the StD pigs than for the FD pigs and was not significantly different between PrD and FD pigs. Thus, dietary protein with dextrose stimulates an increase in mucosal weight. However, protein does not have a substantial effect on small-intestinal barrier function, whereas dietary starch improves small-intestinal barrier function. This suggests that at low feed intake, dietary protein concentrations after weaning are of minor importance for small-intestinal barrier function.

In conclusion, the research reported in this thesis shows that enhancing dietary IP concentrations in broiler starter diets are highly effective in stimulating overall broiler performance. Moreover, dietary protein is a potent stimulator for growth of the proximal small intestine in broilers and of the small-intestinal mucosa in pigs. In young broilers, a greater relative small-intestinal weight is associated with a greater BW gain. However, this thesis does not provide clear insight into the functional changes of the small intestine in broilers after hatch. Although mucosal mass of the small intestine in pigs was enhanced by dietary protein, this did not support the maintenance of barrier function of the tight junctions of the small intestine after weaning. This shows that mucosal mass and luminal protein are of minor importance for small-intestinal barrier function in pigs after weaning. In contrast, luminal carbohydrate supply or energy level is highly important for maintaining small-intestinal barrier function.

Based on the findings of this thesis, 3 areas are suggested for further study. First, it is important to improve our understanding of the physiological nutrient needs in broilers during the first 6 d immediately after hatch. These needs seem to change markedly over a short time frame, and few studies have explored this area. Second, the role of the detrimental effects of an over or incorrectly stimulated immune system on animal health should be investigated in both young broilers and pigs after weaning. Third, the impact of optimal nutritional support through drinking water to pigs after weaning with no or a low feed intake should be investigated in more detail. With this respect, in this thesis research was initiated to explore the role of carbohydrate and protein.

Samenvatting

Tijdens de tweede helft van de 20e eeuw is het prestatieniveau van vleeskuikens en varkens enorm toegenomen. Deze verbeterde dierprestaties zijn vooral gerelateerd aan genetische veranderingen. Voor vleeskuikens is de tijd tussen de uitkomst van het ei en het bereiken van een lichaamsgewicht van ongeveer 1,8 kg afgenomen van 100 dagen in de jaren-50 tot 30 dagen in 2000. Idem, maar in mindere mate, is de gemiddelde daggroei bij varkens bijna verdubbeld tussen het begin en het einde van de vorige eeuw, waarbij het merendeel van de verbeterde groei is gerealiseerd in de tweede helft van de eeuw. Als gevolg hiervan is voor varkens de tijd vanaf de geboorte tot een lichaamsgewicht van 120 kg gedaald van ongeveer 340 dagen in het jaar 1900 tot ongeveer 170 dagen in 2000. Deze gegevens tonen aan dat het relatieve belang van de eerste weken van het leven (uitgedrukt als percentage van de totale levensduur) voor vleeskuikens is verdrievoudigd over een periode van ongeveer 50 jaar en dat voor varkens dit belang is verdubbeld over een periode van ongeveer 100 jaar. Hierdoor is het belang van de verzorging en voeding van deze dieren tijdens de eerste weken na het spenen (het verwijderen van de zeug van de biggen) en na de uitkomst, m.b.t. de prestaties tijdens de totale levensduur, in de afgelopen 50 jaar sterk toegenomen. Dit proces zal zich waarschijnlijk voortzetten in de komende decennia. De verbeterde dierprestaties van vleeskuikens is echter samengegaan met een hogere gevoeligheid voor buikwaterzucht en pootproblemen. Voor varkens is de speenleeftijd gedaald van ongeveer 8 weken in de jaren-50, tot 3 à 4 weken op dit moment. Het spenen van varkens op een dergelijk jonge leeftijd valt samen met abrupte sociale-, milieu- en voerveranderingen, wat resulteert in een hoge mate van stress. Ongeveer tien procent van de varkens vreet niet gedurende de eerste 48 uur na het spenen en de meeste andere varkens hebben een lage voeropname. Als gevolg hiervan zijn varkens na het spenen zeer gevoelig voor darmziekten en infecties.

Bij jonge vleeskuikens en varkens vinden vele fysiologische-, voedings- en milieuveranderingen plaats. Deze veranderingen zijn het opvallendst in de vlokken, het gewicht en de functie van de dunne darm. Uit een literatuurstudie, die is beschreven in hoofdstuk 1, bleek dat bij vleeskuikens het relatieve gewicht van de dunne darm (als percentage van het totale lichaamsgewicht) en de hoogte van de darmvlokken snel stijgt tijdens de eerste week na de uitkomst. Na deze eerste week neemt het relatieve gewicht van de dunne darm geleidelijk af, maar blijft de hoogte van de darmvlokken toenemen. Vier dagen na het spenen is bij varkens de hoogte van de darmvlokken afgenomen tot ongeveer 60% van de hoogte vlak voor het spenen. Twee weken na het spenen is de hoogte van de darmvlokken weer hersteld naar een niveau gelijk aan dat van niet-gespeende controledieren. Dit herstel t.o.v. niet-gespeende controledieren is onafhankelijk van de speenleeftijd van de biggetjes. Bij lage voeropnameniveaus na het spenen of na de uitkomst blijft de ontwikkeling van de dunne darm achter bij de ontwikkeling van de dunne darm van goed vretende dieren. Het relatieve gewicht van de dunne darm en de hoogte van de darmvlokken worden gestimuleerd door verhoging van het eiwitniveau in het voer. Deze effecten van eiwit en voeropname niveau zijn duidelijker tijdens de eerste 2 weken na de uitkomst of na het spenen dan later in het leven. De dunne darm heeft 3 belangrijke functies; (1) het absorberen van voedingsstoffen; (2) voorkomen dat potentiële schadelijke bacteriën en gifstoffen het lichaam binnendringen; (3) als het grootste immunologische orgaan in het lichaam, zorg dragen voor de algemene gezondheid van het dier. Hieruit volgt dat vele gezondheidsproblemen optreden of hun oorsprong vinden in het vroege leven van

kuikens en biggetjes, als de dunne darm nog in ontwikkeling is. Dit benadrukt het belang van een optimale voedingsstrategie in deze levensfasen voor het behalen van de best mogelijke ontwikkeling van de dunne darm.

Studies voor het bepalen van de behoefte aan voedingstoffen bij deze dieren zijn grotendeels voorbijgegaan aan de zeer jonge dieren (de eerste weken na de uitkomst of na het spenen). Het is dus logisch dat er nog steeds leemten zijn in onze kennis over de voeding van kuikens en biggen in deze levensfase. Dit maakt het waarschijnlijk dat de prestaties van jonge vleeskuikens en biggen nog verder kan worden verbeterd door het optimaliseren van de voeding. Men zou echter rekening moeten houden met de vele gezondheidsproblemen bij deze dieren en de grote veranderingen in de darmvlokken en de functie van de dunne darm. Daarom zouden studies naar de behoefte van voedingsstoffen in deze levensfase bij kuikens en biggen zich niet uitsluitend moeten richten op efficiënte productie, maar ook op de gezondheid van de dieren en de functionaliteit van de organen. Dit proefschrift beoogde om de ontwikkeling van de dunne darm en de prestaties van biggen na het spenen en van jonge vleeskuikens na de uitkomst te verbeteren door het optimaliseren van het aanbod van voedingstoffen. Het eerste doel was om meer inzicht te krijgen in de veranderingen van de structuur en het functioneren van de dunne darm in deze levensfasen. Het tweede doel was om het effect van voeropnameniveau en het effect van eiwitvoeding op dierprestaties, ontwikkeling van de dunne darm en diergezondheid te onderzoeken in deze dieren. Bij vleeskuikens concentreerden de studies in dit proefschrift zich op verdere verbetering van de prestaties en het onderdrukken van stofwisselingsstoornissen bij kuikens op een hoog prestatieniveau. Voor varkens na het spenen lag de nadruk op het met de juiste voedingsstoffen ondersteunen van de barrièrefunctie (tegenhouden van schadelijke stoffen en bacteriën) en de absorptiefunctie van de dunne darm bij varkens met een laag voeropname niveau.

In de experimenten die beschreven zijn in hoofdstuk 2 zijn de aminozuurbehoeften bij kuikens onderzocht om groei en voerefficiëntie (gram groei per gram voer) te maximaliseren. Hierbij zijn oplopende gehalten van een uitgebalanceerd eiwit profiel (optimale aminozuur profiel voor de groei van de kuikens) in het voer in de startfase (0–2 weken leeftijd) getest. Daarnaast is een factoriële opzet getest met een standaard of hoog gehalte van een uitgebalanceerd eiwit profiel (voor vereenvoudiging zal hieronder dit worden aangegeven met “eiwit”) in het startvoer en groeivoer (2–4 weken leeftijd) en een laag, standaard, of hoog eiwit gehalte in het afmestvoer (4–5 weken leeftijd) bij zowel mannelijke als vrouwelijke vleeskuikens. Het verhogen van het eiwit gehalte in het startvoer verbeterde de groei van de kuikens in de startfase en in de opvolgende groeifase. Bovendien werd aangetoond dat de effecten op groei van een hoog eiwit gehalte in het startvoer meer uitgesproken zijn dan de effecten van een hoog eiwit gehalte in het groeivoer en afmestvoer.

Vervolgens is een experiment opgezet waarbij de effecten van een verhoging van het gehalte van het uitgebalanceerde eiwit profiel (wederom aangegeven als “eiwit”) in het startvoer en het effect van voeropname beperking bij jonge kuikens in meer detail is bestudeerd (hoofdstuk 3). Een controle behandeling (standaard eiwit gehalte) werd vergeleken met een behandeling met 30% extra eiwit, een behandeling met dagelijkse aanpassing van de aminozuurgehalten en het aminozuurprofiel in het voer en een behandeling met beperking van de dagelijkse voergift. De voeropname beperking werd toegepast van 4–21 dagen leeftijd. De beperking was 15% van 4–14 dagen leeftijd en werd verminderd met gelijke dagelijkse stappen naar 5% op 21 dagen leeftijd. Deze behandelingen werden zowel bij Ross als bij Cobb kuikens toegepast omdat deze rassen

verschillen in de gevoeligheid voor pootproblemen. Op 6, 9, 14 en 36 dagen leeftijd werden de kuikens gewogen en werden de gewichten van de dunne darm bepaald. In lijn met de proeven in hoofdstuk 2 werd de groei in de startfase en de groei in de opvolgende groeifase aanzienlijk verbeterd door het verhoogde eiwit gehalte in het startvoer. De kuikens bleken in de eerste 3 dagen van de startfase nauwelijks harder te groeien op het voer met het verhoogde eiwitgehalte t.o.v. het controlevoer. Echter, in de opvolgende 3 dagen verbeterde de groei aanzienlijk met een hoger eiwitgehalte in het voer. Bovendien resulteerde het met 30% verhogen van het eiwitgehalte in het voer tot een 8% betere voerefficiëntie in de eerste 3 dagen van de startfase, terwijl dit in de eerst volgende 3 dagen een verbetering van maar liefst 20% gaf. Dit toonde aan dat voor het optimaliseren van het voer om de efficiëntie en de dierprestaties te maximaliseren, men de eerste 3 dagen na de uitkomst moet zien als een aparte periode. Dit benadrukt het belang om de behoeften aan voedingsstoffen gedurende deze eerste 3 dagen na de uitkomst in meer detail te onderzoeken. Dertig procent extra eiwit in het voer verhoogde het dunne darm gewicht op 6 en 9 dagen leeftijd. Het toegenomen dunne darm gewicht ging samen met een verhoging van het lichaamsgewicht. Dit is een indicatie dat het dunne darm gewicht van belang kan zijn voor het tot stand komen van deze hogere lichaamsgewichten. Het is echter niet mogelijk te concluderen of het verhoogde eiwitgehalte in het voer de dunne darm ontwikkeling heeft gestimuleerd met als gevolg een hoger lichaamsgewicht, of dat juist het hogere lichaamsgewicht de ontwikkeling van de dunne darm heeft gestimuleerd.

De aan pootproblemen gerelateerde sterfte was sterk gedaald als gevolg van voeropname beperking bij vleeskuikens (hoofdstuk 3). Bovendien daalde de sterfte ten gevolge van pootproblemen in Ross vleeskuikens, maar niet in Cobb vleeskuikens als gevolg van een hoger eiwit gehalte in het voer. In de literatuur werd tot op heden aangenomen dat voornamelijk de verminderde groei van vleeskuikens tijdens het vroege leven zeer effectief is in het verminderen van stofwisselingsstoornissen. In hoofdstuk 3 bleek echter dat voor Ross vleeskuikens de groei toenam als het eiwitgehalte in het voer werd verhoogd, terwijl de sterfte ten gevolge van pootproblemen afnam door deze behandeling. Dit suggereert dat het niet direct de lagere groei is die zorgt voor een vermindering van stofwisselingsproblemen bij de kuikens met voeropname beperking. Het kan een fysiologisch neveneffect zijn van de lagere groei of de lagere voeropname die zorgt voor minder stofwisselingsproblemen. De voeropname beperking verminderde het relatieve gewicht van de milt en de milt is een immunologisch actief orgaan. Dit suggereert dus dat de voeropname beperking de activiteit van het immuunsysteem onderdrukt. In de literatuur is aangetoond dat het immuunsysteem en ontstekingsreacties een belangrijke rol spelen bij stofwisselingsziekten zoals aderverkalking en suikerziekte. Zo kan ook worden verondersteld dat bij vleeskuikens stofwisselingsaandoeningen, zoals buikwaterzucht en pootproblemen mogelijk gerelateerd zijn aan de negatieve neveneffecten van ontstekingsreacties.

In hoofdstuk 4 is de beschikbare literatuur besproken die betrekking heeft op de barrière- en absorptiefunctie van de dunne darm van varkens na het spenen. De barrièrefunctie tussen de cellen van het darmepitheel (laatste laag cellen die de scheiding vormt tussen de darminhoud en de rest van het lichaam) en de actieve absorptie van voedingsstoffen (opname van voedingsstoffen die gereguleerd wordt door specifieke transportsystemen in de epitheelcellen) nemen af wanneer varkens worden gespeend op een leeftijd van 3 weken of jonger. De barrièrefunctie wordt echter in mindere mate aangetast door het speenproces als de varkens gespeend worden op een leeftijd van 4 weken of ouder. Daarnaast wordt op deze hogere speenleeftijd de actieve absorptie van

voedingsstoffen na het spenen niet beïnvloed of gaat zelfs omhoog na het spenen. Deze waarnemingen wijzen erop dat de dunne darm bij 4 weken oude biggen meer gerijpt is dan bij 3 weken oude biggen. Na het spenen daalt meestal de opnamen van grote moleculen door het membraam van de epitheelcellen en daalt de passieve absorptie (opname van voedingstoffen door de epitheelcellen door willekeurige beweging van de voedingsstoffen). Dit kan wijzen op een natuurlijk darmrijpingsproces dat wordt versterkt door het speenproces en voorkomt dat het varken wordt overbelast met antigenen (moleculen die een afweerreactie kunnen opwekken). Dus meestal lijkt de verstoring van de barrièrefunctie van de darm zich te beperken tot de tussen-cel barrièrefunctie. De barrièrefunctie van de membraam van de epitheelcellen lijkt zelfs te verbeteren na het spenen. Uit de literatuur bleek ook dat het schadelijke effect van speenstress op de tussen-cel barrièrefunctie verdween als de werking van mestcellen (specifieke immuuncellen) chemisch werd geblokkeerd. Dit suggereert een negatieve rol van het immuunsysteem met betrekking tot de verstoorde barrièrefunctie van de dunne darm na het spenen. Bovendien bleek uit de literatuur dat de barrièrefunctie van de dunne darm na het spenen niet wordt aangetast als de voeropname voldoende hoog is.

De proef die is beschreven in hoofdstuk 5 was ontworpen om de barrièrefunctie van de dunne darm in de tijd na het spenen te onderzoeken. Het doel van de proef was om te kijken of de doorlaatbaarheid van de dunne darm voor lactulose (een dubbelsuiker) gerelateerd is aan mogelijke oorzaken van infecties en ziekten. Daarom is de doorlaatbaarheid voor lactulose vergeleken met de doorlaatbaarheid van de darm voor een een peroxidase-enzym uit mierikswortel (een groot molecuul) en met de aanwezigheid van bacteriën in de lymfeklieren van de dunne darm en in de lever. De varkens kregen op 2 uur en op 4, 8 en 12 dagen na het spenen via een sonde een oplossing met lactulose toegediend. Vervolgens werd de urine over een periode van een dag verzameld om te kijken hoeveel van de toegediende lactulose in de urine terecht was gekomen. De hoeveelheid lactulose in de urine is een maat voor de doorlaatbaarheid van de darm voor lactulose. De dag na elke lactulose-test werden 10 varkens gedood om de aanwezigheid van bacteriën in de lymfeklieren en in de lever te bepalen en om in het laboratorium de doorlaatbaarheid van een stukje van de dunne darm voor het het peroxidase-enzym te bepalen. Op 4, 8 en 12 dagen na het spenen zat er meer lactulose in de urine dan op de eerste dag na het spenen. De hoeveelheid lactulose in de urine bleek hoger te zijn bij dieren met een lage voeropname dan bij dieren met een hoge voeropname. Het aantal zuurstofbehoevende bacteriën in de lymfklieren was groter op 5 en 13 dagen na het spenen dan op de eerste dag na het spenen. De hoeveelheid lactulose in de urine bleek geen relatie te vertonen met de doorlaatbaarheid van de dunne darm voor het peroxidase-enzym en ook niet met de hoeveelheid bacteriën in de lymfeklieren en in de lever. Hoewel zowel de hoeveelheid lactulose in de urine als het aantal bacteriën in de lymfeklieren na het spenen toenemen, vertoonde de hoeveelheid lactulose in de urine binnen de biggen geen relatie met de hoeveelheid bacteriën in de lymfeklieren en de lever en met de doorlaatbaarheid van de darm voor het peroxidase-enzym. Daarom werd geconcludeerd dat de doorlaatbaarheid van de darm voor lactulose na het spenen niet direct gerelateerd is aan risicofactoren voor infecties.

Uit de resultaten van de proef die is beschreven in hoofdstuk 5 en uit gegevens in de literatuur is gebleken dat een lage voeropname bij varkens na het spenen samengaat met de afbraak van darmvlokken en met een verslechtering van de barrièrefunctie van de dunne darm. Verondersteld wordt dat deze negatieve effecten van lage voeropname op de

kwaliteit van de dunne darm zijn te wijten aan de lage energieopname van de varkens. Er is echter geen literatuur die onderscheid maakt tussen de effecten van niet-eiwit energie en eiwit op de vlokken en op de barrièrefunctie van de dunne darm. In de proef die is beschreven in hoofdstuk 6 zijn de effecten van voeronthouding voor een periode van 4 dagen na het spenen vergeleken met het voeren van zetmeel met dextrose of eiwit met dextrose. Het gewicht van het slijmvlies van de dunne darm werd bepaald op 4 dagen na het spenen. De barrièrefunctie van de dunne darm werd gemeten door het toedienen van lactulose via de bek op 3 dagen na spenen en door vervolgens te meten hoeveel van die lactulose uiteindelijk in de urine van de big is terecht gekomen. Het slijmvlies van de varkens die eiwit met dextrose kregen verstrekt was zwaarder dan het slijmvlies van de varkens zonder voer, terwijl het gewicht van het slijmvlies van de varkens op het zetmeel met dextrose voer hier tussenin zat. De hoeveelheid lactulose in de urine van de biggetjes op het zetmeel met dextrose voer was lager dan voor de gevaste varkens en was niet wezenlijk verschillend tussen de eiwit met dextrose gevoerde en de gevaste varkens. Dus voeding met eiwit en dextrose stimuleert het gewicht van het slijmvlies van de dunne darm. Echter, eiwit heeft geen wezenlijke invloed op de barrièrefunctie van de dunne darm, terwijl het zetmeel in het voer de barrièrefunctie duidelijk verbetert t.o.v. gevaste varkens. Dit suggereert dat bij een lage voeropname na het spenen het eiwitgehalte in het voer van ondergeschikt belang is voor de barrièrefunctie van de dunne darm.

Het onderzoek zoals beschreven in dit proefschrift laat zien dat het verhogen van het eiwit in het startvoer van vleeskuikens zeer effectief is in het verbeteren van de prestaties van de kuikens. Bovendien is eiwit in het voer een krachtige stimulator voor de groei van het begin van de dunne darm bij vleeskuikens en van het darmslijmvlies bij varkens. Bij jonge vleeskuikens blijkt een hoger relatief dunne darm gewicht samen te gaan met een hogere groei van de kuikens. Dit proefschrift geeft echter geen duidelijk inzicht in de functionele veranderingen van de dunne darm van vleeskuikens na de uitkomst van het ei. Hoewel het gewicht van het slijmvlies van de dunne darm bij varkens werd verhoogd door het eiwit in het voer, bleek dit eiwit geen ondersteuning te geven aan de barrièrefunctie tussen de epitheelcellen van de dunne darm. Dit toont aan dat het gewicht van het slijmvlies en het eiwitgehalte in het voer van ondergeschikt belang zijn voor de barrièrefunctie van de dunne darm bij varkens na het spenen. Daarentegen bleken koolhydraten in het voer of een voldoende hoge energieopname wel belangrijk te zijn voor het behouden van de barrièrefunctie van de dunne darm na het spenen.

Op basis van dit proefschrift, worden drie onderwerpen voor verder onderzoek voorgesteld. In de eerste plaats is het belangrijk om voor vleeskuikens onze kennis te verbeteren van de behoeften aan voedingsstoffen gedurende de eerste 6 dagen na de uitkomst. Deze behoeften lijken over deze korte periode sterk te veranderen en dit is tot op heden nog nauwelijks onderzocht. Ten tweede, zou zowel voor jonge vleeskuikens als voor varkens na het spenen, de mogelijk schadelijke gevolgen op de gezondheid van een overmatig of verkeerd geactiveerd immuunsysteem moeten worden onderzocht. Ten derde zou bij varkens met een lage voeropname na het spenen het effect van ondersteuning met voedingstoffen via het drinkwater meer gedetailleerd moeten worden onderzocht.

Curriculum Vitae

About the author

Peter Jan Antonius Wijtten was born on 22 August 1969 on the Dutch island of Texel. He grew up at his parents' farm with sows, fattening pigs, and dairy cows. In 1985 he graduated from the MAVO secondary school in Den Burg. In September of that year, he enrolled at the Secondary Agriculture School in Raalte where he graduated in 1988 in Cattle farming. Subsequently, he obtained his BSc in Cattle farming in 1993 at the Agricultural College in Dronten with a thesis that focused on nutrition of dairy cows. After his graduation, Peter worked at a large dairy farm in Wales for 6 months. In 1994, he started an MSc in Animal Nutrition at Wageningen University, where he graduated *cum laude* in 1997. As a part of his MSc study, he worked on a project at the Department of Human and Animal Physiology with sheep and on a project with broilers for the Animal Nutrition Department that was carried out at Provimi. In January 1997, he started working for Provimi as a research nutritionist, where he was responsible for nutritional research of poultry and pigs. During that time, he became fascinated about the nutrition of young animals, which finally culminated in this thesis. Since January 2010, Peter has had the overall responsibility for the Provimi Research Centre 'De Viersprong' in Velddriel, The Netherlands. Since April 2011, he has combined this function with the position of Associate Science Director Poultry for Provimi's Global Poultry Team.

Peter Jan Antonius Wijtten werd geboren op 22 augustus 1969 op Texel. Zijn ouders hadden een boerderij met zeugen, vleesvarkens en melkkoeien. In 1985 behaalde hij zijn diploma aan de MAVO in Den Burg. In september van dat jaar begon hij aan de Middelbare Landbouwschool in Raalte waar hij in 1988 afgestudeerd in de richting rundveehouderij. Vervolgens studeerde hij in 1993 af aan de Christelijk Agrarische Hogeschool in Dronten met een scriptie over de voeding van melkkoeien. Na zijn afstuderen werkte Peter 6 maanden op een groot melkveebedrijf in Wales. In 1994 begon hij zijn studie aan de universiteit in Wageningen, waar hij in 1997 *cum laude* afstudeerde. Als onderdeel van zijn studie werkte hij aan een afstudeerproject met schapen bij de vakgroep Fysiologie van Mens en Dier en aan een tweede afstudeerproject met vleeskuikens voor de vakgroep Veevoeding. Dit laatste project werd uitgevoerd bij Provimi. In januari 1997 begon hij als voedingsonderzoeker bij Provimi. In die functie was hij verantwoordelijk voor zowel onderzoek met pluimvee als varkens. In die tijd raakte hij geboeid door de voeding van jonge dieren. Uiteindelijk resulteerde dat in dit proefschrift. Sinds januari 2010 heeft Peter de algemene verantwoordelijkheid voor Provimi's Research Centre 'De Viersprong' in Velddriel, Nederland. Sinds april 2011 combineert hij deze functie met de functie van Associate Science Director Poultry voor Provimi's wereldwijde pluimvee team.

Publications

Refereed Scientific Publications

Bonekamp RPRT, Lemme A, Wijtten PJA & Sparla JKWM (2010) Effects of amino acids on egg number and egg mass of brown (heavy breed) and white (light breed) laying hens. *Poult Sci* **89**, 522-529.

Lemme A, Wijtten PJ, Wichen Jv, Petri A & Langhout DJ (2006) Responses of male growing broilers to increasing levels of balanced protein offered as coarse mash or pellets of varying quality. *Poult Sci* **85**, 721-730.

Wijtten PJA, Lemme A & Langhout DJ (2004) Effects of different dietary ideal protein levels on male and female broiler performance during different phases of life: Single phase effects, carryover effects, and interactions between phases. *Poult Sci* **83**, 2005-2015.

Wijtten PJA, Prak R, Lemme A & Langhout DJ (2004) Effect of different dietary ideal protein concentrations on broiler performance. *Br Poult Sci* **45**, 504-511.

Wijtten PJA, Hangoor E, Sparla JK & Verstegen MW (2010) Dietary amino acid levels and feed restriction affect small intestinal development, mortality, and weight gain of male broilers. *Poult Sci* **89**, 1424-1439.

Wijtten PJA, van der Meulen J & Verstegen MWA (2011) Intestinal barrier function and absorption in pigs after weaning: a review. *Br J Nutr* **105**, 967-981.

Wijtten PJA, Verstijnen JJ, Van Kempen TATG, Perdok HB, Gort G & Verstegen MWA (2011) Lactulose as a marker of intestinal barrier function in pigs after weaning. *J Anim Sci* **89**, 1347-1357.

Contributions to Conferences and Symposia

Bonekamp RPRT, Lemme A, Wijtten PJA & Sparla JKWM (2007) Effects of amino acids on egg number and egg mass of brown (heavy) and white (light) hens. *16th European Symposium on Poultry Nutrition*. Strasbourg, France. pp. 113-116.

De Lange LLM & Wijtten PJA (2008) Profiling the microbiota in the gastrointestinal tract and its relation to feed utilisation in broilers. *33rd Animal Nutrition Research Forum*. Wageningen, The Netherlands. pp. 85-86.

De Lange LLM & Wijtten PJA (2010) Microbial profiles in the gastro-intestinal tract of broilers and its relation to feed efficiency. *21st Australian Poultry Science Symposium*. Sydney, Australia. pp. 191-194.

Hangoor E, Wijtten PJA, Perdok HB & Sparla JKWM (2008) The effect of particle size distribution on gizzard development and broiler performance. *33rd Animal Nutrition Research Forum*. Wageningen, The Netherlands. pp. 81-82.

Helmbrecht A, Hangoor E, Wijtten PJA, Sparla JKWM & Lemme A (2010) Optimal sulphur amino acids to lysine ratio in grower phase (day 14-35) in Ross 308 broilers *64th Conference of the Society of Nutrition Physiology*. Göttingen, Germany. pp. 24.

Hoehler D, Pack M, Wijtten PJA, Prak R & Langhout DJ (2001) Effects of varying dietary protein levels based on the ideal amino acid profile on broiler performance. *International Poultry Scientific Forum*. Atlanta, GA. pp. 4.

Hoehler D, Wijtten PJA, Langhout DJ, Lemme A & Petri A (2002) Effects of varying dietary ideal amino acid profile levels on broiler performance in different periods. *The Southern Poultry Science Society, 23rd Annual Meeting*. Atlanta, GA. pp. 4.

Hoehler D, Lemme A, Wijtten PJA & Van Wichen J (2005) Interactions between ideal protein level and feed form in broiler production. *International Poultry Scientific Forum*. Atlanta, GA. pp. 116.

Klein-Hessling H, Langhout DJ & Wijtten PJA (2004) Volatile fatty acids and essential oils (biacid) improve technical performance of broilers. *16th Australian Poultry Science Symposium*. Sydney, Australia. pp. 183-186.

Koch F, Wijtten PJA, Lemme A & Langhout DJ (2002) Impact of a balanced amino acid profile on broiler performance. *Veterinarija ir Zootechnika*. Kaunas, Lithuania. pp. 70-75.

Langhout DJ & Wijtten PJA (2002) Factores Nutricionales y la conformación de la canal. *Tecnologia avipecuaria en latinoamerica / año 15*. Mexico. pp. 6-10.

Langhout DJ & Wijtten PJA (2005) Efeitos da nutrição sobre a qualidade da carne e da gordura. *Conferência Apinco de Aiência e Tecnologia Avícolas*. Santos. pp. 21-32.

Lemme A, Mack S, Wijtten PJA, Langhout DJ, Irish GG & Petri A (2003) Effects of increasing levels of dietary "ideal protein" on broiler performance. *15th Australian Poultry Science Symposium*. Sydney, Australia. pp. 58-61.

Lemme A, Wijtten PJA, Langhout DJ & Petri A (2003) Interactions between ideal protein and dietary energy in growing broiler chickens. *Proceedings of the 14th European Symposium on Poultry Nutrition*. Lillehammer, Norway. pp. 304-305.

Lemme A, Van Wichen J, Wijtten PJA, Langhout DJ & Petri A (2005) The interactions between balanced protein level and feed form in broiler production. *15th European Symposium on Poultry Nutrition*. Balatonfüred, Hungary. pp. 566-569.

Lemme A, Janssen MGT, Wijtten PJA, Sparla JKWM & Redshaw M (2007) Increased dietary balanced protein levels at varying length of application during the starter period of broilers. *Poultry Science Association Annual Meeting*. San Antonio, TX. pp. 153.

Lemme A, Janssen MGT, Wijtten PJA, Sparla JKWM & Redshaw M (2007) Increased dietary balanced protein levels at varying length of application in 1-14 days old broilers. *16th European Symposium on Poultry Nutrition*. Strasbourg, France. pp. 193-196.

Locatelli ML, Hoehler D, Lemme A, Wijtten PJA & Langhout DJ (2003) Effects of varying dietary ideal amino acid levels in the starter diet on performance of male and female broilers. *International Poultry Scienctific Forum*. Atlanta, GA. pp. 28.

Locatelli ML, Lemme A, Hoehler D, Wijtten PJA, Van Wichen J, Sparla JKWM *et al.* (2006) Amino acid requirements in laying hens fed two different energy levels. *Poultry Science Association Annual Meeting*. Edmonton, Canada. pp. 124.

Madsen TG, Wijtten PJA & Lemme A (2008) Effect of increasing dietary levels of balanced protein in male Ross 308 fed either pellets or mash feed. *23rd World's Poultry Congress*. Brisbane, Australia. pp.

Madsen TG, Hangoor E, Wijtten PJA, Sparla JKWM & Lemme A (2010) Optimal sulphur amino acids to lysine ratio in grower phase in Ross 308 broilers. *21st Australian Poultry Science Symposium*. Sydney, Australia. pp. 90-93.

Molenaar R, Reijrink IAM, Meijerhof R, Sparla JKWM & Wijtten PJA (2008) Effect of protein and energy level in feed on posthatch chick performance *International Poultry Scientific Forum*. Atlanta, GA. pp. 144.

Payne RL, Bonekamp PRT, Sparla JKWM, Lemme A & Wijtten PJA (2007) Effects of dietary amino acid level on egg performance of heavy versus light laying hens. *International Poultry Scientific Forum*. Atlanta, Georgia. pp. 769.

Perdok HB & Wijtten PJA (2002) Use of additives to optimize technical performance of food animals. *6th Endo-Neuro meeting*. Doorwerth, The Netherlands. pp.

Petri A, Wijtten PJA, Lemme A & Langhout DJ (2004) Optimizing broiler Nutrition with the ideal Protein concept. *Animal Feed Manufacturers Association Forum (AFMA)*. Sun City, South Africa. pp. 10.

Van den Braak C, Bouritius H & Wijtten PJA (2008) Feed intake during the first week after weaning is critical for maintaining the intestinal barrier function in piglets. *J Physiol Biochem* **64**, 282.

Van Vugt PNA, Wijtten PJA, Perdok HB & Langhout DJ (2001) Provimax improves the technical performance and eggshell quality of laying hens. *13th European Symposium on Poultry Nutrition*. Blankenberge, Belgium. pp. 15-16.

Van Wichen J, Lemme A, Wijtten PJA, Petri A & Langhout DJ (2005) Varying protein and amino acid profiles with changing ballast protein concentration in diets for male broilers. *15th European Symposium on Poultry Nutrition*. Balatonfüred, Hungary. pp. 570-572.

Wijtten PJA, van Eerden GW, Leuvenink HGD & van Bruchem J (1996) Het effect van een gecombineerd infuus van CCK-8 en propionaat op de voeropname bij schapen. *21e Studiedag Nederlandstalige Voedingsonderzoekers*. Lelystad, The Netherlands. pp. 28-29.

Wijtten PJA, Prak R, Langhout DJ, Pack M & Hoehler D (2000) Effect of varying dietary protein levels based on the ideal amino acid profile on broiler performance. *21st World's Poultry Congress*. Montreal, Canada. pp.

Wijtten PJA, Lemme A & Pack M (2001) The effect of enhanced dietary ideal amino acid levels in maize- and wheat-based diets on performance of male and female broilers. *13th European Symposium on Poultry Nutrition*. Blankenberge, Belgium. pp. 41-42.

Wijtten PJA, Lemme A, Petri A & Langhout DJ (2002) Effect of graded dietary ideal protein levels in the starter diet of male and female broilers on performance. *11th European Poultry Conference*. Bremen, Germany. pp.

Wijtten PJA, Lemme A, Petri A & Langhout DJ (2002) Influence of graded dietary ideal protein levels in the starter period and / or the grower period on broiler performance. *11th European Poultry Conference*. Bremen, Germany. pp.

Wijtten PJA, Langhout DJ & Perdok HB (2003) The effect of pelleting, particle size and nutrient density on broiler performance and gastrointestinal tract characteristics. *14th European Symposium on Poultry Nutrition*. Lillehammer, Norway. pp. 117-118.

Wijtten PJA, De Lange LLM, Panneman H, De Goffau KCJ & Van Vugt P (2005) Gut microflora and its relation to broiler performance. *Avian gut function in health and disease*. Bristol, UK. pp. 375.

Wijtten PJA, Lemme A, Van Wichen J, Sparla JKWM & Langhout DJ (2006) Amino acid requirements in laying hens at different energy intake levels. *12th European Poultry Conference*. Verona, Italy. pp. paper 287.

Wijtten PJA, Panneman H, Sparla JKWM & Langhout DJ (2006) Influence of pelleted diets and coarse ground mash diets on broilers gut flora *12th European Poultry Conference*. Verona, Italy. pp. paper 288.

Wijtten PJA, Bouritius H, Bonekamp PRT, Witte GJ, Verstijnen JJ & Van Kempen TATG (2007) Gut function of pigs in relation to weaning. *2nd International symposium on energy and protein metabolism and nutrition*. Vichy, France. pp. 247.

Wijtten PJA, Hangoor E, Perdok HB & Sparla JKWM (2008) The effect of early life feed restriction on performance and mortality of male broilers. *23th World Poultry Congress*. Brisbane, Australia. pp. 234.

Wijtten PJA, Bouritius H, van den Braak CCM, Verstijnen JJ & van Kempen TATG (2009) Delayed eating post weaning is related with an increased urinary lactulose recovery. *11th International Symposium on Digestive Physiology of Pigs*. Barcelona, Spain. pp.

Wijtten PJA, Hangoor E, Sparla JKWM & Verstegen MWA (2009) Dietary amino acid levels and feed restriction affect organ development, mortality and weight gain of male broilers. *34th Animal Nutrition Research Forum*. Melle, Belgium. pp.

Training and supervision plan

The Basic Package	year	credits
WIAS Introduction Course	2007	1.5
Ethics and Philosophy of Animal Science	2008	1.5
Scientific Exposure		
International conferences		
10th Digestive physiology in pigs	2006	0.9
2nd Int. Symp. on Energy and Protein Metabolism and Nutrition, Vichy	2007	1.5
Provimi International Poultry Conference, Brussels	2008	0.9
Seminars and workshops		
WIAS Science Day	2007	0.3
34th ANR forum, Melle	2009	0.3
Presentations		
12th European Poultry Conference, Verona (poster)	2006	1.0
2nd Int. Symp. on Energy and Protein Metabolism and Nutrition, Vichy (poster)	2007	1.0
Provimi International Poultry conference, Brussels (oral)	2008	1.0
34th ANR forum, Melle (oral)	2009	1.0
In-Depth Studies		
Applied statistics course, PHLO	2001	2.4
Internal Provimi statistical workshop	2003	0.6
Mixed models	2008	0.3
Quality assurance course for Good Clinical Practice	2006	0.3
Provimi Poultry Course (PTC+)	2004	2.4
Statutory Courses		
Use of Laboratory Animals (article 9 authorisation)	2007	3.0
Professional Skills Support Courses		
Presentation skills	2009	1.0
Provimi International business week (INSEAD), Crans Montana	2006	2.1
Research Skills Training		
Preparing own PhD research proposal	2007	6.0
Didactic Skills Training		
Provimi additive training: Molecular techniques to study gut microflora	2007	0.3
3 MSc major students	2003-2009	6.0
4 BSc students	2003-2009	4.0
Total		**39.3**

Colophon

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