Intestinal Ecology: Interactions Among the Gastrointestinal Tract, Nutrition, and the Microflora

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Primary Audience: Nutritionists, Veterinarians

SUMMARY

Extremely complex interactions exist between the components of intestinal ecology, including the host intestinal anatomy, intestinal microbial populations, and the nutrition of the animal. The anatomical regions of the gastrointestinal tract can be characterized based on cell type and function and include the epithelial cell layer, lamina propria, muscularis, widespread components of the immune system, and mucus layer. The microflora consists primarily of bacteria, which can be broadly categorized as harmful and commensal populations. Harmful populations may be involved in the induction of infection, intestinal putrefaction, and toxin production. Commensal populations may be involved in vitamin production, stimulation of the immune system via nonpathogenic means, and inhibition of harmful bacterial populations. The nutrition of an animal can directly and indirectly affect each of the aforementioned components and, thus, dramatically affect the health and performance of production animals. A comprehensive understanding of these interactions will provide tools by which animal health and performance can be maximized while the use of pharmacological agents and the excretion of nutrients can be minimized.

Key words: intestine, immune, nutrition, microflora, mucin

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DESCRIPTION OF PROBLEM

The gastrointestinal (**GI**) tract consists of highly organized cells, vasculature, and connective tissue, which function as one of the largest components of the immune system as well as a primary surface through which nutrients and macromolecules enter the body. The GI tract is constantly exposed to ingested materials, including dietary components and microorganisms, and there is an incredibly complex relationship between these inputs and the host tissue. Ultimately, essential nutrients should be digested and absorbed while GI health is maintained by preventing disease challenges.

HOST INTESTINAL ANATOMY

The GI tract of birds can be subdivided into anatomical sections (e.g., beak, esophagus, crop, proventriculus, gizzard, small and large intestines, and ceca). Of all of the anatomical sections, the small intestine is most critically involved in digestion of diet components and absorption of nutrients, and the large intestine and ceca are very important regions for microbial colonization. Therefore, this paper will focus more heavily on

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these regions of the GI tract as compared with the more proximal regions.

Within anatomical sections of the GI tract, 5 major regions can be characterized based upon cell and function. These regions include the epithelial cell layer, lamina propria, underlying muscularis region, widespread components of the immune system, and mucus layer [1]. First, the luminal cell layer of the intestine consists of a single layer of columnar epithelial cells, which are the primary absorptive cells of the intestine (enterocytes). Additionally, goblet cells (secrete mucin glycoproteins), endocrine cells (secrete hormones and neuropeptides), M cells, and intraepithelial leukocytes ($CD8^+$ T lymphocytes and natural killer cells) can be found in the epithelial region. These cells are organized into distinct finger-like (villus) shapes that provide the vast surface area needed for optimal nutrient absorption; the crypts of Lieberkuhn at the villus base are the primary site for proliferation and differentiation of new enterocytes. The rate of enterocyte proliferation is age dependent, is greatest at the time of hatch, and decreases as the bird matures [2]. Unlike in mammals, in which enterocyte proliferation only occurs in the crypts, enterocyte proliferation occurs in the crypt and villus regions in birds [2]. The proportion of proliferating cells in the intestine plateaus at ~50% in the crypt area (72 h posthatch) and 10 to 20% in the villus area (lowest in duodenum, 108 h posthatch) [3]. Next, and just underlying the epithelium within each villus is the lamina propria, a structural network that stabilizes the epithelium and contains nerve fibers as well as an abundance of immune cells including plasma cells (IgA secreting), T lymphocytes (generally $CD4^+$), macrophages, eosinophils, mast cells, and dendritic cells. The third major region of the GI tract consists of the immune system components, which are found within the lamina propria as well as in the regions underlying the mesentery, and consist of aggregated lymphoid tissues and Peyer's patches containing B and T lymphocytes ($CD8^+$ and $CD4^+$). These lymphoid tissues are similar to lymph nodes but do not have a capsule, medulla, or afferent lymphatic ducts [1, 4]. The muscularis layer underlies each of these regions and provides structural support and motility along the GI tract.

The final region of the GI tract is the mucus layer that covers the surface of the intestinal epithelium, provides lubrication of the epithelium, protects against enzymatic degradation of host tissues (auto- and alloenzymes), and presents a diffusion barrier, which is generally permeable to nutrients but not to macromolecules [5]. The mucus layer also plays an important role in the gut-associated lymphoid tissue (**GALT**), allows for fixation of bacteria, and provides a substrate for bacterial fermentation [6]. There are differences in published descriptions of the mucus layer dynamics; many researchers describe a single layer of mucus [7] whereas others describe a mucus bilayer composed of a loosely adherent mucus layer and a firmly adherent mucus layer, the thickness of which varies between anatomical regions of the GI tract [8]. This discrepancy may be due to techniques for measuring the mucus layer, which range from stain absorbance to intravital microscopy techniques, or may reflect differences between species examined. In fact, research suggests that mucus composition and distribution vary between every species of animal that has been studied [9]. In general, the thickness of the mucus layer is greatest in the gastric regions (presumably to protect the host against the acidic and proteolytic environment) and in the intestinal regions (where microbial populations are most numerous) [8]. In chickens specifically, mucin production is greatest in the proventriculus and small intestine, and within the small intestine, the highest mucin levels (determined with staining techniques) were in the distal regions [7].

The mucus layer is made up of mucin proteins that are glycosylated and secreted (rather than membrane-bound mucin) by goblet cells found in the epithelial layer of the GI tract [5]. Mucin proteins range in size from ~1,500 to 4,500 amino acids and are linked to oligosaccharide branches including N-acetyl galactosamine, N-acetylglucosamine, galactose, fucose, or sialic acid. The type of carbohydrate linkage determines whether the mucin is categorized as neutral or acidic (further classified into sulfo- or sialomucin) [5] and can affect the physical properties of mucins, including rigidity, protease resistance, and viscosity [10]. Neutral mucins are located mostly in the gastric mucosa whereas acidic mucins are found in the small and large intestine and tend to be more resistant to proteolytic degradation by bacterial enzymes [5]. Additionally, data indicate that acidic mucins in early life stages are beneficial

to the developing innate and intestinal immune system [11].

The timing of development of the anatomy and the specific digestive capacity of the GI tract vary among avian species and most likely reflect the evolutionary adaptations of that animal in terms of feeding strategy and pathogen load [12]. In chickens, the crypt area has few cells at hatch, but by 48 h posthatch (fed chicks), invagination of crypts is complete in all segments of the small intestine, and by approximately 5 d posthatch the number of cells per crypt, the number of crypts per villus, and the total villus surface area reach a plateau [3]. Similarly, by 24 h posthatch, enterocytes change from a uniform size and shape and gain polarity, increase in length, and develop a brush border [3]. Enterocytes migrate from the crypt to the villus tip in ~72 to 96 h (age dependent) [2], during which enterocytes acquire digestive function and begin to express enzymes such as disaccharidase and alkaline phosphatase [13]. Broiler chicks also synthesize maltase, isomaltase, and sucrase immediately posthatch [13], which allows for digestion of disaccharides. However, apparent digestibility of neutral detergent fiber or total nonstarch polysaccharides tends to be higher in geese and ducks as compared with chickens [12].

INTESTINAL IMMUNE SYSTEM

Virtually every cell type found in the intestine has immune functions. Most apparent are the functions of the Peyer's patches and other aggregated lymphoid follicles, which contain germinal centers where B and T lymphocytes proliferate and become antigen-specific lymphocytes, IgA-secreting plasma cells, and memory cells. In general, the activational state of lymphocytes in these germinal centers is higher compared with that of lymphocytes located in the spleen and thymus [14, 15]. Additionally, the lamina propria and epithelium also contain large populations of lymphocytes; the majority of T cells located in the lamina propria are $CD4^+$, whereas $CD8^+$ T cells and natural killer cells predominate within the epithelial layer [16, 17, 18]. Lymphocytes in the epithelium (often referred to as intraepithelial lymphocytes) directly interact with enterocytes, which provide regulatory signals (inhibitory and stimulatory) to maintain intestinal homeostasis [19, 20, 21]. Intraepithelial leukocytes also provide surveillance of host tissue by removing infected enterocytes [22], monitoring the development of epithelial tumors, protecting the enterocytes from pathogens, and promoting intestinal healing [23].

M cells, found within the epithelial layer, also have immune functions and provide an important route for antigen sampling. Unlike enterocytes, M cells do not have a brush border membrane, and they internalize whole macromolecules and microorganisms. Unique glycosylations on the luminal surface of M cells increase the likelihood of bacterial binding, which is followed by antigen internalization. M cells are then able to interact with lymphocytes via an intraepithelial pocket, a docking site for intestinal lymphocytes to sample the internalized antigen [24, 25] and transport it to a Peyer's patch or other aggregated lymphoid tissue for T- and B-cell priming [26]. Once activated, B cells undergo isotype switching and move throughout the systemic circulation and back to the lamina propria as IgA-secreting plasma cells [27]. Interestingly, the secretion of IgA into the intestinal lumen also involves M cells [28].

Finally, enterocytes and dendritic cells also play a role in the immune defenses of the GI tract. Dendritic cells located in the lamina propria [29] sample bacteria from the GI lumen by extending their dendrites between enterocytes, therefore avoiding the disruption of the barrier integrity [30]. Dendritic cells are hypothesized to distinguish between pathogenic and commensal bacteria via toll-like receptors, which recognize microbial-associated molecular patterns (expressed by commensal) and pathogen-associated molecular patterns (expressed by pathogens) [31, 32].

Enterocytes also participate in the immune system by several mechanisms. First, enterocytes provide a barrier through which there is an organized but limited absorption of food antigens, electrolytes, and water. Second, continuous cell turnover from the mitotic site in the crypts of Lieberkuhn and sloughing of enterocytes at the tip of the villus ensures barrier integrity. Third, enterocytes express toll-like receptors [10, 33, 34], which enables recognition of conserved antigen sequences. Enterocytes also express MHC classes II and I [35, 36, 37], which allows for antigen presentation to other immune cells, al-

though presentation efficiency is lower compared with other antigen presenting cells. Rather than activating $CD4^+$ T-helper cells (as is the case of most antigen presenting cells), enterocytes tend to activate $CD8^+$ T cells, and this disparity may explain the increased level of tolerance or anergy that occurs at the GI tract [36]. Finally, enterocytes can secrete immune mediators including interleukin (**IL**)-6 and nitric oxide [38, 39] and can induce intereukin-1 α and β and tumor necrosis factor [40, 41].

INTESTINAL MICROFLORA

The microflora in the GI tract of nonruminant species are a diverse population of organisms composed primarily of bacteria [42]. These microbial populations in the intestine can be broadly categorized into harmful and commensal populations. Harmful populations may be involved in the induction of infection, intestinal putrefaction, and toxin production. Commensal populations may be involved in vitamin production, stimulation of the immune system via nonpathogenic means and inhibition of harmful bacterial populations [6]. Bacterial populations may also be classified into luminal and mucosal populations, and mucosal microflora may be further divided into epithelial or cryptal [43]. Luminal bacteria are regulated by the influx of nutrients from the diet, the rate of passage of intestinal contents, and the level and activity of antimicrobial substances. Mucosal bacteria are regulated by their ability to bind to the enterocyte, the rate of mucin synthesis and secretion by goblet cells and the level and specificity of IgA secretion [6]. The luminal and mucosal microflora populations can be directly affected by stressors including feed restriction, antibiotic therapy, transportation, and disease [43].

In addition to diversity in bacterial populations within a particular anatomical region of the GI tract, there is also considerable variation in the bacterial populations throughout the GI tract. Most research has focused on interactions between nutrition and the colonic microflora (mammalian research) or cecal microflora (avian research), but the interactions between bacteria and animal nutrition in the small intestine is also crucial for animal health and performance. In either region, bacteria may compete with the animal for nutrients, may produce toxins, and can regulate secretory protein production (e.g., mucin) thus affecting local nutrient requirements [44].

DYNAMIC INTERACTIONS IN INTESTINAL ECOLOGY

It is apparent that there are numerous interactions among intestinal cells, intestinal bacteria communities, and the immune system, many of which are extremely complex and poorly understood. Therefore, the information that follows is merely an attempt to integrate these issues in the context of nutrition of the animal, although much more effort will be needed to understand these interactions.

Interactions Between Nutrition and Intestinal Anatomy

The intestine and its associated cells require nutrients to support their proliferation and differentiation as well as the secretion of enzymes, proteins, and other materials. The epithelial cells of the GI tract are continuously proliferating; although estimates are not available for birds, ~55 million gastrointestinal epithelial cells are proliferated each minute in humans [45]. In addition to cellular proliferation, total intestinal size and capacity can change rapidly as a result of rapid rates of protein synthesis and degradation, and the mean in vivo rate of protein synthesis in the GI tract of chickens has been estimated between 1.3 and 6.6 g/d with a fractional synthesis rate of 49 to 77%/d [46]. Energetic requirements of the gastrointestinal tract have been estimated at about 20% of incoming metabolizable energy [46].

The rates of enterocyte proliferation and migration are affected by a variety of factors. An overt nutrient deficiency will affect intestinal physiology, most notably for zinc [47, 48], vitamin A [49, 50], and cyanocobalamin (B_{12}) [51]. Additionally, glutamine is considered to be a major substrate for energy metabolism in intestinal epithelial cells and by immune cells throughout the body, and it has been hypothesized that when glutamine becomes limiting to these cells (e.g., severe stress), intestinal epithelial turnover, and barrier function is compromised [51, 52]. Fatty acid composition of the diet also affects enterocyte dynamics. For example, feeding broiler chicks oxidized fat (oxidized poultry fat, initial

peroxide value of 212.5 mEq/kg) resulted in increased turnover of intestinal epithelial cells and increased proliferation of hepatocytes [53]. Similarly, n-3 fatty acids affect goblet cell number and mucin production and enhance repair of damaged intestinal tissue in rats, although differential responses were seen to fatty acid sources, depending on whether the animal was challenged with an intestinal disease [54]. Other factors that influence enterocyte physiology include fasting or delay of food in the newly hatched chick [13, 55] and the presence or absence of microflora [56].

The availability of feed to the newly hatched chick is critical for appropriate intestinal development, bacterial colonization [57, 58], ability to digest and absorb nutrients [3, 56, 59], and development of a competent immune system [60]. A newly hatched chick must make a transition between lipid-rich yolk as the primary source of nutrients to carbohydrate and protein-rich feeds, and as the chick ingests feed, auto-enzyme secretion into the duodenum increases [61]. Interestingly, the partitioning of yolk lipids is different between chicks that are fasted or fed. When chicks have access to feed, yolk lipids are transported to the yolk stalk and into the small intestine to a much greater degree than when chicks have no feed access in the first hours to days posthatch [62], and yolk is used more rapidly by fed chicks [63]. In contrast, fasted chicks tend to have more yolk lipids directly transported to the circulation by endocytosis, resulting in greater levels of lipids in the plasma [62]. This altered partitioning likely contributes to reduced intestinal development in fasted chicks. For example, fasting significantly reduces the specific activity and expression of some digestive enzymes and nutrient transporters [51, 63], which lead to reduced growth rates [64], increased mortality, altered intestinal morphology, and increased disease susceptibility [65]. Fasting also affects the microflora populations that first colonize the GI tract and tends to increase proliferation of pathogenic coliform bacteria leading to increased concentration of deconjugated bile acids and reduced short-chain fatty acid (**SCFA**) concentration [66].

Finally, there are significant differences in the development of intestinal anatomy and function among birds of different breed types. Uni et al. [67], found that heavy- and light-strain birds had significantly different passage rates, enzyme secretion, and digestibility, although a significant portion of these differences may be related to the level of food intake. The age of breeder hen may also affect progeny intestinal development, although this may be more apparent for broilers, which are grown for a short time as compared with turkeys and other poultry. Research with turkeys has shown that breeder hen age (34 vs. 48 wk) affects villus height in the jejunum, although effects were only apparent for the first days posthatch [68]. Similarly, duckling intestinal development was similar when hatched from 32- or 44-wk-old hens [69].

Interactions Between Nutrition and Mucin

A major component of the nutrient requirements of the intestine is the investment into the synthesis and secretion of mucin, and mucin synthesis rates are generally higher than that of any other intestinal mucosal proteins [5]. Because secreted mucin will be catabolized by bacteria or excreted through normal intestinal sloughing, much of the nutrient investment into these glycoproteins will be lost to the host. In fact, endogenous protein losses that have high threonine, serine, and proline contents are thought to be indicative of mucin losses, and it has been estimated that endogenous crude protein losses as measured at the ileum consist of 11% mucin glycoproteins [5]. The optimal level of mucin synthesis and secretion is unclear, but it is clear that there is a critical balance between synthesis and degradation that directly impacts animal nutrition. Excessive mucin secretion increases endogenous nutrient losses and impairs nutrient absorption [6]. For example, a 30 to 50% reduction in linoleic acid absorption was observed in rats and humans as mucus thickness increased [5]. Therefore, by reducing the mucus layer, nutrient retention by the animal should be increased. Cowieson et al. [70] demonstrated that reduced sialic acid excretion (indicative of mucin secretion) correlated with reduced excretion of endogenous amino acids. However, although it is desirable that the mucus layer not be too thick, a thin mucus layer may increase the potential for bacterial invasion [6]. Factors that affect mucin dynamics include diet composition (discussed below), bacterial populations, and feed access. Data suggest that through the maintenance of a healthy microflora popula-

tion, fewer nutrients are invested into the production of microbial regulatory proteins such as mucin, and mucus thickness is reduced [71]. Similarly, timely access to feed prevents increases in goblet cell size, mucin mRNA expression, and protein synthesis, and overall reductions in mucin secretion are observed in chicks that are feed restricted for 72 h posthatch [7]. Therefore, some level of mucin synthesis is critical to maintain barrier function, although the optimal amount of mucin synthesis and mucus layer thickness is yet to be determined.

Mucin synthesis and secretion rates are affected by diet composition (nutrients and ingredients). For example, increasing dietary protein increases proteolytic enzyme secretion and mucin degradation, thus mucin secretion rates are expected to increase to maintain homeostasis of the mucin layer as observed in preruminant calves [72]. Therefore, excess dietary N may contribute to excreta N due to limited dietary absorption as well as increases in endogenous N losses. Ingredient choice also affects mucin dynamics via changes in viscosity. Increased viscosity in the lumen of the intestine is associated with reduced rates of nutrient absorption, which could be due to increased thickness of the mucus layer [73] and the subsequent increase in the time it takes for complete mixing of digesta with enzymes and bile acids [74]. At the same time, increased viscosity is hypothesized to decrease luminal oxygenation and increase residence time of the intestinal microflora [75]. Enzyme addition to diets may be one mechanism by which effects of viscosity can be reduced. Xylanase reduced intestinal viscosity in chicks fed wheat-based diets to a level similar to that of chicks fed a corn-based diet and resulted in altered types of secreted mucins [76], whereas phytase addition to wheat-based diets has been shown to reduce goblet cell numbers [77].

Finally, substrates for microbial metabolism (i.e., prebiotics, fiber, and nonstarch polysaccharides) can affect mucin dynamics. The effect of prebiotics is likely due to direct modulation of bacterial populations, which can regulate mucin dynamics. For example, isomaltooligosaccharide has been shown to directly affect cecal bacterial populations in poultry [78], whereas inulin and oligofructose induced alterations in the epithelial anatomy and in mucin when fed to rats [79]. Dietary fiber and nonstarch polysaccharides generally increase mucin secretion to protect against mechanical damage by the fibrous materials. For example, rats fed citrus fiber had an increase of 390% in mucin secretion in the stomach and 210% increase in mucin secretion in the small intestine compared with rats fed a fiber-free diet [5].

Interaction Between Nutrition and Microbial Populations

As previously stated, the bacterial populations in the GI tract represent an enormous contribution to the physiology of the animal. These populations can be regulated in several ways, and, in turn, bacteria can regulate intestinal physiology and nutrition of the animal. The choice of dietary ingredients can dramatically affect the microbial profile of the intestine [80] as can addition of dietary probiotics, prebiotics, or antibiotics [71, 79]. The major hypotheses to explain the beneficial effects of antibiotics on animal performance include 1) inhibition or reduction of subclinical infections, 2) regulation or reduction of toxin production by microflora, 3) reduction of competition for nutrients between animal and microflora, and 4) thinning of intestinal villi thus allowing for enhanced nutrient uptake [44, 82]. Based upon consumer pressure to reduce the use of growth-promoting antibiotics in animal production, alternatives are of considerable interest and include nutrients [83], prebiotics [84, 85], and probiotics. Prebiotics are generally supplements that support the growth of certain microbial populations, whereas probiotics contain live microbial cultures that are normal inhabitants of the gastrointestinal tract, can colonize the GI tract, and directly or indirectly affect other microbial populations [86].

Nutrients have the potential to regulate microbial populations. For their antimicrobial properties, copper and zinc are routinely fed to monogastrics at levels greater than requirements to promote growth. The effects of different sources of these minerals may vary. Dietary copper sources that are more soluble in the GI tract affect intestinal physiology in the anterior portions of the small intestine (i.e., duodenum), whereas sources that are less soluble in the GI tract affect the posterior portions of the small intestine (i.e., jejunum, ileum) [87]. Therefore, selection of dietary copper sources (and likely zinc sources as well)

to modulate GI physiology may be based upon the type and frequency of expected intestinal disease challenge.

Prebiotics can regulate bacterial populations found in the intestine and, thus, affect the nutrition of the animal. Physiological effects of nonabsorbable carbohydrates include increased fecal bulk, increased SCFA production, modification of bacterial populations, and alterations in serum chemistry profiles (e.g., cholesterol and triacylglycerol levels tend to be reduced) [88]. Prebiotics that have notable effects include inulin and oligofructose, which have been shown to increase *Bifidobacteria* populations in the lower intestine [89], and these bacteria produce SCFA, which may be used by the animal [90]. Lactosucrose can also affect microbial populations, resulting in reduced cecal ammonia and increased SCFA production after long-term feeding [91]. Similarly, in chickens the use of lactose to promote growth of specific bacteria can prevent *Salmonella* colonization in the cecum [92, 93, 94].

Probiotics have been studied for many years [95], more recently for efficacy in growing broilers [71, 84, 96], but the results have varied. It is likely that this variation is due to the chosen probiotic strain of bacteria [97] and the conditions under which it was provided to the animal. Some probiotic organisms seem most likely to be efficacious in poultry. Lactic acid bacteria (**LAB**) represent a substantial portion of the intestinal flora of chickens and can reach 10^9 cfu/g of cecal contents (determined by cultivation methods) [98], which meets the first criteria of a probiotic strain (normal inhabitant of the GI tract). Second, most LAB are acid and bile resistant [81, 99], thus fulfilling the second criteria of a probiotics (ability to colonize). The LAB strains have direct and indirect effects on other microbial populations and can reduce colonization by other bacteria, including *Escherichia coli* and *Clostridium perfringens* [100], and *Salmonella typhimurium* [101]. The LAB may also affect other bacterial populations, which is based on work in humans [102], pigs [103], and mice [97]. By regulating the microflora profile (and particularly by reducing certain pathogenic strains of bacteria), LAB (and other probiotics) may enhance food safety [104]. For example, reduction of *Salmonella* colonization might greatly affect the poultry industry. *Salmonella* are a major source of foodborne pathogens in poultry products, which cause severe infection and death in humans [105]. Many trials have examined *Salmonella* colonization in poultry in response to probiotics [81, 106, 107, 108], although many of these trials were conducted in cage systems, making it difficult to determine the microbial effects under commercial conditions. The LAB may also affect parasite invasion by *Eimeria tenella* [109]. LAB can affect local and systemic immune responses and have been shown to reduce production of tumor necrosis factor-α and IL-10 by peripheral blood mononuclear cells [110] and increase systemic antibody titers in humans [111] and chickens [104]. Finally, LAB have been shown to enhance broiler weight gains [112], and recent research has shown that a *Lactobacillus*-based probiotic enhanced weight gains, feed conversions, and bone breaking strength of chicks fed diets containing low levels of essential nutrients (12% reduction in crude protein, methionine, and lysine; 18% reduction in calcium and nonphytate phosphorus compared with a control diet which met NRC requirements) to that of chicks fed at or above NRC requirements. Additionally, apparent P, Ca and CP retention were greater than in chicks fed control diets, indicating that nutrient density may be reduced when animals are fed probiotics [113]. The LAB are not the only probiotic strains of interest. Many studies with other probiotic species have shown very encouraging results [81, 106, 108].

The effects of bacterial populations on the intestinal physiology of the animal are substantial. In general, presence of microbial populations in the GI tract results in smaller enterocytes (based upon protein:DNA ratio) and reduced enterocyte maturity (based upon total enzyme activity) in mammals [114] and birds [115]. Differences between mammals and birds do exist; birds appear to have increased mitotic activity under germ-free conditions [115], whereas mammals appear to have reduced mitotic activity [114]. This difference may be due to the fact that in birds, enterocyte proliferation occurs along the entire villus, which is not the case in mammals [2]. Additionally, germ-free animals tend to have significantly reduced numbers of enterocytes at all levels of the villus [114] and increased rates of enterocyte migration [116]. Faster rates of enterocyte migration coupled with reductions in enterocyte number are associated with reduced capacity to absorb

nutrients [117], and it has been demonstrated that glucose and SCFA absorptions are greater in conventional animals as compared with germ-free animals, resulting in adipocyte hypertrophy and greater body lipid content [118, 119].

Bacteria may also provide nutrition to the host via secretion of nutrients, including vitamins (particularly B vitamins and vitamin K) and amino acids, and SCFA, including lactate, acetate, propionate and butyrate, which can contribute to the energy supply of the animal and can affect bacterial populations as well [117]. These SCFA are absorbed by passive diffusion in the large intestine and may be used for synthesis of ketone bodies (e.g., butyrate), glucose (e.g., propionate), or lipids (e.g., acetate) [117]. SCFA may also directly regulate intestinal function. Butyrate has been shown to affect IL-8 production by epithelial cells [120]. Because IL-8 is involved with neutrophil and monocyte recruitment, butyrate may have direct affects on intestinal immune responses. Bacterial protein is an excellent source of amino acids, but only a small fraction of these amino acids are likely to be absorbed by the host [121], so this protein source is generally lost to the host unless coprophagy is practiced [117]. Metabolites from bacterial fermentation may also provide nutritional value to the animal, although in many cases there is a fine line between beneficial and toxic effects [122]. For example, birds can absorb bacterial-derived amines from decarboxylation of amino acids [123], which can directly affect performance. Laying hens fed putrescine at a low concentration have significant improvements in eggshell weight, but higher putrescine concentrations are associated with reductions in performance [124]. Finally, microbial enzymes may contribute to host nutrition. For example, microbial phytase increases phytate phosphorus disappearance and increases total phosphorus digestibility [125]. Additionally, true ileal amino acid digestibility for several amino acids is greater in the presence of microbial phytase [125].

At the same time that bacteria may provide nutrition to the animal, they may also compete with the host for nutrients or produce toxic metabolites. For example, microbial populations can affect lipid digestion by modulating lipase activity or the level of unconjugated bile salts [126], which directly affect performance of birds by limiting absorption of lipids and lipid-soluble vitamins. This effect is likely to be most notable in younger birds (<6 wk of age); in the very young chick, bile acid synthesis and resorption rates appear to be lower than in older counterparts [127]. Ammonia production by bacteria also results in toxicity to intestinal epithelial cells, as reflected in increased enterocyte turnover and mucin degradation followed by increases in mucin production and secretion [128]. Finally, bacteria can adhere to and degrade mucin, and most bacteria studied can induce mucin gene expression [9] and can enzymatically degrade mucin [129]. Interestingly, the species of bacteria affects its ability to regulate mucin synthesis. Enteropathogenic *E. coli* and *Lactobacillus plantarum* induce MUC2 mRNA expression by intestinal epithelium cells, whereas MUC3 mRNA expression is increased by *L. plantarum* [130] only.

Finally, and of paramount significance, are the recent molecular tools that allow researchers to better characterize the bacterial populations in the GI tract. Until recently, bacterial populations in the intestine were characterized by culture-based methods, which are labor and time intensive, and many bacterial populations do not grow under culture conditions. In fact, in the chicken cecum, approximately 90% of bacteria are not cultivable using current microbiological techniques [131], which makes bacterial profiles determined using culture methods suspect. Recent technologies allow for a more thorough examination of bacterial profiles. The 16S rRNA gene sequence has been used as a tool to determine phylogenetic relationships between bacteria and, more recently, to identify bacterial populations in environmental samples without cultivation [132]. This technique has also been used to determine specific microbial profiles of the chicken intestine [131, 133, 134]. In addition to using species-specific primers to identify precise bacterial populations, terminal restriction fragment length polymorphism (**TRFLP**) has been used to examine the diversity of the intestinal microflora [135], and this tool has been used in chickens to examine changes in microbial populations in response to heat stress [134]. By using TRFLP, the diverse microbial community can be examined rapidly and assessed semiquantitatively for global differences, whereas subsequent quantification of particular populations of interest may be completed

using techniques such as quantitative polymerase chain reaction.

Nutrition and Intestinal Immune Responses

To make issues of intestinal ecology more complex, the GI immune system regulates the degree of inflammation at the intestinal level, thus regulating nutrient digestion and absorption. When inflammatory responses are induced, nutrient metabolism is diverted from growth and development of skeletal muscle [136] to the acute phase response and other catabolic events [137]. Thus, by reducing inflammatory challenges through regulation of microbial populations and promoting intestinal development, the animal will invest nutrients into anabolic rather than catabolic functions [138].

Many nutrients have been shown to have direct effects on immune responses. Important examples in the intestinal tract include fatty acids, because there is evidence that dietary fatty acids directly affect the profile of fatty acids in the GI tract [54]. Additionally, SCFA produced by intestinal bacteria have direct effects on epithelial cells and have been shown to be antiinflammatory in some cases. For example, SCFA have been shown in vitro to inhibit DNA binding by nuclear factor-κB, and butyrate has been shown to regulate IL-8 and IL-6 levels in the intestine during inflammation [51]. The same nutrients that directly affect enterocyte physiology during nutrient deficiency will also affect the intestinal immune response. For example, vitamin A deficiency significantly increases levels of inflammation in the intestine [139].

As is the case for proper nutrition, intestinal bacteria are critical for normal immune system development. The intestinal microflora is involved in induction of mucosal immunity, induction of oral tolerance, and generation of B-cell diversity [140]. In several mammalian species, it is clear that B-lymphocyte proliferation and diversification of the antibody repertoire are reliant on intestinal bacteria. In birds, because these processes occur before hatch, they occur independently of bacterial populations. However, recent evidence suggests that the GI microflora is required for B-cell survival in the bursa after hatching [140].

CONCLUSIONS AND APPLICATIONS

1. It is clear that there are extremely complex interactions among the components of intestinal ecology: host intestinal anatomy, microbial populations, and nutrition of the animal. Understanding these interactions will provide tools by which animal health and performance can be maximized, while the use of pharmacological agents and the excretion of nutrients can be minimized.
2. New technologies will allow researchers to better identify and characterize microbial populations and determine relevant populations for animal health and nutrition. Because bacterial communities work in concert, it is critical that rather than examining a single population of bacteria, experiments should examine the entire framework of microbial ecology.
3. The opportunity to use genetically modified organisms may allow researchers to better understand interactions between environment, anatomical region, development stage, and nutrition.
4. Use of new and traditional research tools to determine the relationship between various parameters of the intestinal ecology and the performance of the animal will allow data to be integrated from multiple experiments.
5. Collaborative efforts will be necessary to critically and effectively examine all of these components.

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