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Pathogenic and Non-pathogenic Factors; Especially Infectious Bursal Disease Viruses; Affect Chicken Digestive System Microbiota and Methods of Its Evaluation and Recovery: A review

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CHICKENS gut have diverse and complex microbial agents that have a critical function in digestability, nutrients uptake, development of immunity and pathogen fight. Then, gut health and bird productivty performance have been linked to falctuation in the intestinal microbiome components. Nevertheless, many factors affect the functionality, integrity and health of the bird's gut including the circumestannces, ration, and the intestinal microbiota. Pathogenesis of enteric and non intestinal illness may result in disturbances in the gut ecosystem; dysbiosis. Gumboro disease is a serious and infectious viral disease that affects chicks and is linked to significant morbidity and mortality rates. The bursa of fabrecius is the target organ of this immunosuppressive virus. Infectious bursal disease (IBD) virus causes histological lesions, alterations in immune cells, and changes in the microbial population by reproducing in gut-associated lymphoid tissue. Our review as part of Master's degree in poultry diseases, mainly focuses on the collection of published data the role of chicken microbiota in gut health, host immunity and chicken productivity in addition to factors affecting microbiota as age, sex, breed, maternal antibody, season, ration composition, antibiotics, relation between pathogen and intestinal microbiota with special reference to effect of IBD on gut microbiota ecosystem with role of probiotic and prebiotic in correction of dysbiosis.

Keywords: Infect, Non-infectious, IBD, Prebiotics, Probiotics.

Introduction

Chickens' intestinal health is influenced by both the intestinal microbiota and host immunological function, and it reflects directly on bird health [1]. The term «state of symbiotic harmony between the microbiota and intestinal tract where animal health and welfare remain unaltered» is a primary factors influencing performance [2]. In the digestive tract of chickens, a complex ecosystem dominated by bacteria, a variety of microbial communities including bacteria, yeasts, archaea, ciliated protozoa, anaerobic fungi, and bacteriophages have been discovered [3,4] . the commensal microbiota, which is affixed to the intestine's epithelium, is crucial for maintaining homeostasis [5,6] and guarding against pathogen colonization and it also plays an important role in the digestion and absorption of feed, health, physiology, as well as immunity in the chicken's[7,8] .

The population of various microorganisms in a specific habitate is referred to "microbiome". In addition, their constituents can be characterized using either genetics and/or circumstances capacities [9]. Ecological diversity is defined as all the microorganisms that make up an ecosystem, whereas the total collection of their genes is termed as genetic diversity [9].

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In addition to the intestinal microbiome, there are many other microbiome sites that can be shared by a community of commensal, pathogenic, or symbiotic microorganisms.[10].

Due to its significance for health as well as its distinctive complexity, the gut microbiome is a subject that receives a lot of research. With 10^8 to 10^{14} bacterial colony/g of digesta, the gut microbiome has one of the densest bacterial communities on the planet [11]. The presence of biochemical and metabolic pathways in the microbiome that are not present in the host genome demonstrates how far the microbiome has developed and it has been discovered that the microbes that make up the gut microbiota have a direct influence on the host health status by peventing the host epithelial damage, digestion improvment, and eliciting the immune system response[12]. The commensal bacteria in an bird's Gastrointestinal tract (GIT) absorb nutrients and increase nutrient utilisation. Additionally, studies to date have demonstrated that earlier colonization of a diverse microbiota promotes better growth and productivity [4].

This review will delve deeper into chicken intestinal ecosystem and agents linked to their dysbisis as part of Master degree in poultry diseases, Faculty of Veterinary medicine, Cairo University.

Influence of Pathogenic and non-pathogenic factors; on chicken digestive system microbiota, and methods of its evolution and recovery

Existence of gut microbiome

The GIT of a newly hatched chick is sterile, and the colonization of microbiota begins firstly by contact with the environment [13]. However, previous studies observed that bacteria can already be found in the caecum of unhatched embryos[14], Other studies confirmed the existence of bacteria in the caecum, liver, and yolk sac at embryonic days 18 and 20 [15]. Also, the GIT of 1-day-old broiler chicks has a community of microorganisms [16]. In the pre-hatching stage, microbiota can be obtained from the chicken reproductive tract [17] or from the surroundings via the eggshell pores [4] .

The microbiota from the hatchery environment or during transport may invade the newborn chicks after hatching before they reach the farm [18,19]. Maternal antibodies delivered through the yolk may also have an impact on the composition of the microbiome, up until two weeks after hatching, maternal antibodies can offer protection against the colonization of specific infections and this may have an impact on the intestinal microbiota of the chicks [21 ,20].

Component of gut microbiome

Chicken intestinal canal microbiota comosed of bacterial, fungal, viral and parasitic

Bacterial composition of microbiome

Up to 100 trillion cells, representing 500–1000 different bacterial species, can be found in the GIT. Different sections have different effects on the GIT as each part has diverse metabolic action that shaped the microbiota composition [3]. *Firmicutes, Tenericutes, Bacteroidetes, Proteobacteria,* and *Actinobacteria* are the five bacterial phyla that make up most of the bacteria in chicken GIT [3].

The main bacterial taxons present in the crop, gizzard, duodenum, and ileum are Lactobacillus [22]. In the crop, Bifidobacterium and Enterobacter are frequently found [22, 23]. There are fewer bacteria in the stomach than in other parts of the GIT because the stomach's acidic environment is unfriendly for most bacteria [24], and only a few enterococci, coliforms, and lactose-negative enterobacteria are present, the gizzard is primarily lactobacilli-rich [25-27].

a. Phylum Firmicutes

Firmicutes are primarily denoted by the genus *Lactobacillus* and to a lesser extent by *Clostridium, Faecalibacterium, Ruminococcus, Bacillus, Eubacterium, Streptococcus*, and *Candidatus Arthromitus* [28,29]. Firmicutes are the most prevalent phylum in the small intestine of chickens according to some authors[22], and are the predominant genera in cecum, cloaca, and excreta [0,3126,3] .

b. Clostridia

Clostridium perfringens (*C. perfringens*), a commensal but potentially virulent bacterium, has also been found in gizzards at a very low level (10^3 bacteria/g of contents) [32]. *Clostridiaceae* are second domain after *Lactobacillus* in gizzard [24, 26, 33] . *Clostridium* was isolated from jejunum [34, 35] . The bacterial community in the caecum varies in different studies. The density of bacteria can reach up to 10^{11} bacterial colony /g. According to Gong et al. [34] the caecum is mainly dominated by *Clostridium* [36].

c. Lactobacillus

Lactobacillaceae are non-spore forming bacteria characterized by small genome. Thes bacteria lowers the gut pH by fermenting carbohydrates. the decrease in gut pH limits the growth of other bacterial species [22]. *Lactobacillus agilis* and *Lactobacillus ruminis* have flagella]37[. The main bacterial taxon present in the crop, gizzard, duodenum, and ileum is *Lactobacillus* (L.) [22, 23].

The density of bacteria in the crop can reach from 10^8 to 10^9 bacterial cells/g contents and it consists mainly of *Lactobacillus* [26,34]. *Lactobacillus* species such as *Lactobacillus antri* (*L. antri), Lactobacillus acidophilus (L. acidophilus), Lactobacillus mucosae (L. mucosae), Lactobacillus reuteri (L. reuteri), Lactobaciilus frumenti (L. frumenti), L. salivarius (Lactobacillus salivarius), Lactobacillus crispatus (L. crispatus), Lactobacillus amylovorus (L. amylovorus), Lactobacillus fermentum (L. fermentum), and, Lactobacillus gallinarum (L. gallinarum)* have been observed in bird GIT 24[, 33] . The density of bacteria in the proventriculus is about 10^4 to 10^6 bacteria cells/g contents, and *Lactobacilli* has been shown to be dominat [32] . In the gizzard, the density of bacteria spans 10^5 to 10^7 bacteria cells/g. It is dominated by Lactobacillus and Clostridiaceae]24,26[. *Lactobacillus* species such as *L. aviaries* and *L. salivarius* are isolated from the duodenum [34]. *Lactobacillus* is the most abundant genus in the duodenum [38]. The jejunum is dominated by *Lactobacillus* species such as *L. aviaries, L. salivarius, L. crispatus, L. reuteri* and *L. johnsonii* [34,35]. While, the ileum is dominated by *Lactobacillus*, followed by *Enterococcus*, *Streptococcus*, *Coliforms*, *Candidatus Arthromitus, Escherichia*, and *Clostridium* [39] . In more detail, *Lactobacillus* species such as *L. salivarius, L. delbrueckii, L. acidophilus,* and *L. crispatus* are isolated from the ileum [29]. The genera *Lactobacillus* and *Ruminococcus* are also have been detected in the caecum [34].

d. Phylum Proteobacteria

Proteobacteria are non-spore forming, gram-negative bacteria, Phylum Proteobacteria members, primarily *Escherichia coli* (*E. coli*) and *Enterococcus*, have been found in the ileum [22,29, 40], and are present in cecum in lesser amounts than *Firmicutes* and *Bacteroidetes.* the caecum of chickens generally colonized by both facultative aerobes as *Escherichia coli* and strict anaerobes as *Sutterrella*, *Parasutterella*, *Desulfovibrio*, *Succinatomonas* and *Anaerobiospirillum* . Furthermore, *Campylobacter* and *Helicobacter* are also belong to chicken GIT ecosystem [40]. .

e. Enterobacteriaceae

It was demonstrated that a small number of *Escherichia* are also found in the crop [24,26] , and in the proventriculus of chicken [32]. *Escherichia* was also isolated from the gizzard [24-26]. *Escherichia* was isolated from jejunum in a number of studies [34, 35]. *E. coli* can be found in the duodenum [38]. *Escherichia* was also detected in cecum in several studies [3,29,41]. Moreover, Enterobacteriaceae and also Bacteroidaceae are common families in cecum of chicken other than Clostridiaceae [42].

f. Enterococcus

Genera other than *Lactobacillaceae* such as *Enterococcus*, *Clostridiaceae, Bifidobacterium*, *Enterobacteriaceae* or *Enterobacter* can be found in the crop [24-26] *in addition,* a small number of *Enterococci* are demonstrated in the proventriculus [32] *Enterococcus* species are also have been recovered from the gizzard [24,26,33]. *Enterococcus*, *Clostridium, Ruminococcus*, *Escherichia,* and *Enterobacteria* were isolated from jejunum [34,35]. Moreover, *Enterococcus* was found to be an abundant family in the caecal microbiota [42] .

2. Fungal component (Mycopiota):

In previous study by using automated repetitive sequence-based PCR, 88 distinct species fungai and yeast, counting species of Aspergillus, Sporidiobolus and Penicillium, as well as 18 unidentified genera, were recognized from samples from cecal which gotten from commercial broiler and layer flocks [43] . Additionally, 50 fungal isolates from seven distinct species including *A. fumigatus*, *A. niger*, *Mucor circinelloides, Chrysonilia crassa*, *Rhizopus oligosporus*, *Rhizopus oryzae*, and Mucor species have been detected in various regions of chickens' GIT [44] . There are currently 125 species and four phyla in the chicken intestine mycobiota, with three genera *Aspergillus, Trichosporon* and *Microascus* accounting for more than 80% of the total population of fungi [45] . It's interesting to note that many species from these genera, *Microascus, Trichosporon*, and *Aspergillus*, are regarded as opportunistic pathogens, especially in immunocompromised host [46,47].

Non pathological Factors affecting microflora:

It is including the following factors:

- *Effect of Age:*

Studies have been demonstrated that the microbiota's maturity and stabilization varies [48]. that cecum microbiota composition stabilized after 21 days of chicken age [49] . However, other researches [29] reported that the stabilization of cecal microbiota happen after 28 days of chicken age under experimental conditions since the microbial communities in the cecum were comparable at days 14 and 28 and distinct at days 49. Furthermore, broilers' cecal microbial populations between days 14 and 28 under experimental settings revealed significant variations [50] . Despite these discrepancies, it is well acknowledged that the environment influences the microbiota maturity .in addition, the age of bird play a critical role in the gut microbial richness and composition [51].

In the same study, *Ruminococcus schinkii, Clostridium indolis (C. indolis)*, and *Clostridium saccharolyticum (C. saccharolyticum)* predominated in the cecal contents at 7 days of age, followed by *Clostridium saccharolyticum (C. saccharolyticum)* and *Clostriudium orbiscindens* (*C. orbiscindens)* from days 14 to 28, and Eubacterium at days 49.[29] . In a study conducted, the relative richness of Enterococcus in ileal contents lowered from 25% at day 8 to 1% at day 15 and stayed the same until day 36, while the levels of *Clostridium* and *Streptococcus* rise from 1% to 18% and 5% to 15%, respectively, from day 8 to day 36 [52] .

Broilers' cecal contents on day zero were dominated by members of the family *Clostridiaceae* [53, 49[. In previous study demonstrated that 25% of the cecal microbiota at 3 days of age were *Lactobacillus* [29]. Comparing d 3 to d 42 of the bird age, the intensity of *Lactobacillus* in cecum was 100 times greater [3]. The main *Lactobacillus* species in the cecum were *L. acidophilus* and *L.s delbrueckii* [29]. In the same study, the cecal contents were dominated by *C. oroticum, C. saccharolyticum* and *C. orbiscindens* at 7 d of age; *Clostridium indolis* and *Ruminococcus schinkii* at d 14 to 28; and *Eubacterium* at d 49 [29]. In other studies, the relative abundance of *Enterococcus* in ileal contents decreased from 25% at d 8 to 1% at d 15 and remained unchanged till d 36 while the level of *Clostridium* increased from 1 to 18% and *Streptococcus* from 5 to 15% from d 8 to 36 52, [84]

- *Effect of maternal antibodies*

Maternal antibodies are known to influence how the immune system and gut bacteria interact in mammals [54] . Although the specific mechanism governing how germs and the immune system interact is not fully understood, the altered immune system development in germ-free animals indicates that the microbiota at least partially influences the immune system's development] 55[. During the first weeks of chicken's life in the gut microbiota richness, for instant, the number of different microbiota taxa, increases [36,16], while by chickens age the individual variation in microbiota composition decreases [13] .

- *Effect of Sex:*

Changes have been noted in broiler chickes and appear to be associated with sex-based alterations in lipid and glycan metabolism by the microbiota of cecum, although the role of sex is more prominent when chickens reach sexual maturity [56-58[. Sexes differ in poultry because men and females are frequently kept together in broiler flocks while males and females are primarily raised separately in layer-type chicken flocks [48] . In general, broiler males grow faster and have a lower feed conversion rate (FCR) than broiler females and because growth rate differences between female and male broilers were not seen until day 21, but modifications in the structure of the intestinal microbiota community were seen as early as day 3, it is likely that non-growth-related variables are also at play [8, 56].

It was found that both, *Oscilospira* and *Tenericutes* were more prevalent in the cecum of 35-day-old Ross broilers, while Bacteroides were more prevalent in the males [57]. In addition, male chickens had larger levels of *Bacteroides, Megamonas, Megasphaera,* and *Phascolarcto bacterium i*n their cecum than female chickens [58]. Moreover, gender had an impact on the total amount of eubacteria, with males having a higher abundance of eubacteria in cecal contents at d 22 and d 42 compared to females [59]. However, male, and female broilers at day three have different microbial communities in the ileum [56]. Although it's unknown how sex generally affects the microbiota in the chicken GIT, Whole-genome profiling of prepubescent mice has revealed intrinsic sex-specific gene regulation in the GIT in mice with identical microbiota, even in the absence of high levels of circulating sex hormones, and that sex differences in the microbiota after puberty are linked with sex differences in the expression of many genes in the GIT [48, 60] .

- *Effect of Breed*

Chicken genotype has an impact on the microbiota, but this influence only appears to apply to certain types [61- 63] . Data from 12 research that reported based on sequencing of 16S rRNA in samples collected from cecum from Ross and Cobb Hyprid were assembled by Kers et al. [8] found that all four research on this breed as well as three out of the eight studies presenting data on Ross chickens found *actinobacteria* in Cobb chickens. Like this, *Bacteroides* were found in Ross chickens in 6 out of 8 trials and in Cobb chickens in all 4 studies [48] . Significant variations in the microbial composition of the cecal fluid of young Hubbard and Ross Hyprid chickens have also been observed [8]. There is a significant differences in the early cecal microbial makeup of Hubbard and Ross chickens [64] . On day zero, the *Enterobacteriaceae* family dominated the Hubbard cecal microbiota while the *Enterococccceae* and *Clostridiaceae* family dominated the Cobb cecal microbiota] 48[. Hubbard chickens had a larger abundance of *Bifidobacteriaceae* but a lower level of *Enterobacteriaceae* on day 3 than Ross chickens , however as of day 7, there was no difference in the microbiological makeup between the two breeds[64] . It is clear from the findings above that age, sex, and breed can affect the gut microbiota of chicken, and specific information about these parameters had better to be included in microbiome studies and it has been recognized that housing conditions also effect the microbiota community in chickens [65].

- *Effect of Season:*

In order to maximise the growth of the birds, intensive chicken rearing necessitates strict control of the environment in the commercial facility, particularly the relative humidity and temperature [66] . Nevertheless, exterior climatic circumstances, particularly excessive heat, can have a deleterious impact on chicken health and productivity even when the internal factors of the poultry house are controlled[67] . In a recent study, there is a significant seasonal change in the cecal microbiota, with summer having twice the species richness of winter]6[. This is consistent with the findings of other authors who examined cecal samples from several flocks of broiler chickens in the USA and found evidence of a similar pattern of seasonal change in alpha diversity [68] . However, numerous studies found variations in the gut microbiota profiles of chickens depending on their geographic location [69,70] . When planning and carrying out experiments about the connotation between the gastrointestinal microbiota and productive performance, it is crucial to take the influence of these climatic and environmental elements into consideration and ignoring this information could result in a significant bias and incorrect findings [6].

- *Effect of Ration:*

The composition of chicken gut microbiota is potentially affected with feed type. In addition, both the physical form of feed and the composition of the diet (mash or pellet) affect digestibility and nutrient absorption as well [11]. A significant rise in bacterial populations can be seen in the chicken gut after the first feed intake following hatch.[18] When newborn chicks have access to food, this encourages villus heightening and an increase in cell proliferation in the crypt [71]. Due to this delays in feed access have an impact on how the gut surface area develops and the microbiota constituents in early chicks]72,73]. Also, temporary feed withdrawal can lead to an increase in intestinal pathogen colonisation, such as with Salmonella, even though feed withdrawal later in life has also been linked to alterations in microbiota composition]74[. Large alterations in the bacterial community were seen in the proximal section of the GIT after 6 hours of meal deprivation [8].

- *Feed additives:*

Different feed additives such enzymes, probiotics, prebiotics, and symbiotics have been utilised to alter intestinal micro-biota and boost the immune system of chickens. The addition of exogenous enzymes raised the butyrate and lactic acid-producing bacteria in the ceca [27, 75, 76].

- *Ration component:*

a. Dietary protein:

The count of *C. perfringens* in the ileum and ceca is influenced by the type and amount of dietary protein consumed. In general, an increase in *C. perfringens* relates to an increase in crude protein levels in poultry diets. This correlation, however, is not always present in birds eating soy-based diets since fish-meal-based diets have greater levels of glycine and methionine.[78]. Additionally, fish meals have greater levels of zinc and glycine, and there is a link between glycine levels and *C. perfringens* abundance[77,78].

b. Non-starch polysaccharides (NSPs):

Non-starch polysaccharides (NSPs), fish meal, and bone meal-rich diets have been linked to a

rise in the growth of *Clostridium perfringens* (*C. perfringens)* and a higher risk of necrotic enteritis in chickens (NE)][9,80]. For instance, NSPs decrease the transit rate, enzymatic activity, and feed conversion efficiency while increasing the viscosity in the intestinal lumen. [81] . Increased digesta retention, particularly in diets high in protein or with imbalanced amino acid profiles, offers substrates and promotes the colonisation of pathogenic bacteria, such as *C. perfringens*, in the small intestine.[82,83].

Gut health and microflora:

The systemic health of birds, animal welfare, production effectiveness, food safety, and environmental impact are all significantly impacted by the intestinal health of poultry. [6]. Even though the link between bird productivity and gut health is well acknowledged, the term "gut health" is not well defined[6]. The term «gut health» refers to the intestinal mucosal intactness, its physiological operations (such as nutrient digestion and uptake), development of barrier functions, cells metabolism, effective immune responses, energy balance, inflammatory respons equilibrium, and specially sufficient microbiom[7] . Disease conditions affect the animal soundness and result in reduction of animal performance, in addition to intestine dysbiosis [6].

Host immunity and microbiota:

The microbial community in the GIT acts as an anti-infectious barrier by preventing pathogen adhesion and subsequent colonization, as well as the production of bacteriocins and other toxic metabolites]6[.It also keeps out pathogenic microorganisms, ferments complex polysaccharides, and gives the host energy. Additionally, the earliest interactions between the intestinal microbiota and the innate immune system of the host may trigger an adaptive immunological response that depends either on B- or T-cells[7]. The creation and development of the gastrointestinal microbiota can be influenced by a variety of host variables, but at certain points, interactions between the gut microbiota and immune system obviously predominate. The innate and adaptive immune systems work together in a complex network of pathways to respond when the host immune system recognizes an infection in the GIT [6].

Beta-defensins are tiny antimicrobial peptides that are found on the surface of the intestinal epithelium and have a significant role in innate immunity [84] . the transcription factors NF-kB and Toll-like receptors TLR-4 and are involved in the production of these peptides in response to challenge by lipopolysaccharide exposure[85] . Beta-defensin expression in the duodenum and ceca of growing birds was reduced as microbial exposure was reduced. Defensin expression in the gut is influenced by microbial exposure [7]. The first response of the innate immune system of host to certain members of the gut microbial community have the potential to alter the makeup of the microbiota and trigger an adaptive immunological response that may or may not be dependent on B- or T-cells. Additionally, the microbial population aids in eliminating infections, fermenting various substrates, and supplying the host with energy [86]. Since GIT is home to numerous, intricate microbial communities, there are numerous, intricate interactions between the microbiota and the host immunity. The outcome of these interactions is in part predetermined at the early developmental statges of embryonic stage of birds. For a healthy gut to grow, early microbial colonization is also crucial. The development and maturity of the gut immune system require microbial exposure, according to preliminary research with germ-free hens[87]. Germ-free animals exhibit decreased vascularity, muscle wall thickness, activity of digestive enzyme, production of cytokines, levels of immunoglobulin, small Peyer's patches and few number of intraepithelial lymphocytes. However, increased enterochromaffin cell area, making them more susceptible to intestinal infections[88]. It is believed that exposure to antigens is a key factor in the immune system's development and maturation. previous studies Compared between normal and germ-free chickens as thay found that at 7d of age, absence of microbiota in the intestine affected the density and number of goblet cell, expression of the MUC2 of intestinal mucin and sulfated & sialylated mucin staining[89], demonstrating that animals free of microbiota have an immature intestinal mucosa .Consequently, the gut microbiome influence the development of immune system[6].

Microbiota and birds' productivity:

a. Meat production

Numerous studies have analysed the microbiota of highly productive and poorly productive birds to uncover members that are much more prevalent in these groups[30]. In the jejunum, there was no distinction between birds with good and poor performance.[35,90] . Differential microbiota was detected in bird's faeces, ileum, and caecum

[35, 91] . Good avian performance was linked to caecal bacterial communities that are known to produce butyrate [92] . The microbiome of a well-performing bird appears to be more likely to have genes involved in flagella movement and sulphur assimilation. There is some proof that a more diverse microbiota is advantageous, although the link between cause and effect is unclear [30] .

b. Egg production:

There have been claims that layer chickens' gut microbiome improved their ability to utilise nutrients[93,94], intestinal barrier [95,96] , production performance [94, 97], as well as egg quality [98,99]. In addition, intestinal microorganisms and their metabolites serve as signalling molecules connecting the digestive tract, liver, brain, and reproductive system[100-102], which in turn has a direct or indirect effect on poultry health and egg quality[103] .

Detection of gut microbiota:

The evaluation of microbiota has relied on culture methods by using selective or non-selective media[104,105]. The fact that culture-based approaches can only evaluate culturable bacteria is one of their key drawbacks. Other culture-independent approaches that depend on molecular technologies have recently been created to analyse microbial populations [65].

a. Culture dependent method, in study for characterization of the gastrointestinal mucosa–associated microbiota [106], as the culture-dependent method was made by make 10-fold Serial dilutions of samples were prepared in PBS, and then sufficient dilutions were plated on different selective agar media . Total anaerobes were cultured on Wilkins-Chalgren agar , *Clostridium* on Reinforced Clostridial Medium supplemented with novobiocin (8 mg/liter) and colistin (8 mg/liter), *Bifidobacterium* on *Bifidobacterium* agar , *Bacteroides* on Schaedler agar supplemented with kanamycin (100 mg/liter), vancomycin (7.5 mg/liter), and vitamin K (0.5 mg/liter), *Staphylococcus* on Baird-Parker agar, *Enterobacteriaceae* on violet red bile dextrose agar, *Enterococcus* on kanamycin-esculine agar, *Lactobacillus* on Rogosa agar, yeast on Bengale Pink agar, and *Propionibacterium* on yeast extract lactate agar .To confirm the selectivity of each medium, 8 to 10 representative colonies from high dilution plates were isolated and examined by Gram staining, cell and colonial morphology, biochemical reactions by Application Programming Interface (API) systems and hybridization with specific group probes. Plate count results were expressed as the log of the number of CFU/ cm^2 [106].

b. Culture-independent methods for characterizing microbiota, sequencing of 16S rRNA gene is major technique to evaluate microbiom in animals and humans, but generally there are two main types of culture-independent methods, fingerprinting and sequencing-based approatches, which are used for characterizing microbiota population [25]. Community-fingerprinting techniques can give a detailed picture of microbiota population by evaluating genomic DNA, these are found to be helpful for comparing of microbial populations and identification of population shifts and include temperature gradient gel electrophoresis (TGGE), denaturing gradient gel electrophoresis (DGGE), single-strand conformation polymorphism (SSCP), terminal-restriction fragment length polymorphism (T-RFLP) automated ribosomal intergenic spacer analysis (ARISA) and temporal temperature gradient gel electrophoresis (TTGE) 25]. All these techniques are quick and economic. nevertheless, they are semiquantitative and lower sensitive, as they can evaluate (>1 %) of microbioal taxa [25]. Other authors have questioned inaccurate abundance estimates [107] or poor reproducibility of the data [108].

To study the chicken microbiome, oligonucleotide microarrays targeting 16S rRNA (PhyloChips) have also been performed[39]. The density and phylogenetic coverage of the probes present on the array have a significant impact on the effectiveness and dependability of the data produced]30[.the sequencing of 16S rRNA genes is the technique of choice for microbiom profiling [30] .

Fluorescent in situ hybridisation (FISH) is often applied alon or with flow cytometry as the next step in microbiota analysis [30]. FISH, nevertheless, is a difficult method [25] . However, it can be used to demonestrat the structured populations such as those found in biofilms. Although there has been a trend toward sequencing techniques over the past two years, most of the chicken 16S-based microbiota research published in the literature to date have used fingerprinting techniques. Although there has been a trend toward sequencing techniques over the past two years, the majority of the chicken 16S-based microbiota research published in the literature to date have used fingerprinting techniques [30] .

Comparison of cultural and Molecular methods of microbiota identification:

Prior to the application of 16S rRNA gene-targeted analysis in the early 2000s, the composition and diversity of poultry intestine microbiomes were predominantly studied using cultivation-based approaches[4, 38,104] . These cultivation-based researchs assisted in the discovery of culturable commensal and pathogenic bacteria, including facultative and aerotolerant anaerobic bacteria. But it quickly became clear that not all gut bacteria can be grown in laboratory media [38, 104].

To define the uncultivable elements of the intestinal microbiomes of poultry, especially chickens and turkeys, DNA-based molecular biology procedures, cloning and sequencing of the 16S rRNA gene, have been used [109,110] to determine chicken microbiota species composition, community and diversity [3].

Recently, Sanger DNA sequencing technology was used to create all reported 16S rRNA gene sequence data sets due to financial limitations, most research only yielded a few hundred or fewer sequences for each sample, indicating only a small part of the intestinal microbiome's full diversity [3]. Additionally, individual studies may be biassed in favour of or against particular bacterial phyla according to the technique used, as was demonstrated for the ruminal microbiome [111, 112].

Effect of pathogens on microflora:

The disterbanc of microbiome composition is referred to dysbiosis which is mainly linked with GIT inflammation [113, 114] . It has been reported that many pathogens have direct impact on gut microbiota composition as qualitative and quantitative changes[115- 117] .

a. Bacteria:

The intestinal microbiota also plays a vital role in the protection of the bird from enteric bacterial infection. However, many intestinal pathogens have established tactics to compete the gut microbiota, result in either both infections and chronic diseases [13]. In commercial production, chicks are housed separately from the adult chickens as soon as they hatch, so they do not have a maternal microbiome.In commercial production, chicks are housed separately from the adult chickens as soon as they hatch, so they do not have a maternal microbiome.Consequently, This makes young chicks highly susceptible to intestinal bacterial infections, such as *Salmonella*, which, depending on the bacterial serovars infecting the host and the host's sensitivity, can lead to varying degrees of illness spectrum, from a subclinical carrier condition to a high mortality rate [118]. By comparing infection of 4-day-old and 16-day-old chicks, the largest changes in microbiota were observed in newly hatched chicks[119] . The infection of young chicks with Salmonella effects on the microbiome community [120] . *S. enteritidis* infection in chichen results in reduction of microbiota. Moreover, the expansion of bacteria of the family *Enterobacteriaceae* disruption of the microbiota community early post infection [118]. Proteobacteria and Firmicutes have a positive correlation in terms of pro-inflammatory cytokine transcription, while the opposite is true for Firmicutes.[121] while genera of *Escherichia/Shigella*, , being positively correlated with expression of IL6 cytokine, a negative correlation with *Firmicutes* (genus *Fecalbacterium*) was also found. Furthermore, Fecal- bacterium is able to secrete metabolites that block activation of NF-kb and production of IL-8 [102].

b. Viruses

Once the intestine is invaded with a virus, the beneficial microbiota decreases while the number of harmful bacteria increases [12] . Inflammation and immunosuppression of T and B cells in chickens may be influenced by viral infection and the microbial makeup of the intestinal tract [123].The core gut flora was affected by the Marek's disease virus (MDV) infection in total faecal samples very soon after infection (2 to 7 D) and in cecal samples in the late stage of viral infection (28 to 35 D), which fits well with the life cycle of MDV both the total faecal and cecal bird samples only included members of the species *Lactobacillus*. A viral infection changed the way the community colonized the core gut flora, which showed up as the enrichment of certain genera during the early and late phases of MDV replication [124]. There are many differences in the metabolic profiles and microbiome community structure between chicken lines that were resistant to MDV and those that were vulnerable to it [124]. Chickens infected with the avian influenza virus (AI) had a change in the composition of their gut microbiota, with *Vampriovibrio, Pseudofalvonifactor, Ruminococcus*, and *Clostridium* cluster XiVb all increasing [125, 126].

Role of Infectious bursal diseases (IBD) viral infection

Infectious bursal diseases (IBD), also known as Gumboro disease, a global serious and infectious viral infection that affects young chickens, and it is linked to significant morbidity and mortality rates. The virus belongs to Birnavirus, which is a double-stranded RNA virus with two segments. The common method for preventing IBD in chickens is vaccination parallel to the application of biosecurity measures [127]. When the bursa of Fabricius reaches its maximum growth at 3 to 6 weeks of age, chickens are particularly vulnerable to the virus[128] . Gumboro virus is an immunosuppressive virus due to the effect of IBDV on lymphoid organs, i.e. lymphoid cells in bursa of Fabricius which is the target organ of the virus, lead to lymphoid depletion and bursa destruction [129] . The IBD virus also multiplicates in gut associated lymphoid tissue (GALT) causing histological lesions, alternation in immune cells, with changes of microbiota community [129-131] . The virus was discovered in the 1970s after the first instances were first noted in the United States in 1957 [132] . It is also known as Gumboro illness since it was identified from broiler chickens in Gumboro, Delaware, in the USA and it creates an acute, extremely contagious illness condition in young hens[133]. It expanded to Australia, the Far East, India, the Middle East, and southern and western Africa in the late 1960s and early 1970s [134-136] . In Egypt, the infectious bursal disease virus was originally discovered in 1974 [137] .

a. Etiology

Infectious bursal diseases virus (IBDV) is the causal agent is a member of the family *Birnaviridae's* genus *Avibirna* virus]138-140 [. A virion-associated RNA dependent RNA polymerase is used in the cytoplasm of the host cell during replication. Fish, poultry, insects, and insects are all affected by the viruses in the family *Birnaviridae* [141] .

Structure of Virus

IBD virus is a small, non-enveloped virus, which is described by its dsRNA structure of size 65-70 nm. IBDV genome is a bi-segmented structure consisting of two segments i.e. A and B of dsRNA virus which is encapsulated between virions consisting of a single shelled icosahedral particle with 32 capsomeres and a diameter of 60-70 nm [142,143] . The large segment consists of 3400 base pairs polyproteins of size 110 kDa and two opening frames (ORFs) which are processed by VP4 to form proteins like VP2, VP3, and VP4. The smaller segment B is of size 2800 bp and encodes for VP1 and the second reading frame partially overlaps the polyprotein gene which encodes for VP5 [142,143] . Viral Protein 1 (VP1), which is encoded in Segment B, oversees polymerase activity. Two Open Reading Frames (ORFs) are present in Segment A, the larger of which encodes a polyprotein made up of VP2, VP3, and VP4. The main antigenic location that is crucial for the development of neutralizing antibodies is present in the viral protein 2 (VP2) [144]. Early neutralizing antibodies target the serine protease activity of VP3 and VP4, which cleaves the polyprotein into its different counterparts. The non-structural protein VP5, which has a role in induced bursal disease, is encoded by the short ORF [145, 146]. The base, shell, and projection domains make up Viral Protein 2, which is crucial for inducing a response from neutralizing antibodies [144].

IBDV antigenicity

Strains of IBDV are categorized into two categories, serotype 1 and serotype 2, based on virus neutralization tests. However, these two serotypes cannot be distinguished via immunofluorescence test or enzyme-linked immunosorbent assay (ELISA) [147,148]. Only 30% antigenic similarity was observed among the serotype 1 strains [149]. The first categories, i.e., serotype 1 virus, are extremely harmful to chickens and divided into divisions depending on antigenicity and pathogenicity. Serotype 1 is further categorized into: classical strains, antigenic variants, classical attenuated strains, and very virulent strains, however, serotype 2 is avirulent and does not generate or produce any type of harm or clinical disease in chickens and turkeys. The mortality rates show huge variation in case serotype 1 viruses ranging from no mortality to 20% in classical strains and 50-90% in vvIBDV strains. Wild and free birds play a huge role in epidemiology for serotype 1 as they act as host reservoirs for the virus whereas, IBDV is said to be more prevalent in the serotype 2 category in many species of free- living birds especially turkeys, but it does not affect poultry health and cause production losses as compared to other strains of IBDV[147]. Serotype two of gumboro cannot shield against challenges with serotype one viruses. Classical strains of serotype one protects against each other and give partial protection against variants strains of serotype one.

Variant strains of serotype one protects against themselves and against the classic viruses [147].

IBDV genotyping

By using revearse transcriptase -PCR PCR (RT-PCR) followed by sequencing of the hyper variable region of VP2 of Gumboro virus to determine phylogenetic relationshipe among international strains of the virus, the Gumboro virus has seven genogroups [150]. Very virulent IBDV isolates that have an amino acid change from alanine to threonine at position 222 while keeping other residues conserved in this genogroup (I242, I256 and I294). The similar isolates clustered into seven major genogroups, which generally related to their serotype or pathotype classification. Genogroup 1 (commonly classical strains) were detected globally, genogroup 2 (mainly antigenic variant viruses) are still commanlly distributed in the Americas, and genogroup 3 (vvIBDV pathotype and vvIBDV reassortants) were detected globally but most often recognized outside North America and some viral isolates, however, did not clearly match into any of the 3 major genogroups and were grouped separately [150].

b. Hosts of IBDV

Chickens serve as the IBDV's natural hosts]147[. Also, eight-week-old ostrich chicks were used to isolate a virus of serotype 1[145]. And the serotype 1 virus was found in both living and dead ducks [151]. Additionally, it was kept separate from penguins, pheasants, partridges, rooks, gulls, shearwater, quails, and guinea fowl kept in captivity[152] .

c. Transmission:

The virus transmitted horizontally via conjunctiva, ingesting, and. After 48 hours post-infection, the infected bird excretes the virus in its droppings, and within 16 days of infection, the sickness is spread by touch and the virus continues to be contagious in the farms where the outbreak occurred for up to 122 days [139] . The virus can persist on the eggshell surface but is not vertically spread [147].

d. Pathogenesis

The BF is the primary target organ of IBDV. IBDV replicates macrophages and B cells in the bursa, and evidence of viral infection was observed within 24 hours post-infection (hpi.) [147]. After oral infection or inhalation of IBDV, mononuclear phagocytic cells and lymphocytes from the intestinal mucosa get infected first. The IBDV initially replicates in lymphocytes and macrophages in the GALT as early as four hours post infection (hpi)[153, 154], and at five hpi, viral antigen can be detected in lymphocytes in the duodenum and jejunum[154] . At the same time, the virus enters the hepatic cells and reaches the blood stream, leading to primary viremia. At 11 hpi, the virus starts replicating in proliferating B lymphocytes of the bursa of fabricius (BF) [154,155]. At 13 hpi, most bursal follicles are virus positive[155]. The virus-infected cells then migrate into the portal circulation or distribute to different tissues via blood circulation, causing secondary viremia [147].

e. Clinical signs

Diseases can incubate for anywhere between two and three days then the inclination of birds to peck at their vents is the first indication of IBDV. Clinical signs in the acute stage include anorexia, prostration, ruffled feathers, vent feather soils with urate depositions, and yellowish watery diarrhea. Death can result from extreme dehydration and low body temperature[156]. Due to the thymus, bursa of Fabricius, and spleen's and thymus' elimination of immature lymphocytes, the hens suffer lifelong and severe immunosuppression in subclinical form [147].

f. Pathological lesions:

Gross lesions

The post-mortem lesions include urate deposits in the kidney, edematous kidney, darkened and discolored pectoral muscles, and hemorrhages in the breast and thigh muscles[157]. Atrophied bursa that occasionally has cheesy exudates in the lumen and slimy exudates in the serosa [158]. Following infection, the bursa, the virus's target organ, goes through a sequence of modifications. Bursa becomes edematous on day three as a result of fluid buildup, doubles in size and weight on day four, and is only one-third its usual size and weight by day eight [159]. Splenomegaly with grey foci on speelenic surface. Petechial hemorrhages on mucosal junction between gizzard and proventriculus [160] .

Microscopic lesions

Microscopic lesions of IBDV infection mostly seen in lymphoid tissues (as cloacal bursa, spleen, thymus, cecal tonsil and Harderian gland), the cloacal bursa had the most drastic changes. There was lymphocyte degeneration and necrosis in the medullary region of bursal follicles as early as one day post infection (PI). Heterophils, pyknotic debris, and hyperplastic reticuloendothelial cells quickly took the role of lymphocytes [147].

According to Wang et al.[161] IBDV could induce a decrease in the villus height in the ileum and jejunum as well as reduction in the number of intestinal intraepithelial lymphocytes and mast cell[. An increase in the number of goblet cells contributes to more mucus production [161].

g. Effects infectious bursal disease virus; on chicken digestive system microbiota, after clinical and subclinical infection

Preliminary study by Li et al [129] who estimte the impact of gumboro viral infection on the bursa of Fabricius (BF), caecal tonsils (CT), and caecum as well as to ascertain the impact on the caecal microbiome population. At 14 (Experiment two) or 15 (Experiment one) days post hatch, a highly virulent (vv) strain of IBDV was administered to commercial broiler chickens (dph) [129]. In Experiment 1 and Experiment 2, they examined the caecal material at three, seven, 14- and 21-days post-infection (dpi), respectively, to gain a fuller understanding of the alterations to the caecal microbiota that take place during vvIBDV infection. The sample with the lowest coverage was defined by 3677 sequences, while 10283 sequences were available for the sample with the maximum coverage. At every time point that was looked at, nine phyla's representatives were found. The majority (over 95 %) of the microbiota was made up of members of the phyla *Firmicutes, Bacteroidetes, Proteobacteria* and *Acitinobacteria* independent of vvIBDV infection. In the caecal samples, the proportional representation of different phyla remained consistent, with *Firmicutes* accounting for more than 90% of the microbiota between 18 and 36 days post hatch (dph) [7] . On the family and genus level, a more thorough examination was conducted. *Lachnospiraceae* and *Ruminococcaceae* made up most bacteria at the family level in both the vvIBDV-infected and virus-free control groups [129] . The prevalence of *Lachnospiraceae* declined throughout time, independent of vvIBDV inoculation. It varied from 54.4 % at 18 dph to 42.2 % at 36 dph. *Ruminococcaceae* displayed a reversal of this tendency, with abundances ranging from 25.6 % at 18 dph to 42.2 % at 36 dph. At the genus level, the abundance of *Faecalibacterium* grew from 0.5 % at 18 dph to 13.2 % at 29 dph, and then fell to 9.3 % at 36 dph. [129] .

Independent of age, vvIBDV changed the intestinal microbiome. Independent of age, vvIBDV inoculation resulted in a reduced abundance of *Clostridium XlVa* at 3 days after infection, which was followed by an increase at 7 and 21 days after infection. Comparing vvIBDV-infected birds to virus-free controls, we found that *Faecalibacterium* was more abundant at seven days post infection (dpi), but less abundant at 14 and 21 dpi. *Escherichia/Shigella* was also less prevalent at three, 14 dpi, and 21 dpi in vvIBDV-infected birds compared to virus-free controls, showing that *Enterobacteriaceae* was less prevalent in the vvIBDV-infected birds than in virus-free controls. At 14 days pi, similar findings were also seen [129] .

However, Daines et al [162], found that at 3 days DPI by IBDV either a very virulent strain (UK661) of IBDV or a classical strain (F52-70) the infection result in lower the percentage of *Bacteroidetes* and an increase in the percentage of *Enterbacteriales* in the caecum, and a reduction in the percentage of *Clostridiales* in the cloaca by using16 s rRNA sequencing on the BF and CT harvested at 3 days post-infection, and a swab was obtained from the cloaca[162] .

Inprevious study to determine the fungul population in IBD infected chichens, a total of 19 purified fungal isolates have been recognized morphologically in immunosuppressed birds. *Aspergillus* isolates were the most identified (42%) fungal isolates from cloacal swabs of IBD infected chickens, followed by *Penicillium* (10.5%), *Trichosporon* (10.5%), *Fusarium* (5%), *Candida* (1%) and non-identified isolates (26%) [163].

h. How IBD virus affects gut microbiota.

According to Daines[162], the depletion of IgM+ B-lymphocyte precursors during Gumboro infection affects both humoral and cellular immune responses and causes a decrease in the percentage of B cells in the cecal tonsil and an increase in the percentage of T cells in the cecal tonsil, as well as the suppression of macrophage function. However, it has been proven that CD45-, Rag-, and CD45Rag-deficient mice had altered gut microbiota compositions compared to wild-type (WT) mice. Few research has examined the impact of both innate and adaptive immune cells on gut microbiota composition in chickens [164]

This implied that the unique mucosal immune system's development might have an impact on the microbiota's make up. The GALT is dispersed throughout the whole gut in chickens. It belongs to

the mucosa-associated lymphoid tissue (MALT), which is made up of structured tissues with one or more lymphoid follicles and freely disseminated lamina propria lymphocytes (LPL) [165] . The lamina propria (LP) of the gastrointestinal tract contains organised lymphoid tissues like the BF, CT, Peyer's patches (PP), Meckel's diverticulum, and other lymphoid aggregates. The GALT is the key immunological system, it is estimated to comprise more immune cells than any other tissue. These associated structures provide a site of stimulation of innate and acquired immune functions through contact with antigens [165]. One of the critical roles of the GALT is to recognise harmless antigens from pathogenic antigens and to elicit an appropriate response. It provides the conditions necessary to induce an appropriate immune response, such as IgA production by B cells. In previous study by Li et al.,]129 [compared to virus-free controls, birds that had been infected with IBDV had considerably more CD4+ and CD8ß+ lamina propria lymphocytes (LPL) and less B cells in the BF, CT, and caecum. In addition, compared to virus-free controls, vvIBDV infection also resulted in a decrease in the number of mast cells, IgA+, besides CD4+ and CD8ß+ intraepithelial lymphocytes (IEL) in the caecum. According to Li et al [129] , vvIBDV infection changed the composition of the gut microbiota in the caecal content. Overall, Li et al.[129] demonstrated that vvIBDV infection had a significant impact on GALT and led to a alteration of the gut microbiota composition and vvIBDV infection led to a systematic and local immunosuppression. According to Daines' theory, the microbiome is changed when IBDV infects the B cell populations in the gut lamina propria and changes the quantity and repertoire of IgA-secreting B cells [162] . Secretory IgA, which is made by B cells in the lamina propria, transcytose into the gut lumen and binds commensal bacteria, changing how mucus removes them. As a result, the sIgA repertoire controls the microbiome's composition [166]

Li et al [129] found that vvIBDV-infected birds had less IgA+ secreting cells in the caecum than virus-free controls. The most prevalent immunoglobulin in mucosal tissue is IgA, which serves as a crucial line of defense for the immune system against invasive enteric infections and IgA also controls the ecological balance of microbiota and is crucial for maintaining mucosal homeostasis[167]. This observed decline in IgA+ cells could be the result of IBDV directly infecting these cells. However, earlier research revealed that IBDV might primarily target receptors found on IgM-bearing cell surfaces. vvIBDV exposure had no effect on IgG and IgA B-cell numbers in the spleen or total serum IgM and IgG concentrations]168].

i. Molecular Diagnosis of IBD:

Molecular methods are quicke techniques to detect Gumboro viruses than virus isolation. These methods can identify the genome of viruses directly from infected bursa after nucleic acid extraction[169].

Reverse-transcriptase polymerase chain reaction (RT-PCR):

Using an IBDV-specific primer, the RT-PCR technique has been utilized to identify IBDV. The hyper-variable region of the VP2 gene, which is known to encode one or more viral neutralizing epitopes, is where most researchers use primer sequences [170]. Reverse transcription (RT) of IBDV RNA into cDNA, followed by polymerase chain reaction (PCR) amplification of the cDNA produced, are the three steps in the RT-PCR procedure [169].

RT-PCR with Restriction Fragment Length Polymorphism

The restriction enzymes were used to break down the RT-PCR (Reverse-transcriptase Polymerase Chain Reaction) products. The enzymes produced RFLP patterns that classified viruses into molecular groups and generated the patterns[170]. The characterization and identification of current and emerging IBDV strains can be done quickly and effectively with RT-PCR RFLP. The virus strains used in vaccines have been grouped into molecular categories thanks to this assay. IBDV strains within a molecular group are connected by descent [171]. Additionally, compared to viruses belonging to distinct molecular groups, viruses within a molecular group contain nucleotide and amino acid sequences that are considerably more similar. The RT-PCR-RFLP techniques used to create molecular groups of Gumboro virus are intended to diversity or nucleotide resemblance between viruses [171].

Real time Reverse-transcriptase polymerase chain reaction:

IBDV-infected chicken samples can have the viral load measured using a real-time RT-PCR technique. It is a very quick and accurate test for IBDV detection. IBDV strains can be distinguished

using real-time RT-PCR with hybridization probe technology [172]. Fluorescence resonance energy transfer (FRET) is used by real-time RTPCR probe systems to recognize RT-PCR products. There are two probes, one with a Red 640 fluorophore and the other with fluorescein isothiocyanate labelling. IBDV-infected chicken samples can have the viral load measured using a real-time RT-PCR technique. It is a very quick and accurate test for IBDV detection. IBDV strains can be distinguished using real-time RT-PCR with hybridization probe technology[172]. Fluorescence resonance energy transfer (FRET) is used by real-time RTPCR probe systems to recognize RT-PCR products. There are two probes, one with a Red 640 fluorophore and the other with fluorescein isothiocyanate labelling. Unlike the TaqMan method, these probes are not destroyed after amplification. They may therefore be utilized to provide a melting temperature for each IBDV strain following RT-PCR amplification. The temperature at which one of the probes will separate from the RT-PCR product, referred to as the mutation probe [173] .

Sequence and Phylogenetic analysis

Because variant and vvIBDV strains exhibit distinctive nucleotide and amino acid alterations, DNA sequencing and phylogenetic analysis may be able to distinguish between classic, variant, and vvIBDV strains after molecular identification [174] . The RT-PCR results were directly sequenced and examined to demonstrate that they were the IBDV genome. This strategy is a useful tool for IBDV molecular epidemiology investigations. Comparative study will show whether these viruses are genetically related to either the traditional strains or vvIBDVs [173] .

Parasites

In birds with a normal intestinal microbiota, infestation with *Eimeria tenella* inhibits the growth of most bacterial species, except for members of the *Enterobacteriaceae* family [175] . In the ceca but not in the ileum, *Eimeria acervulina* infection decreased both bacterial diversity and homogeneity among chicks[176]. Bacterial diversity in the ceca was reduced by mixed infestation with *Eimeria brunetti*, *Eimeria acervulina*, and *Eimeria maxima*[30] . According to 16S rDNA pyrosequencing, the most affected bacteria were Clostridium, *Lactobacillus, Eubacterium, and Ruminococcus*. Additionally, the mixed infection led to a rise in the proportion of culturable enterobacteria and coliform bacteria curiously, the infection also markedly reduced the incidence of *Candidatus savagella* detections, an immune-modulating bacterium]30[. In a related investigation, infection with these three eimeria species resulted in a decrease in Ruminococcaceae groups and an increase in the abundance of three unidentified clostridium species [177] . After contracting *Eimeria tenella* (*E. tenella)*, bacteria-free hens showed less severe clinical symptoms and pathological lesions than standard controls. Both the lesions and the coccidian's rate of replication appeared to be accelerated by the bacteria. Oocyst shedding, however, did not change. Additional findings demonstrated that *E. tenella* infestation drastically changed the quantity of microbiota in the orders *Enterobacteriaceae Lactobacilliales*, *Bacillales* and which were linked to the severity of lesions [116] . *Histomonas meleagridis (H. meleagridis)* only causes moderate atypical lesions in gnotobiotic hens with a single species of gut microbiome or with the combination of *C. perfringens and E. coli* , while only chickens with a natural intestinal microbiota experience typical lesions after infestation . In the intestinal material of commercial chickens infected with *Ascaridia galli (A. galli*) [178]. Studies discovered less diversity in the microbiota and fewer microorganisms than in *A. galli* uninfected hens. In fact, it has been demonstrated that other antimicrobial compounds can be produced by other nematodes [118] .

Antibiotics

Chickens of various ages are affected differently by the administration of antibiotics such as therapeutic or preventative doses. For instance, L. salivarius was increased in the ileum of chickens treated with virginiamycin and bacitracin at 4.4 and 11 ppm each, respectively, while 22 ppm of virginiamycin virtually totally suppressed the existence of these bacteria [179] . In the same experiment, treatment with antibiotics like growth promoters also led to a rise in Enterococcus species[179] . However, in two-week-old chicks, virginiamycin at 11 and 22 ppm inhibited L. salivarius. In the ileum of two- and three-week-old broilers fed a meal supplemented with salinomycin 40 ppm and avilamycin 10 ppm, Lactobacillus population declines[180].

Salinomycin 40 ppm and avilamycin 10 ppm lower the quantity of *C. perfringens*, and this effect seems to be more significant when the feed is supplemented with soy oil than with lard and tallow in birds raised without antibiotics[180].

Tylosin phosphate (100 ppm) has been found to promote *Lactobacillus gasseri* (*L. gasseri)* at the expense of *C. perfringens*, which was found in higher concentrations in control birds [180]. The bacterial community of the small intestine is impacted by food restriction as well and it has been found that the longer the feed withdrawal period, as indicated by a decline in the number of bacterial species seen, the more severe the loss in bacterial uniformity will be[180]. Pedroso et al [181] demonstrated changes in gut microbiome of broilers grown either in battery cages or floor pens and they were given antibiotic treatment (avilamycin, bacitracin methylene disalicylate, and enramycin). In all tests, they discovered that the composition of the intestinal bacterial population of the birds was altered by all antibiotics and the antibiotics used in either environment had no effect on the variety of bacterial genotypes observed in chickens' digestive tracts[180]. Finally, it could be concluded as antibiotic-induced changes to the gut bacterial community's makeup, though, may be connected to enhanced growth capacity [65].

Maintaining gut euobiosis using different dietary additives:

The use of feed additives in animal food production is crucial for improving performance and poultry health, and many of these compounds alter the gut microbial ecosystem in ways that improve immune function, inhibiti colonization of pathogenic microbes, and/or enhancement of nutrient availability and uptake in the gut[182] . Multiple types of infeed additives exist, including antimicrobials, probiotics, coccidiostats, vitamin and mineral oils, organic acids, enzymes, heavy metals and others[182] .

Probiotics:

The gut microbiome is essential for intestinal development, mucosal immunity, feed digestion, and host nutrient absorption [183] . Therefore, gaining an understanding of the characteristics of a highly productive microbiota may help in the creation of antibiotic alternatives that promote growth [184] . Tools like probiotics and prebiotics are being investigated to assist lessen the production's reliance on antibiotics[183] . Probiotics are live bacteria that, after being consumed, have been shown to have positive effects on health. These advantages include improving the efficiency of the host›s intestinal barrier, keeping out possible infections, and preserving GIT homeostasis [185]. Without affecting the entire microbiota, probiotics may directly benefit the host. The ingestion of *Bacillus subtilis* CGMCC 1.1086 increased weight gain and the FCR in male broilers[186] . Another trial involved feeding *L. planatarum*, which improved immunity and boosted blood IgG and secretory IgA levels as well as thymus growth[187]. Because they are both naturally present in large proportions within the bird GIT. *Lactobacillus* and *Enterococcus spp*. are two strains that are frequently used as probiotics in poultry [188] . For poultry flocks, *Lactobacillus spp*. has been linked to improved goblet cell numbers, lowering the *E. coli* in GIT, increased body weight, FCR and broiler growth were enhanced by dietary addition of *Enterococcus* species [89]. The performance parameters of FCR, feed intake, average daily growth, and apparent metabolizable energy were used to compare cecal microbiota differences between the best and worst performing birds to find novel probiotic species [182].The investigation discovered several *Lactobacillus* spp. that significantly connected with poor performance and possible members of the *Lachnospiraceae, Ruminococcaceae*, and *Erysipelotrichaceae* that significantly correlated with high performance [189]. Similar relations between poor bird productivity and *Lactobacillus* spp. were reported [91]. In addition, research suggested that *Clostridium lactatifermentans (C. lactatifermentans)* could be developed into probiotics in the future[182] .

Prebiotics

Prebiotics are feed additives that directly feed beneficial populations of the microbiota to sustain productivity while reducing disease burdens [190]. Dietary fibers like xylooligosaccharides (XOS) and fructooligosaccharides (FOS) , both of which have been more thoroughly discussed in earlier publications are frequently employed as prebiotics in poultry [191] . Depending on the kind of dietary fiber, different prebiotics work in different ways, and many of these ways still need to be completely understood [182]. While studies involving XOS supplementation found improvement of Clostridium cluster XIVa and Lactobacillus concentration in the colon and ceca of broilers, those involving FOS supplementation observed increases in *Bifidobacterium* and *Lactobacillus* populations in the cecum and ileum of broilers along with decreased levels of *E. coli* and *C. perfringens*[192]. Beneficial commensals, such Bifidobacterium, can ferment FOS and XOS into short chain fatty acids (SCFAs), which have

a wide of beneficial impactes on the microbial community and associated health benefits. Later in this essay, the advantages of these SCFAs will be covered in more detail [182] .

Conclusion

One of the primary GIT t defenses against enteric infections is gut microbiota. The disruption of the host-gut microbiota relationship is essential for the emergence of intestinal diseases. As a result, intestinal dysbiosis is related to several pathogenic disorders, including infectious bursitis. So many diseases that are closely related to inflammatory disorders can be controlled by tractable treatments that focus on the manipulation of gut microbiota. Animal performance and health are consequently enhanced. Prebiotics and probiotics as dietary supplements are one of these tactics. Through modification of the intestinal epithelial barrier and enhancement of the gut microbiota, these supplements indirectly exert their effects.

Abbreviations

16S rRNA: 16 small ribosomal RNA
ARISA: automated ribosomal intergenic spacer analysis
BF: bursa of Fabricius
CFU : colony forming unit
CT: caecal tonsils
DGGE: denaturing gradient gel electrophoresis
Dph : post hatch
Dpi: days post-infection
E. coli: Escherichia coli
ELISA: enzyme-linked immunosorbent assay
FCR: feed conversion rate
FISH: Fluorescent in situ hybridisation
FOS : fructooligosaccharides
FRET: Fluorescence resonance energy transfer
GALT: gut-associated lymphoid tissues
GIT: gastrointestinal tract
IBD: Infectious bursal disease
Ig : immunoglobulin
IL: interleukin
MALT: mucosa-associated lymphoid tissue
MDV: Marek's disease virus
NE: necrotic enteritis
NF: nuclear factor
NSPs: Non-starch polysaccharides
PCR: polymerase chain reaction
PI : post infection
SCFAs: short chain fatty acids
SSCP: single-strand conformation polymorphism
TGF: Transforming growth factor
TGGE: temperature gradient gel electrophoresis
TLR: Toll-like receptors
T-RFLP: terminal-restriction fragment length polymorphism
TTGE: temporal temperature gradient gel electrophoresis
vvIBDV: very virulent Infectious bursal disease
XOS: xylooligosaccharides

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العوامل الممرضة وغير الممرضة ؛ خاصه فيروس التهاب غده فبريشي المعدي ؛ التي تؤثر علي ميكروبايوتا الجهاز الهضمي للدجاج وطرق تقييمها واستعادتها.

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يحتوي الجهاز الهضمي (GI) للدجاج على احياء دقيقة متنوعة ومعقدة تلعب دورًا حيويًا في هضم وامتصاص العناصر الغذائية وتطوير نظام المناعة واستبعاد مسببات الأمراض ، لذا ارتبطت التحولات في تكوين المجتمع الميكروبي بصحة الأمعاء والأداء الإنتاجي للدجاج. . ومع ذلك ، فإن سلامة أمعاء الدجاج ووظائفها وصحتها تعتمد على العديد من العوامل بما في ذلك البيئة والأعلاف . تتأثر الميكروبات المعويه بالاضطرابات المعويه و الامراض التي تصيب الاجهزه الحيويه الاخري خارج الامعاء. مرض الجراب المعدي (IBD) ، المعروف أيضًا باسم مرض جمبورو ، هو مرض فيروسي خطير ومعدٍ يصيب الدجاج الصغير ويرتبط بمعدلات مراضة ووفيات كبيرة. جراب فابريسيوس هو العضو المستهدف لهذا الفيروس المثبط للمناعة. يسبب فيروس IBD آفات نسيجية ، وتغيرات في الخلايا المناعية ، وتغيرات في الميكروبيوتا عن طريق التكاثر في الأنسجة اللمفاوية المرتبطة بالأمعاء. تركز هذه المقاله على دور الاحياء القيقة لمعي الدجاج في صحة الأمعاء ، ومناعة العائل وإنتاجية الدجاج بالإضافة إلى العوامل التي تؤثر على الجراثيم مثل العمر والجنس والسلالة والأجسام المضادة للأمهات والموسم وتكوين الحصص الغذائية والمضادات الحيوية والعلاقة بين العوامل الممرضة والميكروبات المعوية مع الاهتمام بتأثير مرض الجراب المعدي على النظام البيئي لميكروبات الأمعاء مع ذكر دور اعطاء الكائنات الحية المجهرية و ما قبل المعزز الحيوي (البريبايوتيك) في تصحيح دسب الاختلال.

الكلمات الأساسية: دجاج ، أمراض ، ميكروبيوتا ، البريبايوتكس، البروبيوتيك ، معي الدجاج ، عدوي الجمبورو.