**Exploring the Role of Postbiotic Metabolites in Enhancing Animal Health and Nutrition: A Comprehensive Review**

**ABSTRACT**

Probiotics result in several health benefits by modulating the gut microbiome, however, various techno-functional limitations such as viability control prevents their full use in the livestock and poultry feed industries. Therefore, the focus is steadily shifting from probiotics to postbiotics. Postbiotics comprise of inanimate microorganisms and/or their components like cell wall, membranes, exopolysaccharides etc. with or without their metabolites that confer a health benefit on the host. Postbiotics confer various health benefits to the host via modulation of resident microbiota, enhancement of epithelial barrier function, modulation of immune response, modulation of systemic metabolic response and systemic signalling via nervous system. Postbiotics can thus improve the growth performance in livestock and poultry as well as improve the meat quality because of probable antioxidant effects. The efficacy of a postbiotic is, however, dependent on the method of deactivation used for its production as some methods may denature the potential metabolites. Postbiotics serve as a safe alternative to live probiotic organisms, but they cannot be presumed to be safe solely based on their progenitor microorganism. There is also a need of more studies for safety assessment of postbiotics for effective results. The review thus, discusses about postbiotics and their potential for successful use in livestock.

**Keywords:** Probiotics, Probiotics, Postbiotics, Inanimate microorganisms, Health and Nutrition

**Graphical abstract**

**Keywords: Postbiotics; metabolites; gut health, production**

**INTRODUCTION**

The gut of an animal is home to a complex ecosystem called the microbiome, which is a site of composite relationship between the gut microbiota and the host. The members of the gut microbiota help with digestion and produce a variety of substances that can be used by both the host and microorganisms. To achieve optimum animal production, the gut microbiota can be modified by different nutritional strategies called biotas. “Prebiotics, probiotics and synbiotics are the commonly used nutritional strategies for modulating the composition and action of the intestinal microbiota. They positively stimulate the host health and nutrition. The proposed benefits of probiotic, prebiotic or synbiotic supplementation depend on the potential production of various metabolites” (Sánchez et al., 2017; O'Grady et al., 2019). However, based on its metabolic and functional phenotype, the composition of the gut microbiota varies and further, the extent to which components are metabolized by microbes shows individual variation. This results in alterations in the health effects of these "biotics" amongst different individuals (Toole et al., 2017). Besides these variations, any temporal changes in gut microbiota composition can also impact the response to interventions. Among the limitations, the common ones are gastrointestinal side effects (Banerjee,2021) and the capability of probiotics to transfer their own antimicrobial resistance genes to other living organisms (Suez et al., 2019; Banerjee,2021). These limitations may compromise the expected health benefits of the probiotic. Thus, it becomes necessary to find safer and effective alternatives to probiotic. This knowledge contributed to a reassessment of the concept of fermentation and gave birth to the concept of postbiotics.

Postbiotics are the newest addition to this family of biotas and refer to bioactive substances produced by microorganisms during fermentation process. The term "postbiotics" was first coined by Tsilingiri and coworkers in 2012. Postbiotics can also be defined as soluble factors secreted by a living probiotic that exert beneficial effects when present in sufficient quantities (Tsilingiri et al., 2012). Other names for postbiotics can be metabiotics, biogens, metabolites or simply cell-free supernatants (CFS). In 2019, International Scientific Association for Probiotics and Prebiotics (ISAPP) selected the term ‘postbiotic’ and defined it as "non-living microorganisms and/or their components that provide health benefits to the host." According to Peluzio et al. (2021), postbiotics are metabolites produced during the anaerobic fermentation of non-digestible dietary components, or even digestible ones (such as complex carbohydrates, proteins, and lipids), as well as those released by bacterial metabolism in the gastrointestinal tract. Additionally, the endogenous components formed through bacteria-host interactions, which may have either beneficial or harmful effects on the organism, are also considered "postbiotics." Bioactive compounds in postbiotics target the host-microbe-pathogen interface, preventing biotic and immune imbalances and inflammation, thus, offering new opportunities for their use in enhancement of animal performance (Puccetti et al., 2020).

**POSTBIOTIC METABOLITES**

“The postbiotic metabolites from different strains of probiotic bacteria create a microbiome ecosystem that supports the growth and proliferation of probiotic bacteria and inhibits the growth of pathogens. Postbiotic may include non-viable microbial cells, microbial cell fragments/structures such as cell walls, membranes, exopolysaccharides, cell wall-associated proteins, pellets, etc., with or without metabolites/end products such as organic acids, peptides, secreted proteins, enzymes, bacteriocins, etc. Postbiotics can be structural compounds, like peptides, teichoic acids and plasmalogens or metabolites generated by the microbiota, like short chain fatty acids (SCFAs), vitamins and enzymes” (Jose and Elena, 2020) (Fig.1). Based on their composition, postbiotic components can also be classified (Tsilingiri and Rescigno 2013) as proteins, carbohydrates, vitamins, fats, organic acids, intricate molecules and cell surface proteins (Fig. 2).

Fig.1: Classification postbiotics (Jose and Elena, 2020)

Fig.2: Classification of postbiotic components based on their composition (Tsilingiri and Rescigno, 2013)

**SYNTHESIS OF POSTBIOTICS**

Probiotics ferment prebiotics eaten by the animal to produce postbiotics. So, these three are part of the same process and complement each other and play a crucial role in gut health (Fig. 3). Prebiotics are special fibers that support beneficial microbes in the gut while probiotics are the live microbes capable of providing health benefits to the consumer. On the contrary, postbiotics are the products produced by microorganisms that affect various physiological processes.



Fig. 3: Relationship between prebiotics, probiotics and postbiotics

Commercial postbiotic production requires probiotic cells as a starter culture and nutrients that support the growth of these probiotics. The mixture of probiotics and nutrients is incubated to obtain a cultured fermentation product containing new probiotic cells formed as a result of the culture and products of the probiotic cells as a result of the culture. This cultured probiotic is then inactivated to obtain a mixture of inactivated probiotic cells and products of transformed probiotic cells formed as a result of the inactivation process. All or part of the inactivated ferment can be used as a postbiotic. Moradi et al*.* (2021) gave the procedure for preparation of postbiotics as described in Fig. 4.

Fig. 4: Procedure for preparation of postbiotics (Moradi et al*.,* 2021)

The different deactivation methods that can be used to prepare postbiotics are: a) heat treatment, b) high pressure treatment, c) formalin inactivation, d) ultraviolet irradiation, e) ionizing radiation and f) ultrasound (Zhong et al.,2022; Chan and Liu, 2022; Almada et al., 2016). “In addition, other methods such as ohmic heating and supercritical CO2, pulsed electric field, pH changes and drying can also be effective in the inactivation and production of postbiotics” (Almada *et al*., 2016). Among the different deactivation methods, the most commonly used are heat treatment and formalin treatment. The main criterion for a good inactivation method is its effectiveness in maintaining the health-promoting properties of beneficial microbes during postbiotic production (Dunand *et al*., 2019). The postbiotic effect is based on microbial metabolites generated in the fermentation matrix (Aguilar-Toalá *et al*., 2018). Postbiotic composition can be influenced by processing methods as microbes involved in the fermentation process can respond differently to different methods. For example, heat treatment can result in denaturation of bacterial proteins, while irradiation may cause mutations in nucleic acids [Almada *et al*., 2016]. Thus, the inactivation methods used in postbiotic production must be chosen taking into account all these factors.

To increase the effectiveness of postbiotics (Fig.5), these compounds (as depicted by coloured geometrical shapes in Fig. 5) can be microencapsulated (Abdolhosseinzadeh *et al*., 2018). In addition, lamination of postbiotic agents in the outer layer, immobilization with a mediator or coating in a polymer matrix can be done to improve the postbiotic effect (Fig.5).



Fig.5: Methods to improve postbiotic efficacy

To qualify as a postbiotic, a preparation must meet several criteria: it should include molecular characterization of the original microorganisms, with identification and screening of any potentially harmful genes; a clear explanation of the inactivation process and confirmation that it has been completed; a detailed composition of the postbiotic preparation; an evaluation of its safety for the target host; and proof of health benefits in the host based on studies (Salminen et al., 2021).

**MECHANISM OF ACTION OF POSTBIOTICS**

Due to the advanced research with respect to probiotics, the probiotic industry regularly goes through continuous evolution and enhancement, resulting in advancement in the concept of probiotics, synbiotics, and integration of probiotics, prebiotics, etc. It has been discovered that the metabolic byproducts of various microbes during the growth or fermentation phase may help in generating beneficial effects on the host independent of the microbes, hence the concept of postbiotics. Postbiotics influence the constituent intestinal microbiota by, further, promoting the growth of beneficial microbes while inhibiting the harmful ones (Fathima et al., 2022). Postbiotics have also been shown to have immunomodulatory, anti-inflammatory as well as antioxidant properties (Vinderola et al*.,* 2022, Scott et al*.,* 2022). They may also help improve the integrity and functioning of the intestinal barrier (Stofilova, 2022), thus enhancing the digestion and nutrient absorption (Rius et al., 2022). These beneficial effects eventually help in improving the health and performance of animals as depicted in Figure 6.



Fig.6: Mechanism of action of postbiotics in animals

1. **Modulation of beneficial resident microbiota –**

Nurturing an optimal gut microbiome is essential for beneficial effects in animal science as a means to enhance the performance and productivity of farm animals (Wiley et al., 2017).

Postbiotic metabolites like lactic acid (Sun et al., 2015) and bacteriocins (Corr et al., 2007) possess direct antimicrobial effects. Lactic acid can be consumed by some members of the gut microbiota, resulting in further synthesis of short chain fatty acids (SCFAs) and butyrate. SCFAs are present in some postbiotic products, resulting in direct impacts or bacterial cross-feeding. These metabolites can compete with indigenous microorganisms for adhesion sites when postbiotics provide adhesins such as fimbriae (Tytgat et al., 2016) and lectins (Petrova *et al*., 2016). Postbiotics of *Lactobacillus plantarum* strains (RI11, RS5 and UL4) increase the total number of caecal bacteria *Lactiplantibacillus* and *Bifidobacterium*, but reduced pathogenic bacteria such as Enterobacteriaceae, E. coli and Salmonella in heat-stressed broilers (Humam *et al*., 2019). *L. plantarum* RG14 0.9% postbiotics increased fiber-degrading bacteria but reduced total protozoa and methanogens in the rumen of lamb (Izuddin *et al*., 2019b). Inulin and different concentrations of postbiotic *L. plantarum* RG14 increases the proliferation and colonization of *Bifidobacterium* while reducing E. coli and *Enterobacteriaceae* and *Bifidobacterium* populations in broilers (Kareem et al., 2016b).

The antimicrobial postbiotic metabolites like organic acids and bacteriocins have show high inhibitory activity against various pathogenic bacteria such as *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, *Pediococcus acidilactici* and Vancomycin-resistant *Enterococci* (Thanh et al., 2010; [Choe](https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-019-2064-9#ref-CR17) et al., 2013).  Godoy *et al*. (2019) isolated Bovicin HC5 bacteriocin from *Streptococcus equinus* HC5 bacteria and found that it inhibited >80% of streptococcal and staphylococcal strains tested in breast milk. Bovicin HC5 concentrations above 100 AU/ml also inhibited the growth of Listeria species in tryptic soy broth. (Nathan et al., 2013). In a study conducted by Sevin et al. (2021), postbiotics secreted by *Lactobacillus sakei* strain EIR/CM-1 isolated from Holstein cow's milk microbiota displayed antibacterial activity against mastitis-causing pathogens in ruminants.They also found that there was a 70% reduction in biofilm formation of ruminant mastitis-causing pathogens when co-incubated with postbiotics at 5 mg/mL and above concentrations (Sevin et al., 2021).

**(2) Improving epithelial barrier functions**

Intestinal barrier function is important to enhance the nutrient utilisation in animal’s digestive system. Exopolysaccharides, one of the postbiotics, promote intestinal barrier function by reducing inflammation (Schiavi et al., 2016). Similarly, mitogen-activated protein kinase and protein kinase B promote tight junction via calcium signaling pathways (Engevik et al., 2019). SCFAs enhance epithelial barrier function and prevnt against lipopolysaccharide-induced disruption (Feng et al., 2018). Butyrate alters tight junction permeability through lipoxygenase activation via histone acetylation in Caco-2 cell lines (Ohata et al., 2005). In a study conducted in broilers,postbiotics from *Lactobacillus plantarum* strains (RI11, RS5 and UL4) were shown to increase the villus height and decrease crypt depth (Humam et al., 2019). The postbiotic combination of *L. plantarum* strains increased the height of the small intestine in broilers (Loh et al., 2010; Thanh et al., 2009; Kareem et al., 2016a) and increased the height of the small intestine in pigs (Loh et al., 2013 Neljap et al., 2016 et al., 2011). 0.9% *L. plantarum* RG14 postbiotics increased villus height in the intestine of post-weaning lambs (Izuddin et al., 2019a).

**(3) Modulation of local and systemic immune responses**

Immune system plays a crucial role in improving the gut as well as overall health of animals. This may eventually result in positive outcome in terms of performance of the livestock. Lipoteichoic acid, a postbiotic, interacts with TLR2 or TLR6 (Mohamadzadeh et al., 2011). Lipopolysaccharide interacts with TLR4 and sometimes with TLR2 (Basic et al., 2018). β-glucans in yeast such as Saccharomyces cerevisiae interact with TLR2 (Jin et al., 2019); and lipoproteins, which mostly interact through TLR2 (Sturm et al., 2005). Indole derivatives of tryptophan produced by *Limosilactobacillus reuteri* can activate the CD4 T cell receptor in the mouse intestine (Cervantes et al., 2017). Histamine (Thomas et al., 2012), branched-chain fatty acids, and SCFAs (Thangaraju et al., 2009) also have potential to influence immune responses.

*L. plantarum* RG14 increased the expression of liver glutathione peroxidase and Cu/Zn SOD genes in post-weaning lambs (Izuddin et al., 2020). Postbiotic RI11 increases MUC2 expression in lambs (Humam et al., 2020). L. plantarum RG14 0.9% postbiotics increased hepatic IL-6 mRNA, TNF, IGF-1 gene expression and rumen MCT-1 gene expression in post-weaning lambs, but decreased IL-1β and IL-10- values. (Izuddin et al., 2019b). The combination of postbiotic (RG14) and prebiotic (inulin) increased IL-6 (interleukin-6) mRNA expression; interferon (IFN) and TNF-α in birds (Kareem *et al*., 2016a). Short-chain fatty acids (SCFA) increased IGF-1 secretion in adipose tissue and liver of mice (Nishitsuji *et al*., 2017).

**(4) Modulation of systemic metabolic responses**

Bile salt hydrolase (BSH) is responsible for bile acid deconjugation, which enables further microbial biotransformation, diversifies the circulating pool of bile acids (Long *et al*., 2017) and affects various metabolic processes in the host, which mainly include glucose, lipid and energy metabolism (Long *et al*., 2017). Succinate, responsible for intestinal gluconeogenesis, was found to improve glycemic control in mice (Wader *et al*., 2016). Propionate can improve insulin sensitivity and glucose tolerance and alter lipid metabolism (Wolever *et al*., 1996). “Butyrate can regulate the antioxidant glutathione and favorably influence oxidative stress in the colon of healthy individual” (Hamer *et al*., 2009).

**(5) Systemic signalling through the nervous system**

**“S**erotonin, dopamine, acetylcholine and GABA, and various compounds that can bind to receptors expressed in the brain (for example, indoles and bile acids). Microbial enzymes can also metabolize various precursors in the feed to synthesise neurotransmitters in the host, such as tryptophan (serotonin) and tyrosine (dopamine)”. (Mahony *et al*., 2015). SCFAs can stimulate enterochromaffin cells to produce serotonin (Iwasaki *et al*., 2019). “SCFAs modulate feeding behavior by stimulating the release of anorexigenic hormones such as glucagon-like peptide 1 and peptide YY” (Chamber *et al*., 2015). In mice, acetate has been shown to enter the brain and regulate appetite through a central metabolic mechanism (Frost *et al*., 2014).

**(6) Detoxification mechanism –**

“Postbiotics can also bind toxic substances. Cell walls can bind to B[a]P” (Shoukat et al., 2019). “The potential detoxification mechanism of *Bifidobacterium* is related to the presence of peptidoglycans and polysaccharides in cell walls. The incubation time and integrity of cell walls affect its ability to detoxify toxins” [Pei-Ren et al., 2002]. “*Bacillus licheniformis* CK1 may degrade ZEN in Lactobacillus broth by producing extracellular chitinase, cellulase, and protease enzymes” [Yi et al., 2011]. Oleic acid promotes Cd excretion by increasing the abundance of *Burkholderia* [Fang et al., 2022]. Postbiotics can have antioxidant properties and scavenge free radicals. Postbiotic supplementation of RI11 in broilers improved antioxidant capacity, catalase and glutathione activity (Humam *et al*., 2020). 0.9% *L. plantarum* RG14 postbiotics affected the activities of glutathione peroxidase and thiobarbituric acid-reactive substances in the blood serum of post-weaning lambs (Izuddin *et al*., 2020).

Oxidative stress, caused by an imbalance between the production of free radicals and the body's antioxidant defenses, can contribute to the accumulation of toxins and cellular damage. By reducing oxidative stress, postbiotics may support the body's natural detoxification mechanisms. Additionally, detoxification is a complex process involving multiple organs and systems in the body, and postbiotics should be seen as a complementary approach rather than a sole solution for detoxification purposes.

**POTENTIAL USE IN LIVESTOCK AND POULTRY**

“Furthermore, postbiotic preparations of *Lactobacillus plantarum* can enhance the antioxidant activity of serum and rumen in weaned lambs, reduce serum lipid peroxidation, and improve growth performance, nutrient utilization efficiency, immune status, and gut health” (Izuddin et al., 2022). “The CFS of *L. acidophilus*, *L. plantarum*, *L. rhamnosus*, and *L. reuteri* can inhibit epithelial cell infection in the vagina and prevent vaginal infection in cows” [Spaggiari et al., 2022). “The addition of postbiotics containing *Lactobacillus plantarum* RG14 increased the number of cellulolytic bacteria (*Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*) in goats, thereby increasing rumen fermentation, improving digestibility, and VFA production” (Izuddin et al., 2018). “Postbiotics can help reduce the environmental impact of ruminant production. For example, certain probiotics can reduce methane production by modulating the rumen microbial population and fermentation patterns. This can contribute to mitigating greenhouse gas emissions associated with ruminant agriculture. The supplementation of lactating goats with postbiotic products from *S. cerevisiae* fermentation improved fiber digestibility and ruminal propionate, thereby enhancing the energy efficiency of milk production and reducing CH4 emissions” (Fernandez et al., 2023).

**Growth:** Thanh *et al*. (2009), investigated the effect of different postbiotic combination on growth performance of broilers and reported that it increased body weight, weight gain, average daily gain and best feed conversion ratio compared to negative control (fed only basic diet). In a research conducted in lambs, 0.9% of *L. plantarum* RG14 postbiotics was provided, increase in nutrient intake and feed digestibility was noted which might have resulted in the better growth performance in the treatment groups. It was found that the addition of postbiotics in the diet improved nutrient digestibility of DM, CP and NDF (Izuddin *et al*., 2019).However, Danladi *et al*. (2022) reported no significant difference in the overall growth performance in terms of the broiler chickens’ final body weight, cumulative weight gain, cumulative feed intake, and feed conversion ratio across all the groups.

**Milk production:** Postbiotic yeast fermentation product supplementation to lactating goats increases the efficiency of milk production by enhancing fiber digestibility and ruminal propionate, and reduces energy losses in methane (Fernández 2023)

**Meat quality:** *Lactobacillus plantarum* strains (RI11, RS5 and UL4) postbiotics increase breast meat pH but reduce shear strength, lightness, drop loss, ripening loss and yellowing in heat-stressed broilers (Humam *et al*., 2020). A combination of postbiotics and inulin reduced drip loss and improved breast muscle pallor in broilers (Kareem *et al*., 2015). Avoiding a drop in pH can improve results, improve antioxidant activity and lower corticoid hormone levels (Sato *et al*., 2010; Hao and Gu 2014; Zaboli *et al*., 2019).

**Methane mitigation**: Postbiotics have the potential to lessen the environmental impact of ruminant farming. For instance, specific probiotics can decrease methane emissions by altering the microbial population and fermentation processes in the rumen. This can help reduce the greenhouse gas emissions linked to ruminant agriculture. Supplementing lactating goats with postbiotics derived from *S. cerevisiae* fermentation enhanced fiber digestibility and ruminal propionate levels, improving the energy efficiency of milk production while also reducing CH4 emissions (Fernandez et al., 2023).

**ADVANTAGES OF POSTBIOTICS OVER PROBIOTICS**

The advantages of postbiotics compared to probiotics are that there is no risk of bacterial transfer from the intestinal lumen to the blood, availability in pure form, ease of preparation and storage, availability of the production process on an industrial scale, specific information about the mechanism. of action, and they are more likely to trigger only targeted responses through specific ligand-receptor interactions. In addition, the results of a recent literature review by Pique et al. (2019) emphasize that postbiotics have several pharmacodynamic properties compared to live bacteria, as listed below:

• Susceptible and immunocompromised patients are not at risk of bacteria being transferred from the intestinal lumen to the blood

• There are less chances of acquiring and transferring antibiotic resistance genes

• More natural to separate, standardize, transport and store

• Loss of vitality due to cell decay can produce additional beneficial effects

• Enhanced interaction of each molecule released by damaged cells with epithelial cells more directly

 From an economic standpoint, the benefits of postbiotics include longer shelf life, easier storage, transport, and a reduced need to maintain a low temperature in comparison to probiotics. The use of a repetitive production process and the possibility of more precise quantitative control (except for BLs) are additional advantages of postbiotics compared to probiotics.

The undoubted advantage of postbiotics is bypassing the problem of acquiring antibiotic resistance genes and virulence factors, which may occur in vivo when probiotics are used (Imperial and Ibana, 2016). Postbiotics eliminate the need for exposure to live microorganisms, which is particularly important in young animals with an immature immune system and a leaky intestinal barrier.

**POSTBIOTIC PROBIOTIC**

**Figure 7. Pre biotic and post biotic**

**SAFETY ISSUES OF POSTBIOTICS**

Postbiotics have a clear chemical structure, safe dosage parameters and a long shelf life, and are considered a safe alternative to live probiotic microbes, but maintaining their biological activity and stability is essential. The safety profile of a stem cell microorganism alone cannot be assumed to be safe. For example, lipopolysaccharides from Gram-negative bacteria can cause sepsis and toxic shock (Opal, 2010). Therefore, the safety of any proposed postbiotic use must be assessed prior to use. Most studies also could not determine a specific dose of postbiotics to ensure beneficial effects of probiotics on 109 living cells. Also, different environmental factors can produce different metabolites by the same probiotics, which need to be further discussed and analyzed in postbiotic functional studies (Liu et al., 2023).

**CONCLUSION**

Postbiotics have the same mechanism of action and potency as probiotics because they contain probiotic-derived secondary metabolites that do not contain living cells. Postbiotics are more effective than probiotics alone, reducing signs of infection faster and supporting a healthy immune system. Production of postbiotics in the laboratory: cultivation, inactivation, collection and concentration of microbes. Microencapsulation, immobilization and lamination can be performed to increase the effectiveness of postbiotics. Postbiotics positively affect growth and host health by modulating the living microbiota, improving epithelial barrier functions, modulating local and systemic immune responses, as well as systemic metabolic responses and systemic signaling through the nervous system. A safety assessment of any postbiotic use is required prior to use. More studies are needed to confirm the specific dose of postbiotic to ensure a beneficial effect.

**Disclaimer (Artificial intelligence)**

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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