

# Chapter 7

## Nutritional and immunological aspects of the intestine

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**This chapter will provide an understanding of:**

- ↳ intestinal metabolism and endocrine regulation
- ↳ the importance of enteral nutrition
- ↳ local immune defence and its influence on weight gain

### 1. Introduction

The digestive process starts in the mouth and continues throughout the gastrointestinal tract as described in [Chapter 5](#). Digestion is the result of enzymatic breakdown of macromolecules and absorption of nutrients to the blood or lymph circulatory systems. The previous chapter mainly described the enzymatic part of the digestive process, and the chapters following the current chapter will describe in more detail the qualitative and quantitative aspects of the absorptive part of the digestive process. This includes absorption of carbohydrates, amino acids, fat, minerals and vitamins. The current chapter will focus more on some of the factors that influence the digestive process such as hormonal regulatory factors, and factors that influence the utilization of nutrients once they are absorbed. The utilization of absorbed nutrients for various purposes may reflect both normal physiological mechanisms as well as pathological mechanisms (eg. activation of the immune system after infection).

One of the normal physiological aspects to consider is how the intestine metabolizes some of the luminal nutritional substrates before they reach the blood circulation. This process, referred to as 'first pass metabolism', shows that growth and maintenance of the digestive tract relies not only on nutrients from the blood, but also on gut luminal nutrients that are directly derived from the diet. This dependency on gut luminal nutrients is unique to the gastrointestinal tract relative to other organs. When pigs and other mammalian species experience periods of low feed intake (starvation periods or periods with reduced appetite due to disease), the integrity and function of the gastro-

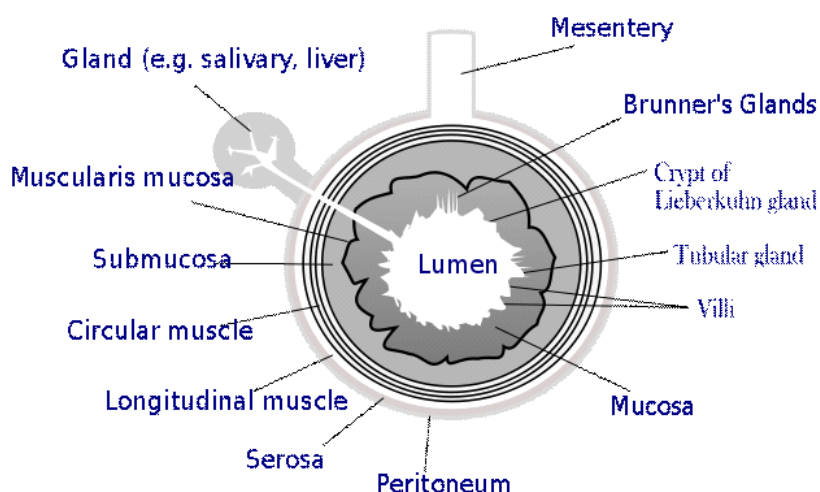
intestinal tract are compromised. When the gastrointestinal tract is compromised it becomes more susceptible to infectious pathogens and the organism as a whole must therefore rely on a well functioning immune defence system to control this.

An active and functioning immune defence system is, however, associated with a 'cost' in growth performance. There is a clear difference in growth performance between conventional pigs that are infected with particular swine pathogens versus specific pathogen-free (SPF) pigs that are free of certain pathogens. This is likely due to the 'costs' of having activated a large amount of immune cells that require energy and other nutrients. It is beyond the scope of this chapter to address all aspects of systemic immune function and how it relates to nutrition. Yet, together with systemic immunity, there is also a very active *local* immune defence system on any mucosal surface in the body (respiratory, urogenital and gastrointestinal surfaces). This mucosal defence system acts as an effective first line of defence and is very important to overall protection against pathogens.

Overall growth performance and feed-to-gain conversion ratios are to a large extent dependent on the activity of the immune system. It follows that commercial feed additives that improve growth performance may reduce rather than activate the immune system. Although commercial feed additives are often claimed to activate the immune system, it is important for animal nutrition students to realize that this may not always be entirely accurate.

## 2. Structures of the intestinal wall and their main cell types

As mentioned in the previous chapter, nutrient absorption takes place mainly in the small intestine and large intestine. A premise for understanding how nutrients are absorbed from the intestinal lumen and transported to blood or lymph, is to understand the histological structure of the intestinal wall and some of the important factors that affect absorptive function. The following sections briefly describe the structures of the small intestine as this is where most of the digestive process takes place.



**Figure 7.1. Structures of the intestinal wall.**  
(source: [http://en.wikipedia.org/wiki/File:Gut\\_wall.svg](http://en.wikipedia.org/wiki/File:Gut_wall.svg))

The mucosa (tunica mucosa) is the innermost cellular layer of the intestine. It mediates digestion of luminal nutrients and simultaneously protects against pathogens. It consists of a one-layered epithelial lining (lamina epithelialis) that rests on a basal membrane and a layer of structural connective tissue (the lamina propria) and a thin muscle layer (lamina muscularis or muscularis mucosae). Within the lamina propria there are stromal cell types including immune cells, fibroblasts and cells lining the blood and lymph vessels. The main cell type of the epithelial layer is referred to as 'enterocytes' in the small intestine and 'colonocytes' in the large intestine. These cells are responsible for the final enzymatic break down of luminal nutrients and for absorption. Enterocytes and colonocytes are derived from a tissue-specific population of stem cells located in the crypt area. Here cells proliferate by asymmetric mitotic division and new cells are gradually matured as they move toward the villus tip.

Most stem cells become digestive cells that display enzymatic and absorptive functions. Other stem cells become enteroendocrine cells or so-called 'goblet' cells (the name is derived from their goblet-like appearance in the microscope). This cell type produces mucin that is excreted from the cell toward the intestinal lumen where it distributes as a protective layer on top of the epithelial lining. Often the layer of mucin (also referred to as 'mucus', and not to be confused with the term 'mucosa') is referred to as the 'first line of defence' due to its ability to allow the passage of luminal nutrients toward the enterocytes, while at the same time reducing the direct contact between luminal bacteria and the epithelium. We will discuss the importance of mucus and how it is affected by nutrition later in this chapter.

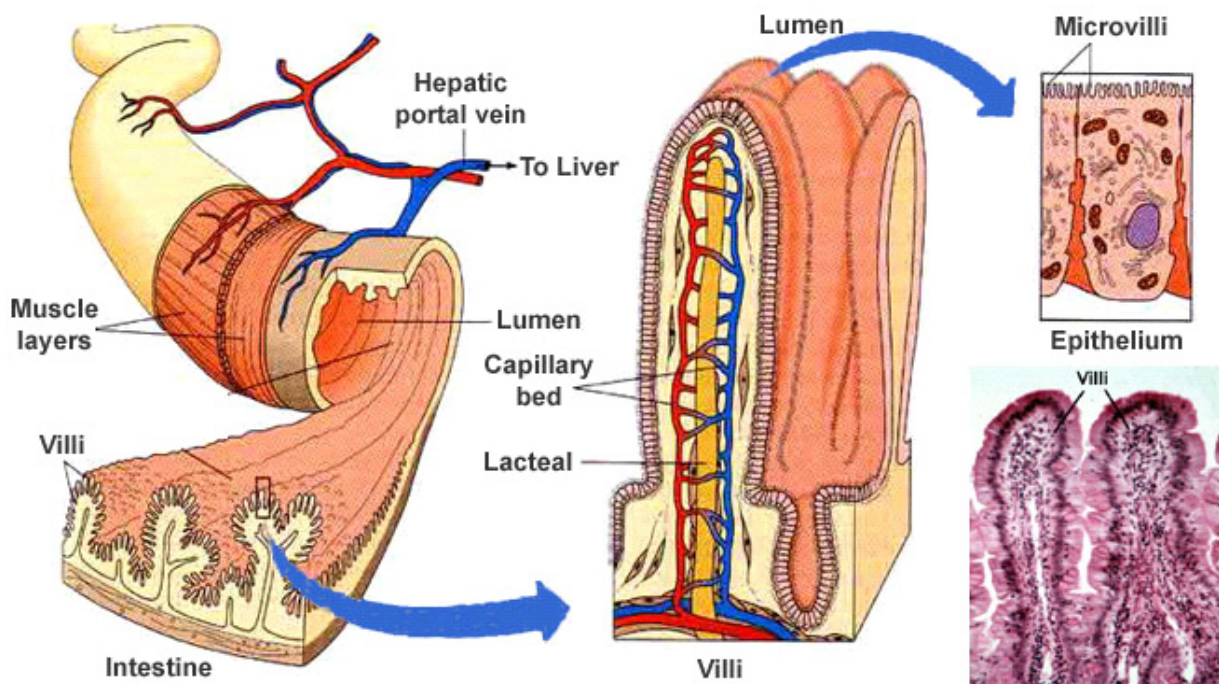
Below the epithelial lining are the architectural structures that support the villi and crypts - i.e. the lamina propria. As mentioned previously, this lamina is composed of a mixture of cell types including fibroblasts, which are responsible for production of connective tissue, and endothelial cells, which line the wall of vascular tissue. Also, within both the lamina epithelialis and the lamina propria, there is a population of immune cells that serves to protect the host from invading intestinal pathogens. The connective tissue mainly consists of collagen fibers that form the network on which epithelial cells and leucocytes can migrate. It gives the structural framework of the finger-like processes (the villi), which gives the small intestine a very large absorptive surface. To produce connective tissue, fibroblasts are dependent on continued supply of nutrients like proline and other amino acids that are important precursors for collagen formation. If supply of enteral nutrients is lacking, one of the best known effects is atrophy of the mucosa, which is due mainly to shortening of the villi.

One of the important factors for preventing mucosal atrophy and maintaining normal mucosal function is blood supply. One arteriole branches from the main artery in the submucosa, penetrates the lamina muscularis and projects into the center of the villi toward the tip (Figure 7.2). Here, the arteriole distributes into a fountain-like capillary network that drains into small veins (referred to as venules) and descends back toward the submucosa. As nutrients are absorbed, blood flow toward the mucosa is markedly increased. As the villi become engorged with blood, muscular fibers within the mucosa (muscularis mucosae) contract and pump venous blood away from the villi.

As nutrients are absorbed by the enterocytes, they are either metabolized or passed directly into the extracellular matrix from which they further diffuse into capillaries. Most nutrients are drained from the capillaries into venules that merge and assemble into larger veins that ultimately drain into the hepatic portal vein. The close proximity of arterial and venous blood in the villi facilitates a countercurrent system where some nutrients are recycled from venous to arterial blood, thereby creating a hyperosmotic environment in the villus tip. This secures a net absorption of water molecules from the gut lumen and thereby contributes to prevention of diarrhoea.

The submucosa (tela submucosa) consists of a thin layer of connective tissue, which may be difficult to differentiate from the lamina propria. Within the submucosa, there are mucin producing glands (referred to as Brunner glands or duodenal glands) that empty their content in the crypt area of the mucosa. This gland type is most abundant in the duodenum where the secreted mucin

distributes and forms a layer that protects the epithelial cells from the acidic chyme from the stomach. Other structures within the submucosa include nerve plexuses, lymphoid tissue and vascular tissue. Arterial branches from the main artery (cranial mesenteric artery) project into the villi as previously described. Some animal species, including the pig, has arterio-venous anastomoses in the submucosa, which can shunt the blood directly from artery to vein, and thereby by-passing the villus capillary network. During digestion, the anastomoses are closed and blood is shunted toward the villus capillary network to facilitate the digestive process. Between meals, the anastomoses are partly open so that only part of the blood enters the mucosa to maintain the mucosal tissue between meals.



**Figure 7.2. Villus structures of the small intestine. (source: [http://www.daviddarling.info/images/small\\_intestine\\_cross-section.jpg](http://www.daviddarling.info/images/small_intestine_cross-section.jpg). reprinted with permission)**

The tunica muscularis consists of two layers: an inner circular and an outer longitudinal layer (Figure 7.1). There is a thin layer of connective tissue between the two muscle layers and embedded within this connective tissue is a myenteric plexus, which plays an important role in peristalsis of the gut.

The tunica serosa is the outermost layer of the intestine. It is formed by loose connective tissue that appears as a very thin layer in a histological cross section of the intestinal wall.

### 3. Development of the digestive process

As already indicated in the previous chapters, the digestive process changes with time as the pig matures. Before birth, nutrition is derived from the placenta and passed into the fetal circulatory system via the umbilical cord. In the last trimester of gestation there is also a considerable amount of oral ingestion of amniotic fluid, which plays an important role in development and protection of the gut. After birth, the intestine has to adapt acutely to enteral nutrition (with colostrum and milk) in contrast to the parenteral nutrition it received in utero via the umbilical cord.

After birth, passive immunization with colostral immunoglobulins is essential for survival. They are absorbed into the enterocytes by endocytosis and provide both local and systemic immunity

through their release to the blood circulation. The ability to absorb intact large molecules gradually decreases as the intestine becomes fully immunized, and is followed by a more normal digestive pattern.

The gut enzymes responsible for digestion at birth are tailored toward digestion of compounds in colostrum and milk. The activity of lactase, which catalyses the hydrolysis of milk lactose into glucose and galactose, is the principal small intestinal carbohydrase at birth, but gradually declines to reach adult levels at around two months of age. Other carbohydrases like  $\alpha$ -amylase, maltase-glucoamylase and sucrase-isomaltase are generally low at birth but increase over time in both a diet-dependent and diet-independent way. The transition towards enzymes that are capable of hydrolyzing more complex carbohydrates than lactose is important in order to prepare the organism towards more adult-type plant-based diets. This may partly explain why it can be beneficial, in regard to the subsequent weaning process, to offer solid food to suckling pigs as it may accelerate gut maturation. It appears, however, that accelerated maturation cannot take place within the first three weeks of life as the organism is largely unresponsive to the stimulatory effect of solid food at this stage.

As for lactase, the specific activity of small intestinal enzymes responsible for digestion of small peptides generally drops with age. However, as the relative activity is typically expressed relative to tissue mass or protein or DNA, the decrease in relative activity is somewhat compensated by an increase in intestinal mass relative to bodyweight.

Proteins from milk are highly digestible and have an ideal amino acid composition to fulfill the requirement for lean growth. However, as the pig is weaned the protein source becomes more or less of plant origin. This poses a challenge to the digestive system as the activity of peptide digesting enzymes is sensitive to infectious and inflammatory conditions like weaning diarrhoea. A contributing factor to this intestinal malfunction may be that many newly weaned pigs experience some degree of anorexia during the first days after weaning. Due to the anorexia, the absence of gut luminal content in the immediate post-weaning phase has a negative impact on gut integrity and functionality. Once the pig resumes a normal eating behavior, the digestive capacity in the small intestine may be exceeded due to reduced gut enzyme activity, and undigested material pass from the small intestine into the caecum and colon where it is fermented by resident bacteria. In pigs with weaning diarrhoea, excess fermentation in the colon may be the result of rapid production of short-chain fatty acids from nutrients not digested in the small intestine. Given this situation, the colon shows an adaptational growth response to meet the increased need for fermentation and absorption.

However, despite the buffer-capacity of the colon, the clinical end result is often weaning diarrhoea. This particular condition has been one of the major health problems in pig production and it has therefore been extensively researched. Generally speaking, there is a distinction between osmotic and secretory weaning diarrhoea. Whereas the reader is referred to other text books for an in-depth description of weaning diarrhoea aetiology and pathology, suffice it to say that osmotic diarrhoea results from loss of absorptive surface whereas secretory diarrhoea is the result of toxins from enterotoxigenic bacteria. Combinations of secretory and osmotic diarrhoea may also occur.

## **4. Nutrition and maintenance of the intestinal wall**

Unlike other organs, the gastrointestinal tract receives its nutrients not only from arterial blood but also directly from the luminal content of nutrients following enteral feeding. It is well known that the mucosa undergoes atrophy during reduced feed intake or starvation. It is also well known that this is not due to the general catabolic condition during negative nutrient balance. In fact, several experimental studies have shown that animals kept in anabolic conditions by total intravenous nutrition also show atrophy of the intestine.

In addition to mucosal atrophy, lack of enteral nutrition has many other negative effects on the intestine, including alteration of the microflora, reduced peristaltic movements and reduced integrity of epithelial tight junctions. This increases permeability of the gut toward not only large molecules, but also bacteria and other large antigenic compounds that may ultimately translocate to deeper tissues and infect the host. Although these mechanisms are well known, it remains a challenge to nutritionists to identify means to avoid gut malfunction and inflammation during critical periods. These aspects will be discussed later in this chapter.

#### *4.1 Cell turnover and gut metabolism*

The life span of intestinal epithelial cells is 2-3 days (and up to 10 days in suckling pigs). Epithelial cells are derived from mitotic division of stem cells in the crypts. The cells differentiate and become progressively more mature as they migrate from the crypt area toward the villus tip. Epithelial cells in the upper half of the crypt-villus axis are therefore the most mature cells and account for the majority of the total nutrient absorption. Consequently, if the upper part of the villi is lost during episodes of diarrhoea, nutrient malabsorption will further contribute to the symptoms.

Under normal physiological conditions, epithelial cells are continuously shed from the villus tip in a controlled fashion referred to as apoptosis (programmed cell death). Unlike necrotic cell death, apoptotic cell death is not pathological and does not elicit an inflammatory response. However, continued loss of epithelial cells must be replaced with new epithelial cells, which explains the relatively short life span of this particular tissue. As a consequence of the short life span, the gastrointestinal tract requires substantial amounts of nutrients to be maintained. It is estimated that approximately 25% of total body protein synthesis takes place in the gut, and 25% of digestible energy in the diet is used for gut metabolism. Given that gut tissue only accounts for approximately 5% of total body weight, these numbers indicate that the gut is a very active metabolic organ.

One of the unique features is the so-called 'first pass metabolism' in the gastrointestinal tissue. This refers to the fact that a substantial amount of orally ingested nutrients are metabolized by the gut before they enter the blood stream. This means that the composition of nutrients in the portal vein only to some extent reflects what was in the diet. From a nutritional perspective, it is important to understand what the gastrointestinal system requires to maintain its function, which is to digest and absorb nutrients and form an effective barrier toward luminal microbes and toxins. Among all nutrients, amino acids and carbohydrates have received most attention in regard to first pass metabolism. When amino acids are absorbed, they are either oxidized or incorporated into protein, or they are converted to other amino acids. The reader is referred to text books on biochemistry for an in-depth description of interconversion of amino acids. However, certain important aspects of amino acid metabolism will be mentioned here. Experimental data have shown that preferential energy substrates for the intestine are glutamate, glutamine, aspartate and glucose. However, whereas glucose as energy substrate for the mucosa is derived mainly from the arterial blood supply, glutamine and especially glutamate are largely derived from the luminal side after oral ingestion. These two amino acids are preferred over glucose as oxidative fuels in the intestine. It appears that the jejunum is the site where first pass metabolism of amino acids is most important. This may reflect the fact that free amino acids are not present to a large extent in neither the stomach nor the colon.

The amount of glutamate and glutamine that is not oxidized can be used for other purposes, including acting as precursors for other amino acids such as alanine, aspartate, proline, citrulline and arginine. In fact, circulating plasma levels of citrulline after citrulline synthesis in the intestine (which is the main site of synthesis) are a reasonably good marker of the amount of functional intestinal mucosa.

Arginine and its precursors citrulline, ornithine and glutamine are equally important to consider. Arginine acts as a substrate in the production of nitric oxide (NO) and citrulline; a reaction that is catalysed by three isoforms of nitric oxide synthase (NOS). These are referred to as endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). eNOS and nNOS are collectively

referred to as constitutive NOS (cNOS), and reflect the level of NOS activity (i.e. NO production) that is required to maintain normal physiological function.

The role of iNOS is less well understood, but may reflect a level of NOS activity that is pathologically high. Although NO has multiple physiological functions, we here focus on those related to vasodilation of intestinal blood vessels only. Histologic lesions associated with conditions like necrotizing enteritis suggest that the tissue is hypoxic. This may result from insufficient intestinal blood supply, and it has been shown that intestinal blood flow increases dramatically in the early postnatal days coinciding with introduction of enteral food and a dramatic intestinal growth. During conditions like necrotizing enteritis, which is a common disease in neonates, it has been shown that plasma arginine is reduced, which may compromise intestinal blood flow leading to hypoxia and necrosis.

It is also interesting that even indispensable amino acids are substrate for oxidation. Studies have shown that threonine, leucine, lysine and phenylalanine are oxidized, although to a much smaller extent than glutamate and glutamine. There is also a significant retention of especially threonine as it is used for mucin synthesis (see later).

#### *4.2 Effects of reduced feed intake on mucosal function and integrity*

Given that the intestine is very metabolically active and dependent on provision of particular gut luminal nutrients, it is not surprising that it is also very sensitive to periods of reduced feed intake. Reduced feed intake can have various reasons, but the best known is the period immediately after weaning, also referred to as weaning anorexia. Glutamate, glutamine and aspartate, which are important energy substrates, cannot adequately support maintenance of the mucosa. Threonine may not adequately support mucin production, and proline may not adequately support collagen formation. These are only some of the effects of reduced feed intake. Fluxes of conversion of one amino acid to another also become affected by low feed intake, and can be seen as the organism's attempt to buffer the lack of important nutrients by using redundant biochemical pathways.

Histological change following reduced feed intake is probably the best described phenomenon. Atrophy of the villi and elongation of crypts is the classical adaptational response of the mucosa. Although this may be associated with maldigestion due to inadequate enterocyte digestive enzyme activity and malabsorption, it cannot be regarded as an inflammatory condition per se. If, however, the integrity of the mucosa is lost as a result of low feed intake, then bacteria, toxins and other compounds with antigenic properties, can translocate into the tissue and elicit an inflammatory response.

Experimentally, the negative effects of anorexia on gut structure and function can be distinguished from the general catabolic condition of the body, by supplying intravenous nutrition. This is commonly referred to as parenteral nutrition to indicate a nutritional route that is different from enteral (and in most cases intra-venous). This way the animal can be kept in energy/protein balance and maintain its weight, or it can be slightly anabolic. If all nutrients are provided parenterally, the term TPN (total parenteral nutrition) is used. If a combination of enteral and parenteral nutrition is used, it is referred to as either PPN (partial parenteral nutrition) or MEN (minimal enteral nutrition). We now know that TPN, where there is no nutritional stimulation from the gut lumen, is associated with mucosal atrophy, reduced digestive capacity, increased permeability, bacterial overgrowth and possibly reduced peristaltic movements of the bowel. This elegantly demonstrates that it is not the general negative energy balance associated with reduced feed intake that is responsible for mucosal atrophy. Rather, the lack of enteral nutrition per se is enough to cause the pathological changes in the gut. This observation emphasizes the importance of first pass metabolism as mentioned in the previous section. However, there is still a lot to learn about the negative effect of lack of enteral nutrition.

Some have suggested that the gastrointestinal microbiota is negatively affected by lack of enteral nutrients. The hypothesis is that in the absence of fermentable substrate from the diet, there is a selection of bacteria that have the capacity to use mucus from the host as alternative substrate. As mucus is used as bacterial substrate and de novo synthesis from mucin producing cells is reduced due to lack of enteral substrate, this compromises gut barrier function and increases the risk of bacterial translocation into deeper tissues. It has been advocated that especially clostridial species have the capacity to digest mucin from the host. Although some clostridial species are pathogenic per se, it may be other types of pathogens that actually translocate into the tissue and initiates the inflammatory response.

There are also other complications associated with starvation, such as cholestasis and fatty liver. This is, however, most pronounced in long-term starvation and, relative to gastrointestinal complications, it is probably of little importance in pig production where normal feeding patterns are resumed relatively fast after a period of anorexia.

## **5. Endocrine signaling in the developing gastrointestinal tract**

Compared with other mammalian species such as the human and the ovine, relatively little research has been conducted to study the endocrinology of pigs. The porcine gestational length is relatively short (114-116 days) and the number of offspring relatively large compared with the human, the bovine and the equine. On the other hand, porcine gestational length is relatively long compared with more altricial species like rodents and carnivores. The intermediate nature of porcine gestational length is somewhat mirrored in the degree of intestinal maturity at birth. However, surprisingly little is known of what regulates intestinal adaptation pre- and postnatally.

Whereas numerous hormones have been proposed as regulators of intestinal adaptation, data from studies with rats suggest that the most important hormones are glucocorticoids. Whereas intestinal maturation in rats primarily takes place postnatally, farm animals like the porcine are more mature at birth. The maturity at birth is associated with a gradual increase in fetal plasma glucocorticoids followed by a peak on the day of parturition. Glucocorticoids play a pivotal role in gut maturation although at the same time they are often referred to as 'stress hormones'. Indeed, stress at moderate levels can be considered beneficial for maturation of intestinal function. Neurons in the fetal brain respond to stress by stimulating the release of cortico-tropin-releasing hormone from the hypothalamus, which subsequently stimulates the release of glucocorticoids from the adrenal cortex via release of pituitary adrenocorticotropin (ACTH). In pigs, it seems that the tissue responsiveness towards ACTH and glucocorticoids is greatest during fetal life. However, significant tissue responsiveness after parturition remains as shown in postnatal intervention studies using exogenous glucocorticoids. These studies showed marked stimulatory effects of glucocorticoids on gut maturation, and it has been shown that a postweaning glucocorticoid surge significantly enhanced intestinal polyamine synthesis. Polyamines stimulate cell proliferation and may therefore play a significant role during weaning-induced remodeling of the intestinal epithelium.

After birth the endocrine system develops rapidly. Ingestion of the first colostrum after birth provides many hormones and regulatory peptides that support the neonate in the early days. The importance of regulatory compounds is likely highest in the first 1-2 days when the intestine absorbs intact proteins and peptides via endocytosis. After this, the newborn animal gradually becomes endocrinologically independent from milk as endogenous levels increase.



The actions of regulatory peptides and hormones can be divided into four categories:

- 👉 regulation of gastric emptying (motilin, CCK, gastrin),
- 👉 regulation of digestive rate (CCK, secretin, GIP, motilin),
- 👉 regulation of transit time (GLP-1, neurotensin),
- 👉 intestinotrophic effect (GLP-2, neurotensin).

These factors are produced in response to ingestion of a meal and provide coordinated regulation of the digestive process that follows. The fat-responsive hormone motilin is secreted from enteroendocrine M-cells in the duodenum and jejunum. As the name indicates, this hormone has its main effect on gut motility and plays a key role in the initiation of the intestinal migrating motor complex. Other regulatory peptides - cholecystokinin (CCK) secretin and gastrin - are simultaneously secreted from the same anatomical location (distal stomach, duodenum and jejunum) and have supplemental effects to motilin. Gastrin is released in response to luminal nutrients and stimulates gastric acid secretion as well as having a trophic effect on the gastrointestinal mucosa. The primary actions of CCK and secretin are to stimulate enzyme and bicarbonate secretion from the pancreas, whereas their secondary actions are to inhibit gastric emptying (CCK) and stimulate biliary secretion. GIP and GLP-1 both inhibit gastric secretion and emptying although they are secreted from widely different anatomical areas of the gut, - GIP is released from enteroendocrine cells in the duodenum and jejunum, whereas GLP-1 primarily is secreted from more distal parts of the small intestine.

GLP-1 is a product of the prohormone proglucagon secreted from enteroendocrine L-cells in the distal small intestine and the colon. It is a 158 amino acid protein that in the posttranscriptional processing is cleaved to form glucagon, glicentin, intervening peptides 1+2 and glucagon-like peptide 1+2. GLP-1 is co-secreted with GLP-2 at an equimolar ratio, and has a stimulating effect on postprandial insulin secretion from  $\beta$ -cells in the pancreas. Further, GLP-1 has a trophic effect on  $\beta$ -cells as well as a reducing effect on  $\beta$ -cell apoptosis. It is unclear whether the coordinated nature of GLP-1 and GLP-2 release from enteric L-cells has an important physiological rationale.

Glucagon-like peptide 2 (GLP-2) is a relatively novel gut hormone. It has potent trophic effects on the intestine (increased cell proliferation and decreased apoptosis) as well as stimulating effects on hexose absorption, and it is secreted in response to presence of nutrients in the distal small intestine. The nutrient-responsiveness may also involve an indirect stimulus of L-cells. The theory is based on enteroendocrine K-cells in the proximal small intestine that respond to presence of luminal nutrients by releasing glucose-dependent insulinotropic peptide (GIP), which via the central nervous system stimulates vagal-induced release of GLP-1 and GLP-2 from L-cells in the distal small intestine.

Because of the very potent trophic effect on the intestine of GLP-2, it has been speculated that this hormone may be an important missing component during episodes of lack of enteral nutrition (like during disease). In fact, studies with neonatal pigs show that in the absence of enteral nutrition there are positive effects on gut growth in response to pharmacological doses of exogenously applied GLP-2. The actions of GLP-2 in regard to anti-apoptosis, cell proliferation and hexose absorption, advocate for the use of GLP-2 as a preventive and/or therapeutic agent in pigs with reduced feed intake. Piglet studies show that the atrophic effects of lack of enteral nutrition are prevented by pharmacological doses of GLP-2 and that this effect is linked to an increase in intestinal blood flow.

## 6. Gastrointestinal defence systems

During gestation the gastrointestinal tract is sterile. At the time of birth, however, rupture of the amniotic membranes as the fetuses are pushed through the birth canal, initiates microbial colonization of the gastrointestinal tract. The first bacteria that are introduced are mainly derived from

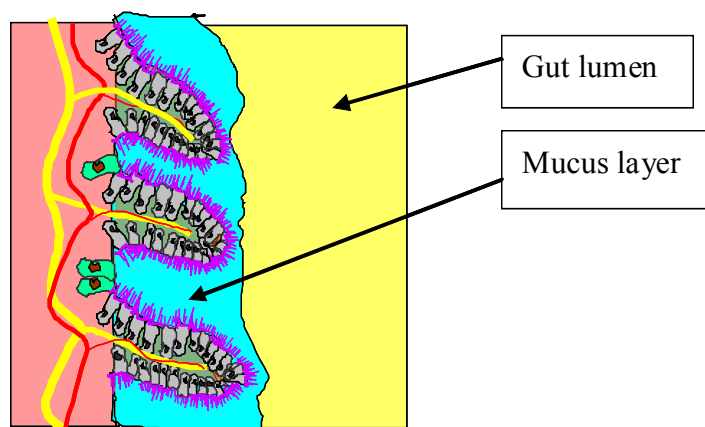
vaginal flora, fecal flora and skin flora from the sow. Within only one day, all parts of the gastrointestinal mucosa are extensively colonized, and it requires a very effective intestinal barrier function to prevent infection. Regardless of the composition of the colonizing microbiota, the gastrointestinal tract and other mucosal surfaces must rapidly adapt to the situation and mount an appropriate immune response that controls pathogens on the one hand, and on the other hand tolerates harmless commensal bacteria.

There are several mechanisms for protecting the host from harmful pathogens. These include the mucus barrier function and other parts of the innate immune system, which together with the adaptive immune system are effective in protecting the host. However, these protective mechanisms require substantial amounts of nutrition to be maintained, and it is well known that periods of malnutrition is a strong risk factor for a breach in the immune system and consequently development of disease. In the following sections, we take a closer look at how the various systems have evolved and what is required to maintain their function.

### 6.1 Mucus barrier function

A key parameter in many studies is visualization of tissue in the microscope and quantification of structures that are considered of importance to gastrointestinal health. Historically, description of morphologic dimensions (villus height, villus width and crypt depth) has been widely used as an indicator of gut health. A reduction in villus height may be a result of apoptosis or necrosis in response to pathogens. But it may also reflect a normal physiological development, like the transition from long slender villi at birth to shorter thicker and more leaf-like villi after weaning. Especially the time around weaning is associated with rapid maturational changes in intestinal morphology. This is presumably a combined effect of 1) absence of sow's milk, 2) introduction of a new diet, and 3) the stress associated with introduction to new environmental conditions. Some textbooks may suggest that gut atrophy following the weaning transition is pathological and directly associated with maldigestion. Although this may be true in some cases, reconstruction of the mucosa following the weaning transition may also be a normal developmental pattern that takes place regardless of the circumstances. Hence mucosal atrophy may not always be a pathognomonic marker of poor gut health, but could also reflect normal physiological reshaping of mucosal structure in the transition from milk to plant-based diets.

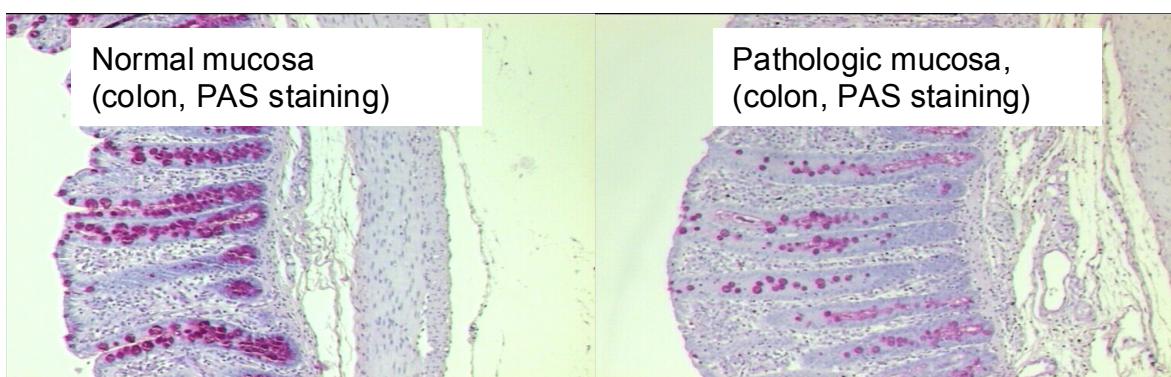
Goblet cells are one of the important structures in the mucosa of the gastrointestinal tract. Goblet cells synthesize and secrete mucus (mucin), which acts as a protective layer on the apical membrane of enterocytes (Figure 7.3).



**Figure 7.3. Schematic presentation of the mucin layer (blue) covering the epithelial lining (modified with permission (Christopher H. Hassell, 9185 Bathurst Street Richmond Hill, ON, L4C 6C2, Canada)).**

The mucus layer protects the enterocytes from acid, pepsin and mechanical damage as well as serving as a barrier towards translocation of bacteria from the lumen to gut tissue. Quantification of the mucin layer would therefore be a reasonable indicator of how well the epithelial layer is protected from the luminal microbial environment. However, visualization and quantification of the amount of free mucin on the apical surface of the epithelial lining are difficult as the layer is lost during preparation of the histological slides. However, mucin contained within the goblet cells is not lost during preparation, and can be visualized with an appropriate staining procedure (Figure 7.4). Goblet cell density is a reasonably good marker of intestinal health. Consequently, it is of interest to know how the mucin layer and the number of goblet cells can be maintained via appropriate nutrition. It appears that the mucin production is influenced by amino acids like glutamine and glutamate (which act as important energy substrates and precursors for other compounds) and threonine and cysteine (which are abundant in mucin glycoproteins). Provision of extra threonine in the diets may, however, not necessarily result in higher mucin production as the mechanisms of synthesis are also under influence of other regulatory factors such as pro- and anti-inflammatory cytokines. This underlines the general concept of 'first limiting factor' where an effect of adding extra threonine would only show if threonine was the limiting substrate and the mechanisms of mucin synthesis were intact. Being an essential amino acid, threonine requirement has been established at a level where growth performance is maximized. The recommended level of threonine reflects overall requirement of the organism, and it is not well known how threonine quantitatively distributes between various tissue compartments and cell types, including goblet cells in the intestine. However, based on data from isotope infusion studies, it is clear that the gut utilizes a large amount of orally ingested threonine. Consequently, in regard to mucin production threonine may become a limiting factor before other nutrients during periods of low feed intake. Threonine is therefore quantitatively the most important amino acid for mucin production.

However, if threonine is provided in sufficient amounts, other amino acids become limiting. Some types of mucin glycoproteins contain relatively large amounts of cysteine. This dispensable amino acid can be derived from the indispensable amino acid methionine. Although relative to threonine, the roles of cysteine and methionine as substrates for mucin production are less well established, it is evident that these amino acids are important substrates for production of sulphur-containing mucins. If cysteine content in the diet is low, methionine may be used as a substrate, which consequently increases methionine requirement in the diet. Compared with threonine, however, it is less well known to which degree cysteine and methionine can be derived from other tissues in situations where dietary intake is lower than the requirement for production of mucin.



**Figure 7.4. Goblet cell density in healthy (left panel) and inflamed (right panel) colon mucosal tissue. Goblet cell density is a good marker of intestinal health. Higher density of goblet cells correlates with better intestinal health.**

As discussed in the previous section on 'first pass metabolism', each nutrient may have unique kinetic features, such that some must be given enterally, whereas others can be derived systemically and still have the same effect as if they were given enterally. This obviously complicates our understanding of 'nutrition' as a scientific discipline. But from a pragmatic point of view, it may not

be important to understand the kinetics of more than two or three of the most important nutrients to be able to formulate a good diet.

## 6.2 *Epithelial integrity*

During disease, the integrity of the epithelial lining is compromised and antigens may penetrate into deeper tissues. Tight junction proteins normally anchor epithelial cells together and provide an effective barrier towards antigens. During disease, however, tight junction proteins are compromised and the epithelial lining loses its integrity allowing antigens to penetrate and elicit an inflammatory response in the mucosa. The inflammatory condition of the intestine is often associated with 'gut permeability'; often referred to as 'gut leakiness'. Increased permeability may lead to translocation of compounds like toxins, allergens, viruses or even bacteria that, under normal circumstances, are effectively kept within the gut lumen. However, if the epithelial lining is penetrated, other defence mechanisms including immune cells in the subepithelial tissue effectively control and neutralize invading pathogens.

## 6.3 *Cells of the immune system*

As the two previous sections have indicated, innate immunity refers to the unspecific defence mechanisms that protect the host from hazardous compounds. Whereas the mucus layer and the integrity of the epithelial lining constitute an effective physical barrier, there is also a large subepithelial population of immune cells that by definition belong to the innate immune system. These cells include neutrophilic granulocytes, macrophages, dendritic cells and natural killer cells. These cell types recognize and neutralize antigens that penetrate through the epithelial layer into the lamina propria. Whereas some of these leucocytes reside in the mucosa, more leucocytes are quickly recruited from the blood if an infectious organism is recognized.

The leucocytes have a fascinating communication system using signaling compounds (generally called 'cytokines' (or 'interleukins' if they signal between leucocytes)). These signaling compounds can be either pro- or anti-inflammatory depending on whether there is a need to neutralize an invading organism (pro-inflammation) or whether the organism is already neutralized and there is a need to resolve the inflamed tissue (anti-inflammation). Blood borne leucocytes that receive a pro-inflammatory signal, penetrate the vascular wall and migrate along collagen fibers in the extracellular matrix toward the site of infection. Here they recognize and phagocytose and neutralize the foreign organism. As invading organisms are neutralized, the pro-inflammatory response shuts down, cellular debris is removed, and repair of damaged tissue begins.

The process of recognition of antigen, signaling to other immune cells, penetration through vascular endothelium and migration towards the inflammatory area is highly specialized. It is beyond the scope of this chapter to address this process in detail, but the reader should understand that it is a long cascade of very specialized biological mechanisms that allows the host to combat foreign antigens. Although this mechanism is very effective, it may be breached by some types of pathogens. In this case, another defence system comes into action. This is the adaptive immune system (also referred to as the specific or acquired immune system). The key cell types in the adaptive immune system are B and T cells. They are present in blood and lymphoid tissue where they play a pivotal role in the systemic immune response. The reader is referred to immunological text books for an in-depth description of systemic immune function, but it is important to give the reader the impression that there are many types of immune cells in the body that become activated in response to invading organisms.

When leucocytes are recruited to a site of inflammation, the tissue quickly becomes infiltrated. The tissue infiltration with leucocytes, which can be visualized in histological sections, is the hallmark of 'inflammation'.

The gastrointestinal tract as an immunological organ is very important. There is no other organ in the body that contains the same amount of immune cells as the GI-tract. This probably reflects the

large size of the GI-tract, and the vast amount of microbes that are contained within the GI-tract. The immune cells are mainly localized within the mucosa, and recent research suggests that there is immunological cross talk between distinct mucosal surfaces such as the gastrointestinal surface- and pulmonary mucosal surfaces. This mucosal surface cross talk is referred to as 'the common mucosal immune hypothesis' to indicate that gut associated lymphoid tissue via the lymphatic drain and blood circulation communicates with other mucosal surfaces.

As gastrointestinal immune function is dependent on continued supply of enteral nutrition, 'the common mucosal immune hypothesis' would suggest that also the immune function of other mucosal surfaces are dependent on enteral nutrition. Hence, any reduction in feed intake may increase the risk of not only enteritis, but also rhinitis and pneumonia.

#### *6.4 Development of the immune system*

Piglets are born relatively immature, and lack of immunity against infectious agents makes them completely dependent on supply of passive immunity from maternal colostral antibodies. The early colostrum is rich in IgG and IgA antibodies and their functions are well known. But in addition to providing immunity, colostrum contains a large number of regulatory compounds such as peptide hormones, growth factors, cytokines, antimicrobial proteins, steroid hormones, nucleotides, enzymes, leucocytes, whey proteins, casein ect. Today, we understand that the composition of colostrum is highly complex, and reflects its ability to provide not only passive immunity, but also immuno-regulation such that an inflammatory response towards foreign antigens happens in a controlled and coordinated way until the pig has acquired its own immune defence. However, much is still to be learned about immuno-regulatory compounds in colostrum and their potential role in the neonatal period.

As all mucosal surfaces become colonized with bacteria, the innate immune system is activated (mainly macrophages, neutrophils, dendritic cells and natural killer cells) to quickly neutralize any invading organisms. Simultaneously, an acquired immune defence evolves based on M-cell mediated transport of antigens from the gut lumen to gut associated lymphoid tissue (GALT). Here the antigens are presented to lymphocytes that differentiate and provide immunity against very specific antigens. As the pig matures, the acquired immune system develops further. It is, however, not fully developed at 4-5 weeks when weaning usually takes place in the industry. Combined with the sudden absence of immunoglobulins from sow's milk, the early post-weaning period is relatively immuno-deficient, making the pig very susceptible to infectious agents.

#### *6.5 'Costs' of having an active immune system*

It is obvious that activation and recruitment of large numbers of leucocytes is associated with a 'cost'. Among the factors that can influence animal growth performance, activation and maintenance of immune cells is probably one of the most important limiting factors. The extent to which the immune system needs to be activated is directly linked to the level of pathogen exposure. There are well known indications of this in the pig industry. The growth promoting effect of reducing bacterial load in the intestine via the use of high sanitary conditions or in-feed antibiotics is one example. Another example is how pigs free of specific pathogens (SPF) grow faster than conventional pigs. This has elegantly been illustrated by counting the number of lymphocytes that reside within the mucosal epithelial layer in conventional pigs, SPF pigs and germ-free pigs respectively. It has been shown that in the jejunum there are approximately 25 lymphocytes per 100 enterocytes in conventional pigs, whereas in SPF-pigs there were only 10-15 lymphocytes per 100 enterocytes, and in germ-free pigs there were less than 5 lymphocytes per 100 enterocytes. These results indicate the impact of not only specific pathogens, but also the presence of a microbiota in general.

There is relatively little information of how the 'cost' of having an active immune defence system, quantitatively translates into nutritional demands. It has been demonstrated that macrophages have a turnover of cellular ATP of 10 times per minute, which is very high. In comparison, the ATP

turnover rate in a maximally working heart cell is 22 times per minute, indicating that large numbers of leucocytes is very energy demanding for the host.

There is not only a cost of activating the immune system during episodes with manifestation of clinical symptoms (eg. diarrhoea and pneumonia), but also during periods where the pigs appear healthy. This is referred to as 'chronic immunological stress' to indicate that an active immune defence is associated with a cost that translates into lower growth performance even in clinically healthy pigs.

It is not well established if/how nutritionists should change diet composition during episodes of disease. Voluntary feed intake during disease is reduced as a consequence of the disease. This has been shown in many studies to be a result of pro-inflammatory cytokines that are released in response to the disease. The cytokines influence the central nervous system and have a reducing effect on appetite. To compensate for a lower voluntary feed intake, diets should consist of highly digestible products that are easily absorbed by the host. Although this may not be enough to compensate for the catabolic condition, even a partial dietary compensation is likely associated with an improved prognosis for the animal.

## **7. Summary**

The gastrointestinal system contains more bacteria, neurons, endocrine cells and leucocytes than any other organ. The number of cells and their function are influenced by several factors including diet. The main epithelial cell type, the enterocyte, has a very short half life relative to other organs. The function and integrity of the gut epithelia and deeper tissue is dependent on supply of nutrients not only from the arterial blood, but also on nutrients derived directly from the diet. Maintenance of epithelial integrity and local defence systems effectively prevents penetration by pathogens and reduces the need for local and systemic immune activation. However, when the barrier is breached, local and systemic immune reactions are initiated, which eliminates the pathogens on the one hand, but on other hand is also associated with a significant consumption of energy and resulting lower weight gain.

## 8. Supplementary reading

1. **Stoll, B. & Burrin, D.G.** (2006) Measuring splanchnic amino acid metabolism in vivo using stable isotopic tracers. *J. Anim. Sci.* 84 (E. Suppl.): E60-E72
2. **Tannock, G.W.** (2005) Microbiota of mucosal surfaces in the gut of monogastric animals. In: *Colonization of mucosal surfaces*. Nataro, J.P., Cohen, P.S. and Weiser, J.N. (Ed). ASM Press Washington, D.C. pp 163-179