

**Review Paper**

**THE ROLE OF INTESTINAL MICROBIOTA IN CHICKEN HEALTH, INTESTINAL PHYSIOLOGY AND IMMUNITY**

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**ABSTRACT**

The actual functioning of the intestinal tract of chicken and its wellbeing are key elements in determining chicken health and performance. Many complicated mechanisms are engaged in the adaptation of gastrointestinal tract functioning and wellbeing. The intestinal tract of chicken is heavily colonized with microbes, which directly interrelate with the host. The gut microbiota has been displayed to principally influence chicken well-being via several functions in relation to diet, immune response, and other physical indices. Intestinal microbiota assists by supplying nutrients from low nutritious feeds and moderating the growth of gastrointestinal tract and immune functions. In response, host delivers a lenient environment and nutrients for microbial establishment and development. Modifying the intestinal microbiota may help the host in terms of good health and proper functioning. The idea of administering favorable microorganisms to the feed has led to the advancement of feed additives especially prebiotics and probiotics. The intestinal microbiota is quickly variable by feed, antibiotics, contamination by microbes, and other host and environmental factors. The capability to deliberately influence the intestinal microbiota by supplying nutritional elements, moderating host immune system, inhibiting microbial gut establishment, or increasing gut barrier function has led to many new techniques to inhibit disease chances, however, it led to enhanced weight gain, carcass production and feed conversion ratio (FCR). Moreover, the application of genomics (next-generation sequencing platforms and sequence database) will be economical, easy to use, and capable of dealing with the nature of poultry and the food safety requirements.

**Keywords:** Intestinal microbiota, intestinal health, interaction, manipulation of intestinal microbiota, chicken.

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**INTRODUCTION**

Optimum intestinal health is of prime importance to animal performance as well as animal health. There seems to be a correlation in animal performance and a “healthy” gut of animal Liu *et al.* (2019). The basic purpose of the healthy gut is to modify physical homeostasis that delivers the host capability to resist pathogenic stimuli Guo *et al.* (2020). Intestinal health includes physical, biological, and physiological processes that collaboratively work together to retain intestinal homeostasis. By maintaining the intestinal homeostasis, a healthy intestine also controls other systems of body as well that enable the animal to resist pathogenic stimuli Celi *et al.* (2017); Bortoluzzi *et al.* (2019). The gastrointestinal (GI) tract of chicken is exposed to exogenous microbes instantly after hatch and subsequently, it turns into a suitable environment for dense microbes containing mostly the anaerobic microorganisms. As chicken matures, these microbes become very divergent until it attains a comparatively active state Yang *et al.* (2019); Blanch *et al.* (2020). In

comparison to other animals, chicken has a smaller gastrointestinal tract and rapid digesta passage. Such anatomical character chooses diverse gut microbes in chicken than other animals Zheng *et al.* (2019); Zhang *et al.* (2020). There are wide correlations of these gut microbes with animal host, diet, and similar relations between specific intestinal microorganisms that have intense impacts on chicken nourishment and well-being Taylor (2014); Zhou *et al.* (2020). Upcoming chicken production is dependent upon understanding the relationship among the intestinal microbes and host performance, that regulate the homeostasis Rubio (2019); Zhou *et al.* (2020). This review described the host-microbe relations, microbe-diet relations, microbe-immunity relations, and microbe-microbe relationship in the chicken intestinal tract.

**Chicken intestinal microbiota and its establishment:** The microbiota in the GI tract of chicken is a diverse population of microorganisms mainly composed of bacteria Kairie *et al.* (2013); Polansky *et al.* (2016); Ding *et al.* (2017). Generally, these microorganisms in the intestine can be classified into pathogenic and commensal

populations. Pathogenic populations may be involved in the induction of infection, intestinal degeneration, and toxin production. Commensal populations may be involved in vitamin synthesis, a stimulus for immune system via non-pathogenic means, and prevent the establishment of pathogenic microorganisms Jeurissen *et al.* (2002); Smith *et al.* (2014). Microbial populations can be categorized into luminal and mucosal populations, and mucosal microbiota may be further subdivided into epithelial or cryptal Ewing *et al.* (1994); Roberts *et al.* (2015). Luminal bacteria are synchronized by the influx of nutrients from the diet, passage rate of intestinal contents, and movement of antimicrobial substances. Mucosal bacteria are synchronized by their capability to attach to the enterocyte, the amount of mucin production and emission by goblet cells, and the quantity and specificity of IgA secretion Jeurissen *et al.* (2002); Smith *et al.* (2014). Normally, the chicken small intestine is occupied by the *Lactobacillus*, *Enterococcus*, and *Clostridium*, with some bacteria from the family *Enterobacteriaceae* Bjerrum *et al.* (2006); Gong *et al.* (2007). The ceca contain a wide variety of bacteria, including *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Enterococcus*, *Escherichia*, *Fusobacterium*, *Lactobacillus*, *Streptococcus* and *Campylobacter* genera Gong *et al.* (2002); Bjerrum *et al.* (2006); Gong *et al.* (2007).

Changes in inter-region environments direct the establishment of particular microbes Bortoluzzi *et al.* (2019). Such environments contain available substances for development, redox potential, pH, digesta passage time, and anti-microbial excretions. Similarly, the intra-region difference in environments participates in alterations detected between, for example, luminal and mucosa-linked inhabitants Awad *et al.* (2016). Epithelial cells, that establish cellular obstruction among tissues and the intestinal microbiota of host, exhibit a diversity of substances, like transmembrane proteins, which can detect microbial adherence Ribet and Cossart (2015); Zanu *et al.* (2020). Several communications among the host cells and microorganisms take place, which, normally, strengthen their mutually beneficial association but may be broken by host or microbes for their personal benefit Allaire *et al.* (2018). For gut bacterial studies, caecum is regularly focused on poultry as caecum has the biggest bacterial inhabitants. There is an overall agreement that pre-caecal GIT areas are mainly colonized by *Lactobacillus* spp. with cell concentrations of up to  $10^9$  per g of digesta. Cell concentrations and bacterial variety must increase over small intestine, predominantly in ileum Oakley *et al.* (2014). Cell concentrations in the caecum may increase  $10^{11}$  per g of digesta and are mainly colonized by *Firmicutes* and *Bacteroidetes* phyla, with minor colonization from other phyla. The representatives of the Firmicutes phylum are *Ruminococcus*, *Clostridium*, and *Eubacterium* genera, but

*Bacteroides* genus colonized by the Bacteroidetes phylum Wei *et al.* (2013); Zuber, Siegert, and Feuerstein (2019). Caecum composition can be affected by several factors including host genetics. Kers *et al.* (2018) defined some alterations among layers and broiler chickens. *Firmicutes* are the supreme abundant phylum in a broiler, whereas in layers, *Proteobacteria* are most dominant up to 7 days of age, afterward, *Firmicutes* becomes abundant.

Age-linked variations in the microbial composition of the poultry intestinal have been defined. Usually, bacterial dominance and variety increase after hatch Clavijo and Vives-Florez (2018). Oakley *et al.* (2014) stated that after hatching on day 7, the intestinal microbiota was abundant by *Flavonifractor*, *Pseudoflavonifractor*, and *Lachnospiraceae* sequence type in broiler caecum. On day 21 after hatch, the *Faecalibacterium* genus was abundant and continued consequently over to day 42 after hatch, while *Roseburia* dominated and the *Lachnospiraceae* type develops. Several host-dependent and ecological aspects can affect the colonization and development of gut microbes Kers *et al.* (2018). The instant rearing conditions severely affect the evolution of chicken intestinal microbes. The study has revealed that day-old chicks that remain with mature hen quickly grow a similar caecum microbiota to the hen at day-7 Prohealth (2017). Genetics and sex may also affect the gut microbial population Kers *et al.* (2018). Moreover, environmental components, like accommodation, litter material, and nutrition, disturb gut bacterial populations Wang *et al.* (2016); Bortoluzzi *et al.* (2019).

**Constituents of healthy gastrointestinal system:** Intestinal health discusses several physical and biological functions that together preserve the intestinal homeostasis Kairie *et al.* (2013); Polansky *et al.* (2016). The principal important role of the healthy intestine is active ingestion and absorption of feed ingredients Kairie *et al.* (2013). The gut must be responsible for an effective obstruction (epithelial lining) which decreases contact with environmental toxins and probable pathogenic microorganisms Zanu *et al.* (2020). The immune system is an additional vital functional element of the gut Wigley (2013); Smith *et al.* (2014). It also makes available a site for a variety of microbiota development which offers another obstruction for the establishment of the pathogen, also, controls the growth and development of immune system and delivers nutrients for nourishment of host Sergeant *et al.* (2014); Roberts *et al.* (2015); Guo *et al.* (2020). Finally, the gut includes an abundance of neurons, hormones, and second messengers, therefore it is believed that it is a major neuroendocrine structure of body Cani and Knauf (2016); Weber (2017). The intestinal microbiota has main functional impacts on each element that assist in retaining intestinal homeostasis. The gut microbiota helps in the breakdown of undigested

feedstuff and delivers essential amino acids and vitamins to the host Blanch *et al.* (2020). Likewise, microbial end products, for example, butyrate deliver energy to the intestinal epithelial cells Sergeant *et al.* (2014); Polansky *et al.* (2016). Healthy intestine offers the microbiota and structural position for development and attachment of microbes, substances and nutrients, microbial breakdown and biological developments, and immunity that enables the gut microbiota to flourish Oakley *et al.* (2013); Oakley *et al.* (2014), Oakley and Kogut (2016). Consequently, healthy intestinal microbiota was essential for optimum growth and development of birds, whereas an unhealthy microbiota could stimulate intestinal pathogens, led to be reduced growth and increased killing rates.

#### **Intestinal microbiota and host interactions:**

Widespread collaborations appear among chicken host and its intestinal microbes (Figure 1). Such collaborations are established mainly by the exchange of nutrients, variety of host intestinal morphology, composition, and immunity.

#### **NUTRITIONAL INTERACTIONS**

**SCFAs production:** The carbohydrates in feed are break down and absorbed in the upper part of gut, leaving indigestible carbohydrates and remaining digestible carbohydrates to microorganisms living in the lower part of gut Sullivan *et al.* (2020). Several gut microbes might break down non-digestible disaccharides, oligosaccharides, and polysaccharides to their constituent sugars, fermented by gut microbes, synthesizing short-chain fatty acids (SCFAs). The SCFAs might be consumed as a source of energy and carbon by the host Tellez *et al.* (2006); Xia *et al.* (2019). Many parts of the chicken gut from crop to cecum have such type of fermentation but mainly proceed in the cecum, which is heavily colonized with microorganisms. Rehman *et al.* (2007). As the birds mature, the above-mentioned fermentation increases. In cecum SCFAs are not observed in one-day old chickens. After the establishment of cecal microbes, the concentrations of these SCFAs become optimum in 15-day old chickens and persist afterward Van Der Wielen *et al.* (2000); Bortoluzzi *et al.* (2019). SCFAs are pass through the epithelium in cecum via passive diffusion and go into several metabolic pathways. However, it is also stated that SCFAs can control colonic (colon) blood flow, enterocyte development, and propagation control mucin production and influence the intestinal immune reactions Wei and Morrison (2013).

**Nitrogen metabolism:** Intestinal microbes also participate in nitrogen breakdown of the host. In chickens, a cloaca, the gut, urinary, and reproductive tracts join where urine and feces mix. Due to backward peristaltic movement in rectum, a little amount of urine could move to the cecum Zhou *et al.* (2020). Then cecum

microbes can convert uric acid into ammonia, which might be utilized and recycled by host for producing amino acids like glutamine Wei and Morrison (2013); Zhou *et al.* (2020). Some amount of alimentary nitrogen is integrated into microbial cell proteins. Consequently, intestinal microbes might be a source of amino acids by themselves Metges (2000); Rubio (2019). However, the bulk of these microbial proteins are vanished to the host with the defecation as most of the gut microorganisms in chickens live in the cecum which cannot break and absorb protein Yang *et al.* (2019).

**Vitamin:** Gut microbes of chicken could also work like a vitamin (particularly B vitamins) supplier to his host LeBlanc *et al.* (2013). Comparable with microbial protein, many vitamins produced by intestinal microorganisms are emitted in feces as they are unable to be absorbed in cecum. But, coprophagic birds (ingest feces) might help from microbial vitamin production Blanch *et al.* (2020). This is proved by a higher vitamin requirement by birds in wire pens, where coprophagy is prohibited, than by birds kept on solid floors Tellez *et al.* (2006).

**Mucin Production:** The study has been shown that the endogenous synthesis of SCFAs stimulates mucus synthesis and release by intestinal microbiota Sakata and Setoyama (1995). The optimum amount of mucin production and emission is indistinct, but it is well known that there is a crucial balance among production and break down which directly influences host nourishment Bortoluzzi *et al.* (2019). Chickens can also deliver some nutritious substances to gut microorganisms. Such as, mucins synthesized by goblet cells are a significant supplier of nitrogen, carbon, and energy for certain beneficial and harmful microorganisms Xia *et al.* (2019). Limited information exists on mucin consuming microorganisms of chicken origin, but in other animal species diversity of microbes can breakdown mucins. These microbes are capable of attaching to the mucus layer and release enzymes for mucin breakdown Derrien *et al.* (2010). While mucin degradation by these microbes has not been proved in chicken until now, but these microbes have been established in GIT of chicken, and it is practical to consider that some of the gut microbes can degrade mucins in chickens. Mucin is a leading source of nutrients for certain intestinal microbes. However, extreme mucin emission increases endogenous nutrient losses and affect nutrient absorption. So, by decreasing the mucus layer, nutrient retaining by the host must be increased Zuber, Siegert, and Feuerstein (2019). By preserving healthy microbiota, fewer nutrients are used into the synthesis of bacterial proteins (mucin), and mucus viscosity is decreased Killer (2011). Nutrients and ingredients of diet influence mucin production and emission rates. For instance, increasing protein in diet increases the production of proteolytic enzyme and mucin

breakdown. Thus mucin emission rates are increases to conserve the homeostatic balance of mucin layer Miyata *et al.* (2011).

**Microbiota affects intestinal physiology:** The premature period is a life-threatening phase for chicken development and well-being. The fast-developing gastrointestinal tract delivers an ideal place for bacterial establishment Zheng *et al.* (2019). Meanwhile, gut microbes also play a vital role in intestinal growth. The gut microbes characterize a link among useful barrier functionality, production of favorable nutrients and proteins, and enhanced energy yield from dietary constituents with low characteristic potential Zhang *et al.* (2020). Removal of the gut microbes entirely is not a reliable method. As an alternative, attention should be on supporting the animal to control the intestinal microbiota so that the fast population fluctuates are avoided, and balance is sustained. The intestinal microbiota is a significant source of vitamins LeBlanc *et al.* (2013) and causes a breakdown of several nitrogenous compounds plus performing a barrier to establishing pathogens. The gut microbes perform the role of corresponding exogenous suppliers. Participants of intestinal microbiota are capable to produce vitamin K and water-soluble vitamins B Davila *et al.* (2013). The gut microbiota participates in many physical processes Marchesi *et al.* (2016). For example defensive functions (pathogen dislodgment, competition for nutrient and receptor, and synthesis of antibacterial factors), fundamental functions (GIT defenses, IgA production, strengthening of tight junctions, and development of immune system) and metabolic functions (ferment indigestible feed deposit, vitamins synthesis, intestinal cell diversity and propagation, absorption of ion) Yitbarek *et al.* (2019). Many metabolites synthesized by the microbes encourage the neuroendocrine cell in the gastrointestinal tract and as a result, the microbes play a significant role in endocrine limitation of GIT functionality. Gut microbes control the homeostasis of host by participating in optimum ingestion and absorption, energy metabolism, mucosal contaminations inhibition, and immune system modification Willing and Van Kessel (2010). Intestinal microbes can also influence the gut morphology of chicken. Villi are smaller, and crypts are thinner in intestine of germ-free birds or those birds inhabited with fewer microorganisms Forder *et al.* (2007). Feed supplementation of probiotic species such as *Bacillus subtilis*, *Lactobacillus acidophilus*, and *Saccharomyces cerevisiae* may increase the height of villi in duodenum and villi height to crypt depth proportion in the ileum of chicken Chae *et al.* (2012); Sullivan *et al.* (2020). Likewise, the addition of prebiotics in a feed like mannan oligosaccharide and fructooligosaccharide or soybean, fermented cottonseed, and rapeseed meal also causes the increased villi length and villus height to crypt depth

proportion in intestine of poultry Sun *et al.* (2013); Xia *et al.* (2019). These changes are not due to direct influence of feed additives but indirectly influenced by the modification of intestinal microbe's structure. Gut structural modification can result in infections caused by intestinal pathogenic microbes. For example, birds with *C. perfringens* and *Eimeria* spp cause necrotic enteritis had considerably decreased villi length and villus height to crypt depth proportion in contrast to controls birds Golder *et al.* (2011). The action of gastrointestinal enzymes can be influenced by intestinal microbes as well. Nutrition that can induce fluctuations in gut microbes can also effect gastrointestinal enzyme action. For example, the functions of amylase and protease enzymes are prominent in chickens fed with diets having cottonseed meal or fructooligosaccharides Sun *et al.* (2013). It was determined that these diets encourage specific microbes like *Bifidobacterium* and *Lactobacillus* which can increase the gastrointestinal enzyme activity, while reducing the number of some microbes like *Escherichia coli* which can either influence the gastrointestinal enzyme emission or release proteolytic enzyme to reduce digestive enzymes Sun *et al.* (2013).

Many factors for example modifications in nourishing practices, imbalanced nutrition like excess of protein, starch or fructose Belanche *et al.* (2012), thermal stress, overloading of birds and bad management and sanitization Schmidt *et al.* (2011), may cause the damage to gut microbiota, that affects the function of host native defense system. Therefore, a healthy, constant, and variable gut microbiota is mandatory to sustain optimum GIT functions. Microbiota configuration and metabolites formed by the microbes are significant for the conservation of optimum intestinal wellbeing Rinttilä and Apajalahti (2013).

**Microbiota and immunity:** As a major structure of the mucosal immune system, gut has developed to perform dual essential roles: nutrient absorption and microbial resistance. Gut immune system comprises of healthy mucosal layer, firmly connected epithelial cells of intestine, released solvable antibody (IgA), and antibacterial peptides Yitbarek *et al.* (2019). It is recognized that a favorable bacterial population plays a significant role in sustaining normal homeostasis, modifying the immune system, and manipulating organ growth and metabolic rate of host Sommer and Backhed (2013). There are limited studies in chickens relating collaborations among microbiota and immune reaction. Forder *et al.* (2007) defined a distinction mucin profile and more quantities of goblet cells in the gut of conventionally raised chickens. Besides, the microbes-free birds have changes in gut lymphocyte and lymphoid cellular subdivisions paralleled to conventional birds. Moreover, the variety of poultry intestinal microbiota has been revealed the effect of the density of the T-cell

receptor in GIT and spleen Mwangi *et al.* (2010). Additionally, to regulating the synthesis of chemokines and cytokines and manipulating the T-cell of gut, gut microbes also modify B-cell reaction and antibody (IgA) synthesis Yitbarek *et al.* (2019). IgA released in lumen perform a significant function in microbes attachment and exclusion, and bacterial modification of IgA homeostasis is, relatively, reliant on host protein programmed cell death 1 (PD1) conveyed on T follicular helper cells in the germinal center Kawamoto *et al.* (2012). Intestinal microbes also control the synthesis of antibacterial peptides in intestinal epithelial cells that contain defenses, which quickly deactivate the microbes. Intestinal immune homeostasis is sustained by a composite cell network and their released solvable products Kamada *et al.* (2013).

Bursa of Fabricius is the dominant position of B-cell growth, which is an exceptional feature of the poultry immune system. In mammals, bone marrow is the site for B cell maturation. The bursa of Fabricius is an extension of GIT and is familiar to be populated with microorganisms just after hatching Sommer and Backhed (2013). These microorganisms can perform as foreign particles or encourage the synthesis of cytokines, enhancing the propagation and development of bursal B-cells. While the bursal duct is ligated before emerging, birds produce the natural antibody, indicating that gut microorganisms might have systemic impacts on pathogenic resistance via this structure Ratcliffe (2006); Zhang *et al.* (2020). The advanced study should be done to conclude if gut microorganisms perform a better function in B cell growth in chickens paralleled to other animals because of their close relationship with the bursa of fabricius. Other constituents of the gut occasionally measured as innate immune resistance. For instance, mucins are generated by the gut materials that assist as lubricants and protectants Ambort *et al.* (2011). Mucins offer nutrition and sites for attachment of favorable microorganisms. Chicken mucins are capable of relieving the infectious properties of *Campylobacter jejuni*, affecting it to accept a beneficial function in poultry tissues Claus *et al.* (2011); Bortoluzzi *et al.* (2019).

**Manipulation of intestinal microbiota:** Manipulating the intestinal microbiota may provide favorable beneficial effects; this is a method for chicken production for example demonstrated through competitive exclusion where newborn chicks might be secure against the establishment by *Salmonella enteritidis* by treating with gut contents obtained from mature healthy birds. This concept of adding beneficial microorganisms to the GIT has led to the development of prebiotics and probiotics Stanley *et al.* (2014); Roto *et al.* (2015). Besides prebiotics and probiotics, several non-antibiotic materials are currently being used in chicken production including exogenous enzymes Kairie *et al.* (2013), essential oils (EO), and organic acids Roberts *et al.* (2015). Prebiotics

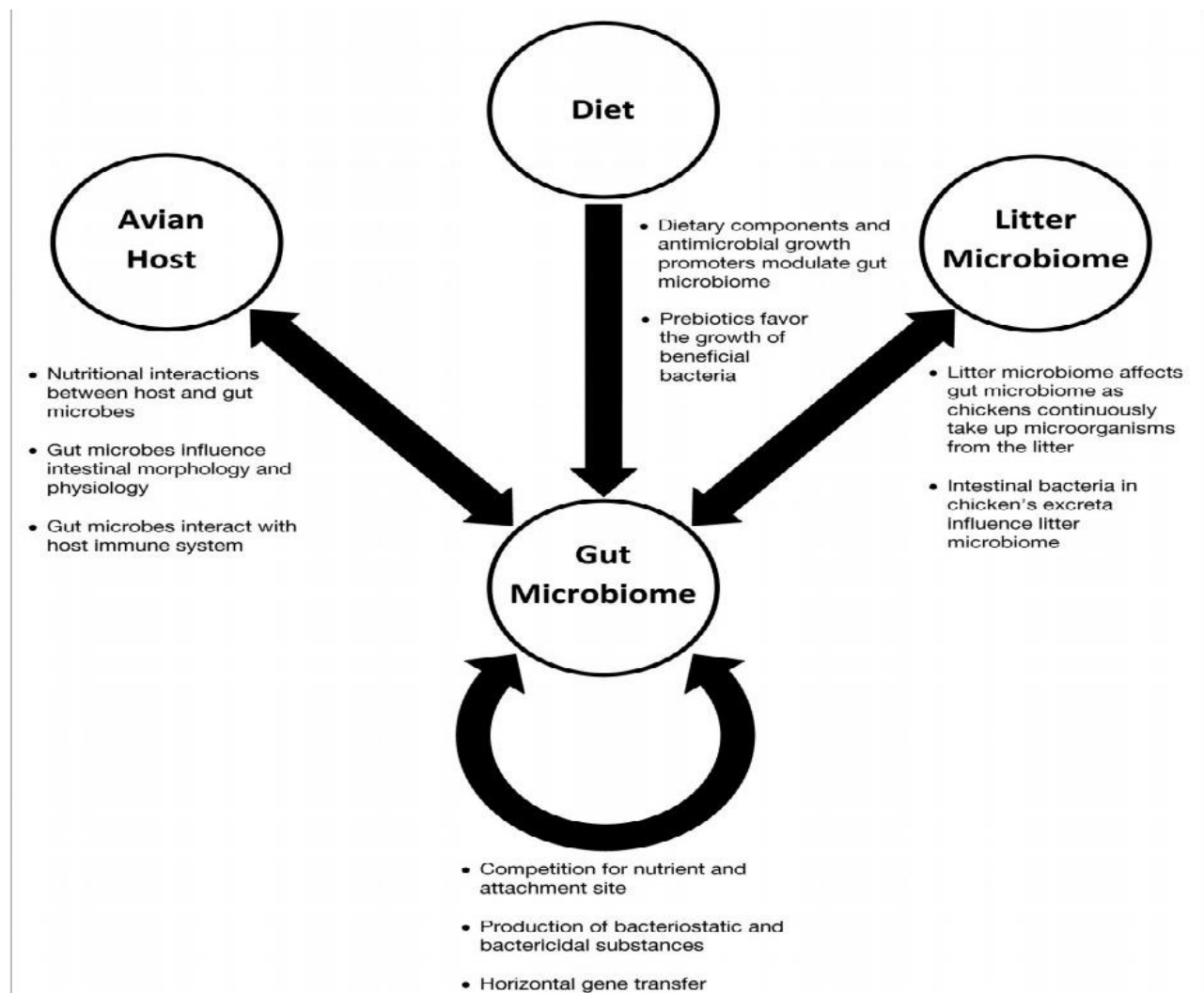
are a non-viable diet component that gives a positive health benefit to chicken linked with a variety of gut microbes.

Mannan oligosaccharides, fructooligosaccharides, xylooligosaccharides, inulin, as well as yeasts, are used as prebiotics Ricke (2015); Xia *et al.* (2019); Blanch *et al.* (2020). Agreeing to the Microbial Compendium Penton (2015), here exist up to fifty prebiotics products on sale in the USA through various do not have a clear mode of action. Prebiotics increased growing performance, gut integrity, immune reaction, and favorable microorganisms Roto *et al.* (2015). Probiotics are live microbes, which, once added in sufficient quantities, give a positive advantage to host. *Lactobacillus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, and *Escherichia* along with mold are mostly used as probiotics Roberts *et al.* (2015); Ricke (2015). Probiotics enhanced immune reaction, increase intestinal blockade function, synthesis of bacteriocins, and increase microbes homeostasis Gaggia *et al.* (2010). Synbiotics are nutritious supplements uniting probiotic and prebiotic which are more beneficial than prebiotic and probiotic. Probiotics cannot survive in the gut without a prebiotic; therefore this is the primary aim of using a synbiotic. Synbiotics are considered not solitary to present valuable bacterial inhabitants, but also to stimulate the propagation of particular endogenous gut microbes Gaggia *et al.* (2010). Synbiotics have been presented to be efficient in aquaculture as a result of improving the development and immune reactions in water animal species Huynh *et al.* (2017). Diet plays a vital role in influencing the microbes. Nutritional variations can cause a significant change in bacterial confirmation in 24 hours. These bacterial changes can be favorable or unfavorable to the normal animal functioning Zheng *et al.* (2019); Zhang *et al.* (2020). Some diet constituents induced special positive effects rather than basic nutrition, indicating the idea of functional diets. Functional diets can increase the health and development of birds which eat them Montalban *et al.* (2015). Some useful diet constituents affect the development and metabolic action of the gut microbes and, thus, its conformation and functions. Exogenous enzymes have been administered in chicken feed to increase the digestion of feed and modify the intestinal microbiota of chicken Adeola and Cowieson (2011). The constant and healthy gut environment reduces the establishment of harmful bacterial populations, improved intestinal blockade function, and enhanced growing performance Roberts *et al.* (2015).

The period of molecular biology and mechanization of the Sanger sequencing technique has led to innovation and progresses in diagnostics and biotechnology. The previous Sanger technology used to study the DNA sequencing, and it ruled the research for more than 2 decades Arulandhu *et al.* (2018); Giannenas *et al.* (2019). To get better of these techniques, next-generation high-throughput sequencing (HT-NGS)

techniques have been developed, as these are fast, cost-effective, and minimize the manual work Haynes *et al.* (2019); Feye *et al.* (2020). Several programs like Illumina (Solexa) sequencing, sequencing-by-synthesis 454 Life Sciences, SOLiD sequencing, and the Ion Torrent semiconductor sequencing techniques were established that use their unique detection principles Hanning, Pendleton, and Souza, (2013); Haynes *et al.* (2019). Nowadays, Nanopore sequencing fluorescent resonant energy transfer and real-time monitoring of PCR are being reported as third-generation sequencing methodologies. These technologies have benefits as they increase the DNA polymerase performance, easily

adaptable, simple, minimize human errors, beneficial for obtaining the real-time results, and to improve the chicken production Hanning, Pendleton, and Souza, (2013); Xing *et al.* (2019). The sequence-based (quantification of intestinal microbiota, genes analysis of metabolic pathway) and function-based (genes selection for antibiotic resistance, vitamins synthesis) analysis may be carried out to determine the intestinal microbiota, and health and disease status of chicken to reduce the number of harmful microorganisms Zahedi *et al.* (2019). As for future aspects, these present technologies and their possible implementations can be used to enhance poultry production along with food safety and public health.



**Fig.1. Relationship between gut microbiota, host, litter microbiome and diet**  
Adapted from Taylor (2014)

**Conclusions:** Intestinal microbiota presently considered an important constituent of the intestinal environment and is devoted to as an ignored structure, which participates

in the welfare of host, particularly in nourishment and disease inhibition. The ability to intentionally manipulate the microbiota by supplying nutrients, modulating host

immunity, inhibiting pathogen intestinal establishment, or improve intestinal barrier function has led to a number of novel methods to prevent disease, but also led to improved body weight, feed conversion, and carcass yield. However, advanced research on chicken gut microbiota and its relation with host and nutrition can provide the information required to develop new approaches that can entirely substitute the antibiotics growth promoters in advanced chicken production.

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## REFERENCES

- Adeola, O., and A.J. Cowieson (2011). Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. *J. Ani. Sci.* 89: 3189-3218.
- Allaire, J.M., S.M. Crowley, H.T. Law, S.Y. Chang, H.J. Ko, and B.A. Vallance (2018). The intestinal epithelium: Central coordinator of mucosal immunity. *Trends Immunol.* 39(9): 677-696.
- Ambort, D., G.C. Hansson, H. Sjövall, K.A. Thomsson, A.M. Rodriguez-Piñeiro, J.H. Bergström, and A. Ermund (2011). Composition and functional role of the mucus layers in the intestine. *Cell. Mol. Life Sci.* 68: 3635–3641.
- Arulandhu, A.J., J.V. Dijk, M. Staats, R. Hagelaar, M. Voorhuijzen, B. Molenaar, R. Hoof, R. Li, L. Yang, J. Shi, I. Scholtens, and E. Kok (2018). NGS-based amplicon sequencing approach; towards a new era in GMO screening and detection. *Food Control.* 93: 201–210.
- Awad, W.A., E. Mann, M. Dzieciol, C. Hess, S. Schmitz-Esser, M. Wagner, and M. Hess (2016). Age-related differences in the luminal and mucosa-associated gut microbiome of broiler chickens and shifts associated with *Campylobacter jejuni* Infection. *Front. Cell. Infect. Microbiol.* 6: 154.
- Belanche, A., M. Doreau, J.E. Edwards, J.M. Moorby, E. Pinloche, and C.J. Newbold (2012). Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation. *J. Nut.* 142: 1684–1692.
- Bjerrum, L., R.M. Engberg, T.D. Leser, B.B. Jensen, K. Finster, and K. Pedersen (2006). Microbial community composition of the ileum and cecum of broiler chickens as revealed by molecular and culture-based techniques. *Poult. Sci.* 85: 1151–1164.
- Blanch, M., J. Herrero-Encinas, J. J. Pastor, A. Mereu, I.R. Ipharraguerre, and D. Menoyo (2020). Effects of a bioactive olive pomace extract from *Olea europaea* on growth performance, gut function, and intestinal microbiota in broiler chickens. *Poult. Sci.* 99: 2–10.
- Bortoluzzi, C., B.S. Vieira, B. Lumpkins, G.F. Mathis, W.D. King, D. Graunard, K.A. Dawson, and T.J. Applegate (2019). Can dietary zinc diminish the impact of necrotic enteritis on growth performance of broiler chickens by modulating the intestinal immune-system and microbiota? *Poult. Sci.* 98: 3181–3193.
- Bortoluzzi, C., B.S. Vieira, C. Hofacre, and T.J. Applegate (2019). Effect of different challenge models to induce necrotic enteritis on the growth performance and intestinal microbiota of broiler chickens. *Poult. Sci.* 98: 2800–2812.
- Cani, P.D., and C. Knauf (2016). How gut microbes talk to organs: the role of endocrine and nervous routes. *Mol. Metab.* 5: 743-752.
- Celi, P., A.J. Cowieson, F. Fru-Nji, R.E. Steinert, A.M. Klunter, and V. Verlhac (2017). Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production. *Anim. Feed Sci. Technol.* 234: 88–100.
- Chae, B., S. Ingale, J. Kim, K. Kim, S. Sen, S. Lee, C. Khong, E.K. Kim, and I.K. Kwon (2012). Effect of dietary supplementation of probiotics on performance, caecal microbiology and small intestinal morphology of broiler chickens. *Anim. Nutr. Feed Technol.* 12: 1-12.
- Claus, S.P., S.L. Ellero, B. Berger, L.Krause, A. Bruttin, J. Molina, A. Paris, E.J. Want, I. de Waziers, O. Cloarec, S.E. Richards, Y. Wang, M.E. Dumas, A. Ross, S. Rezzi, S. Kochhar, P. Van Bladeren, J.C. Lindon, E. Holmes, and J.K. Nicholson (2011). Colonization-induced host-gut microbial metabolic interaction. *MBio.* 2(2): e00271-10.
- Clavijo, V., and M.J. Vives Flórez (2018). The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: A review. *Poult. Sci.* 97(3): 1006-1021.
- Davila, A.M., F. Blachier, M. Gotteland, M. Andriamihaja, P.H. Benetti, Y. Sanz, and

- D.Tomé (2013). Intestinal luminal nitrogen metabolism: role of the gut microbiota and consequences for the host. *Pharmacol. Res.* 68: 95–107.
- Derrien, M., W.J. Van Passel, J.H.B. Van de Bovenkamp, R. Schipper, W. de Vos, and J. Dekker (2010). Mucinbacterial interactions in the human oral cavity and digestive tract. *Gut Microbes.* 1(4): 254-68.
- Ding, J., R. Dai, L. Yang, C. He, K. Xu, S. Liu, W. Zhao, L. Xiao, L. Luo, Y. Zhang, and H. Meng (2017). Inheritance and establishment of gut microbiota in chickens. *Front. Microbiol.* 8: 1967-1967.
- Ewing, W.N., and D.J.A. Cole (1994). *The Living Gut. Context*, Dungannon, Ireland.
- Feye, K.M., D.R. Thompson, M.J. Rothrock Jr., M.H. Kogut, and S.C. Ricke (2020). Poultry processing and the application of microbiome mapping. *Poult. Sci.* 99: 678–688.
- Forder, R.E.A., G. S. Howarth, D.R. Tivey, and R.J. Hughes (2007). Bacterial modulation of small intestinal goblet cells and mucin composition during early posthatch development of poultry. *Poult. Sci.* 86(11): 2396–2403.
- Gaggia, F., P. Mattarelli, and B. Biavati (2010). International J. Food Microbiology Probiotics and prebiotics in animal feeding for safe food production. *Int. J. Food Microbiol.* 141: S15–S28.
- Giannenas, I., E. Bonos, G. Filliouis, I. Stylianaki, P. Kumar, D. Lazari, E. Christaki, and P. Florou-Paneri (2019). Effect of a Polyherbal or an Arsenic-Containing Feed Additive on Growth Performance of Broiler Chickens, Intestinal Microbiota, Intestinal Morphology, and Lipid Oxidation of Breast and Thigh Meat. *J. Appl. Poult. Res.* 28: 164–175.
- Golder, H.M., M.S. Geier, R.E. Forder, P.I. Hynd, and R.J. Hughes (2011). Effects of necrotic enteritis challenge on intestinal micro-architecture and mucin profile. *Br. Poult. Sci.* 52: 500-6.
- 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 ceca and comparison with bacteria in the cecal lumen. *FEMS Microbio. Lett.* 208: 1–7.
- Gong, J., W. Si, 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 Microbio. Eco.* 59: 147–157.
- Guo, Y., Z.H. Zhao, Z.Y. Pan, L.L. An, B. Balasubramanian, and W.C. Liu (2020). New insights into the role of dietary marine-derived polysaccharides on productive performance, egg quality, antioxidant capacity, and jejunal morphology in late-phase laying hens. *Poult. Sci.* 1-8. <https://doi.org/10.1016/j.psj.2019.12.032>.
- Hanning, I., S.D. Sanchez, S. Pendleton, and D.D. Souza (2013). Next-generation sequencing : The future of molecular genetics in poultry production and food safety. *Poult. Sci.* 92: 562–572
- Haynes, E., E. Jimenez, M. Angel, and S.J. Helyar (2019). The future of NGS ( Next Generation Sequencing ) analysis in testing food authenticity. *Food Control.* 101: 134–143.
- Huynh, T.G., Y.L. Shiu, T.P. Nguyen, Q.P. Truong, J.C. Chen, and C.H. Liu (2017). Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: A review. *Fish Shellfish Immunol.* 64: 367–382.
- Jeurissen, S.H., F. Lewis, J.D. van der Klis, Z. Mroz, J.M. Rebel, A.A. ter Huurne (2002). Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity, and functionality. *Curr. Issu. Intes. Microbio.* 3: 1–14.
- Kairie, E., L.F. Romero, and C.M. Nyachote (2013). The role feed enzymes in promoting gut health in swine and poultry. *Nut. Res. Rev.* (1): 71-88.
- Kamada, N., S.U. Seo, G.Y. Chen, and G. Núñez (2013). Role of the gut microbiota in immunity and inflammatory disease. *Nat. Rev. Immunol.* 13: 321–335.
- Kawamoto, S., T.H. Tran, M. Maruya, K. Suzuki, Y. Doi, Y. Tsutsui, and S. Fagarasan (2012). The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. *Science.* 336(6080): 485–489.
- Kers, J.G., F.C. Velkers, E.A.J. Fischer, G.D.A. Hermes, J.A. Stegeman, and H. Smidt (2018). Host and environmental factors affecting the intestinal microbiota in chickens. *Fron. Microbiol.* 9: 235.
- Killer, J., and M. Marounek (2011). Fermentation of mucin by bifidobacteria from rectal samples of humans and rectal and intestinal samples of animals. *Folia Microbiol. (Praha).* 56(2): 85-9.
- LeBlanc, J.G., C. Milani, G.S. de Giori, F. Sesma, D. van Sinderen, and M. Ventura (2013). Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr. Opin. Biotechnol.* 24: 160–168.
- Liu, W., Y. Yuan, C. Sun, B. Balasubramanian, Z. Zhao, and L. An. (2019). Effects of dietary betaine on growth performance, digestive function, carcass traits, and meat quality in indigenous yellow-feathered broilers under long-term heat stress. *Animals.* 9: 506.

- Marchesi, J.R., D.H. Adams, F. Fava, G.D.A. Hermes, G.M. Hirschfield, G. Hold, M.N. Quraishi, J. Kinross, H. Smidt, K.M. Tuohy, L.V. Thomas, E.G. Zoetendal, and A. Hart (2016). The gut microbiota and host health: a new clinical frontier. *Gut*. 65: 330–339.
- Metges, C.C. (2000). Contribution of microbial amino acids to amino acid homeostasis of the host. *J. Nutr.* 130: 1857S-64S.
- Miyata, M., H. Yamakawa, M. Hamatsu, H. Kuribayashi, Y. Takamatsu, and Y. Yamazoe (2011). Enterobacteria modulate intestinal bile acid transport and homeostasis through apical sodium-dependent bile acid transporter (SLC10A2) expression. *J. Pharmacol. Exp. Ther.* 336: 188-96.
- Montalban-Argues, A., P. De Schryver, P. Bossier, G. Gorkiewicz, V. Mulero, D.M. Gaitlin, and J. Galindo-Villegas (2015). Selective manipulation of the gut microbiota improves immune status invertebrates. *Front. Immunol.* 6: 512.
- Mwangi, W.N., R.K. Beal, C. Powers, X. Wu, T. Humphrey, M. Watson, and A. L. Smith (2010). Regional and global changes in TCR $\alpha\beta$  T cell repertoires in the gut are dependent upon the complexity of the enteric microflora. *Dev. Comp. Immunol.* 34: 406–417.
- Oakley, B., and M.H. Kogut (2016). Spatial and temporal changes in the broiler chicken cecal and fecal microbiomes and correlations of bacterial taxa with cytokine gene expression. *Front. Vet. Sci.* 3: 11.
- Oakley, B.B., H.S. Lillehoj, M.H. Kogut, W.K. Kim, J.J. Maurer, A. Pedroso, M.D. Lee, S.R. Collett, T.J. Johnson, and N.A. Cox (2014). The chicken gastrointestinal microbiome. *FEMS Microbiol. Lett.* 360(2): 100-12.
- Oakley, B.B., C.A. Morales, J. Line, M. Berrang, R.J. Meinersmann, G.E. Tillman, M.G. Wise, G.R. Siragusa, K.L. Heitt, and B.S. Seal (2013). The poultry-associated microbiome: network analysis and farm-to-fork characterizations. *Plos. One.* 8(2): e57190.
- Penton Agriculture (2015). Microbial compendium. <http://microbialcompendium.com/>.
- Polansky, O., Z. Sekelova, M. Faldynova, A. Sbkova, F. Sisak, and F. Rychik (2016). Gut health in poultry. *CAB Reviews: Perspectives in Agriculture, Vet. Sci. Nut. Nat. Res.* 15.
- Ratcliffe, M.J.H. (2006). Antibodies, immunoglobulin genes and the bursa of Fabricius in chicken B cell development. *Dev. Comp. Immunol.* 30: 101–118.
- 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: 319-35.
- Ribet, D., and P. Cossart (2015). How bacterial pathogens colonise their hosts and invade deeper tissues. *Microb. Inf.* 17(3): 173-83.
- Ricke, S.C. (2015). Potential of fructooligosaccharides prebiotics in alternative and nonconventional poultry production. *Poult. Sci.* 94: 1411-1418.
- Rintilä, T., and J. Apajalahti (2013). Intestinal microbiota and metabolites—Implications for broiler chicken health and performance. *J. Appl. Poult. Res.* 22: 647–658.
- Roberts, T., J. Wilson, A. Guthrie, K. Cookson, D. Vancraenest, J. Schaeffer, R. Moody, and S. Clark (2015). New issues and science in broiler chicken intestinal health: emerging technology and alternative interventions. *J. Appl. Poult. Res.* 24: 257-266.
- Roto, S.M., P.M. Rubinelli, and S.C. Ricke (2015). An Introduction to the Avian Gut Microbiota and the Effects of Yeast-Based Prebiotic-Type Compounds as Potential Feed Additives. *Front. Vet. Sci.* 2: 28.
- Prohealth (2017). <http://www.fp7-prohealth.eu/knowledge-platform/newsletter/articles/similaritiesand-specificities-gut-microbiota-pigs-and-chickens/>.
- Rubio, L.A. (2019). Possibilities of early life programming in broiler chickens via intestinal microbiota modulation FUNCTIONS OF THE INTESTINAL. *Poult. Sci.* 98: 695–706.
- Sakata, T., and H. Setoyama (1995). Local stimulatory effect of short-chain fatty acids on the mucus releases from the hindgut mucosa of rats (*Rattus norvegicus*). *Comp. Biochem. Physiol.* 111A: 429–432.
- Schmidt, B., I.E. Mulder, C.C. Musk, R.I. Aminov, and M. Lewis (2011). Establishment of normal gut microbiota is compromised under excessive hygiene conditions. *PLoS. One.* 6: e28284.
- Sergeant, M.J., C. Constantinidou, T.A. Cogan, M.R. Beford, C.W. Penn, and M.J. Pallen (2014). Extensive microbial and functional diversity within the chicken cecal microbiome. *Plos. One.* 9: e91941.
- Smith, A.L., C. Powers, and R.K. Beal (2014). The avian enteric immune system in health and disease. In: Shat, K.A., Kaspers, B., Kaiser, P., editors. *Avian Immunology*, 2nd edition. London, UK: Academic Press; pp. 227-250.
- Sommer, F., and F. Bäckhed (2013). The gut microbiota—masters of host development and physiology. *Nat. Rev. Microbiol.* 11: 227–238.
- Stanley, D., R.J. Hughes, and R.J. Moore (2014). Microbiota of the chicken gastrointestinal tract: Influence on health, productivity and disease. *Appl. Microbiol. Biotechnol.* 98: 4301–4310.

- Sullivan, D.J., S. Azlin-hasim, M. Cruz-romero, E. Cummins, J.P. Kerry, M.A. Morris (2020). Antimicrobial effect of benzoic and sorbic acid salts and nano-solubilisates against *Staphylococcus aureus*, *Pseudomonas fluorescens* and chicken microbiota biofilms. *Food Control*. 107: 106786.
- Sun, H., J.W. Tang, X.H. Yao, Y.F. Wu, X. Wang, and J. Feng (2013). Effects of dietary inclusion of fermented cottonseed meal on growth, cecal microbial population, small intestinal morphology, and digestive enzyme activity of broilers. *Trop. Anim. Health Prod.* 45: 987-93.
- Taylor, P. (2014). Intestinal microbiome of poultry and its interaction with host and diet. *Landes Biosci.* 37-41.
- Tellez, G., S.E. Higgins, A.M. Donoghue, and B.M. Hargis (2006). Digestive physiology and the role of microorganisms. *J. Appl. Poult. Res.* 15: 136-44.
- Van Der Wielen, P.W., S.N. Biesterveld, S. Notermans, H. Hofstra, B.A. Urlings, and K.F. Van (2000). Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Appl. Environ. Microbiol.* 66: 2536-40.
- Wang, L., M. Lilburn, and Z. Yu (2016). Intestinal microbiota of broiler chickens as affected by litter management regimens. *Front. Microbiol.* 18(7): 593.
- Weber, W.H. (2017). Overview of gastrointestinal regulatory peptides. *Curr. Opin. Endocrinol. Diabetes Obesity.* 23: 1-2.
- Wei, S., M. Morrison, and Z. Yu (2013). Bacterial census of poultry intestinal microbiome. *Poult. Sci.* 92(3): 671-683.
- Wigley, P. (2013). Immunity to bacterial infection in the chicken. *Dev. Comp. Immunol.* 41: 413-417.
- Willing, B.P., and A.G. Van Kessel (2010). Host pathways for recognition: establishing gastrointestinal microbiota as relevant in animal health and nutrition. *Livest. Sci.* 133: 82-91.
- Xia, Y., J. Kong, G. Zhang, X. Zhang, R. Seviour, and Y. Kong (2019). Effects of dietary inulin supplementation on the composition and dynamics of cecal microbiota and growth-related parameters in broiler chickens. *Poult. Sci.* 6942-6953.
- Xing, R., N. Wang, R. Hu, J. Zhang, J. Han, and Y. Chen (2019). Application of next generation sequencing for species identification in meat and poultry products: A DNA metabarcoding approach. *Food Control.* 101: 173-179.
- Yang, X., S. Liang, F. Guo, Z. Ren, X. Yang, and F. Long (2019). Gut microbiota mediates the protective role of *Lactobacillus plantarum* in ameliorating deoxynivalenol-induced apoptosis and intestinal inflammation of broiler chickens. *Poult. Sci.* 1-12.
- Yitbarek, A., J. Astill, D.C. Hodgins, J. Parkinson, É. Nagy, and S. Sharif (2019). Commensal gut microbiota can modulate adaptive immune responses in chickens vaccinated with whole inactivated avian influenza virus subtype H9N2. *Vaccine.* 37: 6640-6647.
- Zahedi, A., T.L. Greay, A. Paparini, K. L. Linge, C.A. Joll, and U.M. Ryan (2019). Identification of eukaryotic microorganisms with 18S rRNA next-generation sequencing in wastewater treatment plants, with a more targeted NGS approach required for *Cryptosporidium* detection. *Water Res.* 158: 301-312.
- Zanu, H.K., C. Keerqin, S.K. Kheravii, N.K. Morgan, S.B. Wu, M.R. Bedford, and R.A. Swick (2020). Influence of meat and bone meal, phytase, and antibiotics on broiler chickens challenged with subclinical necrotic enteritis: 1. growth performance, intestinal pH, apparent ileal digestibility, cecal microbiota, and tibial mineralization. *Poult. Sci.* 99: 1540-1550.
- Zhang, B., G. Li, M.S. Shahid, L. Gan, H. Fan, Z. Lv, S. Yan, and Y. Guo (2020). Dietary L-arginine supplementation ameliorates inflammatory response and alters gut microbiota composition in broiler chickens infected with *Salmonella enterica* serovar Typhimurium. *Poult. Sci.* 99: 1862-1874.
- Zheng, M., P. Mao, X. Tian, Q. Guo, and L. Meng (2019). Effects of dietary supplementation of alfalfa meal on growth performance, carcass characteristics, meat and egg quality, and intestinal microbiota in Beijing-you chicken. *Poult. Sci.* 98: 2250-2259.
- Zhou, B., L. Jia, S. Wei, H. Ding, J. Yang, and H. Wang (2020). Effects of *Eimeria tenella* infection on the barrier damage and microbiota diversity of chicken cecum. *Poult. Sci.* 99: 1297-1305.
- Zhou, C., P. Xu, C. Huang, G. Liu, S. Chen, G. Hu, G. Li, P. Liu, and X. Guo (2020). Ecotoxicology and Environmental Safety Effects of subchronic exposure of mercuric chloride on intestinal histology and microbiota in the cecum of chicken. *Ecotoxicol. Environ. Saf.* 188: 109920.
- Zuber, T., W. Siegert, A. Camarinha-Silva, D. Feuerstein, and M. Rodehutsord (2019). Effects of protease and phytase supplements on small intestinal microbiota and amino acid digestibility in broiler chickens. *Poult. Sci.* 98: 2906-2918.