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Enzyme-Assisted Extraction of Prebiotic Oligosaccharides from Pomegranate Peel and Their Role in Poultry Gut Health

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Abstract: The poultry industry has traditionally relied on antibiotic growth promoters to improve feed efficiency and productivity, but concerns regarding antimicrobial resistance and food safety have encouraged the use of natural alternatives. Gut health plays a crucial role in poultry performance, with intestinal microbiota influencing digestion, immunity, and disease resistance. Prebiotics have emerged as effective non-antibiotic feed additives due to their ability to selectively stimulate beneficial gut microorganisms. Fruit processing by-products are gaining attention as sustainable sources of prebiotic oligosaccharides, particularly pomegranate peel, which is rich in pectin and dietary fiber. Enzyme-assisted extraction offers an efficient and eco-friendly approach for converting these polysaccharides into bioactive oligosaccharides. These compounds enhance gut microbial balance and short-chain fatty acid production, thereby supporting intestinal health and promoting antibiotic-free poultry production systems.

Keywords **-** Prebiotics, Poultry nutrition, Gut microbiota, pomegranate peel, enzyme-assisted extraction.

Introduction

The rapid expansion of the global poultry industry has been driven by increasing consumer demand for economical and protein-rich animal products. To sustain high productivity under intensive production systems, antibiotics were historically incorporated into poultry feeds as growth promoters. These compounds improved feed efficiency and reduced disease incidence, contributing to significant gains in production performance. However, the long-term and widespread use of antibiotic growth promoters has raised serious concerns regarding antimicrobial resistance, which threatens both animal and human health (*Castanon, 2007; Dibner & Richards, 2005*).

Global assessments have revealed a substantial rise in antimicrobial usage in food animals, particularly in developing and intensive production regions. This trend has been strongly associated with the emergence and dissemination of resistant bacterial strains, which can be transmitted through the food chain and the environment (*Van Boeckel et al., 2015*). As a result, regulatory authorities in many regions have imposed strict limitations or complete bans on the use of antibiotics as growth promoters in poultry production. These policy changes, combined with growing consumer preference for antibiotic-free animal products, have intensified research efforts to identify sustainable alternatives.

Modern poultry nutrition strategies increasingly emphasize gut health management as a foundation for productivity and disease resistance. The gastrointestinal tract is not merely a site for nutrient digestion but also a complex biological system where host–microbe interactions strongly influence growth performance and immune function. Disruption of gut microbial balance can compromise nutrient absorption and increase susceptibility to infections, leading to economic losses (*Choct, 2009; Kogut & Arsenault, 2016*). Consequently, nutritional approaches that support a stable and beneficial gut microbiota are now considered essential.Among the available alternatives, prebiotics have gained prominence due to their targeted mode of action. Unlike antibiotics, prebiotics selectively promote beneficial microbial populations without inducing resistance. Recent attention has focused on plant-derived prebiotics, particularly those obtained from agro-industrial by-products, which offer both functional and environmental advantages (*Gibson et al., 2017; Gadde et al., 2017*). Within this context, pomegranate peel has emerged as a promising substrate for prebiotic oligosaccharide production.

2. Poultry Gut Health and the Role of Microbiota

The poultry gastrointestinal tract harbors a diverse and dynamic microbial community that plays a central role in digestion, metabolism, immune development, and protection against pathogens. The composition of the gut microbiota changes with age, diet, and environmental conditions, reflecting a complex succession process that influences bird performance (*Oakley et al., 2014*). Beneficial microorganisms contribute to the fermentation of undigested feed components, generating metabolites that support intestinal health.

Short-chain fatty acids produced during microbial fermentation, including acetate, propionate, and butyrate, are particularly important for maintaining gut integrity. These metabolites serve as energy sources for intestinal epithelial cells, enhance villus development, and strengthen the intestinal barrier, thereby reducing pathogen colonization (*Stanley et al., 2014; Apajalahti & Vienola, 2016*). In addition, SCFAs modulate immune responses by regulating inflammatory pathways and promoting immune tolerance.

Imbalances in gut microbial populations, often referred to as dysbiosis, can negatively affect nutrient utilization and growth performance. Dysbiosis has been linked to increased susceptibility to enteric diseases and impaired feed efficiency in poultry (*Pan & Yu, 2014*). Therefore, maintaining microbial homeostasis is critical for achieving optimal productivity in antibiotic-free production systems. Nutritional modulation of the microbiota has emerged as an effective strategy to enhance gut health and resilience.

Dietary interventions such as prebiotics support beneficial bacteria including Lactobacillus, Bifidobacterium, and Streptococcus species, while indirectly limiting pathogen growth through competitive exclusion and metabolic interactions (*Clavijo & Flórez, 2018; Kogut, 2019*). By fostering a favorable microbial environment, prebiotics contribute to improved nutrient absorption, immune competence, and overall flock health.

3. Prebiotics as Natural Alternatives to Antibiotic Growth Promoters

Prebiotics are defined as non-digestible dietary components that selectively stimulate the growth and activity of beneficial microorganisms in the gastrointestinal tract, conferring health benefits to the host (*Gibson et al., 2017*). Unlike antibiotics, which exert broad-spectrum antimicrobial effects, prebiotics function by nourishing specific microbial groups that positively influence gut ecology. This targeted mechanism reduces the risk of resistance development and preserves microbial diversity.

In poultry nutrition, the inclusion of prebiotics has been associated with enhanced feed utilization, improved immune responses, and increased resistance to enteric pathogens. Fermentation of prebiotics by gut microbiota leads to the production of SCFAs, which lower intestinal pH and create unfavorable conditions for pathogenic bacteria (*Pourabedin & Zhao, 2015; Ricke et al., 2020*). These changes contribute to improved intestinal morphology, including increased villus height and enhanced absorptive capacity.Prebiotics also interact with the immune system by modulating cytokine production and enhancing mucosal immunity. Such effects are particularly important in intensive production systems, where birds are exposed to multiple stressors that compromise immune function (*Patterson & Burkholder, 2003; Bederska-Łojewska et al., 2018*). By supporting beneficial microbial populations, prebiotics indirectly strengthen host defense mechanisms.

Given these advantages, prebiotics have gained recognition as effective natural alternatives to antibiotic growth promoters. Their integration into poultry feeding programs aligns with global efforts to reduce antimicrobial use while maintaining productivity and animal welfare (*Markowiak & Śliżewska, 2018)*.

4. Fruit By-Products as Sustainable Sources of Prebiotics

The rapid expansion of food and beverage processing industries has led to the generation of large quantities of fruit processing residues, including peels, pomace, and seeds. These by-products are often underutilized or discarded despite being rich in dietary fiber and complex carbohydrates. Valorization of such materials offers an opportunity to reduce environmental waste while creating value-added functional ingredients (*Mirabella et al., 2014*).

Fruit by-products contain substantial amounts of polysaccharides such as pectin, cellulose, and hemicellulose, which can be converted into functional oligosaccharides. Citrus, apple, banana, mango, and pomegranate peels have been extensively studied for their prebiotic potential (*Sharma et al., 2017; Gullón et al., 2013*). These substrates are attractive due to their abundance, low cost, and renewable nature.Oligosaccharides derived from fruit residues have demonstrated the ability to selectively stimulate beneficial gut bacteria and improve intestinal health. Their effectiveness, however, is strongly influenced by extraction efficiency and processing conditions (*Gullón et al., 2016*). Conventional extraction methods often involve harsh chemical or thermal

treatments that can degrade bioactive compounds and reduce functional properties.

Advanced processing approaches are therefore essential to fully exploit the prebiotic potential of fruit by-products. Sustainable extraction strategies not only enhance product functionality but also support circular bioeconomy principles by transforming waste into valuable feed ingredients (*Mussatto & Teixeira, 2010*).

5. Pomegranate Peel as a Functional Prebiotic Substrate

Pomegranate peel constitutes a significant proportion of the fruit and is generated in large volumes during juice and food processing. Chemically, the peel is rich in pectin, cellulose, hemicellulose, dietary fiber, and phenolic compounds, making it a promising source of functional ingredients (*Viuda-Martos et al., 2010; Akhtar et al., 2015*). Among these components, pectin is particularly suitable for enzymatic conversion into bioactive oligosaccharides.

Studies have shown that polysaccharides and oligosaccharides derived from pomegranate peel can support beneficial gut microbiota and enhance intestinal health. The presence of phenolic compounds may further contribute to antimicrobial and antioxidant effects, potentially enhancing functional outcomes through synergistic interactions (*Singh et al., 2018*). These properties make pomegranate peel an attractive candidate for prebiotic production in poultry nutrition.

Despite its potential, pomegranate peel remains underutilized due to challenges associated with extraction efficiency and processing feasibility. Conventional methods often result in low oligosaccharide yield and inconsistent product quality. Addressing these limitations is essential to transform pomegranate peel into a reliable and industry-ready prebiotic ingredient.

6. Enzyme-Assisted Extraction and Microbiota Modulation

Enzyme-assisted extraction has emerged as an efficient and environmentally sustainable method for producing prebiotic oligosaccharides from plant-derived materials. This approach employs specific enzymes, such as pectinase and cellulase, to selectively hydrolyze complex polysaccharides into shorter, bioactive chains under mild conditions (*Pinelo et al., 2008; Ranveer et al., 2021*). Compared with chemical extraction, enzymatic methods reduce energy consumption and preserve functional integrity.

In pomegranate peel, enzyme-assisted hydrolysis enhances the release of pectic oligosaccharides from the cell wall matrix, resulting in improved yield and biological activity. These oligosaccharides resist digestion in the upper gastrointestinal tract and are selectively fermented by beneficial gut bacteria in the lower intestine (*Adeola & Cowieson, 2011*). This fermentation process supports the growth of *Lactobacillus, Bifidobacterium*, and *Streptococcus species*.

Microbiota modulation through enzyme-extracted prebiotics leads to increased SCFA production, reduced intestinal pH, enhanced epithelial health, and improved immune responsiveness. Such effects are closely associated with improved nutrient utilization, growth performance, and disease resistance in poultry (*Choct, 2009; Kogut, 2019*). Fruit-derived prebiotics may further support commensal species such as *Streptococcus salivarius*, contributing to a stable and resilient gut ecosystem.

7. Applications in Poultry Nutrition and Feed Industry

The conversion of pomegranate peel into value-added prebiotic oligosaccharides (POS) through enzyme-assisted extraction offers considerable potential in modern poultry nutrition. With increasing restrictions on antibiotic growth promoters (AGPs), the poultry sector is actively exploring natural feed-based strategies to enhance gut health and productivity. In this context, plant-derived prebiotics obtained from agro-industrial by-products represent a sustainable and scientifically supported alternative.

7.1 Application as a Functional Feed Additive

Prebiotic oligosaccharides extracted from pomegranate peel can be incorporated into broiler rations at controlled inclusion levels, generally between 0.5 and 1% of total feed weight. These oligosaccharides are resistant to enzymatic digestion in the upper gastrointestinal tract and reach the intestine intact, where they selectively serve as substrates for beneficial microorganisms, including Streptococcus salivarius and Lactobacillus spp. Their fermentation promotes a favorable microbial balance, contributing to improved intestinal stability and overall gut health.

7.2 Stimulation of Short-Chain Fatty Acid Production

Selective microbial fermentation of POS leads to the formation of short-chain fatty acids (SCFAs), particularly acetate and butyrate. These metabolites play essential physiological roles, including maintenance of epithelial integrity, modulation of intestinal pH, and suppression of pathogenic bacterial growth.

Enhanced SCFA production is closely associated with improved nutrient utilization efficiency and better feed conversion performance in broiler chickens.

7.3 Replacement Strategy for Antibiotic Growth Promoters

The gradual elimination of AGPs from poultry production systems has intensified the need for safe and effective alternatives. POS supplementation supports immune regulation, strengthens intestinal barrier function, and limits colonization by harmful enteric pathogens. Through these mechanisms, prebiotic supplementation contributes to improved flock health and productivity while minimizing reliance on synthetic growth-enhancing agents.

7.4 Synbiotic Feed Formulations

An important advancement in functional poultry nutrition is the development of synbiotic systems, which combine prebiotics with compatible probiotic strains. Incorporating POS alongside probiotic organisms such as Streptococcus salivarius may enhance microbial colonization and metabolic activity within the gut. This synergistic interaction can further improve intestinal function and growth performance compared to individual supplementation strategies.

7.5 Agro-Waste Utilization and Circular Bioeconomy

The use of pomegranate peel, an abundant fruit-processing by-product, aligns with principles of sustainable resource management and circular bioeconomy. Transforming agricultural residues into high-value functional feed ingredients reduces environmental waste while generating economic opportunities. Such valorization strategies contribute to environmentally responsible livestock production systems.

7.6 Prospects for Industrial Application

Enzyme-assisted hydrolysis employing pectinase and cellulase is adaptable to scalable processing conditions and compatible with established feed manufacturing technologies. The extracted POS can be stabilized through drying or encapsulation processes to produce a shelf-stable powder suitable for commercial feed blending. This scalability enhances the feasibility of translating laboratory findings into practical poultry nutrition applications.

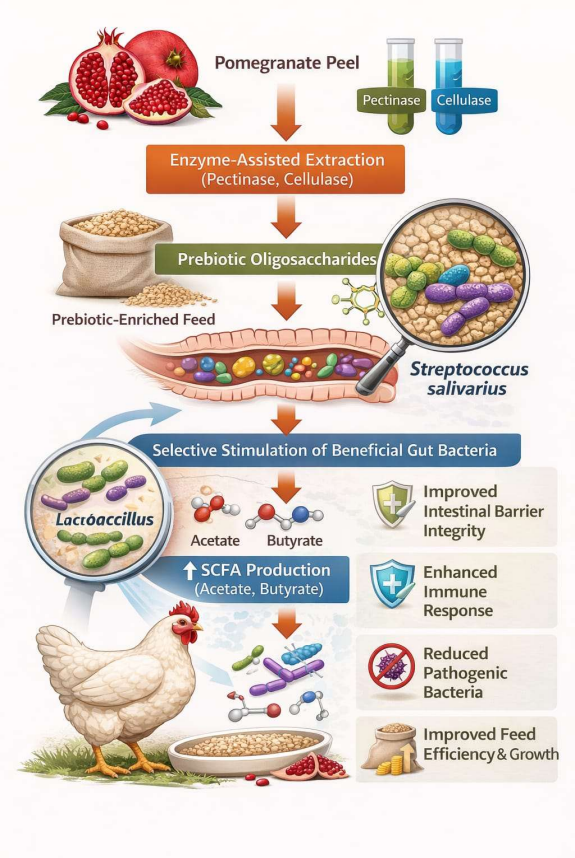

Fig. 1 shows the enzymatic valorization of pomegranate peel into prebiotic oligosaccharides and their role in enhancing gut health and feed efficiency in broilers

8. Conclusion

Prebiotic oligosaccharides derived from fruit by-products represent a sustainable and promising alternative to antibiotic growth promoters in poultry nutrition. This review highlights the potential of pomegranate peel as a valuable source of functional polysaccharides that can be efficiently converted into bioactive prebiotics through enzyme-assisted extraction. Compared with conventional processing methods, enzymatic approaches enhance yield, preserve biological activity, and align with environmentally responsible production practices.

The ability of enzyme-extracted oligosaccharides to modulate gut microbiota, stimulate short-chain fatty acid production, and support beneficial microorganisms underscores their importance in improving poultry gut health and performance. Although challenges related to raw material variability, process optimization, and long-term biological validation remain, continued research is expected to advance their practical application. Overall, enzyme-assisted valorization of

pomegranate peel offers a viable strategy for promoting antibiotic-free poultry production while supporting circular bioeconomy principles

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