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Chapter

The Impact of Heavy Metals on the Chicken Gut Microbiota and Their Health and Diseases

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Abstract

It is important to consider the health and well-being of birds in various production methods. The microbial makeup and function of a bird's gastrointestinal (GIT) system may vary based on the bird's food, breed, age, and other environmental conditions. Gut flora play a critical role in maintaining intestinal homeostasis. Environmental exposure to contaminants such as heavy metals (HMs) has been linked to a wide range of disorders, including the development of dysbiosis in the gut, according to many studies. Changes in the gut microbiota caused by HMs are a major factor in the onset and progression of these illnesses. The microbiota in the gut is thought to be the first line of defense against HMs. Thus, HMs exposure modifies the gut microbiota composition and metabolic profile, affecting HMs uptake and metabolism by altering pH, oxidative balance, and concentrations of detoxifying enzymes or proteins involved in HM metabolism. This chapter will focus on the exposure of chicken to HMs from their feed or water and how these HMs affect the immune system resulting in various diseases.

Keywords: poultry, chicken, broiler, GIT, heavy metals, microbiota, diseases

1. Introduction

Among the most popular types of poultry raised for human consumption are domestic chickens. At 35–40 days of age, a typical broiler chicken will weigh around two kilograms [1]. During this period, they require approximately 3–4 kilograms of feed per day because of their rapid growth. While raising chickens in close proximity is necessary to meet the demand for chicken meat, this practice puts the birds at greater risk of infection and speeds disease transmission [2].

There is a wide range of microorganisms that colonize an animal's digestive system as soon as it is born or hatches, and these microorganisms change over time [3]. The gut microbiota of an animal, a human of the same species, and the location of the host's body all differ [4]. In the gut microbiota, which is a complex, interconnected community of organisms, the actions of all microbial components have a direct effect on its functions [5]. When the host and microbes interact in a way that benefits both of them, an ecological system is created [6]. As with humans, animals' gut microbiome serves many of the same functions: scavenging energy from undigested feed

components through fermentation, creating an immune barrier to keep harmful bacteria out of the digestive tract, and aiding in the absorption of vitamins and amino acids by animals [5]. This is largely true for both species. Farm animals must fulfill environmental and dietary responsibilities, as well as economic ones, in order to be productive [7]. The GIT microbiota has a significant impact on animal performance, particularly in young animals who are exposed to a wide range of stressful situations [8]. Dietary fiber, vitamins, and minerals are all provided by the microbiota that inhabit the GIT. The GIT microbiota may also play an important role in hen health and immunity, according to some evidence [9].

Data shows that heavy metal (HM) exposure may play a role in the etiology of metabolic disease by altering the GIT microbiota [10]. It's important to remember that the gut microbiota protects the body from harmful microbes. Furthermore, HM exposure alters the composition and metabolic profile of the gut microbiota, which in turn affects the uptake and metabolism of these HMs by altering pH, oxygenation, and the concentrations of enzymes or proteins that are involved in the detoxification process [11]. As the intestinal barrier is influenced by gut flora, HM absorption can also be affected.

2. The avian GIT microbiota

There are many different types of microorganisms in the animal microbiota, including those that are beneficial and those that are harmful [12]. The term “microbiota” is used to describe this microbial community. It includes commensal, symbiotic, and pathogenic microorganisms, as well as those that are beneficial or harmful to the host [13]. The microbiome refers to all of these symbionts' genetic material as a whole [14]. When an organism consists of both host and microbial components, it is referred to as “supraorganisms” because of the important role it plays in the health and development of the host [5].

In the chicken intestinal tract, there is a diverse and ever-changing community of microorganisms [15]. When the gut microbiota is first established, it's mostly anaerobic bacteria that take over [16]. The intestinal microbiota of newly hatched chicks is heavily influenced by the surrounding environment, and this is especially true for chicks that have only a small number of bacteria in their system [17]. As animals older, the GIT microbiota evolves from simple to complex and obligate anaerobes, reaching a relatively stable dynamic equilibrium [18]. A variety of functions and microbial compositions are found throughout the chicken GIT, which is divided into numerous sections [19].

The digestion and absorption of nutrients are dependent on the proper functioning of each section of the digestive tract. In chickens, there are two paired ceca, both of which are home to a similar bacterial community [20].

According to Wei *et al.* [21], a total of 915 operational taxonomic units (OTUs), or species (defined as having a phylogenetic distance of 3%), were found in 13 phyla, with *Firmicutes* (70 percent), *Bacteroidetes* (11.3%), and *Proteobacteria* (9.3%) accounting for more than 90% of all sequences analyzed. *Clostridium*, *Ruminococcus*, *Lactobacillus* and *Bacteroides* dominated the 117 genera described in total. *Ethanoligenes* (*Firmicutes*), a genus of bacteria that produces ethanol, was found to be prevalent. The most common *Proteobacterium* was *Desulfohalobium*. The genus *Bifidobacterium* was found in only 1% of the *Actinobacteria* sequences. *Cyanobacteria*, *Spirochaetes*, *Synergistetes*, *Fusobacteria*, *Tenericutes*, and *Verrucomicrobia* were among

the lesser-known phyla. The phylum *Euryarchaeota* was the only Archaea phylum to be found in the chicken GIT, with only 11 out of a total of 3184 sequences. This supports the lack of methanogens in the chicken GIT [22]. There are fewer species of bacteria in chicken GIT than in the GIT of other animals, which may be due to the rapid transit and short retention times of food in the digestive system [5]. For example, a 29-day-old broiler chicken's typical retention time is between 4 and 5 hours, compared to humans' average of 20 hours [23]. *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* are said to be the most common phyla found in the ceca [24]. The presence of Firmicutes and Bacteroidetes in the ceca suggests that the microbiota present is important in the production of essential amino acids, the digestion of non-starch polysaccharides, which stimulates the production of short-chain fatty acids (SCFAs), and nitrogen recycling using uric acid [5]. *Bacteroides* are the most common species in the Bacteroidetes phylum (40%). Others in this family include *Prevotella* (the genus), *Tannerella* (the genus), and *Riemerella*, *Desulfohalobium*, *Escherichia*, *Shigella*, and *Neisseria* are the most common genera of *Proteobacteria* [21].

Clostridium, *Bacteroides*, and *Ruminococcus* are among the obligate anaerobes found in the cecum [25]. The small intestine, which includes the duodenum, jejunum, and ileum and is where nutrients are primarily digested and absorbed, has fewer microorganisms and is primarily colonized by acid-tolerant and facultative anaerobes such as *Lactobacillus*, *Enterococcus*, and *Streptococcus* [17]. The fecal microbiota composition varies greatly depending on the contributions of microbiota from different gut segments [12].

3. Importance of GIT microbiota to broilers

Microorganisms are primarily found in the gastrointestinal tract. Broilers and their intestinal microbiota interact in a variety of ways, with an emphasis on nutrient exchange, immune modulation, digestive system physiology, and pathogen exclusion being the most important [5, 26]. These functions are summarized in the following sections.

3.1 Nutrient exchange

Chickens benefit from the nutrients provided by commensal bacteria in their digestive systems, both directly and indirectly [5, 27]. SCFAs, ammonium, amino acids, and vitamins [12, 15] are all included in this category. Polysaccharides, oligosaccharides, and disaccharides can all be hydrolyzed to primary sugars by the majority of intestinal bacteria [28]. SCFAs such as acetate, propionate, and butyrate are produced by the fermentation of these sugars by intestinal bacteria [12, 15]. Passive diffusion in the ceca allows SCFAs to be absorbed and enter a number of metabolic pathways [29]. SCFAs are a carbon and energy source for chickens [15]. To further enhance their ability to modulate intestinal immune response, they regulate blood flow and stimulate the proliferation of enterocytes [29].

Nitrogen metabolism is also aided by bacteria in the intestines [30]. When uric acid is broken down into ammonium by bacteria in the urinary tract, it can travel from the cloaca to the cecum, affecting the metabolism in the latter and allowing ammonium to be absorbed by the host [29, 31]. This allows the host to use ammonium to synthesize amino acids. However, the same intestinal bacteria can also be a source

of amino acids and vitamins [29], Despite the fact that most of the proteins and vitamins produced by these bacteria are excreted, because most intestinal bacteria are found in the cecum, which cannot digest or absorb proteins [5]. Chickens, on the other hand, may be able to provide nutrients to the intestinal bacteria in a reciprocal manner.

3.2 Immunological modulation

Chickens' immune systems include both innate and acquired immune responses [32]. The microbiota plays an important role in modulating the regulation and activation of both elements [33]. In terms of the innate immune response, the intestinal mucosa is thought to be the first line of defense against infection and a barrier that prevents commensal bacteria from penetrating the intestinal epithelium [32]. The interior surface of the avian intestine is covered in a mucous layer composed of the glycoprotein mucin, which is secreted by calceiform epithelial cells [34]. Mucins containing sialic acid have been found to be more abundant in conventionally reared chickens than mucins containing sulfate, which are found in birds with low bacterial loads. These differences are visible as early as day four (4) after birth, implying that the intestinal microbiota is involved in regulating the establishment of the mucous layer [35]. The intestinal microbiota also regulates the production of antimicrobial peptides on the surface of the intestinal epithelium, which are capable of rapidly killing or suppressing the activity [36]. Some of these peptides are expressed naturally, while others are induced in host cells by bacteria.

Regarding the acquired immune system, it appears that commensal bacteria protect the mucosa membrane by modulating the immune response, controlling the amount of mediators secreted by acquired immune system cells, and stimulating helper T cells [37]. Using germ-free chickens, it was demonstrated that microbiota has a dramatic effect on the repertoire of intestinal T cells and their cytokine expression [38].

3.3 The physiology of the digestive system

After hatching from the egg, the chicks must transition from a yolk-based diet to one rich in carbohydrates and proteins, which is critical to their development and health [39]. So, in this stage of development, the digestive system's organs go through anatomical and physiological changes. An ideal environment for microorganisms to colonize is the rapidly developing digestive tract, and the microbiota also plays an important role in the development of this organ. Compared to conventionally reared chickens, germ-free chickens have smaller intestines and cecas that weigh less and have thinner wall thickness [38]. There is some evidence to suggest that SCFAs increase enterocyte proliferation and growth, which could explain some of the discrepancy [29]. Intestinal microbiota may also influence the enzyme activity in chicken intestines [5]. Compare germ-free and conventionally raised chicken alkaline phosphatase enzyme activity and you'll see that the latter has higher levels of activity [38]. Bifidobacterium and Lactobacillus, which increase the activity of proteases, trypsin and lipases, can be induced by diet as well [40]. Morphological changes can be caused by pathogenic bacteria as well [35]. Co-infection with *Eimeria* and *Clostridium perfringens* has been shown to reduce the length of the intestinal villi [41]. Chickens infected with *Salmonella typhimurium* were also shown to exhibit these symptoms [35].

3.4 Competitive exclusion

Ecologically speaking, two species that compete for the same resources cannot coexist indefinitely [42]. A single competitor will always win out, leading to an evolutionary change, shift to another niche or even the complete demise of the other [5]. To reduce pathogen adhesion and colonization, the intestinal microbiota competes with colonizing pathogenic bacteria [43]. There are a variety of mechanisms that could lead to this reduction, including the physical occupation of space, competition for resources in a specific niche, and even direct physical or chemical confrontation with a potential colonizer [5]. Bacteriocins, for example, have been linked to a reduction in the ability of pathogens to invade the body [44]. No mechanism has been discovered yet to explain the protective effects of the competitive exclusion process on *Salmonella* colonization in broiler chickens' intestinal tracts [5]. It has been shown that the pathogen can be controlled using a variety of products ranging from probiotics to inoculation of bedding with cultures drawn from the fecal matter produced in more productive sheds with better intestinal health [5, 17].

4. Factors affecting the GI tract microbiota

Intestinal microbiota, intestinal environment, and dietary compounds all work together to maintain a delicate equilibrium [45]. Disease can occur if this relationship is out of place [5]. Environmental factors, host age and health, and dietary habits all have the potential to influence microbial populations in either a positive or negative way [5, 45]. Aside from promoting growth and preventing the spread of endemic diseases, the use of low-dose antibiotics in livestock feed is a common practice in intensive farming [46]. Drug-resistant bacteria and public pressure to reduce the use of drugs in food-producing animals have created a need for 'natural' alternatives to boost performance and prevent disease spread [47]. However, these natural alternatives are not without their drawbacks. Intestinal microbiota can be influenced through the use of prebiotics and probiotics [48]. Specific changes in the composition and/or activity of the intestinal microflora, made possible by selective fermentation, that benefit the health and well-being of humans. "Live microorganisms that when administered in adequate amounts confer a health benefit on the host" is defined as [49]. Probiotics, prebiotics, or a combination of the two have been shown to improve the health of broilers in numerous studies [48, 49]. However promising probiotic supplements appear to be in the labs, their effects on commercial broilers vary widely [49]. There are many factors that can affect the intestinal microbiota's composition and must be taken into consideration when trying to manipulate the intestinal microbiota, including the complex relationship between the host and the microbiota [50].

Food is a major source of energy for intestinal bacteria, and as a result, diet has a significant impact on the population of bacteria in the digestive tract [29]. Since different bacterial species have different dietary requirements and preferred substrates, changing one's diet can have an impact on one's gut microbiome [51]. It has been found that when wheat was added to the diet of birds, it promotes the growth of bacteria with 50–55% Guanidine to Cytosine (GC) content and suppressed the growth of those bacteria with 60–79% content [52]. In contrast to diets based on maize, it has been revealed that populations of *Lactobacilli* and *coliforms* increased in response to wheat and barley diets [53]. The *Lactobacilli* population and *C. perfringens* are the most affected by changes in the ileal microbiota due to dietary fat source [54].

When soy oil was substituted for tallow as a source of dietary fat, there was a rise in anaerobes in the intestinal microbiota and an increase in gut transit time [55]. This allows for the manipulation of chicken microbiota through dietary changes and the inclusion of specific components (essential oils, oligosaccharides, enzymes, and specific carbohydrate sources) aimed at enhancing growth and improving intestinal tract conditions for specific commensal bacterial groups [12].

Poultry living conditions and the management that go along with them have a significant impact on their intestinal microbiota as well [56]. As a result of poor hygiene, there will be an increase in food-borne illness and wet litter issues [57]. Since farm litter is a source of bacteria for the birds and a potential source of pathogenic bacteria, proper litter management is essential [56, 57].

Age has been shown to influence the composition of the intestinal microbiota, along with host genotype [58]. The diversity and complexity of the bacterial populations in the intestinal microbiota of older and younger birds are shown to increase as the birds age [59], according to culture-independent molecular profiling techniques [45]. According to Wickramasuriya *et al.* [60], broiler chickens' ileal and caecal microbiota had remarkably similar microbiotas at 3 days of age, but after 2 weeks, these subpopulations had diverged significantly. Many factors are likely to play a role in age-related GIT microbiota changes, including changes in diet, maturation of immune systems, changes in environmental influences, and increased interplay with other animals that expose individuals to a wider range of bacteria [61].

Birds raised in xenobiotic-rich environments are more likely to have a diverse and beneficial GUT microbiota [62]. Heavy metals, plastics, and agrochemicals are just a few of the potentially harmful substances on this list. HMs and the gut microbiota interact in a variety of ways. Exposure alters the normal gut microbiota's metabolism [62].

5. Heavy metals (HMs)

Finding a variety of toxic substances in animal feed or food additives, such as arsenic, lead, cadmium, mercury, and a host of other toxins is very common [63]. In general, it refers to a group of metals with high densities, atomic weights, or atomic numbers that are either not required or only required in trace amounts [64]. As a result of their widespread use in the manufacturing, medical, and agricultural sectors, these chemicals have begun to accumulate in the environment, raising questions about their potential dangers to both human and animal health as well as the environment [65]. Ingestion, inhalation, or dermal exposure to heavy metals can cause a wide range of health issues, including neurological and neurobehavioral disorders, abnormal blood chemistry, cancers, and cardiovascular disease in humans [62].

Poultry can be exposed to a variety of toxic metals from a variety of sources [66]. The application of sewage sludge, the disposal of industrial waste, the use of pesticides and fertilizers, and atmospheric deposition are all methods by which heavy metals can contaminate soil and water [67]. These heavy metals can be found in the air, water, and soil, it is difficult to remove them from animal feed and feed supplies [68]. Heavy metal bioaccumulation and indestructibility raise the possibility of these substances serving as toxins [69]. Metals cannot be catabolized, so chelation is an option for their removal [63].

5.1 Classification of heavy metal

Heavy metals can be classified into four major groups on their health importance.

Essential: Cu, Zn, CO, Cr, Mn and Fe. These metals also called micronutrients [70] and are toxic when taken in excess of requirements [69].

Non-essential: Ba, Al, Li and Zr.

Less toxic: Sn and Al.

Highly toxic: Hg, Cd and Cd.

Heavy metals are also called trace element due to their presence in trace (10 mg Kg^{-1}) or in ultra-trace ($1 \mu\text{g kg}^{-1}$) quantities in the environmental matrices [69, 70].

6. Channels of heavy metals exposure in broiler production

Poultry feed is a common source of heavy metal pollution, as are the majority of animal feeds [71]. Heavy metal contamination in poultry birds can occur from feed or water [66]. Bioaccumulation and the food chain can transfer heavy metals from the soil to plants, animals, and ultimately humans [62]. Due to the use of plants in poultry feeding, contamination of the plant is likely to be found in poultry feed [71]. Rice bran, rice polish, solvent extracted rice and wheat bran, and molasses are all common ingredients in poultry feeds [72]. Calcium, phosphorus, trace minerals (such as Fe, Zn, Mn, Cu, CO, and Me), and vitamins A, D3, E, K, and B complex are among the other minerals and vitamins that can be found [73].

6.1 Feed

Mineral nutrition is required by all animals and heavy metals have been shown to be essential nutrients [73]. It is essential to maintain animal health and productivity because of the numerous enzymes that coordinate many biological processes, such as Co, Cu, Fe, I, Mn, Mo, Se, Zn [74]. Catalysis and regulation are two other important functions that essential metals perform [75]. Minerals are frequently added to commercial feeds to promote optimal growth, functional bioactivity, and antimicrobial properties from the standpoint of mineral nutrition, as well as to prevent mineral deficiencies that could compromise production [73]. There are many factors to consider when it comes to the optimal concentration of essential metals in feed [76]: genetic influences, diet, interactions between nutrients, bioavailability, and subclinical toxic effects [74, 77]. Since soil and climate conditions around the world have a significant impact on farming practices, the levels of heavy metal contamination in feed can vary widely, making it difficult to generalize across locations and legal restrictions [74]. In order to accurately predict the risk of metal exposure, it is necessary to consider the production system [78]. The majority of chicken feed contains trace amounts of heavy metals.

6.2 Water

Water pollution is the term used to describe the process of polluting waterways (e.g. lakes, rivers, oceans and groundwater). This type of pollution happens when contaminants are not properly handled before returning to the environment via rivers [79]. Water pollution has a negative impact on all aquatic life, including individual

species and populations, as well as natural biological ecosystems [80]. “Heavy” or “toxic,” when it comes to metals, is defined as having a density larger than five times the water density. It is important to note that these elements are stable (i.e., those that cannot be digested by the body) and bio-accumulative [63]. Among the heavy metals (the metallic form against the ionic form required by the human body) are mercury, nickel, lead, arsenic and cadmium, aluminum, platinum, and copper.

There are a lot of heavy metals in proteins that have a lot of sulfur in them. The heavy metal concentration in streams, lakes, and rivers is normally less than 0.1 ppm [81]. However, some water sources contained up to 80 ppm of heavy metals. A lack of research has been done on heavy metal concentrations in rainfall and snow [82]. Mono-methyl mercury salts and diethyl mercury salts are the most common water-soluble mercury compounds. Environmental contaminants such as heavy metals have been related to adverse effects on human and animal health [64]. When an animal consumes a large amount of an important metal, it becomes hazardous [66].

A decline in environmental quality can be brought on by the presence of heavy metals in water, soil, or the air [64, 68]. Pollution sources can be traced back to airborne particles. It can be brought to the ground by wind or by raindrops, for example [83]. Contamination of soil layers with Cd is one cause of toxic amounts of Cd in groundwater [83]. Cd will be more concentrated in the water in the pipe duct. Environmental damage occurs when heavy metals in groundwater influence organisms directly or indirectly through adverse effects on human and animal health [84].

HMS can have an impact on our gut microbiota.

In addition to morphological harm, long-term heavy metal ingestion can cause gut flora dysfunction and potentially lead to host metabolic disorders [85]. These germs can impose selection pressure on bacteria that cannot adhere to the mucosal surface [5] and hence affect gut health.

6.3 The impact of heavy metals on the makeup of the gut microbiota

HMs have been shown to limit bacterial growth in several studies [86]. When it comes to microorganisms, Cd has been proven to have harmful effects on growth and development, particularly through disrupting protein synthesis as well as numerous enzymatic processes [83]. Because HMs come into direct touch with the gut microbiota, they have a profoundly negative impact on its composition [85]. After exposure to HM, the majority of studies have shown a drop in Firmicutes and Proteobacteria abundance and a rise in Bacteroidetes abundance at the phylum level. Cd, Pb, Cu, and aluminum (Al) were shown to elicit metal-specific and time-dependent alterations in the gut microbiota of mice, and the quantity of Akkermansia reduced following exposure to these four HMs.

Antibiotics, like heavy metals, may be poisonous to microorganisms as well as dangerous to mammals [5]. As a result, antibacterial metals are being used more frequently in goods. If animals are exposed to heavy metals, their health can be affected both directly and indirectly through their toxicological effects on cells and systems as well as the impact on their animal microbiome [12]. Microbiota imbalance, or dysbiosis, has been associated to several chronic health consequences, including infection [5]. As the immune system matures, the microbiota plays an increasingly important role in ensuring that it stays in a state of homeostasis [13]. Mucus production, epithelial barrier function and inflammation are all affected by beneficial bacteria in the microbiota [27]. The microbiota and the immune system might both be weakened as a result of heavy metal exposure, raising the risk of infection. Furthermore, these

exposures might have a negative influence on health because of the rise in antibiotic-resistant bacteria [85]. Metal resistance, like antibiotic resistance, has been thoroughly documented across many different bacteria for many different metals, despite the fact that heavy metals may be hazardous to microorganisms [36]. Bacteria that are resistant to both metals and antibiotics are often found together. Co-selection of metal and antibiotic resistance genes in bacteria can be caused by a variety of methods. Antibiotic resistance and metal resistance are both coded by two different genes that microbes may have, with one stimulus triggering transcription of both genes either physically or transcriptionally coupled inside a genetic unit like a plasmid. It is also possible that bacteria may have just one gene that makes a protein set that is capable of resisting both metals and antibiotics. As a result of any of these scenarios, bacteria would be able to select for antibiotic resistance as well.

The health impacts of HMs after changes in gut microbiota caused by HMs.

Toxicity-induced gut microbiota alterations have been found to disrupt gut integrity and contribute to a number of downstream consequences [36].

Cucumber toxicity resulted in a deterioration of chicken cecum structure, with the mucosa falling off, vacuoles forming in the lamina propria, and an inflammatory response that was time-dependent. In addition to morphological harm, long-term heavy metal ingestion can cause gut flora dysfunction and possibly host metabolic disorders [11]. Another study found that alterations in the microbiota of the digestive tract have been linked to a number of ailments, including intestinal barrier permeability and inflammation [38]. It is believed that copper exposure might lead to an imbalance in the gut flora, which could have negative consequences for the health of chickens [21].

7. Conclusion

Heavy metals in the broiler chicken production environment affect the gut flora, which in turn affects the health of the animals. In order to minimize or eliminate any impact on the gut microbiota, proper rules for the use of heavy metals in feed and water should be put in place. This is critical for the consumer's health, as heavy metals may build up in the body over time and pose a health risk. Toxic heavy metals may lead to the growth of bacteria that are resistant to heavy metals and antimicrobial resistance at the same time. Regulators and testing should be put in place to limit the discharge and exposure of hazardous materials.

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