

REVIEW

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# The role of probiotics on animal health and nutrition

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## Abstract

**Background:** The constant global need for food has created a demand for colossal food production. Every day the world requires more food than it is capable of growing and harvesting. Antibiotics have been used in healthy food products to promote growth and prevent disease in food-producing animals for a long time. This prolonged use of antibiotics leads to the development of resistant bacteria and the accumulation of antibiotic residue in livestock and fish. To avoid further causalities finding an effective alternative became a dire need. At present, the most suitable alternative for antibiotics is probiotics.

**Main body:** Probiotics are live microorganisms that provide health benefits when consumed or applied to the body with the optimum amount. Probiotics are mainly good bacteria and yeast which fight off the pathogenic bacteria, improve the immune system, and restore the gut microbial balance. Probiotics can eliminate the harmful pathogens following several molecular mechanisms and modulate the immune response of the host animal for the well-being of the animals. This review article aims to describe probiotics as a potential growth promoter in major food sectors (poultry, ruminant, and aquaculture), how probiotics can ensure food safety without harmful effects on animals, and find out some points where more research is required to ensure a positive outcome.

**Conclusion:** The conclusion of this review article highlights the knowledge gaps and how they can be minimized using modern molecular technologies to establish probiotic supplements as an effective alternative to antibiotics.

**Keywords:** Probiotic, Antibiotic, Poultry, Ruminant, Aquaculture, Immunity, Animal health

## Background

The world population is increasing rapidly. It is assumed to extend to over 9.8 billion people by 2050, United Nations Department of Economic and Social Affairs Population Division (2017). This growing population is imposing food security challenges worldwide. Livestock farming is one of the rapidly expanding agricultural sectors. Generally, 75% of rural people and 25% of urban people rely on livestock for their sustenance, Grace (2012). Aquaculture is also developing as a primary agribusiness sector for the ever-increasing global human population. This sector has been advancing swiftly due to

the enhanced cultivation methods in inland water bodies Hai (2015).

To maintain the enormous need for meat and fish production, veterans and farmers use antibiotics in the veterinary sector as antimicrobial growth promoters (AGPs) (Li et al., 2020; Marshall & Levy, 2011). The setback here is that it comes with harmful consequences. People use feed antibiotics as sub-therapeutic drugs for animal growth, but excessive and improper antibiotic use can develop antibiotic resistance in microbial populations (Deng et al., 2020a, 2020b; Han et al., 2020). This unnatural use of antibiotics has turned out into a public concern because of its affiliation with human and animal disorders. Later, the EU Regulation (EC) No 1831/2003 prohibited the unauthorized sale of dietary additives, including probiotics, without precisely labeling the

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product with an exact name and well-functioning group additives. The regulation also stated that the manufacturing company responsible for producing the supplements should mention the name, net weight, and net volume of the specific product appropriately. The product must also have the manufacturing date, including batch number and related information for product usage. The EU regulation also affirmed that companies could only authorize coccidiostats or histomonostats type antibiotics that might serve as dietary supplements, European Parliament and the Council of the European Union (2003).

Apart from antibiotic-caused illness, various foodborne pathogenic bacteria can cause major zoonotic diseases such as salmonellosis, campylobacteriosis, and pathogenic *Escherichia coli* infection in humans. These outbreaks create public health concerns and economic loss around the world. To face the current challenge of producing vast amounts of livestock and fish, probiotics have been found to improve animals' quality and growth without causing adverse effects.

Lilly and Stillwell (1965) first used the word probiotic as the opposite of the word antibiotic. By definition, probiotics are microbial substances capable of stimulating the expansion of another microorganism. Afterward, Fuller AFRC (1989) improved the description significantly, which was pretty near the definition used today. Fuller AFRC (1989) addressed probiotics as alive microbial feed additives that promisingly improve the host animals' intestinal microbial balance. FAO/WHO (2002) provided the current definition of probiotics which affirms that probiotics are active microbes that offer health values for the host animal when appropriately supplemented.

Probiotics can eliminate the pathogenic microorganisms from the GIT and change the host's microbial population density in the intestinal tract. Probiotics subsequently establish a better suited microbial population through a transformation in the equilibrium of beneficial and noxious microorganisms (Mountzouris et al., 2009). After probiotics settle in the gut, it prompts an immunologic response. The intestinal cells can assemble a series of immunoregulatory molecules exhilarated by bacteria (Corcionivoschi et al., 2010). Some probiotic metabolites can modulate various metabolic pathways in cells. Probiotic metabolic components such as bacteriocins, amines, and hydrogen peroxide interact with specific targets of multiple metabolic pathways to regulate apoptosis, cell proliferation, inflammation, and differentiation (Plaza-Diaz et al., 2019).

Nowadays, farmers provide probiotic feed supplements to poultry, ruminants, and fishes. Probiotics are mostly gram-positive bacteria but there are also gram-negative bacteria, yeast, and fungi. The most common probiotics used for animals (Table 1) incorporate *Lactobacillus*,

*Bifidobacterium*, *Lactococcus*, *Bacillus*, *Streptococcus*, and yeast, such as *Candida* and *saccharomyces* (Arora, 2020; Park et al., 2016).

Probiotics have inaugurated a new era in health administration for animals. This study aims to analyze the role of probiotics on animal health and determine the prospects of sustainable probiotics utilization in this sector.

## Main text

### Mode of action of probiotics

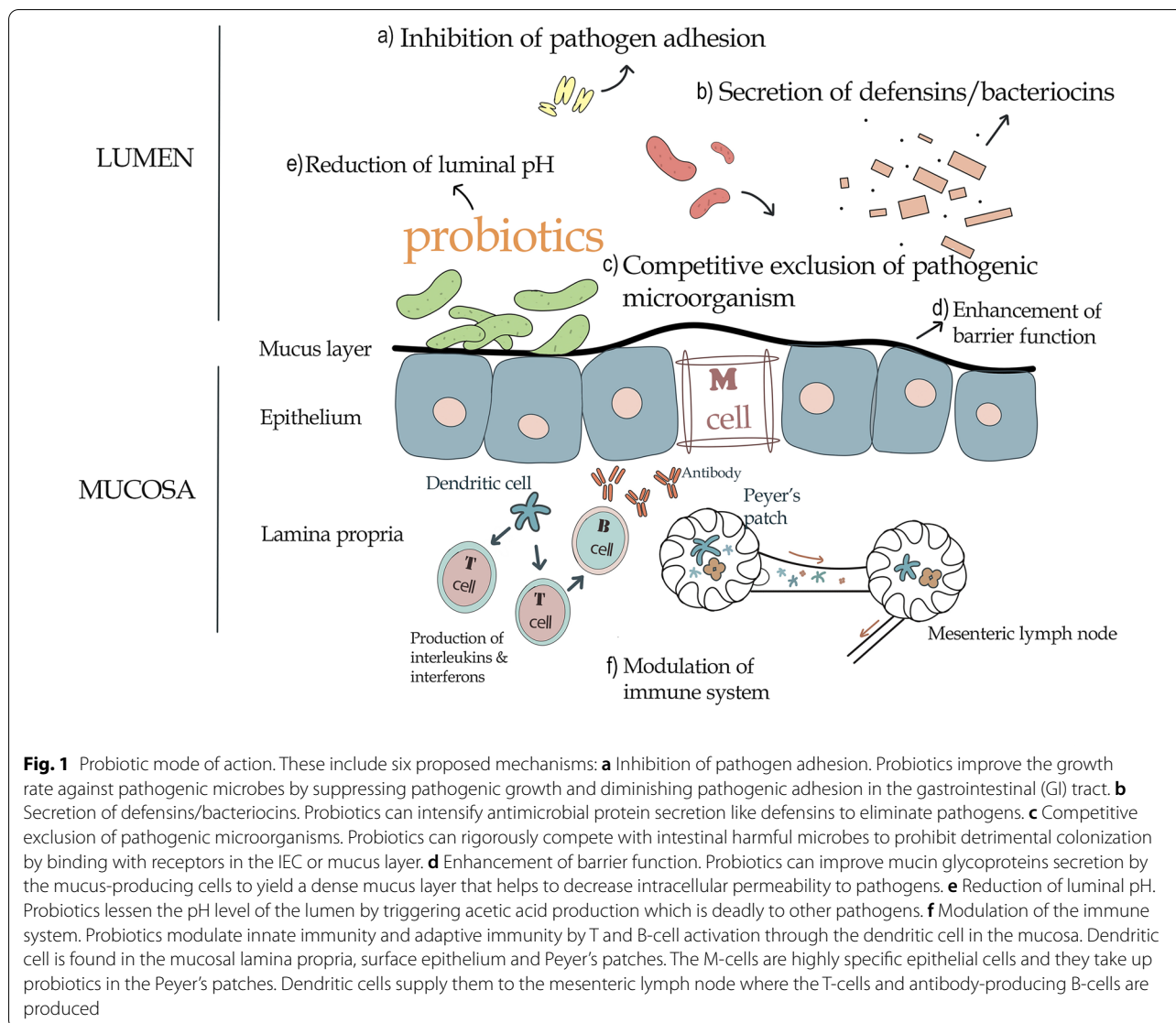
Probiotics exert their effectiveness through diverse mechanisms. Probiotics inhibit and control enteric pathogens along with improving the functioning and production capacity of animals. Basic probiotic mode of action includes (a) inhibition of pathogen adhesion; (b) production of antimicrobial components, i.e., bacteriocins and defensins; (c) competitive exclusion of pathogenic microorganisms; (d) enhancement of barrier function; (e) reduction of luminal pH; and (f) modulation of the immune system (Fig. 1). Probiotics promote health conditions by inhibiting harmful bacteria. For instance, *Lactobacillus rhamnosus* and *Lactobacillus plantarum* can avert *Escherichia coli* adhesion in the intestinal tract (Maldonado Galdeano et al., 2019). Bacteria commonly interact with host cells following the secretion of chemical signals that influence bacterial organisms' approach (Miller & Bassler, 2001; Waters & Bassler, 2005). This communicating technique of bacteria with its host is called quorum sensing (Chen et al., 2020a, 2020b; Deng et al., 2020a, 2020b; Hughes & Sperandio, 2008). Probiotics can influence pathogenicity by modifying the communication process in pathogenic bacteria. Probiotics produce antibacterial substances and impede bacterial adherence and translocation. *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, *Enterococcus*, *Streptococcus*, and *Bifidobacteria* can yield proteins or bacteriocins that minimize the development of closely linked bacterial organisms. These probiotics reduce the number of detrimental microorganisms from the gastrointestinal tract (Kawai et al., 2004; Yildirim & Johnson, 1998). Bacteriocins are bioactive antimicrobial peptides generated in the ribosome of many bacteria and stick to the pathogenic microorganism cells penetrating through the phospholipid membranes. The basic pattern of bacteriocin-mediated pathogen reaction encloses the cytoplasmic membrane penetration of pathogenic bacteria that confers in DNA and RNA synthesis inhibition and cell leakages (van Zyl et al., 2020). Bacteriocins can restrict pathogen cells' capability of GIT colonization and combat antibiotic-resistant strains of bacteria (Kuebutornye et al., 2020; Lajis, 2020). The following figure provides a streamlined illustration of the basic mechanisms of action of probiotics (Fig. 1).

**Table 1** A diverse range of microorganisms commonly used as probiotics in animal

Genus	Species	References
<b>Gram-positive bacteria</b>		
<i>Bifidobacterium</i>	<i>B. animalis</i>	Mountzouris et al., (2010), Giannenas et al., (2012), Wideman et al., (2012)
	<i>B. bifidum</i>	Haghighi et al., (2008a), Daşkiran et al., (2012)
	<i>B. thermophilus</i>	Khaksarzareha et al., (2012); Pedroso et al., (2013)
	<i>B. longum</i>	Seo et al., (2010)
	<i>B. lactis</i>	Seo et al., (2010)
<i>Bacillus</i>	<i>B. amyloliquefaciens</i>	Gracia et al., (2013)
	<i>B. toyonensis</i>	Taras et al., (2005); Kantas et al., (2015)
	<i>B. coagulans</i>	Adami and Cavazzoni, (1999); Hung et al., (2012)
	<i>B. subtilis</i>	Alexopoulos et al., (2004); Davis et al., (2008); Rahman et al., (2013); Afsharmanesh and Sadaghi, (2014)
<i>Lactobacillus</i>	<i>L. acidophilus</i>	Morishita et al., (1997); Haghighi et al., (2008b); Daşkiran et al., (2012); Khaksarzareha et al., (2012); Shim et al., (2012); Rahman et al., (2013); Zhang et al., (2014)
	<i>L. reuteri</i>	Mountzouris et al., (2010); Giannenas et al., (2012); Wideman et al., (2012); Mookiah et al., (2014)
	<i>L. casei</i>	Fajardo et al., (2012); Khaksarzareha et al., (2012); Landy and Kavyani, (2013)
	<i>L. gallinarum</i>	OHYA et al., (2000) Mookiah et al., (2014)
	<i>L. delbrueckii subsp. bulgaricus</i>	Daşkiran et al., (2012)
	<i>L. plantarum</i>	Daşkiran et al., (2012); Rahman et al., (2013)
	<i>L. brevis</i>	Mookiah et al., (2014)
<i>Enterococcus</i>	<i>E. faecium</i>	Mountzouris et al., (2010); Giannenas et al., (2012); Khaksarzareha et al., (2012); Wideman et al., (2012); Abdel-Rahman et al., (2013)(2013); Cao et al., (2013); Chawla et al., (2013); Pedroso et al., (2013); Zhao et al., (2013)
<i>Clostridium</i>	<i>C. butyricum</i>	Zhang et al., (2012); Zhao et al., (2013); Zhang and Kim, (2014)
<i>Streptococcus</i>	<i>S. faecium</i>	Morishita et al., (1997); Rahman et al., (2013)
	<i>S. salivarius subsp. thermophilus</i>	Daşkiran et al., (2012)
<i>Pedococcus</i>	<i>P. acidilactici</i>	Mountzouris et al., (2010), Wideman et al., (2012), Pedroso et al., (2013)
<i>Leuconostoc</i>	<i>L. mesenteroides</i>	Benmechernene et al., (2013)
<i>Lactococcus</i>	<i>L. lactis</i>	Fajardo et al., (2012)
<b>Gram-negative bacteria</b>		
<i>Escherichia</i>	<i>E. coli Nissle 1917</i>	Hashemzadeh et al., (2013)
<i>Megasphaera</i>	<i>M. elsdenii</i>	Seo et al., (2010)
<i>Prevotella</i>	<i>P. bryantii</i>	Seo et al., (2010)
<b>Yeast and Fungi</b>		
<i>Saccharomyces</i>	<i>S. boulardii</i>	Rahman et al., (2013)
	<i>S. cerevisiae</i>	Shim et al., (2012), Abdel-Rahman et al., (2013)(2013, Bai et al., (2013)
<i>Candida</i>	<i>C. pintolepesii</i>	Daşkiran et al., (2012)
<i>Aspergillus</i>	<i>A. oryzae</i>	Daşkiran et al., (2012); Shim et al., (2012)
	<i>A. niger</i>	Seo et al., (2010)

Probiotic microbes can withstand pathogenic bacteria by lowering luminal pH. Probiotic *Bifidobacterium breve* can lower the luminal pH. *Bifidobacterium breve* can trigger acetic acid production in higher concentrations in a deadly Shiga toxin-yielding *Escherichia coli* O157:H7 mouse experimental model (Asahara et al., 2004). Probiotics can shape the cell–cell communication of bacteria and host and maintain cellular consistency by

strengthening the intestinal barrier function. Such consistency is achieved through the modulation of cytoskeletal and tight junctional protein phosphorylation. *Lactobacillus* can abide by the epithelial cells of the ileum of chickens (Jin et al., 1996). Probiotics can competitively exclude pathogenic microorganisms from the host intestine by strengthening this intestinal communication system (Mookiah et al., 2014). This anti-pathogenic



mechanism (competitive exclusion) demonstrates that bacterial species rigidly battle for attaching to receptors at particular binding sites in the GIT and might integrate antimicrobial substances secretion and competition for accessible nutrients (van Zyl et al., 2020).

Probiotics can improve the immunity of the host by modulating the immune system. The consumed probiotics play a crucial role in stimulating the mucosal immune system (MIS) and induce a network of signals. The reaction of diverse probiotic microbes on dendritic cells (DC) has been investigated in different experimental approaches. Dendritic cells are antigen-displaying cells that have crucial roles in innate and adaptive immunity. Dendritic cells can identify and react to bacterial components besides launching

primary immune responses, which lead to the straight development of T- and B-cell responses. Probiotics can directly govern intestinal dendritic cells with pathogen recognition patterns (PRPs) displayed on the surface, which can precisely recognize the pathogen-associated molecular patterns (PAMPs) on the bacterial organism. This acknowledging method stimulates DC maturation regarding up-regulation of co-stimulatory molecular expression. As the immune system becomes active, cytokine secretion incites T-cell activation (Langenkamp et al., 2000; Mellman & Steinman, 2001). DC-originated signals ascertain the type of T-cells responses such as T helper cells polarization or T regulatory response, which determines the B-cell responses against pathogenicity, Kapsenberg (2003).

### Probiotics increase digestibility

Probiotics increase the rate of digestion in animals. Probiotics can ameliorate cecal microorganism constitution and nutrient digestion in broilers (Khalid et al., 2021). Probiotics boost the ileal digestibility of essential amino acids, with a 5 percent enhancement in chicken body weight, Zhang and Kim (2014) and can ameliorate the bioaccumulation of calcium in poultry (Chawla et al., 2013). (Maas et al., 2021) experimented with enzymes xylanase, phytase together with probiotics. They reported the impact of *Bacillus amyloliquefaciens* on digestion or metabolism and found better calcium absorption and improved microorganisms' interactions in the gut.

Probiotics increase enzyme activity in the GIT and improve the digestibility of the food eaten by the host. For example, a study in buffalo calves showed that probiotic feed containing *Lactobacillus acidophilus* could ensure more dry matter intake, daily feed conversion efficiency, and apparent digestibility of nutrients compared to the control group (Sharma et al., 2018).

### Probiotics improve the immune system

Probiotics can enhance immunity in the host in many ways. Multiple studies have proven the immunostimulatory properties of probiotics (Bilal et al., 2021; Kong et al., 2020; Punetha et al., 2018; Terada et al., 2020). Probiotics comprising *Lactobacillus fermentum* and *Saccharomyces cerevisiae* excited the gut T-cell immunity, highlighted by the enhanced yield of CD3+, CD4+, and CD8+ T-lymphocytes in the gastrointestinal tract of chickens (Bai et al., 2013). In neonatal chicks of three-day- and seven-day-old expression of CD3+, IL-2, and IFN- $\gamma$ -genes were higher in the small intestine when provided food with probiotics *Lactobacillus jensenii* TL2937 and *Lactobacillus gasseri* TL2919 than without probiotics (Sato et al., 2009).

Probiotics can also boost serum immunoglobulin levels in chickens. A probiotic feed supplement containing *Lactobacillus acidophilus*, *Bacillus subtilis*, and *Clostridium butyricum* enhanced IgA and IgM serum levels in chickens, Zhang and Kim (2014).

The LAB (Lactic Acid Bacteria) can generate a broad range of antimicrobial compounds to reduce pathogenic attacks. These include antimicrobial peptides (AMPs), such as defensins, organic acids, bacteriocins, ethanol, carbon dioxide, and diacetyl, Liao and Nyachoti (2017). Organic acids like short-chain fatty acids, formic acids, and lactic acid have been proved to suppress potential harmful microbes of importance in farm animals. *Lactobacillus* bacteria can generate lactic acid as the main glucose metabolism product (Russo et al., 2017). Organic acids work on the bacterial cell wall, cytoplasmic membrane, and precise metabolic functions such

as replication and protein synthesis, which lead to the destruction and death of pathogenic microorganisms (Surendran Nair et al., 2017; Zhitnitsky et al., 2017). *Salmonella Enteritidis*, *Lactobacillus monocytogenes*, and *Escherichia coli* could be effectively inactivated after exposure to 0.5% lactic acid for two hours (Wang et al., 2015).

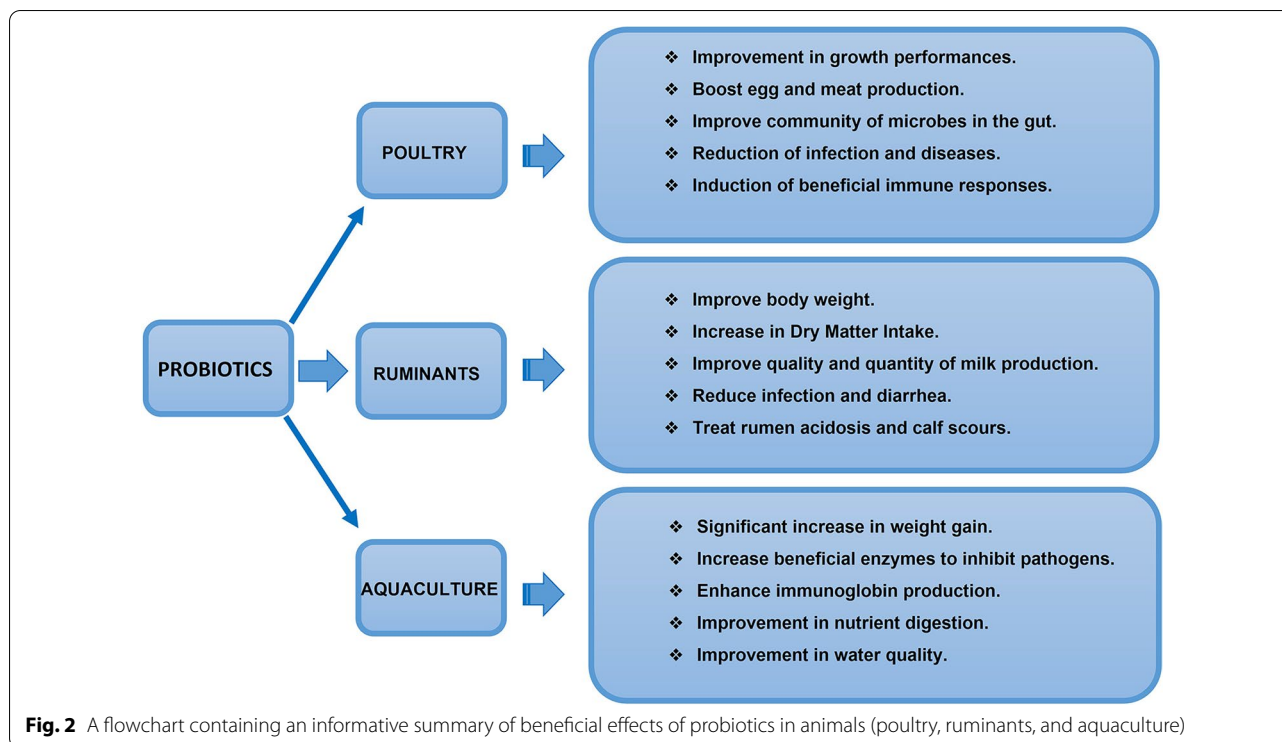
Probiotics can influence both the innate and adaptive immunity of the host. Several immune cell types, comprising granulocytes, dendritic cells, macrophages, T lymphocytes, and B lymphocytes, are engaged with inflammatory responses, which are regulated by cytokines like TNF $\alpha$ , IL-8, IL-1 $\beta$ , IL-15, and IL-6 interleukins. The anti-inflammatory reactions are mediated by TGF $\beta$ , IL-10, IL-12 (Hardy et al., 2013). Innate immunity provides physical and chemical barriers against pathogens for the host organism. For example, intestinal epithelial cells (IECs) prevent the spread of harmful microbes so that infections don't occur. It has been reported that *Lactobacillus* bacteria such as *Lactobacillus gasseri*, *Lactobacillus salivarius*, *Lactobacillus fermentum*, and *Lactobacillus crispatus* can regulate favorably the secretion level of the pro- and anti-inflammatory interleukins IL-6, IL-8, and IL-10 to manage the inflammation and rebuild the physiological balance in animals (Luongo et al., 2013; Pérez-Cano et al., 2010; Rizzo et al., 2015; Sun et al., 2013). *Lactobacillus delbrueckii* strain can stimulate anti-inflammatory properties and capable of downregulating the pro-inflammatory cytokines IL-8 levels. These inflammatory responses were seen in *Aeromonas hydrophila* contaminated carp (*Cyprinus carpio* Huanghe var.) when supplemented with  $1 \times 10^7$  CFU g/L *Lactobacillus delbrueckii* probiotics (Zhang et al., 2017). The adaptive immune response is dependent on B-lymphocytes and T-lymphocytes, which stimulate an antigen-specific response. In poultry, feed supplementation with  $1 \times 10^9$  CFU/kg of *Lactobacillus acidophilus* LA5 elevated the amount of CD8+, CD4+, TCR1+ T-cell in the gastrointestinal tract as well as in the peripheral blood system (Asgari et al., 2016).

### Role of probiotics in poultry

The world's poultry production now is five times higher than the poultry production of 50 years ago, FAOSTAT (2016). Evaluation from the 2016 "Global Livestock Environmental Assessment Model" FAO (2018) discloses that around 73 million tons of eggs and approximately 100 million tons of poultry meat have been required worldwide. Probiotics can help to meet this massive need without exerting harmful effects (Fig. 2).

Probiotics can improve the growth rates of broiler chicken (Abd El-Hack et al., 2020; Afsharmanesh & Sadaghi, 2014; Lei et al., 2015; Mookiah et al., 2014; Zhang





& Kim, 2014). Probiotics can prevent gastrointestinal diseases like salmonellosis (Biloni et al., 2013; El-Sharkawy et al., 2020; Fazelnia et al., 2021; Tellez et al., 2012); necrotic enteritis (Jayaraman et al., 2013; Rajput et al., 2020; Xu et al., 2020); as well as coccidiosis (El-Sawah et al., 2020). Broiler chickens fed with *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium* at about 1 percent of the food amount comprising over  $5 \times 10^9$  CFU/g can improve the chickens' growth rate, immune system, and antioxidant quantity (Zhang et al., 2021).

Probiotics can modify the histology of the intestinal mucosa of poultry. The intestinal mucosa framework is a crucial factor for both digestive and absorptive intestinal function and affects the poultry's growth. The villus height and the villus-to-crypt ratio of intestinal mucus layer were enhanced by *Bacillus subtilis* (Afsharmanesh & Sadaghi, 2014; Jayaraman et al., 2013); *Bacillus coagulans* (Hung et al., 2012), *Lactobacillus salivarius*, *Pediococcus parvulus* (Biloni et al., 2013), and *Enterococcus faecium* (Abdel-Rahman et al., 2013; Cao et al., 2013). Along with the improvement in villus height and villus height-to-crypt ratio, the assimilation of nutrients also increases owing to a greater surface area, Afsharmanesh and Sadaghi (2014). The use of *Lactobacillus sakei* Probio-65 ameliorated villi height likewise crypt depth in the jejunum of broiler chicken compared with chickens

supplemented with or without antibiotics (Wlodarska et al., 2011).

Probiotics can prevent salmonellosis in chickens. Hatched chicks that were vaccinated with  $1 \times 10^9$  CFU *Lactobacillus plantarum* LTC-113 strain can prevent salmonellosis. *Lactobacillus plantarum* can limit the gut colonization of harmful bacteria and stabilize the expression of tight junction genes in gut epithelial cells, thus make the chickens better tolerant of the infection (Wang et al., 2018). As broiler chickens get affected by APEC and *Salmonella*, the latest research indicated that when probiotics and RASV are provided in a combination for the White leghorn chickens that can decrease the rate of infection in leghorn chickens (Redweik et al., 2020). *Bacillus licheniformis* and *Bacillus subtilis* containing feed supplement can reduce the shedding of *Escherichia coli* in laying hens (Upadhaya et al., 2019).

Campylobacteriosis is another major illness of poultry caused by *Campylobacter jejuni*. In chicken, in vitro experiments with probiotic bacterial strains such as *Pediococcus acidilactici*, *Lactobacillus reuteri*, *Enterococcus faecium*, and *Lactobacillus salivarius* showed that the probiotics could impede the growth of *Campylobacter jejuni* (Ghareeb et al., 2012). Feeding the artificially infected chicken a commercial mixture of probiotic *Lactobacillus acidophilus* and *Enterococcus faecium* decreased the shedding of *Campylobacter* by 70

percent and the gut colonization of *Campylobacter* by 27 percent (Morishita et al., 1997).

Coccidiosis is another crucial parasitic disease of poultry resulting from a protozoan, *Eimeria*. *Eimeria* colonizes the intestinal tract. When probiotics supplementation is provided including *Bifidobacterium animalis*, *Enterococcus faecium*, *Bacillus subtilis*, and *Lactobacillus reuteri*, either individually or in combination, it can lower down the infection rate (Giannenas et al., 2012).

Probiotics improve the laying performance of hens and egg quality. Lohmann pink laying hens which are nourished with *Clostridium butyricum*, *Saccharomyces boulardii*, and *pediococcus acidilactici* have improved the gastrointestinal state and egg quality (Xiang et al., 2019). When brown laying hens are given *Bacillus subtilis* ATCC PTA- 6737 at  $1 \times 10^8$  CFU/kg feed, the laid eggs have better yolk color, albumen quality, shell thickness, and breaking strength than the eggs laid by control group hens, Sobczak and Kozłowski (2015). Probiotics can efficiently minimize the egg yolk cholesterol level. *Bacillus* spores, lactic acid bacteria, and yeast can minimize the cholesterol level of the egg yolk (Haddadin et al., 1996; Kurtoglu et al., 2004; Panda et al., 2003; Yousefi, & Karkoodi, 2007).

Probiotics improve the meat quality of the chicken. *Bacillus subtilis* can upgrade chicken meat quality (Mohammed et al., 2021). *Bifidobacterium bifidum* and *Bacillus toyonensis* can enhance the growth rate and meat quality in quails (Abou-Kassem et al., 2021). *Lactobacillus casei* can elevate the high-density lipoprotein (HDL) level and mitigate the low-density lipoprotein (LDL) level in broiler chicken (Yulianto et al., 2020). *Lactobacillus acidophilus* together with *Streptococcus faecium* enhances the protein amount in meat by lowering high-density lipoprotein cholesterol levels and plasma protein concentrations, Pietras (2001). Additional works based on *Lactobacillus* spp. supplementation offered similar results, with minimization in the whole cholesterol levels and low-density lipoprotein cholesterol levels (Kalavathy et al., 2003; Taherpour et al., 2009), as well as triglycerides level in blood serum of broilers (Kalavathy et al., 2003).

Probiotics provide immunomodulatory responses. Probiotics supplementation helps broiler chickens to cope up with stress conditions. Lactic acid bacteria (LAB) probiotics addition as a food supplement can rectify heat-stress-relevant diseases in chickens (Abd El-Hack et al., 2020). Lactic acid bacteria can improve antibody production (Zulkifli et al., 2000). Probiotics can enhance immunity by increasing the Toll-like receptor (TLR) signaling. Toll-like receptor (TLR) plays a crucial role in the activation of T-cells in the intestinal immune system (Asgari

et al., 2018). *Lactobacillus fermentum* and *Saccharomyces cerevisiae* can strengthen the degree of mRNA expression of TLR-2 and TLR-4 in the foregut of the chickens in respect of those treated with or without an antibiotic (Bai et al., 2013).

#### Role of probiotics in ruminants

The rumen has an intricate microbial population. Host animals ingest carbohydrates and proteins, which are degraded by rumen microorganisms. The commonly used probiotics for ruminants are *Saccharomyces cerevisiae* (Chaucheyras-Durand et al., 2008; Elghandour et al., 2020), Lactic acid bacteria (LAB) (Chiquette et al., 2008; Weimer, 2015); *Aspergillus oryzae* (Jouany et al., 1998; Mathieu et al., 1996); *Bacillus* and *Enterococcus* (Uyeno et al., 2015) which confer significant well-being for the ruminant health (Fig. 2).

Probiotics can enhance milk production in dairy cattle. Probiotic microorganisms such as *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Enterococcus faecalis* can enrich milk secretion (Ma et al., 2020), and also *Bifidobacterium bifidum* can inhibit milk allergy reaction (Jing et al., 2020). Cows supplemented with  $5 \times 10^9$  CFU of *Enterococcus faecium* and  $2 \times 10^9$  CFU *Saccharomyces cerevisiae* cells enhanced milk production by 2.3 L per cow each day, Nocek and Kautz (2006). A rigorous quantitative study on yeast probiotics' implications in ruminants demonstrated that ruminants fed with active yeast probiotics increased milk production by nearly 1.2 g/kg of body weight. Dry matter intake (DMI) by the farm animals was elevated nearly 0.44 g/kg of bodyweight though there was no effect on milk protein amount (Desnoyers et al., 2009).

Probiotics can increase the ruminants' body weight. For instance, a probiotic combination of *Lactobacillus reuteri* DDL 19, *Lactobacillus alimentarius* DDL 48, *Enterococcus faecium* DDE 39, and *Bifidobacterium bifidum* DDBA collected from a fine goat and fed to other goats for about two months. This caused an improvement in the goat's standard bodyweight by nine percent (Apás et al., 2010). *Bacillus subtilis* and *Bacillus amyloliquefaciens* can upgrade intestinal maturation and growth competency by stimulating GH/IGF-1 hormone (Du et al., 2018).

Probiotics can increase food digestibility in ruminants. Using a mixture of *Lactobacillus acidophilus* NP51 and *Propionibacterium freudenreichii* NP24 as cow feed has improved the digestion of neutral detergent fiber, crude protein, and milk yield by nearly 7.6 percent (Boyd et al., 2011).

Probiotics can enhance the immune system in ruminants. The supplementation of *Lactobacillus acidophilus*, *Lactobacillus salivarius*, and *Lactobacillus plantarum* at

a pace of  $10^7$ – $10^8$  CFU/g bring down the occurrence of diarrhea in juvenile calves (Signorini et al., 2012). Nisin is an antimicrobial peptide generated from *Lactococcus lactis*. Nisin infusion in the intra-mammary gland can treat mastitis, which is caused by *Staphylococcus aureus* in dairy cows (Cao et al., 2007). Lactobacillus base teat spray can improve mammary gland condition and strengthen the functions of the teat sphincter (Alawneh et al., 2020). Probiotics supplementation can alleviate rumen acidosis in cows and improve immunity in young stressed calves (Krehbiel et al., 2003).

Probiotics can strengthen rumen fermentation. Multiple probiotic strains have been proved to yield antimicrobial components that can decrease zoonotic pathogens and control ammonia production. *Rhodopseudomonas palustris*, a photosynthetic bacteria, have been considered a feasible probiotic in the animal feed sector (Chen et al., 2020a, 2020b) reported that *Rhodopseudomonas palustris* containing feed supplements can promote the viability of rumen microorganisms. They also noticed that *Rhodopseudomonas palustris* addition rendered high growth performance of rumen microorganisms and increased microbial fermentation to keep up the microbial balance. Application of *Megasphaera elsdenii* can ameliorate butyrate production and improve dietary intake in newborn calves (Muya et al., 2015).

### Role of probiotics in aquaculture

In 2018, universal fish production was around 179 million tons, coupled with a total sale value estimated at USD 401 billion, FAO (2020). Antibiotics are extensively used to meet the increasing demand in aquaculture. However, this extensive use of antibiotics gives rise to the drug-resistant bacteria that transmit through the food web to human (Cabello, 2006; Da Costa et al., 2013; Hassoun-Kheir et al., 2020; Kim et al., 2004; Tanwar et al., 2014; Wanja et al., 2020).

Probiotics confer several beneficial effects to aqua-animals (Fig. 2). Probiotics promote the growth and reproduction of water-dwelling animals, safeguard from pathogens, strengthen immunity, help in digestion, improve water quality, and work as an alternative to antibiotics, Banerjee and Ray (2017).

Farmers use a broad range of probiotics in fish farming. *Bacillus subtilis* from *Bacillus* genera is frequently used in aquaculture (Hong et al., 2005; Olmos et al., 2020). *Bacillus* probiotics alone can mitigate various harmful microorganisms in fish such as *Vibrio*, *Pseudomonas*, *Aeromonas*, *Clostridium*, *Streptococcus*, *Flavobacterium*, *Acinetobacter*, and white spot syndrome virus (Kuebutornye et al., 2020). Other bacterial strains commonly used as probiotics in aquaculture are the LAB bacteria such as *Lactococcus lactis* (Balcázar et al.,

2007) and *Lactobacillus plantarum* VSG-3 (Giri et al., 2013). A broad range of Gram-negative bacteria performs a vital role in fish farming. Several microalgae such as *Dunaliella tertiolecta*, *Dunaliella salina*, *Isochrysis galbana*, *Phaeodactylum tricornutum*, and *Tetraselmis suecica* have enhanced the development and survival rate of aquatic animals (Cahu et al., 1998; Marques et al., 2006; Naas et al., 1992; Reitan et al., 1997; Supamattaya et al., 2005). Yeast (*Saccharomyces cerevisiae*) has been proven useful for aquatic animals (Mo et al., 2020; Wu et al., 2020).

Farmers supply the fishes with probiotic feed supplement either through water circulation or dietary supplements (Moriarty, 1998; Skjermo & Vadstein, 1999). A single bacterial strain or a combination of several bacterial strains can be used as probiotics along with other prebiotics or immunostimulants (Hai et al., 2009a, 2009b). Achieving expected outcomes in fish culture depends on the proper dosage and timing.

Probiotics improve the health conditions of fish and other water-dwelling creatures *Bacillus pumilus* (Aly et al., 2008) and *Lactobacillus plantarum* (Van Doan et al., 2020) can ameliorate the health conditions of Nile tilapia. To mitigate the adverse impact of the Tilapia Lake Virus, dietary supplements of 1 percent *Bacillus* spp were given orally for the red hybrid Tilapia fishes (Waiyamitra et al., 2020). This feed supplement can reduce the fatality rate by Lake Virus infection. Probiotic *Pseudomonas* I-2 can inhibit disease-causing *vibrio* microorganisms (Chythanya et al., 2002). Probiotic *Pediococcus acidilactici* can resist vibriosis in white leg shrimp (Castex et al., 2008). Feeding White leg shrimp larvae with a variety of probiotic feed supplements (*Lactobacillus*, *Saccharomyces*, *Bacillus*, effective microorganisms such as gram-positive cocci and *Bifidobacterium*, and Photosynthetic Bacteria) can accelerate the growth speed and larval metamorphosis. It also encumbers incomplete molting during the development and minimizes the number of vibrio pathogens (Wang et al., 2020). *Pediococcus pentosaceus* and *Staphylococcus hemolyticus* can decrease the frequency of white spot syndrome virus in white leg prawns (Leyva-Madriral et al., 2011). *Saccharomyces cerevisiae* can be used as a substitute for live food in the cultivation of clownfish (Gunasundari et al., 2013); Catla (Mohanty S.K.; Tripathi, S.D., 1996); hybrid striped bass (Li & Gatlin, 2004, 2005); Japanese flounder (Taoka et al., 2006) and Nile tilapia (Lara-Flores et al., 2003). *Bifidobacterium animalis* and *Lactobacillus acidophilus*-rich feed additives can ennoble the growth and lifespan of *Hypophthalmichthys molitrix* fingerlings (Noor et al., 2020).

Probiotics can enhance immune responses in fish. *Bacillus* activates the humoral and cell-mediated immunologic response in fish (Kuebutornye et al., 2020).



*Bacillus pumilus* and *Bacillus licheniformis* strengthen the immunity of Nile tilapia (Aly et al., 2008) and *Bacillus pumilus* improves the immunity of rohu fish (Ramesh et al., 2015). *Bacillus licheniformis* assists in performing the immunomodulatory activities and raises *Oreochromis niloticus* production by up-regulating the Toll-like receptors (TLR-2) and anti-inflammatory cytokines (Midhun et al., 2019).

Probiotics can improve growth performance, feed intake, and digestive enzyme processes in aquatic animals. Probiotics generate extracellular enzymes such as protease, carbohydrase, and lipase and efficiently engage in the nutrient digestion of aquatic animals (Arellano-Carbajal & Olmos-Soto, 2002; Leonel Ochoa-Solano & Olmos-Soto, 2006). *Bacillus* generates different hydrolytic enzymes such as  $\beta$ -1,3-glucanases, proteases, and cellulases to improve the digestion in fish (Kuebutornye et al., 2020). In a sea snail, *Haliotis midae*, the probiotic *Vibrio midae* SY9 can reinforce digestive protease activity, protein digestion, and growth rate activities, Huddy and Coyne (2015). *Bacillus* spp. and photosynthetic bacteria can improve white leg prawns' growth through an increase in lipase and cellulase activity, Wang (2007). *Pseudomonas aeruginosa* and *Pseudomonas synxantha* can enhance the growth rate of western king prawns (van Hai et al., 2010, van Hai et al., 2009a, 2009b).

Probiotics can improve water quality. Probiotics have shown their potency in nourishing water properties, controlling disease, and thus upgrading fish habitat (Chen et al., 2020a, 2020b; Kewcharoen & Srisapoom, 2019; Soltani et al., 2019). Probiotics enhance water quality by reducing the number of harmful microbes (Dalmin et al., 2001; Park et al., 2000), mitigate nitrogen (Wang et al., 2005) and reduce phosphate contamination in the sediments, Wang and He (2009). Probiotics can alleviate metabolic wastes at the time of the fish school's transportation, cardinal tetra (*Paracheirodon axelrodi*) (Gomes et al., 2009).

#### Use of probiotics on miscellaneous animals

Probiotic feed supplements confer health values in farm animals such as sheep, lamb, pigs, rabbits, ducks, and turkeys. Scientists worldwide proposed different probiotic feed additives for the safe production of meat, egg, and milk maintaining these animals well-being. Probiotics can improve the health condition of sheep and lambs. *Bacillus licheniformis* and *Bacillus subtilis* can improve body weight, upgrade the intestinal microbiome, boost immunity, and preserve regular metabolic actions in two-month-old sheep and lambs (Devyatkin et al., 2021).

Probiotics can improve milk quality in ewes. Feed supplements including *Bacillus subtilis* and *Bacillus licheniformis* can lower mortality, improve milk protein content,

and increase the production of milk in ewes (Kritas et al., 2006).

Probiotics can ameliorate the health condition of birds. When white Pekin ducks were given a dietary feed supplement containing *Lactobacillus acidophilus* and *Lactobacillus casei* that increased total weight gain, protein content, intestinal enzyme activity, bactericidal activity, and decreased cholesterol, glucose, and cortisol level (Khattab et al., 2021). *Lactobacillus* can produce lactic acid and reduce pathogenic infection maintaining intestinal microorganism balance in geese (Dec et al., 2014). In Cherry Valley Pekin ducks, *Bacillus subtilis* and *Bacillus licheniformis* can enhance LXR $\alpha$  and CYP7 $\alpha$ 1 enzyme activities in the liver and reduce lipid concentrations and fat deposition (Huang et al., 2015).

In neonatal turkey birds, lactic acid bacteria can fight back against *Salmonella enteritis* and *Clostridium jejuni* and improve GIT condition (Yang et al., 2018). Modified probiotic bacteria, *Escherichia coli* Nissle 1917, can secrete the antimicrobial peptide, Microcin J25 which diminishes *Salmonella enteritidis* in GIT of turkey (Forkus et al., 2017).

Probiotics can improve the health condition of rabbits. *Clostridium butyricum* can improve growth rate, gut microbial condition, and intestinal immunity in Rex rabbits. When healthy female rabbits were fed with *Clostridium butyricum*, it considerably ameliorated body weight, the action of digestive enzyme, improved the immune system by enhancing the abundance of beneficial bacteria (Liu et al., 2019). *Bacillus subtilis* containing feed supplements can improve growth performance, meat quality, and immune response in rabbits brought up in a heated environment (Fathi et al., 2017). Growing rabbits fed with *Aspergillus awamori* demonstrated improved body weight, nutrient digestibility, and better antioxidative responses (El-Deep et al., 2021).

Probiotics can improve the health status of pigs. *Bacillus amyloliquefaciens* is considered a useful dietary supplement against antibiotics in pigs and piglets for fattening (Cao et al., 2020). Pigs face much trouble during weaning which leads to immune and intestinal system malfunctions, upsets the gut microbial environment, and hampers the growth rate of piglets. Duan-Nai-An, an engineered *Saccharomyces cerevisiae* strain, can significantly improve body weight, feed intake, and decrease the rate of diarrhea and death in early-weaned piglets (Xu et al., 2018).

#### Conclusions

The world is being densely populated day by day. To meet the need for meat and fish production of this ever-growing population, some effective yet harmless solutions became a crying need. Probiotics have a vital role

to solve this food production problem and replace the harmful antibiotic use in farm industries. Nonetheless, every unique probiotic cannot be guaranteed to provide safety with conventional strains. Some probiotics might have undesirable properties such as transmittable antimicrobial resistance, virulence factors, hemolytic potential, and unwanted yield of toxic biochemical substances. To avoid any causalities, distinct probiotic strains must be determined for individual species in a particular environment. The efficiency and reactions for every probiotic are dissimilar. So, the optimum condition for a probiotic to survive, colonize, expand, and render its effects to the hosts in a particular environment needs to be identified.

Modern molecular methods such as quorum sensing, different staining methods, polymerase chain reaction (PCR), scanning electron microscope, fluorescent in situ hybridization (FISH), and genome-wide association study (GWAS) could be applied. Implementing these techniques will successively help to find out the detail about the adherence and colonization of probiotic and pathogenic bacteria, interaction with the host and breeding environment, the communication process between probiotics and host mucosa, gene exchange or transfer horizontally or vertically. Proper knowledge of the immune-modulatory effects of different probiotics and their viability before probiotics which are added in farm animals' dietary feed is essential. Moreover, dosage-dependent studies should be done in greater detail by confirming the organism's identity using molecular testing at a reference laboratory. Additional investigations are required before providing guidelines for probiotics with any degree of confidence.

#### Abbreviations

GIT: Gastric Intestinal Tract; IgA: Immunoglobulin A; IgM: Immunoglobulin M; TNF $\alpha$ : Tumor necrosis factor alpha; TGF $\beta$ : Transforming growth factor beta; IFN $\gamma$ : Interferon gamma; IL: Interleukin; TcR: T-cell receptor; IEC: Intestinal epithelial cell; GH: Growth hormone; IGF-1: Insulin-like growth factor 1; APEC: Avian pathogenic *Escherichia coli*; RASV: Recombinant attenuated Salmonella vaccines; CD: Cluster of differentiation; CFU: Colony-forming unit.

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