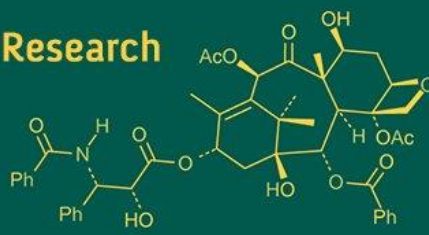
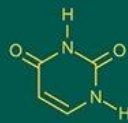


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Postbiotics and parabiotics: Emerging functional ingredients in animal feed for gut health, immunity, and productivity

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Abstract

The focus on next-generation functional feed additives continues to rise due to the increasing demand for sustainable livestock production and less dependence on antibiotics, as they can cause antibiotic resistance. The ability of postbiotics and parabiotics to improve immunity, gut health, and overall performance without the risks associated with administering live microbes has helped them stand out from others. This review highlights their modes of action, including immunoregulation, metabolic optimisation, manipulation of the gut microbiota, and restoration of epithelial barrier integrity. Existing evidence of improved growth performance, disease resistance, and nutritional utilisation in companion animals, ruminants, poultry, swine, sheep, & goat and aquaculture. The bioactive compounds producing these effects are additionally determined using metabolomic techniques, while standardisation, cost, and inactivation procedures continue to be issues. Postbiotics and parabiotics are considered future-oriented feed improvements due to their combination of safety, effectiveness, and multifunctionality. To fully utilise them in animal health and production systems, it will be essential to link translational applications with mechanistic advances.

Keywords: Postbiotics, Parabiotics, Gut health, Immunomodulation, Functional feed additives

Introduction

By 2050, there will likely be nine billion people on the planet, and scientists are looking for ways to produce more food while keeping prices down and quality high. Improved meat safety while maintaining animal comfort is the goal of new animal breeding techniques. Long-term use of antibiotics resulted in drug-resistant bacteria, despite their ability to alter microbiota and increase production. Probiotics and prebiotics are examples of alternatives that are being investigated.

The ideal state of the gut is important to the productivity and health of production animals. It's not only about a prompt digestion and absorption of nutrients but also about proper energy homeostasis and metabolism, a steady state and diverse intestinal microbiota population, an intact mucus layer, a strong barrier integrity, and a balanced mucosal immune response. These, in concert, contribute to growth performance, feed efficiency, disease resistance in animals, and nutrient utilisation (Kogut and Arsenault, 2016). The preservation of the general health and well-being of an animal is mainly maintained by a complex collection of trillions of microorganisms harboured in the gut, which has gained importance. In order to maintain good digestive function, immune system functioning, and mental health, the gut response is essential (Li *et al.*, 2021). The composition of the host's gut microbiota may have a major impact on host wellbeing, and disturbing the equilibrium of this microbiota is linked to neurological diseases, behavioural and developmental disorders, including autism, irritable bowel syndrome (IBS), obesity, allergies, and diarrhoea (Thakur *et al.*, 2024). Numerous illnesses, such as depression, colorectal cancer, cardiovascular disease, obesity, and type 2 diabetes, have also been linked to altered amounts of microbial metabolites (Caspani *et al.*, 2019).

It depends on maintaining a delicate balance of microbial populations. Disturbances in the gut microbiota have led to Many metabolic disorders, highlighting the need for successful approaches to support and maintain this complex ecosystem of microbes. In the present day, the functional dietary components like prebiotics, probiotics, symbiotics, and postbiotics

(PPSPs) have gained a lot of importance (Thorakkattu *et al.*, 2022). The table given below (Table 1) discusses the definitions of biotics.

2. The evolution of functional feed additives

2.1 Probiotics

Vergin, when he was researching the negative effects of antibiotics and other microbial compounds on the gut microbial community, coined the term "probiotics" (Pandey *et al.*, 2015) ^[68, 69]. Probiotics are crucial for preserving the host's microbial balance, boosting the numbers of good intestinal microbes, and boosting the activity of digestive enzymes, all of which enhance nutrient absorption while shielding the host from harmful bacteria and regulating the immune system (Sumon *et al.*, 2022) ^[93]. Probiotics' immunomodulatory method of action, which is based on the innate or adaptive immune system, is very significant in relation to animal health and illnesses. In addition to disease microbes, poisons, and some foreign antigens, the gastrointestinal lumen also includes vital nutrients and helpful microbes (Markowiak and Śliżewska, 2018) ^[60]. Between the internal body tissues and the luminal environment, the GIT mucosa's epithelial cells form a selectively permeable barrier (Liao and Nyachoti, 2017) ^[55]. Gut innate immunity, the host's initial line of defence against dangerous microorganisms in the GIT, might be compromised by stress or medical conditions (Bajagai *et al.*, 2016) ^[7]. Furthermore, these methods are less dangerous than traditional chemical therapy (Sanches *et al.*, 2022) ^[82]. The most recent accepted definition, jointly given by the FDA and WHO, is "live microorganisms which, when administered in adequate amounts, confer a health benefit to the host. Probiotic microorganisms, primarily *Bacillus* spore formers, are commonly used in meals, particularly fermented milk products. New strains are constantly discovered, and products can be one or a combination of multiple strains. However, the benefits of probiotics are highly strain-specific and cannot be broadly applied (Pandey *et al.*, 2015) ^[68, 69]. These health advantages include increasing intestinal barrier protection, reducing lactose intolerance or diarrhoea, inflammatory bowel disease, anti-mutagenic action, and suppressing gastrointestinal microbes that are pathogenic by changing their immunogenicity and promoting the breakdown of enteric antigens. When administering probiotics to elderly and immunocompromised individuals, consideration should be given to their safety (Nambiar *et al.*, 2017) ^[65]. Keeping the probiotics viable is the biggest obstacle when giving them to the host. Probiotics are also susceptible to acidity and heat. Researchers attempt to combine prebiotics in order to overcome this difficulty, leading to the development of synbiotics (Wisastra *et al.*, 2025) ^[113]. It is advised to include prebiotics and carrier materials such as maltodextrin, fructooligosaccharide, and inulin to preserve the vitality of probiotics (Nambiar *et al.*, 2018) ^[66]. Synbiotics are indigestible dietary elements that promote the growth of beneficial microorganisms (Swanson *et al.*, 2020) ^[97].

2.2 Prebiotics

"Prebiotics are produced from indigestible carbohydrates that can specifically increase the growth and metabolism of beneficial bacteria, thereby enhancing the gut microbiota (Davani *et al.*, 2019) ^[17]. Prebiotics are a type of plant-

derived oligosaccharides that have selective action towards advantageous targeted bacterial species in the gut flora, including *Bifidobacterium* and *Lactobacillus*, according to Gibson and Roberfroid's 1995 study. Prebiotics are made up of a variety of substances, such as oligosaccharides made of fructose (FOS), such as inulin, Galactose (GOS), Isomaltose (IMO), and Xylose (XOS), as well as lactulose, polydextrose, lactitol, vegetable protein hydrolysates, Chinese herbal medicines, and wild plants (Yang *et al.*, 2020) ^[117]. Prebiotics' main purpose is to provide a food source for beneficial bacteria, encouraging their development and activity in the gastrointestinal system. Prebiotics often reduce the relative number of harmful bacteria and choline metabolizers and show resistance to heat and acid (Zang *et al.*, 2024) ^[118]. Recently, it has been discovered that the positive impact on the gut microbiota may be produced without the microorganism's viability (Sarkar, 2018) ^[83]. Therefore, it paved the way for a new concept where probiotics or their non-viable cells' metabolites also referred to as postbiotics or parabiotics become a feasible and promising substitute to provide positive benefits (Gurunathan *et al.*, 2023) ^[33].

2.3 Post and Para biotics

Biotic supplements have been used in animals, especially as microbial feeds or additives for aquatic species. In 1986, Kozasa was the first to propose the concept of using the spore of *Bacillus toyoi* to boost the growth of yellowtail fish (*Seriola quinqueradiata*) and also lowered the mortality of Japanese eels infected with *Edwardsiella species* (Wisastra *et al.*, 2025) ^[113]. The term paraprobiotics was created by Taverniti and Guglielmetti in 2011 ^[101] and defined them as "in active microbial cells (altered or unaltered) or raw cell extracts (with complex chemical composition) that provide health benefits to human or animals when given (orally or topically) in adequate amounts." Furthermore, paraprobiotics have been referred to as "ghost probiotics," which is why the prefix "para," which is Greek for "alongside of," has been used to denote both similarities and differences with the conventional idea of probiotics. Recent research has demonstrated that non-viable microbes can improve health and welfare (Kumar *et al.*, 2023) ^[48].

3. Need for alternatives to antibiotics in animal production

The massive use of antibiotics, previously regarded as miracle drugs that saved lives, is acting as a quick fix and has given rise to multi-drug resistance (MDR) and extreme drug resistance (EDR) among the pathogens (Gomaa, 2020) ^[32] which will have a net negative effect upon animal health and has raised concerns with respect to residues in meat and egg products (Ajuwon, 2016). From the perspective of the intestinal microbiome, the antibiotics do not discriminate between good and bad bacteria in the gut microflora (Gomaa, 2020) ^[32] and the selection of antibiotic-resistant bacterial populations (Shazali *et al.*, 2014). In an attempt to limit this dangerous practice, the EU prohibited the use of antibiotics to promote the growth of livestock in 2006 (Martin *et al.*, 2015) ^[61]. In the United States of America, the Food and Drug Administration suggested farmers discontinue using antibiotics voluntarily (Teillant and Laxminarayan, 2015) ^[103]. With the ban on antibiotics as growth promoters, there is a need to find natural alternatives to antibiotics. Strains of probiotics, prebiotics, synbiotics,

paraprobiotics, and postbiotics, known as "biotic" feed additives, are likely the most promising substitutes for antibiotics. They have been used to avoid the harmful effects of antibiotics, improve animal productivity, support intestinal health, and fight enteric infections (Jahromi *et al.*, 2016) ^[38].

3.1 Why postbiotics and parabiotics are gaining attention

Even though probiotics have many health promotion and disease prevention benefits, concerns have been raised about their practical applicability and functionality. For example, we don't fully understand the working mechanism at the molecular level, and their effects depend on the specific strain used, colonisation patterns, viability in food, gut persistence, and the possibility of virulence gene acquisition from pathogenic bacteria through horizontal gene transfer (Nataraj *et al.*, 2020; Choudhury and Kamilya, 2019) ^[15, 67].

Some probiotics can develop resistance to antibiotics or transfer harmful genes to good gut bacteria; they may stop the natural gut bacteria from growing well. In rare cases, they can cause infections, especially in people with weak immunity. These infections can include inflammation, heart infections (infective endocarditis), sepsis, or the spread of bacteria from the gut to the blood, leading to bacteraemia (Suez *et al.*, 2019) ^[92]. However, factors like food acidity, water activity, specific chemical compounds, and storage conditions such as moisture, temperature, packaging, and shelf life can affect the survival of probiotic strains. As a result, the poor survival of certain probiotics in some foods has been frequently reported (Johnson *et al.*, 2019) ^[39]. All these are major challenges that limit their safe use.

Because of these limitations, the probiotics are being replaced with safer and recently advanced postbiotics and parabiotics, which are derived from probiotics.

4. Terminology and conceptual basis

Table 1: definitions of different 'biotics'

Term	Definition	Note	Examples
Probiotic (Li <i>et al.</i> , 2021; Fiore <i>et al.</i> , 2020; Salminen <i>et al.</i> , 2021) ^[28, 81]	live microorganisms that, to enhance the host's health, must be present in appropriate quantities at the time of administration.	-Viable bacteria are necessary. -sufficient amounts, they have positive health effects.	Lactobacillus acidophilus, Bifidobacterium bifidum
Prebiotic (Lockyer and Stanner, 2019; ^[58])	indigestible dietary components, primarily fibres, that specifically promote the development and activity of good gut microorganisms	-Give good microorganisms the nourishment they need. -Improve the equilibrium of intestinal microbes.	Inulin, Fructooligosachharide (FOS), Galatooligosachharide (GOS)
Symbiotic (Pandey <i>et al.</i> , 2015; Thorakkattu <i>et al.</i> , 2022) ^[68, 69, 107]	A mix of prebiotics and probiotics that work together to improve the activity, colonisation, and survival of good gut microorganisms	Enhances gastrointestinal health, increases host immunity, and improves probiotic viability.	Lactobacillus + inulin
Postbiotic (Vinderola <i>et al.</i> , 2022 ^[109] ;	Inactivated microbial cells and/or their metabolites are examples of postbiotics, which are bioactive substances created during the fermentation of probiotics.	-Preparations of non-viable or inert microorganisms -Connected to immunological, metabolic, and gastrointestinal health advantages.	Short-chain fatty acids (SCFAs), bacteriocins, Vitamins, Peptides.
Paraprobiotic (Taverniti and Guglielmetti, 2011) ^[101]	non-viable microbial cells or microorganisms (intact or broken) or crude cell extracts (i.e. with complex chemical composition), which, when administered (orally or topically) in adequate amounts, confer a benefit on the human or animal consumer's health.	cell wall components of the probiotics are referred to as paraprobiotics (Posadas)	Heat-killed Lactobacillus

4.1 Postbiotics: non-viable bacterial products and metabolic byproducts

While purified elements from microbial metabolism and vaccines are not regarded as postbiotics, postbiotics can be thought of as a complex mixture of metabolic products secreted by probiotics in cell-free supernatants (CFSs),

including enzymes, secreted proteins, short-chain fatty acids, vitamins, secreted biosurfactants, amino acids, peptides, organic acids, etc. (Zendeboodi *et al.*, 2020) ^[119]. The tables given below (Table 2) and (Table 3) discuss the benefits and pharmacodynamic advantages of postbiotics over probiotics.

Table 2: Benefits of postbiotics over probiotics (Wei *et al.*, 2024; Vinderola *et al.*, 2022) ^[09, 112]

Benefits	Description
Pure availability	Postbiotics are readily available in their pure form.
Ease of production and storage	They are simple to produce and store.
Industrial scalability	The production process is available for industrial scale-up.
Specific mechanism of action	They act through a defined mechanism.
Improved MAMP accessibility	Enhanced accessibility of Microbe Associated Molecular Patterns (MAMPs) during recognition and interaction with Pattern Recognition Receptors (PRRs).
Targeted biological response	Higher probability of eliciting only the targeted responses through specific ligand-receptor interactions.

Table 3: pharmacodynamic advantages of postbiotics over live bacteria (Piqué *et al.*, 2019; Nataraj *et al.*, 2020) [67, 72]

No risk of translocation	Postbiotics decrease the risk of systemic infection since they are non-viable, indicating they are unable to pass the circulation from the intestinal lumen.
No concern about antibiotic resistance	Antimicrobial resistance genes can't be acquired or transmitted horizontally when living cells are eliminated.
Ease of handling	Compared to live probiotics, it is simpler to extract, organise, transport, and store.
Beneficial effects from cell lysis	When viability is lost during processing, intracellular components with extra functional benefits can be released.
Enhanced host interactions	Local effects are enhanced by close interactions among intestinal epithelial cells and bioactive chemicals released from damaged cells.

4.1.1 Classification of postbiotics

4.1.1.1 Short-chain fatty acids

Short-chain fatty acids (SCFAs) are created when plant fibres are fermented by the good bacteria in our stomach. (Lee *et al.*, 2017) [49]. The SCFAs have a wide range of positive effects on the host, such as providing energy sources for colonic epithelium cells, regulation of T regulatory cells, maintenance of metabolic homeostasis, and anti-inflammatory effects (Teame *et al.*, 2020) [102]. Short-chain fatty acids (SCFAs), which are known to stimulate growth, can be produced by lactobacilli and bifidobacteria. They can also release peptides (such as endopeptidases) and/or cationic species into the medium, which may subsequently enter cells or interact with the outer layers (Matera, 2024) [62]. Acetic acid, propionic acid, and butyric acid are the common carbon-based negative ions that make up SCFAs (Gill *et al.*, 2018) [31]. Host species, diet, and gut environment, the type and amount of SCFAs produced during fermentation, which determine the specific function of SCFAs. In improving livestock and poultry productivity, acetate salt plays a crucial role by regulating appetite in the central nervous system (Rodrigues *et al.*, 2023) [78]. A high proportion of propionate salts helps in regulating the activity of colon histone deacetylase 3 (HDAC3), which, as a result, promotes the immune cell differentiation (Xie *et al.*, 2022) [114]. Propionate salt, specifically sodium propionate, which is the primary source of carbon for glucose synthesis involved in hepatic gluconeogenesis, regulates carbohydrate metabolism and inhibits cholesterol synthesis by interfering with the utilisation of acetate (Liu *et al.*, 2023). Propionate salt has an anti-inflammatory property due to which it helps in the inhibition of immune-related gene expression, NF-kappa B reporter gene activity, and cytokine release *in vivo*, which results in the treatment of inflammatory diseases (Li *et al.*, 2021). Butyrate salts participate actively in intestinal cell nutrient metabolism, which helps in promoting the intestinal epithelial regeneration and boosts the production of cytokines that suppress the immune system and lower the expression of genes that cause the inflammation (Kasahara *et al.*, 2018) [45]. Butyrate provides a very high amount of energy to the intestinal lining cells for their effective growth and repair. Butyrate can affect gene function by blocking specific enzymes known as histone deacetylases, and butyrate possesses immunosuppressive qualities, which regulate the immune responses (Lee *et al.*, 2017) [49]. Lactobacillus strains can produce SCFAs, like *L. rhamnosus* GG and *L. gasseri* PA 16/8, which produce propionate (Teame *et al.*, 2020) [102]. Mice on supplementation with *L. plantarum* increased acetate and butyrate levels and decreased intestinal permeability and monoamine oxidases in the brain (Dhaliwal *et al.*, 2018) [21]. The strain of *L. plantarum* CECT 7529, which has a greater ability to produce higher quantities of propionic and butyric acids, has shown reducing cholesterol levels in the animal

body (Bosch *et al.*) [8]. Probiotic strains *L. salivarius* FP25 and FP35, and *L. reuteri* NCIMB are characterised by having excellent inhibitory and suppressive effects on colon cancer cell proliferation, which is mainly due to the production of SCFAs (Kahouli *et al.*, 2015) [44]. SCFAs, along with probiotic *L. johnsonii* L531 used in the treatment of Salmonella infection, have shown good results and maintain metabolic homeostasis in pigs (He *et al.*, 2019). Roseburia intestinalis in a mouse model with atherosclerosis produces high quantities of butyrate salt, which helps in inhibiting plaque formation by reducing inflammation throughout the body.

4.1.1.2 Secreted biosurfactants

The various polymeric compounds known as biosurfactants (BS) are produced in the late log or early stationary phase of an organism's growth cycle and are released either extracellularly or attached to the cell wall. By expanding the surface area, BS helps its own cell with (i) nutrition uptake, (ii) hydrophobic substrate metabolism, and (iii) cellular defence mechanisms (Kaczorek *et al.*, 2018) [42]. BS are amphiphilic compounds with hydrophilic and hydrophobic components, offering surface-active and emulsifying properties. They interfere with biofilms, stop pathogenic microbes from forming them, and prevent bacteria from adhering and interacting. Research suggests that BS's antagonistic effects on bacterial cells may be due to its hydrophobic interactions with lipids found in the membrane, creating pores and inhibiting enzymes, leading to intracellular cytoplasmic leakage (Sharma, 2016) [85]. Compared to commercial detergents, BS has many benefits, including being food-grade, having increased biodegradability, low toxicity, and stability at different processing parameters, including temperature, pH, and salt concentrations.

4.1.1.3 Enzymes

Enzymes are proteins that are grouped into six categories based on their activity or function oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases and are accountable for catalysing biological reactions. The catalase from genetically modified *Lactobacillus lactis* has the potential to prevent chemically induced colon cancer in mice. Enzymes are mostly produced from fungal strains like *Aspergillus Niger* and *Aspergillus oryzae*, as well as strains of bacteria like *Bacillus subtilis* and *Bacillus licheniformis*. Glutathione peroxidase, an antioxidant, is found in *Lactobacillus fermentum*. The proteolytic enzymes secreted by the *Bacillus* genus are extremely stable (Thorakkattu *et al.*, 2022) [107].

4.1.1.4 Exopolysaccharides

Lactic acid bacteria (LAB) produce long-chain, high molecular weight polymers known as exopolysaccharides

(EPS). They belong to one of two distinct groups: homopolysaccharides, which include one monosaccharide unit (examples include cellulose, levan, curdlan, pullulan, and dextran, etc.), and heteropolysaccharides, which have many monosaccharides (examples include xanthan, gellan, galactan, and kefiran, etc.) (Chaisuwan *et al.*, 2020) ^[13]. Immunomodulation, tumour suppression and antimutagenicity, antioxidant, anti-inflammatory, antihypertensive, antiviral, antibacterial, lowering cholesterol, and anti-gastrointestinal characteristics are only a few of the bioactivities that EPSs possess (Gezginç *et al.*, 2022) ^[30]. They might also enhance lipid metabolism. EPS from *Lactococcus lactis* and *Lactobacillus reuteri* Mh-001 exhibit antioxidant and immunomodulatory properties, with anti-inflammatory and antioxidant properties studied extensively. EPS is used in the food industry for emulsifying, stabilizing, and water-binding agents (Thorakkattu *et al.*, 2022) ^[107].

4.1.1.5 Amino acids, phenolic-derived metabolites, and Peptides

The aromatic amino acids, dietary polyphenols, which are bioactive molecules of the brain, kidney, and cardiovascular system, are produced and metabolized by gut microbes. The interaction of CFS from *L. plantarum* and *Enterococcus faecium*, a positive impact was also observed for CFSs produced by lactobacilli, indicating that the species may create a bifidogenic factor with a promoting effect. Although the composition of these substances is not known, some studies point to the existence of amino acid supernatant, including proline and glutamic acid (Tan *et al.*, 2024; Altieri *et al.*, 2024) ^[5, 98]. Bioactive peptides that are derived from food proteins can show immunomodulatory and anticancer properties (Liu *et al.*, 2020).

4.1.1.6 Bacteriocins

Lactic acid bacteria (LABs) bacteriocins are secreted extracellularly, making them useful in preserving food products like cheese, paneer, meat, and vegetables (Dridar *et al.*, 2016) ^[22]. Proteinaceous CFS is used for isolation and purification. Bacteriocins are antimicrobial peptides produced by Gram-positive and Gram-negative bacteria. They are classified into two categories: Class I, which contains unusual amino acids like lanthionine and β -methyl lanthionine, and Class II, which does not (Field *et al.*, 2018) ^[27]. There are five different classes of bacteriocins. Lactic acid bacteria (LAB) create bacteriocins, which are cationic peptides that cause intracellular components to seep out of cytoplasmic membranes. FDA-approved bio-preservatives Nisin and Micro-GARD are class A nisin and pediocin-like bacteriocins that act on lipid-bound cell wall precursor lipid II. LAB bacteriocins may find use in the therapeutic field as they show the ability to inhibit a variety of urogenital, antibiotic-resistant bacteria and food preservation fields (Nataraj *et al.*, 2020) ^[67].

4.1.1.7 Vitamins

Vitamins are chemical substances naturally present or added externally to food to support normal body functions. B-complex group vitamins participate in metabolic processes of energy production, while vitamin K is the only fat-soluble vitamin that has properties of a coenzyme (Giori and LeBlanc, 2018) ^[19]. Most vitamins are obtained exogenously, with some produced by gut bacteria and

probiotics. Because B-group vitamins are easily damaged during food processing, some vitamins must be added for fortification (Thakur and Tomar, 2016) ^[104]. One safe way to treat a vitamin deficiency is to use in situ vitamin-producing LAB. A cost-effective substitute for chemically produced pseudo-vitamins is industrial microbial fermentation, which enables the production of foods with higher vitamin content without adverse consequences. High-performance liquid Chromatography and spectrophotometric methods can be used for the evaluation and identification of biosynthesized vitamins (Deptula *et al.*, 2017) ^[20].

4.2 Paraprobiotics

4.2.1 Classification of paraprobiotics

4.2.1.1 Peptidoglycans

peptoglycan (PG) is an essential component of Gram-positive bacteria's cell walls. N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) residues are attached by beta 1-4 links to produce this linear glycan strand, which is cross-linked by peptides. The peptidoglycan structure of *Lactococcus lactis* (LAB), consisting of a di-amino acid (γ) and the amino acid sequence L-Ala-g-D-Glu- γ -D-Ala. In some LAB, D-Lac takes the place of D-alanine to provide intrinsic resistance to vancomycin (Nataraj *et al.*, 2020). Peptoglycan, a peptide found in probiotic bacteria, has been shown to have various health benefits. It has been found to have anti-cancer, immunomodulatory, and anti-inflammatory effects. The process involves delipidation of the cell wall, purification through proteases and nucleases, and confirmation of amino acids and carbohydrates using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and chromatographic or Nuclear magnetic resonance (NMR) techniques. However, only the immunomodulatory, anti-proliferative, or anti-tumour effects of probiotics mediated by peptidoglycan have been explored (Wang *et al.*, 2018) ^[111].

4.2.1.2 Teichoic acid

The presence of anionic glycopolymers known as wall teichoic acids (WTAs) is what drives the surface charge on the peptidoglycan layer of gram-positive bacteria. These are essential for establishing the structure of cells, controlling cell division, and regulating other basic metabolic processes in cell physiology. Furthermore, teichoic acids give gram-positive bacteria pathogenicity and resistance to antibiotics (Sumrall *et al.*, 2019) ^[94]. Lipoteichoic acids (LTAs) and wall teichoic acids (WTAs) are the two main forms of teichoic acids, which are glycopolymers containing phosphodiester-linked polyol repeat units. WTAs are covalently bound to peptidoglycan, whereas LTAs are amphiphilic molecules with a hydrophilic portion composed of glycerol phosphate or ribitol phosphate that has been replaced with d-alanine or sugars (Nataraj *et al.*, 2020) ^[67]. Studies on the functional properties of LTA have been carried out, with immunomodulatory potential serving as a crucial foundation. to initiate an inflammatory cytokine response, the toll-like receptors (TLR-2) on the surface of gut enterocytes recognize LTA, a microbe-associated molecular pattern (MAMP), and transduce cellular signals (Shiraishi *et al.*, 2016) ^[86] LTA of *L. plantarum* can inhibit the production of biofilms without influencing *S. aureus* development (Jung *et al.*, 2019) ^[40]. However, removing the d-alanine moieties from the LTA structure did not have the same impact. In the formation of *S. aureus* biofilms, poly-N-

acetylglucosamine is produced by the LTA-controlled *ica*-operon. *Streptococcus mutans*, *S. aureus*, and *E. faecalis* are among the oral and intestinal pathogens that are effectively broken up by lactobacilli-derived tetrapeptide (LTA). Promoting the release of autoinducer-2 prevents the growth of *S. aureus* biofilms. Biofilms were more prone to macrophages and antibiotics after LTA treatment (Ahn *et al.*, 2018)^[2].

4.2.1.3 Cell wall polysaccharides

The adhesive qualities of exopolysaccharides (EPS), such as anti-pathogenic effects, may facilitate interactions between bacteria and the environment. EPS that are derived from the Probiotic strain can alter mucosal immune responses and intestinal homeostasis (Lee *et al.*, 2023)^[50].

4.2.1.4 Cell surface proteins

The valuable component of the LAB cell envelope is the surface layer proteins. Cell surface proteins are covalently or non-covalently attached to the cell surface. According to a recent study, LAB strains generate a variety of surface proteins, including moonlight proteins, LPxTG proteins, pili proteins, and S-layer proteins (Lee *et al.*, 2023)^[50].

5. Production and sources

5.1 Postbiotic production methods

The process of producing postbiotics is very similar to that of producing probiotics, as both require bacterial metabolism that produces a variety of metabolites. The removal of leftover bacteria and preservation of metabolites are crucial when preparing postbiotics. The release intracellular metabolites into the extracellular medium, microbial cells can go through many processes that alter the cell membrane's structure. High pressure, pulsed electric fields, heat treatment, and sonication are often employed techniques (Pimentel *et al.*, 2023)^[71]. The particular bacterial strain, as well as the stability and sensitivity of the metabolites under various circumstances, determine which approach is best. Furthermore, to guarantee the total elimination of living organisms, centrifugation and microfiltration are frequently used. Centrifugation and microfiltration techniques are sufficient for postbiotics that are released by living cells that already exist in the extracellular medium (Rafique *et al.*, 2023; Liang and Xing

2023)^[54, 75, 76]. *Bifidobacterium* spp. strains were cultivated in De Man, Rogosa, and Sharpe (MRS) broth (Oxoid, Milan, Italy) with cysteine (0.05% w/v) (cMRS) (Sigma-Aldrich, Milan, Italy) at 37 °C for 48 hours, whereas *L. plantarum* strains were cultivated in MRS broth (Oxoid, Milan, Italy) at 30 °C for a duration of 48 hours. Each strain was grown separately. This procedure was followed by two washes with sterile saline solution (NaCl 9 g/L) and centrifuged at 3000× g at 5 °C for 15 minutes to separate the cells from the broths. The cell-free hydro-soluble fractions were extracted from the supernatants by passing them through a 0.22 µm Millipore filter (Whatman, Dassel, Germany). As a result, 6 different cell-free supernatants were produced (Altieri *et al.*, 2024)^[5].

Bioactive substances of postbiotics that are produced from probiotics should be free of impurities and contain beneficial microbial byproducts like peptides or short-chain fatty acids (SCFAs). The precursor microbe should also have been molecularly characterized. It is compulsory to validate the inactivation processes and conduct a thorough study on their safety and health advantages (Salminen *et al.*, 2021)^[81].

5.2 Parabiotic production methods

Parabiotics are inactivated microorganisms that still provide health benefits. The desired strain, even after inactivation (by heat or pressure), should still have its beneficial properties. It must also be stable and safe to store. The inactivation process should be standardized to maintain bioactive components, and the parabiotic should work effectively with the host's cells or microbiota (Almada *et al.*, 2018). The table given below (Table 4) discusses the various sources for the production of post and parabiotics.

Parabiotics are nothing less than probiotic microbes that have undergone an inactivation process. Heat treatment is the most commonly used technique for inactivation; among other techniques, such as chemical exposure, irradiation, and heat treatment, can also be used (Nataraj *et al.*, 2020)^[67]. In this method of inactivating probiotics, two different kinds of biotic products are produced: parabiotics, the non-viable microbial cells that provide health advantages, and postbiotics, which exclusively use microbial metabolites (Wisastra *et al.*, 2025)^[113].

5.3 Sources

Table 4: Source and production procedure of post and parabiotics

Probiotic source (strain/example)	Product type	How it's produced
<i>Lactiplantibacillus plantarum</i> RG14 (Izuddin <i>et al.</i> , 2019) ^[37]	Postbiotic (CFS)	Inoculate 10% (v/w) active culture (~10 ⁹ CFU/mL) into MRS; incubate 30 °C, 10 h → centrifuge 10,000 ×g, 4 °C, 15 min → 0.22 µm filtration → store -20 °C until use.
<i>Lactobacillus acidophilus</i> (poultry isolate) (Monika <i>et al.</i> , 2024) ^[63]	Postbiotic (CFS)	Grow in MRS 37 °C, 48 h → centrifuge 6,000 ×g, 10 min → filter through 0.22 µm membrane; store 4 °C.
<i>Lactiplantibacillus plantarum</i> PD18 (Butrungrod <i>et al.</i> , 2023) ^[9]	Postbiotic (CFS)	Culture in MRS; centrifuge 10,000 ×g, 10 min → 0.22 µm filtration; pH may be adjusted depending on assay.
<i>Limosilactobacillus reuteri</i> (reuterin producer) (Sun <i>et al.</i> , 2022) ^[96]	Postbiotic (specific metabolite: reuterin)	Two-step/re-streak or resting-cell process: grow cells, wash; incubate dense cell suspension anaerobically with glycerol (often 37 °C, 1-3 h) to convert glycerol → 3-hydroxypropionaldehyde (reuterin); remove cells by centrifugation/filtration; quantify 3-HPA.
<i>Saccharomyces cerevisiae</i> (yeast culture / SCFP) (Maina <i>et al.</i> , 2024) ^[59]	Postbiotic (fermentation product)	Controlled yeast fermentation on defined carbohydrate substrate → inactivation (drying/heat) → drying (spray- or drum-dry) to yield metabolite-rich fermentate (no viable yeast required in product).
<i>Lactobacillus plantarum</i> L-137 (HK L-137) (Tartrakoon <i>et al.</i> , 2023) ^[100]	Paraprobiotic (heat-killed cells)	Prepare biomass; heat-kill per established method (e.g., 100 °C for 30 min in aqueous suspension) → dry/blend into carrier;

Lactobacillus paracasei K71 (Thi <i>et al.</i> , 2022) ^[106]	Paraprobiotic (heat-inactivated cells)	commercial ingredient examples contain defined % HK cells. Culture; heat-sterilize to inactivate (temperatures/time per manufacturer/protocol); dry and blend; used as inactivated biomass.
Enterococcus faecalis FK-23 (paraprobiotic lysate) (Lee <i>et al.</i> , 2023) ^[50]	Paraprobiotic (heat-killed/lysate)	Grow <i>E. faecalis</i> ; heat inactivate (e.g., autoclave or 100-121 °C) to kill; optionally lyse cells to obtain lysate; dry for inclusion.
Pediococcus acidilactici (e.g., K9-3) (Izuddin <i>et al.</i> , 2019) ^[37]	Paraprobiotic (heat-killed cells)	Culture; heat-inactivate (study uses a commercial heat-killed preparation); administered as powder to pets; (methods papers commonly report autoclave 121 °C 15 min or 80-100 °C killing for <i>Pediococci</i>).
General lactic acid bacteria (LAB) (multiple species) (Salminen <i>et al.</i> , 2021) ^[81]	Postbiotics & Paraprobiotics	Postbiotics (ISAPP): deliberate inactivation of microbes, often after fermentation, retaining cells/cell-fragments with or without metabolites; common steps: centrifugation → 0.22 µm filtration, pH adjusts, spray-dry/lyophilize. Paraprobiotics: inactivation without major structural disruption via heat (pasteurization/tyndallization), HPP, irradiation, sonication, then dry and formulate.

6. Applications in animal feed

Multiple health benefits, safe use in vulnerable populations, no risk of antibiotic resistance, emergence, or transfer, and safe transmission during antibiotic or antifungal therapy. Because of their unique mechanisms of action, postbiotics are cutting-edge fields in research on nutrition that offer significant health advantages. Probiotic bacterial fermentation generates postbiotics, which have been connected with better gut health, immunological regulation, and potential illness prevention (Al-Habsi *et al.*, 2024)^[3]. The table (Table 5, Table 6, Table 7, Table 8, Table 9, and Table 10) highlights the benefits of functional foods along with their doses in ruminants, poultry, swine, companion animals, and aquatic species. Future functional foods improved with non-viable bioactive cells may be able to get

past many of the obstacles that are currently limiting the widespread use and advancement of probiotics (Sionek and Gantner, 2025)^[88].

6.1 Ruminants

In the dairy sector, Paraprobiotics can be of significant value because of their ability to hold stability over a wide range of pH and temperature, allowing their incorporation into foods with the highest levels of acidity and before thermal processing without negatively affecting their function. Their addition helps in modifying gut microbiota while preserving the taste and texture of the finished goods, reducing negative effects such as high acidity in yoghurt during fermentation (Siciliano *et al.*, 2021)^[87].

Table 5: Benefits of functional food in ruminant nutrition

Species	Functional food	dosage	Benefits
Holstein dairy calves (Centeno <i>et al.</i> , 2023) ^[12]	Saccharomyces cerevisiae fermentation product (SCFP) (yeast fermentation metabolites)	Milk replacer: 1 g/day SmartCare®; Starter/Grower: 0.8%, then 0.44%, then 0.275% of diet DM (NutriTek®)	↑ ADG, BW, hip width; ↓ respiratory disease post-weaning; fecal microbiome maturation effects
Dairy calves	SCFP / yeast fermentation metabolites (commercial SCFP products)	Not mentioned	Increased ADG, improved health, and microbiome maturation
Dairy cows (mid-lactation)	Saccharomyces cerevisiae fermentation product (SCFP; NutriTek/NTK)	19-38 g/day	Improved NDF digestibility, resilience to feed restriction, and inflammatory marker modulation
Beef cattle	SCFP (yeast fermentation product)	Not mentioned	Improved performance under production stressors and accelerated recovery

6.2 Poultry

Table 6: Benefits of functional food in poultry nutrition

Species	Functional food	dosage	benefits
Broilers (Kadekar <i>et al.</i> , 2024) ^[43]	Cell-free culture supernatant (postbiotic) from <i>Lactobacillus acidophilus</i>	0.2% of drinking water	Improved BWG and FCR; reduced necrotic enteritis lesions
Broilers (Tukaram <i>et al.</i> , 2022) ^[108]	Heat-killed / tyndallized <i>Lactobacillus</i> mixture (paraprobiotic)	0.6% (w/v) inactivated culture in water/feed	Better growth, gut morphology and immune markers; alternative to antibiotics
Broilers (Monika <i>et al.</i> , 2024) ^[63]	Cell-free supernatant (CFS) from <i>Lactobacillus acidophilus</i> (no live cells; CFS prepared by centrifugation + 0.22 µm filtration)	0.2%, 0.4%, 0.6% (v/w) mixed into feed daily (best overall response at 0.2%)	↑ body weight & better FCR; ↑ villus height and crypt depth; ↑ jejunal antioxidant activity; ↓ caecal coliform counts (pathogen pressure); improved humoral & cell-mediated immunity
Broilers (Tao <i>et al.</i> , 2023) ^[99]	<i>Bacillus subtilis</i> postbiotic metabolites (in-feed metabolite mix)	Not mentioned	Improved growth, immune response, and stress resilience
Broiler (Chang <i>et al.</i> , 2022) ^[14]	<i>L. plantarum</i> CFS RS5	Included in feed, exact percent not given; postbiotic RS5	↑ growth, gut health, antioxidant, mucin production, immune response, mucosal integrity

		improved BWG, reduced FCR.	
Broiler (Li <i>et al.</i> , 2024)	Bacillus subtilis ACCC 11025 postbiotic	0.015% (best effects), also tested 0.03%, 0.045% (w/w in feed)	↑ ADG, ↓ FCR, ↓ mortality, ↑ antibody titers vs NDV/H9 AIV, improved tibia strength
layer pullets (Saeed <i>et al.</i> , 2023) ^[80]	SCFP (yeast postbiotic)	1.5 kg/ton feed (day 0-21); 1.0 kg/ton (22-32)	↓ <i>Salmonella Enteritidis</i> colonization in ceca
Broiler (Atan and Kırkpınar, 2025) ^[6]	Encapsulated postbiotics ± inulin	Various: 0.15% - 0.60% postbiotic + 1% inulin in feed	Improved carcass, breast & thigh yields; best at 0.15% postbiotic + inulin
Broiler (Humam <i>et al.</i> , 2019) ^[35]	<i>L. plantarum</i> RI11 CFS	0.2%, 0.4%, 0.6%, 0.8% in feed	↑ GSH, catalase activity; ↑ mucin, ZO-1, IL-10; ↓ HSP70, TNF-α, IL-8, acute-phase proteins

6.3 Swine

Table 7: Benefits of functional food in swine nutrition

Species	Functional food	Dose	Benefits
Nursery piglets (Xu <i>et al.</i> , 2022) ^[115]	Lactobacillus fermentate (LBF; Adare Biome) postbiotic fermentate	2 kg/ton of feed (challenge model compared to bacitracin 30 g/t)	↓ post-weaning diarrhea; ↑ intestinal health & microbial diversity; improved growth efficiency
Starter/finisher pigs (Tartrakoon <i>et al.</i> , 2023) ^[100]	Heat-killed <i>Lactobacillus plantarum</i> L-137 (HK L-137, paraprobiotic)	~20 mg/kg feed	Stimulated immune responses in suckling/starter pigs; improved finishing performance
Weaned piglets (Zhao <i>et al.</i> , 2024) ^[120]	Postbiotic blends / Lactobacillus-derived metabolites	Not given	Modulation of TLR/NOD pathways, enhanced mucosal immunity, reduced pathogen effects
Nursery pigs (Duarte <i>et al.</i> , 2024) ^[23]	Lactobacillus postbiotics (Lactobacillus-derived metabolites; no live cells)	Not given	↑ IFN-γ & PGLYRP4; ↓ TLR4/NOD1/CD14 → enhanced mucosal immunocompetence vs. F18+ ETEC
Finishing pigs (~77 kg) (Ryu <i>et al.</i> , 2022) ^[79]	Heat-killed <i>L. salivarius</i> LS 189 (postbiotic)	0.2% (w/w) of basal diet	↑ final BW, ADG, ADFI and better G:F trend; altered gut microbiota beta-diversity
Post-weaning piglets (Zhong <i>et al.</i> , 2022) ^[121]	Postbiotic mix (metabolites of <i>L. plantarum</i> TL1, RG14, RS5)	0.5% (w/w) of metabolites in the diet	↑ ADG and daily feed intake; ↓ diarrhea incidence; gut: ↓ Enterobacteriaceae, ↑ LAB, and SCFA levels

6.4 Sheep & Goat

Table 8: Benefits of functional food in sheep & goat nutrition

Species	Functional food	Dosage	Benefits
lambs (Izuddin <i>et al.</i> , 2020) ^[36]	<i>L. plantarum</i> RG14 postbiotic (cell-free metabolites)	0.9% (v/w) in feed	Improved BWG, ruminal fermentation, antioxidant status and barrier function
Lambs (post-weaning) (Zhao <i>et al.</i> , 2024) ^[120]	<i>L. plantarum</i> postbiotic (RG14)	0.9% (v/w) in feed	Reduced leukocyte counts (indicative of reduced systemic inflammation) and improved rumen health
Lactating goats (Murciano-Granadina)	Probiotic Ruminants (yeast fermentation product/postbiotic)	3.75 g/day added to the control diet	↑ fiber digestibility, ↑ ruminal propionate, ↓ methane output more efficient milk production
Ewes (peripartum dairy sheep)	Yeast live + postbiotic fraction (MOS + beta-glucans) + organic-Se yeast	Supplement added from -6 to +6 weeks around lambing (dose per diet)	↓ β-hydroxybutyrate; ↑ milk yield postpartum; improved energy and oxidative status
Hu sheep (growing lambs) (Wang <i>et al.</i> , 2024) ^[110]	Compound postbiotic mixture (multi-strain <i>Aspergillus oryzae</i> 1, <i>Aspergillus oryzae</i> 2, and <i>Candida utilis</i> ; precise composition in methods)	Optimal CP dose: 800 g/t (Group C)	↑ ADG, ↑ DM, EE, Ca digestibility; ↑ GSH, IgG, IL-2, IL-6, IFN-γ; enhanced rumen fermentation enzymes

6.5 Companion animals

Table 9: Benefits of functional food in companion animal nutrition

Species	Functional food	Dosage	Benefits
Dogs (Sordillo <i>et al.</i> , 2025) ^[90, 91]	Indole-rich postbiotic (canine immune health postbiotic)	7.5-15 g/day (size-based allotment as specified) for 6 weeks	Reduced itching/pruritus; improved skin condition in randomized trials
Dogs (Cauquil and Olivry, 2025) ^[11]	Heat-killed <i>Lactobacillus rhamnosus</i> SGL01 & <i>L. reuteri</i> SGL06	2 × 10 ⁵ peripheral blood mononuclear cells (PBMCs), they added 1 × 10 ⁶ heat-killed (Tyndallized) (in vivo study)	↑ IL-12, IFN-γ, IL-10 (anti-allergic/Th1-Treg tilt) → supports rationale for feed inclusion

Dogs (halitosis) (Sordillo <i>et al.</i> , 2025) ^[90, 91]	Superculture® Pet Oral (heat-inactivated <i>Pediococcus pentosaceus</i> + <i>Bacillus subtilis</i> postbiotics in carrier)	250 mg/day top-dressed on food for 14 days	Reduced volatile sulfur compounds (halitosis); improved breath quality
Cat (Sun <i>et al.</i> , 2025) ^[95]	Inactivated <i>Pediococcus lactis</i> in yogurt	yogurt: 50 g/day; postbiotic content: 2% by weight of yogurt	↓ TBIL, TBA, TG, glucose, BUN, CREA, TNF-α, IL-6; ↑ fecal sIgA; modulated fecal flora (↑ <i>Bifidobacterium</i> , <i>S. thermophilus</i>)

6.6. Aquaculture

Table 10: Benefits of functional food in aquatic species

Species	Functional food	Dosage	Benefits
Juvenile gilthead seabream (<i>Sparus aurata</i>) (Gómez <i>et al.</i> , 2025)	Postbiotics derived from <i>Vibrio proteolyticus</i> DCF12.2	Included in feed for 62 days (specific inclusion not stated)	Healthier intestinal morphology (more goblet cells), α-diversity ↑, beneficial gut microbiota shifts, inflammation genes ↓ (e.g., <i>tnfa</i> , <i>cox2</i>), epithelial integrity gene (<i>cdh1</i>) ↑ after LPS challenge
Nile tilapia juveniles (<i>Oreochromis niloticus</i>) (Abdollahzadeh and Pourmozaffar, 2023) ^[1]	Heat-inactivated (<i>paraprobiotic</i>) <i>Bacillus</i> sp. NP5	Feed with 1% inclusion at densities 10 ⁸ , 10 ⁹ , 10 ¹⁰ CFU/mL-equivalent for 30 days	Enhanced growth performance, improved immune responses, survival rate ↑ post challenge with <i>S. agalactiae</i> ; best at 10 ¹⁰ CFU mL ⁻¹
Common carp (<i>C. carpio</i>) (Feng <i>et al.</i> , 2020) ^[25]	<i>Exopolysaccharides Lactococcus lactis</i> Z-2	Each fish received 0.1 mL of the respective EPS-2	Immunity enhancement Resistance against <i>A. hydrophila</i>
Grouper (<i>Epinephelus coioides</i>) (Yang <i>et al.</i> , 2021) ^[116]	Cell wall components (peptidoglycan and lipoteichoic acid) from <i>Bacillus pumilus</i> SE5	1.0 × 10 ⁸ CFU/g of feed	Improved growth performance; strengthened innate and adaptive immunity
Silvery pomfret (<i>Pampus argenteus</i>) (Gao <i>et al.</i> , 2016) ^[29]	Lipoteichoic acids <i>L. plantarum</i> LTA	A 30 mg/mL concentration was used <i>in vitro</i>	Resistance against <i>V. anguillarum</i> -caused vibriosis

7. Challenges and limitations: lack of standardized definitions and production protocols

Because they are made of either inactivated microbial cells or microbial metabolites, postbiotics and parabiotics provide a possible safer substitute by removing the hazards associated with live organisms. Their use is still in its infancy, though, and more study is required to confirm their effectiveness. Metabolomic analysis is an essential part of postbiotics since it allows the identification and measurement of bioactive substances made by microorganisms. Targeted or untargeted metabolomics, depending on the purpose of the study (Kumar *et al.*, 2024) ^[47]. The untargeted metabolomics aims to detect and evaluate every metabolite present in a sample, including unknown or unexpected substances. Targeted metabolomics concentrates on determining and measuring specifically, well-characterized metabolites of importance. The main challenge in this case is the high cost of the equipment and the necessity for skilled workers, especially when using advanced tools (Qiu *et al.*, 2023) ^[74]. Finding the best inactivation technique for parabiotics that maintains biological activity while guaranteeing safety is important (Almada *et al.*, 2021) ^[4].

8. Conclusion

Postbiotics and parabiotics in recent times are becoming more popular as future-generation functional feed additives in an attempt to address the limitations of probiotics and the overuse of antibiotics in animal husbandry. Their bioactive compounds, including short-chain fatty acids, bacteriocins, exopolysaccharides, cell wall components, and enzymes, cell surface proteins, are essential for balancing microbiota, protecting gut integrity, and boosting the immune system. Cattle, poultry, aquaculture, and companion animals have

shown their potential for improving nutrient utilisation, growth performance, feed utilisation, and resistance to disease without risk of pathogen transfer, resistance to antibiotics transmission, or viability loss. Prices, metabolomic validation, manufacturing methods, dosage, and standardisation, however, remain to be concerns. With increasing demand for sustainable farming, these biotics provide a safe, effective, and multifunctional strategy, promising further research for precision applications and large-scale use.

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