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Review

MYCOBIOTA OF CHICKEN GASTROINTESTINAL TRACT – COMPOSITION, IMPACT AND METHODS OF STUDY

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Summary

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Investigating the fungal elements within the chicken gastrointestinal tract (GIT) is essential for enhancing poultry health and productivity. This understanding may facilitate the creation of specific probiotics or dietary strategies aimed at improving gut health and overall performance in poultry. Bacterial and viral infections in chickens can significantly affect the fungal aspects of the gut microbiome, potentially resulting in fungal overgrowth, mycotic infections, and various health and production challenges. By implementing dietary strategies that focus on improving the fungal component of the chicken GIT, it is possible to enhance gut health, nutrient absorption, and overall poultry performance. Certain fungi within the chicken GIT play diverse roles, ranging from beneficial to harmful. Beneficial fungi, such as *Saccharomyces cerevisiae* and specific *Aspergillus* species, promote nutrient absorption and gut health, while pathogenic fungi like *Candida* and mycotoxin-producing *Fusarium* can pose serious health threats. Environmental factors including temperature, humidity, feeding composition, litter management, ventilation, and seasonal variations significantly affect fungal diversity in poultry. Additionally, microbial balance, immune suppression, increased pathogenicity, and mycotoxin production are critical considerations. To study the fungal component of the chicken GIT microbiome, special techniques are necessary for accurate assessment of the diversity and composition of fungal communities. Recent advancements in sequencing technologies and metagenomic methods are particularly beneficial for gaining a deeper understanding of the intricate fungal communities present in poultry. Future research should aim to further elucidate these interactions, which will aid in the developing of effective management strategies to reduce risks to poultry health.

Key words: beneficial fungi, chickens, composition, environmental factor, gastrointestinal tract, mycobiota

INTRODUCTION

The gastrointestinal tract (GIT) of chickens hosts a diverse community of microorganisms, known as chicken gut microbiome. It includes bacteria, protists, archaea, viruses, and fungi, but is dominated by bacteria (about 1×10^{14}) (Sergeant *et al.,* 2014; Peixoto *et al*., 2021). The fungal GIT component is gaining increasing

attention due to its potential role in gut health, nutrient absorption, and overall poultry productivity. Poultry mycobiome studies were culture based, and mostly restricted to the caecum (Hume *et al.,* 2012; Byrd *et al*., 2017), where *Candida* or *Aspergillus* were predominant. Surprisingly, in a culture-independent pyrosequencing approach study, only two *Cladosporium* species were detected in the cecum of broilers fed an unmedicated diet, although another seventeen fungal species were detected in birds supplemented with essential oils (Hume *et al*., 2012).

A study on the fungal diversity of chicken caeca reported *Aspergillus* as the most frequently found fungal genus, which constitutes 18.4% of all fungal species, followed by *Penicillium* (15.6%), *Verticillium* (6.2%), and *Sporidiobolus* (5.2%) (Byrd *et al.,* 2017). In addition, *Fusarium*, *Wallemia*, and *Aspergillus* species contribute about 84% of the total ileal fungal mycobiota in chickens (Yang *et al.*, 2021). However, the relative abundance of different fungal genera within a region of the gut varies depending on the sources of feed ingredients and the housing environment (Robinson *et al.*, 2022). The fungal load in broilers ranges from 1.6×10^3 to 3.9×10^3 CFU/g of digesta in broilers (Robinson, 2019). The abundance of gut fungal mycobiota in poultry is highest in the crop (9.1×10^5 CFU/g), followed by the ventriculus (6.2×10^5 CFU/g) and lowest in the duodenum (0.1×10^5 CFU/g) (Robinson *et al.*, 2022). The higher abundance of mycobiota in the upper GI tract might be due to the lower enzymatic activity and higher pH (Yang *et al*., 2021). Moreover, fungal metabolites serve as signalling molecules that exert anti-inflammatory, antibacterial, and trophic effects (Mogilnicka & Ufnal, 2019), thus preventing enteric infections. Probiotic yeast species secrete metabolites including amino acids, which promote the growth of beneficial gut bacteria such as *L. lactis* and *L plantarum* (Ponomarova *et al*., 2017). *Lactobacillus* in turn produces lactose, which can serve as a carbon source for the yeasts (Ponomarova *et al.,* 2017). Robinson *et al.* (2020) found that out of 124 sequenced samples, a total of 468 unique fungal features that belong to four phyla and 125 genera were identified. Ascomycota and Basidiomycota were represented by 90% to 99% of the intestinal mycobiota, with three genera (*Microascus*, *Trichosporon*, and *Aspergillus*) accounting over 80% of the total fungal population in most GIT segments.

This review aims to shed light on the fungi of the chicken digestive system as a components of the complex community that plays an essential role in the life of chickens by collecting published data mostly from PubMed Central (PMC) website, journals in veterinary science and veterinary medicine, Poultry Science, Poultry Research and Avian Diseases journals. The data were classified as being available for interested students, researchers, and specialists.

CLASSIFICATION OF GASTRO-INTESTINAL TRACT MYCOBIOTA

The GIT fungal community (mycobiota) can be classified according to the incubation conditions into two major groups: aerobic and anerobic:

Aerobic fungi

- Yeasts

In chickens, *Candida* spp. can cause opportunistic infections like candidiasis, leading to symptoms like diarrhoea and poor feed conversion (Ibrahim *et al.*,

2020). The incidence of *Candida* in the crops ranged from 17.4–51.5%. In 573 birds examined, the *Candida* population in the crops was less than 1%, with visible lesions associated with C*andida*. *C. albicans* made up 95% of the isolates, while *C. ravautii, C. salmonicola, C. guilliermondi, C. parapsilosis, C. catenulata,* and *C. brumptii* accounted for the rest. The prevalence and quantity of *Candida* spp. in the crop were associated with the management practices on the farm (Wyatt & Hamilton, 1975). *Candida* spp. are part of the normal gut flora but can cause opportunistic infections with transition between yeast and hyphal forms depending on environmental conditions (Köhler *et al.,* 2009). *Saccharomyces* spp. can aid in the fermentation of feed components, improving nutrient utilisation and gut health (Jha & Mishra, 2021). To explore the prevalence of yeast species colonising the GIT of 5-week-old healthy turkeys, Sokół *et al.* (2018) collected and cultured samples from the beak cavity, crop, and cloaca. The isolates were morphologically and biochemically classified, as well as submitted to genetic analysis based on ITS1-5.8rRNA-ITS2 fragment sequencing. The yeast positive samples were 12.4%. The highest frequency of strains was 50% obtained from the crop, followed by 30.8% from the beak cavity and 19.2% from the cloaca. The most frequently isolated yeast belonged to the *Candida* spp., accounting for 88.5%, followed by *Trichosporon* at 7.7% and *Rhodotorula* at 3.8%. Among the species, *C. catenulata* was the most common (30.7%), followed by *C. albicans* (21.7%), *C. palmioleophila* and *C. rugosa* both with 17.4%, and *C. glabrata* (8.7%) (Sokół *et al*., 2018).

- Molds

Filamentous fungi such as *Aspergillus, Penicillium*, and *Cladosporium* identified in the GIT may be transient members of the gut mycobiota or play a role in certain GIT disorders (Underhill & Iliev, 2014). *Aspergillus* spp. and *Fusarium* spp. can produce mycotoxins, which can cause immunosuppression and liver damage in chickens (Vörösházi *et al.,* 2024).

The predominant mold species in the chicken GIT include *Aspergillus* spp., particularly *A. flavus* and *A. fumigatus* (Bano *et al*., 2019; Pires *et al*., 2024). Other fungi, such as *Penicillium* spp. and *Fusarium* spp., have been also isolated from the intestines of chickens (Gibbons *et al*., 2018). The prevalence of molds in chicken intestines can vary based on geographical location and environmental conditions. Higher fungal contamination rates in poultry farms located in humid climates and areas with poor sanitation and ventilation were detected (Bano *et al*., 2019; Pires *et al.*, 2024). Species like *Histoplasma* and *Blastomyces* can exist in both yeast and mold forms, depending on environmental cues.

Anaerobic fungi

Anaerobic fungi of the phylum Neocallimastigomycota are found in the GIT of chickens and other ruminants (Gruninger *et al*., 2014). These fungi play a significant role in the degradation of complex plant polysaccharides, improving feed utilisation and nutrient availability.

COMPOSITION OF GASTRO-INTESTINAL TRACT MYCOBIOTA

Research on the mycobiome in avian species remains limited, with most studies being culture-based and focused solely on the caecum (Byrd *et al.* 2017, Sokół *et al*., 2018). In culture-dependent studies, *Candida* and *Aspergillus* are the predominant genera found in the chicken caecum. Con-

versely, a culture-independent pyrosequencing study identified only two *Cladosporium s*pecies in the caecum of broilers fed an unmediated diet (Hume *et al*., 2012). The fungi were cultivated using potato dextrose agar (PDA) medium.

A total of 50 pure fungal isolates were obtained from chickens of three different ages: 4 days, 1 week, and 2 months, with 5, 10, and 35 isolates, respectively. The highest concentration of isolates was found in the ileum, followed by the caecum, jejunum, and duodenum. These fifty fungal isolates represented seven species: *A. fumigatus, A. niger, C. crassa, M. circinelloides, Mucor sp, R. oligosporus,* and *R. oryzae* (Yudiarti *et al*., 2012). Illumina sequencing conducted to analyse the intestinal mycobiome in 28-day-old chickens revealed that the genus *Microascus* was predominant, and a significant difference in caecal mycobiome composition was seen between 14-days and 28-days old chickens (Robinson *et al*., 2020). Robinson *et al*. (2022) employed Illumina sequencing to investigate the internal transcribed spacer 2 (ITS2) region of fungal rRNA genes, focusing on spatial and temporal variations in the mycobiota. Their research emphasised the biogeography and succession of the GIT mycobiota in broiler chickens, showing the fungal sources responsible for both initial colonisation and long-term establishment in the GIT. The results showed that mycobiota was more diverse in the upper GIT compared to the lower tract up to 42 days of age. The dominant phyla in the intestinal mycobiota were Ascomycota and Basidiomycota, with *Gibberella, Aspergillus*, and *Candida* being the most prevalent genera. A core mycobiome was found, comprising twenty-six fungal taxa that representing over 85% of the fungal population in each GI location. The total fungal population varied, ranging from 1.0×10^4 to 1.1×10^6 /g of digesta along the GIT, accounting for less than 0.06% of the bacterial population in 42-day-old broilers. The mycobiota from the hatchery environment was identified as the primary source for the first colonisation of GIT in newly hatched chicks, with *F. pseudonygamai* consistently recognised as the dominant fungal taxon across all broiler chickens ages (Robinson *et al*., 2022). Davies *et al*. (2022) examined the fungal communities, or mycobiomes, in the mucosa of the chicken ileum and caecum from hatching till the 14 days of age. All mycobiomes were composed of *Gibberella*, although *Aspergillus, Cladosporium, Sarocladium, Meyerozyma*, and *Penicillium* were also prevalent. In the caecal and ileal lumens, *Penicillium* was found in extremely low quantities or was absent during the first two days but increased over time, while *Meyerozyma* and *Wickerhamomyces* also showed gradual increases in luminal sites.

IMPORTANCE OF CHICKEN GASTROINTESTINAL TRACT FUNGI

The presence of beneficial fungi can enhance nutrient availability and improve the overall gut environment by producing metabolites that inhibit pathogenic organisms. A balanced gut microbiome that includes beneficial fungi is associated with improved digestion, better feed conversion ratios, and enhanced immune function. Conversely, an imbalance or overgrowth of pathogenic fungi, such as *Candida*, can lead to gastrointestinal disorders and negatively affect growth rates and overall health (Bai *et al*., 2021a).

GIT mycobiota is not just a collection of individual species but a complex community that interacts with bacteria, vi-

ruses, and other microorganisms. The composition and dynamics of this fungal community play a role in keeping gut homeostasis and influencing human health and disease (Richard & Sokół, 2019).

The fungal component of the GIT mycobiota of chickens plays crucial roles in nutrient digestion, feed utilisation, and maintain gut homeostasis. However, dysbiosis or overgrowth of certain fungal species can also contribute to gastrointestinal diseases and health issues in poultry (Aruwa & Sabiu, 2024). Fungi in the GIT mycobiota of chickens play several important roles.

- Normal flora and digestion

Fungi, particularly yeasts, are part of the normal gut microbiota in chickens, aiding in the digestion of complex carbohydrates and contributing to nutrient absorption. *Saccharomyces* species can enhance the fermentation of feed components, improving overall gut health (Jha & Mishra, 2021).

- Pathogenic potential

Aspergillus is opportunistic pathogens in chickens, particularly under stress conditions or in immunocompromised birds. *Candida* spp. is often linked to candidiasis in poultry, resulting in symptoms such as diarrhoea and poor feed conversion (Ibrahim *et al*., 2020). The intestinal mycobiota of chickens is dominated by Ascomycota, followed by Basidiomycota, which together account for 97–99% of the ileal mycobiota in broilers. In cases of necrotic enteritis (NE), *Wallemia* and *Aspergillus* are reduced. Additionally, *A. vitricola, A. magnivesiculatus,* and several unidentified *Aspergillus* species show a negative correlation with the severity of NE (Yang *et al*., 2021).

- Impact on gut health

Recent research shows that healthy intestinal mycobiota plays a crucial role in maintaining host homeostasis, modulating immune responses, and competitively excluding pathogens (Li *et al*., 2019). For instance, colonisation by *C. albicans* helps protect mice from infections caused by virulent fungi and bacteria by promoting the expansion of Th17 cells and activating neutrophils, thereby strengthening the host defense against extracellular pathogens (Shao *et al*., 2019).

- Mycotoxicosis

Chickens are susceptible to mycotoxins produced by molds such as *Aspergillus* and *Fusarium*. These toxins can contaminate feeds, leading to severe health issues, including immunosuppression and liver damage (Vörösházi *et al*., 2024). Mycotoxins can alter the gut microbiota composition in chickens, leading to a shift towards a dysbiotic community (Li *et al*., 2018). Aflatoxin B1 (AFB1), a common mycotoxin found in feed, has been shown to decrease the abundance of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium,* while increasing the abundance of pathogenic bacteria, such as *Escherichia coli* and *Salmonella* (Chen *et al*., 2020). Similarly, ochratoxin A (OTA), another mycotoxin, has been found to alter the gut microbiota composition, leading to a decrease in short-chain fatty acid production and an increase in inflammatory markers (Wang *et al*., 2019).

- Role in immune response

Fungi in the GIT can modulate the immune response of chickens. Certain fungal components can stimulate the immune system, enhancing the host ability to resist infections (Aruwa & Sabiu, 2024).

INTERACTIONS OF CHICKEN GASTROINTESTINAL TRACT MYCOBIOTA

The interaction between fungal and bacterial populations in the chicken GIT is complex. Fungi can influence bacterial communities through the production of enzymes and metabolites, which can either promote or inhibit the growth of specific bacterial species. For instance, research has shown that certain fungi can help the growth of beneficial bacteria, thereby enhancing the overall microbial balance in the gut (Zhang *et al*., 2019). Anaerobic fungi interact with the bacterial community in the chicken GIT, forming complex symbiotic relationships that influence overall gut health and function. The fungal-bacterial interactions can change the production of volatile fatty acids, which are essential for gut health and immune function (Song *et al*., 2023).

The balance and interactions between beneficial and pathogenic fungal species in the chicken GIT can have significant implications for the overall health and productivity of the birds. A diverse and balanced fungal community can support nutrient absorption, enhance immune function, and suppress the growth of harmful microorganisms. In contrast, an imbalance favoring pathogenic fungi can lead to increased susceptibility to infections, reduced growth performance, and economic losses for poultry producers (Ruff & Finkler, 2018).

Monitoring and managing the fungal composition in the chicken GIT through techniques including probiotics, feed additives and environmental control, can help maintain a healthy and productive gut microbiome (Félix *et al*., 2020). Further research is needed to elucidate the complex interactions between various fungal species and their effects on chicken health and productivity.

Viral infection risk on the fungal component in the chicken GIT

Viral infections can have significant impacts on the fungal part of the microbiome, potentially leading to various health and production-related issues in chickens. Viral infections can disrupt the balance of the gut microbiome, including the fungal community (Chiang *et al*., 2019). Certain viruses, such as avian influenza virus (AIV) and infectious bursal disease virus (IBD), have been shown to alter the diversity and abundance of gut fungi in chickens (Ruff & Finkler, 2018; Gong *et al*., 2019). This disruption can lead to overgrowth of opportunistic fungal species, such as *Candida* and *Aspergillus*, which can cause mycotic infections in GIT (Félix *et al*., 2020). Cloacal swabs were collected from immunosuppressed chickens affected by IBD to isolate fungal species on specific media. A total of 19 fungal isolates were morphologically found. Among these, *Aspergillus* isolates were the most prevalent, accounting for 42%, followed by *Trichosporon* and *Penicillium*, each at 10.5%, *Fusarium* at 5%, *Candida* at 1%, and unidentified isolates making up 26%. The study further classified the eight *Aspergillus* isolates, with A. fumigatus being the most often identified species, occurring in 4 out of all 19 isolates and 4 out of the 8 *Aspergillus* isolates. The other identified species included *A. flavus* (2 isolates), *A. niger* (1 isolate), and *A. terreus* (1 isolate). Molecular identification of five representative *Aspergillus* isolates confirmed the presence of *A. fumigatus* (n=2), *A. flavus* (n=2), and *A. niger* (n=1) (Ghetas *et al*., 2022). A total of 88 different fungal and yeast species were identified from caecal

samples from commercial broiler and layer flocks by using automated repetitive sequence-based PCR, including *Aspergillus, Penicillium,* and *Sporidiobolus* species, and 18 unknown genera (Byrd *et al*., 2017). Furthermore, 50 fungal isolates belonging to seven species (*Aspergillus fumigatus, Aspergillus niger, Chrysonilia crassa, Mucor circinelloides, Mucor* spp., *Rhizopus oligosporus* and *Rhizopus oryzae*) have been isolated from various parts of the chicken GIT (Yudiarti *et al*., 2012).

Recently, the next-generation sequencing of chicken intestinal mycobiota declared that three fungal genera represented by *Scopulariopsis brevicaulis* (the anamorph form of *Microascus*), *Trichosporon asahii*, and two species of *Aspergillus* were predominant. These three genera accounted over 80% of the total fungal population in most segments of the GIT (Robinson *et al*., 2020). Interestingly, many species belonging to these genera (*Microascus, Trichosporon,* and *Aspergillus*) are considered opportunistic pathogens particularly in immunocompromised subjects (Sugui *et al*., 2014).

Fungal overgrowth and infections in the chicken GIT can have several negative consequences. Mycotic infections can lead to reduced nutrient absorption, impaired gut barrier function, and increased inflammation, all of which can affect the overall health and performance of the chickens (Félix *et al*., 2020; Dumas *et al*., 2021). Additionally, some fungal species can produce toxins, such as aflatoxins, which can be harmful to the chickens and potentially enter the food chain (Antonissen *et al*., 2014).

Furthermore, viral infections can weaken the chickens' immune system, making them more susceptible to fungal infections and reducing their ability to mount an effective response (Ruff & Finkler, 2018). This can lead to more severe and persistent fungal infections, further worsening the negative impacts on the birds.

Bacterial infection risk on the fungal component in the chicken GIT

Certain pathogenic bacteria can form symbiotic relationships with specific fungi, leading to enhanced virulence and increased risk of infection. For example, the opportunistic pathogen *C. albicans* has been shown to help the invasion and colonisation of the intestinal tract by *S. enterica* in chickens (Sam *et al.,* 2017). Furthermore, disruptions to the delicate balance of the chicken GIT mycobiome, such as by antifungal medications, can create opportunities for pathogenic bacteria to proliferate. This may increase the likelihood of bacterial infections, including those caused by *C. perfringens*, a major contributor to NE in poultry. In addition, some fungal species in the chicken GIT may produce mycotoxins, which can have immunosuppressive effects and make the host more susceptible to bacterial infections (Antonissen *et al*., 2014).

It was reported that the total fungal population and relative abundance of fungal species in the poultry GIT were altered by inflammatory conditions and infections, for instance in case of severe NE, the total fungal population decreased 5-fold compared to non-infected controls. Additionally, the diversity of the ileal mycobiota was tended to decrease in chickens suffering from NE (Yang *et al*., 2021).

DIETARY INTERVENTIONS ENHANCING THE FUNGAL COMPONENT IN CHICKEN GASTROINTESTINAL TRACT

Enhancing the fungal component of the chicken GIT can be achieved through targeted dietary interventions. Several studies have explored the use of various feed additives and supplements to modulate the fungal population and promote balanced gut microbiome.

- Prebiotics

Prebiotics are non-digestible food ingredients that selectively stimulate the growth and activity of beneficial microorganisms, including fungi, in the gut. Butyrate, inulin and fructooligosaccharides (FOS) are commonly studied prebiotics that have been shown to increase the abundance of beneficial fungi, such as *Saccharomyces* and in the chicken GIT (Hosseini *et al*., 2016; Onrust *et al*., 2021).

- Probiotics

Probiotic supplements containing beneficial fungal strains, such as *Saccharomyces cerevisiae*, have been demonstrated to enhance the fungal part of the chicken gut microbiota. These probiotic fungi can outcompete pathogenic fungi, produce antimicrobial metabolites, and promote the growth of other beneficial microorganisms (Fathi *et al.*, 2017).

- Fermented feed

Incorporating fermented feed ingredients, such as fermented soybean meal or corn, into the chicken diet can increase the abundance of beneficial fungi in the GIT. The fermentation process promotes the growth of fungal species that can improve nutrient availability and gut health (Zhang *et al*., 2019).

- Dietary fibre

Dietary fibres, such as beta-glucans and mannan-oligosaccharides, have been shown to selectively stimulate the growth of beneficial fungi in the chicken gut. These fibres serve as a substrate for fungal fermentation, leading to the production of metabolites that can enhance gut health and support the overall microbial balance (Shao *et al*., 2021).

FUNGAL DIVERSITY IMPLICATIONS FOR CHICKEN HEALTH AND PRODUCTIVITY

Fungal diversity in the GIT of chickens plays a crucial role in their overall health and productivity. The presence and balance of various fungal species can have significant effects on nutrient absorption, immune response, and disease resistance:

- Nutrient absorption and digestion

Fungi contributes to the breakdown of complex carbohydrates and other nutrients in the GIT, which can enhance nutrient availability for chickens. For example, certain fungi can produce enzymes that aid in the digestion of dietary components, leading to improved feed efficiency and growth rates (Félix *et al*., 2020). A diverse fungal community can help improve these processes, ensuring that chickens obtain maximum nutritional benefits from their feed.

- Immune system modulation

Fungal diversity can influence the immune response of chickens. Beneficial fungi may stimulate the immune system, enhancing the birds' ability to fight off infections. Conversely, a lack of diversity or an overabundance of pathogenic fungi can lead to immune dysfunction and increased susceptibility to diseases (Ruff & Finkler, 2018). Maintaining a balanced

fungal population is essential for supporting the gut-associated lymphoid tissue (GALT), which plays a key role in immune function.

- Pathogen competition

A diverse fungal community can help suppress the growth of pathogenic fungi and bacteria through competitive inhibition. For instance, beneficial fungi can outcompete harmful species for resources and space in GIT, reducing the likelihood of infections (Chiang *et al*., 2019). This competitive dynamic is critical for supporting gut health and preventing disease outbreaks in poultry.

- Mycotoxin production

Not all fungi are beneficial; some can produce mycotoxins that are harmful to chickens. Fungal diversity includes both non-toxigenic and toxigenic species, and an imbalance favoring toxigenic fungi can lead to mycotoxin contamination in feed. This can result in serious health issues (Antonissen *et al*., 2014). Monitoring and managing fungal diversity are thus vital to mitigate these risks.

- Impact on productivity

Fungal diversity has direct implications for poultry productivity. Healthy chickens with a balanced gut microbiome, including a diverse fungal population, tend to have better growth performance, feed conversion ratios, and overall health (Gong *et al*., 2007). On the other hand, disruptions in fungal diversity can lead to increased morbidity and mortality, poor production efficiency and economic viability for poultry producers (Dumas *et al*., 2011).

ROLE OF SPECIFIC FUNGI IN CHICKEN GASTROINTESTINAL TRACT

Fungi play a crucial role in the GIT of chickens, influencing various physiological processes that affect their health and productivity. They can improve the nutrient absorption of feed grains, thereby enhancing the overall nutritional quality of the chicken's diet (Solaiman *et al*., 2005).

Beneficial fungi

According to a recent research, those mycorrhizal fungi that establish symbiotic relationships with plant roots, may also benefit poultry nutrition. Incorporating mycorrhizal fungi into chicken diets as yeasts and its products could offer more advantages for growth and health. (Fathima *et al*., 2023):

- *Saccharomyces cerevisiae*

Saccharomyces cerevisiae, commonly known as brewer's yeast, is a beneficial fungus often added to poultry diets. It supports gut health by fostering the growth of beneficial bacteria, thus enhancing the diversity of the gut microbiome (Kyoung *et al*., 2023). This yeast species is widely used as a probiotic in poultry nutrition. It has been shown to improve nutrient absorption, boost the immune system, and inhibit pathogenic bacteria in the GIT (Shanmugasundaram *et al*., 2013). The yeast cell wall contains bioactive compounds, including various polysaccharides such as mannans, chitin, β-1,3-glucans, and 1,6-glucans. These compounds can enhance the host's immune response, reduce pathogen loads, and mitigate the negative effects of enteric infections, resulting in health benefits for the chickens (Fathima *et al*., 2023). Additionally, this yeast facilitates nutrient absorption, particularly of proteins and vitamins, which improves growth performance and efficiency (Perricone *et al.,* 2021).

- *Aspergillus* spp.

Certain species of *Aspergillus* (*A. nidulans*) are recognised for their ability

to break down complex carbohydrates and produce various industrial enzymes such as cellulases, β-glucosidases, hemicellulases, laccases, lipases, proteases, β-galactosidases, tannases, keratinase, cutinases, and aryl-alcohol oxidase (Kumar, 2020). These enzymes assist in digesting fibrous feed components, thereby increasing nutrient availability for the chickens. Improved fibre digestion leads to better energy utilisation and overall health (Gong *et al.*, 2007).

- *Bacillus subtilis*

Certain *Bacillus* species, including *B. subtilis*, are known to produce enzymes that aid in the digestion of complex carbohydrates, proteins, and lipids. This can lead to improved feed utilisation and growth performance in chickens (Latorre *et al*., 2016).

- *Trichoderma* spp.

Fungi from the *Trichoderma* genus can produce a variety of enzymes that break down cellulose, hemicellulose, and other plant-based components in the chicken feed. This can enhance the availability of nutrients and improve feed efficiency (Shabani *et al*., 2012).

Pathogenic fungal species

- *Aspergillus* spp.

Aspergillus is a genus of fungi that can cause aspergillosis, a respiratory disease in chickens. Aspergillosis in poultry is mainly related to *A. fumigatus,* while *A. teneus, A. glaucus*, *A. nidulans, A. niger*, *A. amstelodami*, and *A. nigrescens* are rarely isolated. Outbreaks occur when the organism is present in sufficient quantities to establish disease or when the host resistance is lowered by environmental stresses, immunosuppressive agents, inadequate nutrition, or other infectious diseases (Charlton *et al.,* 2008). Chute *et al.* (1956) observed that *A. fumigatus* is found frequently and is not always pathogenic in young broiler chicks. Certain *Aspergillus* species can also produce mycotoxins (Dönmez *et al*., 2016).

- Candida spp.

Candida species are opportunistic fungi that can cause candidiasis, a fungal infection in the GIT of chickens. Candidiasis can lead to diarrhoea, malabsorption, and reduced growth performance (Rossi *et al*., 2020). Candida species can be opportunistic pathogens in chickens. Under conditions of dysbiosis or immune suppression, *Candida* spp. can proliferate, leading to infections that may result in gastrointestinal disturbances, reduced nutrient absorption, and overall health decline (Ruff & Finkler, 2018). Managing fungal diversity is crucial to preventing the overgrowth of such pathogenic species.

- Mycotoxin-producing *Fusarium* spp.

Fusarium spp. fungi are known to produce a wide range of mycotoxins, such as deoxynivalenol (DON) and zearalenone (ZEN), which can negatively impact the health and productivity of chickens (Antonissen *et al*., 2014), as well as trichothecenes, which can contaminate feed and pose serious health risks to chickens. These mycotoxins can lead to immunosuppression, reduced growth rates, and increased susceptibility to infectious diseases (Antonissen *et al*., 2014; Dönmez *et al*., 2016).

ENVIRONMENTAL FACTORS INFLUENCING FUNGAL DIVERSITY IN POULTRY

Fungal diversity in poultry is significantly influenced by various environmental factors, which can impact the health and productivity of poultry.

- Temperature and humidity

Environmental conditions such as temperature and humidity play a crucial role in shaping fungal communities. High humidity levels can create favourable conditions for fungal growth, leading to increased colonisation by both beneficial and pathogenic fungi (Pires *et al.*, 2024). Warmer and more humid environments tend to favor the growth of opportunistic fungi, such as *Aspergillus* and *Candida*, which can lead to increased risk of mycotic infections (Dönmez *et al*., 2016). *Aspergillus* species thrive in warm, humid environments, which can lead to mycotoxin production and associated health risks in poultry (Ochieng *et al*., 2021).

- Feed composition

The type and quality of feed significantly affects fungal diversity in poultry. Nutrient-rich feeds can promote the growth of beneficial fungi, while contaminated or poor-quality feeds can lead to the proliferation of harmful fungi (Antonissen *et al*., 2014). The presence of specific substrates in feed can also select for fungal species, influencing the overall gut microbiome composition as diets high in carbohydrates or containing mycotoxin-contaminated ingredients can promote the growth of specific fungal species, such as Fusarium can produce harmful mycotoxins (Antonissen *et al*., 2014).

- Litter management

The management of litter in poultry housing can affect the fungal diversity in the environment. Poor litter management increases moisture and nutrient accumulation, fostering fungal growth and potentially leading to pathogenic outbreaks (Ochieng *et al.,* 2021). Regular cleaning and the use of litter additives can help maintain a balanced microbial environment, reducing harmful fungal populations. Litter with high moisture content, poor drainage, or contamination with organic matter can provide a favorable environment for the growth of pathogenic fungi (Dumas *et al.,* 2011).

- Ventilation and air quality

Good ventilation in poultry housing is essential for maintaining optimal air quality and reducing humidity levels. Poor ventilation can lead to the accumulation of ammonia and other gases, creating an environment conducive to fungal growth (Ochieng *et al.,* 2021). Improved air quality not only reduces fungal contamination but also supports overall poultry health and performance.

- Seasonal changes

Seasonal variations can also influence fungal diversity. For example, warmer seasons may promote the growth of certain fungi, while colder seasons may favour others. This seasonal shift can impact on the overall microbial balance in the gut and the surrounding environment (Gong *et al.*, 2007).

- Biosecurity and sanitation

Proper biosecurity measures and sanitation practices in poultry facilities can significantly impact fungal diversity. Poor hygiene, inadequate cleaning and disinfection, and the introduction of contaminated equipment or personnel can facilitate the spread of pathogenic fungi (Ruff & Finkler, 2018).

- Antibiotic use

The use of antibiotics in poultry production can disrupt the delicate balance of the gut microbiome, including the fungal community. Indiscriminate or excessive antibiotic usage can lead to a decrease in beneficial fungi and an overgrowth of opportunistic species, making birds more susceptible to fungal infections (Félix *et al*., 2020).

- Stress and immunosuppression

Factors that induce stress or weaken the immune system in poultry, such as poor management practices, disease outbreaks, or environmental challenges, can also contribute to changes in fungal diversity. Immunocompromised birds are more vulnerable to fungal overgrowth and infections (Ruff & Finkler, 2018).

METHODS TO STUDY FUNGAL DIVERSITY IN CHICKEN GASTROINTESTINAL TRACT

Understanding the fungal diversity in the GIT of chickens is crucial for optimising poultry health and productivity. Fungi can be isolated from chicken intestines using various techniques, including culture-based methods and molecular-based approaches. The choice of method(s) depends on the specific research objectives, available resources, and the level of detail required in understanding the fungal diversity and dynamics within the chicken GIT microbiome.

Sample collection

Fungal samples are typically collected from different segments of the chicken GIT, including the crop, caecum, and colon. Cloacal swabs and intestinal digesta are common sources for fungal isolation (Ghetas *et al*., 2022). About 0.20–0.3 g of digesta is collected aseptically from the crop, ventriculus, colon, mid-duodenum, mid-jejunum, mid-ileum, and left cecum of each bird by gently squeezing it into sterile tubes (Robinson *et al*., 2022).

Direct plating. Intestinal contents or faecal samples can be directly plated onto fungal-specific selective culture media such as Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA). These media are supplemented with antibiotics to inhibit bacterial growth, allowing for the selective growth of fungi (Abd El Tawab *et al*., 2015, Ghetas *et al*., 2022).

Enrichment culture. Intestinal samples can be enriched in a broth medium, such as brain heart infusion (BHI) or yeast extract peptone dextrose (YPD), to promote fungal growth before plating onto agar media (Yudiarti *et al.*, 2012). Isolation and cultivation of fungal strains from chicken GIT samples on selective media (Aiyegoro & Okoh, 2009).

Culture-dependent studies

This approach allows for the identification of viable fungal species. However, it may underestimate diversity, as not all fungi can be cultured in laboratory settings. Common media used include SDA, which is conducive to yeast and mold growth (Ghetas *et al.,* 2022). Fewer than 20 fungal species have been identified, with *Candida* spp. often noted as the most prevalent genus in the GIT of chickens and turkeys, although the dominant species of *Candida* may differ (Robinson *et al.,* 2020). In chickens*, Trichosporon*, *Geotrichum, Rhodotorula*, and *Saccharomyces* are commonly isolated (Sokół *et al.,* 2018). Additionally, one culture-dependent study identified eighty-eight fungal species from over 3,000 caecal samples of both broiler and layer chickens, with *Aspergillus, Penicillium, Sporidiobolus*, and *Verticillium* being the four most abundant genera (Byrd *et al*., 2017).

- Incubation conditions

Samples are incubated at specific temperatures (usually between 25 °C and 37 °C) for several days to promote fungal growth. Observations for colony morphology and characteristics (Yudiarti *et al*., 2012; Abd El Tawab *et al*., 2015; Ghetas *et al*., 2022).

- Identification of isolated fungi

Morphological characteristics: Initial identification of fungal colonies is often based on the morphological characteristics of the fungal colonies, including colour, texture, and growth patterns. Based on morphology of fungal cells, the microscopic examination of conidia and hyphal structures e.g. their colour, shape, and size helps to identify and differentiate fungal species (Abd El Tawab *et al*.,2015). The spore features include shape and size, which also aid the identification (Yudiarti *et al*. ,2012).

Scanning electron microscopy (SEM) or transmission electron microscopy (TEM): SEM is a highly versatile technique used to obtain high-resolution images and detailed surface information of samples, while TEM uses transmitted electrons (electrons passing through the sample) and is therefore a quantitative method to determine the particle size, shape and distribution in the created image. Both techniques are useful in visualisation and ultrastructural analysis of fungal cells and their interactions within the chicken GIT (Niu *et al*., 2022).

Biochemical tests: Fungi can be identified based on their ability to utilise specific nutrients or produce certain compounds, such as enzymes or pigments (Funder, 1961).

Culture-independent (molecular-based) approaches

Molecular methods have revolutionised the study of fungal diversity by allowing for the identification of fungi that are difficult to culture. Metagenomics involves sequencing all the genetic material present in a sample, allowing for the detection of both culturable and non-culturable fungi. This approach provides a broader understanding of the fungal community structure and its functional potential within the gut ecosystem (Choi *et al*.,2015). Molecular techniques and bioinformatic analysis to identify and classify fungal taxa proved that to date, only >7% (144,000) fungal species have been named and classified, while the majority are still unknown (Willis, 2018). Wang *et al.* (2020) created a bioinformatics tool for classifying fungal ITS barcodes down to the species level. This tool utilises an ITS database that includes over 25,000 species across various fungal taxa, and it is anticipated that the Its2vec model will aid in identifying fungal species. For fungal species identification several rRNA genes have been employed, including the small and the large ribosomal subunit, the RNA polymerase II binding protein, and the ITS. Among these, the ITS (including ITS1 and ITS2 separated by the 5.8S genic region) has been widely used as a useful marker for fungal identification and exploration of fungal diversity.

Molecular techniques and bioinformatic analysis are:

- Polymerase chain reaction (PCR)

Fungal DNA can be extracted from samples and amplified using PCR. For identification of fungi, many PCR techniques were adopted including PCR-denaturing gradient gel electrophoresis (PCR-DGGE) results showed that the DGGE band patterns of arbuscular mycorrhizal (AM) fungal strains differed according to their phylogenetic positions, allowing for the rapid and easy identification of the proliferated fungal strains. PCR-restriction fragment length polymorphism (PCR-RFLP) allows the resolution of mixtures of PCR products of several different fungi, as well as products resulting from mixed-template amplifications, into distinct banding patterns, so it is used to identify fungi by amplifying specific

DNA regions (e.g., the internal transcribed spacer region) for fungal identification (Abd El Tawab *et al*., 2015).

- Quantification of specific fungal species or groups using quantitative PCR

A range of target genes have been used in development of quantitative PCR (qPCR) assays, but the ribosomal DNA gene region (consisting of the 18S, 5.8S, and 28S domains, separated by the *ITS1* and *ITS2* regions), has been shown to be a promising target to detect and identify clinically important molds i.e., Mucorales, *Aspergillus* spp. and *Fusarium* spp. up to genus level by melting curve analysis (Pandey *et al.,* 2022).

- Next-generation sequencing

Next-generation sequencing (NGS) high-throughput sequencing technologies enable researchers to obtain comprehensive profiles of fungal communities in GIT. This method provides insights into the diversity and relative abundance of different fungal taxa (Bai *et al*., 2021b). Fungal DNA can be extracted and sequenced using NGS technologies, such as Illumina or PacBio, to identify fungal communities in the chicken intestine. Sequencing specific genes, such as the ITS region was used (Abd El Tawab *et al*. 2015). The use of Illumina HiSeq sequencing of the ITS2 region for investigation of the chicken intestinal mycobiome revealed the presence of 468 unique fungal amplicon sequence variants (ASVs) with *Microascus* (*S. brevicaulis*) being predominant, followed by *Trichosporon asahii*, and *Aspergillus* (Robinson *et al*., 2020). Robinson *et al.* (2022) found that *Gibberella (F. pseudonygamai*), *Candida* (*C. albicans*), two *Aspergillus* species (*A. flavus* and *A. amstelodami*), and *T. asahii*) were the five most abundant fungi while the occurrence of *S. brevicaulis* was much lower (from 0.2 to 2.8%). Sokół *et al*. (2018) Collected used genetic analysis based on ITS1-5.8rRNA-ITS2 fragment sequencing to identify candida isolates from various parts of healthy turkey GIT.

- Nanopore sequencing

The method involves amplifying and sequencing of longer stretches of rRNA operons using nanopore long-read sequencing technology. The resulting reads were with 99.5–100% accuracy (Ohta *et al*., 2023).

- Fungal barcoding

Fungal barcoding involves using specific genetic markers to identify fungal species. This method often employs DNA barcodes like the ITS region, which is widely used for fungal identification. Barcoding can be combined with NGS for a more detailed analysis of fungal diversity (Naranjo-Ortiz & Gabaldón, 2019).

- Environmental DNA analysis

Environmental DNA (eDNA) techniques involve extracting DNA directly from GIT samples without prior culturing. This method allows for the identification of fungi present in the gut environment, providing insights into the overall fungal biodiversity (Huang *et al*., 2022).

- Fungal community profiling

Denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE) separate amplified fungal ITS regions based on their sequence differences, allowing for community-level analysis (Bai *et al*., 2021b).

- Terminal restriction fragment length polymorphism

The terminal restriction fragment length polymorphism (T-RFLP) generates fingerprints of fungal communities by analysing the terminal restriction fragments of amplified ITS regions (Fathi *et al*., 2017).

CONCLUSIONS

Understanding the fungal component of chicken GIT biota is crucial for optimising poultry health and productivity and for the development of targeted probiotics or dietary interventions that enhance gut health and performance in poultry. Understanding disruption of mycobiota and its role in microbial balance, immune suppression, increased pathogenicity of infections, mycotoxin production, as well as economic impacts in poultry production is becoming increasingly important.

Viral infections in chickens can have significant impacts on the fungal component of the gut microbiome, potentially leading to fungal overgrowth, mycotic infections, and other health and production-related issues.

Implementing dietary interventions and environmental factors have a significant influence on fungal diversity in poultry and overall poultry performance.

Specific fungi in the chicken GIT play various roles, from beneficial to pathogenic. Beneficial fungi like *Saccharomyces cerevisiae* and certain *Aspergillus* species enhance nutrient absorption and gut health, while pathogenic fungi like *Candida* spp. and mycotoxin-producing *Fusarium* spp. can pose significant health risks.

Advances in sequencing technologies and metagenomic approaches are particularly valuable in providing a more comprehensive understanding of the complex fungal communities in poultry, which can supply information to build up strategies to improve gut health. Future research should focus on better understanding of these interactions, which can help in developing effective management strategies to mitigate risks in poultry health.

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