

# Informal nutrition symposium: leveraging the microbiome (and the metabolome) for poultry production

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**ABSTRACT** Knowledge of gut microbiology of poultry has advanced from a limited ability to culture relatively few microbial species, to attempting to understand the complex interactions between the bird and its microbiome. The Informal Nutrition Symposium 2021 was intended to help poultry scientists to make sense of the implications of the vast amounts of information being generated by researchers. This paper represents a compilation of the talks given at the symposium by leading international researchers in this field. The symposium began with an overview of the historical developments in the field of intestinal microbiology and microbiome research in poultry. Next, the systemic effects of the microbiome on health in the context of the interplay between the intestinal microbiota and the immune system were presented. Because the microbiome and the host communicate and influence each other, the novel

field of kinomics (the study of protein phosphorylation) as used in the study of the poultry microbiome was discussed. Protein phosphorylation is a rapid response to the complex of signals among the microbiome, intestinal lumen metabolites, and the host. Then, a description of why an understanding of the role of microbial endocrinology in poultry production can lead to new understanding of the mechanisms by which the gut microbiota and the host can interact in defined mechanisms that ultimately determine health, pathogenesis of infectious disease, and behavior was given. Finally, a view forward was presented underscoring the importance of understanding mechanisms in microbiomes in other organ systems and other species. Additionally, the importance of the development of new -omics platforms and data management tools to more completely understand host microbiomes was stressed.

**Key words:** poultry, microbiome, immunometabolism, kinomics, microbial endocrinology

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## INTRODUCTION

Momentous changes have occurred in the last 2 decades in our understanding of the interactions of poultry with microbial populations in the intestinal tract. We have gone from studying intestinal microbial populations and

trying to identify individual species to describing them as a microbiota or the living organisms in a specific ecosystem. The focus in the last few years has been toward the microbiome concept, which views the microbiota behavior or function within a particular space (see Berg et al., 2020 for review). Functional interactions between the microbes that constitute the microbiota and their environment are the main focus of this type of research. This overview will focus on the history of these developments as well as on different host–microbiota interactions. The areas that will be covered are immune metabolic interactions between the microbiome and the host; kinomics, or the regulation of the metabolome defined as the small molecule

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intermediates of metabolism; microbial endocrinology that tackles the crosstalk between the microbiota and the host through, in part, neurotransmitters. Current and potential applications of the new knowledge being derived in these areas are also discussed. What is clear from this overview is that a holistic integration of the different areas of focus will be necessary to move forward in this search for understanding of the numerous interactions between the hosts; this sometimes being called a “new organ” the metabolome.

## HISTORICAL OVERVIEW OF THE DEVELOPMENT OF POULTRY MICROBIOME RESEARCH

Use of microbiome transfer (transfaunation) has been practiced for centuries in veterinary medicine in ruminants to restore nutrient function (DePeters and George, 2014; Niederwerder, 2018). Fecal transfaunation was described centuries ago in Chinese medicine to treat intestinal and other diseases (Leung and Cheng, 2019). However, the research in this field is much more recent. Because pathogens are commonly members of environmental or vertebrate microbiomes, the growth of microbiome research essentially began with the growth of medical microbiology and its methodology. Medical microbiology was introduced in the First Renaissance of Microbiology in the 1700 and 1800s with the recognition of the role of microbes in health and disease. While Edward Jenner gets much of the credit for initiating vaccination for prevention of smallpox in 1757, it was a common practice in the 16th century in the Middle East and likely spread through Africa and Asia prior to its use in Europe (Gross and Sepkowitz, 1998; Riedel, 2005). Medical hygiene came to Europe in the 1840s with Ignaz Semmelweis’ observation that childbirth fever was commonly associated with physicians who did not wash their hands between autopsies and delivering babies (Tyagi and Barwal, 2020). Therefore, recognition that microbes could be transmitted was formally established leading to a better understanding of their roles. This First Renaissance included revelations by some of the most recognizable names in microbiology, Leeuwenhoek (visualization), Spallanzani (refuted spontaneous generation of microbes), Jenner (vaccination), Pasteur (sterilization and vaccination), as well as Koch (postulates for verifying infectious cause of disease) (Guardino, 2005).

The growth of microbiology methods and revelations regarding the microbial causes of some diseases flourished in the 1880s. Robert Koch was the first to isolate bacteria in pure culture (anthrax) and demonstrate its role in the disease. Julius Petri worked in Koch’s lab and developed the tools and methodology for bacterial culture that we largely use today. These discoveries led to an expansion of pathogen detection methodology that contributed to the growth of public health infrastructure in the early 1900s and “the great sanitary awakening” to reduce infectious diseases transmitted by sewage

(Martin, 1926). Many of the microbiologists in the First Renaissance had broad focus including environmental, food and fecal samples that enabled development of sample specific methodologies (Bertrand et al., 2015).

The *Microflora of the Rumen* (McGaughey and Sellers, 1948), for example, was one of the early works of microbiome research; however, a survey of the published literature shows a long gap in time because most of the cultivation-based microbiome studies occurred in the 1970-80s with the bloom of anaerobic culture methods that evolved from methodology of the First Renaissance. Many of these are described in *Intestinal Microbiology* (Drasar and Barrow, 1985) published as a series of short books commissioned by the American Society of Microbiology. Coauthor Paul Barrow was a poultry microbiologist known for his work on *Salmonella* colonization; however, much of his work focused on the role of the microbiome in competitive exclusion (Barrow, 1992). Cultivation-based studies established a baseline for describing the microbiome and its impact on bird development and performance. Studies from the 1970s revealed a very high abundance of bacteria in the chicken cecum at approximately  $10^{11}$  bacteria per gram, which is nearly the physical capacity of a cubic centimeter (Salanitro et al., 1974b). Avian and ruminant samples were a significant focus of labs seeking to develop anaerobic culture methodology because only a small portion of viable cells quantified by microscopy could actually be cultured. This became known as the “great plate count anomaly” illustrating the limitations of culture methodology (Staley and Konopka, 1985).

Poultry microbiologists discovered that an elevated CO<sub>2</sub> anaerobic atmosphere allowed colony counts approaching 80% of the cecal microbiome illustrating the rapid advancement of culture methodology for poultry samples (Salanitro et al., 1974a). During the 1970s-1980s, Ella M. Barnes and colleagues published a series of elegant studies of avian anaerobes including a description of the early microbiome of young birds, manipulation of its composition and describing novel species making up the anaerobic community (Barnes and Impey, 1968; Barnes, 1979; Barnes et al., 1980). These cultivation-based reports were the foundation of interpreting the studies of avian intestinal physiology, function, and development in the absence of the microbiome with the use of gnotobiotic (germ-free) birds (Furuse and Okumura, 1994). Furuse and Okumura’s review of the literature (1994) included a number of studies that described characteristics of germ-free chickens including growth rates, feed consumption, and body weight compared to conventional birds. Interpretation of the differences between conventional birds and germ-free included assessment of intestinal structure and development in an effort to explain the role of the microbiome in gut development. These studies included comparisons of metabolic and brush border enzyme activity (Siddons and Coates, 1972; Palmer and Rolls, 1983), morphometric parameters such as microvillus length, villus area, crypt depth and proliferative cellular pool in the crypt (Cook and Bird, 1973), goblet cell counts

(Cheled-Shoval et al., 2014), and nutrient absorption (Boyd and Edwards Jr., 1967; Ford and Coates, 1971; Yokota and Coates, 1982).

In particular, these studies illustrated that growth disadvantage was evident in conventional chickens colonized with specific bacterial species leading to the finding that weight gain and feed conversion improved with antimicrobial-amended feed (Lev and Forbes, 1959). This improvement, however, was not observed in germ-free animals and it was most pronounced in chickens raised in heavily contaminated environments (Lev and Forbes, 1959). The mechanism of action of growth-promoting antibiotics has been ascribed to suppression of microbiome density allowing better host access to feed nutrients. However, it may also be mostly due to the suppression of certain pathogenic bacterial species, including *Clostridium perfringens* that causes intestinal damage (Fukata et al., 1991; Hofacre et al., 1998). The earliest studies in transfaunaation were done to suppress disease (pathogens) therefore is not surprising that the poultry intestinal microbiome itself can also exhibit “competitive exclusion,” a concept important in disease control (Barrow, 1992). The challenge was to identify the microbes that exhibited the ability to control pathogen colonization or pathogen behavior (Pedroso et al., 2021).

The Second Renaissance of microbiology has been technology-driven and arrived with the discovery of DNA sequencing and the understanding that a molecular approach could augment classical methods in classifying microbes. Molecular ecology was born from the finding that comparative cataloging of 16S ribosomal RNA provided a database of prokaryotic systematics (Fox et al., 1977). Thus was born the prokaryotic phylogenetic tree using 16S rDNA fingerprinting, which launched rapid growth of environmental molecular ecology that was eventually adopted by microbiologists studying the gut microbiome. Initially the methods were applied to cultured isolates from varied environments; however, new techniques of separating DNA and RNA molecules (cloning and then microfluidics) allowed cultivation-free molecular analysis. These initial poultry studies focused on acquiring a census of the intestinal bacterial community and documenting the effects of age, breed, and feed ingredients on the composition. Cultivation-based studies showed the bacterial microflora of chickens’ cecum is primarily gram positive (Barnes and Impey, 1968; Salanitro et al., 1974b). Prior to the 16S rDNA studies in birds, Apajalahti demonstrated treatment effects on the intestinal microbiome of chickens using G+C profiling of DNA extracted from the intestinal contents (Apajalahti et al., 2001). Following Apajalahti’s intriguing findings, a group of studies using 16S rDNA methods in 2002-2003 showed that the major components are *Lactobacillus* and *Clostridia* with compositional differences in the small and large intestines and ecological changes correlated with age (Gong et al., 2002a,b; Lan et al., 2002; Lu et al., 2003; Zhu and Joergers, 2003). Comparable analysis of the turkey intestinal microbiome would occur a few years later with

similar findings as in chickens (Lu and Santo Domingo, 2008; Santos Jr. et al., 2008).

Of course, the assumption was that a census of the members of the microbiome would be meaningful; that describing changes would be illuminating in regards to feed conversion and competitive exclusion. With the exception of detecting pathogens in the microbiome, compositional studies have been less than helpful. For monogastric vertebrates, we have slowly and reluctantly accepted that correlation is not causation. Many changes detected by culture or molecular methods were due to developmental changes in the bird and not from experimental conditions. This disappointing revelation means that the Third Renaissance of microbiology must be function-driven. Monogastric microbial ecology has struggled with the transition to functional assays that are the hallmark of environmental and ruminant microbial ecology. In the 1930s-1980s, Robert Hungate studied the termite gut and cattle rumen therefore describing the fermentative microbial communities and rumen methanogens (Bertrand et al., 2015). This was the first fully characterized microbial ecosystem and resulted in a culture system for strict anaerobes. The study of the rumen ecosystem was supported by rumen protein assays, volatile fatty acid assays, amino acid composition, and strong culture methodology correlating the contributions of specific microbial species.

Ruminant and termite microbiology illustrated the first well described animal/microbe symbiosis in nutrition which resulted in the concept of microbiome that we use today. However, recent studies of the role of specific bacterial interactions illustrated by the bioluminescent Hawaiian squid/*Vibrio* symbiosis indicate greater roles for animal development related to symbiosis (Nyholm and McFall-Ngai, 2004). Therefore, the Fourth Renaissance in microbiology, specifically related to animal health and food production will likely be tending the microbial farm rather than our antiquated methods of pathogen control and animal husbandry.

Over the centuries and especially in recent decades, a greater understanding of the field has been gained, both in terms of advances in techniques and an appreciation for the limitations of our current knowledge. A growing understanding of the fundamental biology will allow researchers to make further discoveries regarding the inter-relationship between the microbiome and the host. Nutritionists will be able to leverage that knowledge into practical use and optimized production approaches.

## THE LINK AMONG IMMUNOMETABOLISM, METABOLIC HEALTH, AND GUT MICROBIOTA

### *Immunometabolism and Metabolic Health*

The development and survival of multicellular organisms like poultry depend on the crosstalk

between immunologic and metabolic pathways. The traditional role of such immunometabolic interplay is to divert nutrients, most notably energy, toward the immune system and away from growth and other physiological functions during the setting of infection. In addition to fueling leukocyte activity and synthesis of immune mediators, reallocated nutrients sustain metabolic futile cycles that account for a large proportion of the infection-induced losses in poultry productivity (Klasing, 2017). In recent years, studies in mammals and the model organism *Drosophila melanogaster* have also revealed that conserved immunometabolic processes assist immune cells infiltrated in metabolic organs like the liver, white adipose tissue (WAT), and pancreas to drive inflammatory events intending to repair or renew neighboring cells stressed by metabolic anomalies like chronic nutrient excess (Hotamisligil, 2017). In this way, immunometabolic interactions safeguard the function of metabolic tissues and thereby the “metabolic health” of the organism (Zmora et al., 2017). However, immunometabolic dysfunction triggered by excessive supply of nutrients and energy has been causally linked to most noncommunicable metabolic diseases (i.e., unrelated to infections) currently affecting millions of human beings (Hotamisligil, 2017; Zmora et al., 2017). Even though the incidence, prevalence, and implications for poultry production of noncommunicable metabolic disorders are currently unclear, the described evolutionarily conserved mechanisms also seem to govern the inflammatory tone and metabolic competence of modern poultry breeds raised under prevailing production schemes (Kogut et al., 2018). In either context (infection or metabolic stress), it should be noted that inflammation is a transient physiological process intended to facilitate organismal adaptation to fluctuations in environmental factors and nutrient availability. More specifically, a short-lived and spatially limited immune response is vitally important not only for eliminating invading pathogens, maintaining tissue integrity, and rapidly resolving an inflammatory episode but also for restoring the transiently compromised metabolic homeostasis. Yet, systemic inflammatory states originating in the intestine that are persistent, low grade, and cause metabolic dysfunction as observed in humans (termed metabolic inflammation or metaflammation) (Hotamisligil, 2017) are increasingly recognized as major challenges compromising the health and productivity of poultry (Kogut et al., 2018; Cardoso Dal Pont et al., 2020) and livestock species (Liehr et al., 2017; Cangiano et al., 2019).

### **Immunometabolic Instructions by the Gut Microbiome**

Along with host genetics and diet, the intestinal microbiome is an essential regulator of immunometabolism and metabolic health. In mammals, metabolic

pathology induced by exogenous factors like high-fat diets, antibiotics, or circadian disruption is associated with chronic inflammation of insulin-sensitive tissues and altered composition or activity of gut microbiota (also referred to as gut dysbiosis) (Dabke et al., 2019; Scheithauer et al., 2020). Although gut dysbiosis is a context-specific singularity, it frequently entails deviations in the interaction between usually underrepresented microbes (e.g., opportunistic pathogens) and epithelial/immune cells, shedding of microbial-associated molecular patterns in the intestinal lumen (e.g., lipopolysaccharides), and availability of metabolites either produced (e.g., volatile fatty acids) or transformed (e.g., trimethylamine, secondary bile acids) by gut microbiota. These alterations eventually contribute to systemic metabolic incompetence by dysregulating enteroprotective mechanisms in charge of preventing mucosal inflammation and hyperpermeability (i.e., leaky gut) and remotely affecting metabolic organs that become inflamed and resistant to the canonical function of metabolic hormones like insulin, glucagon, and fibroblast growth factor 21 (Zmora et al., 2017; Dabke et al., 2019; Scheithauer et al., 2020). In this scenario, the immunometabolic program underlying metabolic-tissue dysfunction not only includes leukocytes but also stromal components and metabolic cells (hepatocytes, adipocytes,  $\beta$ -cells) where integration of inflammatory and metabolic signaling is mediated by receptors (immune, hormone, and nutrient sensors), organelle (mitochondria, endoplasmic reticulum), kinase pathways (IKK, ERK, JNK, AMPK, and mTOR among others) and gene expression (most notably cytokines, adipokines, and lipid mediators) (Buck et al., 2017; Hotamisligil, 2017).

Arguably, proof-of-concept and suitability of the described microbe-immunometabolism link and its implications for short-lived animals like poultry are proven by experimental models on the growth-permitting action of subtherapeutic doses of antibiotics. Briefly, a series of studies showed that the intestinal colonization of germ-free mice with microbiota from donor counterparts treated with low doses of antibiotics 1) increased weight gain and fat deposition when fed standard diets and, particularly, when offered an energy-rich chow, 2) reduced biomarkers of mucosal immune activation, but aggravated inflammatory signals in the liver of animals fed high levels of fat, and 3) caused multiple shifts in pathways implicated in glucose and lipid metabolism, mainly in liver and WAT (Cho et al., 2012; Cox et al., 2014; Schulfer et al., 2019). These reports, along with several others not cited herein, establish a causal connection among gut microbiota, immunity, metabolism, and the growth-promotion phenotype. Furthermore, they provide correlational evidence suggesting that altered microbial production of metabolites, including volatile fatty acids and bile acids (BA), is mechanistically involved in the molecular dialogue mediating their integration.

## **The Gut Microbiota-Bile Acid Axis in Immunometabolism and Metabolic Health**

The fitness of the mutualistic relationship between host and intestinal microbes is partly dictated by a wide array of molecular interactions between them. An expanding number of molecules either produced or transformed by gut microbiota is known to operate as signals in the host-microbe interplay and through their cognate receptors influence host metabolism and immunity, among other vital functions (Donia and Fischbach, 2015). One group of such molecules synthesized by the liver and later modified by gut microbiota are BA, which homeostasis and pleiotropic functions are under the control of the nuclear receptor farnesoid X receptor (FXR) and the G-protein-coupled sensor TGR5. Because BA structure and concentration dictate their ability to signal through FXR and TGR5, microbial bioconversions are a major regulator of the multifaceted influence of BA on immunometabolism (Wahlstrom et al., 2016). In parallel, BA shape the assembly, composition, and activity of gut microbes directly via their antimicrobial properties and indirectly through modulation of the mucosal immune repertoire (Chen et al., 2019; van Best et al., 2020). In part, this is possible because FXR and TGR5 are expressed in a wide spectrum of tissues and cell types, including the intestine, liver, immune cells, adipocytes, pancreas, kidneys, muscle, nervous system, and brain (Duboc et al., 2014; Mazuy et al., 2015). Given their broad expression, these BA receptors intervene in multiple signaling pathways implicated in the homeostatic regulation of metabolism (glucose, lipids, and amino acids) and immunity (innate and adaptive) (Di Ciaula et al., 2017). In short, BA interact with FXR and TGR5 to 1) promote metabolic competence primarily by restraining lipogenesis, gluconeogenesis, and insulin resistance during the postprandial phase, and 2) prime a tolerogenic state by downregulating immune reactivity in the face of enhanced flow of nutrients, dietary xenobiotics, and microbial antigens that follows a meal (Fiorucci et al., 2018; Molinaro et al., 2018). Consequently, factors that impinge on the BA-mediated dialogue between intestinal microbes and host, like high-fat diets and antimicrobial compounds, have the potential to cause immunometabolic defects and metabolic inflexibility by altering BA metabolism by gut microbiota and impairing pathways controlled by BA receptors (Wahlstrom et al., 2016; Chavez-Talavera et al., 2017).

The above observations suggest that alterations in BA biology may indeed underlie the growth-promoting effect of antimicrobials. This hypothesis was recently examined using a model of agricultural growth promotion to nurse early-weaned pigs (Ipharraguerre et al., 2018). Briefly, authors observed that customary combinations of antimicrobial agents (i.e., antibiotics and zinc oxide) converged in modifying BA biosynthesis by colonic microbiota, which led to increased production of 7 $\alpha$ -dehydroxylated BA species (especially of

lithocholic acid). In turn, this change increased the potency of the BA pool to activate FXR and TGR5 in multiple tissues and induced growth-supporting adaptations in endocrine, metabolic, and immune pathways regulated by BA. Of particular interest, the antimicrobial-remodeled BA pool repressed lipogenesis and fat accumulation in the liver and downregulated inflammatory tone in subcutaneous and visceral WAT. These effects were associated with increased expression and/or activity of hepatic FXR and WAT TGR5, suggesting that increased BA signaling in those tissues improved metabolic fitness of antimicrobial-fed animals (Ipharraguerre et al., 2018). In line with this proposition, supplementation of broiler diets with porcine BA enhanced weight gain, feed conversion, and percentage of thigh muscle in the carcass, whereas reduced the deposition of abdominal fat (Lai et al., 2018). Furthermore, the feeding of high-fat starter (6.1 vs. 2.6%), grower (10.2 vs. 2.7%), and finisher (11.4 vs. 3.0%) diets to broiler chicks resulted in higher BW and a remarkable enrichment of BA pools (bile, plasma, cecum, and feces) with cholic acid (CA), a 12-hydroxylated BA produced by the liver (Techna France Nutrition, unpublished). Importantly, the circulating levels of this BA are abnormally high in obese mammals because of reduced microbial transformation of CA into secondary BA (Wei et al., 2020).

In the cited study with antimicrobial-fed pigs; however, the most profound changes were observed in the gut where increased BA signaling through FXR and TGR5 strengthened mucosa protection against bacterial infection and pathological secretion of fluids and electrolytes, which were otherwise deteriorated by weaning stress (Ipharraguerre et al., 2018). Supporting these findings, recent evidence demonstrates that microbial metabolites of lithocholic acid have potent antimicrobial action against multidrug-resistant pathogens (Sato et al., 2021) and drive differentiation of T cells toward regulatory T cells in colonic lamina propria (Hang et al., 2019), which jointly support intestinal homeostasis. Furthermore, research in broiler chicks revealed that deoxycholic acid, a 7 $\alpha$ -dehydroxylated metabolite of CA abundantly present in birds, protects chickens against colonization by *Campylobacter jejuni* (Alrubaye et al., 2019) and necrotic enteritis induced by *Clostridium perfringens* (Bansal et al., 2020).

Collectively, available evidence identifies BA as integrators and modulators of the host metabolic and immune-inflammatory responses to alterations in gut microbial ecology and activity, making them a promising target for the development of interventions to improve animal health and performance. Certainly, this suggestion is partly supported by results from recent studies exploring the use of natural agonists of BA receptors in experimental settings of chronic inflammation (Liehr et al., 2017; Cangiano et al., 2019) or intestinal dysfunction (Lin et al., 2019) in farm animals, including poultry (Herrero-Encinas et al., 2020). Additionally, BA may be useful biomarkers of intestinal microbiota assembly, composition, and activity as well as the influence of

such microbial traits on host metabolism and immunity. Beyond the proposed therapeutic and diagnostic value of BA, understanding the still-obscure molecular basis that underlie the immunometabolic interplay between host and its microbiomes may illuminate ways for safeguarding the health, productivity, and welfare of animals like poultry.

## KINOMICS: REGULATION OF THE METABOLOME

The history of microbiology research and its application to animal physiology and nutrition has been dependent on advances in analytical techniques. As previously discussed, it is not only important to understand what microbial species may be present in the intestine, but it is likely more critical to understand what physiological processes are taking place. The metabolome, the small molecule intermediates of metabolism within a biological system (EMBL-EBI, 2021), has a complex regulation that involves a number of fundamental biological principles. One of the predominant regulatory mechanisms in eukaryotes is post-translationally modifying protein to alter its function in response to stimuli (Graves and Krebs, 1999). The most prevalent post-translational modification is phosphorylation. Indeed, Nobel Prize winner Edmond Fischer stated that, “Phosphorylation is one of the most prevalent mechanisms of regulation and it is clear that it would be very difficult to find a physiological reaction that was not directly or indirectly affected by protein phosphorylation” (Fischer, 2015). Kinases are enzymes that carry out protein phosphorylation; kinomics is “the study of kinases, phosphatases and their targets, and has been used to study the functional changes in numerous diseases and infectious diseases with aims to delineate the cellular functions affected” (Berard et al., 2018). Considering the above and the relative paucity of tools to carry out high-throughput metabolomics, especially in the chicken, the study of phosphorylation-dependent regulation of the metabolome via kinomics is an opportunity for poultry scientists.

Advantages of kinomics over other omics techniques (genomics, transcriptomics, etc.) are that phosphorylation of protein can occur nearly instantly and alters host function very near the observable phenotype (Albertin et al., 2013). For example, the constituent enzymes that are required to carry out glycolysis are present in the cell in the absence of glucose or an insulin signal. These enzymes are activated when energy is required via glycolysis by a variety of biochemical signals, such as allosteric activation or phosphorylation. The activation of glycolysis is not dependent on the transcription and subsequent translation of the glycolytic enzymes, as this would not allow for the rapid adaptation to the ever-changing metabolic environment (Albertin et al., 2013). Indeed, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme that catalyzes a step of glycolysis, has a transcription rate

that is so stable that it is often used as a housekeeping control gene for PCR experiments (Silver et al., 2006). Analyzing the transcriptional change of such a gene would yield very little information regarding the metabolic state of a bird.

On the other hand, by understanding the phosphorylation state of several critical metabolic regulating proteins, one can elucidate a large amount of metabolic data for an organism. A classic example is AMP-activated protein kinase (AMPK). A major function of AMPK is to act as an energy sensor for the cell, sensing the ratio of ADP and AMP to ATP (Hardie et al., 2012). When there is a reduction in ATP, it signals that the cell is in need of energy, AMPK is phosphorylated and a number of metabolic changes are induced in the cell. These changes include, increases in glucose uptake, glycolysis, mitochondrial biogenesis and fatty acid oxidation, in addition to decreases in protein synthesis, fatty acid synthesis, sterol synthesis, and glycogen synthesis (Srivastava et al., 2012). It becomes clear that understanding the phosphorylation status of just a single kinase can provide a wide range of information on the metabolic state of a cell, tissue or organism. Combine this with the knowledge of the phosphorylation status of other key metabolic proteins regulated by phosphorylation such as, PI3K, AKT, mTOR, HIF, MAPK, GSK3B among others, and a quite comprehensive view of metabolism can be obtained. Metabolites can also act as signaling molecules themselves, binding to receptors, and initiating phosphorylation-dependent signal transduction cascades that alter cellular function (Liu and Wellen, 2020). Examples include lactate, butyrate, alpha-ketoglutarate, and succinate (Liu et al., 2018; Liu and Wellen, 2020). By understanding the signaling induced by metabolites one can infer their presence and determine their effects.

## Techniques

A number of well-established techniques have been developed to study protein phosphorylation. Likely the most widely used is western blotting using phosphospecific antibodies. These antibodies will only bind to the phosphorylated protein and will not bind to the nonphosphorylated form. Well-characterized metabolic regulatory proteins such as AMPK have been studied using this method (Bäckhed et al., 2007). A high-throughput technique that uses the similar principles to the western blot is antibody microarrays (Alhamdani et al., 2009). With these arrays, phosphospecific antibodies are immobilized on an array surface. Labeled proteins from the sample of interest are then applied to the array and the antibodies will capture their complementary protein. Visualizing the label or adding a second labeled antibody allows one to determine the presence of the phosphorylated protein of interest. Mass spectrometry has been used to study both metabolomics (Alseikh et al., 2021) and post-translational modifications (Breitkopf and Asara, 2012). With this technique, digested and

separated sample fragments are run through a tandem mass spectrometer and the fragment spectrum is analyzed to identify metabolites or proteins containing specific modifications. Finally, the species-specific kinome peptide array has been used extensively in the past several years to analyze the kinome of chicken, and other agricultural species (Daigle et al., 2014). The technique involves printing kinase enzyme target sequences on an array, when exposed to a biological sample the active kinases will recognize and phosphorylate their target on the array. This phosphorylation can be recognized by phosphospecific fluorescent dye and the data can be analyzed to characterize the phosphorylation-based signaling occurring in the sample. Each of these techniques has their pros and cons that should be considered when choosing the best technique for your application.

### Immunometabolism

Immunometabolism is a research perspective that considers the interconnection between immunity and metabolism and studies their changes as an integrated response (Mathis and Shoelson, 2011). Comprehensive studies of metabolic diseases such as diabetes and cancer along with infectious diseases began to illuminate how much cross talk occurred in these diseases between immunity and metabolism. In animal production, an incidence of disease has often correlated with a decrease in productivity (Klasing, 1984; Colditz, 2004; Klasing, 2007; Greer, 2008). Given what has been discussed above regarding phosphorylation and how it regulates nearly every physiological response, a study of phosphorylation can illuminate our understanding of an integrated field-like immunometabolism as well (Arsenault and Kogut, 2015).

### Applications

The applications of kinomics are as wide and varied as any of the other omics disciplines like transcriptomics or genomics. Related to metabolomics, gut health and poultry, there have been key successes in applying a kinomic and metabolic approaches to poultry production and health issues. From characterizing the changes in muscle metabolism due to a *Salmonella* infection of the poultry gut (Arsenault et al., 2013), to understanding the immunometabolic effects of postbiotic supplementation during a *Clostridium perfringens* challenge (Johnson et al., 2019). As all fields of poultry science have advanced, we have come to realize how important it is to understanding the mechanism of action of our interventions. We know that antibiotics improve growth, but how? We know heat stress reduces weight gain, but why? We know some feed additives work in some flocks under some conditions but not others, what is the difference?

Poultry scientists, and scientists in other fields, are defining mechanisms for both diseases and treatments.

By studying mechanism of action and better defining molecular physiology, this work can be better applied to the field. If we know disease X causes acute gut inflammation, and we know antibiotic alternative Y directly modulates that response, we can rationally design a treatment regime. Probiotics such as *Bacteroides fragilis* (*B. fragilis*) containing polysaccharide A are known to reduce the Th17 response and induce regulatory T cells, and are broadly anti-inflammatory (Mazmanian et al., 2008; Round and Mazmanian, 2010), whereas Segmented Filamentous Bacteria (SFB) activate the Th17 response and are broadly proinflammatory and immune stimulating (Ivanov et al., 2009). Which probiotic to use will depend on your disease of interest and its pathogenesis. Treatments may include *B. fragilis* for *Clostridium perfringens* infection and SFB for *Salmonella* infection. Starting with the scientifically determined mechanism of action may get us to the solution that much faster. The field of immunometabolism has opened up a new world of interventions to improve poultry health and production. Drugs that alter key metabolic pathways like glycolysis, fatty acid oxidation can shift immune responses from effector to regulatory or vice versa (Mockler et al., 2014). Much like the above probiotics example, drugs such as metformin or rapamycin can be used, once the mechanism of action of a disease is understood, to modulate the immune response.

### Future Directions

The modulation of the microbiome to improve poultry health and production requires knowledge of how the intervention will impact the host. We have significant detail on which microbes are present in the various segments of the poultry gut (Yeoman et al., 2012). What we are now uncovering is the functions provided by those microbes to the host (Sergeant et al., 2014). This may include what signals are provided by the microbiome to the host to alter host physiology. As applied to kinomics, research into the regulation of bacterial function, the utilization of serine, threonine and tyrosine kinases by bacteria seems to have been underestimated (Pagano and Arsenault, 2019). Applying the same kinomic techniques to the microbiome that we have to date applied to the eukaryotic host, may provide a wealth of information on the functional changes induced by modulation of the microbiome and the signals provided by the microbiome to the host.

Understanding the gut-microbiome system can be seen as a signals intelligence problem. Signals are provided from the microbiome to the host, lumen metabolites to the host, the host to the microbiome and from the host gut cells to the host immune system. By measuring and understanding these signals, we can provide a more comprehensive view of the state of gut health and disease, and rationally design intervention strategies based on the signals we have observed. The ultimate goal being a healthier, disease-free gut, and a healthy, productive bird.

## **MICROBIAL ENDOCRINOLOGY: HOW EVOLVED INTERSECTIONS OF MICROBIOLOGY AND NEUROBIOLOGY MATTER TO POULTRY WELL-BEING, SUSCEPTIBILITY TO INFECTIOUS DISEASES AND NUTRITION**

The connection between the intestinal microbiota and the host has perhaps most intuitively focused on what happens at the level of the gut. Within the lumen, microbes utilize ingested nutrients, and produce molecules that can have a detrimental, neutral, or positive effect on the host. The presence of physical barriers and the gut-associated lymphoid tissue normally keep intestinal microbes within the lumen, and prevent disease. Systemic effects of intestinal immune activation can reduce poultry performance, but the number and importance of host physiological systems in communication with the intestinal microbiome is a rapidly advancing field.

### ***How Should We Think of the Bacteria That Constitute the Microbiome and Their Relationship to Poultry?***

In learning about bacteria in our school years, we were taught that bacteria are essentially “dumb bugs.” By “dumb bugs,” we mean that we were taught that bacteria are simple organisms that given the proper nutrition and environment divide from 1 to 2, 2 to 4, 4 to 8 in numbers and so on. They, the bacteria, do not concern themselves with the health and wellbeing of the host, in the case of the chicken gut, that they inhabit. That the chicken may derive benefit from the presence of gut bacteria through the provision of certain vitamins, for example, is incidental but not inconsequential to the health of the chicken.

This “dumb bugs” view is one that must now be abandoned in view of the numerous reports that have been accumulating over the last 2 decades which show how bacteria interact and respond to the changing environmental conditions present in the intestinal tract. Changes in nutrition, stress (e.g., fighting) and environmental temperature (e.g., heat stress) are some of the conditions that can dramatically influence the composition and function of the microbiota in the gut that in turn can have either beneficial or deleterious effects on poultry health.

As such, a holistic evolutionary-based approach is needed if we are to understand how harnessing the microbiota can lead to improvements in poultry management. In this brief review, such a holistic approach, which is embodied by the evolutionary-based theory of microbial endocrinology, will demonstrate that integrating the crucial elements found in the intestinal tract is necessary to devise ways to manipulate the microbiota to obtain defined effects on poultry health and behavior. Microbial endocrinology, by definition, represents the integration of the fields of microbiology

and neurobiology. It is based on the use of neurochemistry as a shared evolutionary language between the microbiota and the host (e.g., chicken) (Lyte, 2013b; Lyte, 2016a; Lyte, 2016b).

### ***Introduction and Recognition of Bacteria as Neuroendocrine Organisms – Why a Microbial Endocrinology-Based Approach Matters***

That neurochemicals should represent the “words” of such a shared evolutionary language may not at first consideration be immediately apparent to the vast majority of scientists. However, an even cursory examination of the literature dating back over 100 yr will provide example after example where bacteria not only recognize, but also produce, the very same neurochemicals that we typically only associate with animals. For example, the complete biosynthetic pathway for the production of the stress-associated neurochemicals norepinephrine and epinephrine has been shown to exist in bacteria and in fact, bacteria produce both of the neurochemicals which constitute the “fight or flight response” (Iyer et al., 2004). Acetylcholine is another example of a neurochemical that is produced in copious amounts by lactic acid bacteria and has been known since the 1940s (Stephenson and Rowatt, 1947). Additionally, bacteria also possess receptors for neurochemicals that, once bound, alter the physiological function of the bacterium.

The demonstration that members of the chicken microbiota recognize and produce the same neurochemicals that are produced by the chicken’s neurophysiological system means that the gut microbiota and the chicken share a common evolutionary-based language that enables each to “talk” to each other. Such cross-talk ultimately means that the production of neurochemicals from the chicken into the gut, as occurs during stress when the nerves that innervate the chicken’s intestinal tract secrete fight or flight neurochemicals into the gut lumen, can influence how members of the microbiota function. And in turn, the production of neurochemicals by the microbiota can influence aspects of chicken health and even behavior through what is known as the microbiota-gut-brain axis.

### ***Relevance of the Enteric Nervous System to Microbial Endocrinology***

The intestinal tract of the chicken is highly innervated by nerves that belong to a division of the nervous system known as the enteric nervous system (ENS). It is generally not well appreciated by scientists in general who are concerned with the study of the gut microbiome and its interaction with its host that in addition to immune elements within the gut that help to regulate the diversity of the microbiota, there is additionally another system, that being the ENS, which is crucial to the regulation of the gut microbiota and interactions with the immune system.



The degree of ENS innervation in the chicken gut is still an area that lags far behind in understanding when compared to other animal species. In humans, it is believed that the ENS is composed of over 500 million neurons innervating the entire length of the alimentary tract (Furness et al., 2014). The degree of ENS innervation is so extensive that individual villi have nerve projections extending through them from the base to the tips. This means that a system for gathering information concerning the status of the various elements that comprise intestinal physiology as well as intestinal contents within the lumen exists and a means to communicate that information to the central nervous system and ultimately the brain (Green et al., 2003; Powley et al., 2011). Such communication to the brain occurs via the vagus nerve which is the longest nerve in the body and provides one of the means by which the ENS maintains constant communication with the CNS. The role of the ENS should be envisioned, in part, as a sensory organ that plays a critical role in maintaining health as has been amply demonstrated (for review see Furness et al. (2013)).

It can therefore be hypothesized that a shared evolutionary-based language between microbe and host would also potentially involve elements of the host's nervous system and by extension host behavior which is under the control of the neuronal elements including those coming from the gut. Such interaction of microbiota with host gut and brain neurophysiology (Goyal et al., 2015) would have implications extending from brain development to the behavior of adult animals including those in farm production settings.

### **Role of Microbial Endocrinology in the Pathogenesis of Infectious Disease**

The role of neurochemicals, in particular the catecholamines which are involved in the fight or flight stress response, has been known for nearly 3 decades (Lyte, 2016a). These reports, which have extended over a number of animal species, have shown that micromolar amounts of norepinephrine can increase the growth of enteric pathogens such as *Yersinia* spp. and *Salmonella* spp. by log orders within hours (Freestone et al., 2007; Karavolos et al., 2008; Bearson, 2016; Hiller et al., 2019; Lucca et al., 2020). It should be noted that the neurochemicals themselves do not provide any nutritive value to the infectious pathogen, but instead provide an environment signal that informs the bacterium to initiate the pathogenic process for its own survival.

Interestingly, a publication examining infectious disease susceptibility in poultry asked in the title "Are happy chickens, safer chickens?" (Humphrey, 2006). From a microbial endocrinology-based perspective the answer to that question would have to be "yes" (as was also concluded by the author). The reasons can be found in the recognition of stress neurochemicals by infectious bacteria. For example, *E. coli* possesses the complete biosynthetic pathway for the production of the stress-

related neurochemicals including strains found in chickens (Iyer et al., 2004; Villageliu and Lyte, 2017). While it is not yet known why *E. coli* has the capacity to produce the exact same fight-or-flight neurochemicals as animals, it has been shown that exposure of *E. coli* to physiologically-relevant concentrations of stress-related neurochemicals that would be present in the intestinal tract of a stressed bird due to production by ENS neurons that innervate the gut (as discussed above) results in orders of magnitude increase in growth (Barker et al., 1977; Kinney et al., 2000; O'Donnell et al., 2006; Toscano et al., 2007; Lyte, 2016a) and production of virulence-related factors, such as pilus adhesion (Lyte et al., 1997), as well as production of autoinducer metabolites that regulate growth (Lyte et al., 1996) and facilitate interkingdom signaling (Moreira and Sperandio, 2016). The ability of stress neurochemicals (i.e., norepinephrine and others) to initiate changes in gene expression has also been demonstrated (Oneal et al., 2008; Bearson and Dowd, 2010; Bearson, 2016). Further, in vivo production of stress-related neurochemicals has been shown to facilitate bacterial attachment to mucosal surfaces (Lyte et al., 2003; Sandrini et al., 2014) and to increase proliferation within the intestinal tract (Vlisidou et al., 2004). And while many reports understandably target the ability of stress-related neurochemicals to influence known *E. coli* pathogens such as *E. coli* O157:H7 (Sharma and Casey, 2014; Bearson, 2016), similar findings have been shown for *Salmonella* spp. as well as *Campylobacter* spp. (Hoffman et al., 1979; Cogan et al., 2007; Pullinger et al., 2010; Bearson, 2016; Hiller et al., 2019; Liu and Lyte, 2020; Truccollo et al., 2020).

Critically, the overwhelming majority of the reports demonstrating the ability of stress-related neurochemicals to influence bacterial physiology as it relates to infectious disease pathogenesis have been carried out in non-avian animal species ranging from cattle to humans. As such, the utilization of microbial endocrinology represents a new approach by which to identify mechanistic pathways by which stressors that poultry encounter during production, such as heat stress, may influence the ability of infectious bacteria, notably avian pathogenic *E. coli*, to infect chickens. Thus, the use of microbial endocrinology-based approach holds the potential to help account for the known lack of a specific *E. coli* pathotype that causes infection in chickens.

### **The Microbiota-Gut-Brain Axis and Chicken Behavior**

The ability of the gut microbiota to influence behavior through what has become known as the microbiota-gut-brain axis has been the subject of intense interest in clinical medicine and has recently made it into discussions of how the gut microbiota can influence behavior in farm production animals. In brief, this axis is a bidirectional one in which the gut microbiota through the production of metabolites such as neurochemicals as well as

direct interaction with enteric elements such as immune cells and the ENS, can influence behavior principally through the gut ENS neurons that connect to the vagus nerve that runs to the brain (for reviews as well as other pathways see (Lyte and Cryan, 2014; Cryan et al., 2019)).

The first demonstration that the gut can sense the bacteria present in the gut and communicate that information to the brain was shown in mice that had been exposed to a novel bacterium not normally found in its gut (Lyte et al., 1998). In these animals, an anxiety-like state of behavior was produced following feeding of this bacterium. Interestingly, while the bacterium in the gut did not produce any immunological response, it did result in the gut ENS sensing its presence and communicating that information to the brain via the vagus nerve. Cutting the vagus nerve completely abrogated the ability of the bacterium to induce an anxiety-like state thereby demonstrating the existence of a microbiota-gut-brain axis (Goehler et al., 2005).

The microbiota-gut-brain axis can also influence higher functions in the brain that benefit the animal as well. Mice fed a beef-supplemented diet experienced change in gut microbial diversity that ultimately resulted in improvement of memory function (Li et al., 2009). To what degree the manipulation of this axis would benefit poultry is still unknown. Social conflict among chickens is well-recognized to be detrimental to the industry. The possibility therefore exists that manipulation of the gut microbiota through a microbial endocrinology-based approach could provide a means to effect changes in the gut microbiota that alter behavior that results in fighting. Understanding all the elements that comprise the microbiota-gut-brain axis in chickens represents a new frontier in poultry-based research.

### **Role of Microbial Endocrinology in Nutrition and Potential to Benefit Poultry Production**

As already discussed, the bacterial production and recognition of neurochemicals that are structurally identical to those found in poultry is not well recognized. In a similar fashion, it is not well appreciated that plants also possess many of the same neurochemicals. For example, *Mucuna pruriens* (velvet bean) is a legume that has been employed worldwide for many decades as a feed ingredient to provide protein and fat for numerous farm production animals including poultry (Carew and Gernat, 2007). In fact, it was highly employed in the United States and recommended by the United States Department of Agriculture as a source of animal nutrition before it was supplanted in the 1900s by the increasing use of soybeans (Piper and Morse, 1938; Carew and Gernat, 2007). Interestingly, *M. pruriens* can contain 4–8% by weight of the neurochemical L-dopa. Another example among many of the plants that are capable of neurochemical production are potatoes which have been shown to produce stress-related neurochemicals (Swiedrych et al., 2004).

Why plants contain neurochemicals is not fully understood although their role in growth and cell-to-cell communication has been the subject of investigation for many years (Kuklin and Conger, 1995; Soares et al., 2014; Akula and Mukherjee, 2020). As interestingly, the question needs to be asked to what extent does poultry feed, which is composed of plant-based material, also contain neurochemicals that can affect both host and gut microbiota. The answer is largely unknown as little research, if any, has yet been published. However, the intriguing possibility exists that manipulation of the plant sources that go into poultry feed can serve as a means by which to influence the gut microbiota to the benefit of poultry health and even behavior. This would not have to be done through any GMO-based modification of the plant as different cultivars of many plants can contain different levels of neurochemicals. Thus, the careful selection of food plant sources to include a neurochemical profile that benefits the gut microbiota and its interaction with the host may be one of the cutting-edge approaches to the next generation of poultry feedstuffs (Lyte, 2013a).

### **Conclusions and Future Directions**

This brief review is intended to introduce the theory of microbial endocrinology and why an understanding of interkingdom cross-talk between the microbiota and the chicken plays a critical role in poultry health. As discussed, there are a number of pathways, including the use of nutritive means, by which microbial endocrinology may play a role in mediating the interaction of the gut microbiota with the host. The existence of multiple mechanistic pathways is to be fully expected when a more holistic approach based on a shared common evolutionary pathway of communication between microbe, host and nutrition is employed.

The manipulation of the gut microbiome is seen by many in academia and industry as one of the next generation of poultry management means by which to improve poultry health and behavior. How that microbiome manipulation will occur is still a matter of continuing investigation. The control of the nutritive input into the chicken represents perhaps the most promising way to do so given the elimination of antibiotics from the diet (Lyte, 2013a). However, such use of nutritive means must be guided by an understanding of the involved mechanisms of action. It is herein suggested that microbial endocrinology represents one, but certainly not the only, approach to achieve that goal by manipulation of the cross-talk that occurs between the microbiota and the chicken.

### **APPLYING THE KNOWLEDGE: WHERE IS THE RESEARCH LEADING US?**

Our understanding of the impact of the microbiome on animal performance and health continues to grow and is moving at a fast pace. Translating this knowledge

into application offers a lot of promise given the implications of the microbiome in health and performance; however, this will require a significant effort and coordination between those involved in basic and applied research. A few critical areas should be considered to ensure successful application and future microbiome research. First, going beyond the gut and bacteria in order to understand all microbial populations and their interactions in other tissues to ensure a more complete and integrated view of how the microbial system fits within animal physiology. Second, we must strengthen our focus on microbial function and not microbial signatures alone, as the former is better associated with animal outcomes. Finally, there are such exciting opportunities to take more hypothesis-driven questions into the microbiome space to better leverage this system to achieve the desired animal outcomes and to allow for more targeted measures, or biomarkers, to help monitor and ensure progress.

The overwhelming research focus to date with the microbiome has been to catalogue microbial signatures within the intestine. These data have helped us to understand the temporal and spatial changes in microbial communities, as well as how different interventions, such as diet, disease challenges, genetics and more, are impacted. Only recently are we starting to gain an understanding of microorganisms beyond bacteria and how these communities are potentially interacting with one another in the intestine. [Robinson et al. \(2020\)](#) characterized fungal microorganisms, or the mycobiome, in the broiler intestinal tract and similar to bacteria profiles found dominant fungi genera across intestinal compartments. However, unlike bacteria the diversity of mycobiota was greater in the upper regions of the intestinal tract relative to the lower intestinal tract. Additionally, most of the species were associated with soil or cereal grains, indicating their environmental presence in the intestinal tract originated from the diet or surrounding environment. In fact, as it relates to the microbiome community and the surrounding environment, there continues to be interest in understanding the contribution of external variables on host microbiome outcomes, such as ingredients, housing conditions and other potential management factors ([Marcolla et al., 2019](#)). The ability to identify microbiome signatures unique to external factors can provide a more holistic view to managing the host microbiome.

In addition to understanding microorganisms beyond bacterial populations, there is a need to explore microbiota communities in other tissues. [Johnson et al. \(2018\)](#) found that potential respiratory pathogens in the trachea were negatively correlated with performance. Using metagenomic sequencing techniques, [Mulholland et al. \(2021\)](#) measured bacteria, virus, bacteriophage and fungi signatures in the trachea and found that infectious laryngotracheitis resulted in microbiome dysbiosis of the trachea virome. Furthermore, strong correlations were detected between bacteria and bacteriophage families in the trachea, showing how these different microorganisms may be interacting with one another. In swine,

fecal microbiota from healthy sows to barrows reduced morbidity, mortality and percent infected with lung lesions after exposure to a respiratory challenge ([Niederwerder et al., 2018](#)). The ability of the microbiome in the intestine to influence microbiome outcomes in other tissues, such as the gut-lung axis, provides exciting opportunities to deliver in feed solutions that impact the microbiome beyond the intestine ([Zheng et al., 2020](#)).

The rapid development of next generation sequencing and metaomics is ushering in a new wave of microbiome discovery. These culture-independent methods not only provide microbiota signatures, but also insight into microbiome function ([Bikel et al., 2015](#); [Cao et al., 2017](#); [Shakya et al., 2019](#)). These methods enable a shift in understanding from who is there to what are they doing to allow for a better understanding of host-microbiome interactions. Human microbiome samples were collected from healthy subjects from multiple locations and clearly show rich diversity in microbial communities across compartments and subjects; however, the metabolic pathways of these very diverse microbial species were stable ([Human Microbiome Project Consortium, 2012](#)). [Qi et al. \(2019\)](#) also compared broiler and layer cecal microbiomes and found microbial signatures to be different and diverse while microbiome function was very similar between these meat and egg laying breeds. As it relates to applying microbiome knowledge, these data point to microbiome pathways generating more stable and reliable outcomes compared to microbial populations, and this can have big implications on potential biomarkers. It appears that for a particular microbiome process the profile of the microbiota may be less critical to achieving the desired activity or outcome. In ruminants, animals with high feed efficiency have decreased rumen microbial richness, increased species dominance, and increased dominance of microbial activities and metabolites ([Shabat et al., 2016](#)). Taken together these data suggest a more specific rumen metabolic phenotype that aligns with improved feed efficiency. The microbiome metabolic capacity in the broiler foregut and hindgut was characterized by [Huang et al. \(2018\)](#), with maximum capacity being reached at approximately 15 to 28 d and for increased performance to be associated with enriched amino acid, bile acid and vitamin metabolic pathways. By understanding how microbiome functions are tied to desired host responses, such as increased feed efficiency, more hypothesis-driven questions can be asked for future research and more targeted interventions can be applied to achieve desired microbiome impacts on host response. For example, [Walsh et al. \(2021\)](#) utilized a glycan ingredient to modify microbiome genes involved in propionate production in the cecum that was associated with increased feed conversion.

While there appears to be considerable functional redundancies in metabolic pathways of the microbiome, more thought and consideration should be given to host immune outcomes. To answer this and other questions, and ultimately to develop and apply more robust

solutions, integrative analysis will be critical to achieve some of the next key breakthroughs in understanding. For example, [Mon et al. \(2020\)](#) inoculated layer chicks with *Salmonella* Enteritidis and evaluated the cecal microbiome and metabolome. *S. Enteritidis* downregulated microbiome pathways involved in purine metabolism, particularly arginase. In addition, metabolome analysis identified *S. Enteritidis* enrichment in arginine and proline metabolic pathways. Taken together the integrative approach suggests potential microbiome targets to help enhance for improved *Salmonella* protection. As mentioned previously, -omics platforms continue to usher in large scale data sets to help better understand host-microbiome interactions; however, the potential exists in their integration into multivariate models to help build more robust solutions and diagnostics to help apply and measure microbiome applications.

The study of interactions between the host and the intestinal microbiome has evolved from a rather basic knowledge of relatively few microbial inhabitants of the gut, to an ever-expanding appreciation for the scope of host physiological systems communicating with, and affected by the microbiota of the intestinal tract. In this review, the immune and neuroendocrine systems were given as examples of the complex relationships that exist between the chicken and the intestinal microbiota. Kinomics is an analytical approach to quantify changes in protein functions as influenced by the complex interplay between the host and the microbiome. Using this and other novel analytical techniques, researchers can further understand how the microbiome can have positive or negative effects on the productivity of animals. Gaining an appreciation for and understanding of the relationship of the microbiome in other physiological systems and other animal species will be useful in comprehending these complex interactions. Ultimately, for this knowledge to be used to optimize bird health, well-being and productivity requires communication between scientists and field nutritionists.

## DISCLOSURES

The authors do not have any conflicts of interest to declare.

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