CONNECTION OF GUT MICROBIOM, GUT HEALTH AND PRODUCTIVITY IN CHICKEN

S. S. PAUL^{1*}, R. N. CHATTERJEE¹, S. V. RAMA RAO¹, M.V. L. N. RAJU¹,
A. KANNAN¹ AND B. PRAKASH¹

¹ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad-500 030, Telangana, India

Abstract

With increasing menace of antimicrobial resistance, demand for organically grown chicken is increasing and the concept of gut health is attracting huge interest among all stakeholders of chicken production system. Chicken gut microbiome plays a key role in the maintenance of gut health and productivity of host through competitive exclusion of pathogens, contribution to the development and function of host immune system and gut epithelial barrier, besides contributions to host nutrition. Recently research focus has shifted towards defining gut microbial community composition in relation to performance variables in different breeds or lines of chicken using high throughput sequencing technologies and towards development of feed additives to support development and maintenance of healthy gut. Host, diet and environment play key role in succession of gut microbial community of chicken, although some evidence suggest that some form of microbial inheritance occurs naturally from dam through the pores of the eggshell and that early interventions can influence intestinal colonization of birds with a healthy microbiota. This review compiles information about the connection of gut microbiome with gut health and performance of chicken and advances in technologies related to dietary interventions to modulate gut health in chicken favorably.

Key words: Antibiotics alternative, Chicken, Growth, Gut health, Gut microbiome

Introduction

Among traditional livestock species, chicken is the most efficient converter of feed, with a feed conversion ratio in modern commercial broiler chicken being in the range of 1.5-1.7. Feed efficiency and faster growth rate are the crucial goals in highly competitive poultry production system. For attaining these goals maintaining a healthy gut is an important prerequisite. The intestinal mucosa is responsible for absorption of nutrients. Intestinal tract in chicken consumes about 19.3 to 50% of oxygen utilized in whole body. A substantial proportion of this consumption is due to the higher rate of turnover of cellular protein, which permits the intestine to expand in size and activity or nutrient uptake capacity

(Cant *et al.*, 1996). Further, intestine is one of the most active organs of immune system with more than 70% resident immune cells of the entire body (Vighi *et al.*, 2008). Over the last decade, the concept of “gut health” is attracting significant interest amongst all stakeholders of food animal production (Kogut and Arsenault, 2016). This can be attributable to general urge for developing nutritional interventions for modulating gut health towards optimal health and production so as to facilitate rearing chickens organically. Poor gut health in broilers has been associated with gut wall morphology changes, inflammation, systemic infection and malabsorption. The importance of gut health in chicken can be gauged from the fact that more

*Corresponding Author

than 50% of the therapeutic use of antibiotics in broilers in the European Union is to control intestinal pathologies (Persoons *et al.*, 2012). The gastrointestinal tract of chickens is densely populated with complex microbial communities (bacteria, fungi, archaea, protozoa and virus) with bacteria being the most dominant one. The interactions between the host and the chicken gastrointestinal (GI) bacterial microbiome are considered to play important roles in bird nutrition, physiology and gut development. To maintain a good intestinal barrier function, an optimal balance between the mucus layer, the intestinal epithelium and the microbiota is crucial. In this review we will focus mainly on connection of gut microbiome with gut health and performance of chicken and advances in technologies related to dietary interventions to modulate gut health favorably.

Colonization and succession of chicken gut microbiome

The initial colonization of the GI tract of birds begins by passing of microorganisms from dam through the pores of the eggshell (Lee *et al.*, 2019). After the initial colonization of the intestine a succession of microorganisms is observed in which the species richness and diversity of the community structure of the microbiota increase as the birds grow, until microbiota reaches a state of maturation and stabilizes. In case of commercial broiler chickens this stabilization period has been shown to be around three weeks of life (Diaz Carrasco *et al.*, 2019). However, development times and succession patterns of gut microbiome species can vary greatly depending on the genetic makeup of the birds, nature of feed (feed processing, feed components, feed buffering activity and feed additives) and farm management factors (Choi *et al.*, 2014). Lots of environmental microbes try to get established within gut of chicken but host plays very important role in selection of its symbionts in gut through immune response and other

attributes like pH, etc. Already inhabited microbes also play important role in selection of other microbes (Baumgartner *et al.*, 2020). Also some microbes have evolved mechanisms for evading host immune response (de Jong and Alto, 2018) and have developed complex metabolic profile so as to make them fit into particular environment to make them compatible with other microbes within the ecosystem. Such complexities involved in shaping microbial community structure are frontline areas of current research in human nutrition. Recently focus on such studies in chickens has also gathered momentum (Saxena *et al.*, 2016; Pandit *et al.*, 2018; Paul *et al.*, 2019). It has been suggested that early colonization of the intestine is of great importance for poultry health and productivity, since it can alter the morphology and physiology of the intestine by contributing to the construction of intestinal barrier function and susceptibility to infectious diseases by modulating development and function of host immune system (Kers *et al.*, 2018). The inoculations with prebiotics, probiotics and consortia of microorganisms applied directly in ovo have been shown to favor the early intestinal colonization of birds with a “healthy” microbiota from the moment of hatching (Simon *et al.*, 2016; Baldwin *et al.*, 2018). The reuse of litter during consecutive flocks is a common practice that is usually applied to reduce cost in broiler production. Since the first day of life, chicks start pecking and ingesting litter materials and hence litter microbial load may influence gut microbiome of chickens. In one study, it has been shown that reused litter does not lead to higher abundances of *C. perfringens* or *Salmonella* (Wei *et al.*, 2013). However, chickens raised on fresh litter showed an increasing colonization with beneficial microbes such as *Lactobacillus* spp. (Cressman *et al.*, 2010). Wang *et al.* (2016) reported that reuse of litter increased the prevalence of halotolerant/alkaliphilic bacteria

in litter, but also altered the intestinal microbiota of the birds, increasing the levels of *Faecalibacterium prausnitzii*, a commensal butyrate-producing species, in the cecum of young chickens. More studies are needed to assess how litter reuse can influence chicken gut health.

Costs and benefits of gut microbial community

A gut microbial community has benefits as well as costs to the host. The main benefits that are provided by commensal microbiota are competitive exclusion of pathogens or non-indigenous microbes (Dibner and Richards, 2005), immune stimulation and programming of host immune system development, and contributions to host nutrition. It was shown that a germ-free mouse can be killed with 10 cells of *Salmonella enteritidis*, although it requires 10⁶ cells to kill a conventional mouse (Collins and Carter, 1978). It has been shown that commensal gut microbes can stimulate the development of immune system including the mucus layer, epithelial layer, the intestinal immune cells (e.g., cytotoxic and helper T cells, immunoglobulin producing cells and phagocytic cells), and the lamina propria (Oakley *et al.*, 2014). These tissues work as barriers between the host and the microbes, and prevent invasion of opportunistic pathogens. In the hindgut, the microbiota also produces short chain fatty acids (SCFA), vitamins (e.g., vitamin K and vitamin B groups) and amino acids from the undigested feeds, which eventually become available for the host. SCFA produced in hindgut acts as source of energy for host. Data on exact contribution of SCFA to chicken nutrition could not be traced. However, studies with pigs have shown that the SCFA produced in hindgut may provide approximately 20 - 30% of their energy requirement for maintenance (Rérat *et al.* 1987; Bergman, 1990; Yen *et al.*, 1991). SCFA can also kill or suppress pathogens, such as *Salmonella* spp. (Ricke *et al.*, 2003) and can further stimulate gut

epithelial cell proliferation, thus increasing the area of absorption surface of villi. In addition, gut microbiota also contributes to metabolism of host nitrogenous compounds and they can convert uric acid to ammonia, which is subsequently absorbed by the bird and further used to produce amino-acids such as glutamine (Singer, 2003) and influence bile salt metabolism and recycling (Suzuki, 1970). The gut microbiota can form a protective barrier by attaching to the epithelial walls of the enterocyte and thus reduce the opportunity for the colonization of pathogenic bacteria (Yegani and Korver, 2008).

On the other hand, commensal microbiota also incurs cost to the host. The gut microbiome can also be a source of bacterial pathogens including those having potential zoonotic role such as *Salmonella* and *Campylobacter* which can harbor antibiotic resistance genes and therefore may pose a serious threat to public health. In the proximal gut, microbes compete with the host for energy and protein. In entire gut, microbes may produce toxic metabolites (e.g., amino acid catabolites) and catabolize bile acids, which may depress growth and decrease fat digestibility of the birds, respectively (Gaskins *et al.*, 2002). Further, microbes in gut increases mucin secretion, increases rate of epithelial cell turnover and increases secretion of IgA. While generally beneficial, these processes do increase the demand for energy and protein from the host and therefore have an influence on the growth performance of the birds.

Relation of gut microbial community composition and performance

The relationship between the diversity of species in the intestinal microbiota and the feeding efficiency of poultry is not very clear. But recent use of high throughput sequencing technologies and real time PCR has helped to understand gut microbiome composition comprehensively in

relation to different performance levels. Based on such analysis, potential performance-related phylotypes were assigned to some bacteria species such as *Lactobacillus salivarius*, *Lactobacillus aviarius*, *Lactobacillus crispatus*, *Clostridium lactatifermentans*, different members of family *Ruminococcaceae*, *Bacteroides vulgatus*, *Akkermansia*, and *Faecalibacterium*, among others (Torok *et al.*, 2011; Han *et al.*, 2016; Yan *et al.*, 2017). In another study involving analysis of feces samples, Singh *et al.* (2012) identified eight families (*Fusobacteriaceae*, *Flavobacteriaceae*, *Rhizobiaceae*, *Vibrionaceae*, *Xanthomonadaceae*, *Comamonadaceae*, *Campylobacteraceae* and Incertae Sedis XIII) linked to high feed conversion ratio (FCR) birds and six families (*Victivallaceae*, *Synergistaceae*, *Prevotellaceae*, *Rikenellaceae*, *Enterobacteriaceae* and *Ruminococcaceae*) associated with low FCR birds. In another study, broiler chickens with a high FCR exhibited a higher abundance of the genera *Actinobacter*, *Bacteroides*, *Streptococcus*, *Clostridium*, and whereas in low FCR chickens *Escherichia*, *Shigella* and *Salmonella* were more abundant (Singh *et al.*, 2014). *Campylobacter* colonization of broiler chickens has been shown to be associated with reduced economic performance in terms of an increase in cumulative FCR (Awad *et al.*, 2015). The abundances of *Escherichia*, *Shigella* were found to correlate negatively with growth and fat digestibility in broiler chickens (Rubio *et al.*, 2015).

Feed additives with potential to modulate gut microbiota favourably

The potential restriction of antibiotics as growth promoters in different countries has led to the search for alternatives to improve growth performance and to prevent gut diseases such as gut dysbiosis, necrotic enteritis, diarrhea, loss of appetite and underdevelopment of the immune system.

Poultry diets have a tremendous impact on the gut microbial community structure. Diets with high levels of non starch polysaccharides (NSP), such as rye, barley and wheat-based diets, increase gut viscosity, increase the retention time of feed and reduce nutrient digestibility. Such conditions favor the colonization of *Clostridium perfringens* and increases occurrence of necrotic enteritis (Pan and Yu, 2014). Use of enzymes such as xylanase and β -glucanase are known to reduce gut viscosity and stimulate the growth of lactic acid bacteria having the ability to adhere to the gut epithelium and compete with pathogens for its colonization (Rodríguez *et al.*, 2012). The NSP enzyme market is globally dominated by endo-1,4- β -xylanase and endo-1,3(4) β -glucanase (Adeola and Cowieson, 2011). This corresponds with arabinoxylan and glucan substrates as the predominant NSP types in common broiler diet raw ingredients. It has been shown that use of xylanases and beta-glucanases in high NSP containing diets such as diets rich in wheat and barley reduced the viscosity of the digesta by 30 to 50% and 300%, respectively (Juanpere *et al.*, 2005). Reduced viscosity results in improvements of protein digestibility, apparent metabolizable energy, feed consumption, body weight gain, and feed conversion. Xylanases also increased the apparent ileal digestibility of amino acids by 15% in a corn-based diet and for wheat- and rye-based diets by 16% and 30%, respectively (Cowieson and Bedford, 2009). However, several studies have reported no effect of enzymes on performance of birds (Olgun *et al.*, 2018; Walters *et al.*, 2018) probably due to variation in structure of NSP and that of ideal substrate required for the enzymes.

Phytase is commonly supplemented in poultry diet to increase utilization of dietary phosphorus. In the crop, phytase supports to increase in abundance of *Aeromonadaceae* and *Flavobacteriaceae* while reducing the dominance of *Lactobacillus* (Witzig *et al.*,

2015). In another study it has been shown that phytase supplementation increases the abundance of *Lactobacillus* sp., *Clostridium leptum* and *Enterococcus* sp. in the ileum (Ptak *et al.*, 2015). The authors stated that these findings might be associated with the greater availability of P in the lumen of the gastrointestinal tract and that such effects of phytase are influenced strongly by Ca and P content of a diet and that excessive P supply, perhaps achieved by the combination of the high Ca:P diet with phytase addition, may negatively influence some bacterial populations.

Organic acids (OA) have been reported to exert antimicrobial activities that are similar to antibiotics and which induce significant benefits in poultry. Similar to that of antibiotics, OA also have a specific antimicrobial activity. The main modes of action of OAs are: dissociating into ions and protons, reducing the cytoplasmic pH, causing cell death by inhibiting enzymatic reactions and denaturing proteins and DNA (Cherrington *et al.*, 1991). Organic acids are particularly effective against acid-intolerant species such as *Escherichia coli*, *Salmonella* and *Campylobacter* (Dibner and Buttin, 2002). Organic acids improve animal performance due to their wide ranging effects, such as; improvement of protein and energy utilization, (which may be attributed to their ability to reduce microbial competition with the host for nutrient and endogenous nitrogen loss), reduction in bacterial toxin, ammonia production and other growth-depressing microbial metabolites (in order to lower the incidence of subclinical infections), and the regulation and secretion of immune mediators (Dibner and Buttin, 2002). Besides antibacterial action, OA causes reduction in digesta pH, increase of pancreatic secretion, and have trophic effects on the GI mucosa and changing the morphology of the intestinal wall in a way that reduces colonization of pathogens, thus

preventing damage to the epithelial cells (Langhout, 2000; Dibner and Buttin, 2002). Sodium butyrate has been shown to favor the establishment of lactic acid producing bacteria including *Lactobacillus* spp. and *Bifidobacterium* spp., while reducing the colonization by harmful bacteria such as *Salmonella* and *Campylobacter* (Van Deun *et al.*, 2008). Effectiveness of different OA against different pathogens depends on pKa and chain length of the OA. A combination of different OAs can also expand the spectrum of activity against pathogens. Organic acid mixtures (fumaric acid, calcium formate, calcium propionate, potassium sorbate, calcium butyrate, calcium lactate) were found to be more effective than the antibiotic growth promoter (enramycin) in decreasing intestinal *E. coli* and *Salmonella* spp. (Hassan *et al.*, 2010). Paul *et al.* (2007) reported that organic acid salt (ammonium formate or calcium propionate) reduced coliform count in broiler feed compared to control, whereas the *Clostridium* count was not affected. The authors also showed that ammonium formate supplementation also lowered *E. coli* count in the gut but the *Clostridium* count was unaffected. In another study, Mikkelsen *et al.* (2009) showed that 0.45% potassium diformate in diet reduced mortality associated with necrotic enteritis (*Clostridium perfringens*). Fernandez-Rubio *et al.* (2009) reported that sodium butyrate (in both partially protected and unprotected forms) was able to prevent *Salmonella* colonization in the crop and caeca of broilers, whereas only the partially protected source of the butyrate salt reduced internal organ colonization (liver). Gheisari *et al.* (2007) showed that supplementation of 0.2% encapsulated OA to the diet might improved the proliferation of *Lactobacillus* spp. and reduced the population of *Clostridium perfringens*, *E. coli* and *Salmonella* spp. in poultry gut contents.

Antimicrobial activities of plant extracts and

essential oils (EO) have been demonstrated in several animal experiments. A mixture of EO containing carvacrol and thymol reduced the shedding of *E. coli* and *C. perfringens* in broilers (Jang *et al.*, 2007). Siragusa *et al.* (2008) reported that adding extracts of *Humulus lupulus* into drinking water decreased the number of *C. perfringens* in the broiler intestine. Essential oils are shown to be more effective against Gram-positive bacteria than Gram-negative bacteria. Among Gram-negative bacteria *E. coli* are inhibited by low dose of EOs whereas *Salmonella* inhibition require high dose of most of the EOs. Sensitivity of microbes to particular EO may differ under aerobic and anaerobic growth condition and strains or species of same genus may differ in sensitivity to EOs (Singh *et al.*, 2011). Several recent studies show that mugwort, fennel, or oregano EO, as well as their components, including eugenol, isopulegol, carvacrol, carvone and thymol, can be used in the prevention of coccidiosis in poultry (Drăgan *et al.*, 2014; Murakami *et al.*, 2014). Essential oil compounds and their combinations also show antifungal activity (Ownagh *et al.*, 2010). Saderi and Abbasi (2011) reported that thyme oil is effective against adenoviruses. Oregano oil and one of its components, carvacrol, have been demonstrated to be effective against entero- and rotaviruses (Sánchez *et al.*, 2015).

The components of EOs permeate the cell membranes and mitochondria of the microorganisms and inhibit the membrane bound electron flow and there with the energy metabolism. High concentrations may also cause lysis of the cell membranes and denaturation of cytoplasmic proteins (Gopi *et al.*, 2013; Nazzaro *et al.*, 2013).

In a review (Adaszyńska-Skwirzyńska and Szczerbińska, 2017) of data from 11 feeding trials on broiler chickens involving different EOs (dose rate 75 to 1200 mg/kg feed) the

increase in ADG ranged from -4 to 20.6% and improvement in FCR ranged from -23.5 to +13.2%.

In a study involving broiler chicken, Bozkurt *et al.* (2012) supplemented the corn and soybean based-basal diet with three doses of one of the following additives: Antibiotic growth promoter (AGP) (Avilamycin, 10 mg/kg diet), organic acid blend (OAB) (0.9, 1.8, 2.7 g/kg diet, respectively), EO mixture (EOM) (12, 24, 36 mg/kg diet, respectively) and OAB – EOM combination. The study indicated that addition of EOM to the diets of broiler either alone or in combination with OAB significantly improved body weight or feed efficiency of broilers without affecting mortality. Results obtained with EOM or OAB were comparable to that of AGP. In other study of Basmacioğlu-Malayoğlu *et al.* (2016) it was shown that the use of the combination of OA and EO was more effective, in some respects, than their individual use.

Prebiotics are oligosaccharides such as fructooligosaccharides (FOS), galactooligosaccharides (GOS), mannanoligosaccharides (MOS), and related carbohydrate polymers that can be utilized by beneficial bacteria such as *Bifidobacterium* and *Lactobacilli* but not by common pathogens. Prebiotics have 2 major modes of action. Initially, the prebiotic remain undigested in the upper part of the GI tract and are selectively utilized by some of the beneficial bacteria in intestine and hind gut. Secondly, prebiotic fermentation results in generation of short-chain fatty acids and lactic acid and a decreased rate of pathogen colonization (Ricke *et al.*, 2020). In the gut, xylo-oligosaccharides (XOS) were shown to increase the abundance of *Lactobacillus* and *Clostridium* cluster XIVa which is known to possess genes related to butyrate production (De Maesschalck *et al.*, 2015). Beta-D-glucan and MOS, components of yeast cell wall was shown to bind to the

receptor mannose-specific type-1 fimbriae and prevent pathogen colonization while favoring the genus *Faecalibacterium* which is considered as beneficial bacteria for gut health (Park *et al.*, 2016).

Probiotics are living microorganisms that improve gut health and animal performance by competing with pathogenic bacteria for adhesion sites at the intestinal epithelium. Mechanisms of action from probiotics include enhancement of activity of digestive enzymes like proteases, lipases and amylases, the improvement of mucosa ultrastructure, thus also increasing nutrient absorption (Borda-Molina *et al.*, 2018). Various strains used as probiotic include *Lactobacillus plantarum*, *Enterococcus faecium*, *Bacillus* spp., *Bifidobacterium* and yeast such as *Saccharomyces cerevisiae* which were shown to impact the small intestinal microstructure with an increase of villous height and *Lactobacillus* and *Bifidobacterium* counts in the ceca. Its supplementation decreases the presence of harmful bacteria such as *E.coli* and *Salmonella* sp. (Yang *et al.*, 2017).

Synbiotics denotes combined effects of pre- and probiotics. Synbiotics have been shown to improve the implantation and survival of the supplemented bacteria in the GIT (Awad *et al.*, 2009). Synbiotics was shown to be effective in the reduction of *C. jejuni*, which causes zoonosis frequently and causes a strong inflammatory effect (Humphrey *et al.*, 2014). The combination of *Bifidobacterium longum* PCB133 with a XOS successfully reduced the load of *Campylobacter* spp. and *C. jejuni* (Baffoni *et al.*, 2017). Humam *et al.* (2019) reported that supplementation with postbiotics improved growth performance, intestinal morphology and immune response in broiler chickens under heat stress.

Metabolites synthesized from probiotics are

referred to as “postbiotics” and represent an alternative to antibiotic growth promoters since exerting the positive effect of probiotics without applying living cells (Sobolewska *et al.*, 2017). Kareem *et al.* (2014) reported that *Lactobacillus* sp. is able to produce OA and bacteriocins in their culture medium that promote the presence of lactic acid bacteria. On administration of metabolites produced by *L. plantarum*, there was a decrease of pH and counts of enterobacteria, an intensification of mRNA IGF1 expression which is an indicator for stimulation of body growth. Humam *et al.* (2019) reported that supplementation with postbiotics improved growth performance, intestinal morphology and immune response in broiler chickens under heat stress.

Bacteriophages are a group of viruses whose replication is dependent on invasion of bacterial cells. Bacteriophages exhibit strong bactericidal activity. Some phages display specific affinity for single types of bacteria, while others have a broad range of activity. Phages have been shown to be effective in preventing infections and in treatment of colibacteriosis in poultry (Barrow *et al.*, 1998). Phage therapy has also been shown to be effective in necrotic enteritis infections of broiler chickens by anaerobic *Clostridium perfringens* (Miller *et al.*, 2010). Fiorentin *et al.* (2005) observed that single oral dose of a blend of phages (CNPSA1, CNPSA3 and CNPSA4) at a dosage of 10^{11} plaque forming unit (PFU) decreased the occurrence of *Salmonella enteritidis* strains by 3.5 log units and shown that application of a single dose of a bacteriophage suspension with a high titre was more effective in reducing the population of pathogenic bacteria in the gut than long-term application of a lower titre.

Besides EO, various plant bioactive compounds can stimulate growth and immunity in chickens and may also influence gut microbial community structure. Inclusion of sea weed

extract rich in plant phycobillins was recently shown to increase growth of broiler chickens significantly along with intensification of gene expression of gut immunity and porosity related genes (Paul *et al.*, 2020).

Conclusion

The increasing use of molecular biology tools has helped in studying succession of gut microbiome and factors influencing microbial community composition in chicken gut in

relation to performance comprehensively. Detail understanding of such factors has helped in development of several feed additives with potential to modulate gut microbiome favorably. Various innovative dietary supplements as outlined in this document have the potential to offer environmental friendly solution as alternative to antibiotic growth promoters to increase growth and feed efficiency by helping to maintain healthy gut in chickens.

REFERENCES

- Adaszyńska-Skwirzyńska M and Szczerbińska D, 2017. Use of essential oils in broiler chicken production- A review. *Ann Anim Sci*, 17(2): 317-335, doi: 10.1515/aoas-2016-0046
- Adeola O and Cowieson AJ, 2011. Board-invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. *J Anim Sci*, 89(10): 3189-3218, doi: 10.2527/jas.2010-3715
- Awad WA, Smorodchenko A, Hess C, Aschenbach JR, Molnár A *et al.*, 2015. Increased intracellular calcium level and impaired nutrient absorption are important pathogenicity traits in the chicken intestinal epithelium during *Campylobacter jejuni* colonization. *Appl Microbiol Biotechnol*, 99(15): 6431-6441, doi: 10.1007/s00253-015-6543-z
- Awad WA, Ghareeb K, Abdel-Raheem S and Böhm J, 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poult Sci*, 88(1): 49-56, doi: 10.3382/ps.2008-00244
- Baffoni L, Gaggli F, Garofolo G, Di Serafino G, Buglione E *et al.*, 2017. Evidence of *Campylobacter jejuni* reduction in broilers with early synbiotic administration. *Int J Food Microbiol*, 251: 41-47, doi: 10.1016/j.ijfoodmicro.2017.04.001
- Baldwin S, Hughes RJ, Van TTH, Moore RJ, Stanley D *et al.*, 2018. At-hatch administration of probiotic to chickens can introduce beneficial changes in gut microbiota. *PLoS One*, 13(3): e0194825, doi: 10.1371/journal.pone.0194825
- Barrow P, Lovell M and Berchieri Jr A, 1998. Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. *Clin Diagn Lab Immunol*, 5(3): 294-298, doi: 10.1128/CDLI.5.3.294-298.1998
- Basmacioğlu-Malayoğlu H, Ozdemir P and Bađriyanik HA, 2016. Influence of an organic acid blend and essential oil blend, individually or in combination, on growth performance, carcass parameters, apparent digestibility, intestinal microflora and intestinal morphology of broilers. *Br Poult Sci*, 57(2): 227-234, doi: 10.1080/00071668.2016.1141171
- Baumgartner M, Bayer F, Pfrunder-Cardozo KR, Buckling A and Hall AR, 2020. Resident microbial communities inhibit growth and antibiotic-resistance evolution of *Escherichia coli* in human gut microbiome samples. *PLoS Biol*, 18: e3000465, doi: 10.1371/journal.pbio.3000465
- Bergman EN, 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev*, 70(2): 567-590, doi: 10.1152/physrev.1990.70.2.567
- Borda-Molina D, Seifert J and Camarinha-Silva A, 2018. Current perspectives of the chicken

- gastrointestinal tract and its microbiome. *Comput Struct Biotechnol J*, 16: 131-139, doi: 10.1016/j.csbj.2018.03.002
- Bozkurt M, Küçükyılmaz K, Çatli AU, Çınar M, Çabuk M *et al.*, 2012. Effects of administering an essential oil mixture and an organic acid blend separately and combined to diets on broiler performance. *Arch Geflügelkd*, 76(2): 81-87
- Cant JP, McBride BW and Croom WJ, 1996. The regulation of intestinal metabolism and its impact on whole animal energetics. *J Anim Sci*, 74(10): 2541-2553, doi: 10.2527/1996.74102541x
- Cherrington CA, Hinton M, Mead GC and Chopra I, 1991. Organic acids: chemistry, antibacterial activity and practical applications. *Adv Microb Physiol*, 32: 87-108, doi: 10.1016/S0065-2911(08)60006-5
- Choi JH, Kim GB and Cha CJ, 2014. Spatial heterogeneity and stability of bacterial community in the gastrointestinal tracts of broiler chickens. *Poult Sci*, 93: 1942-1950, doi: 10.3382/ps.2014-03974
- Collins FM and Carter PB, 1978. Growth of *salmonellae* in orally infected germfree mice. *Infect Immun*, 21(1): 41-47
- Cowieson A and Bedford M, 2009. The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: complimentary mode of action? *Worlds Poult Sci J*, 65(4): 609-624, doi: 10.1017/S0043933909000427
- Cressman MD, Yu Z, Nelson MC, Moeller SJ, Lilburn MS *et al.*, 2010. Interrelations between the microbiotas in the litter and in the intestines of commercial broiler chickens. *Appl Environ Microbiol*, 76(19): 6572-6582, doi: 10.1128/AEM.00180-10
- de Jong MF and Alto NM, 2018. Cooperative immune suppression by *Escherichia coli* and *Shigella* effector proteins. *Infect Immun*, 86: e00560-17, doi: 10.1128/IAI.00560-17
- De Maesschalck C, Eeckhaut V, Maertens L, De Lange L and Marchal L *et al.*, 2015. Effects of xylo-oligosaccharides on broiler chicken performance and microbiota. *Appl Environ Microbiol*, 81: 5880-5888, doi: 10.1128/AEM.01616-15
- DiazCarrasco JM, Casanova NA and FernándezMiyakawa ME, 2019. Microbiota, gut health and chicken productivity: what is the connection?. *Microorganisms*, 7(10): 374, doi: 10.3390/microorganisms7100374
- Dibner JJ and Buttin P, 2002. Use of organic acid as a model to study the impact of gut microflora on nutrition and metabolism. *J Appl Poult Res*, 11(4): 453-463, doi: 10.1093/japr/11.4.453
- Dibner JJ and Richards JD, 2005. Antibiotic growth promoters in agriculture history and mode of action. *Poult Sci*, 84(4): 634-643, doi: 10.1093/ps/84.4.634
- Drăgan L, Gyorke A, Ferreira JF, Pop IA, Dunca I *et al.*, 2014. Effects of *Artemisia annua* and *Foeniculum vulgare* on chickens highly infected with *Eimeria tenella* (Phylum Apicomplexa). *Acta Vet Scand*, 56: 22-28, doi: 10.1186/1751-0147-56-22
- Fernandez-Rubio C, Ordonez C, Abad-Gonzalez J, Garcia Gallego A, Honrubia MP *et al.*, 2009. Butyric acid-based feed additives help protect broiler chickens from *Salmonella enteritidis* infection. *Poult Sci*, 88(5): 943-948, doi: 10.3382/ps.2008-00484
- Fiorentin L, Vieira ND and Barioni Jr W, 2005. Oral treatment with bacteriophages reduces the concentration of *Salmonella enteritidis* PT4 in caecal contents of broilers. *Avian Pathol*, 34(3): 258-263, doi: 10.1080/01445340500112157
- Gaskins HR, Collier CT and Anderson DB, 2002. Antibiotics as growth promotants: mode of action. *Anim Biotechnol*, 13(1): 29-42, doi: 10.1081/ABIO-120005768
- Gheisari AA, Heidari M, Kermanshahi RK, Togani M and Saraeian S, 2007. Effect of dietary supplementation of protected organic acids on ileal microflora and protein digestibility in

- broiler chickens. In: Proceedings of the 16th European Symposium on Poultry Nutrition. Strasbourg, France, pp519-522
- Gopi M, Karthik K, Manjunathachar HV, Tamilmahan P, Kesavan M *et al.*, 2013. Essential oils as a feed additive in poultry nutrition. *Adv Anim Vet Sci*, 2(1): 1-7, doi: 10.14737/journal.aavs/2014.2.1.1.7
- Han GG, Kim EB, Lee J, Lee J-Y, Jin G *et al.*, 2016. Relationship between the microbiota in different sections of the gastrointestinal tract, and the body weight of broiler chickens. *SpringerPlus*, 5: 911
- Hassan HMA, Mohamed MA, Youssef AW and Hassan ER, 2010. Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. *Asian-Aus J Anim Sci*, 23(10): 1348-1353, doi: 10.5713/ajas.2010.10085
- Humam AM, Loh TC, Foo HL, Samsudin AA, Mustapha NM *et al.*, 2019. Effects of feeding different postbiotics produced by *Lactobacillus plantarum* on growth performance, carcass yield, intestinal morphology, gut microbiota composition, immune status, and growth gene expression in broilers under heat stress. *Animals (Basel)*, 9: 644, doi: 10.3390/ani9090644
- Humphrey S, Chaloner G, Kemmett K, Davidson N, Williams N *et al.*, 2014. *Campylobacter jejuni* is not merely a commensal in commercial broiler chickens and affects bird welfare. *mBio*, 5(4): e01364-14, doi: 10.1128/mBio.01364-14
- Jang IS, Ko YH, Kang SY and Lee CY, 2007. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. *Anim Feed Sci Technol*, 134(3): 304-315, doi: 10.1016/j.anifeedsci.2006.06.009
- Juanpere J, Pérez-Vendrell AM, Angulo E and Brufau J, 2005. Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. *Poult Sci*, 84(4): 571-580, doi: 10.1093/ps/84.4.571
- Kareem KY, Ling FH, Chwen LT, Foong OM and Asmara SA, 2014. Inhibitory activity of postbiotic produced by strains of *Lactobacillus plantarum* using re-constituted media supplemented with inulin. *Gut Pathog*, 6: 23, doi: 10.1186/1757-4749-6-23
- Kers JG, Velkers FC, Fischer EAJ, Hermes GDA, Stegeman JA *et al.*, 2018. Host and environmental factors affecting the intestinal microbiota in chickens. *Front Microbiol*, 9: 235, doi: 10.3389/fmicb.2018.00235
- Kogut MH and Arsenault RJ, 2016. Editorial: gut health: the new paradigm in food animal production. *Front Vet Sci*, 3: 71, doi: 10.3389/fvets.2016.00071
- Langhout P, 2000. New additives for broiler chickens. *World Poult*, 16(3): 22-27
- Lee S, La TM, Lee HJ, Choi IS, Song CS *et al.*, 2019. Characterization of microbial communities in the chicken oviduct and the origin of chicken embryo gut microbiota. *Sci Rep*, 9(1): 6838, doi: 10.1038/s41598-019-43280-w
- Mikkelsen LL, Vidanarachchi JK, Olnood CG, Bao YM, Selle PH *et al.*, 2009. Effect of potassium diformate on growth performance and gut micro-biota in broiler chickens challenged with necrotic enteritis. *Br Poult Sci*, 50(1): 66-75, doi: 10.1080/00071660802613252
- Miller RW, Skinner EJ, Sulakvelidze A, Mathis GF and Hofacre CL, 2010. Bacteriophage therapy for control of necrotic enteritis of broiler chickens experimentally infected with *Clostridium perfringens*. *Avian Dis*, 54(1): 33-40, doi: 10.1637/8953-060509-Reg.1
- Murakami AE, Eyng C and Torrent J, 2014. Effects of functional oils on coccidiosis and apparent metabolizable energy in broiler chickens. *Asian-Australas J Anim Sci*, 27(7): 981-989, doi: 10.5713/ajas.2013.13449
- Nazzaro F, Fratianni F, deMartino L, Coppola R and deFeo V, 2013. Effect of essential oils on pathogenic bacteria. *Pharmaceutics*, 6(12): 1451-1474, doi: 10.3390/ph6121451
- Oakley BB, Lillehoj HS, Kogut MH, KimWK, Maurer

- JJ *et al.*, 2014. The chicken gastrointestinal microbiome. *FEMS Microbiol Lett*, 360(2): 100-112, doi: 10.1111/1574-6968.12608
- Olgun O, Altay Y and Yildiz AO, 2018. Effects of carbohydrase enzyme supplementation on performance, eggshell quality, and bone parameters of laying hens fed on maize-and wheat-based diets. *Br Poult Sci*, 59(2): 211-217, doi: 10.1080/00071668.2018.1423677
- Ownagh A, Abbas H, Mardani K and Samira E, 2010. Antifungal effects of thyme, agastache and satureja essential oils on *Aspergillus fumigatus*, *Aspergillus flavus* and *Fusarium solani*. *Vet Res Forum*, 2: 99-105
- Pan D and Yu Z, 2014. Intestinal microbiome of poultry and its interaction with host and diet. *Gut Microbes*, 5(1): 108-119, doi: 10.4161/gmic.26945
- Pandit RJ, Hinsu AT, Patel NV, Koringa PG, Jakhesara SJ *et al.*, 2018. Microbial diversity and community composition of caecal microbiota in commercial and indigenous Indian chickens determined using 16S rDNA amplicon sequencing. *Microbiome*, 6: 115, doi: 10.1186/s40168-018-0501-9
- Park SH, Lee SI and Ricke SC, 2016. Microbial populations in naked neck chicken ceca raised on pasture flock fed with commercial yeast cell wall prebiotics via an Illumina MiSeq platform. *PLoS One*, 11: e0151944, doi: 10.1371/journal.pone.0151944
- Paul SK, Halder G, Mondal MK and Samanta G, 2007. Effect of organic acid salt on the performance and gut health of broiler chicken. *J Poult Sci*, 44(4): 389-395, doi: 10.2141/jpsa.44.389
- Paul SS, Chatterjee RN, Raju MVLN, Prakash B, Rama Rao SV *et al.*, 2019. Diversity and community structure of gut microbiome in commercial and indigenous Indian chickens determined using high throughput sequencing. *Int Anim Nutr Conf, INCAN 2019, Kolkata, Dec 17-19*, pp126, abst no PSN 050
- Paul SS, Venkata HGRV, Raju MVLN, Rama Rao SV, Nori SS *et al.*, 2020. Dietary supplementation of extracts of red sea weed (*Kappaphycus alvarezii*) improves growth, intestinal morphology, expression of intestinal genes and immune responses in broiler chickens. *J Sci Food Agric*, doi: 10.1002/jsfa.10708
- Persoons D, Dewulf J, Smet A, Herman L, Heyndrickx M *et al.*, 2012. Antimicrobial use in Belgian broiler production. *Prev Vet Med*, 105(4): 320-325, doi: 10.1016/j.prevetmed.2012.02.020
- Ptak A, Bedford MR, Swiatkiewicz S, Zyla K and Jozefiak D, 2015. Phytase modulates ileal microbiota and enhances growth performance of the broiler chickens. *PLoS One*, 10(3): e0119770, doi: 10.1371/journal.pone.0119770
- Rérat A, Fiszlewicz M, Giusi A and Vaugelade P, 1987. Influence of meal frequency on postprandial variations in the production and absorption of volatile fatty acids in the digestive tract of conscious pigs. *J Anim Sci*, 64(2): 448-456, doi: 10.2527/jas.1987.642448x
- Ricke SC, 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poult Sci*, 82(4): 632-639, doi: 10.1093/ps/82.4.632
- Ricke SC, Lee SI, Kim SA, Park SH and Shi Z, 2020. Prebiotics and the poultry gastrointestinal tract microbiome. *Poult Sci*, 99(2): 670-677, doi: 10.1016/j.psj.2019.12.018
- Rodríguez ML, Rebolé A, Velasco S, Ortiz LT, Treviño J *et al.*, 2012. Wheat- and barley-based diets with or without additives influence broiler chicken performance, nutrient digestibility and intestinal microflora. *J Sci Food Agric*, 92(1): 184-190, doi: 10.1002/jsfa.4561
- Rubio LA, Peinado MJ, Ruiz R, Suárez-Pereira E, Ortiz Mellet C *et al.*, 2015. Correlations between changes in intestinal microbiota composition and performance parameters in broiler chickens. *J Anim Physiol Anim Nutr*, 99(3): 418-423, doi: 10.1111/jpn.12256
- Saderi H and Abbasi M, 2011. Evaluation of anti-adenovirus activity of some plants from *Lamiac-eae* family grown in Iran in cell culture. *Afr J Biotech*, 10(76): 756-760, doi: 10.5897/AJB10.1686

- Sánchez C, Aznar R and Sánchez G, 2015. The effect of carvacrol on enteric viruses. *Int J Food Microbiol*, 192: 72-76, doi: 10.1016/j.ijfoodmicro.2014.09.028
- Saxena S, Saxena V, Tomar S, Sapkota D and Gonmei G, 2016. Characterization of caecum and crop microbiota of Indian indigenous chicken targeting multiple hypervariable regions within 16S rRNA gene. *Br Poult Sci*, 57: 381-389, doi: 10.1080/00071668.2016.1161728
- Simon K, Verwoolde MB, Zhang J, Smidt H, de Vries Reilingh G *et al.*, 2016. Long-term effects of early life microbiota disturbance on adaptive immunity in laying hens. *Poult Sci*, 95(7): 1543-1554, doi: 10.3382/ps/pew088
- Singer MA, 2003. Do mammals, birds, reptiles and fish have similar nitrogen conserving systems? *Comp Biochem Physiol B Biochem Mol Biol*, 134(4): 543-558, doi: 10.1016/S1096-4959(03)00027-7
- Singh BR, Singh V, Singh RK and Ebibeni N, 2011. Antimicrobial activity of lemongrass oil (*Cymbopogon citratus*) oil against microbes of environmental, clinical and food origin. *Int Res Pharm Pharmacol*, 1(9): 228-236
- Singh KM, Shah T, Deshpande S, Jakhesara SJ, Koringa PG *et al.*, 2012. High through put 16S rRNA gene-based pyrosequencing analysis of the fecal microbiota of high FCR and low FCR broiler growers. *Mol Biol Rep*, 39(12): 10595-10602, doi: 10.1007/s11033-012-1947-7
- Singh KM, Shah TM, Reddy B, Deshpande S, Rank DN *et al.*, 2014. Taxonomic and gene-centric metagenomics of the fecal microbiome of low and high feed conversion ratio (FCR) broilers. *J Appl Genet*, 55: 145-154, doi: 10.1007/s13353-013-0179-4
- Siragusa GR, Haas GJ, Matthews PD, Smith RJ, Buhr RJ *et al.*, 2008. Antimicrobial activity of lupulone against *Clostridium perfringens* in the chicken intestinal tract jejunum and caecum. *J Antimicrob Chemoth*, 61(4): 853-858, doi: 10.1093/jac/dkn024
- Sobolewska A, Bogucka J, Dankowiakowska A, Elminowska-Wenda G, Stadnicka K *et al.*, 2017. The impact of synbiotic administration through *in ovo* technology on the microstructure of a broiler chicken small intestine tissue on the 1st and 42nd day of rearing. *J Anim Sci Biotechnol*, 8: 61, doi: 10.1186/s40104-017-0193-1
- Suzuki R, 1970. Influence of intestinal microorganisms on the metabolism of bile acids in mice. *Keio J Med*, 19(2): 73-86, doi: 10.2302/kjm.19.73
- Torok VA, Hughes RJ, Mikkelsen LL, Perez-Maldonado R, Balding K *et al.*, 2011. Identification and characterization of potential performance-related gut microbiotas in broiler chickens across various feeding trials. *Appl Environ Microbiol*, 77(17): 5868-5878, doi: 10.1128/AEM.00165-11
- Van Deun K, Haesebrouck F, Van Immerseel F, Ducatelle R and Pasmans F, 2008. Short chain fatty acids and L-lactate as feed additives to control *Campylobacter jejuni* infections in broilers. *Avian Pathol*, 37(4): 379-383, doi: 10.1080/03079450802216603
- Vighi G, Marcucci F, Sensi L, Di Cara G and Frati F, 2008. Allergy and the gastrointestinal system. *Clin Exp Immunol*, 153(Suppl 1): 3-6, doi: 10.1111/j.1365-2249.2008.03713.x
- Walters HG, Brown B, Augspurger N, Brister R, Rao S *et al.*, 2018. Evaluation of NSPase inclusion in diets manufactured with high-and low-quality corn on male broilers. *J Appl Poult Res*, 27(2): 228-239, doi: 10.3382/japr/pfx063
- Wang L, Lilburn M and Yu Z, 2016. Intestinal microbiota of broiler chickens as affected by litter management regimens. *Front Microbiol*, 7: 593, doi: 10.3389/fmicb.2016.00593
- Wei S, Gutek A, Lilburn M and Yu Z, 2013. Abundance of pathogens in the gut and litter of broiler chickens as affected by bacitracin and litter management. *Vet Microbiol*, 166(3-4): 595-601, doi: 10.1016/j.vetmic.2013.06.006
- Witzig M, Camarinha-Silva A, Green-Engert R, Hoelzle K, Zeller E *et al.*, 2015. Correction: spatial variation of the gut microbiota in

- broiler chickens as affected by dietary available phosphorus and assessed by T-RFLP analysis and 454 pyrosequencing. PLoS One, 10(12): e0145588, doi: 10.1371/journal.pone. 0143442
- Yan W, Sun C, Yuan J and Yang N, 2017. Gut metagenomic analysis reveals prominent roles of *Lactobacillus* and cecal microbiota in chicken feed efficiency. Sci Rep, 7: 45308, doi: 10.1038/srep45308
- Yang JJ, Qian K, Dong WU, Zhang W, Yi-jing WU *et al.*, 2017. Effects of different proportions of two *Bacillus* sp. on the growth performance, small intestinal morphology, caecal microbiota and plasma biochemical profile of Chinese Huainan Partridge Shank chickens. J Integr Agric, 16(6): 1383-1392, doi: 10.1016/S2095-3119(16)61510-1
- Yegani M and Korver DR, 2008. Factors affecting intestinal health in poultry. Poult Sci, 87(10): 2052-2063, doi: 10.3382/ps.2008-00091
- Yen JT, Nienaber JA, Hill DA and Pond WG, 1991. Potential contribution of absorbed volatile fatty acids to whole animal energy requirement in conscious swine. J Anim Sci, 69: 2001-2012, doi: 10.2527/1991.6952001x