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Current Perspectives of the Chicken Gastrointestinal Tract and Its Microbiome

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ABSTRACT

The microbial communities inhabiting the gastrointestinal tract (GIT) of chickens are essential for the gut homeostasis, the host metabolism and affect the animals' physiology and health. They play an important role in nutrient digestion, pathogen inhibition and interact with the gut-associated immune system.
Throughout the last years high-throughput sequencing technologies have been used to analyze the bacterial communities that colonize the different sections of chickens' gut. The most common methodologies are targeted amplicon sequencing followed by metagenome shotgun sequencing as well as metaproteomics aiming at a broad range of topics such as dietary effects, animal diseases, bird performance and host genetics. However, the respective analyses are still at the beginning and currently there is a lack of information in regard to the activity and functional characterization of the gut microbial communities. In the future, the use of multi-omics approaches may enhance research related to chicken production, animal and also public health. Furthermore, combinations with other disciplines such as genomics, immunology and physiology may have the potential to elucidate the definition of a "healthy" gut microbiota.

1. Introduction

The global population is increasing continuously and is estimated to comprise about 9.6 billion individuals by 2050. Correspondingly, poultry production has intensified during the last years and is predicted to produce about 130 million tons of chicken meat in 2020 (OECD/FAO) to match the demands of a growing world population. Such extreme growth is only feasible with proper strategies for disease control and prevention to minimize the impact of bacterial, parasitic or viral infections of the animals and simultaneously reduce associated ecological damage and waste of resources.

Chicken breeders focused on high performance, fast growth, breast meat yield, efficiency of feed conversion rates, skeletal quality, heart and lung functionality and as well on egg production and quality. Looking for the preferred phenotypic traits and selecting the most superior individuals influenced the animals' genetics [1]. However, selection for a single trait may also affect other traits. For example, broiler chickens that were selected for meat production gained a higher body weight (~3 kg) within 42 days. On the other hand, ascites and/or lameness occurred in the animals [2]. Thus, a balanced selection across the different traits might improve the animals' well-being.

Besides breeding and selection, optimized nutrition of broiler chickens is a fundamental component of efficient poultry production. The animals' fodder accounts for 70% of the total costs in chicken production [3] and poultry diets are expensive since egg and meat production require high amounts of energy and protein sources. Diets contain energy and protein, mineral supplements, specific amino acids and vitamins in a defined formulation providing all nutrients necessary for the bird's health and adequate performance. Diets with imbalanced mineral supplementation may lead to health problems and result in inefficient use of the natural resources. Consequently, high amounts of valuable nutrients such as nitrogen, phosphorus (P), calcium (Ca) and zinc get lost by defecation and urination [4].

Gut microorganisms are mainly responsible for the degradation of complex substrates such as non-starch polysaccharides which requires highly specialized, hydrolytic enzymes [5]. The discovery of novel enzymatic tools depends on metagenomic data for instance from the broiler caeca. Recently, a xylanase gene from the chicken caecum has been isolated and overexpressed which emphasizes the potential for the development of new, optimized feed additives for industrial application [6]. Close interactions between the intestinal microbiome and the animals' diet are well established since dietary factors are known to alter the gut microbiota. Bacteria are able to hydrolyze indigestible

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carbohydrates and polysaccharides allowing further fermentation by other members of the gut ecosystem that produce short chain fatty acids (SCFA) which in turn become available for the host.

Moreover, microorganisms growing on poultry litter have an influence on the gut microbiome and may constitute a source of infection. Since the first day of life, chicks start pecking and ingesting litter materials including the adhered microorganisms that are usually detected in feces and soil. In this way, microbes of other habitats can be transferred to the gastrointestinal tract [7]. Previous studies have shown that *Salmonella* and *Clostridium perfringens* decrease in abundance in reused litter and *Campylobacter jejuni* and *Escherichia coli* become more prevalent [7]. Wang et al. compared the microbiota of fresh and reused litter and its effects on the chickens' gut microbiota finding an increase of halotolerant/alkaliphilic bacteria in reused litter and a stronger effect of the litter on the microbiota of the ileum in comparison to the caecal microbiota. Caecal samples of young birds raised in reused litter showed a higher bacterial diversity when compared to mature animals that were kept under the same conditions. The reuse of litter is a common practice in broiler production. Despite studies showing that reused litter does not exhibit higher abundances of *C. perfringens* or *Salmonella* [8], chickens raised in fresh litter revealed an increasing colonization with beneficial *Lactobacillus* spp. [9]. Proper litter management may reduce pathogen activity, promote a balanced gut microbiome and improve the chickens' health status.

This review will focus on the methodologies that were used in the past years to characterize the microbial communities within the chickens' gut to provide insights into the effects of different feeding strategies and host genetics on the gut microbiome. New perspectives will elucidate yet unknown aspects of the chickens' gut microbiome.

2. Exploring the Composition and Function of the Chicken Gut Microbiome

2.1. Targeted Amplicon Sequencing of the 16S rRNA Gene

Next-generation sequencing revolutionized the characterization of microbial communities. The respective studies are mainly based on amplifying the small subunits of the 16S ribosomal gene of Bacteria and Archaea, the 18S rRNA gene of eukaryotic species and the nuclear ribosomal internal transcribed spacer (ITS) regions of Fungi [10]. In this way, deep characterization of microbial communities and quantification of relative abundances of the different microorganisms can be achieved. Most of the studies available aim at the bacterial 16S rRNA gene. Even though this method has been used in other scientific disciplines for several years, the first study characterizing the chickens' gastrointestinal microbiota was published in 2011 [11]. The 16S rRNA gene comprises nine hypervariable regions [12]. However, so far microbial studies of the chickens' gut have covered the V1–V3, V3–V4, V4–V5, V1, V3 or V4 regions [5,7,11,13–18]. The sequencing technologies of choice are Roche 454-pyrosequencing, Illumina MiSeq, HiSeq and Ion PGM systems [19]. Bioinformatic processing of the generated sequences can be achieved by employing open sources platforms such as QIIME [20] and mothur [21] that, in order to perform taxonomic assignments, depend on public databases like GreenGenes [22], the ribosomal database project (RDP) [23] and SILVA [24]. The latter represents the most recent database. Functional prediction algorithms such as PICRUSt and Tax4Fun can be used to obtain further information from 16S rRNA gene sequencing data. PICRUSt is based on the GreenGenes database and uses an algorithm with proved accuracy regarding humans, soils and mammalian guts [25]. However, the GreenGenes database was last updated in 2013. Tax4Fun employs the SILVA database and claims to reach higher correlations regarding the functional predictions since the link association is based on the nearest neighbor with a minimum sequence similarity. Despite the promising information that can be obtained by functional prediction processing, caution is advised when drawing strong conclusions since there are large numbers of operational taxonomic units (OTUs) that cannot be assigned to a specific genus and not even to a family level [31]. Moreover, the respective approaches should be validated thoroughly in particular for avian species since their deviating organism may imply different functions and associations between microorganisms and the host.

More than 900 bacterial species inhabit the GIT of broilers being involved in the digestion of food, breakdown of toxins, stimulation of the immune system, exclusion of pathogens and endocrine activity. Interactions between microorganisms and the GIT influence the stability of the microbial communities, the animals' health, growth and consequently also feed conversion rates [26]. As feed is ingested and moves through the GIT, different groups of microbes start the digestion. The chickens' GIT is divided into three parts: the upper segment, small intestine and large intestine that are colonized by microbes in their entire length. Due to the enormous diversification of each GIT section, they are commonly studied as independent ecosystems. However, it is known that the different sections are highly interconnected and thus also influence each other's community composition [27]. Variations regarding the protocols for DNA extraction, choice of the amplified 16S rRNA gene regions and overall microbial community characterization make comparison between studies difficult. The study design strongly influences the microbial profiles of each gut section due to the differences between individual birds, species, gender, age, genetics, diets and housing. Microbiota studies in individual chickens showed a high inter-individual variation, disregarding the identical diet composition or housing conditions [5,13,16].

In the crop, breakdown of starch and lactate fermentation are initiated by several *Lactobacillus* sp. and *Bifidobacterium* sp. as well as by members of the Enterobacteriaceae family that were also detected within this section [28]. Lactobacilli also appear in high abundances in the proventriculus and gizzard. Nutrient absorption occurs in the ileum which exhibits high numbers of *Lactobacillus* sp. and to a lesser extend bacteria with butyrate producing activities such has *Clostridium*, *Streptococcus* and *Enterococcus* [28]. Fermentation and digestion of complex substrates such as cellulose, starch and other polysaccharides occur in the caecum, which is the most diverse gut section characterized by the longest feed retention time (12—20 h). In contrast, only 2.5 h are required to pass through the upper parts of the intestine [36]. The most abundant families within the caecum are Clostridiaceae, Bacteroidaceae, Lactobacillaceae and butyrate producers like Lachnospiraceae. The caecum is highly dominated by not yet characterized bacteria and exhibits the highest concentrations of short chain fatty acids (SCFA) [28]. As broilers age, their caecal microbiota becomes more diverse. Out of 50 genera detected on day zero post-hatching the caecal genera increased to above 200 on day 42 post-hatching [29]. Temporal fluctuations occur particularly in the fecal microbiota due to the random emptying of the GIT section [30].

Previous studies of chicken broilers focused on lumen samples neglecting the mucosa that is mainly composed of mucins and glycans which promote colonization by distinct groups of microorganisms. Studies in humans, mice, rats, macaques, pigs and cows showed a divergence between lumen- and mucosa-associated microbiota structures [38–41]. In contrast to the continuous flux of nutrients in the lumen, the mucosa is expected to show a more stable balance of nutrients which may represent a selective criterion for certain bacterial species [39]. A recent comparison between lumen and mucosa associated microorganisms revealed a much greater microbial community richness in the mucosa, particularly in the ileum and caecum of broiler chickens [13]. *Pseudomonas* spp. were detected in the ileal mucosa but not in the lumen. These species have the ability to hydrolyze phytate, degrade starch and in soils they are known to improve plant phosphorus availability [31]. Species belonging to the genera of *Clostridium* XI and *Ralstonia* were present in higher abundance in mucosa samples, while *Lactobacillus* sp. were three times more abundant in the ileal lumen. High abundance of commensal *Clostridium* XI species might induce a greater bacterial translocation from the ileal mucosa to the lymph

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