# SYMPOSIUM: CURRENT AND FUTURE DIRECTIONS FOR NEXT GENERATION SEQUENCING OF POULTRY MICROBIOMES

## Influential factors on the composition of the conventionally raised broiler gastrointestinal microbiomes

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**ABSTRACT** The microbiome has entered the vernacular of the consumer as well as broiler production and is, therefore, becoming increasingly important to poultry producers to understand. The microbiome is, by definition, compositional and relates to how the microbiological organisms within the gut inhabit that ecological niche. The gut is diverse, flexible, and data acquired requires a greater understanding of the host-microbiome axes, as well as advanced bioinformatics and ecology. There are numerous microbial populations that define the gut microbiome; however, there are even more effects that can influence its composition. As management practices vary between producers, documenting these influences is an essential component of beginning to understand the microbiome. This review targets broiler production and concatenates the currently understood compositional ecology of the broiler gastrointestinal tract microbiome as well as its influences.

**Key words:** conventional broiler production, gastrointestinal tract, microbiome, bioinformatics, composition

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## INTRODUCTION

The microbial communities within the intestinal tract play a significant role in the overall health and digestion in avian species. Determining the predominant bacterial species within each section of the intestinal tract is an important first step in understanding the role the microbiome contributes to physiology. Compared to other food animals, broilers have a short intestinal tract and significantly faster passage rate that limits bacterial populations and outgrowth (Pan and Yu, 2014). The formation and stability of the microbiome is guided by the host. Peripheral consequences to this relationship include the regulation of intestinal morphology, which in turn impacts nutrient digestion and absorption. Evidence indicates that bacteria may increase the digestibility of the diet, which may increase dietary bioavailability to the host and could lead to better skeletal-muscular accretion. Additionally, the stability of the microbiome can impact the immune response as well as be exploited by some pathogens. Collectively, this can impact feed efficiency, which is ultimately the bottom-line for the poultry industry.

*Bacteroidetes*, *Firmicutes*, *Proteobacteria, Actinobacteria,* and *Tenericutes* are the predominant phyla in the avian gastrointestinal tract (**GIT**) (Waite and Taylor, 2014; Choi et al., 2015). Regardless of age or section of the intestinal tract, the most abundant phylum in turkeys and chickens includes *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* (Wei et al., 2013; Wilkinson et al., 2017). However, the proportion of each phylum differs between turkeys and chickens. Specifically, in the intestine of the chicken, *Firmicutes* is the most predominant (70%), *Bacteroidetes* (12.3%), *Proteobacteria* (9.3%), which is proportionally different from that of the turkey microbial composition (Wei et al., 2013). While turkeys have their own nuances, this review will focus primarily on the broiler chicken. By doing so, a comprehensive view of the populations within different sections of the GIT, how feed amendments can impact those populations, and the impact the microbiome has on chicken physiology will be discussed.

## CROP

The crop is an enlarged portion of the esophagus and is responsible for moistening the food prior to enzymatic digestion and storing food during times of low food



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resources, harboring approximately $10^8$ to $10^9$ bacteria per gram of crop contents (Rehman et al., 2007; Yeoman et al., 2012; Svihus, 2014; Kiero'nczyk et al., 2016). The colonization of this organ begins at 1-h post-hatch, and the environmental conditions and diversity of this organ are not mutually exclusive (Fuller, 2001). The most prevalent bacteria within the crop of commercial broilers include *Lactobacillus*, *Bifidobacterium*, and *Enterobacter* (Bolton, 1965; van der Wielen et al., 2002; Yeoman et al., 2012; Saxena et al., 2016). However, indigenous chicken from India that are reared in backyard conditions, under different housing conditions, while being fed grain as well as insects and household scraps resulted in *Bacillus* becoming more dominant throughout the life of the chicken (56 to 73%) (Saxena et al., 2016). This indicates that the poultry gut microbiota composition observed in birds raised in the U.S. is not necessarily universal and may be influenced by diet. It also appears that while the crop is typically colonized at 1-h post-hatch, the environment dictates the overall microbial composition of the organ.

The crop is an organ unique to avian species and plays a vital role in the broiler chicken where feed availability and composition directly impacts crop function and physiology (Svihus, 2014). Overall the crop maintains a temperature of 40°C and anaerobic conditions, which makes fermentation likely (Bolton, 1965). Importantly, starch catabolism begins in the crop via host enzyme hydrolysis. Microbial fermentation by the microbiota is also able to convert sugar into organic acids (Bolton, 1965; van der Wielen et al., 2002). The lactic acid production within the crop reduces the pH and limits the growth of other bacteria (Bolton, 1965; Rehman et al., 2007; Stanley et al., 2014).

## PROVENTRICULUS AND GIZZARD

The proventriculus and gizzard are considered the "true stomach" of the broiler chicken. The proventriculus is the section of the intestinal tract that secretes pepsin and hydrochloric acid and is responsible for the chemical digestion and the production of chime while the gizzard mechanically breaks down food (Bedford, 2006). Together the proventriculus and gizzard house fewer bacteria as the local environment is acidic (Rehman et al., 2007). The gizzard has approximately $10^7$ to $10^8$ bacteria per gram of chyme (Yeoman et al., 2012). Predominant bacterial populations in the broiler gizzard include (in order from most abundant to least abundant): *Lactobacillus*, *Enterobacteriaceae,* and coliform bacteria (Engberg et al., 2002, 2004; Rehman et al., 2007; Yeoman et al., 2012).

## INTESTINAL TRACT

In *Aves*, the digestive tract is responsible for the digestion and absorption of most nutrients and undergoes consistent peristalsis. Within the small intestine, there are three distinct sections, the duodenum, the jejunum, and the ileum with the duodenum and ileum being the primary focus of this review. The duodenum has a short transit time with a low pH that functions to activate the enzymes. The pancreatic and bile secretions aid in digestion, ultimately diluting the chyme and limiting the number of bacteria able to colonize the intestinal tract (Rehman et al., 2007). However, when the chyme enters the ileum, there is a drop in digestive enzyme activities, and bile acids are deconjugated, both of these events facilitate bacterial colonization (Rehman et al., 2007). The small intestine has approximately $10^8$ to $10^9$ bacteria per gram of digesta (Apajalahti et al., 2001, 2002; Gong et al., 2002b; Yeoman et al., 2012). In chickens, 3 D post-hatch, the bacterial density (number of microorganisms per gram of digest) in the ilea and ceca usually peaks, plateaus and remains stable; however, the composition of the microbiome varies beyond this time point (Apajalahti et al., 2001, 2002). Digesta passage rate in the small intestine is more rapid than the ceca and is likely one of the reasons the ceca contains more bacteria (Apajalahti and Kettunen, 2006).

Bacterial growth within the small intestine is limited by chemical inhibitors such as acid and bile, nutrient competition with the host, the high passage rate of the intestinal contents, constant epithelial cell turnover, and host immune defenses (Apajalahti and Kettunen, 2006). The predominant phylum in the broiler small intestine is the *Firmicutes,* which is largely represented by *Lactobacillus*, *Candidatus, Arthromitus*, *Clostridium*, *Streptococcus,* and *Ruminococcus* spp. (Jin et al., 1997; Yeoman et al., 2012; Munyaka et al., 2015; Wang et al., 2016). Members of *Proteobacteria* have also been detected in the ileum, specifically *Escherichia* and *Enterococcus* (Jin et al., 1997; Yeoman et al., 2012; Wang et al., 2016). In the ileum of broilers consuming a standard industry corn-soy-based diet, the most abundant sequences were *Lactobacillus* (70%), followed by *Clostridiaceae* (11%), *Streptococcus* (6.5%), and *Enterococcus* (6.5%) (Lu et al., 2003). While occasionally reported, the presence of various bacteria in the ileum, such as *Clostridia* Cluster XIVa, is likely the result of reverse peristalsis and reflects the microbial community of the ceca (Apajalahti and Kettunen, 2006). There is limited microbial carbohydrate metabolism in the small intestine due to the short digesta transit time; therefore, there is a lower concentration of short chain fatty acids (**SCFA**) in the ileum compared to the ceca (Rehman et al., 2007).

## CECA

While pairs of enlarged ceca are unique to domesticated species, they exist universally in all of *Aves* with the exception of the pigeon (Svihus, 2014). Unlike the digestive tract that has constant peristalsis, the ceca will empty twice a day as well as exhibit anti-peristalsis (Svihus, 2014). Anti-peristalsis reverses the movement of the digesta, resulting in the deposition of fecal material from the colon into the ceca. The ceca contribute to numerous functions in avian physiology, such as water and electrolyte absorption as well as nitrogen recycling (Svihus, 2014). The cecal microbial populations are also capable of fermentation, which results in metabolites that are used by the host.

The ceca have the highest bacterial density in broiler chickens (Rehman et al., 2007). Additionally, fermentation occurs in the ceca, where numerous microorganisms are capable of degrading non-digestible starches and produce SCFA (Beckmann et al., 2006).

The anaerobic environment, long transit time, and partially digested metabolites entering the ceca are ideal for microbial fermentation and the production of SCFA (Rehman et al., 2007). The average number of bacteria in ceca ranges from $10^{10}$ to $10^{11}$ bacteria per gram of digesta (Apajalahti et al., 2001, 2002; Gong et al., 2002a; Yeoman et al., 2012; Borda-Molina et al., 2018). There is also stratification, where the cecal mucosa houses $10^{11}$ recoverable bacterial cells (Gong et al., 2002a). Nutrient availability and inhibitory metabolites serve as limiting factors that can control microbial outgrowth (Apajalahti and Kettunen, 2006).

The cecal tonsils have the highest abundance and diversity of bacteria, with reports of 2,200 operational taxonomic units (**OTUs**) (Wei et al., 2013) and 3,522 genotypes (Qu et al., 2008; Yeoman et al., 2012). In the ceca, more than 90% of the bacteria are Gram positive (Gong et al., 2002a). The most dominant phyla included *Firmicutes*, followed by *Bacteroidetes* and *Actinobacteria*, in that order (Qu et al., 2008). The majority of the bacteria identified were from the *Firmicutes* phylum (44 to 54%) and included the following genera: *Ruminococcus*, *Faecalibacterium*, *Pseudobutyrivibrio*, *Subdoligranulum*, *Acetanaerobacterium*, *Peptococcus*, *Sporobacter*, *Megamonas*, *Oscillospira*, *Oscillibacter*, *Lactobacillus*, *Blautia*, *Heliobacterium Eubacterium,* and *Clostridium* (Jin et al., 1997; Apajalahti et al., 2001; Yeoman et al., 2012; Sohail et al., 2015). The next most abundant phylum was *Bacteroidetes*, which was 23 to 46% of the bacteria within the ceca and consisted predominantly of the genera *Bacteroides* (Yeoman et al., 2012). *Proteobacteria* is also a phylum that has been identified in the ceca and ranges from 1 to 16% of the microbiota and includes the *Escherichia* and *Bilophila* populations (Jin et al., 1997; Yeoman et al., 2012). The minor populations that likely have significant impacts physiologically include Archeans (0.81%), which are primarily represented by *Methanobrevibacter*, *Methanobacterium*, *Methanosphaera*, *Methanothermus*, *Methanothermobacter*, *Methanopyrus,* and *Methanococcus* (Yeoman et al., 2012). Identification of bacterial populations within the ceca collected from different states within the U.S. and in countries in Europe revealed that the majority of the bacteria were members of the *Clostridia* clusters IV and XIVa, *Bacteroides*, *Lactobacillus,* and *Bifidobacterium* (Lu et al., 2003; Holben et al., 2004; Apajalahti and Kettunen, 2006). This suggests that regardless of geographical location, broiler chicks contain similar bacterial populations and perhaps feeding programs affect proportions of bacterial populations within the intestinal tract (Lu et al., 2003; Holben et al., 2004; Apajalahti and Kettunen, 2006).

In the ceca, the mucosal-associated microbiome is vital for pathogen control, immune modulation, biotransformation, and host nutrient absorption (Gong et al., 2002a). Mucosal bacterial populations can vary from the luminal populations, which may be due to the microenvironment of the gut lumen vs. the mucosae (Gong et al., 2002a; b). Mucosa cecal bacteria are dominated by butyrogenic species (25%) such as *Enterococcus cecorum* and *Fusbacterium prausnitzii* (6%), as well as lactic acid producers *Lactobacillus* (3%) (Gong et al., 2002a). This population of butyrogenic bacteria is more abundant in the mucosa as compared to the lumen of the chicken ceca (Gong et al., 2002a). Butyrate is considered an indicator of optimal gut health, stable symbiotic microbial populations, and is linked to reducing foodborne pathogens such as *Salmonella* (Gantois et al., 2006).

## FECES

As interest in microbiome research has expanded, measuring microbial diversity and composition through fecal swabs and sampling is emerging as a tool for producers to spot check the gut health of the bird. However, the relationship between the ceca microbiome and the feces is not direct (Stanley et al., 2015). Not only is anti-peristalsis possible, but the microenvironments of both locations are also significantly different. Microbial diversity in the feces increases as chicks age and develop, specifically the number of OTUs, the OTU richness (Abundance-based Coverage Estimator and Chao1) and diversity indices (Shannon) (Lim et al., 2015). At day of hatch, the fecal bacteria community consisted mainly of *Enterococcus* (52%), followed by *Escherichia* (26%), *Clostridium* (14%), *Zea* (5%), and *Lactobacillus* (2%) (Lim et al., 2015). At 35 D of age, *Lactobacillus* was the most predominant bacteria (72%), followed by *Clostridium* (15%), *Turicibacter* (2%), *Arthomitus* (1%), and other minor bacteria which collectively comprised 10% of the population (Lim et al., 2015). Therefore, in order to determine gut health, it may be difficult to directly link the fecalbiome to the microbiome.

## FACTORS AFFECTING THE INTESTINAL MICROBIAL COMPOSITION

The previous sections describe the prevalent bacteria within each section of the intestinal tract. However, these populations are not static as the microbiome is dynamic and is influenced by numerous factors such as rearing practices, age, sex, diet, endemic and episodic disease states, antibiotic use and other growth or health promotors, geographical location, and environmental stress (Qu et al., 2008; Waite and Taylor, 2014). As a result, it is crucial to consider these factors when comparing microbiome populations across different farms or experimental units.

### *Age and Sex*

Although previous research has indicated that the intestinal microbiota peaks within 3 D post-hatch and matures by day 21, the microbial composition still varies with age, although variation decreases as the bird ages

over time (Ballou et al., 2016). There have also been reports that there is variation in the microbial communities between sexes (Lee et al., 2017). Females had a higher abundance of anaerobic *Firmicutes* such as *Oscillospira* and *Tenericutes*, while males exhibited a higher abundance of *Bacteroidetes*, specifically *Bacteroides* in the ceca (Lee et al., 2017).

Pedroso and colleagues (2016) tracked bacterial populations prior to hatching through the first week post-hatch. At hatch, the ceca of the chicks were positive for *Clostridium*, *Escherichia coli*, and *Salmonella* species (Pedroso et al., 2016). There was also a high diversity of bacterial phyla in the ileum and ceca, which suggested that the colonization post-hatch was rapid (Awad et al., 2016). At 3 D post-hatch, compared to the at-hatch time point, chicks had a unique bacterial community structure in the ileum and ceca which indicated that the early microbial community development and population structure is transient (Lu et al., 2003). This has been emphasized by the difference in the bacterial profiles between the ileum and ceca, which were very distinct by 3 D post-hatch (Ballou et al., 2016). Initial colonization begins with facultative anaerobic bacteria followed by the colonization of obligate anaerobic bacteria (Lu et al., 2003). Importantly, the data suggest that the predominant early colonizers in the chicken intestinal tract include *Proteobacteria*, specifically the family *Enterobacteriaceae,* while the anaerobic *Firmicutes*, namely *Lachnospiraceae*, *Ruminococcaceae*, *Clostridiaceae*, and *Lactobacillaceae*, were the dominant populations in older birds (Lu et al., 2003; Awad et al., 2016).

Overall, differences in microbial community structure change with age, which in turn changes the competitive structure of the gut. The structure refers to the overall population density, core community, and populations of the gut and how they potentially interact with one another. In a perfect setting, this stable population dynamic can exclude bacterial populations, such as *Salmonella*. Stability of the gut reduces the likelihood of dysbiosis, and may ultimately be the definition of a healthy microbiome. Similar to production stages, the microbial community structure was stable during skeletal growth (14 to 28 D of age) and then changed during the finisher stage, when the bird is rapidly gaining weight (day 28 to 49) (Lu et al., 2003). Controversy sometimes exists as to when the microbiome is ultimately stable, whether it is at the end of the broiler production cycle, at 42 D of age, or around 15 to 22 D post-hatch (Ranjitkar et al., 2016). Some of the controversy may exist depending on what metric is used to determine microbiome maturity, and perhaps the environment ultimately plays a more significant role in the stability of that microbiome than previously thought.

The goal of the majority of the studies linking the gut microbiome with the physiology and development of broiler chickens includes identifying populations that may be exploitable by the bird during development. In turn, researchers hope to manipulate these relationships for production gains. For example, *Lactobacillus* was 100-fold higher in the ceca in 3 D old broiler chicks compared to 42 D old chickens indicating its importance during early bird growth (Gong et al., 2008). Populations that show up earlier in other species are usually favored for probiotics and gut health (Gao et al., 2017). Overall, the proportion of either human or avian pathogenic bacteria, such as *Campylobacter*, *Clostridium*, *E. coli,* and *Salmonella*, is approximately 1.5% and varies as chicks became older based on environment, health, and dietary factors (Oakley et al., 2014). The decrease in *E. coli* is associated with changes in microbial community structure affecting pathogen colonization and host immune response has also been noted (Awad et al., 2016).

## Diet, Feeding Patterns, and Feed Additives

Decreasing microbial outgrowth reduces the energy tax on the immune system, which in turn partitions the energy toward musculoskeletal growth. In order to identify feed management strategies for producers that exploit the harmony of the gut-microbiome axis, feed additive companies strive to provide microbiome modulating compounds including probiotics, organic acids, enzymes, yeast fermentates, and other additives to increase competitive exclusion and reduce foodborne disease. The totality of that literature is all-encompassing and ultimately not reviewed here. However, what is interesting is that while efficacy varies among commercial company products, how feed is prepared by the mills can also change the microbiome drastically, independent of feed amendments, whether it is differences in the basal diet composition or even how the feed is pelleted.

Feed manufacturing choices, such as processing temperature, pellet size, and feed availability, alter nutrient availability (Apajalahti et al., 2001, 2004). There are distinct differences in the microbiome when comparing the microbiome of chicks fed pelleted vs. mash starter feed. Evidence suggests that pelleting feed increases the number of coliforms and *Enterococci* in the ileum (Engberg et al., 2002). *Clostridium perfringens* was also significantly higher in the ceca and rectums of the pellet fed chicks compared to the mash fed birds (Engberg et al., 2002). However, while drawbacks may exist, there are potentially some benefits to pelleting as well. It has been demonstrated that the ceca of pellet fed birds exhibited a greater amount of total volatile fatty acids, especially butyrate and acetate (Engberg et al., 2002).

Interestingly, mash fed chicks had a higher amount of lactogenic bacteria throughout the intestinal tract than pellet fed chicks (Engberg et al., 2002). This may be due to changes in nutrient availability. For example, the addition of an enzyme increases the bioavailability of the feed ingredients and results in an increase in *Ruminococcus*, *Lachnospiraceae*, *Lactobacillaceae*, *Peptostreptococcaceae*, *Clostridiales*, *Acidovorax*, and *Blautia* in the ceca, all of which are relying on butyrate or lactic acid metabolism (Munyaka et al., 2015).

As with the influence of age, the basal diet in poultry production can dramatically shift the microbiome and may lead to significant production gains. Including whole

wheat into the diet results in a decrease in *C. perfringens* and *Enterococci* when compared to the pellet fed chicks in the ceca, while increasing *Bifidobacterium* and diversity (Apajalahti et al., 2001; Engberg et al., 2004). Chicken gizzard contents sequenced for the microbiome also had a greater abundance of *Lactobacillus*, which resulted in a lower pH and a higher concentration of acetate and lactate (Engberg et al., 2004). This likely indicates that bacterial fermentation is occurring within the gizzard (Engberg et al., 2004). Interestingly, chickens and turkeys fed a rye-based diet had significantly greater numbers of lactic acid bacteria in all three sections of the intestinal tract (Tellez et al., 2014, 2015). However, the consumption of a rye-based diet also increased the number of coliforms in the duodenum and ileum (Tellez et al., 2014, 2015). Feeding chickens a barley based diet supplemented with exogenous enzymes to increase nutrient bioavailability, altered the microbial communities in the ileum and ceca, and were associated with improved apparent metabolizable energy (Torok et al., 2008). It is likely that diets containing grains with high non-starch polysaccharides can affect the microbial profile of the avian intestinal tract, and this is in contrast to the microbiome responses observed using the standard corn-soy based basal diet.

## Antibiotics

Antibiotics have previously been used to improve performance from 4 to 8% and enhance feed utilization by 2 to 5%, as well as reduce morbidity and mortality (Butaye et al., 2003; Apajalahti and Kettunen, 2006). Currently, there are 11 antibiotics or antimicrobials often added to feed as growth promotants, including bacitracin, chlorotetracycline, decoquinate, diclazuril, naracin, nicarbazin, monensin, penicillin (if dermatitis or clostridial disease is in the flock), robenidine hydrochloride, tylosin, and virginiamycin (VFD, 2015). As of 2019, approximately 50% of poultry in the United States were raised antibiotic-free (National Chicken Council, 2019). However, some companies still use antibiotics as long as they are prescribed within the guidelines of the Veterinary Feed Directive (VFD, 2015).

Antibiotics kill or halt the replication of bacteria in the gut, but the selection is not universal. Even though an antibiotic is broad-spectrum and by definition will target a variety of bacterial species, it still does not kill every species of bacteria present and is therefore selective. As with humans, numerous studies have studied the effects of antibiotics on the chicken GIT microbiome. Apajalahti and Kettunen, (2006) found that the ionophore monensin decreased the total number of ileal microorganisms, which reduced competition for nutrients between microorganisms and the host, thus partitioning the nutrition to the host. The historically beneficial effects of monensin on the body weight gain of broiler chickens may not be solely based on the prevention of coccidiosis, but also the reduction of overall bacterial growth in the small intestine (Apajalahti and Kettunen, 2006). When bacitracin methylene disalicylate, a standard antimicrobial in poultry production, and phenoxy methyl penicillin was tested in combination with monensin, alpha diversity in the small intestine was reduced vs. monensin alone (Apajalahti and Kettunen, 2006). Interestingly enough, these changes corresponded to an increase in total body weight that was significantly different from monensin alone (Apajalahti and Kettunen, 2006). There also were drastic changes to the microbial composition, including a decrease in *Lactobacilli* and an increase in *E. coli* abundance. Lee and Newell (2006) observed similar changes in the numbers of *Lactobacilli* and *E. coli* with antibiotics.

When an antibiotic is included in the feed, the overall dynamics of the GIT microbial community change. For instance, in Europe, the removal of antibiotic growth promoters from feed resulted in an increase in necrotic enteritis (Bedford, 2000). Therefore, besides production losses, there are legitimate concerns that removing antibiotics will create other issues. For example, the removal of antibiotics from feed may change the antibiotic resistance profile of critical foodborne pathogens (Dibner and Richards, 2005; Johnson et al., 2009).

## Environment

Environmental factors such as temperature, litter quality, and maternal interaction likely play a role in the development of the microbiota within the intestinal tract. At hatch, chicks are exposed to bacteria from the surface of the eggshell that is covered in bacteria from the breeder hen and the hatching cabinet (Apajalahti and Kettunen, 2006). The initial microbiota impacts host immunity and intestinal health, ultimately directing the development of the gut-associated lymphoid tissue (Apajalahti and Kettunen, 2006). The influence on the gut-associated lymphoid tissue dictates immunological tolerance and aids in the establishment of the intestinal microbiota (Apajalahti and Kettunen, 2006). To minimize pathogen exposure, hatching cabinets are fumigated with formaldehyde, which ultimately dictates the first bacterial species to colonize the intestinal tract (Apajalahti and Kettunen, 2006).

In the United States, litter is often reused between broiler flocks, whereas countries such as Canada require fresh litter for every production cycle. The broilers will peck and ingest litter, which in turn will have an effect on their microbiome and may be exploitable if the litter is properly primed with potentially beneficial bacteria. Wang et al. (2016) compared the microbial composition of used and fresh litter to determine how they respectively influenced the intestinal tract of the flock. The used litter consisted of more alkaliphilic and bile tolerant bacteria, suggesting that used litter could contain a higher sodium content that may impart an advantage to some bacteria (Wang et al., 2016). They also noted that fresh litter contained bacteria that are not found in high proportions within the GIT of chickens such as *Acinetobacter*, *Devosia*, *Luteimonas*, *Trichococcus*, and *Yaniella* (Wang et al., 2016). At 10 D of age reused

litter had a significantly greater abundance of *Blautia*, *Faecalibacterium*, *Anerotruncus* in the ceca; however, fresh littler had a significantly higher level of *Escherichia*, *Lactobacillus*, *Bacteroides*, *Subdoligranulum*, and *Clostridium XIVb* (Wang et al., 2016). At day 35, the broilers set on re-used litter exhibited a significantly higher abundance of *Faecalibacterium* and *Oscillibacter* while also carrying a significantly lower abundance of *Subdoligranulum* in the ceca (Wang et al., 2016). These results indicate that litter management can influence the microbiota in an age-dependent fashion (Wang et al., 2016).

## CONCLUSIONS

It is evident that the diet, genetics, and environment play a vital role in the avian intestinal microbial population. However, while conventionally reared birds are housed in similar conditions, significant variation continues to exist and contribute to some of the inconsistencies in production gains that continue to remain a challenge to overcome. Opportunities exist now more than ever before to use the "omics" data to understand these conditions and differences that may change how the broiler bird grows, despite the strong line-bred genetics. Improving how the microbiome data are assessed and quantified by improving the bioinformatics interface used for interpretation will also be required in the future. However, the poultry industry, as a whole, must become much more aware of the strengths and limitations of these approaches as well as the appropriate interpretations without unwarranted extrapolations. This may entail supporting basic research to link the desired production traits with biomarkers in the gut. Ideally, academic peer-reviewed research will establish the standard benchmarks for the development of accurate and reliable criteria to not only understand this data, but interpret it in an appropriate manner. Detecting the presence of bacteria is no longer enough, nor is selectively highlighting microbial populations that lack foundational scientific evidence to support their actual roles in the GIT microbiome. Understanding this will allow for an appropriate as well as productive application strategy of microbiome research for optimizing bird performance as well as reduction of veterinary and foodborne diseases.

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