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REVIEW

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Probiotics in poultry: a comprehensive review

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Abstract

Background The increase in global population has elevated the food demand which in turn escalated the food animal production systems, especially poultry industries. For a long time, antibiotics are used worldwide to safeguard animals from diseases and for high production performances. Over usage of antibiotics has led to severe side effects such as antibiotic resistance among pathogenic bacteria, harming the beneficial bacteria in the gut, and stacking up of residuals in animal food products. It is the need of the hour to find a competent alternative to antibiotics. Probiotics have gained major attention as safe, feasible, and efficient alternatives to commercial antibiotics.

Main body Probiotics meaning "*prolife*" are live, non-pathogenic microorganisms that when given in sufficient amount confer an advantage to the host health and well-being. Probiotics are reported to improve growth, production performance, immunity, and digestibility, safeguard gut microflora, and enhance egg and meat quality traits in poultry. Proper selection of probiotics strains is crucial before their commercialization. This systematic review focuses on the mechanism of action of probiotics and summarizes the potential role of different probiotics supplementation for enhancing the production and shielding the health and immunity of poultry flocks.

Conclusions Probiotics has got a beneficial impact on the health and immunity of poultry, showing their competence as an alternative to commercial antibiotics. Modern experimental techniques are required to shed more light on the capabilities of probiotics and their usage for animal health.

Keywords Probiotics, Poultry, Gut health, Immunity

Background

The poultry industry has emerged as an efficient sector contributing significantly to livelihood and nutritional security to the growing global demand for large-scale industries. As per the latest data, the world poultry population is over 26.8 billion (FAO, 2020). According to the Food and Agriculture Organization (FAO), between 1961 and 2019 the annual global poultry meat production was estimated to be 132 million tonnes, which is 37% share of the global meat production (FAO, 2020). Demand for animal-derived food is increasing because of rapid growth in the human population, rise in income, and urbanization (FAO, 2020). To overcome the huge need for meat and egg, the poultry flocks are regularly under high pressure and stress. So, for disease prevention, growth improvement, and better immunity in poultry flocks, antibiotics usage has escalated causing the evolution of antibiotic resistance among various pathogenic bacteria (Garcia-Migura et al., 2014; Roth et al., 2019). World Health Organization explained antibiotic resistance as "a serious threat to public health worldwide that requires action across all government sectors and society" (WHO Factsheets, 2015). Countries like Denmark, France, Italy, Sweden, Norway, the Netherlands, Germany, Belgium, and Finland have initiated national

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antimicrobial resistance monitoring programmes (Garcia-Migura et al., 2014). Research is ongoing for many years to enlighten the prospects of probiotics and their competence as an option to safeguard antibiotic resistance in the poultry industry.

In an investigation held between 1992 and 1998, the Department of Public Health, Minnesota reported fluoroquinolone resistance in diseases caused by *Campylobacter* spp. (Smith et al., 1999). Many reports showed that Staphylococci were found in poultry products for human use (Manie et al., 1998; Mead & Dodd, 1990). The surrounding environments, food products, and direct or indirect contact can be a factor in the transmission of antibiotic-resistant bacteria from animals to humans (Graham et al., 2009; Price et al., 2005). Antibiotic-resistant *Salmonella* was isolated from commercially available ground meat, and it was also observed that the over usage of antibiotics for preventing disease had led to the existence of antibiotic residue in poultry products which could indirectly harm humans (White et al., 2001). Overutilization of antibiotics created a significant threat to human health as resistant organisms propagate into the food cycle and extensively spread in animal food products (Cui et al., 2005; Garofalo et al., 2007; Kim et al., 2005; Parveen et al., 2007; Ramchandani et al., 2005). Fresh meat products can act as a reservoir for antibiotic-resistant genes that can be transmitted to humans on regular intake (Diarrassouba et al., 2007; Gundogan et al., 2005; Mena et al., 2008). As antibiotic resistance among pathogenic bacteria is increasing rapidly, the livestock industry is in search of an efficient alternative over the years, and probiotics is used worldwide as an efficient alternative to filling this gap.

The history of probiotics started long back during the 1900s when the concept was first presented by Russian-born scientist Ellie Metchnikoff. He was a Nobel Prize winner whose pioneering studies discovered that few beneficial bacteria could make difference in the gastrointestinal tract of humans when ingested regularly (Metchnikoff, 1907). He came to this theory after observing the peasants living in the mountains drinking fermented milk products on daily basis and living a long and healthy life. He suggested that beneficial microorganisms in human microflora had higher resistance to pathogenic organisms. The word probiotics is derived from Greek, meaning "*prolife*" (Shokryazdan et al., 2017a, 2017b). The definition of probiotics by the FAO/WHO is given as a "live organism that when administered in adequate amount confer a health benefit on the host" (FAO/WHO joint report, 2001). Probiotics are known for their capability to improvise the gut microflora and immunity of the living being (Chen et al., 2012). These are widely used in clinical therapeutics and veterinary purposes (Abushelaibi et al., 2017; Srinivas et al., 2017). The use of probiotics in the animal diet has improved the growth, production performance, prevalence of diseases, immunity, digestibility, faecal microflora, etc. in livestock (Cavalheiro et al., 2015; Zhao et al., 2015; Lan et al., 2017).

In the past few years, non-specific immunomodulators like probiotics, prebiotics, synbiotics, postbiotics, polysaccharides, organic acids, enzymes, essential oils, etc. have emerged as a great alternative to commercial antibiotics and are also used to enhance the gut microbiota of poultry birds (Callaway et al., 2017; Shi et al., 2019). Prebiotics, synbiotics, and postbiotics are compounds that help in the development and actions of beneficial gut bacteria (Allen et al., 2013; Vyas & Ranganathan, 2012). The most frequently used probiotics in the poultry feed industry are *Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus, Pediococcus, Enterococcus,* and *Weissella* (Guarner et al., 2003; Azad et al., 2018).

The International Scientific Association of Probiotics and Prebiotics has reviewed and published a consensus statement on the definition of prebiotics "a substrate that is selectively utilized by host microorganism conferring a health benefit" (Gibson et al., 2017). Synbiotics are also described as a "synergistic combination of probiotics and prebiotics that are beneficial for the host by improving the development and colonization of live microorganisms in the gut" (FAO/WHO joint report, 2002). The International Scientific Association of Probiotics and Prebiotics has also reviewed and published a consensus statement on the definition of postbiotics as "the preparation of inanimate microorganism and/or their components that confer a health benefit on the host" (Salminen et al., 2021). Probiotics have taken major attention and are believed to be a safe and feasible alternative to commercial antibiotics.

Therefore, this review focuses on the significance of probiotics in poultry health and production majorly focusing on the poultry industry. This review will help to understand the current scenario, mechanism of action, and the need for probiotics as an essential alternative to commercial antibiotics in the global poultry industry.

Main text

Mechanism of action of probiotics

Probiotics place a key role in gut microbial health. The mechanisms of action of probiotics mainly include two, i.e. competitive exclusion and immune system modulation. Competitive exclusion of pathogens by probiotics includes: (a) production of inhibitory compounds like bacteriocins, mucins, defensins, etc. (b) preventing the adhesion of pathogens, (c) competition for nutrients, (d) reduction of toxin bioavailability, and (e) modulation of the host immune system including the enhancement of both innate and adaptive immunity (Hernandez-Patlan et al., 2020).

a) Secretion of inhibitory compounds

Probiotics produce different types of inhibitory compounds that help in reducing pathogen invasion. These include "antimicrobial peptides (AMPs)"such as bacteriocins, hydrogen peroxide, organic acids, ethanol, and diacetyl (Liao & Nyachoti, 2017). Bacteriocins are ribosomal synthesized AMP that can eliminate or inhibit pathogenic bacterial strains. They are segregated depending on their size, structure, post-translational modifications (Cotter et al., 2013).

It has been reported that pediocin A produced by *Pediococcuspentosaceus* and divercin of *Carnobacteriumdivergens* has improved the broiler performance in a field trial when challenged with *C. perfringens* (Grilli et al., 2009; Józefiak et al., 2012). An inhibitory compound—nisin produced by *L. lactis*—affects the cells and spores of *C. perfringens* in *in vitro* conditions (Udompijitkul et al., 2012).

Bacteriocin secreted by gram-positive bacteria such as lactic acid bacteria (LAB), kill the pathogens by disrupting their cell wall synthesis or by making pores in them (Belguesmia et al., 2010). LAB-bacteriocin does not affect other bacterial population in the microbiota as it targets specific species in the GI tract (Hernandez-Patlan et al., 2020).

Synergistic effects of LAB-bacteriocins together with a few biomolecules are reported such as enterocin AS-48 and ethambutol against *M. tuberculosis* (Aguilar-Pérez et al., 2018), beta-lactams with nisin against *S. enterica* serovar *Typhimurium* (Rishi et al., 2014; Singh et al., 2014), Garvicin KA farnesol against the gram-negative and gram-positive bacteria (Chi & Holo, 2018), citric acid with nisin against *L. monocytogenes* and *S. aureus* (Zhao et al., 2017). A strain of *Brevibacillus borstelensis* active in GI tract has anti-*C. perfringens* activity and is linked with a thermostable bacteriocin-like inhibitory substance (BLIS) of 12 kDa (Sharma et al., 2014).

Organic acids exhibited inhibitory activities against pathogenic bacteria. They reduce the intracellular pH and stop the movement of the internal protons which in turn deplete the cellular energy (Ricke, 2003). Organic acids directly target the cytoplasmic membrane, cell wall, and particular metabolic functions of harmful bacteria leading to its disruption and depletion (Nair et al., 2017; Zhitnitsky et al., 2017).

Lactic acid bacteria produce lactic acid which generates an unfavourable condition in the gut of pathogenic bacteria (Dittoe et al., 2018). Reports showed that lactic acid bacteria in a concentration of 0.5% (v/v) could inhibit the growth of pathogens such as *Salmonella* spp., *L. Monocytogenes,* or *E. coli* (Wang et al., 2015). However, these acids do not affect the IEL as the pH is maintained by the mucus (Allen & Flemström, 2005).

Ethanol causes cell death by creating a leakage in the plasma membrane as it alters the membrane integrity (Ingram, 1989). Diacetyl hampers with the arginine-binding protein of gram-negative bacteria (Lindgren & Dobrogosz, 1990). Carbon dioxide generates an anaerobic condition that is not favourable for aerobic bacteria to grow (Singh, 2018).

b) Inhibition of pathogenic adhesion

Probiotics helps in blocking the adhesion of pathogenic bacteria to the intestinal epithelial binding sites by competitive inhibition (Bermudez-Brito et al., 2012). Adhesion is among one of the major criteria for the selection of efficient probiotics strains (Collado et al., 2005). It activates mucosal immunity and helps in the production of mucins and defensins which enhances the epithelial barrier (Bermudez-Brito et al., 2012). Mucins are heavily glycosylated glycoproteins which are produced by intestinal epithelial cells to shield and lubricate the epithelial cell surfaces (González-Rodríguez et al., 2012). These are major macromolecular element of mucus which inhibits the adhesion and colonization of pathogenic bacteria (Collado et al., 2005). The specific interaction between the IEL and surface proteins of probiotics bacteria are accountable for the possible exclusion of pathogenic bacteria (Ouwehand et al., 2002a, 2002b; Van Tassell et al., 2011). Mucins are also involved in modulating immune responses. Defensins are from a family of membrane-disrupting peptides (Ayabe et al., 2000). These are small cationic peptides that can kill or inhibit bacterial growth by either direct membrane disruption or inhibition of bacterial cell wall synthesis (Kagan et al., 1990). The electrostatic interaction of anionic phospholipid groups of the epithelial membrane creates pores in the membrane which cause its disruption and lysis of the harmful bacteria (Kagan et al., 1990). It also helps in neutralizing the secreted toxins by pathogenic bacteria (Schlee et al., 2008; Tiwari et al., 2012).

c) Competition for nutrients

The adhesion of probiotics to the IEL creates a competitive depletion of essential nutrition (Callaway et al., 2008). It also constricts the pathogen-binding or adhesion sites in the GI tract (Callaway et al., 2008). This leads to a rapid decline in the proliferation and colonization of the pathogenic population (Callaway et al., 2008; Liao & Nyachoti, 2017; Vieco-Saiz et al., 2019). Probiotic bacteria create unfavourable surroundings for the pathogens for their survival (Schiffrin & Blum, 2002). Competitive exclusion of pathogenic bacteria has been showcased *in* *vitro* using chicken intestinal mucosa (Hirn et al., 1992). *Salmonella* colonization was reduced in chicks when *lactobacillus*-based probiotics were given at the age of 1 to 7 days at the concentration of 1×10^5 CFU/ml (PenhaFilho et al., 2015). Data revealed that for reducing the colonization of *S. enteritidis* S1400 in the chicken gut, a mixture of 5×10^7 CFU/ml of *L. salivarius* 59 and *E. Faecium* PXN33 was used (Carter et al., 2017).

d) Reduction in toxin bioavailability

Probiotics like *lactobacillus* help the reduction in the uptake of pathogenic toxins in the intestinal cells. The positive effects of LAB-based probiotics had helped in the reduction of toxin expression in the gut (Liao & Nyachoti, 2017). Lactic acid bacteria are known for their natural barriers against mycotoxins which are harmful compounds for animals (Peng et al., 2018; Tsai et al., 2012). A few strains can also eradicate the detrimental reactions of aflatoxins on human and animal health (Abbès et al., 2016; Li et al., 2017).

e) Modulation of the host immune system

Probiotics are known for their immunomodulatory effects (Ashraf & Shah, 2014; Bermudez-Brito et al., 2012; Tellez et al., 2012; Tsai et al., 2012). These bacteria are said to be capable of interacting with epithelial, dendritic cells, macrophages, and lymphocytes (Bermudez-Brito et al., 2012). Innate immune response enhances the IEL cells to prevent the proliferation of pathogens and the spreading of infections (Vieco-Siaz et al., 2019). Probiotics also helps to improve the epithelial barrier by enhancing mucus and AMPs production (Bermudez-Brito et al., 2012). Intestinal epithelial cells and dendritic cells interact with the pattern recognition receptor (PRR) of gut microorganisms (Gómez-Llorente et al., 2010; Lebeer et al., 2010). The activation of adaptive immunity starts with the interactions among the PRR of antigen-presenting cells like dendritic cells (DC) which lead to the release of T and B-cells (Iwasaki & Medzhitov, 2015; Lebeer et al., 2010). These activations of immunity play an important role in pointing out the efficiency of LAB as a probiotic candidate (Hardy et al., 2013; Kiczorowska et al., 2017; Wells, 2011).

Chemical and physical barriers like mucus and IEL act as the first line of defence in innate immunity (Riera-Romo et al., 2016). Probiotics strains modulate the immune system of the host organism in different ways such as secretion of mucus and AMPs, elevated immune responses, variation in cytokine levels, and also safeguarding the epithelial layer cells from pathogenic bacteria (Anderson et al., 2010; Madsen, 2012). *L. brevis* ZLB004 is reported to downregulate the proinflammatory cytokines TNF-*α* and IL-8 in an *in vivo* animal experiment (Li et al., 2016). Probiotics are capable of suppressing intestinal inflammation by downregulating the TLR expression and restricting the NF-κB in enterocytes (Joo et al., 2011).

The B and T lymphocyte cells are induced to produce antibodies for antigen-specific reactions as an adaptive immune response (Kabir et al., 2009). In broiler chickens, an elevation of IgG and IgA responses was observed when they were fed with a mixture of *Clostridium butyricum* (1×10^6 CFU/kg of feed) and *L. plantarum* (1×10^7 CFU/kg of feed) (Han et al., 2018). *Lactobacillus* spp. can effectively initiate mucosal immunity in chicken by elevating the IgA and IgG levels when it is administered at a concentration of 10^{10} CFU/ml (Rocha et al., 2012).

Single- and multi-strain probiotics

Probiotics are broadly classified into single strain and multi-strain. Single-strain probiotics contain an individual bacterium in a certain concentration that confers health benefits to the host and the commonly used genera for probiotics are *Lactobacillus, Bifidobacterium, Streptococcus, Pedicoccus, Enterococcus, Bacillus, Saccharomyces, Micrococcus*, etc. (Babot et al., 2018; Sanders et al., 2019) (Fig. 1).

Multi-strain probiotics are a mixture of more than one strain of the same species or multiple genera of bacteria which are beneficial for the well-being and immunity of the host (Kwoji et al., 2021). The body weight of chickens increased when they were supplemented with multi-strain *Lactobacillus* probiotics at the concentration of 1×10^9 CFU/g or combined with prebiotics or synbiotics (Mookiah et al., 2014). Growth performance, feed intake, and gut health were improved in broiler chicken challenged with *Pasteurella multocida* when they were supplemented with multi-strain probiotics containing *S. cerevisiae, L. fermentum, P. acidilactici, L. plantarum*, and *E. faecium* (Lambo et al., 2021).

A few examples of commercial multi-strain probiotics are PrimaLac containing *Bifidobacterium thermophilum, Enterococcus faecium*, and *Lactobacillus* spp., PoultryStar ME containing *Pediococcus acidilactici, Lactobacillus reuteri, Lactobacillus salivarius, and Enterococcus faecium*, Bifilac containing *Streptococcus faecalis, Clostridium butyricum, Bacillus mesentericus*, and *Lactobacillus sporogenes*, and Microgaurd containing different species of *Bacillus, Lactobacillus, Saccharomyces, Bifidobacterium*, and *Streptococcus* (Lambo et al., 2021).

A study shows that single-strain and multi-strain probiotics mechanism of action varies from depending upon their viable concentration of bacterial count, and hence, multi-strain probiotics had more beneficial effects in the maintenance of a healthy gut in different conditions

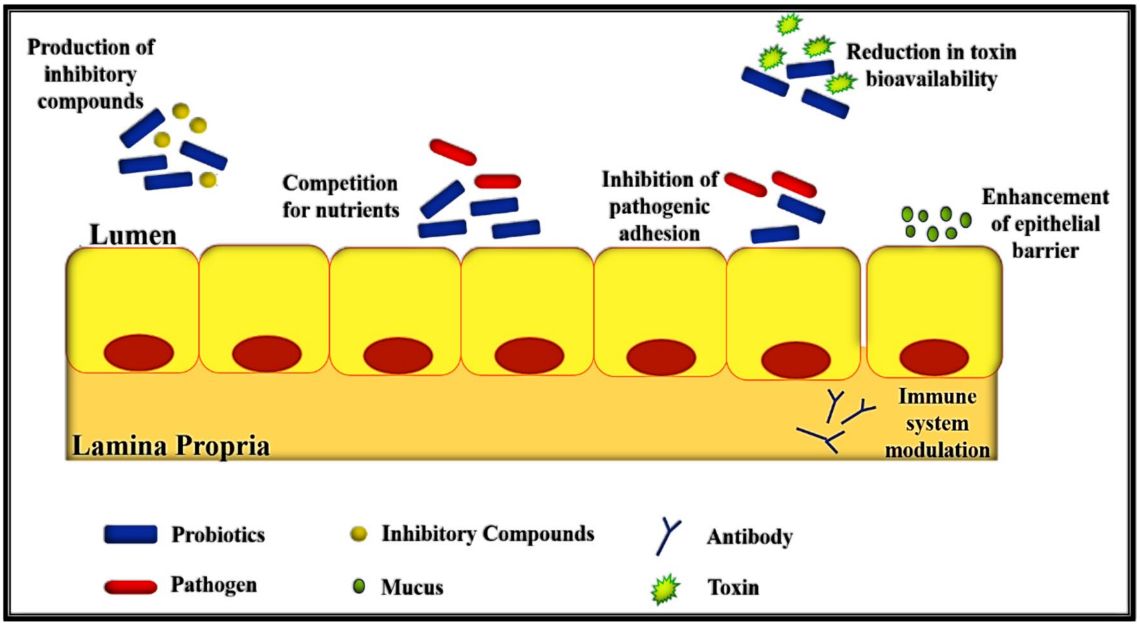

Fig. 1 Mode of action of probiotics. It starts with the secretion of inhibitory compounds leading to inhibition of the pathogen adhesion to the epithelial layer of the GI tractbesides creating competition for nutrients among pathogens thereby reducing their colonization. Also it helps in diminishing the toxin bioavailability and modulates the immune system of the host by activating adaptive and innate immunity

(Konieczka et al., 2022). Conversely, there are finding from a few studies that showed dietary treatments of single- or multi-strain probiotics had no significant effects on broiler breeder performance, egg production, egg quality, and hatchability (Aalaei et al., 2018). So in-depth research is needed to specify whether single-strain or multi-strain probiotics are more beneficial for poultry birds. Probiotics have emerged as an effective alternative to antibiotics in livestock industries. The action mechanism of probiotics, their different strains, metabolic activities, colonizing in the GI tract, etc. have played a major role in their selection as probiotics candidate in various livestock industries, especially poultry enterprise.

Lactic acid *bacteria* (LAB)

Lactic acid bacteria are good probiotics candidate as they show antimicrobial activities and beneficial effects on the host (Caly et al., 2015). *Lactobacillus* spp. reduced the *C. perfringes* population in the chicken gut without affecting their gut flora (Gérard et al., 2008). Lactic acid bacteria as probiotics promote the overall growth, production performance, and well-being of animals (Seal et al., 2013). *Lactobacillus paracasei* sub *paracasei* and *L. rhamnosis* have a positive influence on broiler's growth performance and the health of their gut (Fesseha et al., 2021). Lactic acid bacteria produce different inhibitory compounds to decrease pathogen invasion such as bacteriocin, ethanol, organic acids, hydrogen peroxide, diacetyl (Liao & Nyachoti, 2017). Lactic acid bacteria have immunomodulatory properties as they initiate the production of cytokines and impact the changes in the immune system of the host by modulating the innate or adaptive responses (Kiczorwska et al., 2017).

Lactic acid bacteria-based probiotics at a concentration of 1×10^5 CFU efficiently reduces *Salmonella* colonization in chicks at their early ages (PenhaFilho et al., 2015). *Lactobacillus acidophilus* improves broiler production performance, intestinal health, and metabolic functions (De Cesare et al., 2020). The supplementation of *L. acidophilus* increased the BWG of broilers infected with *C. perfringens* and decreased the mortality (Li et al., 2018). *Lactobacillus salivarius* supplementation enhances the growth performance of white leghorn chickens, decreases heat stress, and reduces their organ injury and mortality by *E. coli* infection. Lactic acid bacteria supplementation also enhances lymphocyte proliferation and immune responses after IBD vaccine immunization (Wang et al., 2020).

Lactobacillus acidophilus used for lying hens efficiently lowered the egg yolk and liver cholesterol as well as plasma triglycerides levels (Alaqil et al., 2020). *Lactobacillus acidophilus* D2/CSL (CECT 4529) supplementation (0.2 g and 0.02 g) in drinking water improved the beneficial microbes and functional genes in broiler crops and caeca (De Cesare et al., 2020). The addition of 0.10% of *L. plantarum* had beneficial effects on growth, excreta microbiota, and gas emission, and also reduces a significant number of *E. coli* counts from chicken excreta (Sampath et al., 2021). Ruminal acidiosis can be prevented by LAB supplementation and by creating a suitable condition for lactic acid-consuming good bacteria (Chaucheyras-Durand & Durand, 2010).

Lactic acid bacteria act as a natural barrier of the gastrointestinal tract as it reduces the bioavailability of mycotoxins and neutralizes the side effects. It also facilitates the excretion of mycotoxins by faeces (Zoghi et al., 2014; Damyanti et al., 2017). Inclusion of *L. reuteri, E. faecium, B. animalis, P. acidilactici*, and *L. salivarius* in the concentration of 10^9 and 10^{10} CFU/kg of feed changed the composition of caecal flora of broiler at 14 and 42 days of age (Mountzouris et al., 2007). *Lactobacillus crispatus, L. salivarius, L. fermentum, L. gasseri* were investigated to have a positive influence on IL-6, IL-8, and IL-10 (Luongo et al., 2013; Pérez-Cano et al., 2010; Rizzo et al., 2015; Sun et al., 2013).

Supplementation of 1×10^9 CFU/kg *L. acidophilus* LA5 has elevated the levels of $CD4^+$, $CD8^+$, and $TCR1^+$ T cells in the gastrointestinal tract and peripheral blood of chickens (Asgari et al., 2016). 1×10^9 CFU of *L. plantarum* LTC-113 strain was inoculated into hatched chicks which restricted the intestinal colonization and managed the expression of tight junction genes which led to anti-*salmonella typhimurium* protection (Wang et al., 2018).

Bacillus

Many strains of Bacillus have potential against pathogenic bacteria. A group of researchers isolated 200 Bacillus strains from the faeces of broiler chicken and many strains among them showed activity against *C. perfringens* in *in vitro* conditions (Barbosa et al., 2005). A study suggested that *B. subtilis* strain SP6 when used in a field trial, the mortality of chicken infected with Necrotic enteritis was reduced to half. It also reduced the number of *C. perfringens* and enhanced the intestinal health of chickens (Jayaraman et al., 2013). Regular use of *B. licheniformis* supplementation reduced mortality and increased the performance among the chicks (Knap et al., 2010).

Yeast

Yeast has shown antimicrobial properties, and among them, many types of yeast have *β-glucans* which are accountable for the immunomodulatory responses of the host (Hatoum et al., 2012; Novak & Vetvicka, 2008; Paul et al., 2012). It protects against pathogenic bacteria by producing mycocins, secreting inhibitory substances which degrade the toxins, preventing the adhesion of pathogens to epithelial cell surfaces, and creating competition for nutrition (Hatoum et al., 2012). A study showed that chickens supplemented with *S. boulardii* had a beneficial impact on intestinal health (Rajput et al., 2013) and also have good results on chickens infected with *S. enteritidis* (Gil de Lossantos et al., 2005). A recombinant strain of *Pichia pastoris* carry a gene that codes for *C. perfringens-α* toxin which is responsible for the secretion of anti-*C. perfringens* antibodies and improved performance of broilers (Gil de Lossantos et al., 2012).

Enterococci

Enterococci are actively known for its range of bacteriocins, named enterocin, which acts against gram-positive and gram-negative bacteria (Franz et al., 2007). A report showed that *C. perfringens* was reduced by supplementation of *E. faecium* in chicks on the day of hatch (Cao et al., 2013). A strain of *E. faecium* was isolated from the intestines of the broiler chicken which showed in vitro activity against *C. perfringens* (Shin et al., 2008). *Enterococci faecium* supplementation (0.5 g/L) in broiler chickens reduced the detrimental effects of coccidiosis in turn it improved the growth performance (El-Sawah et al., 2020). An increase in IgA production and change in the faecal biome was also observed in chickens fed with *E. faecium* (Beirao et al., 2018). *E. faecalis*-1 supplementation in broilers had increased growth performance and was beneficial for immunity and caecal microbiome modulation (Shehata et al., 2019). Supplementation of *E. faecium* in broiler chicken results in better nutrient utilization and improves metabolic efficiency (Zheng et al., 2016).

Association between antibiotics usage and antibiotic-resistant *bacteria* in food animals

Antibiotics are widely used feed additives to enhance the growth rate, feed conversion, poultry immunity, and productivity besides preventing infections (Gadde et al., 2017). However, the application of antibiotics is linked to the increasing occurrence of antibiotic resistance and antibiotic residue in livestock and its products (Marshal et al., 2011). Evidence showed that antibiotic-resistant genes could be transmitted from animals to humans (Greko, 2001). Few researchers had reported the over usage of antibiotic treatments among food animals that are used by humans has raised a matter of concern (Lanzas et al., 2010; Lhermie et al., 2016). This significant threat of transmission of antibiotic resistance to humans has increased over the years through the direct intake of food and antibiotic treatment failure in humans (Lanzas et al., 2010).

A report suggested that the *E. coli* strains isolated from farming families and their livestock showed highly associated resistance patterns (Fein et al., 1974). It was observed from 1982 to 1989 that quinolone resistance in *Campylobacter* subspecies increased from 0 to 14% in poultry products during which the use of fluoroquinolones increased in veterinary and human use (Endtz et al., 1991).

A group of researchers found that there was a prevalence of Vancomycin-resistant *Enterococcusfaecium* (VRE) in turkeys, turkey farmers, turkey slaughterers, and neighbouring residents (Van den Bogaard et al., 1997). The same group of researchers has also found that antibiotic-resistant *Enterococci* were present in faecal isolates of broiler chicken and their farmers (Van den Bogaard et al., 2002). The use of quinolone in food animals has increased antibiotic resistance among *C. jejuni* and *C. coli* (Engberg et al., 2001). Antibiotic-resistant *Salmonella* and *Campylobacter* were extracted from both organic and conventional retail chicken (Cui et al., 2005). *Salmonella aureus* isolated from retail chicken, calf, and lamb products showed that 88% of them were bacitracin-resistant, 68% were methicillin-resistant, 53% were penicillin-resistant, and 7% isolates were erythromycin-resistant (Gundogan et al., 2005). *K. pneumonia* isolated from turkey and chicken farms as well as from commercial poultry and meat products were resistant to ampicillin, tetracycline, streptomycin, gentamycin, and kanamycin (Kim et al., 2005). About 79.8% of *salmonella* isolated from chilled and non-chilled processed chicken carcasses showed antibiotic resistance (Parveen et al., 2007). Isolates of *E. coli* from poultry, retail poultry products, hospitalized adults, and outpatient vegetarians were similar (Johnson et al., 2007).

Agricultural workers and farmers are the ones majorly affected by antibiotic-resistant bacteria (Smith et al., 2013). Reports suggested that children living in a household having poultry tend to have more antibiotic resistance among them (Brogdon et al., 2021). Resistant *Enterococci* and *Staphylococci* were frequently retrieved from flies captured near poultry farms that had similar traits to the ones isolated from poultry litter of the same farm (Graham et al., 2008). A trial on human urinary tract infection in a few states of the USA showed that the trimethoprim–sulfamethoxazole-resistant *E. coli* was suggested to be sharing similar traits with those isolated from food animals and their products (Ramchandani et al., 2005). Several other reports also revealed the indirect transfer of many antibiotic-resistant strains such as *S. aureus,* and *Campylobacter* to humans via the food web (Bengtsson et al., 2014). *Enterococcus coli* showed interconnection between food animals and human, and it also suggested that antibiotic-resistant *E. coli* isolates that causes bloodstream diseases in humans are majorly acquired from animal food products (Vieira et al., 2011).

In 1986, Sweden was the first country to ban antibiotics usage in animal feed followed by countries like South Korea, Denmark, Germany, and Taiwan (Ziggers, 2011). According to the Fish and Animal Feed Act 2010, Bangladesh imposed a complete ban on the use of antibiotics in animal feed (Kiers & Connolly, 2014; Maron et al., 2013). In 2006, the European Union banned the use of subtherapeutic antibiotics in animal diets (Franz et al., 2010; Huyghebaert et al., 2011; Maron et al., 2013; Ziggers, 2011). In 2012, the US Food and Drug Administration (FDA) prohibited the use of certain antibiotics on food animals. In 2017, World Health Organization launched a guideline that directed to reduce the use of all classes of antibiotics for growth promotion and disease prevention without diagnosis (Maron et al., 2013; Ziggers, 2011). Public awareness of the health risks of commercial antibiotics has taken a huge turn in the use of antibiotics in the poultry industry. More trends have been seen in the industry using optional approaches, so the quest for an efficient alternative to antibiotics has escalated in past years. Researchers are concentrated on searching for a competent product that can help advance poultry health, and performance, and in turn, improve food safety for humans. Competitive exclusion of pathogens, secretion of antimicrobial substances like bacteriocin, and adherence to gastrointestinal mucosa help probiotics to stand out as an effective alternative to commercial antibiotics (Collins et al., 1998; Ouwehand et al., 2002a, 2002b).

Beneficial effects of probiotics on poultry

a) Effects on growth performance and productivity

Probiotics enhance BWG, DWG, FCR, feed intake, and also increase productive performances. In chicken, feeding of *P. acidilactici* (10^8 CFU/kg of feed) had a beneficial effect on egg quality by improving their eggshell thickness, weight and reduced the cholesterol level in egg yolk (Mikulski et al., 2012). In broilers, feeding probiotics has elevated the microbiological quality of meat and reduced the contamination by *S. enteritidis* in carcasses, which saves the consumer from food-borne infections (Bailey et al., 2000). *Bacillus subtilis* at 1×10^8 CFU/kg was found to have a positive influence on egg quality, performance, and the cholesterol levels of the yolk (Sobczak & Kozłowski, 2015). Multi-strain probiotics (0.4%) supplementation in layers increased egg production, enhanced the quality of eggs, and was cost-effective (Ribeiro et al., 2014). An experiment showed that when 1 g/kg of *B. subtilis* was fed under heat stress, the effects of stress on growth performance were reduced and the colonization of beneficial bacteria in the gut was enhanced (Abdelqader, 2020).

Lactic acid bacteria-based probiotics are used therapeutically to cure infections by pathogenic bacteria like *E. coli, Salmonella spp., Clostridium spp.*, etc. (Park et al., 2016; Tellez et al., 2012). Dietary supplementation of *L. Salivarius* mixture at the concentration of 0.5 or 1 g/kg has enhanced the FCR and BWG in broilers (Shokryazdan et al., 2017a, 2017b). In broilers, commercially available probiotics (Primalac) had improved the FCR, and BW Gas compared to the control groups (Taherpour *et al.*, 2009). The FCR, DWG, and BWG of chicks at the age of 3 to 6 weeks had increased significantly when fed with probiotics at a concentration of 1 g and 0.8 g/kg diet (Alkhalf et al., 2010). Lactic acid bacteria have been reported to play a crucial role in nutrient metabolism and absorption (Burgain et al., 2014).

b) Effects on serum biochemistry

Evidence showcased that probiotics in adequate quantity have effects on host serum biochemistry. In broilers, alkaline phosphatise and creatine kinase activity were significantly reduced when supplemented with both *Lactobacillus plantarum* 16 (Lac16) and *Paenibacillus polymyxa* 10, and uric acid and LDL cholesterol levels were significantly reduced in the broiler group supplemented with only *P. polymyxa* (Wu et al., 2019). Total cholesterol, glucose, LDL cholesterol, and triglycerides levels were reduced and protein level was increased by the supplementation of probiotics in broilers (Reuben et al., 2021). LAB also showed antioxidant activity against *in vitro* oxidation of LDL in chickens (Ito et al., 2015). Supplementation of *Lactobacillus* spp. such as *L. sporogenes* helped to reduce cholesterol levels in the broiler chicken (Fathi, 2013; Panda et al., 2006). The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are reduced after the intake of *Lactobacillus* culture as probiotics in broilers (Fathi, 2013).

Researchers found that there was significant upregulation of protein, and calcium levels and downregulation of total cholesterol, LDL cholesterol, VLDL cholesterol, and triglycerides in broiler serum by dietary supplementation of *L. sporogenes* at 100 mg/kg of diet (Arun et al., 2007). Levels of cholesterol, triglycerides, and ALT were reduced by the dietary supplementation of *L. acidophilus* D2/CSL CECT 4529 and *B. Subtilis* PB6 ATCC-PTA 6737 (Forte et al., 2016). Cholesterol absorption in the intestine was reduced in broiler chicken by supplementation of *Lactobacillus* cultures as probiotics (Mohan et al., 1995; Alkhalf et al., 2010; Fathi *et al*., 2013). In broiler chicken, serum cholesterol level was reduced when supplemented with *L. rhamnosus* (Hashemzadeh et al., 2013).

Supplementation of *E. faecium* M74 reduced the cholesterol, lipid, and calcium levels in ISA Brown laying hens (Capcarova et al., 2010). In broiler chicken, a reduction of total cholesterol, triglycerides, and LDLcholesterol were observed by the intake of the *L. salivarius* mixture (Shokryazdan et al., 2017a, 2017b). An experiment on male broiler chickens revealed that the dietary supplementation of commercially available probiotics (Primalac) had reduced the level of total cholesterol, LDL cholesterol, and cholesterol/HDL ratio in the serum (Taherpour *et al.*, 2009). The intake of *Lactobacillus* spp. reduces the level of total cholesterol, LDL cholesterol, and triglycerides in the blood serum of broilers (Kalavathy et al., 2003). The cholesterol level in eggs was reduced to 10.4% than the control hens at 28 weeks which were fed with *Lactobacillus* spp. (Ramasamy et al., 2010).

c) Effect on health and immunity

Probiotics act as novel feed supplement which improves the health and immunity of poultry flocks. A study shows that LAB affects the proinflammatory cytokine expressions, i.e. IL-6, IL-10, IL-1β, INF-γ, and TNF-α, and helped in reducing the inflammation in broiler chickens (Chen et al., 2012; Park et al., 2014). In broilers, *C. butyricum* when given at 2×10^7 CFU/kg or 3×10^7 CFU/kg feed, the gut flora and immune responses were boosted (Yang et al., 2012).

In broiler chicks, mixture of *L. fermentum* (1×10^7 CFU/g) and *S. cerevisiae* (2×10^7 CFU/g) at 0.1 and 0.2% concentration uplifted the T-cell generation (Bai et al., 2013). *Lactobacillus* spp. has shown a positive influence on fatty acid composition in the host (Kishino et al., 2013). *Lactobacillus plantarum* 10hk2 and *Lactobacillus johnsonii* HY7042 effectively suppressed the proinflammatory cytokine production by restricting the NF-κB activation in broilers (Chon et al., 2010; Joo et al., 2011; Li et al., 2015). *Lactobacillus* spp. reduced the IL-1β expression and enhanced TLR4 mRNA abundance when compared with the control group in broilers (Li et al., 2015). *Lactobacillus lactis* showed a proinflammatory response on PBMC by upregulating the IL-6, IL-8, IL-1b, and IL-12p40 mRNA abundance (Slawinska et al., 2021). *Lactobacillus casei* in a concentration of 10^8 CFU/g had elevated the intraepithelial lymphocytes and their migration by chemokine signalling pathway and also, modulated the mucosal immunity by upregulating the cytokine expression in chicks (Tian et al., 2021) (Fig. 2).

Risk assessment for probiotics

Probiotics have clinically proved efficiency still, the nature of the microorganisms to be used must be secured. Thus, assessment of risk factors for various strains of probiotics is necessary for commercial use. Due to the history of global probiotic usage, major strains are recognized as safe but like any other organism, probiotic strains may carry unwanted properties such as transferable antimicrobial resistance, virulence factors, and the

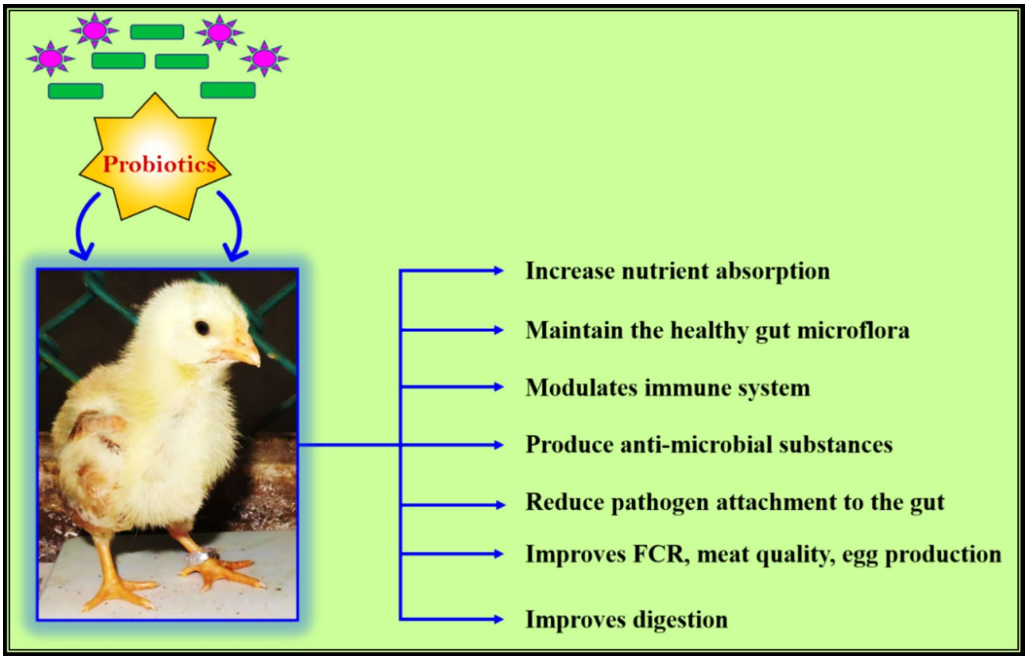

Fig. 2 Positive effects of probiotics on poultry

ability of toxin production (Donohue, 2006; Lee et al., 2017). A guideline for the evaluation of probiotic safety, validated by the European Union of Scientific Committee on Animal Nutrition, states that "(i) the assessment of strain identity; (ii) in vitro tests to screen potential probiotic strains; (iii) assessment of safety: requirement of proof that a probiotic strain is safe and without contamination in its delivery form; and (iv) in vivo studies for the substantiation of the health effects in the target host" (FAO/WHO Joint report, 2002; Kim et al., 2018) (Table 1).

Conclusions

It has been proved in different ways that probiotics are promising alternatives to commercial antibiotics. The application of different probiotics has a beneficial influence on the health and production of animals. These give both immunomodulatory and economic benefits. Probiotics safeguards meat, egg, and other edible products for human use. Various strains of probiotics have various benefits as LAB-based probiotics has been documented to have an enhanced growth rate with better production performance, improves the quality of meat, egg, and amplify growth and immunity of the host. These probiotics helps in controlling host–microbe interactions and pathogenic infections by secreting various inhibitory compounds, also undergo competitive exclusion, reduce toxin bioavailability, strengthen the IEL, and positively influence the immune system. To achieve the maximum benefit, the appropriate strain, their forms of supplementation, probiotics concentration, and the mode of delivery have to be analysed thoroughly. More benefits have to be explored to make standardized protocols for their applications worldwide. Gaining insights into probiotics will help form different strategies for the prevention or treatment of various gastrointestinal diseases. Although much research shows the positive influence of probiotics in animal farming, still more clarity is needed regarding the probiotics strains and their potential use as an alternative to commercial antibiotics.

Table 1 Few studies showing the potentials of probiotics in poultry

S. No.	Species used as Probiotics	Details	Authors
1	*Lactobacillus spp.*	*Lactobacillus* species reduced the number of *C. perfringens* in chickens but did not affect the gut flora of the host	Gérard et al. (2008)
2	*Lactobacillus spp.*	1×10^5 CFU of LAB-based probiotics helps in reducing *Salmonella* colonization at the early age of chicks	PenhaFilho et al. (2015)
3	*L. acidophilus*	*L. acidophilus* improved broiler production performance, intestinal health & metabolic functions	De Cesare et al. (2020)
4	*P. acidilactici*	Supplementation of *P. acidilactici* (10^8 CFU/kg of feed) enhanced the egg quality and reduced the cholesterol level in the yolk	Mikulski et al., 2012
5	*B. subtilis*	1 g/kg of *B. subtilis* reduced the effect of heat stress on growth performance and improved the colonization of beneficial bacteria in the gut	Abdelqader, 2020
6	*L. salivarius*	Supplementation of *L. salivarius* mixture (0.5 or 1 g/kg) enhanced body weight and FCR	Shokryazdan et al., 2017a, 2017b
7	*L. sporogenes*	Supplementation of *L. sporogenes* (100 mg/kg) increased FCR and BWG	Arun *et al.*, 2007
8	*L. plantarum, P. polymyxa*	The levels of ALT and creatinine kinase were reduced when supplemented with *L. plantarum* 16 and *Paenibacillus polymyxa* 10 in broiler chicken	Wu et al., 2019
9	*Lactobacillus spp.*	ALT and AST levels were reduced by the intake of *Lactobacillus* culture as probiotics in broilers	Fathi, 2013
10	*L. sporogenes*	Supplementation of *L. sporogenes* helped to reduce cholesterol levels in broiler chickens	Panda et al., 2006; Fathi, 2013
11	*L. acidophilus*	The levels of cholesterol, triglycerides, and ALT were reduced by supplementation of *L. acidophilus* D2/CSL CECT 4529 and *B. subtilis* PB6 ATCC-PTA 6737	Forte et al., 2016
12	*E. faecium*	The levels of cholesterol, lipid, and calcium were reduced Supplementation of *E. faecium* M74 in ISA Brown laying hens	Capcarova et al., 2010
13	*L. salivarius*	Reduction of total cholesterol, triglycerides, and LDLc was observed in broiler chicken by intake of *L. salivarius* mixture	Shokryazdan et al., 2017a, 2017b
14	Multi-strain probiotics (Primalac)	Supplementation of commercial probiotics (Primalac) reduced the level of total cholesterol, LDLc, and cholesterol/HDL ratio in male broiler chickens	Taherpour *et al.*, 2009
15	*Lactobacillus spp.*	The level of cholesterol in eggs was reduced to 10.4% than the control hens at 28 weeks of age which were fed with *Lactobacillus spp.*	Ramasamy et al., 2010
16	*Lactobacillus spp.*	LAB supplementation affects the proinflammatory cytokine expressions, i.e. IL-6, IL-10, IL-1β, INF-γ, and TNF-α, and reduced the inflammation in broiler chicken	Chen et al., 2012; Park et al., 2014
17	*C. butyricum*	Supplementation of *C. butyricum* at 2×10^7 CFU or 3×10^7 CFU/kg feed, the intestinal microflora and immune responses were boosted	Yang et al., 2012
18	*L. fermentum*	Probiotics with a mixture of *L. fermentum* (1×10^7 CFU/g) and *S. cerevisiae* (2×10^7 CFU/g) at 0.1 and 0.2% in the feed uplifted the intestinal T-cell in broiler chicks	Bai et al., 2013
19	*L. plantarum, L. johnsonii*	*L. plantarum* 10hk2 and *L. johnsonii* HY7042 supplementation suppressed the proinflammatory cytokine production by restricting the NF-κB activation in broilers	Chon et al., 2010; Joo et al., 2011; Li et al., 2015
20	*Lactobacillus spp.*	Intake of *Lactobacillus spp.* reduced the IL-1β expression and enhanced TLR4 mRNA abundance more than the control group in broilers	Li et al., 2015
21	*L. lactis*	*L. lactis* showed a proinflammatory response on PBMC by upregulating the IL-6, IL-8, IL-1b, and IL-12p40 mRNA abundance	Slawinska et al., 2021

Table 1 (continued)

S. No.	Species used as Probiotics	Details	Authors
22	*L. casei*	*L. casei* (10^8 CFU/g) supplementation elevated the intraepithelial lymphocytes and their migration by chemokine signalling pathway and modulated the mucosal immunity by upregulating the cytokine expression in chicks	Tian et al., 2021
23	Multi-strain probiotics	Growth performance, feed efficiency, and intestinal health were improved when supplemented with multi-strain probiotics containing *S. cerevisiae, L. fermentum, P. acidilactici, L. plantarum,* and *E. faecium* in broiler chicken challenged with *Pasteurella multocida*	Lambo et al., 2021
24	*B. subtilis*	*B. subtilis* strain SP6 reduced the mortality in chickens infected with Necrotic enteritis, also reduced the number of *C. perfringens,* and enhanced gut health	Jayaraman et al., 2013
25	*B. licheniformis*	Reduction in mortality and increase in performance among the chicks treated with *B. licheniformis* when used in a large amount and for long periods	Knap et al., 2010
26	Yeast	Yeast has antimicrobial properties and among them, many types of yeast have *β-glucans* which are responsible for immunomodulatory effects on the host	Novak & Vetvicka, 2008; Hatoum et al., 2012
27	*S. boulardii*	Chickens supplemented with *S. boulardii* had a positive influence on intestinal health	Rajput et al., 2013
28	*E. faecium*	Supplementation of *E. faecium* reduced the number of *C. perfringens* in chicks on the day of hatch	Cao et al., 2013
29	*E. faecium*	A strain of *E. faecium* extracted from the broiler intestines showed in vitro activity against *C. perfringens*	Shin et al., 2008
30	*E. faecium*	*E. faecium* supplementation (0.5 g/L) reduced the effects of coccidiosis and improved the growth performance in broilers	El-Sawah et al., 2020
31	*E. faecium*	Supplementation of *E. faecium* in broiler chicken resulted in better nutrient utilization, in turn, improves metabolic efficiency	Zheng et al., 2016

Abbreviations

FAO	Food and Agriculture Organization
WHO	World Health Organization
FDA	Food and Drug Administration
IEL	Intestinal epithelial layer
GIT	Gastrointestinal tract
AMPs	Antimicrobial peptides
BLIS	Bacteriocin-like inhibitory substance
PRR	Pattern recognition receptor
DC	Dendritic cell
PBMC	Peripheral blood mononuclear cell
IL	Interleukins
TNF-α	Tumour necrosis factor-alpha
TLR	Toll-like receptor
NF-κB	Nuclear factor-kappa B
INF-γ	Interferon-gamma
Ig	Immunoglobulin
IBD	Infectious bursal disease
VRE	Vancomycin-resistant *E. faecium*
CFU	Colony forming unit
BWG	Body weight gain
DWG	Daily weight gain
FCR	Feed conversion ratio
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
VLDL	Very low-density lipoprotein

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