End of text
and excreta; however, despite the large number of research groups dedicated to finding ideal biomarkers, the use of these tools outside research settings has been limited (Ducatelle et al., 2018).

The main objective of this review is to highlight the existing literature on the chicken digestive microbiota and its function, factors affecting the gut microbiota and emergence of enteric diseases, and methods to evaluate gut health and their association with performance. The focus of this literature review is on broilers, although some of the information presented is relevant for other poultry industries.

**THE AVIAN DIGESTIVE SYSTEM**

The gastrointestinal tract (GIT) of the chicken is short compared to mammals so that the passage rate of the digesta in the poultry GIT is faster. Although the type of feed given to the chickens determines the retention time of the digesta, the transit of feed in the GIT is in general between 3 and 4 h (Hughes, 2008). The major organs of the GIT and the main microbiota in each organ are presented in Figure 1.

The digestive tract of birds starts with the beak which is used for prehension. Salivary glands in the mouth then secrete mucus which provides lubrication, antiviral and antibacterial protection to the mucosa, and the mucus also facilitates the transport of feed from the mouth to the esophagus (Scanes and Pierzchala-Koziec, 2014). Primary peristalsis in the esophagus moves the feed into the crop or directly into the stomach (Rodrigues and Choct, 2018). The chicken stomach contains a glandular part, named proventriculus, and a muscular part, named gizzard. The contraction and relaxation of the gizzard determine the feed flux which will bypass the crop when the gizzard is empty and will enter the crop when the gizzard is fully or partially filled (Denbow, 2015). The crop is a distended pouch that plays a major role in fermentation (Fuller and Brooker, 1974; Scanes and Pierzchala-Koziec, 2014). Contraction of the crop wall results in the evacuation of feed into the gizzard via proventriculus (Denbow, 2015). Protein digestion is initiated in the proventriculus aided by the secretion of hydrochloric acid and pepsinogen in this organ (Scanes and Pierzchala-Koziec, 2014). The volume of the proventriculus is small so the feed rapidly transits into the gizzard; however, the feed can reflux back into the proventriculus several times for enzymatic digestion (Rodrigues and Choct, 2018). The gizzard is responsible for mixing and grinding the feed until particles are smaller than 0.1 mm when it is then passed into the small intestine (Hetland et al., 2002; Jacob et al., 2011).

The small intestine, which is composed of the duodenum, jejunum, and ileum, is a major site of chemical digestion and absorption after which the feed is passed into the 2 large ceca which are responsible for fermentation and digestion of starch, cellulose, and indigestible...
carbohydrates (Mead, 1989; Stanley et al., 2014, 2015). The lower passage rate of digesta in the ceca compared to other sections of the GIT allows for increased fermentation of the digesta resulting in increased concentration of short-chain fatty acids (SCFAs) production by the resident microbiota and water absorption (Clench and Mathias, 1995; Rehman et al., 2007; Rodrigues and Choct, 2018). Emptying of the cecum takes place every 24 to 48 h (Clench and Mathias, 1995). Digesta move through the ileo-cecal junction into the colon, from which the digesta can move to the ceca by retrograde antiperistalsis or to the cloaca by peristaltic contraction (Duke, 1989). The colon is very small and little to no digestion or absorption takes place in it. From the colon, the digesta is passed into the cloaca and mixed with uric acid before being expelled out.

THE MICROBIOTA OF THE CHICKEN INTESTINAL TRACT

The microbiota of the gastrointestinal tract is a complex ecosystem predominantly comprised of bacteria, but also contains viruses, archaea, fungi, and protozoa (Smulikowska, 2006; Wei et al., 2013). This section focuses on the bacterial communities that are part of the intestinal microbiota and for simplicity; the term “microbiota” will refer to the bacterial microbiota hereafter.

The Role of the Intestinal Microbiota

The importance of intestinal microbiota in the nutrition, health, physiology, and immunity of the chicken has become evident in recent years (Apajalahti et al., 2004; Sekirov et al., 2010; Pan and Yu, 2014; Kers et al., 2018). The commensal microbiota is attached to the epithelium of the intestine and plays an important role in maintaining homeostasis and in the protection against colonization by pathogens (Yegani and Korver, 2008; Diaz Carrasco et al., 2019). The microbiota interacts with the chicken immune system assisting the training and maturation of the immune cells to ensure that commensal microorganisms are tolerated, pathogens are recognized and curbed and those potentially pathogenic commensal microorganisms such as Clostridium perfringens and Escherichia coli are kept in check (Adil and Magray, 2012; Klindworth et al., 2013). It also helps in the development of the lamina propria, mucus layer, and epithelial monolayer through the production of metabolites such as SCFAs, indoles, vitamins, and antimicrobial compounds, increasing the epithelial absorptive surface (Dibner and Richards, 2005; Yegani and Korver, 2008; Shakouri et al., 2009; Oakley et al., 2014). An in-depth review of microbiota and chicken immune interactions can be found at Kogut et al. (2020).

Microbiota of the cecum also plays a role in host nitrogen metabolism which is highly associated with the efficiency of chickens to extract energy from feed (Oakley et al., 2014). Uric acid and feces mixed in the cloaca may go to the cecum due to the retrograde peristalsis where bacteria convert uric acid to ammonia which is utilized by the host to synthesize amino acids such as glutamine (Vispo and Karasov, 1997). However, the microbiota may also compete for energy and protein and reduce the availability of nutrients for the host (Pan and Yu, 2014). Some of the intestinal microbiota can catabolize bile acids which retards fat digestion and absorption (Furuse and Yokota, 1985; Engberg et al., 2000). When protein is not fully digested and absorbed in the small intestine, it undergoes further fermentation in the large intestine with the potential production of toxic amines which can decrease bird growth and performance (Gaskins et al., 2002). Detailed reviews of the interaction between gut microbiota and diet have been recently published (Pan and Yu, 2014; Yadav and Jha, 2019).

Microbiota in the Different Sections of the Gut

Initial Colonization of the Gut After chicken eggs are laid they are usually sent to hatcheries that adhere to strict hygienic conditions. Bacteria can penetrate through the eggshell while it is moist (Berrang et al., 1999) and although transmission of bacteria such as Salmonella enterica serovar Enteritidis from hens to chicks has been described, there is somewhat limited evidence of transovarial transmission of bacteria (Tankson et al., 2002; Cortés et al., 2004; Kubasova et al., 2019; Akinjewo et al., 2020). Therefore the majority of colonization of the chick gut with bacteria begins right after hatching via contact with environmental sources at the hatchery, mainly people handling the eggs, transportation boxes, and any feed and water or bedding material available (Kizzeretter-Swida and Binek, 2008; Stanley et al., 2014). Young chickens at 1 and 2 d of age are delivered to commercial farms where they are usually placed in bedding material, although rearing of chicks in plastic nets is also practiced in some countries such as China (Chen et al., 2020; Yan et al., 2021). Several microbiota taxa from new or reused bedding material and other environmental sources such as feed continue the initial colonization of the gut (Wang et al., 2016). As the chickens grow, the microbiota undergoes temporal successions and become more complex and diverse (Wei et al., 2013).

Microbiota in Different Parts of the Gastrointestinal Tract Overall, the 5 most dominant bacterial phyla found in the chicken GIT are Firmicutes, Tenericutes, Bacteroidetes, Proteobacteria, and Actinobacteria (Wei et al., 2013) (Figure 1). Lactobacillus is the primary bacterial taxa found in the crop, gizzard, duodenum, and ileum. Bifidobacterium and Enterobacter are also commonly detected in the crop (Yeoman et al., 2012; Saxena et al., 2016). The acidic nature of the stomach provides an unfavorable environment for most bacteria so it harbors fewer bacteria than other regions of the GIT (Rehman et al., 2007). The gizzard has primarily lactobacilli with few enterococci, coliforms, and
lactose-negative enterobacteria (Zoetendal et al., 2004; Sekelja et al., 2012; Yadav and Jha, 2019). *Clostridium perfringens*, a commensal but potentially pathogenic bacterium, has also been found in gizzard at a very low level (10^3 bacteria/g of contents) (Engberg et al., 2004).

The most dominant phylum in the small intestine of chickens is *Firmicutes* (Yeoman et al., 2012), and this phylum is largely represented by the genus *Lactobacillus* and to a lesser extent by *Clostridium, Ruminococcus, Streptococcus*, and to a lesser extent by *Enterococcus* (Jin et al., 1997; Lu et al., 2003; Yeoman et al., 2012; Munyaka et al., 2016; Wang et al., 2016). Bacterial members of the phylum *Proteobacteria*, mainly *Escherichia* and *Enterococcus* have also been reported in the ileum (Jin et al., 1997; Lu et al., 2003; Yeoman et al., 2012; Wang et al., 2016).

The highest bacterial density and diversity in the chicken intestinal tract are in the cecum (Dibner and Richards, 2005; Shakouri et al., 2009; Oakley et al., 2014). The most abundant phyla found in the cecum are *Firmicutes* followed by *Bacteroidetes* while *Proteobacteria* and *Archaea* are present in lesser amounts (Figure 1). The most dominant genera in the cecum within the phylum *Firmicutes* were found to be *Lactobacillus, Ruminococcus, Faecalibacterium*, and *Clostridium* (Jin et al., 1997; Gong et al., 2002; Qu et al., 2008; Wang et al., 2016) (Figure 1).

*Lactobacillus, Clostridium, Faecalibacterium, Ruminococcus, Bacillus*, and *Eubacterium* (phylum *Firmicutes*) are the predominant genera in cloaca and excreta (Sekelja et al., 2012; Stanley et al., 2014; Lim et al., 2015); however, the microbiota found in cloacal mucosa and excreta are not stable and abrupt temporal fluctuations occur due to the cyclical emptying of different regions of the GIT (Deusch et al., 2015).

**Factors Affecting the Intestinal Microbiota Composition**

This section covers some of the major factors that influence intestinal microbial composition such as age, sex, and breed, diet, and litter conditions (Kers et al., 2018).

**Age, Sex, and Breed** The maturation of the microbiota which is defined as the age at which microbial community matures and does not change over time has been shown to vary between studies. In a study in commercial broiler farms, Kers et al. (2020) found that cecum phylogenetic diversity stabilized after 21 d of chicken age. However, a study by Lu et al. (2003) found that microbial communities in the cecum were similar at d 14 and 28 and different compared to d 49 suggesting that the microbiota stabilized after 28 d of chicken age under experimental conditions. Torok et al. (2009) also showed large differences in cecal microbial communities between d 14 and 28 in broilers under experimental conditions. Despite those differences, it is accepted that the GIT microbial richness increases and the microbial composition changes with age, that the microbiota becomes more stable as age increases, and that the environment plays a role in when the stabilization will occur (Feye et al., 2020).

At the beginning of the production cycle, on d 0, the family Clostridiaceae was dominant in cecal contents in broilers (Kers et al., 2019a; Kers et al., 2020). Lu et al. (2003) showed that at 3 d of age *Lactobacillus* represented 25% of the total bacteria in the cecal contents. The concentration of *Lactobacillus* in cecal contents at d 3 was 100 times higher compared to d 42 (Gong et al., 2008). Among the *Lactobacillus, Lactobacillus delbrueckii* and *Lactobacillus acidophilus* were the dominant species in the cecum (Lu et al., 2003). In the same study, the cecal contents were dominated by *Clostridium saccharolyticum*, *Clostridium oroticum*, and *Clostridium orbiscindens* at 7 d of age; *Ruminococcus schinkii* and *Clostridium indolis* at d 14 to 28; and *Eubacterium* at d 49 (Lu et al., 2003). In a study by Ranjitkar et al. (2016), the relative abundance of *Enterococcus* in ileal contents decreased from 25% at d 8 to 1% at d 15 and remained unchanged till d 36 while the level of *Clostridium* increased from 1 to 18% and *Streptococcus* from 5 to 15% from d 8 to 36.

Although the effect of sex is more pronounced when chickens undergo sexual maturity, differences have also been observed in younger broilers and seem to be correlated with sex-based differences in glycan and lipid metabolism by the cecal microbiota (Lumpkins et al., 2008; Lee et al., 2017; Cui et al., 2021). In the cecum of 35-day-old Ross 308 broilers, *Oscilospira* and *Tenericutes* were higher in females and *Bacteroides* were higher in males (Lee et al., 2017). In a study by Cui et al. (2021), the abundance of *Bacteroides, Megasphaera, and Phascolarctobacterium* were higher in males while *Akkermansia* was higher in females in the cecum of 35-day-old chickens. Torok et al. (2013) also found that sex influences total eubacterial numbers with males having an increased abundance of eubacteria compared to females in cecal contents at d 22 and 42. Lumpkins et al. (2008) showed that microbial communities of the ileum also differed between male and female broilers at d 3. While the overall effect of sex on the chicken GIT microbiota is unclear, whole-genome profiling of prepubertal mice has shown that even in the absence of high levels of circulating sex hormones, mice with identical microbiota have intrinsic sex-specific gene regulation in the GIT and that sex differences in the microbiota after puberty are correlated with sex differences in GIT expression of multiple genes (Vemuri et al., 2019).

Chicken genotype affects the microbiota (Pandit et al., 2018; Ji et al., 2020; Tumova et al., 2021), although this effect appears to be limited to specific taxa (Chintoan-Uta et al., 2020; Wen et al., 2021). Kers et al. (2018) compiled data from 12 studies reporting 16S rRNA sequencing of cecal samples of Cobb and Ross breeds. *Actinobacteria* was present in Cobb chickens in all 4 studies of this breed, and in 3 out of 8 studies reporting data on Ross chickens. Similarly, *Bacteroidetes* were present in Cobb chickens in all 4 studies and
6 out of 8 studies in Ross chickens. Large differences in the cecal microbial composition between chickens of Hubbard and Ross breeds at an early age have also been reported (Richards et al., 2019). On d 0, Hubbard cecal microbiota was dominated by Enterobacteriaceae while Cobb cecal microbiota was dominated by Enterococccaeae and Clostridiaceae. On d 3, a higher abundance of Bifidobacteriaceae but a lesser level of Enterobacteriaceae was seen in Hubbard compared to Ross chickens but from d 7, no differences were observed in the microbial composition between breeds (Richards et al., 2019).

From the above findings, it is evident that age, sex, and breed can influence the microbiota of the poultry, and detailed information should be provided in microbiota studies regarding these parameters. **Diet** Diet has a major influence on the gut microbiota composition and both the composition of the diet and the physical form of feed (pellet or mash) affect digestibility and nutrient absorption in the gut (Apajalahti et al., 2001, 2004). When broilers were fed a wheat-based diet in pellet form there was an increased concentration of coliforms and enterococci in the ileum and C. perfringens in the cecum compared to the same feed in mash form (Engberg et al., 2002). Compared to grounded wheat in pellet form, whole wheat feeding resulted in the reduction of C. perfringens and lactose negative enterobacteria and increased numbers of Bifidobacterium and bacterial diversity in ceca (Apajalahti et al., 2001; Engberg et al., 2004). Higher levels of Lactobacillus spp were found throughout the intestinal tract of chickens fed whole wheat compared to chickens fed with pellets of grounded wheat (Engberg et al., 2004).

Chickens fed a rye-based diet had increased levels of coliforms in the duodenum and ileum and increased levels of lactic acid bacteria in the cecum, duodenum, and ileum compared to chickens fed a corn-based diet (Tellez et al., 2014). Diets rich in nonstarch polysaccharides (NSPs), fish meal, and bone meal have been associated with the increased proliferation of C. perfringens and predispose chickens to NE (Williams et al., 2003; Williams, 2005; M’Sadeq et al., 2015). NSPs increase the viscosity in the intestinal lumen, decrease the passage rate and enzymatic activities, and reduce the feed conversion efficiency (Chcott and Ammo, 1992). The increased retention time of the digesta, especially in diets of high protein or with unbalanced amino acid profiles, provides substrates and facilitates colonization of pathogenic bacteria in the small intestine, including C. perfringens (Waldenstedt et al., 2000; Annett et al., 2002; Timbermont et al., 2011; Loh and Blaut, 2012). Fish meal also contains higher amount of zinc and glycine and a positive correlation between the C. perfringens abundance and glycine concentration has been shown (Wilkie et al., 2005; Daihya et al., 2007). In addition to feed ingredients and physical feed form, feed deprivation and withdrawal also influence gut health and microbiota composition leading to increased Salmonella colonization (Burkholder et al., 2008; Thompson et al., 2008; Lamot et al., 2014).

Torok et al. (2008) investigated the difference in gut microbial communities in broilers fed barley or barley supplemented with exogenous enzymes and found that the bacterial community in cecum and ileum was different between treatments. The addition of exogenous enzymes increased the butyrate and lactic acid-producing bacteria in the ceca (Munyaka et al., 2016). Different feed additives such as enzymes, probiotics, prebiotics, and symbiotics have been used to modulate gut microbiota and improve the immune system of poultry (Jha and Berrocoso, 2015; Yadav and Jha, 2019).

**Litter Type and Conditions** Litter, which is a mixture of excreta, bedding material, feather, dander, feed, and various microorganisms, can have a direct influence on gut microbiota composition. Studies describing the microbial composition of litter are scarce and usually focus on the detection of pathogenic microorganisms such as Salmonella, E. coli, Campylobacter, and C. perfringens (Bennett et al., 2005). In some countries such as the United States and Brazil, it is common to reuse litter in broiler flocks up to 6 cycles of grow-out (Coufal et al., 2006; Roll et al., 2011) while in Canada and Australia, fresh bedding materials is usually used in every grow out period (Cockerill et al., 2020; Feye et al., 2020). As chickens consume up to 4% of their diet as litter during the first 2 wk of age (Malone et al., 1983), litter type and microbial composition directly influence the colonization and development of gut microbiota in chickens (Torok et al., 2009).

Using culture-based techniques, Terzich et al. (2000) investigated total bacteria, coliforms, Staphylococcus, Gram-positive, and Gram-negative bacterial counts in samples of litter that had been used for at least 3 chicken grow-out cycles in the United States. The average total number of bacteria was $2.54 \times 10^{11}$ colony forming units (CFU)/g while the average count of Gram-negative or Gram-positive was $1.60 \times 10^{11}$ CFU/g; Staphylococcus and coliforms were commonly detected (Terzich et al., 2000). Reused wood shaving litter was found to enable Campylobacter jejuni and Campylobacter coli to survive for at least 20 d while fresh litter inhibited the growth of these pathogens (Kassem et al., 2010).

Using a sequencing-based technique, Wang et al. (2016) found that reused pine shaving litter contained mostly halotolerant alkaliphiles and Faecalibacterium prausnitzii while fresh litter was dominated by Devisoa, Yaniella, Acinetobacter, Trichococcus, and Luteimonas. In the same study, ideal mucosa of broilers raised on reused litter had a higher abundance of Enterococcus at d 10 while Lactobacillus was higher at d 35 compared to birds raised on fresh litter. Similarly, in cecal contents, Blautia, Anaerotruncus, and Faecalibacterium were at higher levels in birds raised on the reused litter while Lactobacillus, Escherichia, Subdoligranulum, Bacteroides, and Clostridium XIVb were higher in cecal contents of birds raised on fresh litter at d 10 (Wang et al., 2016). On d 35, chickens raised on reused litter had a higher abundance of Faecalibacterium and Oscillibacter in cecal contents while the abundance
of Subdoligranulum was higher in cecal contents of birds raised on fresh litter (Wang et al., 2016). Cressman et al. (2010) described that 7-day-old broilers raised on reused litter had unclassified Clostridiales group of bacteria as the dominating feature in the ileal mucosa while the Lactobacillus were dominant in chickens raised on fresh litter.

The microbial communities also vary depending on the bedding material (Torok et al., 2009). The microbial communities in the cecum of 28-day-old broilers raised on chopped straw were significantly different from broilers raised in sawdust; similarly, there were differences in the cecal microbial communities of broilers raised on rice hulls, chopped straw, or shredded paper (Torok et al., 2009). Total aerobic mesophilic bacteria, enterococci, Enterobacteriaceae, and Staphylococcus aureus counts were found to be higher in pine wood shavings compared to dry litter (Dumas et al., 2011; Oakley et al., 2013). Although not consistently, wet litter is associated with poor gut health in poultry (Collett, 2012). These results show that litter quality, type, and management have a direct impact on gut microbiota composition, although the relationship of early microbial colonization of the gut in various litter management conditions and chicken productive performance is unknown.

Some Methodological Aspects of Studying Microbiota

Although 16S rRNA gene sequencing has become a popular approach to study microbiota in humans and animals, direct comparison between microbiota detected in different studies could be hampered by differences in sample processing. Some of the technical aspects that could bring potential bias to microbiota studies are discussed below. In-depth reviews have been recently published covering sample size calculation (Casals-Pascual et al., 2020), laboratory methodological aspects (Pollock et al., 2018), and bioinformatics (Bharti and Grimm, 2021).

**Sample Size** Because of the high bird-to-bird microbiota variability, selecting the appropriate sample size is crucial to detect differences between groups in microbiota studies (Stanley et al., 2014; Bharti and Grimm, 2021). In humans, Falony et al. (2016) described that over 500 samples per group are required to accurately identify microbial shifts and sample sizes of 100 individuals per group are considered relatively small. In contrast, chicken microbiota studies have often less than 10 samples per treatment at each time point (Kers et al., 2020). Appropriate sample sizes should be calculated considering a high population variance and weak treatment effect sizes to exclude bias in the results (Bharti and Grimm, 2021). Different statistical models have been developed for power test and sample size calculation in microbiome studies (Xia et al., 2018), ideally using data from preliminary experiments to calculate the expected variances and treatment effect sizes (Qian et al., 2020).

**Sample Collection** In a given GIT region, samples can be collected directly from digesta, mucosa, or a swab of digesta and/or mucosa. Although the impact of the choice of sample type has not been fully studied in chickens, a comparison between the microbiota of cecal luminal contents and cecal mucosa in broilers showed a higher abundance of Bacteroides and a lower level of Lachnospiraceae in luminal contents compared to the mucosa (Richards et al., 2019), suggesting that the sample type may have an impact on microbial communities.

**Storage Condition of Samples** Ideally, gut contents or excreta should be stored at -80°C immediately after collection to prevent changes in the microbial community composition, but the maintenance of cold chain from sample collection until transference to a laboratory is not always possible (Gorzelaak et al., 2015). It has been established that the microbial composition of human stools stored at 4°C for up to 72 h was similar to the control samples stored at -80°C (Choo et al., 2015; Tedjo et al., 2015). However, in stool samples stored at room temperature for 3 d, there was a decrease in abundance of the phylum Firmicutes, mainly genus Anaerostipes, Ruminococcus, Faecalibacterium, and Lachnospiraceae; and an increase in the abundance of phylum Actinobacteria mainly the genus Bifidobacterium compared to samples stored at -80°C (Roesch et al., 2009; Choo et al., 2015). Preservatives such as RNAlater have been shown to decrease DNA purity and lower microbial diversity compared to samples stored at -80°C for 3 d (Dominanni et al., 2014). Similarly, buffers containing EDTA have been shown to increase the abundance of Proteobacteria and reduce the abundance of Firmicutes and Actinobacteria in stool samples stored in this buffer for 3 d compared to samples stored at ~80°C (Choo et al., 2015). Other preservatives such as OMNIgene.GUT (DNA Genotek, OMRI-200, Ontario, Canada) decreased the relative abundance of Lachnospiraceae and increased the abundance of Sutterella and Faecalibacterium when samples were stored for 3 d compared to storage at ~80°C without buffer (Chen et al., 2019). Consequently, all samples in an experiment should undergo to the same cryopreservation protocol to avoid the introduction of biases in the analysis.

**DNA Extraction Methods** The methods of DNA extraction employed have a direct impact on the microbiota composition and diversity (Scupham et al., 2007; Costea et al., 2017; Abundo et al., 2021). Different commercially available extraction kits use different protocols for bacterial cell lysis such as enzymatic and chemical digestion, and/or mechanical disruption of bacterial cell walls such as bead-beating (Videnska et al., 2019). Mechanical disruption, particularly bead-beating following chemical digestion is recommended for lysis of the
cell walls of Gram-positive bacteria as it increases the DNA yield and microbial diversity (Lu et al., 2015; Fidler et al., 2020). Complete absence of the Gram-positive *Bifidobacterium* spp was observed upon extracting the DNA without a bead-beating step while the same genus was well represented after a bead-beating step was added (Walker et al., 2015). However, bead beating for a long time results in the shearing of DNA into small fragments and the protocol needs to be optimized depending upon the sample matrix (von Wintzingerode et al., 1997; Dihlari et al., 2017). Once the extraction method is optimized, the same protocol should be consistently followed to allow for direct comparison of the microbiota (Boers et al., 2019). The use of mock bacterial communities of known composition is a recommended technical control in every study to account for the bias introduced by the DNA extraction protocol (Salter et al., 2014).

**Choice of Primers** 16S rRNA gene amplicon sequencing is based on the amplification of a hypervariable region of the bacterial 16S rRNA gene by PCR (Adhikari, 2019). The 16S rRNA gene is around 1550 nucleotides long and contains 9 hypervariable regions (V1-V9) that allow the differentiation between bacterial species (Van de Peer et al., 1996), and different studies target different hypervariable regions for amplification and different primers have been used to amplify the same hypervariable region (Bergmann et al., 2011; Walker et al., 2015; Barb et al., 2016; Pollock et al., 2018). Studies have shown that some of the commonly used universal primers may underestimate the abundance level of certain bacterial taxa or may not amplify some biologically relevant bacterial taxa (Bergmann et al., 2011; Walker et al., 2015). Barb et al. (2016) compared the efficacy of 6 universal primers designed to target V2, V3, V4, V6-7, V8, and V9 regions of 16S rRNA gene on the identification of the bacteria present in mock samples and found that the highest divergence from the original mock microbial community was found for primer sets targeting the V9 region, and the least divergence was found for primer sets targeting V2, V4, and V6-7. Fouhy et al. (2016) also found differences in genus and species detected using 3 different universal primer sets illustrating that the choice of primer could impact microbiota studies. The choice of primers should allow high phylogenetic resolution and low bias (Klindworth et al., 2013; Fukus et al., 2018).

**PCR Cycles and DNA Polymerase Types** Further biases could also be introduced in the sequencing results because of the number of PCR cycles and the type of DNA polymerase used. Increased PCR cycles (15–30) lead to the formation of artifacts and a large number of chimera sequences which could lead to misinterpretation of results (Suzuki and Giovannoni, 1996; von Wintzingerode et al., 1997; Ishii and Fukui, 2001; Hongoh et al., 2003). This effect is much more pronounced when using high-fidelity DNA polymerase with proofreading activity compared with normal Taq DNA polymerase (Ahn et al., 2012). In a study by Hongoh et al. (2003), 2 high-fidelity polymerases, namely PfuUltra II Fusion HS DNA Polymerase (Stratagene, La Jolla, CA) and Ex Taq (Takara, Ontario, Canada) produced significantly different microbial community structures. It is recommended to optimize the cycling conditions and to evaluate the effect of different polymerase enzymes in samples of mock bacterial communities before the commencement of the study and keep the same conditions throughout the same study (Pollock et al., 2018).

**MICROBIOTA PROFILES AND PRODUCTIVE PERFORMANCE**

Identifying and understanding the role of specific microbiota profiles associated with efficient feed conversion is of utmost importance to develop and test dietary interventions (Stanley et al., 2016). Numerous studies have been performed to identify GIT microbiota associated with high and low-performance in chickens with often nonreproducible or contradictory results among trials as demonstrated by Stanley et al. (2016), with additional literature recently reviewed by Diaz Carrasco et al. (2019).

Stanley et al. (2016) investigated the differences in cecal microbiota of high- and low-performance birds across 3 experimental trials using the same experimental facility, feed batch, and source of birds. In that study, specific microbiota was differentially abundant in high and low performing birds in each trial but some of the microbiota present in high performing birds in one trial was absent in other trials and none of the microbial taxa was consistently present in high or low performing birds across the 3 trials (Stanley et al., 2016). Although specific microbial taxa could not be conclusively associated with a production outcome, high performance, as measured by feed conversion ratio (FCR), were in general enriched with bacterial communities known to degrade cellulose and resistant starch, mainly members of the families *Lachnospiraceae* (e.g., *Clostridium lactifermentans*), *Ruminococcaceae* (e.g., *Clostridium islandicum*, *Faecalibacterium prausnitzii* *Erysipelotrichaceae* (e.g., *Clostridium spp*), *Bacteroides fragilis* and *Clostridium cellulosi* while bacterial communities negatively correlated with performance belonged to uncultured members of an unknown class of *Firmicutes* and species of clostridium belonging to the family *Clostridiaceae* (Stanley et al., 2013a; Stanley et al., 2016).

Those findings were similar to previous studies in experimental chickens in which Ruminococcus *torques* and *Clostridium lactatifermentans* isolated in ceca were associated with high performance (Torok et al., 2013) while Lactobacillus *salivarius*, *L. aviaris*, and *L. crispatus* in ileum were associated with decreased performance (Torok et al., 2011). As evidenced by the findings above, although some *Lactobacillus* strains can be a major energy source for the host via their ability to produce SCFAs not all species or strains are beneficial. Lactobacillus gasseri SBT2055 has been
reported to reduce body weight in obese humans (Kadooka et al., 2010) while *L. salivarius* has been associated with bile deconjugating activity which is associated with a decrease in the growth rate of chickens (Knarreborg et al., 2002). *Streptococcus faecium* and *C. perfringens* are also strong bile deconjugating bacteria (Stutz and Lawton, 1984; Knarreborg et al., 2002). In a study in commercial broiler flocks, Johnson et al. (2018) found that the microbiota in the cecum and ileum positively correlated with bird weight was *Bacteroides*, *Bilophila*, and *Butyricimonas* while the taxa negatively correlated with bird weight included *Anaerotruncus*, *Bacteroides*, *Blautia*, *Clostridium*, *Coprobacillus*, *Coprococcus*, *Eggerthella*, *Enterococcus*, *Lactobacillus*, and *Oscillospira*. In a study by Singh et al. (2012) in experimental broilers, excreta of low-performance birds, as measured by FCR, were enriched with *Acinetobacter*, *Anaerobacter*, *Arcobacter*, *Dysgonomonas*, *Ignatzschineria*, *Megamonas*, *Myroides*, *Providencia*, and *Wohlfahrtiimonas* and excreta of high performing birds were enriched with *Alistipes*, *Barnesiella*, *Cloacibacillus*, *Escherichia/Shigella*, *Faecalibacterium*, *Helicobacter*, *Oscillibacter*, and *Phascolarctobacterium* while the abundance of *Lactobacillus*, *Enterococcus* and *Bacteroides* were found to be similar in both low- and high-FCR birds. As revised above, the majority of the studies have focused on the association of cecum and ileum microbiota with production. The development of practical and noninvasive tools to monitor microbiota associated with production would greatly enhance this field of research (Diaz Carrasco et al., 2019). Monitoring the microbiota at flock-level using samples such as pooled excreta, litter or poultry dust would be perhaps an alternative to individual bird sampling as a way to reduce the noise generated by the within bird variation observed in GIT microbiota studies. Another approach for overcoming the variability of the GIT microbiota is to further interrogate the functionality of the observed microbial population, either by doing predictive functional bioinformatics analysis or by directly measuring microbial metabolites of interest in the cecum using high-performance liquid chromatography, which is particularly useful for measuring microbial fatty acids and lactic acid outputs that are related to gut health, or using liquid-chromatography-tandem mass spectrometry, which captures the microbial metabolomic profile (Kers et al., 2019b; Niu et al., 2020; Park et al., 2020; Slizewska et al., 2020).

**DISEASES OF THE ENTERIC TRACT:**

**Coccidiosis and Necrotic Enteritis**

*Coccidiosis*

Coccidiosis is an enteric disease that can be caused by any of 7 species of *Eimeria* in chickens. From those, *Eimeria mitis* and *E. praecox* are less pathogenic compared to *E. brunetti*, *E. maxima*, *E. acervulina*, *E. necatrix*, and *E. tenella* (Williams, 2005). Globally the annual cost of coccidiosis was estimated to be US$3 billion (Williams, 1999) but this figure is largely outdated. Coccidiosis can occur in clinical or subclinical forms. In the clinical form, it is associated with mortality, bloody diarrhea, and weight loss whereas subclinical coccidiosis is manifested by reduced feed efficiency and poor weight gain (Williams, 1999). The infection with *Eimeria* usually affects the growth and feed conversion for 2 to 3 wk, after which a period of compensatory growth occurs (Voeten et al., 1988). Subclinical coccidiosis may have no effect on FCR and chicken growth when birds are infected in the first week of life because of compensatory growth, or when birds are infected on the last week of grow-out because in this time-frame there is not enough damage to the intestinal mucosa (Voeten et al., 1988; Kipper et al., 2013).

Traditional diagnosis of coccidiosis is based on counts of oocysts in excreta or litter, which is costly, labor-intensive, and requires skilled personnel to differentiate the oocysts based on the morphology (De Gussem, 2007). Based on this method, an *Eimeria* oocyst count of more than 50,000/g of pooled excreta has been associated with loss of performance in chicken flocks when more than 50% of oocysts are of medium size (corresponding to infections with *E. necatrix*, *E. tenella*, or *E. praecox*) or oocysts of large size (*E. brunetti* or *E. maxima*); while the presence of small oocysts (*E. acervulina*) was not associated to significant production losses (Haug et al., 2008). Molecular based approaches, mainly using PCR, have been effective to subtype and quantifying *Eimeria* species in excreta and litter in both experimental and commercial flocks (Morris and Gasser, 2006; Morris et al., 2007; Morgan et al., 2009; Vrba et al., 2010; Godwin and Morgan, 2015). More recently, DNA of *C. perfringens* and *Eimeria* species have been detected in poultry house dust of experimental and commercial flocks (Ahaduzzaman et al., 2021a; Bindari et al., 2021a).

In commercial broiler flocks, monitoring of coccidiosis is usually performed by scoring several intestinal sections based on the predisposition of the *Eimeria* species; a score of 0 is given for the absence of gross pathology in an intestinal segment and a score of +4 is given for severe gross lesions (Johnson and Reid, 1970). This method can be subjective and requires a high level of expertise to correctly score lesions as other diseases with similar pathologies may co-occur, for example, NE (Shirley et al., 2005). Coccidiosis is an important predisposing factor for NE and different *Eimeria* ssp administration regimens have been used to induce NE experimentally (Van Immerseel et al., 2009; Dierick et al., 2021).

Coccidiosis also alters gut microbiota. Ceca of healthy birds were found to be dominated by the genera *Subdoligranulum*, *Coprococcus*, *Alistipes*, *Lactobacillus*, and *Faecalibacterium* while ceca from *Eimeria* ssp (*E. acervulina*, *E. maxima*, and *E. tenella*)
infected birds were dominated by *Escherichia/Shigella* and *Bacteroides* (Kley et al., 2012). In a study by Macdonald et al. (2017), Bacillales and Lactobacillales were decreased while *Enterobacteiraceae* was increased following *E. tenella* infection in the cecum; however, the overall diversity of the microbial taxa was not changed. Wu et al. (2014) reported a reduction in the number of *Ruminococcaceae* groups and an increase in 3 unknown *Clostridium* species in birds challenged with *E. acervulina, E. maxima,* and *E. brunetti* compared to unchallenged groups. Hume et al. (2006) also observed a clear shift in the microbiota of duodenal, ileum, and cecal contents following *E. acervulina, E. maxima,* and *E. tenella* infection compared to unchallenged birds.

**Necrotic Enteritis**

Necrotic enteritis (NE) is an economically important enteric disease affecting broilers (M’Sadeq et al., 2015). Strains of *C. perfringens* type A carrying necrotic enteritis B-like toxin (*netB*) plasmid are associated with increased risk of NE, although the presence of *netB* does not seem sufficient nor necessary to cause clinical disease (Rood et al., 2016). Similar to coccidiosis, NE can manifest clinically or subclinically. The clinical presentation includes diarrhea and associated wet litter, decreased appetite, ruffled feathers, anorexia, depression, and death (Al-Sheikhly and Al-Saieg, 1980; Williams, 2005). In NE-affected flocks, the mortality rate is about 1% per day and birds usually die within a few hours of displaying clinical signs (Helmboldt and Bryant, 1971). The subclinical form may result in reduced feed intake and poor FCR (Lovland and Kaldhusdal, 2001; Morris and Gasser, 2006) which may lead to great economic losses (Dahiya et al., 2006).

*Clostridium perfringens* is found in the intestinal tract of healthy chickens usually in lower amounts ($10^2$–$10^4$ CFU/g digesta) without causing disease (Kondo, 1988; Caly et al., 2015). Changes in the GIT environment via feed, disease, stress, and immunosuppression can facilitate *C. perfringens* proliferation to concentrations of $10^7$ to $10^9$ cfu/g digesta in NE affected chickens (Kondo, 1988; McDevitt et al., 2006). Birds that survive NE outbreaks have impaired digestion and absorptive capacity of nutrients (Paiva and McElroy, 2014); however, microbiota shifts in those birds remain to be investigated.

The onset of NE is associated with a clear shift of microbiota but it is unclear if the microbial shift was a predisposing factor or a consequence of the disease (Antonissen et al., 2016). Stanley et al. (2012) found that birds with NE induced by a wheat-based diet with 50% fish meal starting on d 15, followed by a *C. perfringens* in-feed challenge on d 19 to 22 had decreased abundance of the butyrate-producing bacteria *Weissella confusa* and an increase in the abundance of unclassified *Mollicutes* compared to healthy birds examined at d 23. Feng et al. (2010) tested the difference in abundance of *Enterococcus, Enterobacteriaceae, Clostridium,* and *Lactobacillus* between birds fed with and without *C. perfringens* contaminated feed and found that only lactobacilli were reduced 2 d postinfection compared to the controls. Antonissen et al. (2016) conducted an extensive review on microbial shifts associated with NE and concluded that different predisposing factors for NE reduced the abundance of segmented filamentous bacteria, butyric acid and lactic acid producing bacteria.

Diagnosis of NE is usually performed by the scoring of intestinal lesions (Keyburn et al., 2006; Vidanarachchi et al., 2013), while the culture of digesta for detection of *C. perfringens* and toxins and histopathological techniques are also available (Smyth, 2016). One of the major issues with the scoring of intestinal lesions in the field is misidentifying autolytic changes or coccidiosis lesions, especially from *E. necatrix,* as NE (Smyth, 2016). Monitoring of both *Eimeria* species and *C. perfringens* using noninvasive easy to collect population-level samples would perhaps provide useful research or on-farm tools to evaluate the management interventions of both diseases at flock-level (Ahaduzzaman et al., 2020; Bindari et al., 2021a).

**METHODS FOR MONITORING SHIFTS IN CHICKEN INTESTINAL STATUS**

A wide range of tools have been evaluated in their ability to accurately evaluate healthy or diseased enteric states (Ducatelle et al., 2018; Celi et al., 2019) but to date, no standardized, reliable and practical method is available. Currently, the gold standard to measure gut health consists of microscopic examination of small intestine sections for evaluation of the villus height and crypt depth ratio (de Verdal et al., 2010). It is, however, time-consuming, and requires the collection of samples and a specialized laboratory, which is not readily available outside of research settings. In commercial settings, scoring of different intestinal sections is usually performed when an enteric disease is observed or anticipated but this method is time-consuming, requires skilled staff and the diagnosis can be confounded as NE, coccidiosis and dysbiosis produce similar gross lesions and these conditions may overlap.

To overcome these limitations, biomarkers that are indicative of gut inflammation or disruption of the gut barrier permeability have been widely investigated. Gut permeability, a term describing the capacity of the intestinal epithelium to allow the passage of molecules through nonmediated diffusion, is affected by several factors including infectious and noninfectious diseases, stress, hormones, diet, and others (Travis and Mennen, 1992). Biomarkers for gut health can be grouped into 3 broad categories, 1) Host protein biomarkers that are indicative of gut barrier damage such as plasma protein detection in gut contents (e.g., hemoglobin subunit beta); or detection of gut epithelium proteins in the
blood (e.g., enzyme diaminodioxidase); or detection of acute-phase proteins (e.g., C-reactive protein, ovotransferrin, alpha-1 antitrypsin) in blood or excreta produced in response to inflammation or as a result of translocation of bacteria to the liver; 2) Microbial metabolites (e.g., D-lactate) and macromolecules (e.g., fatty acid-binding protein) in blood as evidence of gut barrier failure, or the profile of microbiota metabolites in the cecum (e.g., fatty acids or lactic acid); 3) Biomarkers related to microbiota patterns.

This section will focus on the third category, while biomarkers from categories 1 and 2 above that have been used in poultry are summarized in Table 1 and recent reviews by Ducatelle et al. (2018) and Celi et al. (2017). Among those biomarkers, reference values indicative of gut health are available for microscopic examination of villus height and depth and villus/crypt ratio in broilers at d 23 (de Verdal et al., 2010); while reference values for other biomarkers have not been established.

**Biomarkers Related to the Microbiota Patterns**

In poultry, microbial shifts have been mainly studied in NE models (Antonissen et al., 2016). Some of the important microbial signatures of dysbiosis identified in both humans and poultry are described herein.

Depleted level of family Ruminococcaceae and Lachnospiraceae, mainly butyrate-producing Clostridiales, has been reported in stool samples of humans with Crohn’s disease (Duvallet et al., 2017). The same family was seen to be depleted in experimental chickens following the feeding of fish meal and Eimeria inoculation (Wu et al., 2014). Most of the bacterial species under the family Ruminococcaceae and Lachnospiraceae are butyrate producers that activate glucagon-like peptide 2 (GLP-2) secretion which maintains intestinal epithelial cells integrity. Butyrate supplementation in feed has been shown to decrease feed efficiency and aid in the protection against NE in challenged broilers (Eeckhaut et al., 2016; Keerqin et al., 2021).

While the abundance of the phylum *Proteobacteria* has been regarded as a signature of dysbiosis and disease in humans (Shin et al., 2015). Many opportunistic bacteria are under the phylum *Proteobacteria* such as *Escherichia*, *Salmonella*, *Campylobacter*, and *Proteus*. Therefore, an increase in the abundance of *Proteobacteria* could be a useful indicator of dysbiosis. More specifically family *Enterobacteriaceae* was found to increase in intestinal epithelial dysfunction as a result of noncommunicable disease (cardiac disease, colorectal cancer, metabolic diseases) and diarrhea in humans, and the same family was found to be depleted in the ileal contents of birds with NE induced by supplementation of *Salmonella Typhimurium*, *E. maxima* and *C. perfringens* (Hernandez-Patlan et al., 2019b).

While a clear shift in microbiota has been shown in clinical NE and coccidiosis, it is often difficult to detect changes in subtle gut health conditions (Shin et al., 2015; Duvallet et al., 2017; Ducatelle et al., 2018). In poultry research, several attempts have been made to define the microbiota associated with gut health and productivity but the microbiota consistently associated with high or low-performing flocks has not been identified (see Section 4). Considerable bird to bird and flock to flock variations in microbiota resulted in the lack of reproducibility of bacterial profiles related to production across trials (Stanley et al., 2013b; Stanley et al., 2016). Although a sequence-based approach has been useful to characterize the GIT microbiota, a more comprehensive analysis of microbial composition combined with the microbial and host metabolites (metabolomics) using liquid chromatography and mass spectroscopy would be more insightful on defining host-gut microbiome interactions (Aggrey et al., 2019; Kers et al., 2019b; Cao et al., 2020; Gonzalez-Uarquin et al., 2020; Niu et al., 2020; Park et al., 2020; Slizewska et al., 2020; Wang et al., 2021; Zhang et al., 2021). Although metabolomics offers great promise for biomarker discovery and mechanistic investigation on in-feed additives, they are time-consuming and expensive which limits their application in commercial settings.

**The Use of Noninvasive Sampling as a Proxy for Gut Microbiota**

In human gut microbiota research, stools are considered a reference sample type despite their limitations compared to the detection of bacteria in the intestinal mucosa (Tang et al., 2020). In poultry, the majority of studies are conducted using cecum microbiota although some of the research has been performed to identify
### Table 1. Potential biomarkers for monitoring gut health in poultry.

| Biomarkers                                                                 | Biological samples for examination | Tools used to measure biomarker | Pros                                                                 | Cons                                                                 | References                                                                 |
|---------------------------------------------------------------------------|-----------------------------------|-------------------------------|----------------------------------------------------------------------|----------------------------------------------------------------------|----------------------------------------------------------------------------|
| **Microscopic examination of the intestine (measurement of villus length/crypt depth ratio)** | Direct microscopic evaluation of intestinal wall | Histology                      | The gold standard method to evaluate intestine health. Reference values are available. | Requires skilled personnel to collect samples and a specialized laboratory for sample processing. For diagnostic purposes, additional tests are required increasing costs. | (de Verdal et al., 2010; Chen et al., 2015; Ducatelle et al., 2018) |
| **Intestinal inflammation and gut wall appearance**                        | Direct macroscopic evaluation of intestine | Gross evaluation of tissues    | Direct measurement of gut appearance, scores are available to determine the extent of intestinal inflammation for necrotic enteritis, coccidiosis, and dysbiosis | It is subjective, requires a skilled veterinarian for lesion scoring and terminal sampling of birds | (Johnson and Reid, 1970; Keyburn et al., 2006; De Gussem, 2010). |
| **Acute-phase proteins**                                                   |                                    |                               |                                                                      |                                                                      |                                                                           |
| Ovotransferrin                                                             | Blood and excreta                  | Immunoassay                    | Cost-effective, no need to sacrifice chickens for sample collection | The biomarker is susceptible to proteolysis and samples should be tested from fresh excreta. Has only been reported to be increased in experimental meat chickens in a necrotic enteritis model, *E. maxima*, *E. tenella*, and *E. coli* infection. | (Rath et al., 2009; Goossens et al., 2018) |
| **Host protein biomarkers**                                                |                                    |                               |                                                                      |                                                                      |                                                                           |
| Citrulline                                                                 | Blood                              | Immunoassay                    | Cost-effective, no need to sacrifice chickens for sample collection | Decreased level of plasma citrulline was correlated with reduced enterocyte mass in chickens fed a rye-based diet compared to chickens fed a corn-based diet after 20 d. Need to be tested in other conditions of intestinal disturbance. | (Baxter et al., 2019) |
| Fibronecctin                                                               | Blood and excreta                  | Immunoassay                    | Cost-effective, no need to sacrifice chickens, and has the potential to be used as a marker of intestinal inflammation in poultry | Increased levels on gut leakage model induced by administration of dexmethasone and *E. maxima* and *E. acervulina*. Need to be tested in other conditions of intestinal disturbance. | (Owen et al., 2008; De Meyer et al., 2019; Barekatain et al., 2020) |
| Diaminoxidase                                                              | Blood                              | Immunoassay                    | Cost-effective, no need to sacrifice chickens for sample collection | Only studied in laying hens as a marker of gut barrier failure after feed withdrawal for 12 h. Not reported in broilers, needs to be tested in other conditions of intestinal disturbance. | (Lei et al., 2013) |
| Fatty acid-binding protein                                                 | Blood and excreta                  | Immunoassay, PCR               | Cost-effective, no need to sacrifice chickens for sample collection | Due to the higher molecular weight of fatty acid-binding protein (15000 Da), severe damage of intestinal epithelium is required for its passage from the intestine to the blood. In excreta, one study showed no difference between gut barrier damage induced by dexmethasone using ELISA. However, in another study, the DNA of this biomarker was increased in blood after birds were administered twice the dose of a coccidiosis vaccine containing a mixture of *E. acervulina*, *E. maxima*, and *E. tenella*. Needs further investigation. | (Iizuka and Konno, 2011; Chen et al., 2015; Barekatain et al., 2020) |

*(continued)*
### Table 1 (Continued)

| Biomarkers                                                                 | Biological samples for examination | Tools used to measure biomarker                                                                 | Pros                                                                                                           | Cons                                                                                                                                                                                                 | References                                                                 |
|----------------------------------------------------------------------------|----------------------------------|------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| Myeloid protein-1, Alpha-actin-4, Apolipoprotein A-1, Hemoglobin subunit beta, Nucrophiomnin, Ovoinhibitor, Transthyretin     | Colon contents                   | PCR                                                                                          | Cost-effective, colon contents can be collected without sacrificing chickens                                   | Only tested in gut leakage model induced by coccidia administration (E. maxima and E. acervulina). Needs testing in other conditions of intestinal disturbance                                                                 | (De Meyer et al., 2019)                                                                                                               |
| Metabolome profiling                                                       | Blood                            | Ultra-high performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS)            | No need to sacrifice chickens for sample collection. Characterizes biochemical and metabolic changes in the host, can be used for biomarker discovery and characterization of metabolites and metabolic changes of the host and microbiota-associated products in broilers after inoculation with Eimeria spp., eating restriction patterns, and supplementation with enzymes, antibiotics, prebiotics, and probiotics. | Expensive, time-consuming, requires specialized equipment and intensive bioinformatics                                                                                                            | (Aggrey et al., 2019; Cao et al., 2020; Gonzalez-Uarquin et al., 2020; Wang et al., 2021; Zhang et al., 2021) |
| Microbial shift or detection of bacterial metabolites as biomarkers        | Gut contents and excreta         | Sequencing based approach/PCR                                                                | Identification of microbial shifts could be used for testing of management interventions or occurrence of antibiotic resistance genes | Expensive, time-consuming, and difficult to find consistent microbial taxa associated with health and disease due to extensive bird to bird variation                                                                 | (Ducatelle et al., 2018; Hernandez-Patlan et al., 2019a)                                                                 |
| Bacterial metabolite ‘D-lactate’ in blood                                  | Blood                            | Immunoassay                                                                                  | No need to sacrifice chickens for sample collection                                                       | Only studied in laying hens after feed restriction for 12 h. Needs testing in meat chickens and other conditions of intestinal disturbance.                                                                                         | (Lei et al., 2013; Gilani et al., 2016)                                                                                                           |
| Bacterial count in liver                                                  | Liver                            | Culture                                                                                      | A more accurate approach to evaluate translocation of bacteria as a result of gut barrier failure            | Need to sacrifice birds, time-consuming, and expensive. Has been reported to be increased in birds fed with the rye-based diet compared to the corn-based diet, not studied in intestinal disease models.                                      | (Tellez et al., 2014; Ducatelle et al., 2018)                                                                                           |
| Genes encoding bacterial enzymes in the butyrate production pathway       | Excreta and gut contents         | PCR                                                                                          | Cost-effective, no need to sacrifice chickens for sample collection                                       | Has not been studied in leaky gut models but reported to be increased amounts in improved feed conversion efficient birds fed with xylooligosaccharides and wheat-rye based diet compared to only wheat-rye based diet.                     | (De Maesschalck et al., 2015; Ducatelle et al., 2019)                                                                                   |
| Endotoxin production by gram-negative bacteria                            | Blood                            | PCR                                                                                          | Cost-effective, no need to sacrifice chickens for sample collection                                       | Has only been reported to increase in chickens administered twice the recommended dose of coccidiosis vaccine containing a mixture of E. acervulina, E. maxima, and E. tenella | (Chen et al., 2015)                                                                                                                      |
| Fatty acids and lactic acid profiles, other bacterial metabolites          | Gut contents (cecum)             | High-performance liquid chromatography (HPLC). Liquid chromatography-tandem mass spectroscopy (LC-MS/MS) | Monitor real-time, dynamic changes in bacterial metabolites, providing direct analysis of bacterial metabolic activity. | It is expensive and requires specialized equipment. If LC-MS/MS is used intensive bioinformatics is needed. Requires the sacrifice of chickens for sample collection, maintenance of cold chain after sample collection, and rapid processing of samples. | (Kers et al., 2019b; Niu et al., 2020; Park et al., 2020; Slizewska et al., 2020)                                                         |
| Others                                                                    | Fluorescein isothiocyanate–dextran (FITC-d) | Blood                                                                                        | Measures epithelial permeability                                                                         | Limited to research settings since the compounds need to be gavaged and monitored in blood as a tool to determine gut barrier failure                                                                 | (Niewold, 2014; Immerseel, 2019; Barekatain et al., 2020; Vuong et al., 2021)                                                       |
suitable alternatives. The nonterminal sampling method most commonly used is excreta or cloacal swabs (Stanley et al., 2014). However, the microbiota of excreta varies at different time points due to the periodic emptying of different GIT sections (Sekelja et al., 2012). Although excreta has been shown to contain microbial taxa present in the cecum, there are quantitative differences in the microbial composition between sample types (Stanley et al., 2015). Similarly, microbial communities in excreta are more similar to litter compared to cecum or ileum (Johnson et al., 2018). Therefore, using excreta as a proxy of gut contents would likely miss some important core gut microbial taxa (Stanley et al., 2014; Videvall et al., 2018).

Cecal droppings and boot sock samples have been suggested as suitable alternatives to cecal contents for studying longitudinal microbiota composition (Pauwels et al., 2015; Kers et al., 2018). However, the collection of cecal droppings is difficult as chickens produce one cecal dropping after the production of 7-8 excreta droppings (Williams, 1995), and the use of boot sock sampling needs to be further evaluated (Kers et al., 2019a).

A potential noninvasive sample is poultry house dust. Previous research has shown that the poultry dust is composed mostly of dried excreta, and to a lesser extent from feed and feather dander (Ahaduzzaman et al., 2021b), and nucleic acid of viruses, bacteria, and protozoa can be readily detected in the dust of commercial chicken flocks (Walkden-Brown et al., 2013; Ahaduzzaman et al., 2020; Assen et al., 2020; Bindari et al., 2021a). More recently the use of poultry dust has been proposed as an alternative sample type to study microbiota at flock-level but further investigation is needed (Bindari et al., 2021b, c). Combining a sequencing approach with quantitative PCR for absolute measurements of microbial communities would assist in more accurately analyzing changes in microbial taxa and further investigating the microbiota function (Barlow et al., 2020; Jian et al., 2020). This approach may be more suitable for use on routine monitoring of commercial broiler farms.

CONCLUSIONS

Phasing out of in-feed antibiotics in many countries has been associated with the re-emergence of enteric diseases such as coccidiosis, necrotic enteritis, and dysbiosis. The establishment of practical tools to monitor gut health has the potential to provide timely information which would help to identify, implement, and evaluate targeted interventions quickly. Monitoring of the chicken gut microbiota has been the most widely used research tool to infer gut health; however, microbial signatures related to production remain elusive despite great research efforts in this area. Many host and environmental factors have been shown to influence the microbial community structure, including nutritional and husbandry management, chicken breed, and age, in addition to various methodological factors such as sample storage and data analysis. Microbiota studies must provide details on these variables for accurate interpretation of the results. Efforts should be made by the research community to standardize the methodology used in chicken microbiota studies to allow for a direct comparison of data sets among studies, which ideally should include functional analysis of the microbiota.

The use of noninvasive and easy-to-collect population-level samples, such as boot socks and poultry dust, do not require terminal sampling and offer promise to provide flock-level metrics on microbial composition and burden of enteric pathogens using a reduced number of samples. This would therefore reduce costs and potentially facilitate on-farm studies which are crucial to fully assess management interventions including testing of in-feed additives as the outcomes observed in experimental studies under controlled conditions do not always translate to findings in commercial settings. Further research on the usefulness of population-level sampling approaches to monitor microbiota composition and their association with flock production is warranted.

ACKNOWLEDGMENTS

We thank PoultryHub Australia for kindly providing the chicken digestive tract schematic figure used in this review.

DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

Abundo, M. E., J. M. Ngunjiri, K. J. Taylor, H. Ji, A. Ghorbani, M. KC, B. P. Weber, T. J. Johnson, and C.-W. Lee. 2021. Assessment of two DNA extraction kits for profiling poultry respiratory microbiota from multiple sample types. PLoS One 16:e0241732.

Adhikari, B. 2019. Investigation of microbiota in health and disease of poultry. University of Arkansas, Fayetteville. Thesis and Dissertations. 3371.

Ayd, S., and S. Magray. 2012. Impact and manipulation of gut microflora in poultry: a review. J. Anim. Vet. Adv. 11:873–877.

Aggrey, S. E., M. C. Milfort, A. L. Fuller, J. Yuan, and R. Rekaya. 2019. Effect of host genotype and Eimeria acervulina infection on the metabolome of meat-type chickens. PLoS One 14:e0223417.

Ahaduzzaman, M., C. Keerqin, A. Kumar, S. Musigwa, N. Morgan, S. K. Kheravii, S. Sharpe, S. Williamson, S.-B. Wu, and S. W. Walkden-Brown. 2021a. Detection and quantification of Clostridium perfringens and Eimeria spp. in poultry dust using real-time PCR under experimental and field conditions. Avian Dis. 65:77–85.

Ahaduzzaman, M., L. Milan, C. L. Morton, P. F. Gerber, and S. W. Walkden-Brown. 2021b. Characterization of poultry house dust using chemometrics and scanning electron microscopy imaging. Poult. Sci. 100:101188.

Ahaduzzaman, M., S. W. Walkden-Brown, P. F. Gerber, C. Keerqin, A. Kumar, S. Musigwa, N. Morgan, S. K. Kheravii, S. Sharpe, and S. Williamson. 2020. Detection and quantification of Clostridium perfringens and Eimeria spp. in poultry dust using real-time PCR under experimental and field conditions. Avian Dis. 65:77–85.

Ahn, J.-H., B.-Y. Kim, J. Song, and H.-Y. Weon. 2012. Effects of PCR cycle number and DNA polymerase type on the 16S rRNA
performance are related to cecal microbiota in chicken. Microb. Pathog. 150:104710.
Dahiy, J., D. Hoehler, A. G. Van Kessel, and M. D. Drew. 2007. Dietary encapsulated glycine influences Clostridium perfringens and Lactobacilli growth in the gastrointestinal tract of broiler chickens. J. Nutr. 137:1408–1414.
Dahiy, J., D. Wilkie, A. Van Kessel, and M. Drew. 2006. Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Anim. Feed Sci. Technol. 129:60–88.
Danzeisen, J. L., H. B. Kim, R. E. Isaacon, Z. J. Tu, and T. J. Johnson. 2011. Modulations of the chicken cecal microbiome and metagenome in response to anticoccidial and growth promoter treatment. PLoS One 6:e27949.
De Gussem, M. 2007. Coccioidosis in poultry: review on diagnosis, control, prevention and interaction with overall gut health. Proceedings of the 16th European Symposium on Poultry Nutrition (pp. 253–261) 253–261.
De Gussem, M. 2010. Macroscopic scoring system for bacterial enteritids in broiler chickens and turkeys. World Veterinary Poultry Association Meeting.
De Maesschalck, C., V. Eeckhaut, L. Maertens, L. De Lange, L. Marchal, C. Nezer, S. De Baere, S. Croubels, G. Daube, and J. Dewulf. 2015. Effects of xyla-oligosaccharides on broiler chicken performance and microbiota. Appl. Environ. Microbiol. 81:5880–5888.
De Meyer, F., V. Eeckhaut, R. Ducatelle, M. Dhaenens, S. Daled, A. Dedruwerdaer, M. De Gussem, F. Haesebrouck, D. Deforce, and F. Van Immerseel. 2019. Host intestinal biomarker identification in a gut leakage model in broilers. Vet. Res. 50:1–14.
De Verdral, H., S. Mignon-Grasteau, C. Jeulin, E. Le Bhâan-Duval, M. Leconte, S. Mallet, C. Martin, and A. Narcy. 2010. Digestive tract measurements and histological adaptation in broiler lines divergently selected for digestive efficiency. Poult. Sci. 89:1955–1961.
Denbow, D. M. 2015. Gastrointestinal anatomy and physiology. Sturkie's Avian Physiology. Elsevier, New York, NY, 337–366.
Densch, S., B. Tilicca, A. Camarini-Silva, and J. Seifert. 2015. News in livestock research—use of Omics-technologies to study the microbiota in the gastrointestinal tract of farm animals. Comput. Struct. Biotechnol. J. 13:55–63.
Díaz Carrasco, J. M., N. A. Casanova, and D. W. Polson, D. Ritter, J. Ravel, J. Gelb Jr, G. E. Duke, G. E. 1989. Relationship of cecal and colonic motility to diet, habitat, and cecal anatomy in several avian species. J. Exp. Zool. 252:38–47.
Duve, T. S., D. W. Polson, D. Ritter, J. Ravel, J. Gelb Jr, R. Morgan, and K. E. Wommack. 2011. Impacts of poultry house environment on poultry litter bacterial community composition. PLoS One 6:e24758.
Duvall, C., S. M. Gibbons, T. Gurry, R. A. Irizarry, and E. J. Alm. 2017. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. Nat. Commun. 8:1–10.
Eeckhaut, V., J. Wang, A. Van Parys, F. Haesebrouck, M. Joossens, G. Falony, J. Raes, R. Ducatelle, and F. Van Immerseel. 2016. The probiotic Butyricicoccus ureibacterium reduces feed conversion and protects from potentially harmful intestinal microorganisms and necrotic enteritis in broilers. Front. Microbiol. 7:1416.
Engberg, R., M. Hedemann, and B. Jensen. 2002. The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. Br. Poult. Sci. 43:569–579.
Engberg, R., M. Hedemann, T. Leser, and B. Jensen. 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poult. Sci. 79:1311–1319.
Engberg, R., M. Hedemann, S. Steinfeldt, and B. Jensen. 2004. Influence of whole wheat and xylan on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83:925–938.
Falony, G., M. Joossens, S. Vieira-Silva, J. Wang, Y. Darzi, K. Faust, A. Kurilshikov, M. J. Bonder, M. Valles-Colomer, and D. Vandeputte. 2016. Population-level analysis of gut microbiome variation. Science 352:560–564.
Feng, Y., J. Gong, H. Yu, Y. Jin, J. Zhu, and Y. Han. 2010. Identification of changes in the composition of ileal bacterial microbiota of broiler chickens infected with Clostridium perfringens. Vet. Microbiol. 140:116–121.
Feye, K., M. Baxter, G. Tellez-Iasais, M. Kogut, and S. Rieke. 2020. Influential factors on the composition of the conventionally raised broiler gastrointestinal microorganisms. Poult. Sci. 99:653–659.
Fidler, G., E. Tohnai, A. Stagel, J. Remenyik, L. Stundl, F. Gal, S. Biro, and M. Paholcsek. 2020. Tendentious effects of automated and manual metagenomic DNA purification protocols on broiler gut microbiome taxonomic profiling. Sci. Rep. 10:1–16.
Fonky, F., A. G. Clooney, C. Stanton, M. J. Claesson, and P. D. Cotter. 2016. 16S rRNA gene sequencing of mock microbial populations-impact of DNA extraction method, primer choice and sequencing platform. BMC Microbiol. 16:1–13.
Fukus, G., M. Elgart, A. Amir, A. Zeisel, P. J. Turnbaugh, Y. Soen, and N. Shental. 2018. Combining 16S rRNA gene variable regions enables high-resolution microbial community profiling. Microbiome 6:1–13.
Fuller, R., and D. Brooker. 1974. Lactobacilli which attach to the crop epithelium of the fowl. Am. J. Clin. Nutr. 27:1305–1312.
Furuse, M., and H. Yokota. 1985. Effect of the gut microflora on chick growth and utilisation of protein and energy at different concentrations of dietary protein. Br. Poult. Sci. 26:97–104.
Gaskins, H., C. Collier, and D. Anderson. 2002. Antibiotics as growth promotants: mode of action. Anim. Biotechnol. 13:29–42.
Gilanli, S., G. Howarth, S. Kitessa, C. Tran, R. Fordier, and R. Hughes. 2017. New biomarkers for increased intestinal permeability induced by dextran sodium sulphate and fasting in chickens. J. Anim. Physiol. Anim. Nutr. 101:e237–e245.
Gilanli, S., G. S. Howarth, S. M. Kitessa, R. E. Fordier, C. D. Tran, and R. J. Hughes. 2016. New biomarkers for intestinal permeability induced by lipopolysaccharide in chickens. Anim. Prod. Sci. 56:1984–1997.
Godwin, R. M., and J. A. Morgan. 2015. A molecular survey of Eimeria in chickens across Australia. Vet. Parasitol. 214:16–21.
Gong, J., R. J. Forster, H. Yu, J. R. Chambers, P. M. Sabour, R. Wheatcroft, and S. Chen. 2002. Diversity and phylogenetic analysis of bacteria in the mucosa of chicken cecum and comparison with bacteria in the mouse cecum. Microb. Ecol. Lett. 208:1–7.
Gong, J., W. S. Li, R. J. Forster, R. Huang, H. Yu, Y. Yin, C. Yang, and Y. Han. 2007. 16S rRNA gene-based analysis of mucosa-associated bacterial community and phylogeny in the chicken gastrointestinal tracts: from crops to ceca. FEMS Microbiol. Ecol. 59:147–157.
Gong, J., H. Yu, T. Liu, J. Gill, J. Chambers, R. Wheatcroft, and P. Sabour. 2008. Effects of zinc bacitracin, bird age and access to range on bacterial microbiota in the ileum and ceca of broiler chickens. J. Appl. Microbiol. 104:1372–1382.
Gonzalez-Urzua-Fru, F., A. Remon, M. Rodehutsdord, and K. Huber. 2020. Dietary phytate and myo-inositol supplementation are associated with distinct plasma metabolome profile in broiler chickens. Animal 14:549–559.
Goossens, E., G. Debyser, C. Callens, M. De Gussem, A. Dedruwerdaer, B. Decreuve, F. Haesebrouck, M. Flitgel, S. Pelzer, and F. Thiennmann. 2018. Elevated faecal ovotransferrin concentrations are indicative for intestinal barrier failure in broiler chickens. Vet. Res. 49:1–8.
strong correlations with performance. Appl. Environ. Microbiol. 84:e00362-18.
Kadooka, Y., M. Sato, K. Imazumi, A. Ogawa, K. Ikuyama, Y. Akai, M. Okano, M. Kagoshima, and T. Tsuchida. 2010. Regulation of abdominal adiposity by probiotics (Lactobacillus gasseri SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur. J. Clin. Nutr. 64:636–643.
Kassem, I. L., Y. Sanad, D. Gaugain, M. Liburn, J. Lejeune, and G. Rasjahakara. 2010. Use of bioluminescence imaging to monitor Campylobacter survival in chicken litter. J. Appl. Microbiol. 109:1988–1997.
Kecören, C., L. Rhatay, Z.-H. Zhang, K. Gharib-Naseri, S. Kheravii, E. Devillard, T. Crowley, and S.-B. Wu. 2021. Probiotic Bacillus subtilis 29.784 improved weight gain and enhanced gut health status of broilers under necrotic enteritis condition. Poult. Sci. 100:109981.
Kers, J. G., J. E. de Oliveira, E. A. Fischer, M. H. Tersteeg-Zijdeveld, P. Konstanti, J. A. Stegeman, H. Smidt, and F. C. Velkers. 2020. Associations between phenotypic characteristics and clinical parameters of broilers and intestinal microbial development throughout a production cycle: a field study. Microbiol. Open 9:e1114.
Kers, J. G., E. A. Fischer, J. A. Stegeman, H. Smidt, and F. C. Velkers. 2019a. Comparison of different invasive and non-invasive methods to characterize intestinal microbiota throughout a production cycle of broiler chickens. Microorganisms 7:431.
Kers, J. G., F. C. Velkers, E. A. Fischer, G. D. Hermes, J. A. Stegeman, and H. Smidt. 2018. Host and environmental factors affecting the intestinal microbiota in chickens. Microbiol. 9:235.
Kers, J. G., F. C. Velkers, E. A. J. Fischer, G. D. A. Hermes, D. M. Lamot, J. A. Stegeman, and H. Smidt. 2019b. Take care of the environment: housing conditions affect the interplay of nutritional interventions and intestinal microbiota in broiler chickens. Anim. Microbiome 1:10.
Keyburn, A. L., S. A. Sheedy, M. E. Ford, M. M. Williamson, M. M. Awad, J. I. Rood, and R. J. Moore. 2006. Alpha-toxin of Clostridium perfringens is not an essential virulence factor in necrotic enteritis in chickens. Infect. Immun. 74:6496–6500.
Kipper, M., I. Andretta, C. R. Lehnen, P. A. Lovatto, and S. G. Monteiro. 2013. Meta-analysis of the performance variation in broilers experimentally challenged by Eimeria spp. Vet. Parasitol. 196:77–84.
Kizzerwetter-Swida, M., and M. Binek. 2008. Bacterial microflora of the chicken embryos and newly hatched chicken. J. Anim. Feed Sci. 17:224–232.
Kley, M.-V., E. Oviedo-Rondón, S. Dowd, M. Hume, and A. Nalian. 2012. Effect of Eimeria infection on cecal microbiome of chicken embryos and gut flora of newly hatched chicken. J. Anim. Feed Sci. 17:224–232.
Kogut, M. H., A. Lee, and E. Santin. 2020. Microbiome and pathogen interaction with the immune system. Poult. Sci. 99:1906–1913.
Kondo, F. 1988. In vitro lecithinase activity and sensitivity to 22 anti-coccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasitol. 28:30–36.
Kubasova, T., M. Kollarcikova, M. Cghanova, D. Karasova, D. Cejkova, A. Sekhova, J. Matiasiovcová, M. Faldynova, A. Pokorna, A. Cizek, and I. Rychlik. 2019. Contact with adult haematobia affects development of caecal microbiota in newly hatched chicks. PLoS One 14:e021246.
Kadooka, Y., M. Sato, K. Imazumi, A. Ogawa, K. Ikuyama, Y. Akai, M. Okano, M. Kagoshima, and T. Tsuchida. 2010. Regulation of abdominal adiposity by probiotics (Lactobacillus gasseri SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur. J. Clin. Nutr. 64:636–643.
Kassem, I. L., Y. Sanad, D. Gaugain, M. Liburn, J. Lejeune, and G. Rasjahakara. 2010. Use of bioluminescence imaging to monitor Campylobacter survival in chicken litter. J. Appl. Microbiol. 109:1988–1997.
Kecören, C., L. Rhatay, Z.-H. Zhang, K. Gharib-Naseri, S. Kheravii, E. Devillard, T. Crowley, and S.-B. Wu. 2021. Probiotic Bacillus subtilis 29.784 improved weight gain and enhanced gut health status of broilers under necrotic enteritis condition. Poult. Sci. 100:109981.
Kers, J. G., J. E. de Oliveira, E. A. Fischer, M. H. Tersteeg-Zijdeveld, P. Konstanti, J. A. Stegeman, H. Smidt, and F. C. Velkers. 2020. Associations between phenotypic characteristics and clinical parameters of broilers and intestinal microbial development throughout a production cycle: a field study. Microbiol. Open 9:e1114.
Kers, J. G., E. A. Fischer, J. A. Stegeman, H. Smidt, and F. C. Velkers. 2019a. Comparison of different invasive and non-invasive methods to characterize intestinal microbiota throughout a production cycle of broiler chickens. Microorganisms 7:431.
Kers, J. G., F. C. Velkers, E. A. Fischer, G. D. Hermes, J. A. Stegeman, and H. Smidt. 2018. Host and environmental factors affecting the intestinal microbiota in chickens. Microbiol. 9:235.
Kers, J. G., F. C. Velkers, E. A. J. Fischer, G. D. A. Hermes, D. M. Lamot, J. A. Stegeman, and H. Smidt. 2019b. Take care of the environment: housing conditions affect the interplay of nutritional interventions and intestinal microbiota in broiler chickens. Anim. Microbiome 1:10.
Keyburn, A. L., S. A. Sheedy, M. E. Ford, M. M. Williamson, M. M. Awad, J. I. Rood, and R. J. Moore. 2006. Alpha-toxin of Clostridium perfringens is not an essential virulence factor in necrotic enteritis in chickens. Infect. Immun. 74:6496–6500.
Kipper, M., I. Andretta, C. R. Lehnen, P. A. Lovatto, and S. G. Monteiro. 2013. Meta-analysis of the performance variation in broilers experimentally challenged by Eimeria spp. Vet. Parasitol. 196:77–84.
Kizzerwetter-Swida, M., and M. Binek. 2008. Bacterial microflora of the chicken embryos and newly hatched chicken. J. Anim. Feed Sci. 17:224–232.
Kley, M.-V., E. Oviedo-Rondón, S. Dowd, M. Hume, and A. Nalian. 2012. Effect of Eimeria infection on cecal microbiome of chicken embryos and gut flora of newly hatched chicken. J. Anim. Feed Sci. 17:224–232.
Kogut, M. H., A. Lee, and E. Santin. 2020. Microbiome and pathogen interaction with the immune system. Poult. Sci. 99:1906–1913.
Kondo, F. 1988. In vitro lecithinase activity and sensitivity to 22 anti-coccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasitol. 28:30–36.
Kubasova, T., M. Kollarcikova, M. Cghanova, D. Karasova, D. Cejkova, A. Sekhova, J. Matiasiovcová, M. Faldynova, A. Pokorna, A. Cizek, and I. Rychlik. 2019. Contact with adult haematobia affects development of caecal microbiota in newly hatched chicks. PLoS One 14:e021246.
Lei, K., Y. Li, D. Yu, I. Rajput, and W. Li. 2013. Influence of dietary inclusion of Bacillus licheniformis on laying performance, egg quality, antioxidant enzyme activities, and intestinal barrier function of laying hens. Poult. Sci. 92:2389–2395.

Lim, S., S. Cho, K. Caetano-Anolles, S. G. Jeong, M. H. Oh, B. Y. Park, H. J. Kim, S. Cho, S. H. Choi, and S. Ryu. 2015. Developmental dynamic analysis of the excreted microbiome of chickens using next-generation sequencing. J. Mol. Microbiol. Biotechnol. 25:262–268.

Loh, G., and M. Blaut. 2012. Role of commensal gut bacteria in inflammatory bowel diseases. Gut Microbes 3:544–555.

Lopez-Siles, M., S. H. Duncan, L. J. Garcia-Gil, and M. Martinez-Medina. 2017. Faecalibacterium prausnitzii: from microbiology to diagnostics and prognostics. ISME J. 11:841–852.

Lovland, A., and M. Kählhusdal. 2001. Severely impaired production performance in broiler flocks with high incidence of Clostridium perfringens-associated hepatitis. Avian Pathol. 30:73–81.

Lu, J., U. Idris, B. Harmon, C. Hofacre, J. J. Maurer, and M. D. Lee. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 69:6816–6824.

Lu, Y., P. Hugenholtz, and D. J. Batstone. 2015. Evaluating DNA extraction methods for community profiling of pig hindgut microbial community. PLoS One 10:e0142720.

Lumpkins, B., A. Batal, and M. Lee. 2008. The effect of gender on the bacterial community in the gastrointestinal tract of broilers. Poult. Sci. 87:964–967.

M’Saadeq, S. A., S. Wu, R. A. Swick, and M. Choct. 2015. Towards the metabolic cross-talk. Appl. Microbiol. Biotechnol. 104:4345–4357.

Oakley, B. B., B. S. Lillehoj, M. H. Kogut, W. K. Kim, J. J. Maurer, A. Pedrosa, M. D. Lee, S. R. Collett, T. J. Johnson, and N. A. Cox. 2014. The chicken gastrointestinal microbiome. FEMS Microbiol. Lett. 360:100–112.

Oakley, B. B., C. A. Morales, J. Line, M. E. Berrang, R. J. Meinersmann, G. E. Tillman, M. G. Wise, G. R. Siragusa, K. L. Hiett, and B. S. Seal. 2013. The poultry-associated microbiome: network analysis and farm-to-fork characterizations. PLoS One 8:e57190.

Ohi, S.-J. 2011. Meta analysis to draw the appropriate regimen of probiotics and probiotic supplementation to pigs and chicken diets. Asian-Austral. J. Anim. Sci. 24:573–586.

Owen, H. C., S. J. Roberts, S. F. Ahmed, and C. Farquharson. 2008. Dexamethasone-induced expression of the glucocorticoid response gene lipocalin 2 in chondrocytes. Am. J. Physiol. Endocrinol. Metab. 294:E1023–E1034.

Paiva, D., and A. McElroy. 2014. Necrotic enteritis: applications for the poultry industry. J. Appl. Poult. Res. 23:557–566.

Pan, D., and Z. Yu. 2014. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes 5:108–119.

Pandit, R. J., A. T. Hinsu, N. V. Patel, P. G. Korina, S. J. Jakesara, J. R. Thakker, T. M. Shah, G. Limon, A. Pefidji, J. Guittain, D. A. Hume, F. M. Tomley, D. N. Rank, M. Raman, K. G. Tirumurugan, D. P. Blake, and C. G. Joshi. 2018. Microbial diversity and community composition of caecal microbiota in commercial and indigenous Indian chickens determined using 16s rDNA amplicon sequencing. Microbiome 6:115.

Park, I., N. P. Zimmerman, A. H. Smith, T. G. Rehberger, E. P. Lillehoj, and H. S. Lillehoj. 2020. Dietary supplementation with Bacillus subtilis direct-fed microbials alters chicken intestinal metabolite levels. Front. Vet. Sci. 7:123.

Patterson, A. M., I. E. Mulder, A. J. Travis, A. Lan, N. Cerf-Bensussan, V. Gaboriu-Routhiau, K. Garden, E. Logan, M. I. Delday, and A. G. Coutts. 2017. Human gut symbiont Roseburia hominis promotes and regulates innate immunity. Front. Immunol. 8:1166.

Pauwels, J., B. Taminiau, G. Jaansens, M. De Beenhouwer, L. Delhalle, G. Danhe, and F. Coopman. 2015. Cecal drop reflects the chickens’ caecal microbiome, fecal drop does not. J. Microbiol. Methods 117:164–170.

Pollock, J., L. Glendinning, T. Wisedchanwet, and M. Watson. 2018. The madness of microbiome: attempting to find consensus “best practice” for 16s microbiome studies. Appl. Environ. Microbiol. 84:e02627-17.

Qian, X.-B., T. Chen, Y.-P. Xu, L. Chen, F.-X. Sun, M.-P. Lu, and Y.-X. Liu. 2020. A guide to human microbiome research: study design, sample collection, and bioinformatics analysis. Chin. Med. J. 133:1544.

Qu, A., J. M. Brule, M. K. Wilson, B. F. Law, J. R. Theoret, L. A. Joens, M. E. Koukel, F. Angly, E. A. Dinsdale, and R. A. Edwards. 2008. Comparative metagenomics reveals host specific metavirulomes and horizontal gene transfer elements in the chicken caecum microbiome. PLoS One 3:e2945.

Ranjitkar, S., B. Lawley, G. Tannock, and R. M. Engberg. 2016. Bacterial succession in the broiler gastrointestinal tract. Appl. Environ. Microbiol. 82:2399–2410.

Rath, N., N. Anthony, L. Kannan, W. Huff, G. Chapman, G. Erf, and P. Wackel. 2009. Serum ovotransferrin as a biomarker of inflammatory diseases in chickens. Poult. Sci. 88:2069–2074.

Ravindran, V. 2013. Feed enzymes: The science, practice, and metabolic realities. J. Appl. Poult. Res. 22:628–636.

Rehman, H. U., W. Vahjen, W. A. Awad, and J. Zentek. 2007. Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. Arch. Anim. Nutr. 61:319–335.

Richards, P., J. Fothergill, M. Bernardau, and P. Wigley. 2019. Development of the caecal microbiota in three broiler breeds. Front. Vet. Sci. 6:201.

Roeber, L. F., G. Casella, O. Simell, J. Krischer, C. H. Wasserfall, D. Schatz, M. A. Atkinson, J. Neu, and E. W. Triplett. 2009. Influence of fecal sample storage on bacterial community diversity. Open Microbiol. J. 3:30.
Roll, V., M. Dai Prá, and A. Roll. 2011. Research on Salmonella in broiler litter reused for up to 14 consecutive flocks. Poult. Sci. 90:2257–2262.

Rood, J. I., A. L. Keyburn, and R. J. Moore. 2016. NetB and necrotic enteritis: the hole movable story. Avian Pathol. 45:295–301.

Saengkerdsub, S., R. C. Anderson, H. H. Wilkinson, W.-K. Kim, D. J. Nisbet, and S. C. Ricke. 2007a. Identification and quantification of methanogenic archaea in adult chicken ceca. Appl. Environ. Microbiol. 73:353–356.

Saengkerdsub, S., P. Herrren, C. Woodward, R. Anderson, D. Nisbet, and S. Ricke. 2007b. Detection of methane and quantification of methanogenic archaea in faves from young broiler chickens using real-time PCR. Lett. Appl. Microbiol. 45:629–634.

Salter, S. J., M. J. Cox, E. M. Turek, S. T. Calus, W. O. Cookson, Scupham, A., J. Jones, and I. Wesley. 2007. Comparison of DNA extraction methods for analysis of turkey cecal microbiota. J. Microbiol. Meth. 71:153–160.

Scanes, C. G., and K. Pierzchala-Koziec. 2014. Biology of the gastrointestinal tract in poultry. Avian Biol. Res. 7:193–222.

Scupham, A., J. Jones, and I. Wesley. 2007. Comparison of DNA extraction methods for analysis of turkey cecal microbiota. J. Microbiol. Meth. 71:153–160.

Saxena, S., V. Saxena, S. Tomar, D. Sapeota, and G. Gommei. 2016. Characterisation of cecum and crop microbiota of Indian indigenous chicken targeting multiple hypervariable regions within 16S rRNA gene. Br. Poult. Sci. 57:381–389.

Scanes, C. G., and K. Pierzchala-Koziec. 2014. Biology of the gastrointestinal tract in poultry. Avian Biol. Res. 7:193–222.

Sekelja, M., I. Rud, S. Knutsen, V. Denstadli, B. Westereng, T. Naes, and K. Rudi. 2012. Abrupt temporal fluctuations in the chicken fecal microflora are explained by its gastrointestinal origin. Appl. Environ. Microbiol. 78:2941–2948.

Sekirov, I., S. L. Russell, L. C. M. Antunes, and B. B. Finlay. 2010. Gut microbiota in health and disease. Physiol. Rev. 90:859–904.

Shakouri, M., P. Iji, L. L. Mikkelsen, and A. Cowieson. 2009. Intestinal function and gut microbiota of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. J. Anim. Physiol. Anim. Nutr. 93:647–658.

Shin, N.-R., T. W. Whon, and J.-W. Bae. 2015. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 33:496–503.

Shirley, M. W., A. L. Smith, and F. M. Tomley. 2005. The biology of avian Eimeria with an emphasis on their control by vaccination. Adv. Parasitol. 60:285–330.

Singh, K., T. Shah, S. Deshpande, S. Jakhesara, P. Koringa, D. Rank, Shakouri, M., P. Iji, L. L. Mikkelsen, and A. Cowieson. 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 12:1–12.

Stanley, D., M. S. Geier, S. E. Denman, and R. J. Moore. 2012. Changes in the caecal microflora of chickens following Clostridium perfringens challenge to induce necrotic enteritis. Vet. Microbiol. 159:155–162.

Stutz, M., and G. Lawton. 1984. Effects of diet and antimicrobials on growth, feed efficiency, intestinal Clostridium perfringens, and ileal weight of broiler chicks. Poult. Sci. 63:2036–2042.

Suzuki, M. T., and S. J. Giovannoni. 1996. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. Appl. Environ. Microbiol. 62:625–630.

Tang, Q., G. Jin, G. Wang, T. Liu, X. Liu, B. Wang, and H. Cao. 2020. Current sampling methods for gut microbiota: a call for more precise devices. Front. Cell. Infect. Microbiol. 10:151.

Tankson, J., J. Thaxter, and Y. Vizzier-Thaxter. 2002. Bacteria in heart and lungs of young chicks. J. Appl. Microbiol. 92:443–450.

Tedjo, D. I., D. M. Jonkers, P. H. Savelkoul, A. A. Maslee, N. van Best, M. J. Pierik, and J. Penders. 2015. The effect of sampling and storage on the fecal microbiota composition in healthy and diseased subjects. PLoS One 10:e0126085.

Telegz, G., J. D. Latorre, V. A. Kuttanappan, M. H. Kogut, A. Wolfenden, X. Hernandez-Velasco, B. M. Harris, W. G. Bottje, L. R. Bieleke, and B. F. Faulkner. 2014. Utilization of rye as energy source affects bacterial translocation, intestinal viscosity, microbiota composition, and bone mineralization in broiler chickens. Front. Genet. 5:339.

Terzich, M., M. J. Pope, T. E. Cherry, and J. Hollinger. 2000. Survey of pathogens in poultry litter in the United States. J. Appl. Poult. Res. 9:287–291.

Thompson, K., K. Burkeholder, J. Patterson, and T. Applegate. 2008. Microbial ecology shifts in the ileum of broilers during feed withdrawal and dietary manipulations. Poult. Sci. 87:1624–1632.

Tilg, H., and S. Danese. 2014. Roseburia hominis: a novel guity player in ulcerative colitis pathogenesis? Gut 63:1204–1205.

Timberlont, L., F. Haesebrouck, R. Ducatelle, and F. Van Immerseel. 2011. Necrotic enteritis in broilers: an updated review on the pathogenesis. Avian Pathol. 40:341–347.

Timberlont, L., A. Lanckriet, J. Dewulf, N. Kollet, K. Schwarzer, F. Haesebrouck, R. Ducatelle, and F. Van Immerseel. 2010. Control of Clostridium perfringens-induced necrotic enteritis in broilers by target-released butyric acid, fatty acids and essential oils. Avian Pathol. 39:117–121.

Torok, V., C. Dyson, A. McKay, and K. Ophel-Keller. 2013. Quantitative molecular assays for evaluating changes in broiler gut microbiota linked with diet and performance. Anim. Prod. Sci. 53:1260–1268.

Torok, V., R. Hughes, K. Ophel-Keller, M. Ali, and R. MacAlpine. 2009. Influence of different litter materials on cecal microbiota colonization in broiler chickens. Poult. Sci. 88:2474–2481.

Torok, V. A., R. J. Hughes, L. L. Mikkelsen, R. Perez-Maldonado, K. Balding, R. MacAlpine, N. J. Percy, and K. Ophel-Keller. 2011. Identification and characterization of potential performance-related gut microbiotas in broiler chickens across various feeding trials. Appl. Environ. Microbiol. 77:5868–5878.

Van Immerseel, F., J. I. Rood, and R. J. Moore. 2009. Rethinking our understanding of the pathogenesis of necrotic enteritis in chickens. Trends Microbiol. 17:32–36.
Van Limbergen, T., S. Sarrazin, I. Chantziaras, J. Dewulf, R. Ducatelle, I. Kyriazakis, P. McMullin, J. Méndez, J. K. Niemi, and S. Papasolomontos. 2020. Risk factors for poor health and performance in European broiler production systems. BMC Vet. Res. 16:1–13.

Vemuri, R., K. E. Sylvia, S. L. Klein, S. C. Forster, M. Plebanski, R. Eri, and K. L. Flanagan. 2019. The microgendarome revealed: sex differences in bidirectional interactions between the microbiota, hormones, immunity and disease susceptibility. Semin. Immunopathol. 41:265–275.

Vidanaarachchi, J. K., L. L. Mikkelsen, C. Constantinou, M. Choct, and P. Iji. 2013. Natural plant extracts and prebiotic compounds as alternatives to antibiotics in broiler chicken diets in a necrotic enteritis challenge model. Anim. Prod. Sci. 53:1247–1259.

Videnska, P., K. Smerkova, B. Zwinsova, V. Popovic, L. Micenkova, K. Sedlar, and E. Budinska. 2019. Stool sampling and DNA isolation kits affect DNA quality and bacterial composition following 16S rRNA gene sequencing using MiSeq Illumina platform. Sci. Rep. 9:1–14.

Vispo, C., and W. H Karasov. 1997. The interaction of avian gut microbes and their host: an elusive symbiosis. Gastrointestinal Microbiology, Springer, London, UK, 116–155.

Voeten, A. C., W. W. Braunius, F. W. Orthel, and Van Limbergen, T., S. Sarrazin, I. Chantziaras, J. Dewulf, R. Ducatelle, I. Kyriazakis, P. McMullin, J. Méndez, J. K. Niemi, and S. Papasolomontos. 2020. Risk factors for poor health and performance in European broiler production systems. BMC Vet. Res. 16:1–13.

Walkden-Brown, S. W., A. Islam, P. J. Groves, A. Rubite, S. M. Sharpe, Waldenstedt, L., K. Elwinger, A. Lunden, P. Thebo, M. Bedford, and Williams, R. 1999. A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. Int. J. Parasitol. 29:1209–1229.

Walker, A. W., J. C. Martin, P. Scott, J. Parkhill, H. J. Flint, and K. P. Scott. 2015. 16S rRNA gene-based profiling of the human infant gut microbiota is strongly influenced by sample processing and PCR primer choice. Microbiome 3:1–11.

Wang, L., M. Lilburn, and Z. Yu. 2016. Intestinal microbiota of broiler chickens as affected by litter management regimens. Front. Microbiol. 7:593.

Wei, S., M. Morrison, and Z. Yu. 2013. Bacterial census of poultry intestinal microbiome. Poult. Sci. 92:671–683.

Wen, C., W. Yan, C. Mai, Z. Duan, J. Zheng, C. Sun, and N. Yang. 2021. Joint contributions of the gut microbiota and host genetics to feed efficiency in chickens. Microbiome 9:126.

Wienen, P., D. Keuzenkamp, L.V. Lipman, F. Knape, and S. Biesterveld. 2002. Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. Microb. Ecol. 44:286–293.

Wilkie, D. C., A. G. Van Kessel, L. J. White, B. Laarveld, and M. D. Drew. 2005. Dietary amino acids affect intestinal Clostridium perfringens populations in broiler chickens. Can. J. Anim. Sci. 85:185–193.

Williams, R. 1995. Epidemiological studies of coccidiosis in the domesticated fowl (Gallus gallus): II. Physical condition and survival of Eimeria acervulina oocysts in poultry-house litter. Appl. Parasitol. 36:90–96.

Williams, R. 1999. A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. Int. J. Parasitol. 29:1209–1229.

Wu, S.-B., D. Stanley, N. Rodgers, R. A. Swick, and R. J. Moore. 2014. Two necrotic enteritis predisposing factors, dietary fishmeal and Eimeria infection, induce large changes in the caecal microbiota of broiler chickens. Vet. Microbiol. 169:188–197.

Xia, Y., J. Sun, and D.-G. Chen. 2018. Power and sample size calculations for microbiome data. Statistical Analysis of Microbiome Data With R. Springer, Singapore, 129–166.

Yadav, S., and R. Jha. 2019. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. J. Anim. Sci. Biotechnol. 10:1–11.

Yan, L., Z. Lv, S. An, K. Xing, Z. Wang, M. Lv, M. Choct, Y. Guo, and G. Zhou. 2021. Effects of rearing system and narasin on growth performance, gastrointestinal development, and gut microbiota of broilers. Poult. Sci. 100:108048.

Yegani, M., and D. Korver. 2008. Factors affecting intestinal health in poultry. Poult. Sci. 87:2052–2063.

Yeoman, C. J., N. Chia, P. Jeraldo, M. Sipos, N. D. Goldenfeld, and B. A. White. 2012. The microbiome of the chicken gastrointestinal tract. Anim. Health Res. Rev. 13:89.

Zhang, T., H. Ding, L. Chen, Y. Lin, Y. Gong, Z. Pan, G. Zhang, K. Xie, G. Dai, and J. Wang. 2021. Antibiotic-induced dysbiosis of microbiota promotes chicken lipidogenesis by altering metabolomics in the cecum. Metabolites 11:487.