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REVIEW

Surfactin: a multifunctional lipopeptide with antimicrobial, immunomodulatory, and nutritional applications in animal production

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ABSTRACT. Surfactin is a potent cyclic lipopeptide produced by *Bacillus* species that demonstrates broad-spectrum antimicrobial, antiviral, anti-inflammatory, antiparasitic, and immunomodulatory activities. Its amphiphilic structure enables membrane disruption, nutrient emulsification, and immune stimulation, making it a promising alternative to antibiotics in animal agriculture. Surfactin-rich fermented products have been shown to improve growth performance, gut health, nutrient absorption, and disease resistance in poultry, pigs, and aquaculture species. Additionally, surfactin functions as an effective vaccine adjuvant and shows synergistic effects with antibiotics and plant extracts, improving therapeutic outcomes. Collectively, these multifunctional properties position surfactin as a sustainable feed additive and biomedical agent with broad application potential in livestock production and related biomedical fields.

Introduction

Surfactin is a *Bacillus*-derived metabolite that plays an important role in gut microbiota–host interactions by combining antimicrobial activity against pathogens with growth-promoting effects at low inclusion levels. Recent studies have clarified the functional relevance and biological basis of surfactin activity. Lee et al. (2022) identified high surfactin secretion as a biomarker for high-throughput probiotic screening, whereas Ritter et al. (2018) described its biosynthesis via the *sfp* gene, which encodes 4′-phosphopantetheinyl transferase, and is typically located in the operons of *Bacillus subtilis* strains widely used in commercial feed additives. In addition, Tran et al. (2023) confirmed the presence of surfactin in the gastrointestinal tract using liquid chromatography-mass spectrometry (LC–MS) in antibiotic growth promoter (AGP) replacement trials, validating its role as an active antimicrobial metabolite.

Moreover, Lee et. al (2023) demonstrated that dietary supplementation with surfactin obtained from high-yielding *Bacillus subtilis* fermentation improved broiler growth performance, intestinal villus development, and elevated *Bacillus*-like populations and protease activity in the gut. These findings highlight the therapeutic potential and importance of surfactin for animal production. However, suboptimal gastrointestinal conditions, including high nutrient availability, low oxygen tension, and competition from resident microbiota, can suppress *in vivo* surfactin secretion by *Bacillus* spp. (Arjes et al., 2020; Tran et al., 2023). Several approaches have been proposed to maintain effective intestinal surfactin levels. First, rational strain selection combined with high-throughput screening of naturally high-producing *Bacillus* isolates can increase constitutive surfactin generation in the gut environment (Lee et al., 2022; Ritter et al., 2018). Second, formulation and delivery approaches, including protected or spore-based carriers and encapsulation, can shield probiotic *Bacillus* cells from gastric stress and allow targeted germination with surfactin release in the small intestine (Gao et al., 2013; Patel et al., 2015). Third, dietary co-strategies, such as prebiotic supplementation or controlled inclusion of fermentable substrates, can modulate intestinal redox conditions and nutrient availability to stimulate *in situ* surfactin biosynthesis (Tran et al., 2023). Finally, exogenous delivery of surfactin through controlled fermentation of feed ingredients or the inclusion of surfactin-enriched fermented products offers a practical means of supplying consistent functional doses that bypass the constraints of *in vivo* production (Cheng et al., 2018; Chen and Yu, 2021). Taken together, these approaches, applied individually or in combination, provide feasible options to overcome physiological limitations associated with intestinal surfactin production and support reliable antimicrobial and nutritional effects when surfactin is administered as a feed additive.

Surfactin

This compound is a potent cyclic lipopeptide biosurfactant produced by *Bacillus subtilis*. It consists of a seven-amino acid peptide loop (comprising Lasparagine/aspartate, L-leucine, L-glutamate, L-valine, and two Dleucines) linked to a hydrophobic fatty acid chain that varies between isoforms. First identified in 1968 as a fibrin clot inhibitor with cell-lytic properties (Arima et. al, 1968), surfactin was subsequently recognised for its broader ecological and industrial application.

Recent studies have indicated that surfactin isoforms differ in biological activity depending on fatty acid chain length and amino acid substitutions within the heptapeptide ring. To clarify these structure–activity relationships (SAR), Table 1 summarises the main surfactin isoforms, highlighting key structural differences and their corresponding antimicrobial, antiviral, and surface-active properties reported in the literature. This comparison shows how molecular variation among isoforms influences bioactivity and functional specialisation, supporting the selection of isoform-specific applications in food, pharmaceutical, and feed contexts.

Table 1. Structure–activity relationships of surfactin

Isoform / structural feature	Typical molecular variants (examples)	Reported effect on bioactivity / physicochemistry	References
Fatty-acid chain length: C13	C13 (β-OH-C13)	Generally lower antiviral activity and often lower hemolytic potency vs C14–C1	Kracht et al. (1999)
Fatty-acid chain length: C14	C14 (β-OH-C14) — most common in many strains	Balanced: good antiviral inactivation, moderate–high hemolysis and membrane activity; strong surface-activity and emulsification	Zhen et al. (2023)
Fatty-acid chain length: C15 / C16	C15 (β-OH-C15), C16	Often highest antiviral potency (C15 frequently top) and stronger membrane-disrupting activity; increased hydrophobicity lowers CMC and increases micelle stability	Kracht et al. (1999)
Branching / methylation of FA	iso-/anteiso, methylated FA	Branching can modulate packing and membrane insertion — effects reported as isoformspecific	Liu et al. (2015)
Peptide-ring substitutions	Common L/D Leu↔Val (e.g., Leu^2 vs Val^2), Glu/Asp conserved at positions 1 & 5	Amino-acid changes alter charge distribution, amphipathicity and target selectivity; charged residue changes modulate binding to membranes/viral envelopes	Théatre et al. (2021)
Natural isoform mixtures	Natural extracts contain C13–C16 + peptide variants	Often synergy or broadened activity vs single purified isoforms (antibiofilm, antiviral)	Bochynek et al. (2023)

FA – fatty acids, L/D – left/right, Leu – leucine, Val – valine, GLU/Asp – glutamic acid/aspartic acid, CMC – critical micelle concentration

Unlike related *Bacillus* lipopeptides, such as iturins and fengycins, which are primarily antifungal, surfactin uniquely combines broad-spectrum antimicrobial and antiviral activity with strong surface-active and emulsifying properties. These complementary functions make surfactin particularly suitable for applications where both microbial control and efficient lipid dispersion are required, such as feed additives and mucosal adjuvants.

As facultative anaerobes, *Bacillus* species can influence the luminal redox environment through oxygen consumption. Arjes et al. (2020) have shown that surfactin supports *Bacillus* survival under hypoxic conditions by inducing membrane depolarisation in subpopulations and facilitating oxygen diffusion, while complete anoxia stimulates sporulation to enable long-term survival.

The amphiphilic structure of surfactin, together with its high stability across a wide range of pH and temperature conditions, indicates its broad applicability. It has been applied in oil recovery, heavy metal remediation, and biodegradation, and has also been studied for antimicrobial, antiviral, and anticancer activity in biomedical research. In agriculture, surfactin enhances disease resistance and stress tolerance, while in the food and cosmetics sectors it is used as a natural preservative, emulsifier, and anti-inflammatory agent. Table 2 provides an overview of surfactin applications in animal production by summarising its reported effects on growth performance, intestinal development, immune-regulation, as well as antimicrobial and anti-inflammatory responses in livestock.

2023). Feeding trials demonstrated improvements in gut microbial balance and reduced incidence of post-weaning diarrhoea in piglets (Hung et al., 2019; Lin and Yu, 2020), as well as enhanced growth performance and modulation of faecal microbiota in sows and broilers (Chen et al., 2020; Yu et al., 2020; Chen and Yu, 2020).

Recent broiler studies further confirmed the protective effects of surfactin under pathogen challenge. In a necrotic enteritis (NE) model, dietary surfactin (0.01%) improved feed efficiency, reduced intestinal lesions, and restored gut barrier integrity, restoring overall performance close to parameters of healthy, uninfected birds. These findings demonstrate that surfactin can partially replace antibiotic growth promoters by supporting recovery and maintaining intestinal health under NE stress (Kumar et al., 2025).

Table 2. Applications, mechanisms of action, and observed effects of surfactin in livestock production

Application in Livestock	Mechanism / Mode of Action	Observed Effects
Growth Promotion in Poultry	Modulates gut microbiota, enhances nutrient absorption, improves intestinal morphology	↑ Body weight gain, ↑ Feed conversion efficiency, ↑ Villus height
Antimicrobial Activity	Disrupts bacterial cell membranes, inhibits Gram-positive bacteria and some Gram-negative bacteria	↓ Pathogenic bacteria load, ↓ incidence of diarrhea
Immunomodulation	Stimulates innate immunity, enhances macrophage activity and cytokine production	↑ Immune response, ↑ antibody titer, ↓ inflammation markers
Feed Efficiency / Digestibility	Improves fat emulsification, enhances enzyme activity, modulates gut microbiota	↑ Nutrient digestibility, ↑ enzyme activity, ↓ fecal nitrogen excretion
Stress & Pathogen Challenge Mitigation	Reduces oxidative stress, modulates intestinal barrier function	↓ Stress-related biomarkers, ↓ intestinal lesions under pathogen challenge

Pathogenic control

Surfactin exerts antibacterial effects primarily by disrupting bacterial membranes through pore formation (Sheppard et al., 1991). Fermented products of *Bacillus subtilis* and *Bacillus licheniformis* containing surfactin were reported to show antimicrobial properties against *Clostridium perfringens* (Horng et al., 2019; Cheng et al., 2021b). In broiler models, dietary supplementation with surfactin-rich fermented products alleviated necrotic enteritis, reduced intestinal lesions, and improved gut morphology (Cheng et al., 2018).

Moreover, surfactin has shown activity against *Vibrio parahaemolyticus*, a key pathogen in shrimp aquaculture (Zhou et al., 2025). It disrupts bacterial membranes, increases generation of reactive oxygen species (ROS), and interferes with bacterial metabolism. Transcriptomic data have indicated downregulation of virulence genes and essential metabolic pathways, leading to oxidative stress and reduced bacterial viability (Zhou et al., 2025).

As a feed additive, surfactin not only suppresses pathogen development but also prevents their adhesion and colonisation, contributing to a healthier gut microbiome (Liu et al., 2019a; Andric et al.,

Anti-inflammatory effects

Surfactin exerts strong anti-inflammatory effects by targeting nuclear factor kappa-B (NF-κB), mitogen-activated protein kinase (MAPK), and inflammasome signalling pathways (Giri et al., 2020). In lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages, surfactin C (50 μg/ml) significantly downregulated pro-inflammatory mediators such as interleukin-1 beta (IL-1β) and inducible nitric oxide synthase (iNOS), as well as reduced nitric oxide (NO) production in a dose-dependent manner (Hwang et al., 2005).

Mechanistically, surfactin blocks IκB kinase/inhibitor of κB alpha (IKK/IκBα) phosphorylation and NF-κB p65 nuclear translocation (Byeon et al., 2008), while suppressing MAPK and phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathways (Park and Kim, 2009; Datta and Chattopadhyay, 2024). In BV-2 microglial cells exposed to amyloid-β, surfactin reduced ROS, prostaglandin E_2 (PGE_2), and inflammatory cytokine levels, including tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Park et al., 2013).

In vivo, oral administration of surfactinrich *B. licheniformis* products alleviated colitis in mice by protecting the gut lining and inhibiting inflammasome activation of neutrophil-lymphocyte ratio (NLR) family pyrin domain containing 3 (NLRP3) (Tsai et al., 2022). In diabetic models, surfactin reduced pancreatic IL-1β levels and improved glycaemic control (Chen et al., 2022). Additionally, surfactin was shown to decrease endotoxin and cytokine concentrations in septic shock models by interfering with LPS–binding protein interactions (Seydlová and Svobodová, 2008). Among surfactin isoforms, surfactin C was most effective in suppressing NO production and cytokine expression (Kim et al., 2006).

Further evidence from a cyclophosphamide-induced immunosuppression model shows that oral surfactin enhances systemic immunity and mucosal barrier function. It was shown to increase immune-organ indices, expression of sIgA and tight-junction proteins, and improved microbial balance by elevating *Lactobacillus* and reducing *Escherichia coli* abundance. These findings clearly indicate that surfactin is a potent modulator of host immunity and intestinal homeostasis (Jia et al., 2024).

Anti-parasitic and anticoccidial effects

Surfactin has demonstrated broad anti-parasitic activity. Shebl et al. (2022) reported that it inhibited *Plasmodium falciparum* development and reduced *Nosema ceranae* spore infection potential, showing prospective benefits for honeybee health maintenance (Baffoni et al., 2022). These effects are largely attributed to its amphipathic structure, which disrupts protozoan membranes (Seydlová and Svobodová, 2008; Datta and Chattopadhyay, 2024).

In poultry, surfactin was shown to inhibit *Eimeria* sporulation and sporozoite development, effectively blocking the coccidial life cycle (Wang et al., 2021). Supplementation with 1.25 g/kg of *B. licheniformis*-fermented product resulted in anticoccidial effects comparable to those induced by conventional drugs (Cheng et al., 2021a). Surfactin from *B. licheniformis* (ATCC 12713) inhibited the complete *Eimeria* cycle *in vivo* (Yu et al., 2021). Additionally, it has been reported that surfactin improves gut microbiota composition and mucosal health, providing additional resistance to parasitic infection (Tran et al., 2023).

Antiviral activity

Surfactin shows broad-spectrum activity against enveloped viruses by disrupting lipid membranes, resulting in >4.4 $\log_{10}$ reductions of herpes simplex virus type 1, simian immunodeficiency virus, vesicular stomatitis virus, and Semliki Forest virus at concentrations of 25–80 µM (Vollenbroich et al., 1997). Its antiviral properties are associated with fatty acid chain length, with C15 isoforms showing the highest activity (Kracht et al., 1999). In addition, surfactin interferes with membrane fusion, blocking entry of porcine epidemic diarrhoea virus (PEDV) and transmissible gastroenteritis virus (TGEV) by disturbing lamellar lipid phases and competing with host receptors like epidermal growth factor receptor (EGFR) and aminopeptidase N (APN) (Wang et al., 2017; Yuan et al., 2018). Surfactin also reduced incidence of infections with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Vero E6 cells without detectable cytotoxicity, and inhibited related coronaviruses, including SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV) (Johnson et al., 2019; Crovella et al., 2022). Moreover, surfactin-rich fermented *Bacillus* products alleviated PEDV symptoms and reduced viral shedding in piglets, suggesting the potential of feed-based antiviral applications (Peng, et al., 2019).

Emulsifier and nutritional enhancer

Surfactin possesses strong surface-active properties, and can act as a natural emulsifier in animal nutrition. At concentrations as low as 20 µM, it was shown to lower water surface tension from 72 mN/m to approximately 27 mN/m and form stable oil-in-water emulsions even at 0.01% (w/w) (Zhen et al., 2023). These properties improve lipid dispersion and absorption in the gastrointestinal tract, contributing to better nutrient digestibility and energy utilisation.

In aquaculture, dietary supplementation with surfactin (100 mg/kg feed) in orange spotted grouper has been shown to regulate lipid metabolism by lowering serum cholesterol and triglyceride levels, hepatic lipid accumulation, as well as altering lipogenic enzyme activity, including fatty acid synthase (FAS), hepatic lipase (HL) and lipoprotein lipase (LPL) (Chen et al., 2017). Similarly, tilapia fingerlings fed diets containing 50 mg/kg surfactin showed improved growth performance, digestive enzyme activity, and favourable serum biochemical profiles (Zhai et al., 2016).

In poultry, incorporation of *Bacillus*-fermented products (BFP) rich in surfactin in broiler diets has been shown to improve performance and nutrient use. Supplementation at 0.3% (approximately 4.7 mg/g surfactin) significantly improved feed conversion ratio, fat digestibility, and ileal absorption of dry matter, crude protein, and fat by 8–12% compared to control diets (Cheng et al., 2018 and Chen and Yu, 2020). Addition of BFP at 1.25 g/kg also exerted anticoccidial effects and supported gut health and overall performance (Chen and Yu, 2020). Recent nutritional trials have optimised the inclusion rate of surfactin-enriched fermented products (at 11.23 mg/g) in broiler feed to 1.9%, which corresponds to about 213 mg/kg dietary surfactin. This dosage maximised growth rate, improved intestinal villus height, morphology, and tibial bone strength in broilers (Lee et al., 2024).

Surfactin has a low critical micelle concentration (CMC) and remains stable over a wide range of pH, which makes it well suited for use in different feed formulations. By improving lipid emulsification, digestive efficiency, and intestinal health, surfactin serves not only as an effective emulsifier but also as a functional feed additive in modern livestock systems.

Adjuvant effects

Surfactin enhances both innate and adaptive immunity, acting as a vaccine adjuvant when administered via intramuscular, oral, and intranasal routes. It induces ROS generation in macrophages, activating MAPK, NF-κB, and inflammasome signalling pathways (Gan et al., 2016). Surfactin also promotes dendritic cell maturation via Toll-like receptor 2 (TLR2) signalling (Xu et al., 2016) and strengthens mucosal immunity by inducing mast cell degranulation (Sato et al., 2018). Hepatitis B vaccines formulated with surfactin elicit stronger and prolonged humoral and T cell responses compared to conventional adjuvants (Pan et al., 2014; Gao et al., 2012). In addition, surfactin protects antigens from degradation and improves gastrointestinal absorption, supporting its potential as an oral vaccine adjuvant (Gao et al., 2012; Patel, et al., 2015).

Surfactin also shows synergistic interactions with antibiotics and botanical compounds. Co-administration with amoxicillin was shown to reduce minimum inhibitory concentration (MIC) against *E. coli* and improve survival in infected chicks (Liu et al., 2019b). Synergy has also been reported with plant extracts, including *Achyrocline satureioides*, where antimicrobial activity was up to 32-fold higher (Sabaté et al., 2009). Comparable effects observed for surfactant-stabilised nanoemulsions confirm the role of surfactin in enhancing the effects of essential oil-based antibacterial agents. (Donsì et al., 2011).

Dosage determinants and cross-species conversion of surfactin

The effectiveness of surfactin supplementation in livestock depends on several interacting factors that affect its bioavailability, antimicrobial activity, and immunomodulatory effects. Species-specific dosage ranges are frequently reported, but they highly vary due to differences in digestive physiology, age, diet composition, health status, and administration method. Digestive tract characteristics, such as length, passage time, and microbial composition can markedly impact surfactin availability. In ruminants, foregut fermentation may partially degrade surfactin, whereas monogastric species are likely to have higher intestinal exposure. Younger animals, with immature gut microbiota and immune systems, may respond to lower absolute doses but are generally more sensitive to bioactive effects. Diet composition also plays an important role: high fat or fibre levels can alter surfactin solubility and emulsification, requiring dose adjustments. Moreover, animals exposed to pathogens or environmental stress may benefit from higher supplementation, which supports gut barrier function and immune responses. Finally, the mode of delivery, including feed, water, or encapsulated formulations, influence the effective intestinal dose, emphasising the need to consider these factors jointly when optimising supplementation strategies.

Safety and regulation

Surfactin, a lipopeptide produced by *Bacillus* species that is generally recognised as safe (GRAS), still lacks a well-defined safety profile for use as a feed additive. Although its biological origin suggests a favourable safety background, available data on long-term exposure and safe dietary inclusion levels remain limited. Potential issues such as haemolytic activity, cytotoxicity, and immunogenic responses at higher doses require careful evaluation (Wahab and Al-Sahlany, 2025).

Recent work from the authors' laboratory has provided encouraging evidence in this regard. a tolerance trial in broiler chickens together with toxicological assessments in rodent models, showed no adverse effects on animal health, growth performance, or organ function at the tested doses. Although these data have not yet been published, they strongly suggest that surfactin can be safely applied within practical dosage ranges for animal feeding.

In addition to *in vivo* safety data, established analytical methods for residue detection are available. Validated liquid chromatography-mass spectrometry (LC–MS) and LC–MS/MS techniques enable sensitive and specific quantification of surfactin in fermented products and animal tissues. A validated LC–MS/MS method for the simultaneous quantitation of surfactin and iturin has been reported and is suitable for residue monitoring. Moreover, high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC–MS^n) allow detailed characterisation of individual surfactin isoforms. These analytical tools are ready for inclusion in regulatory submissions to support residue monitoring and safety assessment (Tang et al., 2010).

Future research should build on these findings through comprehensive toxicological evaluations, including long-term feeding trials in different livestock species, to define acceptable daily intake (ADI) values and no-observed-adverse-effect levels (NOAEL).

To ensure regulatory compliance and facilitate approval by relevant national feed safety agencies, it is crucial to develop standardised protocols for assessing haemolytic activity, allergenicity, and cumulative exposure risks. Establishing these safety benchmarks will provide the scientific basis for setting appropriate inclusion rates and support the safe and effective use of surfactin as a functional feed additive in animal production.

Conclusions

Surfactin, a multifunctional lipopeptide biosurfactant produced by *Bacillus* species, demonstrates a wide range of beneficial properties relevant to animal health and nutrition. Amphiphilic structure of surfactin is responsible for its potent antimicrobial, antiviral, anti-inflammatory, antiparasitic, and immunomodulatory activities. In livestock systems, surfactin-rich fermented products improve growth, performance, disease resistance, and gut health. Surfactin has also been investigated as a vaccine adjuvant, with evidence of its capacity to stimulate both innate and adaptive immune responses. Together, these characteristics support its use as a natural alternative to antibiotics, chemical additives, and synthetic emulsifiers, showing its potential for broader application in veterinary medicine, animal nutrition, and related biopharmaceutical fields.

Conflicts of Interest

The Authors declare that there is no conflict of interest.

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