Veterinary Integrative Sciences 2027; 25(1): e2027001-1-33. DOI: 10.12982/VIS.2027.001

Veterinary Integrative Sciences
ISSN; 2629-9968 (online)



Review article

Encapsulated defensins as antimicrobial alternatives for poultry: A review

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Abstract

Foodborne zoonotic infections are primarily caused by *Salmonella enterica* and *Clostridium perfringens*. Encapsulated defensins offer a promising strategy for controlling both pathogens through their antibacterial and immunomodulatory properties. This review evaluates the strategies for delivering encapsulated defensins and their effectiveness in controlling both pathogens. Relevant studies were identified through systematic searches of the PubMed, Scopus, and Web of Science databases, covering publications from the last 15 years. Studies that evaluate the functional performance of defensin encapsulation systems were considered eligible, while those that do not provide relevant functional outcomes or sufficient methodological information were excluded. A total of 35 articles meets the eligibility criteria and constitute the primary evidence base for this review. Encapsulation improves defensin stability under acidic, enzymatic, and thermal conditions when enabling targeted release in the lower intestine, thereby enhancing antimicrobial efficacy, strengthening intestinal barrier function, and promoting beneficial gut microbiota. Polysaccharide-based carriers, including alginate, chitosan, and pectin, further support microbial homeostasis, immune responses, and reproductive performance through the production of short-chain fatty acids. Overall, encapsulated defensins represent a promising and practical strategy for pathogen control and immune enhancement in poultry production.

Keywords: Antimicrobial peptides, Antimicrobial resistance, Encapsulation, Gut microbiota, Immune modulation

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Funding; *The research received funding from the Faculty of Animal Science, Diponegoro University, under the 2025-2026 fiscal year.*

Article history; *received manuscript: 8 May 2026,*
revised manuscript: 8 August 2026,
accepted manuscript: 11 August 2026,
published online: 25 August 2026,
Academic editor; *Promporn Piboon*

INTRODUCTION

Global demand for poultry meat and eggs has increased due to population growth and changing dietary preferences. Consequently, intensive broiler and layer production systems have expanded, and many producers adopting antibiotic growth promoters (AGPs) as a primary strategy to improve production efficiency (Mehdi et al., 2018; Sholikin et al., 2021). The AGPs reduced the colonization of pathogens such as *Salmonella enterica* and *Clostridium perfringens*; however, their prolonged use in animal feed disrupted gut microbiota composition and accelerated the development of antimicrobial resistance (Zhu et al., 2021).

Many countries have implemented stringent regulations on antibiotic use in animal production. At the same time, the poultry industry faces increasing pressure from growing consumer demand for antibiotic-free poultry products. These circumstances have accelerated the search for safer, and more effective, and more sustainable alternatives to conventional AGP (Wibawati et al., 2023). Several alternatives have emerged, including probiotics, prebiotics, synbiotics, phytogenics, organic acids, enzymes, antimicrobial peptides (AMPs), and bacteriophages (Rahman et al., 2022). Among them, defensins have attracted considerable attention because of their broad-spectrum antimicrobial activity and immunomodulatory properties, which can support intestinal health, enhance immune function, and improve overall poultry performance (Adli et al., 2025; Sugiharto et al., 2025).

Alternatives to AGPs originate from diverse sources, including medical plants, microbial products, and animal-derived bioactive compounds. Evidence from several meta-analysis studies demonstrates that these alternative effectively improve poultry health and production performance. Dietary supplementation with 22.6–27.6 g/kg clay minerals improves mucosal immunity, growth performance, and feed efficiency in broilers (Sholikin et al., 2023). Likewise, fermented herbal supplementation at approximately 26.3 g/kg increases body weight gain and average daily gain while improving the feed conversion ratio (Adli et al., 2024). The same meta-analysis also reports improvements in villus height, crypt depth, and the villus height-to-crypt depth ratio, indicating enhanced intestinal development. Although emulsifiers differ mechanistically from conventional AGP alternatives, lecithin- and lysolecithin-based formulations also improve feed efficiency (Priyatno et al., 2025). In addition, oligosaccharide-based prebiotics enhance poultry performance by improving protein digestibility (Adli et al., 2023a). Beneficial effects of probiotics have also been documented in broiler finisher and breeder chickens, particularly following supplementation with lactic acid bacteria and yeast (Adli et al., 2023b, 2025). Collectively, findings from multiple meta-analyses demonstrate that alternative feed additives with diverse mechanisms of action consistently improve poultry health and production performance.

The efficacy of defensins in maintaining gastrointestinal health in poultry is strongly influenced by feed processing conditions and the physiological environment of the gastrointestinal tract. Thermal exposure during feed processing, fluctuations in gastrointestinal pH, and proteolytic enzyme activity can reduce peptide stability and compromise biological activity (Xu and Lu, 2020; Ko et al., 2021). Therefore, the development of effective encapsulation and targeted delivery systems is essential to preserve defensin stability, protect bioavailability, and ensure controlled release throughout the gastrointestinal tract (Hong et al., 2012; Jiang et al., 2019).

Defensin exhibit broad-spectrum antimicrobial activity against bacteria, fungi, and viruses, making it a promising feed additives for sustainable poultry production (Li et al., 2025). Compared with conventional antibiotics, including avilamycin, bacitracin, chloramphenicol, oxytetracycline, and tetracycline, defensins have a substantially lower potential to promote antimicrobial resistance (Nazeer et al., 2021; Zhao et al., 2022; Alabi et al., 2024). Based on the arrangement of conserved cysteine residues, defensins are classified into three major families:

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α-, β-, and θ-defensins (Figure 1; Fazly Bazzaz et al., 2021). Each family possesses a distinct disulfide bond formation and a unique three-dimensional structural, which contributes to differences in biological functions (Kumaresan et al., 2024). Among them, θ-defensins are distinguished by a rare cyclic structure that set them apart from the α- and β-defensin families (Negahdaripour et al., 2022).

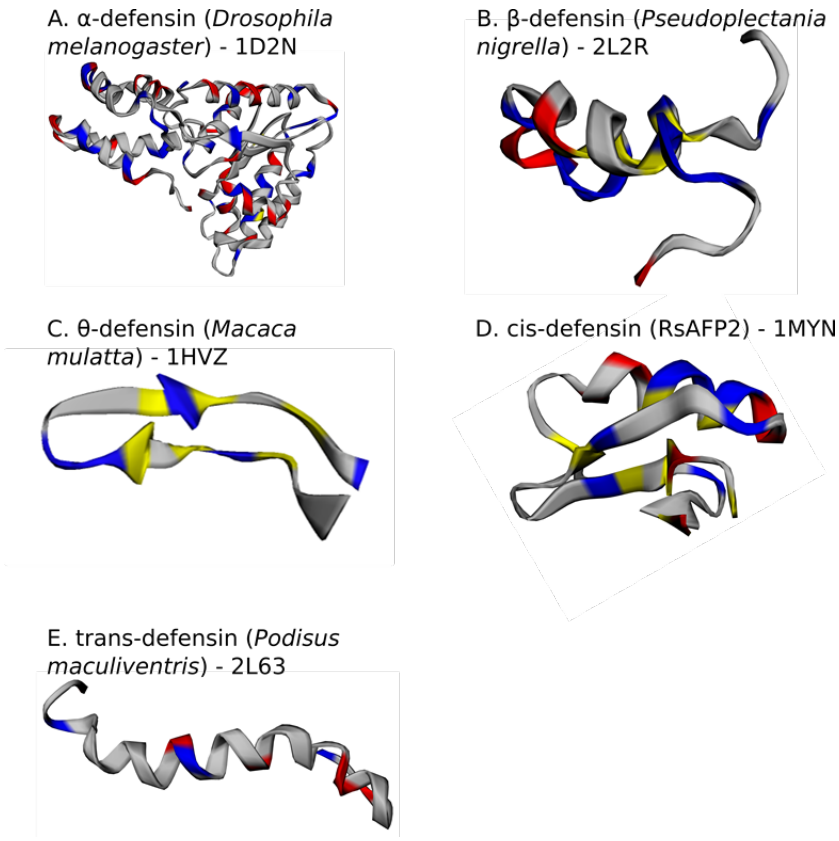


Figure 1 Peptide structures of several defensin types. A) α-defensins (*Drosophila melanogaster*; 1D2N), B) β-defensins (*Pseudoplectania nigrella*; 2L2R), C) θ-defensins (*Macaca mulatta*; 1HVZ), D) Cis-defensins (RsAFP2; 1MYN), and E) Trans-defensins (*Podisus maculiventris*; 2L63). Blue color positively charged amino acids (arginine, lysine, and histidine); red indicates negatively charged amino acids (aspartic acid and glutamic acid); and yellow highlights cysteine residues. The illustration was produced using Inkscape (Version 1.1.1) and r3dmol (Version 0.1.2).

Plectasin, a member of the defensin family, retains potent antibacterial activity under the acidic conditions of the gastrointestinal tract and demonstrates high efficiency when administered through poultry diets (Zhang et al., 2021a). Beyond its direct antimicrobial activity, defensins modulate the composition of the intestinal microbiota by increasing the abundance of beneficial bacteria, such as *Bacteroides*, while reducing colonization of enteric pathogens, including *C. perfringens*, *Escherichia coli*, and *S. enterica* (Nazeer et al., 2021). Such changes in the intestinal microbial community improve gut health, enhance nutrient utilization, and reduce the incidence of enteric infections (Zhao et al., 2022). Collectively, these beneficial effects may reduce reliance on antibiotics and help mitigate the development of antimicrobial resistance (Nazeer et al., 2021).

Current evidence supports the biological efficacy of defensins; however, maintaining their stability and functionality during feed processing and throughout gastrointestinal transit remains a major challenge for practical poultry applications. Defensins are highly susceptible to degradation under acidic conditions and by proteolytic enzymes in the poultry gastrointestinal tract. In addition, fluctuations in

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gastric pH, digestive enzyme activity, and thermal stress during feed processing can compromise peptide structural integrity, thereby reducing antimicrobial activity (Zhou et al., 2022). To address this limitation, micro- and nanoencapsulation strategies have been developed to protect defensins from premature degradation while enhancing their stability, bioavailability, and controlled released throughout the digestive tract (Zhang et al., 2019b). Such delivery systems provide an effective strategy for maximizing the functional efficiency of defensin in poultry nutrition (Zhang et al., 2019b). Although recent advances have substantially improved encapsulation technologies for bioactive compounds in poultry, current evidence remains fragmented, with most studies evaluating either formulation characteristics or biological efficacy rather than integrating both aspects (Alabi et al., 2024; Dityana et al., 2024). Consequently, a comprehensive review integrating defensin structure, encapsulation strategies, stability during feed processing and gastrointestinal digestion, controlled intestinal release, antimicrobial mechanisms, microbiota modulation, immune signaling, and poultry production performance has yet to be established (Thuy, 2024; Thuy and Tuu, 2026).

Encapsulation protects defensins from degradation in the upper gastrointestinal tract and enhances their bioavailability, thereby facilitating targeted delivery to the distal small intestine. Following release, defensins interact with the negatively charged membranes of pathogenic bacteria, including pore formation and membrane disruption that ultimately result in bacterial cell death while supporting microbiota homeostasis and innate immune function (Zhang and Wong, 2019; Fusco et al., 2022). Advanced encapsulation systems platforms further incorporate controlled, site-specific release mechanisms that minimize nonspecific interactions and potential toxicity while promoting stimulus-responsive release under the intestinal pH and enzymatic conditions of the poultry gastrointestinal tract (Ujilestari et al., 2023). Collectively, these features maximize the antimicrobial efficacy and biological activity of defensins, thereby improving gastrointestinal health and diseases resistance in poultry (Roque-Borda et al., 2021a; Ujilestari et al., 2024).

This review aims to evaluate the effectiveness of encapsulated defensins as multifunctional agents for controlling the colonization of *S. enterica* and *C. perfringens* in poultry production, with particular emphasis on structural protection during feed processing and gastrointestinal digestion as well as targeted release in the small intestine. In addition, this review systematically examines the roles of defensins in modulating the gut microbiota, activating immune signaling pathway, and improving reproductive performance. It also discusses the major limitations associated with gastrointestinal delivery and evaluates current micro- and nanoencapsulation technologies as strategies to enhance peptide stability, bioavailability, and functional efficiency for sustainable poultry production.

METHODOLOGY

Literature Sources and Search Approach

This article was prepared as a systematic review. A structured literature search was conducted between January 20 and February 8, 2025, to ensure that the review incorporated relevant and current scientific evidence. The search covered major indexed databases, including PubMed/MEDLINE, Scopus, and Web of Science, to identify publications that investigated encapsulation strategies, including microencapsulation and nanoencapsulation, for defensin against *C. perfringens* and *S. enterica*, or other pathogenic bacteria. Keywords: encapsulation, defensin, alginate, chitosan, pectin, cellulose, gelatin, Eudragit, lecithin, antimicrobial activity. The search terms were developed using the Population–Intervention–Comparison–Outcome (PICO) framework solely to facilitate the identification of relevant literature rather than to perform a formal systematic review (Cumpston et al., 2021; Sugiharto et al., 2025). The P =

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"antimicrobial peptide" OR defensins OR "bioactive peptide"; I = encapsulation OR microencapsulation OR nanoencapsulation OR alginate OR chitosan OR pectin OR cellulose OR gelatin OR Eudragit OR lecithin; C = "non-encapsulated defensins" OR "free defensins"; and O = "antimicrobial activity" OR "antibacterial activity" OR stability OR bioavailability. The review considered only only articles published between 2015 and 2025 to capture recent advances in encapsulation technologies and antimicrobial peptide research.

A publication period covering the past 15 years was applied to capture recent advances in encapsulation technology. The search identified 357 articles. Researchers evaluated the retrieved publications by reviewing the titles, abstracts, and full-texts to identify studies relevant to the scope of this systematic review. After screening of titles and abstracts, 97 articles met the eligibility criteria for for full-text evaluation.

Selection of Relevant Literature

Publications were selected according to their scientific relevance to the objectives of the review. Eligible studies evaluated encapsulation materials for antimicrobial peptides and reported at least one relevant outcome, including antimicrobial activity, pH tolerance, thermal stability, enzymatic protection, or short-chain fatty acid (SCFA) production. Studies were excluded when they did not align with the review objectives, failed to report outcome data, focused on non-encapsulated peptides, or represented duplicate or non-peer-reviewed publications.

The study selection process followed the PRISMA 2020 guidelines and is presented in the PRISMA flow diagram (Figure 2; Page et al., 2021). A total of 35 publications met the eligibility criteria and provided the evidence for comparative analysis presented in Table 1. Selected publications comprised eight studies on alginate, seven on chitosan, four on pectin, four on cellulose, five on gelatin, four on Eudragit, and three on lecithin.

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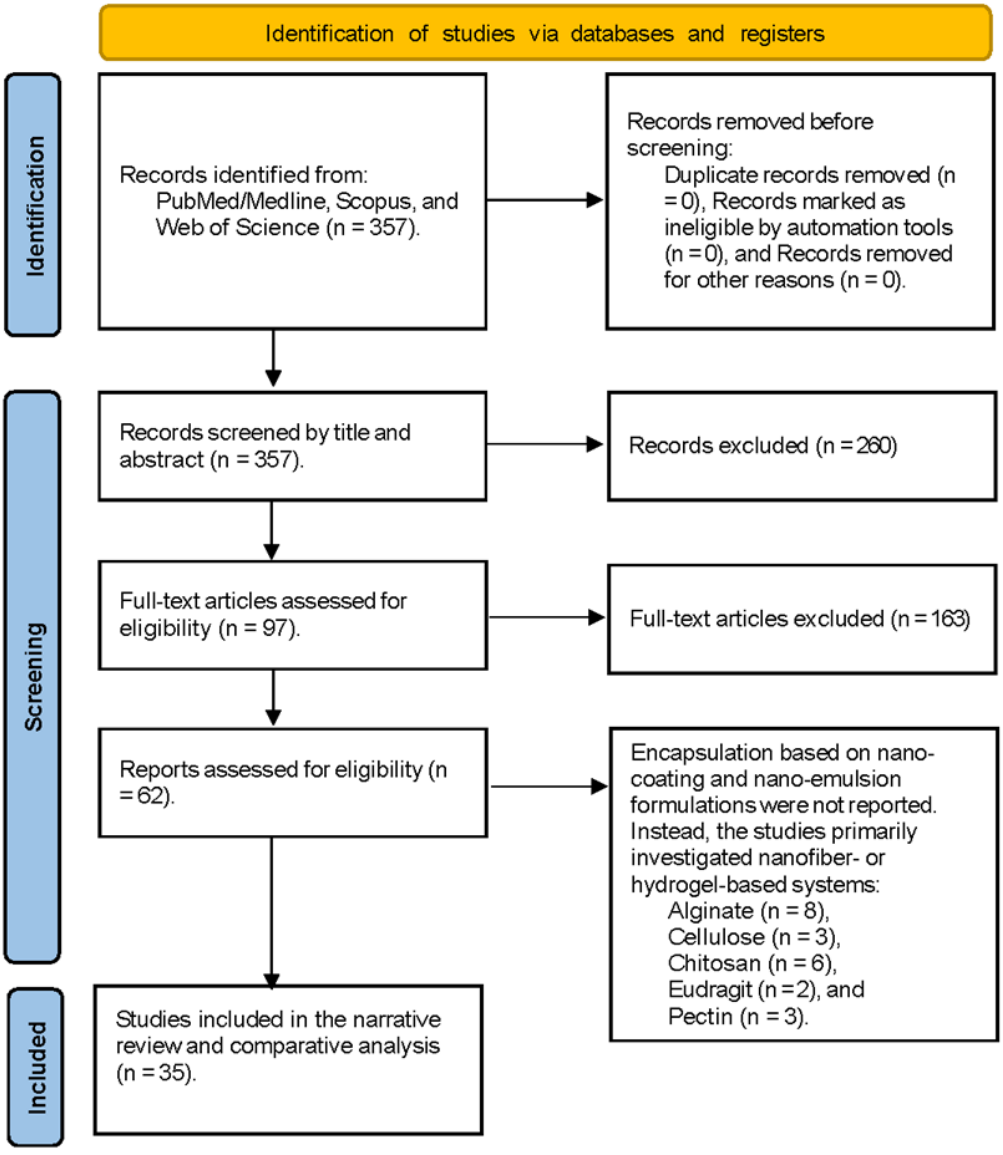


Figure 2 Flowchart of the article selection process for studies investigating coating materials used in defensin encapsulation.

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Table 1 Effective encapsulation materials for reducing *C. perfringens* and *S. enterica* in encapsulated defensins

Encapsulant	Key formulation	Resilience (pH and temperature)	Beneficial effects	Estimated limitations	Encapsulant antibacterial properties	AMP
Alginate	Pectin-alginate blend, alginate-Ca^{2+} spheres, alginate-chitosan microcapsules, alginate-κ-carrageenan, microcapsules film with nanoparticles, AOS, and alginate-inulin-chitosan microspheres	Stable in pH 1.5–3 and 70–121 ℃	Stable against pepsin and bile salts, stable during gastrointestinal transit, antipathogen effect (*S. enterica* Enteritidis, *Campylobacter jejuni*, *S. enterica* Typhimurium, *C. perfringens*), promotes the growth of LAB and bifidobacteria population, increases SCFA production (acetate, propionate, and butyrate)	Low mechanical strength, high porosity, sensitive to chelating agents, and unstable in alkaline condition	Alginate–chitosan microcapsules, alginate-inulin-chitosan microspheres, and AOS	Osmeceutical pentapeptide-4, human β-defensin 2, IbM1-M6, nisin, odorranain A, ε-polylysine, and polymyxins
Reference (Soto et al., 2018; Flórez-Castillo et al., 2019; Mizuno et al., 2020; Stokniene et al., 2020; Roque-Borda et al., 2021a; Osorio-Alvarado et al., 2022; Yaghoubi et al., 2024; Da Silva et al., 2025)						
Chitosan	Chitosan (crustacean and insect-derived; 85-95 deacetylation), microparticle chitosan, and COS (666-1,638 Da)	Stable at pH 4.5–5	Effective against pathogen (*E. coli*, *Listeria*, *S. enterica*, *C. perfringens*), increase SCFA production (acetate, propionate, and butyrate), and increase beneficial microbe (*Bacteroides*, *Roseburia*, *Prevotella*, *Phascolarctobacterium*)	Poor solubility at neutral and alkaline pH, exhibits physicochemical variability associated with the degree of deacetylation, and potential for inconsistency for batch-to-batch	Chitosan low molecular and COS	Ciprofloxacin, CysHHC10, octominin, and recombinant LL37
Reference (Pranantyo et al., 2018; Zhang et al., 2018; Ibañez-Peinado et al., 2020; Fahimirad et al., 2021; Huan et al., 2022; Jayathilaka et al., 2022; Rao et al., 2017)						
Pectin	Methyl-esterified POS, Crude pectin, citrus pectin (*Citrus unshiu* Marc.), and High-purity citrus pectin (>84% purity)	Stable at pH 2.5 and temperature 40–100 ℃	Suppresses pathogenic bacteria (*E. coli*, *S. aureus*, and *S. enterica*), also increase acetate, propionate, and butyrate production	Rapid dissolution, weak strength as single blend, highly variable structure, and weak in pectinases degradation	POS	Nisin
Reference (Mao et al., 2019; Zofou et al., 2019; Pachaleva et al., 2023; Wang et al., 2023)						
Cellulose	HPMC, Chitosan-HPMC blend, micro-fibrillated cellulose, and functionalized cellulose	Effective at pH <6 and stable at 70 ℃	Inhibits pathogens, including *Bacillus cereus, E. coli, S. aureus, Campylobacter*, and *Candida albican,* also resistant to cellulase, and enhances butyrate production	Lack of bioactivity, poor solubility, slower degradation properties, and high viscosity	Chitosan-HPMC blend	Ctx (Ile21) - Ha and nisin
Reference (Wu et al., 2019; Roque-Borda et al., 2021b; Khater et al., 2023; Uddin et al., 2023)						

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Table 1 Cont.

Encapsulant	Key formulation	Resilience (pH and temperature)	Beneficial effects	Estimated limitations	Encapsulant antibacterial properties	AMP
Gelatin	Gelatin (220 bloom), gelatin-hyaluronic acid blend, and gelatin-pullulan blend	pH stable >50 ℃, up to >100 ℃	Inhibit growth of *E. coli*, *S. enterica*, and *S. aureus*	Poor moisture barrier, thermal sensitive, and brittle (dry condition)	-	Cys-LL37, Lfcin β, LL37, MBP-1, mellitin, Pac-525, and RW-4
Reference (Abarca et al., 2022; Yang et al., 2022; Ji and Chang, 2025)						
Eudragit	Eudragit L, S, RS, and Eudragit-pullulan film	Resistant at pH 1.2–5.5 and stable at 70–330 ℃	Resistant to pepsin & pancreatin and inhibits pathogens (*E. coli*, *S. aureus*, *C. albicans*, *Porphyromonas gingivalis*)	Lack of bioactivity, undegradable, and regulatory issue	-	Alyteserin-1c and tyrothricin
Reference (Cantor et al., 2019; Kaffash et al., 2021; Mahadlek et al., 2021; Kielholz et al., 2022; Nie et al., 2024)						
Lecithin	Lecithin-montmorillonite and alginate-lecithin blend	pH 3–6.5 and stable at 110–242 ℃	Inhibit pathogen bacteria such as, *E. coli* and *S. enterica* Typhimurium	Susceptible to oxidation, produce off-flavors, low mechanical strength, and variable sources	-	MccJ25
Reference (Gomaa et al., 2017; Wang and Phillips, 2023; Socha et al., 2024)						

Abbreviation: AMP, antimicrobial peptide; AOS, alginate oligosaccharide; COS, chitosan oligosaccharide; HPMC, hydroxypropyl methylcellulose; POS, pectin oligosaccharide.

MECHANISMS OF INCREASING DEFENSINS STABILITY

The stability of defensins may improve, particularly passage through the gastrointestinal tract. Protective delivery systems have been developed to reduce degradation of active compounds, and encapsulation remains one of the most promising options (Figure 3). Encapsulation strategies aim to reduce defensin exposure to harsh gastric conditions, including low pH and proteolytic enzyme such as pepsin, rather than provide complete protection (Zong et al., 2016). A wide range of encapsulation technologies has been explored and researchers generally select each approach according to the physicochemical properties of defensins and the conditions encountered in the digestive tract (Cado et al., 2013).

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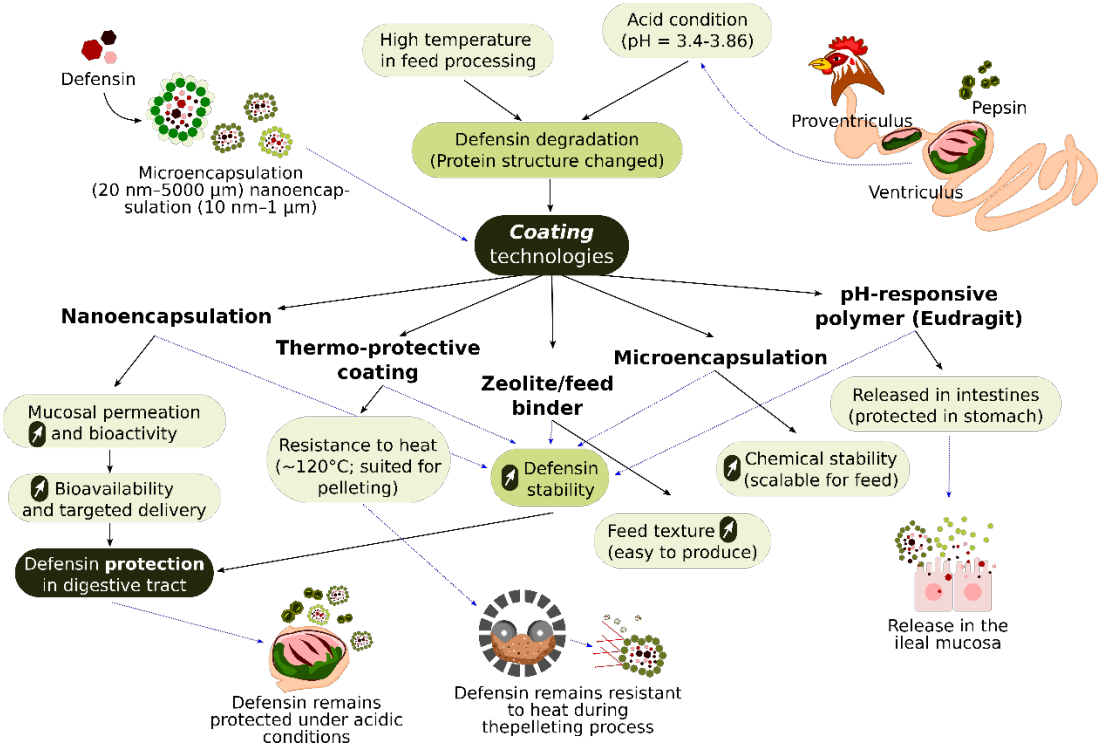


Figure 3 The use of encapsulation materials to reduce defensins degradation in the foregut intestine under to low pH. Alternatives protection strategies, including nanoencapsulation and thermo-protective coatings can enhance bioavailability, specific release, and heat resistance. The illustration was created using Inkscape (Version 1.1.1).

Microencapsulation

Microencapsulation maintains the biological properties of active compounds under acidic conditions. Notably, the AMP Ctx (Ile^{21})-Ha formulated with hydroxypropyl methylcellulose phthalate (HPMCP) and hydroxypropyl methylcellulose acetate succinate maintain bioavailability under simulated gastrointestinal tract conditions (Roque-Borda et al., 2021a). Enteric coating prevent premature peptide release in the acidic stomach environment, with HPMCP-coated microparticles showing less than 15% peptide release under gastrointestinal conditions (Roque-Borda et al., 2021a). Subsequently, the system successfully delivers more than 85% of the peptide payload for targeted release in the intestinal environment (Roque-Borda et al., 2021a, 2021b). The advantage is particularly relevant to defensins, which are susceptible to degradation in the harsh gastric conditions. Microencapsulation protects defensins against pH-induced damage and enzymatic degradation during digestion. The protective effect enhance stability and preserves defensins activity until the compounds reach the target site, such as the intestine. Supporting evidence demonstrates that this nisin microencapsulation using a W/O/W system with gum arabic as the primary encapsulating material increases mortality of *E. coli* and *S. aureus* (81.33% and 89.75%, respectively) while maintaining stability at pH 3.85 (Batool et al., 2025).

Microencapsulation provides several benefits, including improved oxidative stability, enhanced heat resistance, extended shelf-life, preservation of biological

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activity, controlled volatility, protection against chemical and physical degradation, and increased formulation efficiency of defensins (Bakry et al., 2016).

However, several studies identified key limitations, including variable encapsulation efficiency, risks of uncontrolled release, and insufficient *in vivo* for evaluating long-term efficacy and safety (Yan et al., 2022). Scientific evidence regarding consumer acceptance of derivative products and potential toxicity remains limited, requiring further investigation, particularly for defensin-based microencapsulation. In addition, several reports have emphasized the need for stricter handling and storage procedures, highlighted challenges in scaling up microencapsulation processes for industrial applications, and indicated that the materials required for microencapsulation remain relatively expensive (Calderón-Oliver and Ponce-Alquicira, 2022).

Nanoencapsulation

Furthermore, nanoencapsulation offers greater advantages than microencapsulation. Key benefits include improved stability of active compounds, enhanced bioavailability, prolonged shelf-life, deeper penetration into smaller pores, and more precise delivery of bioactive compounds (Figure 4). Previous study reported that chitosan nanoparticles encapsulating the LfcinB peptide (228 nm) remained stable under highly acid conditions (pH = 2.0; time = 8.5 h), as shown in Table 1 (Rao et al., 2017; Kulawik et al., 2023). Such findings suggest that nanoencapsulation provides a more effective strategy for protecting active defensins during passage through the upper digestive tract of poultry, where feed typically remains for approximately 90 min (Rodrigues and Choct, 2018).

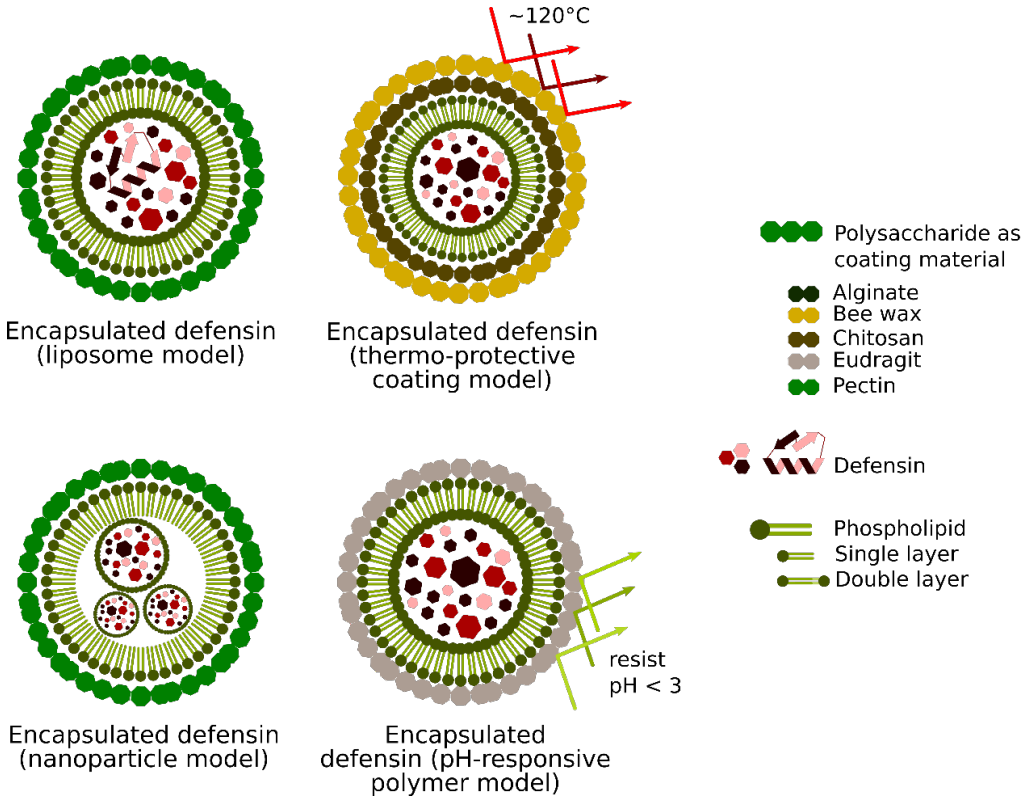


Figure 4 Protection mechanisms of defensins using encapsulation strategies, including liposomes, nanoparticles, thermos-protective coatings, and pH-responsive polymer matrices. Phospholipid-based and composite outer layers improve resistance to heat and extreme pH conditions. Alginate, beeswax, chitosan, Eudragit, and pectin enhance encapsulation stability. The illustration was created using Inkscape (Version 1.1.1).

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Nanoencapsulation and microencapsulation differ primarily in particle size, stability, bioavailability, diffusion rate, and technological complexity. Nanoencapsulation generally produces particle ranging from 50 to 500 nm, with an upper limit of 1,000 nm, whereas microencapsulation generate particles between 1 and 1,000 μm (Jeong et al., 2020). Smaller particle size enables nanoencapsulation to provide superior protection and stability for active compounds while improving resistance to storage-related stress. Microencapsulation generally offers lower protective efficiency under comparable conditions (Jeong et al., 2020; Roque-Borda et al., 2021b). Nanoencapsulation also improves bioavailability and absorption because smaller particles increase the surface area available for interaction with biological tissues. Moreover, nanoencapsulated materials diffuse more rapidly than microencapsulated counterparts (Betala et al., 2018). Despite such advantages, nanoencapsulation requires more sophisticated processing technologies and higher production costs (Lalarukh et al., 2025). Both encapsulation strategies remain effective delivery systems, and the optimal approach depends on the intended application and available resources.

Experimental evidence showed that nanoencapsulation defensins exerted strong inhibitory activity at concentrations ranging from 1.56 to 6.17 μg/mL (Marcelino-Pérez et al., 2021). Nanoencapsulated defensin particles have average size of approximately 140 nm and a pore diameter ranging from 4.8 to 5.4 nm (Marcelino-Pérez et al., 2021). Particle characteristics supported a defensin loading efficiency of up to 99% (Marcelino-Pérez et al., 2021). Evidence also demonstrated that encapsulated defensins resisted pepsin degradation and remained efficiently retained within the mesoporous matrix, thereby preserving peptide stability during gastrointestinal exposure (Rishi et al., 2015).

The main limitations of the nanoparticle-based encapsulation system included high production costs for practical applications, expensive processes required to generate uniform nano-sized particles, proteolytic instability *in vivo*, unresolved safety concerns, and the absence of official evaluations for nanoencapsulation drug products (Biswaro et al., 2018).

Thermo-Protective Coating

Extreme temperature conditions also require careful consideration and support the use of thermos-protective coatings to preserve bioactive compounds. The protective material used in this technique is resistant to the temperatures encountered during feed processing, where temperatures can reach 80–100 ℃ (Zhang et al., 2019b). Examples of thermo-protective agents include beeswax, carnauba wax, shellac, gelatin, hydrogenated palm oil, and maltodextrin, all of which have been investigated for enhancing the thermal stability of bioactive compounds during processing (Iñiguez-Moreno et al., 2021; Hosseini et al., 2023; Kumar et al., 2025). Heat-resistant materials simplify the poultry feed processing process in large-scale manufacturing industries. However, to date, there has been no application of thermo-protective coatings for bioactive peptides, particularly defensins. An example of a promising coating material is a coffee-beeswax composite, which has been shown to form a stable, intact layer even at 120 ℃ (Figure 4; Zhang et al., 2019b).

Limited compatibility between coating materials and bioactive compounds remains the primary limitation of thermos-protective coatings. Some coating materials may hinder defensin release and reduce bioavailability during gastrointestinal transfer, ultimately limiting biological efficacy (Stawarz et al., 2019). In addition, coating materials may hinder defensins release because the coating matrix tends to be rigid and difficult to degrade (Szczepaniak et al., 2021).

pH-Responsive Polymer and Feed Binder using Zeolite

Another encapsulation strategy involves low-pH-resistant materials such as Eudragit S100, which dissolves at pH 6.0. Eudragit S100 preserves the structural

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integrity of defensins during passage through the proventriculus and ventriculus, allowing targeted release in the small intestine (Figure 4; Zhang et al., 2019a; Abdollahy et al., 2024). An Eudragit S100-zeolite composite also improves thermal stability, making the formulation more compatible with conventional feed pelleting conditions while supporting consistent defensin delivery (Jablan and Jug, 2015; She et al., 2015). Despite those advantages, pH-responsive polymer systems combined with zeolites present several challenges. Polymer-based materials raise safety concerns because of limited biodegradability and the potential accumulation of polymer residues (Nikam et al., 2023). Zeolites also exhibit non-specific adsorption, which can decrease loading selectivity and reduce delivery efficiency (Sholikin et al., 2023; Ujilestari et al., 2025). Moreover, manufacturing procedures remain relatively complex, limiting large-scale production and increasing production costs (Sang et al., 2024).

Characteristics and Limitations of Encapsulating Materials

Numerous materials can function as encapsulating agents, but based on the literature summarized in Table 1, only seven materials appear suitable for defensins encapsulation. Each material has distinct characteristics and limitations. Alginate functions as a gel-forming agent that undergoes easy, simple, mild, rapid, and non-toxic gelation. It is biocompatible, biodegradable, and exhibits bio-adhesion to the intestinal mucosa (Milivojević et al., 2023). However, alginate presents several limitations. Macro-scale alginate delivery systems often show low bio accessibility, and the formation of alginate structures is strongly influenced by the molecular composition of the alginate and the kinetics of the cross-linking process (Ching et al., 2017).

Cellulose as an encapsulant shows characteristics similar to alginate. It offers several advantages, including its ability to form structural networks or matrices, its hydrophilic nature, and its flexibility to be modified into derivatives such as carboxymethyl cellulose, hydroxypropyl methylcellulose, and cellulose acetate (Bhatia, 2020; Ahmadi et al., 2022). These modifications aim to achieve different levels of viscosity, solubility, or permeability. However, cellulose also presents drawbacks (Bhatia, 2020). High mechanical strength hinders defensin release, while thermal sensitivity limits application at elevated temperatures, making the material unsuitable for high-temperature encapsulation (Li et al., 2018).

Chitosan offers key advantages as an encapsulating material. Chitosan degrades gradually in the proventricular environment of poultry, which makes it suitable for targeted defensins encapsulation (Maleki et al., 2022). Chitosan also provides controlled release due to the polymer structure of D-glucosamine. In addition, it remains stable at moderate temperatures and shows good resistance to oxygen (Maleki et al., 2022). However, chitosan has several limitations. Chitosan shows low solubility under neutral and alkaline pH conditions, which complicates encapsulation when the target site is located in the intestine (Aranaz et al., 2021). Low chitosan solubility in normal pH, becomes more severe due to its low mechanical and physical stability. Researchers often overcome those limitations by converting chitosan into functional derivatives, including chitosan oligosaccharide, carboxymethyl chitosan, quaternized chitosan, and hydroxypropyl chitosan (Aranaz et al., 2021). Another option is dissolving chitosan in weak acids that are safe for poultry (Maleki et al., 2022).

Eudragit offers several advantages as a pH-responsive polymer. L100-55 dissolves at pH levels above 5.5, S100 dissolves at pH levels above 7, and RS100 is pH-independent (Calderón-Oliver and Ponce-Alquicira, 2022; Miranda-Calderon et al., 2022). Although it is not toxic, Eudragit can influence defensins release because the polymer forms acid-base interactions and hydrogen bonds with

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defensins (Kaffash et al., 2021). Apart from that, its characteristics are comparable to the previously described encapsulant materials.

Gelatin differed from other encapsulating materials because aromatic amino acids absorbed ultraviolet radiation, which enhanced the protective effect against photo-oxidative damage (Abarca et al., 2022; Yang et al., 2022). However, its major drawback was its hydrophilic nature, which produced poor resistance to water vapor, so defensins microcapsules tended to absorb more moisture from the environment (Abarca et al., 2022).

Lecithin provides additional functionality as an encapsulating agent because its surfactant properties stabilize mixtures of hydrophilic and lipophilic components while improving film homogeneity and structural integrity (Wang and Phillips, 2023). Such properties enhance the stability of defensin encapsulation by promoting stronger interfacial adhesion. However, lecithin contains fatty acids that readily undergo oxidation. Oxidative degradation can cause rancidity and reduce the overall quality and stability of defensin encapsulation (Socha et al., 2024).

Pectin provides flexibility for structural modification that many other encapsulating agents. Gel-based encapsulation systems allow control over particle size and release characteristics, while alcohol-induced precipitation facilitates capsule formation (Zofou et al., 2019). However, pectin has several limitations, including sensitivity to extraction conditions, limited resistance to hydrolysis because enzymatic and chemical reactions readily degrade the polymer, and inconsistent in raw material quality that affects product performance (Wang et al., 2022).

MECHANISM OF ACTION OF ENCAPSULATED DEFENSINS IN POULTRY

Encapsulated Defensins Suppress *C. perfringens*

Broiler health plays a critical role in determining productivity and feed efficiency. Gut strongly influences intestinal function and overall phycological performance. Abd El-Hack et al. (2022) reported a high prevalence of necrotic enteritis caused by *C. perfringens*. Infection with *C. perfringens* damages the intestinal epithelium, impairs nutrient absorption, suppresses growth, reduces feed efficiency, and represents a major cause of mortality in broiler (Abd El-Ghany et al., 2022).

Defensins inhibit *C. perfringens* through multiple antimicrobial mechanisms. Several plectasin variants, including NZ2114, MP1102, and MP1106, exhibit low minimal inhibitory concentration and minimum bactericidal concentrations. Plectasin exerts antimicrobial activity primarily by disrupting the bacterial cell membrane and subsequently damaging bacterial DNA (Zheng et al., 2017). The MP1102 variant promotes bacterial lysis by increasing membrane permeability, whereas both NZ2114 and the MP1102 bind to the genomic DNA of *C. perfringens*, resulting in bacterial growth inhibition (Zheng et al., 2017).

Immunomodulatory Effect of Encapsulated Defensins to *C. perfringens*

In the innate immune system, defensins protect the intestinal mucosa against *C. perfringens* (Figure 5). Signaling through the mitogen-activated protein kinase (MAPK) pathway, particularly the extracellular signal-regulated kinase and p38 MAPK pathway, allows defensins to enhance macrophage chemotaxis (Cieślik et al., 2021; Guryanova and Ovchinnikova, 2022). In addition, defensins and MAPK signaling initiate and adaptive immune responses while regulating inflammatory activity (Zhou and Deng, 2021).

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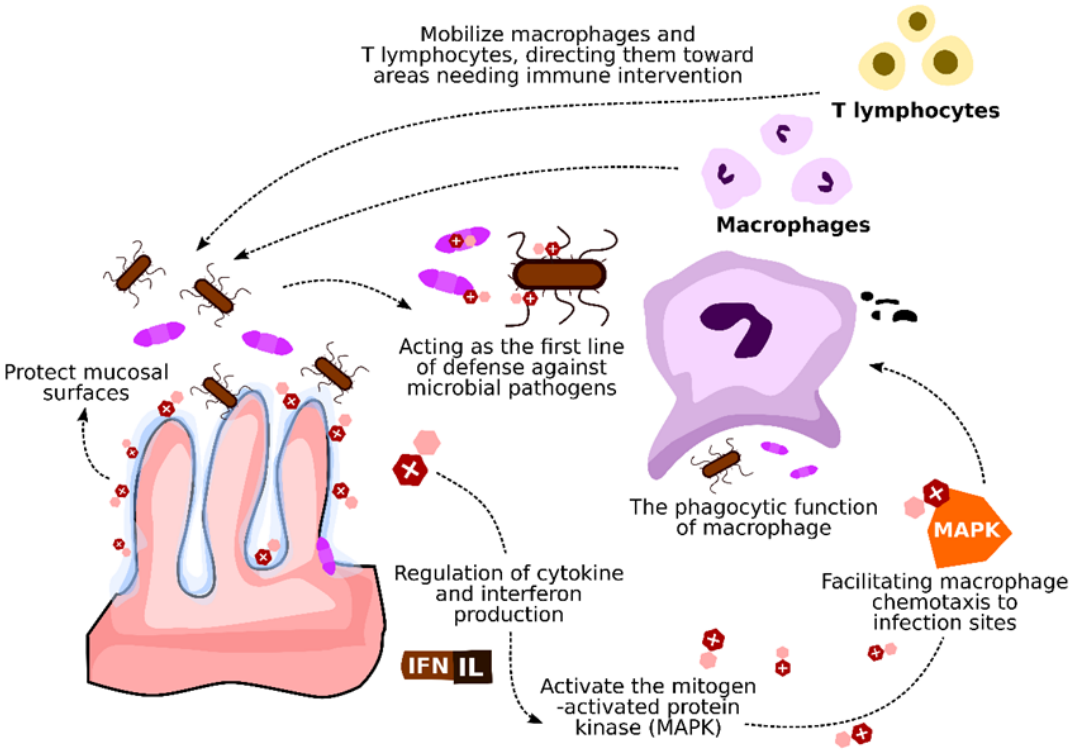


Figure 5 Immunomodulatory effects of defensins: key roles in innate immunity and cytokine regulation. Defensins activate the mitogen-activated protein kinase (MAPK) signaling pathway to facilitate the migration of macrophages to infection. Defensins also promote the production of interferons (IFN), which play essential roles in combating infections via interleukin (IL) intervention, also, regulating inflammatory responses. The illustration was produced using Inkscape (Version 1.1.1).

Defensins regulate immune cell receptor activity through protein kinase C, thereby preventing excessive immune responses and reducing tissue damage (Meng et al., 2018). Defensins also recruit macrophages, T lymphocytes, and mast cells to the site of infection. In turn, those immune cells stimulate plasmacytoid dendritic cells to release interferon-alpha, a cytokine that play an essential role in regulating inflammatory responses (Guryanova and Ovchinnikova, 2022). Antimicrobial properties, together with immunomodulatory functions, enable defensins to control *C. perfringens* infection while preserving the balance between innate and adaptive immunity. Recent studies also demonstrate that defensin-1 and defensin-2 are essential components of the honeybee immunity (Ilyasov et al., 2013; Al-Rubaie et al., 2024). Defensin-1 concentrations range from approximately 0.04 to 5.17 µg/g, whereas defensin-2, which is produced in the honeybee fat body, exhibits broad-spectrum antimicrobial activity (Ilyasov et al., 2013).

Encapsulant Material and Reduction of *C. perfringens* Population

Defensins encapsulation is expected to significantly reduce *C. perfringens* populations in the jejunum and ileum. (Pietruska et al., 2023). β-defensin-2 promotes the formation of endogenous ligands for toll-like receptor 4 (TLR-4) and activates dendritic cell through the MyD88 and NF-κB-mediated signaling pathways (Barabas et al., 2013). Likewise, human β-defensin-3 (HBD-3) activates monocytes through TLR-1/2, demonstrating the ability of defensins to interact with

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multiple TLRs and regulate the host immune response (Fesahat et al., 2023). Activation of immune signaling pathways enhance host defense and suppresses *C. perfringens* colonization in the gastrointestinal tract (Fesahat et al., 2023).

Chitosan with a 95% degree of deacetylation and a molecular weight of 220 kDa serves as the encapsulating material. Chitosan exhibits bactericidal activity at a concentration of 220 ppm and actively inhibits *C. perfringens* at concentrations ranging from 50 to 100 ppm (Chang et al., 2020). In addition, a concentration of 250 ppm reduces the heat resistance of *C. perfringens* spores (Chang et al., 2020). Chitosan oligosaccharides also demonstrate antibacterial activity against *C. perfringens* in broiler chickens, as summarized in Table 1 (Zhang et al., 2018).

Other encapsulation materials, including carrageenan, gum arabic, and konjac glucomannan, stimulate intestinal mucosal production and inhibit pathogen colonization (Kim and Lillehoj, 2019; Changchien et al., 2020). In addition, pectin and inulin function as fermentable prebiotics that serve as substrates for intestinal microbial metabolism, leading to the production of SCFAs, particularly butyrate. Butyrate exhibits antimicrobial activity against *C. perfringens*, as summarized in Table 1 (Tawfick et al., 2022; Guo et al., 2024). Formulation defensin peptides with appropriate encapsulation materials can enhance antimicrobial efficacy and may generate synergistic effect that improve gut health while reducing *C. perfringens* colonization.

Encapsulated Defensins Inhibit *S. enterica* Colonization

Salmonellosis, caused by *S. enterica*, remains a major challenge in the laying hen industry (Braden, 2006). *S. enterica* infection reduces egg production by causing internal egg contamination (Namata et al., 2008). Among the *S. enterica* serovars, Enteritidis (SE) and Pullorum (SP) present major epidemiological concerns. Host specificity distinguishes both serovars. SE is a zoonotic pathogen that can infect humans, whereas SP is host-adopted and infects only poultry and other avian species (Barrow et al., 2012).

Mechanism of Action of Encapsulated Defensins Against *S. enterica*

It has been reported that in vitro, porcine β-defensin-2 (PBD-2) inhibits S. enteric Typhimurium (STH) at concentrations from 0 to 64 μg/mL after 30 min of exposure. PBD-2 also induces morphological and structural damage in STH cells (Li et al., 2025). In addition, gallinacin-6 defensins exhibit bacteriostatic activity against STH, although both the minimal inhibitory concentration and minimal bactericidal concentration values exceed 128 μg/mL (Tai et al., 2014).

Defensin suppress of *S. enterica* colonization through several specific mechanisms. *S. enterica* infection triggers defensins expression following the recognition of polysaccharides on the bacterial cell membrane. Electrostatic interactions between cationic defensins and the anionic bacterial cell envelope, particularly lipopolysaccharide, mediate the initial binding process, as shown in Figure 6A (Zhang et al., 2021b). Following lipopolysaccharide binding membrane integrity by damaging the membrane structure and inducing pore formation. This membrane damage model is divided into carpet, barrel-stave, and toroidal-pore models, all of which will trigger destabilization and cause cell death refer to Figure 6B (Azmi and Hussain, 2021). Moreover, defensins exert bactericidal effects by inhibiting vacuole maturation, inducing cytoplasmic membrane retraction, and cause septal damage. The combined effects of those mechanisms synergistically disrupt bacterial cellular integrity and ultimately lead to cell death (Nguyen et al., 2011).

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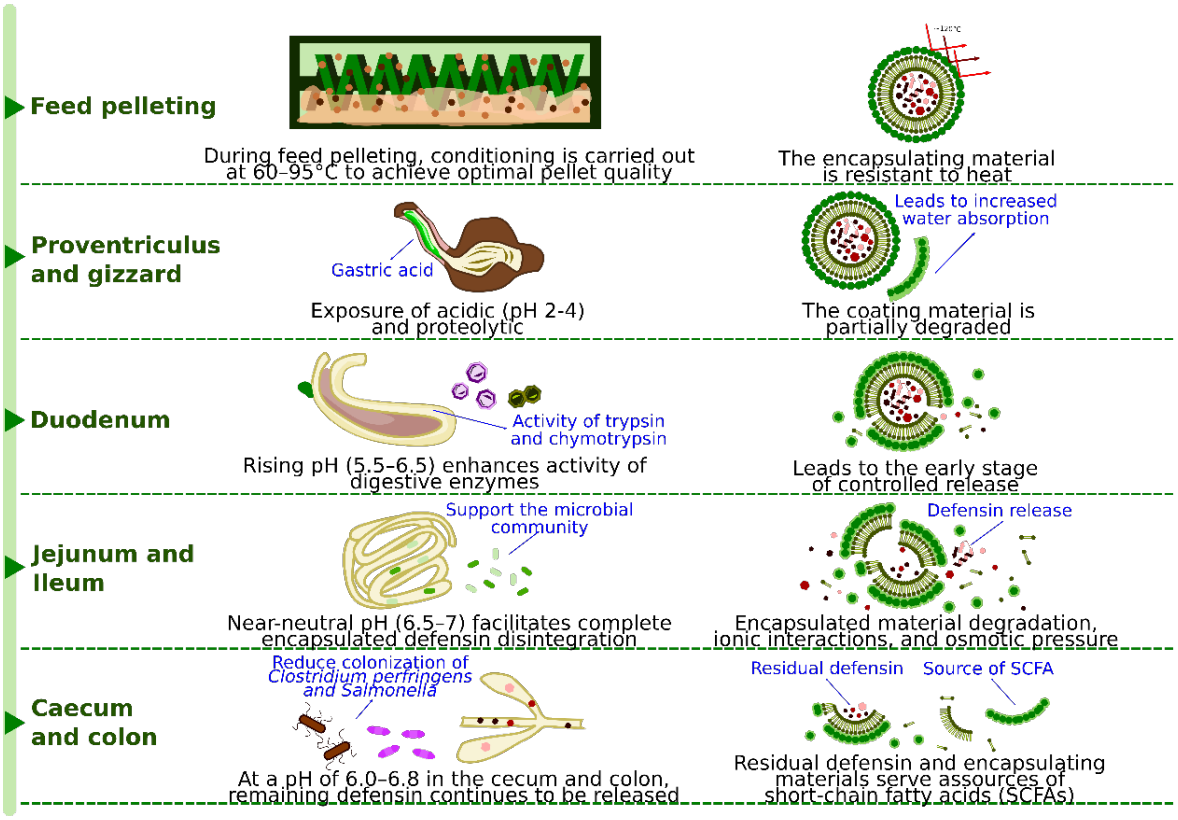


Figure 6 Overview of defensins recognition, membrane interaction models, and cellular impact on pathogenic microbes. A) Defensins recognize pathogens through both receptor-mediated and non-receptor-mediated mechanisms. B) Defensins disrupt the structural integrity of microbial cell membranes via several interaction models, including the carpet model, the barrel-stave model, and the toroidal-pore model. C) In fungal pathogens, defensins exert their effects by targeting the cell membrane, inducing reactive oxygen species (ROS) production, and causing mitochondrial dysfunction. The illustration was produced using Inkscape (Version 1.1.1).

Several defensins exhibit antifungal activity. Similar to other defensins, cis-defensins effectively disrupt fungal cell membranes and induce cell death through the production of reactive oxygen species (ROS). Cis-defensins also exhibit potent antimicrobial activity against *S. enterica* (Cools et al., 2017; Meng et al., 2025). They have also been reported to disrupt intracellular functions such as mitochondrial activity and cell-cycle regulation, and similar mechanisms contribute to their antibacterial action against *S. enterica* as illustrated in Figure 6C (Cools et al., 2017). However, the acidic conditions in the avian gastrointestinal tract can reduce defensin activity. Protective materials, such as polysaccharides, and nanoencapsulation techniques can help overcome such limitations by improving defensin stability and targeted delivery. Consequently, encapsulation strategies can enhance the antimicrobial efficacy of defensins within the avian gastrointestinal tract.

Targeted Delivery Techniques for Encapsulated Defensins Against *S. enterica*

Chitosan-alginate beads and cellulose acetate phthalate remain stable under acidic conditions but at neutral pH because water absorption causes structural swelling and promotes the controlled release of defensins, as shown in Figure 7 (Li et al., 2024). The properties of chitosan-alginate beads make them suitable for targeted delivery systems.

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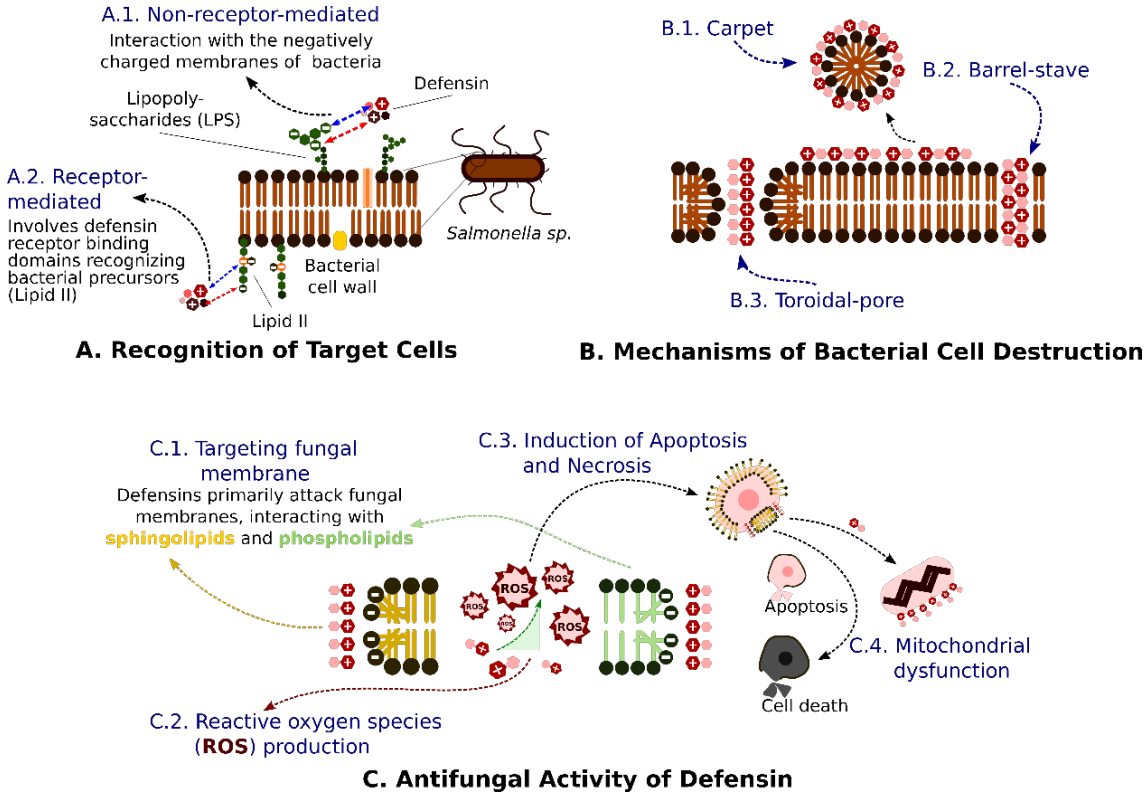


Figure 7 Release process of encapsulated defensins in various sections of the poultry digestive tract. Encapsulated defensins aims to preserve its release until reaching the small intestine, caecum, and colon. The illustration was produced using Inkscape (Version 1.1.1).

Another approach, enzyme-responsive delivery (ERD), uses microbial enzyme activity in the colon to achieve targeted delivery. Complex polysaccharides from ERD systems (including pectin, guar gum, xylan, and arabinogalactan) are specifically degraded by pectinases and xylanases (Zeybek et al., 2025). An alternative polysaccharide to ERD, the amylose-lipid complex, holds promise because it is more resistant to enzymatic degradation, but amylose degrades in the small intestine, resulting in the gradual release of defensins (Fatima et al., 2024).

ERD encapsulation prevents defensin release in the stomach and small intestine, allowing defensins to reach the cecum and colon, where SE colonization occurs, as shown in Figure 7 (Feng et al., 2024). This targeted approach maximizes antimicrobial efficacy while minimizing potential side effects at off-target sites.

Another alternative approach involves receptor-mediated targeting. Receptor-mediated targeting involves the presence of specific ligands on protective materials for cellular targeting, such as M-cell receptors and mucin surface receptors. Ligands such as lectins, dectins, and galectins, can enhance bio-adhesion and promote selective accumulation at target site, thereby prolonging the residence time of defensins on the small intestinal epithelium (Liu et al., 2024).

Time-based delivery systems use chronotropic polymers to achieve the slow and sustained released of active substance. Chronotropic polymers, such as Eudragit and HPMCP, gradually swell after absorbing fluid imbibition, which facilitates the controlled release of defensins over an extended period (Roque-Borda et al., 2021b; Ujilestari et al., 2023). The controlled release prolongs the activity of antimicrobial agents and helps maintain their therapeutic concentrations for longer periods.

Some polymers provide sustained-release properties. In particular, Eudragit is semi-permeable and poorly soluble in water, which allows defensins to diffuse

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gradually from the encapsulation matrix (Kakunje et al., 2024). Ethyl cellulose can further prolong the stability of the defensin encapsulation structure by forming a water-insoluble matrix, thereby extending the duration of defensin release (Nie et al., 2024).

To enhance intestinal residence time and local mucosal absorption, polymers can be engineered with mucoadhesive properties. Chitosan is suitable material for such applications because its cationic nature promotes strong adhesion to the negatively charged intestinal mucosa (Kulawik et al., 2024). Mucoadhesive modification can improve site-specific delivery and increase the bioavailability of therapeutic peptides.

Further surface modification of encapsulant materials involves incorporation of thiol groups (-SH) to form reversible disulfide bonds (-S-S-) with cysteine residues in mucins. Although the thiolated structure can gradually lose stability because of the reversible nature of the polymer (Mfoafo et al., 2023), several polymers can undergo thiolation to improve mucoadhesive properties. Common polymers include alginate (-COOH/-OH), chitosan (-NH2), cyclodextrin (-OH), gelatin (-NH2), hyaluronic acid (-COOH), and poly(acrylic acid) (-COOH) (Mao et al., 2023; Mfoafo et al., 2023).

Directed delivery through advanced approaches, such as stimuli-responsive delivery, is particularly useful when the desired delivery properties depend on environmental conditions, including low pH, temperature fluctuations, redox conditions, and specific enzymes. SR delivery shares similarities with ERD, but SR systems respond to a broader range of environmental stimuli. Another approach is microbiota-targeted method, which involves designing a direct interaction between the defensin-encapsulant material and specific microbes (Qiu et al., 2023). Controlled defensin release can therefore be designed to target susceptible receptors or cellular sites in specific strains of *S. enterica* and *C. perfringens*.

PH-DEPENDENT DUALITY OF DEFENSINS ACTIVITY IN THE DIGESTIVE TRACT

The potential of defensins as antimicrobials and immunomodulatory agents has been extensively evaluated in experimental studies. However, existing research does not fully define their potential as feed additives. A comprehensive evaluation is needed to determine their stability in the poultry digestive tract, particularly in the proventriculus and ventriculus, where acidic conditions predominate, with a pH ranging from 3.4 to 3.86 (Han et al., 2023). Maintaining the structural integrity of the peptide and its function in these acidic areas is crucial, given that defensins are believed to function most effectively in the mucosa of the small intestine (Fu et al., 2023; Li et al., 2025). Consequently, enhanced biological activity within the small intestine may contribute to improved growth performance and reproductive outcomes, highlighting the need to clarify the mechanisms through which defensin act under physiological conditions (Li et al., 2025). Furthermore, several studies report that acidic conditions can strengthen the biological function of certain defensins, emphasizing the need for a bidirectional assessment of defensins activity. Such an approach can characterize defensin function as a context-dependent process rather than assuming that acidic conditions uniformly impair peptide activity (Sanapalli et al., 2023).

Defensins derived from purple beans retain antifungal activity across a broad temperature range of 0–100 °C and an exceptionally wide pH range of 0–14 (Wei et al., 2009). Furthermore, studies on CpoBD13, a defensins identified from crocodiles, have shown structural stability between pH 5.5 and 7.5 (Williams et al., 2023). This type of peptide contains approximately 13% histidine, which, when combined with arginine and lysine, provides a fairly large net positive charge (Williams et al., 2023). At pH 3, histidine residues remained fully protonated because their pKa was 6. This condition markedly increased the peptide's cationic

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character (Martins et al., 2021). Protonation of peptides or enhancement of positive charge increases antibacterial effectiveness. Notably, gaduscidin-1 and gaduscidin-2 from cod fish show increased antimicrobial activity at low pH (McDonald et al., 2015; Malik et al., 2016). Similarly, clavanins (*Styela clava*), styelins *(S. clava)*, and hebraein *(Amblyomma hebraeum)* exhibit comparable effectiveness under acidic conditions (In et al., 2001; Lai et al., 2004; Malik et al., 2016). Acidic conditions promote pore formation in microbial membranes and increase the positive charge of peptides containing histidine and aspartic acid residues, thereby enhancing peptide-membrane interaction and disrupting bacterial membrane integrity (Malik et al., 2016).

Positively charged peptides strengthen electrostatic interactions with phospholipids in microbial membranes, thereby enhancing antibacterial adsorption. However, excessive protonation can disrupt the three-dimensional structure of proteins and compromise their functional integrity, particularly under highly acidic conditions (Williams et al., 2023). In contrast, low pH can stabilize disulfide bonds within peptides, thereby maintaining peptide structure and reducing conformational perturbations caused by proteolytic enzymes (Sholikin et al., 2021).

Previous evidence indicates that low pH enhances defensins stability and may increases their affinity for membrane surfaces. However, empirical evidence remains relatively limited, particularly in the avian digestive tract. Further experimental studies and comprehensive evaluations under both *in vitro* and *in vivo* conditions are required to clarify and validate the underlying mechanisms. Therefore, to mitigate potential damage under acidic conditions while preserving beneficial effects, feasible alternative approaches include the use of encapsulation techniques to protect active compounds from degradation caused by excessive temperature and pH fluctuations. Encapsulation techniques have great potential to increase defensins bioavailability.

ANTIMICROBIAL RESISTANCE EFFECT AND ALLERGIC POTENTIAL

Potential Microbial Resistance to Defensins

The ultimate goal of defensins encapsulation is to improve poultry growth performance (Figure 8). However, the potential development of antimicrobial resistance remains a major concern. Therefore, researchers need to evaluate the safety of encapsulated defensins and their potential impact on consumers. A rigorous assessment of the defensin's ability to induce resistance is also needed.

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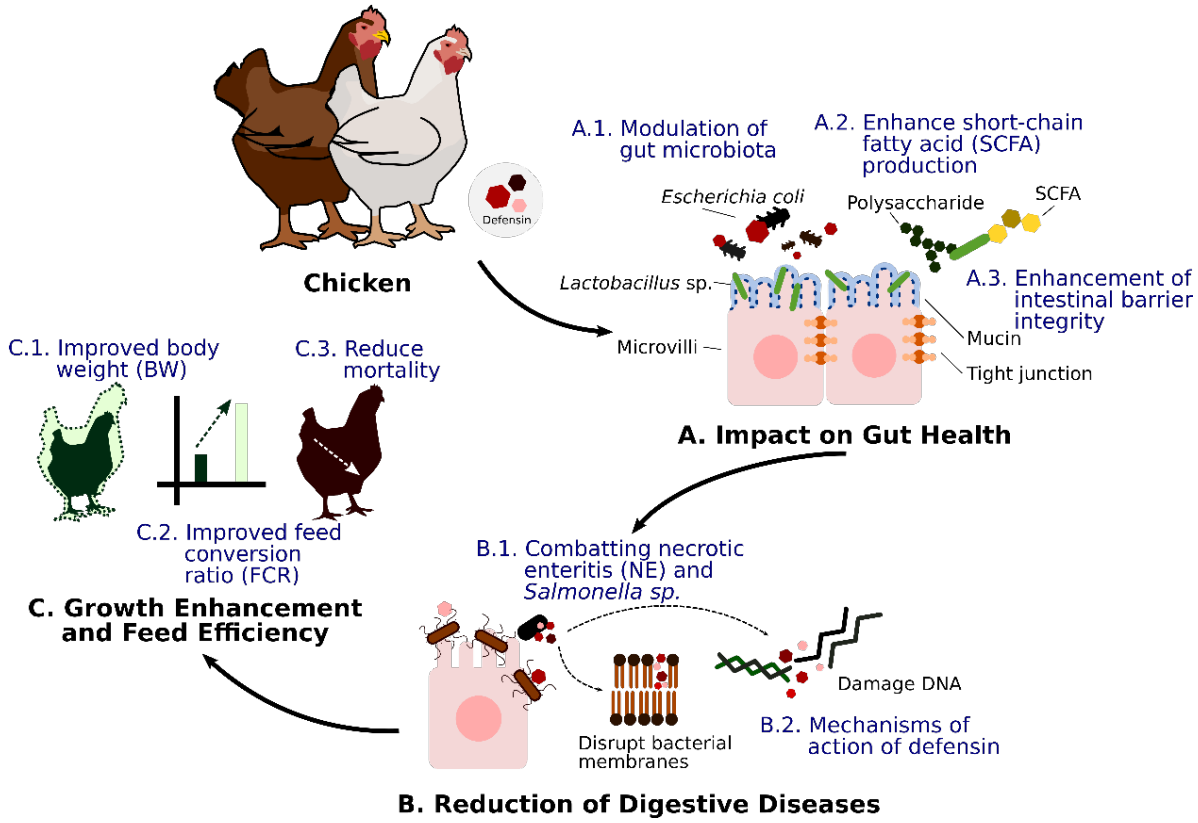


Figure 8 Schematic representation of the role of defensins in enhancing growth performance and feed efficiency in chickens. Defensins improve intestinal health and mitigate digestive diseases such as necrotic enteritis and *S. enterica*, thereby optimizing nutrient absorption in the intestines. Enhanced nutrient absorption contributes to improved growth and feed efficiency in chickens. The illustration was produced using Inkscape (Version 1.1.1).

Reports on of defensin-induced resistance remain limited. One example is the reported resistance of non-typeable *Haemophilus influenzae* to recombinant chinchilla beta-defensins (RCBD-1; Mason et al., 2005). This evidence suggests that *H. influenzae* relies on the AMP sap operon of RCBD-1 for bacterial protection. Significant mortality was recorded in the sapA mutant strain (carrying a dysfunctional sap operon) compared to the inbred strain, strengthening the suspicion of RCBD-1 resistance (Mason et al., 2005). The findings of this study indicate the possibility that defensins induce antimicrobial resistance, which is highly undesirable.

Cationic antimicrobial peptides (CAMPs), including defensins, can also induce resistance in group B *Streptococcus* (GBS; Saar-Dover et al., 2012). GBS has been reported to induce anionic teichoic acid through D-alanylation of CAMPs (Saar-Dover et al., 2012). D-alanylation of the lipoteichoic acid (LTA) region is what confers GBS resistance (Saar-Dover et al., 2012). The resistance mechanism does not involve reduced peptide binding to the bacterial cells. Instead, LTA modification alters the conformation in the cell wall and CAMP penetration. Observations using transmission electron microscopy confirmed that LTA modification triggers CAMP penetration through the cell wall (Saar-Dover et al., 2012). This further strengthens the potential for defensin-induced resistance.

Other studies also report potential resistance to defensins both Gram-positive and Gram-negative bacteria (Andersson et al., 2016). In Gram-positive bacteria, proteolytic degradation and binding of intracellular polysaccharide adhesins to poly-γ-glutamate contribute to defensin resistance (Joo et al., 2016). Wall teichoic acid modification also promotes resistance by causing steric

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hindrance from L-rhamnosylation and altering lipid II (Saar-Dover et al., 2012). In addition, peptidoglycan undergoes aminoacylation and active ATP-binding cassette transporters are synthesized (Joo et al., 2016). Meanwhile, in Gram-negative, self-defense strategies use proteolytic degradation pathways, utilization of alginate and polyciliate, and modification of phosphate groups in lipid A to increase the rigidity of the outer membrane (Maria-Neto et al., 2015). Also, peptidoglycan acylation strengthens the inner membrane (Gallo and Hooper, 2012). Efflux pumps belonging to the resistance-nodulation-division family further enhance resistance by actively exporting defensins from bacterial cells (Joo et al., 2016).

Implications of Resistance and Prevention Strategies

Reports of potential defensin resistance raise concerns about the long-term effectiveness of defensins as alternatives to conventional AGPs. Related reports include data from *E. coli* isolates in poultry, which showed 20.6% resistance to ampicillin, 85.3% resistance to nalidixic acid, and 82.4% resistance to tetracycline (Miles et al., 2006). In comparation, bacterial isolates from the human gastrointestinal tract showed lower resistance rates of 35.4%, 10.4%, and 43.8% resistance to ampicillin, nalidixic acid, and tetracycline, respectively (Miles et al., 2006).

The use of encapsulated defensins in poultry feed aims to increase production performance and feed efficiency by modulating microbial and mitochondrial function (Fernández Miyakawa et al., 2024). No scientific reports have documented antimicrobial resistance associated with encapsulated defensins in broiler and laying hen. However, preventive measures remain important. Strict control of the withdrawal period for encapsulated defensins and determination of optimal dosages that balance biological efficacy with residual safety are recommended (Maria Cardinal et al., 2019).

A targeted encapsulation system effectively reduces the development of antimicrobial resistance. Antimicrobial resistance that arises from interactions between active compounds (antibiotics) and non-target gut microbiota can be mitigated through encapsulation strategies (Moravej et al., 2018). In contrast, conventional antibiotics generally distribute systemically and exhibit broad-spectrum activity, which increases the risk of widespread resistance development. Recent studies report that ZnO-alginate-based nanoparticles effectively combat antibiotic-resistant bacteria, including *E. coli* and *Pseudomonas aeruginosa*, and maintain their efficacy across multiple usage cycles without significant loss of activity (Baek et al., 2019). Furthermore, nanoparticle encapsulation employ multiple mechanisms to target bacteria, which limits bacterial adaptation (Pandey et al., 2021). Precise intracellular delivery reduces the required drug dose and overall exposure, thereby limiting the development of resistance (Pandey et al., 2021). Finally, ROS-based targeting systems induce oxidative stress that bacteria cannot easily overcome through a single resistance mechanism (Baek et al., 2019).

More specifically, strategies to minimize resistance to encapsulated defensins, in line with current recommendations regarding antibiotic resistance, consist of three main steps (Capuozzo et al., 2024). The first step involves rational use and stewardship. Farmers follow standardized dosage guidelines, apply consistent administration protocols, implement antimicrobial stewardship at the farm level, rotate and combine encapsulated defensins with other organic additives such as probiotics and prebiotics, and conduct strict withdrawal and residue monitoring (Capuozzo et al., 2024).

The second step focuses on strengthening biosecurity, hygiene, and infection control. It involves improving on-farm biosecurity and infection management, optimizing hygiene through proper sanitation, ventilation, and litter management, and implementing routine poultry vaccination programs (Goulas et al., 2020).

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The third step focuses on monitoring, surveillance, and evaluation. Routine monitoring and surveillance track bacterial resistance patterns, document and report the use of feed additives, validate resistance biomarkers to detect bacterial adaptation, and evaluate the long-term effectiveness of additives on poultry performance (MacNair et al., 2024).

A controlled encapsulation policy for defensins can maximize their benefits while minimizing resistance risks to animal or human health. Responsible use under stewardship, stronger biosecurity and infection control, and continuous monitoring form an integrated strategy that reduces microbial pressure, ensures responsible application, and maintains the long-term effectiveness of encapsulated defensins in poultry production.

Safety, Allergenic Risks, and Regulatory Toxicity Oversight

The safety of encapsulated defensins primarily depends on safety and suitability of encapsulant materials, which are predominantly derived from polysaccharide sources. To date, no specific reports have indicated that defensins, particularly HBD, cause adverse effects in the gastrointestinal tract (Nguyen et al., 2021; Guryanova and Ovchinnikova, 2022). Polysaccharides commonly used as encapsulant agents, such as cellulose and chitosan, are known to possess strong water-binding capacities, reaching approximately 1,250 g/g (Narayanan et al., 2018). These characteristic warrants attention because excessive moisture retention may promote wet litter conditions in broilers (Dunlop et al., 2016). Therefore, establishing an optimal inclusion level for polysaccharide-based materials, such as cellulose, is essential. Current recommendations indicate that dietary cellulose inclusion at 4–5% can improve amino acid and protein digestibility in broilers (Tejeda and Kim, 2020). However, interactions between cellulose and other feed additives, including probiotics, prebiotics, enzymes, and antibiotics, require careful evaluation.

It is important to recognize that not all defensin types are suitable as feed additives because some exhibit allergenic properties. For examples, polyproline defensins from Asteraceae pollen caused allergic reactions in approximately 95% of sensitized patients (Pablos et al., 2018). Similarly, the food-derived defensin Pru-du-1 functions as major allergen in almonds and can trigger systemic reactions, including urticaria and anaphylaxis (Petersen et al., 2015). Current evidence indicated that allergenic defensins identified to date originated primarily from plant sources.

The toxicity observed in encapsulated defensins most likely originates from the encapsulant material. Most encapsulating agents consist of polysaccharides that are generally considered relatively safe and are classified as “Generally Recognized as Safe” (Rehman et al., 2021). However, certain Eudragit monomers requires careful consideration because some reports indicate that they may irritate the mucosal lining (Rehman et al., 2021; Nikam et al., 2023).

OPTIMAL CONDITIONS AND ECONOMIC IMPLICATION

Optimal Conditions for Defensins Encapsulation

A key practical consideration concerns the stability of encapsulated defensins during storage. Recent research reported that *Lactobacillus acidophilus* and *Bifidobacterium longum* encapsulated with whey protein and fructose as protective agents maintained significant stability and viability during storage at 4 ℃ for one yeat (Juárez-Trujillo et al., 2024). The encapsulated cells also retained their inhibitory activity after gastrointestinal simulation. Similar results were reported for peptide-based additives, including defensins (Yan and Kim, 2024). Post-storage

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evaluations conducted after nearly one year showed no significant reduction in effectiveness compared with the initial condition (Juárez-Trujillo et al., 2024). Defensins encapsulation aims to improve its functional performance and protect it during passage through the gastrointestinal tract, which makes long-term stability an essential factor (O'Brien et al., 2022). Overall, recent evidence indicates that appropriate protective agents and controlled storage temperature can improve the stability of encapsulated defensins products.

Recent findings indicate that nanoencapsulated defensins remained stable under gastric conditions for approximately four hours (Betala et al., 2018). Mucoadhesive nanoparticle systems maintain for up to ten hours, whereas raft-forming and solid lipid nanoparticles gastro-retentive systems exceeded twelve hours (Akbarbaglu et al., 2025). In-situ gelling system can prolong gastric retention for more than twenty-four hours (Betala et al., 2018). The retention period depends on the materials, formulation composition, and the formulation technique used to maintain defensins stability in the stomach through encapsulation (Raja et al., 2023).

Economic Impact of Defensins Encapsulation

Research on the economic impact of defensins encapsulation remains limited, especially regarding its application in the poultry industry. Cost estimates generated at the laboratory scale are not reliable for industrial evaluation because they tend to be excessively high (Lafarga et al., 2021). Current reports indicate that the bioactive peptide production coast approximately USD 88.5 per gram (Gnasegaran et al., 2017). Such a cost is exceptionally high and approaches 21,000 times the price of conventional additives such as zinc bacitracin.

Defensin production alone may not be economically feasible for the industrial application because the process involves multiple stages before peptide reaches a useable form. Recent reports on the production of plant-derived AMPs and recombinant expression in *E. coli* indicate that production costs remain high. Cloning design and templating require approximately USD 200–500 per attempt (Wibowo and Zhao, 2019). Transformation and initial culture require approximately USD 50–150. Scale-up production costs range from USD 5–20 for plant-derived peptides, whereas expression in *E. coli* may reach nearly USD 2,000. Extraction and initial purification require approximately USD 5–800, and final AMP purification adds another USD 100–400 (da Cunha et al., 2017; Gaglione et al., 2019). The estimates above reflect laboratory-scale testing, based on production per kilogram for plant-derived peptides and per liter for E. *coli* cultures (Wibowo and Zhao, 2019; Ghidey et al., 2020). The production of encapsulated peptides with materials such as cellulose or chitosan required matrix components that ranged from 5 to 100 USD per 5–100 g. Stabilizers cost 2–10 USD, while solvents required approximately 30 USD. Buffers and additional solvents ranged from 5 to 20 USD. Quality analysis reached up to 250 USD per sample.

These estimates above apply to the production of 100 mg of encapsulated peptide. However, the feasibility assessment excludes labor, fixed costs such as facility rental and equipment use, and post-production waste treatment.

The current production cost makes defensin encapsulation economically unfeasible. However, potential benefits and the development of alternative production methods may reduce production costs in the future. Despite demonstrated effectiveness, further economic evaluation is necessary to determine the feasibility of defensin encapsulation for the poultry industry.

CONCLUSIONS

Encapsulated defensins show promise as functional feed additives for broilers and laying hens because they may provide broad-spectrum antibacterial activity, modulate immune responses, and benefit from structural protection that

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enhances stability and bioavailability. Micro- and nanoencapsulation technologies may facilitate targeted release in the ileum and cecum-colon regions, thereby suppressing *C. perfringens* and *S. enterica*, restoring mucosal integrity, stabilizing gut microbiota, protecting against acidic pH, and improving production performance. In addition, polysaccharide-based encapsulating materials may promote short-chain fatty acid fermentation, which plays an important role in gut health and mineral metabolism.

Despite these encouraging findings, the application of encapsulated defensins in poultry feed requires further rigorous evaluation. Optimization of dosage and release profiles remains necessary, together with careful assessment of potential risks, including antimicrobial resistance development and allergenicity, to establish long-term safety and sustained efficacy under commercial production conditions. Although existing in vitro studies and simulation-based models provide an important foundation, additional in vivo studies are needed to validate their biological effects and practical applicability. Future research should also evaluate growth performance, feed conversion efficiency, gastrointestinal health, gut microbiota, long-term safety, and residue profiles under commercial conditions. In parallel, techno-economic analyses, including scalable production processes, detailed cost assessments, and production-cycle evaluations, are needed to determine industrial feasibility and support informed decisions regarding the practical adoption of encapsulated defensin additives in the poultry industry.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Faculty of Animal Science, Diponegoro University, for providing publication funding support, which was received by Sugiharto.

CONFLICT OF INTEREST

The authors declare that no potential conflicts of interest exist that could compromise the integrity of this study. The authors confirm that ChatGPT was used solely as a language editing tool to improve grammar, clarity, readability, and English expression during the preparation of the manuscript. ChatGPT was not used to generate or manipulate any data, images, results, references, or scientific interpretations. All AI-assisted suggestions were carefully reviewed, revised where necessary, and verified by the authors. The authors take full responsibility for the accuracy, integrity, originality, and scientific content of the manuscript.

AUTHOR CONTRIBUTIONS

Sugiharto Sugiharto: Resource, Funding Acquisition, Data Curation, Writing – Original Draft Preparation, and Writing – Review & Editing.
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How to cite this article;
Sugiharto Sugiharto, Mohammad Miftakhus Sholikin, Danung Nur Adli, Mochamad Dzaky Alifian, Tri Ujilestari and Bilal Ahmed. Encapsulated defensins as antimicrobial alternatives for poultry: A review. Veterinary Integrative Sciences. 2027; 25(1): e2027001-1-36.

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