Unravelling the mysteries of the gut

by Ralph Stonerock and Todd Applegate, Biomin GmbH, Industriestrasse 21, 3130 Herzogenburg, Austria.

The gastro-intestinal tract (GIT) of the chick begins its development shortly after epithelial cell involutes to form a tube in the embryo. For the next 20 days prior to hatch the embryo must provide the GIT all of the necessary supportive organs that will ensure proper functional digestion. Immediately after hatching the intestinal tract must be ready to take in nutrients that will sustain its life and provide a barrier to external challenges.

The small intestine and caecum increase in size and weight as much as five times more rapidly than most other organs with growth rate maximised at 6-10 days. Essential for absorption of nutrients is the rapid development of the intestinal villi, crypts and enterocytes. The proventriculus, gizzard, pancreas, liver, gall bladder, and lymphoid tissue must develop in synchrony with these changes. Food ingested shortly after hatch enhances subsequent development and growth.

For the GIT to maximise nutrient uptake and minimise any antigenic insult from ingested food, it consumes approximately 20% of dietary energy with a protein turnover rate of 50-75% per day. Nearly 25% of daily protein synthesis is secreted into the GIT to support digestive and barrier functionality. The GIT has 10 times more bacterial cells than our own cells, resulting in greater than 70% of all immune cells in the body. The physical barrier separating the system that will deliver nutrients to all cells of the body and of the GIT is both specific and non-specific; and is far from remaining static. There is much to learn about gut barrier function, and we are just beginning to grasp the complexities.

Birds experiencing necrotic enteritis (top) versus control birds (bottom).

Non-immune barriers

The first line of non-immune defences associated with gut barrier function is the mucus layer overlying the epithelial cells. Mucins contain a diverse array of carbohydrates (glycoprotein) structures and are secreted by goblet cells. These goblet cells are located along the villi within the intestinal epithelium.

Viscosity of the mucus varies throughout the GIT. It functions to lubricate intestinal surfaces, trapping and neutralising bacteria, detoxification of heavy metals, interacts with the intestinal immune system, acts as a diffusion barrier for nutrients and macromolecules, and protects underlying epithelial cells.

Because mucins contain a diverse array of carbohydrate structures, they provide numerous potential attachment sites for both commensal and pathogenic bacteria, becoming a colonisation niche. The tightly adherent mucus layer prevents access of most bacteria to epithelial receptors.

However, it may also allow some specific adapted pathogens to migrate through the mucin towards the epithelium where colonisation may occur. Protection is then reliant upon accumulation of bactericidal and bacteriostatic compounds, as well as secretory immunoglobulin-A found in the mucus. A loosely adherent mucus layer is constantly sloughed, carrying any resident or invading bacteria from the GIT.

Although mucus is an important factor in maintaining a strong intestinal barrier, it is difficult to predict how the mucus layer inhibits or aids specific pathogens. Mucin composition, quality, quantity, digesta flow, and gut motility all interact with the success or failure of the pathogen.

Intestinal epithelia

Intestinal cells are derived in the crypt and migrate along the length of the crypt-villus axis. In the crypt, they can differentiate into a digestive/absorptive enterocyte, a mucus secreting goblet cell, a peptide hormone producing enteroendocrine cell, or an antimicrobial peptide/protein secreting paneth cell.

In the event of an invading pathogen enterocytes migrate faster along this axis and increase the rate of programmed cell death (apoptosis) in order to remove the infected enterocytes from the villus. This causes the enterocytes to be more 'immature'. It can become detrimental to the animal, as with expression of carbohydrases. The upper 40% of the villus expresses 30-40% more sucrase and maltase activity per enteroctye than the lower crypt/villus axis.

During enteric stress, enteroctyes do not have time to express these enzymes, thus, undigested disaccharides may enter the lower digestive tract permitting microbial growth resulting in osmotic and secretory diarrhoea.

Tight junctions

The intestinal epithelium contains tight junctions, intermediate junctions, and desmosomes. These serve as a regulated barrier

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between the apical and basolateral ends of the enterocyte. Anderson and Cereijido report 90% of substances (ions and nutrients) are absorbed via this paracellular transport and are highly regulated by tight junctions discriminating against different ions depending on the surrounding pH.

Enteric pathogens and associated virulence factors can change the functionality of these tight junctions.

For example, they can cause direct alteration of junction proteins (for example Clostridium perfringens enterotoxin), modification of actin cytoskeleton (for example Clostridium difficile toxins A and B) or activation of cell signal transduction (for example enteropathogenic E. coli causes occluding dephosphorylation via changes in intracellular [Ca^{2+}]), thus increasing permeability of these tight junctions.

Immune defences

It is estimated the GIT contains greater than 70% of all immune cells found in the body with nearly one-fourth of the intestinal mucosa composed of lymphoid tissue. The protection that is provided by gastrointestinal-associated lymphoid tissue (GALT) includes both non-specific (innate) and adaptive components. Innate immunity is a non-specific defence which includes: physical barriers such as epithelial cells and secretions of mucus, antibacterial peptides, phagocytes, and macrophages that destroy bacteria by engulfing them. Adaptive or acquired immunity is far more specific to individual antigens and is characterised by the development of T cells, B cells, and antigen-specific antibodies.

The connective tissue that underlies the epithelium of the gut, the lamina propria, is a primary component of the GALT. It is highly vascularised, richly innervated by the enteric nervous system, and contains sizeable populations of immune B cells and T lymphocytes, immunoglobulins, macrophages, mast cells, and plasma cells.

Additionally, intraepithelial lymphocytes (IEL) produce numerous cytokines that mediate an immune response.

Sampled bacteria are presented to immune cells within Peyer's patches and lamina propria which initiates recognition and response to bacterial antigens. An antibody specific to the bacteria is then produced (immunoglobulin A, IgA), the main immunologic intestinal defence.

Secreted into the intestinal lumen, sIgA provides protection to the intestine by attaching, neutralising, and preventing the bacteria from adhering, injuring or destroying epithelial cells. Additionally, the production of IgA within the intestine stimulates the production of memory T and B-cells, or cells that automatically recognise the specific bacteria in subsequent encounters.

This recognition decreases the response time if the incidence reoccurs.

Intestinal microbiota

After hatching the GIT begins colonisation by an active microbial population that will have a major impact on further development. Its precise contribution of microflora will be dependent upon exposure to a range of microflora beginning in the hatchery and continue through placement.

This can be a random event with random consequence or a less random event with the addition of a probiotic. Once established, the resident intestinal microbiota provides a wide range of benefits to the host, including the ability of the microbiota to inhibit colonisation by pathogenic bacteria.

Inhibition is achieved by competing for epithelial attachment sites, nutrients, and the production of bacterocins, short chain fatty acids, and modified bile acids.

In birds, the largest concentrations of bacterial populations are found in the distal ileum, caeca, and colon.

Bacterial populations within the crop are dominated by the lactobacilli species, and may contain low concentrations of other organisms (Clostridium perfringens, micrococci, staphylococci, and yeast). The proventriculus and gizzard are relatively inhospitable to bacteria due to a low pH and rapid transit of feed, thus only a few

species of bacteria are located within these organs.

Among those bacteria reported to be found in the gizzard and proventriculus are Lactobacillus with low populations of E. coli, enterococci, and yeasts. The duodenal and jejunal sections have relatively low microbial densities which increase considerably at the distal ileum, caeca, and colon to densities of 1011 bacteria per gram (wet weight) of caecal content.

Our knowledge of factors affecting horizontal populations and functionality in poultry is very limited and borne primarily from mammalian studies.

Nutrient use by the GIT

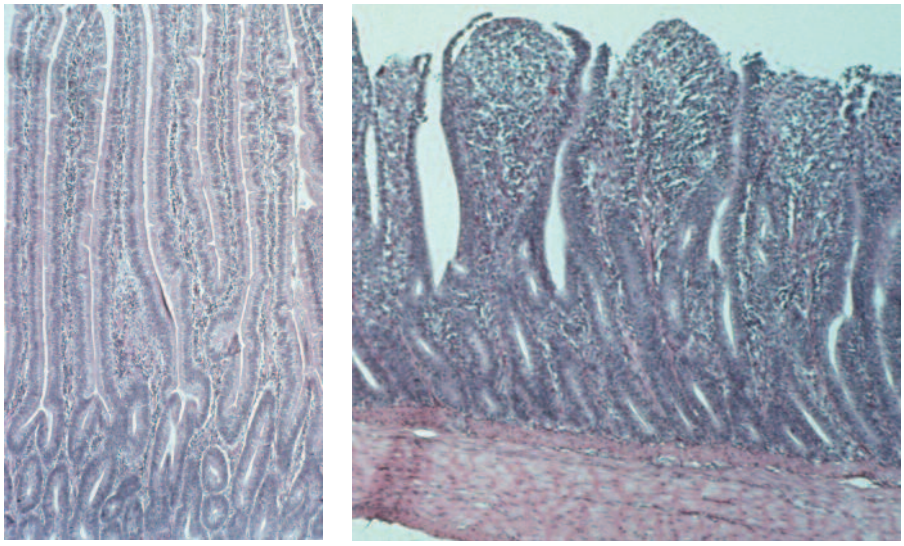
For poultry, the small intestine appears morphologically and functionally immature at hatch in terms of digestive and absorptive capabilities. Controversy has existed as to the barrier functionality of the intestine at hatching. Karcher and Applegate however observed the presence of both tight junctions and desmosomes between enterocytes in both the crypt and throughout the length of the villus as early as seven days prior to hatching in the duckling, turkey poult, and chick.

Early in the bird's life, functional utilisation of nutrients is limiting.

Maturation of amino acid and energy digestion does not occur until 10 and 14 days, respectively. This is in part due to changes in pH of the proventriculus and endogenous secretion differences.

The pH of the chick drops from 5.2 at two days of age to 3.5 at 10 days of age. An estimated 60-80% of the differences in amino acid utilisation during the first week and later are attributable to differences in basal endogenous amino acid losses.

In summary, maintenance of the barrier functions of the GIT comes at a considerable price. We have only begun to learn about communication among bacteria, bacterial modulin signalling to the enterocyte, commensal relationships with the mucosal epithelia, and conditions that perpetuate or refute induction of virulence among bacterial species. Further work is required to identify conditions permitting us to fully maintain barrier functionality while minimising nutritional 'cost' associated with them. ■



Necrotic enteritis can cause a severe shortening and blunting of intestinal villi, thus limiting the effective digestive and absorptive surface area of the intestine.