



Mare's Milk as a Natural Probiotic-Antimicrobial Ecosystem: Composition, Microbial Ecology, and Functional Implications

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Abstract | Mare's milk has been increasingly recognized as a distinctive non-ruminant dairy matrix with intrinsic functional properties linked to its biochemical composition and spontaneous fermentation ecology. Compared with ruminant milk, mare's milk is characterized by high lactose availability, a whey-dominant protein fraction, low casein content, and the presence of host derived antimicrobial proteins such as lysozyme and lactoferrin. This article presents a narrative integrative review that synthesizes peer reviewed evidence on mare's milk as an intrinsically organized probiotic antimicrobial system by integrating milk composition, microbial architecture during spontaneous fermentation, and multilayered antimicrobial defense mechanisms. Particular emphasis is placed on Sumbawa mare's milk as a tropical, low input traditional production system that provides a comparative model to classical Central Asian koumiss fermentation. Across diverse studies, lactic acid bacteria (LAB) are frequently reported as dominant microbial groups, supported by ecological selection pressures imposed by the milk matrix and reinforced by LAB mediated fermentation metabolites. Antimicrobial functionality is shown to arise from the combined action of host derived proteins, LAB produced organic acids and bacteriocins, and auxiliary bioactive components rather than from isolated factors alone. However, most available evidence is derived from *in vitro* assays, culture-based studies, and observational analyses, highlighting important limitations in clinical and *in vivo* validation. By integrating fragmented findings into a system level framework, this review identifies key conceptual linkages, methodological constraints, and future research priorities relevant to food safety, non-thermal processing, and clean-label functional dairy innovation.

Keywords | Mare's milk, Lactic acid bacteria, Spontaneous fermentation, Antimicrobial activity, Functional dairy

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INTRODUCTION

Growing consumer interest in functional foods has intensified the search for dairy matrices that inherently deliver bioactive compounds, probiotic microorganisms, and antimicrobial functionality. This trend is closely linked to increasing concerns regarding antibiotic stewardship, food safety, and clean-label processing within

the dairy sector. Within this context, non-ruminant milks particularly mare's milk have attracted growing scientific attention as alternative functional dairy sources. Compared with conventional ruminant milk, mare's milk exhibits a distinct biochemical profile, including lower fat and casein contents, a whey-dominant protein fraction, and a relatively high abundance of innate immune components. Collectively, these characteristics suggest considerable

potential for the development of health oriented dairy products in fresh, fermented, or dried forms (Musaev *et al.*, 2021; Malacarne *et al.*, 2002).

Beyond its nutritional composition, mare's milk contains a range of bioactive proteins involved in innate antimicrobial defense, notably lysozyme, lactoferrin, lactoperoxidase, and immunoglobulins. These components have been widely reported to exhibit antimicrobial and immunomodulatory activities, providing a biochemical rationale for the long standing consumption of fermented mare's milk products such as koumiss in Central Asia and Eastern Europe. In these systems, spontaneous fermentation has historically been associated with enhanced safety and functional value, as suggested by repeated observational and experimental studies (Wlazlo *et al.*, 2025). Together, these compositional and functional attributes position mare's milk not merely as a conventional dairy substrate, but as a biologically active matrix with intrinsic protective potential.

Despite this growing interest, research on mare's milk remains conceptually fragmented. Existing studies generally fall into three largely disconnected streams: (i) compositional characterization of milk constituents, (ii) isolation and functional screening of lactic acid bacteria (LAB) with probiotic potential, and (iii) antimicrobial assays targeting selected pathogens using individual milk fractions or fermented products. While each approach has generated valuable insights, they are rarely integrated within a unified framework that explains how milk composition, spontaneous fermentation ecology, and antimicrobial functionality interact as an interconnected system. This lack of integration is particularly evident under tropical, low input production conditions, where fermentation dynamics, microbial succession, and functional outcomes may differ substantially from those reported in temperate or industrialized dairy systems. An initial integrative perspective has recently been proposed to conceptualize mare's milk as a biologically organized probiotic–antimicrobial system; however, such frameworks have not yet been systematically expanded to incorporate ecological context, critical appraisal of evidence strength, and explicit discussion of limitations across diverse production systems (Faradila *et al.*, 2025).

In Indonesia, Sumbawa mare's milk (SMH) represents a culturally significant and scientifically relevant example of such a system. Produced under traditional low input management practices in West Nusa Tenggara, SMH provides a natural context for studying spontaneous fermentation under tropical conditions, where indigenous microbiota develop without technological standardization. Previous studies have frequently reported that naturally fermented SMH is enriched in Firmicutes, particularly LAB genera such as *Lactobacillus* and *Streptococcus*,

alongside yeasts including *Kazachstania*, reflecting a complex fermentation ecology observed across multiple investigations (Jatmiko *et al.*, 2019). LAB isolated from SMH have been shown to reach high cell densities and exhibit probiotic relevant traits, suggesting substantial indigenous functional potential (Detha, 2019).

Complementary evidence indicates that SMH exhibits antimicrobial activity against several major foodborne pathogens, an effect attributed to synergistic interactions among organic acids, antimicrobial proteins (e.g., lysozyme and lactoferrin), and bacteriocin-producing LAB (Hermawati *et al.*, 2004). However, most available evidence is derived from *in vitro* antimicrobial assays, which demonstrate inhibitory potential rather than guaranteed pathogen absence. In parallel, household level spontaneous fermentation of SMH has been associated with acceptable organoleptic properties and relatively stable physicochemical characteristics, supporting its long-standing consumption as a traditional fermented beverage (Ardiansyah *et al.*, 2021).

Nevertheless, the absence of an integrated, mechanism-oriented synthesis limits the translation of existing knowledge into dairy processing strategies, safety oriented design, and functional product innovation, particularly for non thermal and clean-label applications. Interactions among milk composition, microbial dynamics during spontaneous fermentation, and multilayered antimicrobial mechanisms remain insufficiently conceptualized as a coherent probiotic antimicrobial system.

Therefore, this review aims to synthesize and critically evaluate current evidence on (1) the natural probiotic potential of mare's milk, with emphasis on LAB diversity and functional attributes, and (2) antimicrobial activity associated with bioactive proteins, peptides, and microbial metabolites. Using Sumbawa mare's milk as a focal model for tropical traditional production systems, this review integrates compositional, microbiological, and bioactivity data to identify conceptual gaps, methodological limitations, and future research directions relevant to the safe and scalable development of fermented or minimally processed mare's milk products (Shokrollahi *et al.*, 2025).

This manuscript is a narrative integrative review that synthesizes peer reviewed evidence on mare's milk as an intrinsic probiotic antimicrobial system. Literature was identified through searches of major scholarly databases (e.g., Scopus, PubMed, Web of Science, and Google Scholar) using combinations of relevant keywords related to mare's milk, lactic acid bacteria, antimicrobial components, spontaneous fermentation, and microbial ecology. The scope prioritized studies published within approximately the last two decades, while retaining key

historical references where foundational. Studies were included if they reported milk compositional features, microbial communities or isolates (culture based or sequencing based), and antimicrobial outcomes relevant to mare's milk systems, including evidence from Sumbawa mare's milk (SMH).

MICROBIAL ARCHITECTURE OF MARE'S MILK: LAB DOMINANCE AS A FREQUENTLY OBSERVED ECOLOGICAL OUTCOME

Across diverse geographical regions, the microbiota of mare's milk has frequently been reported to display a convergent ecological pattern characterized by enrichment of lactic acid bacteria (LAB), particularly during spontaneous fermentation. Rather than representing a random assemblage of microorganisms, this LAB-enriched community is plausibly shaped by intrinsic biochemical features of mare's milk. High lactose availability, a whey protein dominant fraction, and the presence of antimicrobial proteins such as lysozyme may collectively impose selection pressures that favor LAB proliferation while constraining the growth of competing or potentially pathogenic microbial groups. However, the relative contribution of each factor varies across studies and production contexts.

Evidence from traditional fermented mare's milk products, including koumiss and airag, frequently reports the predominance of LAB genera such as *Lactobacillus*, *Leuconostoc*, *Lactococcus*, and *Enterococcus*, which contribute to acidification, lactic acid production, and the generation of bioactive metabolites. Comparative microbial surveys from both temperate and tropical systems have repeatedly identified LAB taxa such as *Lactobacillus plantarum*, *L. fermentum*, *L. helveticus*, *L. rhamnosus*, and *L. casei* across regions, despite differences in climate, management, and cultural practices (Brumini *et al.*, 2016; Martuzzi *et al.*, 2024; Tang *et al.*, 2020). This recurring taxonomic pattern suggests that LAB enrichment in mare's milk is not incidental, although controlled comparative studies remain limited.

This ecological pattern is further supported by observations from Sumbawa mare's milk (SMH), where metagenomic analyses have reported enrichment of the phylum Firmicutes, particularly LAB genera such as *Lactobacillus* and *Streptococcus*, alongside functionally relevant yeasts including *Kazachstania* and *Kluyveromyces* (Jatmiko *et al.*, 2019). Similar microbial community profiles have been described across multiple production areas (Dompu, Bima, Sumbawa), suggesting a degree of ecological consistency under comparable low input fermentation practices. Nevertheless, variability related to sampling timepoints, hygiene, and analytical methods should be considered

when interpreting these findings.

Functional characterization of LAB associated with mare's milk further supports their ecological relevance. Species commonly isolated from fermented mare's milk, including *L. plantarum*, *L. fermentum*, and *L. casei*, have been shown to exhibit traits associated with probiotic candidacy, such as tolerance to acidic conditions and bile salts, adhesion capacity, aggregation properties, and bacteriocin production (Martuzzi *et al.*, 2024). These traits may enhance both gastrointestinal resilience and competitive fitness within the milk environment. Taken together, available evidence suggests that mare's milk can support LAB enriched fermentation systems; however, the stability and reproducibility of these outcomes depend on contextual and methodological factors.

Table 1 summarizes LAB taxa frequently reported across fermented and raw mare's milk systems, including their identification methods, ecological roles during fermentation, and types of functional evidence. Reported functions are primarily derived from culture based and *in vitro* studies unless otherwise stated. The presence of certain taxa (e.g., *Enterococcus* spp.) highlights the need for strain level safety assessment prior to application.

ECOLOGICAL FILTERING MODEL: DRIVERS SHAPING LAB ENRICHMENT IN MARE'S MILK

The predominance of LAB in mare's milk can be conceptually interpreted through an ecological filtering model, in which environmental and management related drivers influence milk biochemical traits that may, in turn, impose selective pressures on microbial communities. Within this framework, LAB enrichment is proposed as an emergent outcome of interconnected ecological processes rather than a universally demonstrated mechanism. At the primary ecological level, feeding regimes under extensive grazing systems may play an important role. Horses typically consume diverse pasture vegetation rich in fermentable carbohydrates, phytochemicals, and polyunsaturated fatty acids (PUFA). Due to the monogastric digestive physiology of equids, plant derived lipids particularly α -linolenic acid (18:3n-3) are absorbed with limited biohydrogenation, contributing to relatively elevated PUFA proportions in mare's milk (Blanco Doval *et al.*, 2024). These compositional features may influence milk physicochemical properties and indirectly enhance fermentability, creating conditions favorable for LAB growth and metabolic activity.

Climatic pressures may act as additional ecological filters. Environments characterized by temperature fluctuations, seasonal variability, and resource constraints can impose selective pressure on stress tolerant microorganisms.

Table 1: Lactic acid bacteria reported in mare's milk and fermented mare's milk systems.

LAB taxon (species/ strain)	Sample source	Identification method	Ecological role in fermentation	Functional evidence (reported)	Evidence type	Reference
<i>Lactobacillus casei</i> Zhang	Koumiss (fermented mare's milk)	Culture-based isolation; phenotypic and molecular characterization	Rapid acidification during early fermentation	High viability; tolerance to simulated gastrointestinal conditions	<i>In vitro</i> strain-level assays	Guo <i>et al.</i> , 2009
<i>Lactobacillus plantarum</i>	Fermented and raw Sumbawa mare's milk	Culture-based isolation; RAPD-PCR; metabolite analysis	Dominant lactose-fermenting LAB; competitive exclusion	Production of antimicrobial metabolites	<i>In vitro</i> metabolite assays	Kusdianwati <i>et al.</i> , 2020; Kusdianawati <i>et al.</i> , 2023
<i>Lactobacillus rhamnosus</i>	Fermented Sumbawa mare's milk	Culture-based isolation	Reinforcement of antimicrobial fermentation environment	Inhibition of enteric pathogens	<i>In vitro</i> inhibition assays	Fajriani <i>et al.</i> , 2023
<i>Lactobacillus helveticus</i>	Central Asian koumiss	Culture-based isolation	Proteolysis and peptide release during fermentation	Bioactive peptide generation	Observational and <i>in vitro</i>	Brumini <i>et al.</i> , 2016; Shokrollahi <i>et al.</i> , 2025
<i>Lactobacillus fermentum</i> SM-7	Koumiss	Culture-based isolation; strain characterization	Organic acid production; contribution to microbial stability	Bile salt tolerance; antimicrobial activity	<i>In vitro</i> strain-level assays	Pan <i>et al.</i> , 2010
<i>Leuconostoc</i> spp.	Central Asian koumiss	Culture-based isolation	Exopolysaccharide production; matrix structuring	Contribution to texture and fermentation stability	Observational studies	Brumini <i>et al.</i> , 2016
<i>Enterococcus</i> spp.	Central Asian koumiss	Culture-based isolation	Microbial competition via enterocin production	Antimicrobial activity with safety considerations	<i>In vitro</i> assays	Sanam, 2022

Note: Quantitative metrics and statistical parameters are reported where available in the original studies; however, many studies provide qualitative or semi-quantitative outcomes typical of *in vitro* screening assays. Functional attributes should therefore be interpreted as indicative rather than definitive.

Mare's milk produced under such conditions, particularly in Central Asian and tropical extensive systems, has often been reported to maintain LAB enriched microbial communities. Ecological adaptation of horses to marginal environments may also influence milk composition, including the relative abundance of bioactive whey proteins such as lysozyme and lactoferrin (Panzaru *et al.*, 2024), which can inhibit competing or pathogenic microbes while remaining compatible with LAB growth.

Management practices constitute a third potential filtering layer. Low input, extensive production systems are typically characterized by minimal antibiotic use and greater exposure to environmental microbiota. Grazing behavior increases contact with soil and plant associated microorganisms, which may modulate gastrointestinal and mammary microbial populations and, consequently, influence the microbial composition of milk (Miraglia *et al.*, 2020). Variations in pasture quality, botanical diversity, and herd management have also been associated with differences in milk protein fractions, lipid profiles, and bioactive components factors known to affect fermentability and the persistence of LAB during fermentation (Ronzaud *et al.*, 2022).

Collectively, these ecological drivers diet, climate, and

extensive management are likely to shape the biochemical traits of mare's milk in ways that influence microbial selection during spontaneous fermentation. From this perspective, mare's milk may be viewed as the emergent product of a complex ecological network that contributes to LAB enrichment under specific conditions. While this conceptual model is consistent with observations from both Central Asian koumiss and tropical Sumbawa mare's milk systems, targeted experimental validation remains necessary to confirm causal relationships.

MULTILAYERED ANTIMICROBIAL DEFENSE IN MARE'S MILK

Unlike conventional dairy systems in which antimicrobial functionality is often introduced exogenously through processing aids, preservatives, or selected starter cultures, mare's milk has been widely described as an intrinsically antimicrobial matrix. In this system, host-derived bioactive proteins and metabolites produced by indigenous lactic acid bacteria (LAB) may act in a complementary manner to create a layered antimicrobial defense. Rather than complete microbial sterilization, this layered architecture appears to selectively suppress pathogenic or competing microorganisms while permitting the persistence of beneficial LAB populations, a feature that underlies the

traditional use of mare's milk in spontaneous fermentation systems.

HOST DERIVED PROTEIN DEFENSE: INNATE ANTIMICROBIAL LAYER

At the primary defense layer, mare's milk contains several whey-associated bioactive proteins that contribute to innate antimicrobial protection. Among these, lysozyme has been reported at substantially higher concentrations than in most domesticated dairy species and exhibits bacteriolytic activity through hydrolysis of peptidoglycan in Gram-positive bacterial cell walls. The antimicrobial spectrum of lysozyme may be broadened through synergistic interactions with lactoferrin, which destabilizes the outer membrane of Gram-negative bacteria and increases lysozyme accessibility to peptidoglycan substrates (Brumini *et al.*, 2016; Khan *et al.*, 2024).

Lactoferrin also contributes independently to antimicrobial defense through iron sequestration, depriving microorganisms of essential growth factors, as well as through direct membrane disruptive and immunomodulatory effects. Additional host derived components, including immunoglobulins and the lactoperoxidase system, further enhance this protein based defense by neutralizing pathogens and generating bactericidal oxidative stress. Importantly, these protein mediated mechanisms impose selective antimicrobial pressure rather than broad-spectrum elimination, thereby allowing LAB populations to persist within the milk matrix.

PROBIOTIC MEDIATED DEFENSE: FERMENTATION DRIVEN ANTIMICROBIAL LAYER

Superimposed upon the protein-based defense is a dynamic, fermentation driven antimicrobial layer mediated by LAB commonly associated with mare's milk, including *Lactobacillus plantarum*, *L. fermentum*, and *L. casei*. During spontaneous or controlled fermentation, these organisms produce antimicrobial metabolites such as lactic and acetic acids, hydrogen peroxide, exopolysaccharides, and bacteriocins that collectively reduce pH, disrupt proton gradients, compromise membrane integrity, and inhibit cell wall synthesis in susceptible microorganisms (Pan *et al.*, 2010; Jena and Choudhury, 2025).

Unlike host derived proteins, which provide baseline antimicrobial activity in fresh milk, LAB-derived metabolites typically intensify antimicrobial effects over time as fermentation progresses. This temporal differentiation highlights the dynamic nature of antimicrobial defense in mare's milk, in which probiotic activity does not replace protein mediated defense but may amplify and extend it through microbial metabolism.

EVIDENCE FROM SUMBAWA MARE'S MILK: SUPPORT FOR A LAYERED ANTIMICROBIAL MODEL

Evidence from Indonesia, particularly from Sumbawa mare's milk (SMH), is consistent with the proposed multilayered antimicrobial framework under tropical traditional production conditions. Hermawati *et al.* (2004) reported that SMH inhibited the growth of several foodborne pathogens, including *Salmonella* spp., *Staphylococcus aureus*, *Shigella* spp., *Pseudomonas* spp., and *Escherichia coli*. These findings, primarily derived from *in vitro* inhibition assays, suggest that antimicrobial activity is intrinsic to the milk matrix and not solely dependent on industrial processing or exogenous starter cultures.

Subsequent studies indicate that antimicrobial functionality may persist beyond the fresh state. Amara *et al.* (2016) reported that spray dried SMH encapsulated with gum arabic retained antimicrobial activity while improving the stability of bioactive proteins such as lysozyme and lactoferrin. While these results support the feasibility of preserving antimicrobial properties during processing, their functional implications remain largely supported by laboratory-scale evidence.

Additional support is provided by probiotic fermentation studies in which SMH based beverages inoculated with *Lactobacillus rhamnosus* inhibited enteric pathogens, reinforcing the role of LAB as contributors to antimicrobial activity during fermentation (Fajriani *et al.*, 2023). These observations are aligned with findings by Sanam (2022), who demonstrated that both whey fractions and LAB isolates from mare's milk inhibited *Salmonella enteritidis* and *Staphylococcus aureus*, further suggesting synergistic interactions between protein derived antimicrobial factors and LAB derived metabolites.

In addition to protein and microbe derived mechanisms, bioactive fatty acids naturally present in mare's milk, including linoleic and lauric acids, may contribute an auxiliary antimicrobial layer by disrupting pathogen lipid membranes and increasing cellular permeability. Although often considered secondary contributors, these lipid associated effects may further reinforce the multilayered nature of antimicrobial defense in mare's milk.

SYSTEM LEVEL SYNTHESIS: FROM COMPONENTS TO LAYERED DEFENSE ARCHITECTURE

Collectively, host derived antimicrobial proteins, fermentation driven probiotic metabolites, and bioactive lipid components appear to form a multilayered antimicrobial defense system in mare's milk. These layers are functionally distinct yet potentially synergistic: protein based defenses provide baseline microbial filtering, while LAB mediated fermentation dynamically amplifies

antimicrobial pressure over time. Although much of the available evidence is derived from *in vitro* and laboratory scale studies, this system level organization distinguishes mare's milk from conventional dairy matrices and supports its relevance for food safety considerations, non thermal preservation strategies, and clean-label functional dairy applications.

SUMBAWA MARE'S MILK AS A TROPICAL TRADITIONAL MODEL SYSTEM

Sumbawa mare's milk (SMH) is discussed in this review not as an isolated regional product, but as a tropical traditional analogue of classical Central Asian mare's milk fermentation systems. Under low-input production conditions, SMH provides a valuable context for examining probiotic ecology and antimicrobial functionality in mare's milk across contrasting agroecological environments.

LOW INPUT PRODUCTION SYSTEM AS A COMPARATIVE MODEL

Sumbawa mare's milk is produced within a low input

equine dairy system characterized by extensive grazing, minimal supplementary feeding, and limited technological intervention. This production context shares key features with traditional equid milk systems reported across Central Asia and Eurasia. Accordingly, SMH may be considered a tropical counterpart to temperate koumiss producing systems, allowing comparative evaluation of mare's milk functionality under different climatic and environmental conditions (Musaev *et al.*, 2021).

Low input equine dairy systems are generally associated with a conserved biochemical profile, including high lactose content, whey-dominant protein fractions, and low casein levels. These attributes are consistently reported across mare populations and are primarily determined by species-specific lactation physiology rather than intensive nutritional manipulation (Malacarne *et al.*, 2002; Kondybayev *et al.*, 2021). Although some compositional variability exists, this shared biochemical framework supports spontaneous fermentation across geographically distinct systems.

Table 2: Multilayered antimicrobial components of mare's milk and types of supporting evidence.

Defense layer	Component	Primary mechanism of action	Functional role within the system	Representative assay / evidence type	Main micro-bial targets (reported)	Reference
Host derived protein defense (baseline layer)	Lysozyme	Peptidoglycan hydrolysis; enhanced activity with lactoferrin	Early microbial filtering in fresh milk	<i>In vitro</i> lysis assays; agar diffusion	<i>Staphylococcus</i> , <i>Listeria</i>	Brumini <i>et al.</i> , 2016
	Lactoferrin	Iron sequestration; outer-membrane destabilization	Broadening antimicrobial spectrum	<i>In vitro</i> growth inhibition; iron-binding assays	<i>E. coli</i> , <i>Salmonella</i>	Brumini <i>et al.</i> , 2016; Khan <i>et al.</i> , 2024
	Immunoglobulins	Pathogen binding and neutralization	Target-specific microbial interaction	Immunological assays; observational studies	Enteric pathogens	Brumini <i>et al.</i> , 2016
	Lactoperoxidase system	Generation of OSCN ⁻ ; oxidative stress	Continuous antimicrobial pressure in raw milk	Enzymatic activity assays	Gram-positive and Gram-negative bacteria	Brumini <i>et al.</i> , 2016
Probiotic mediated fermentation defense (dynamic layer)	Organic acids (lactic, acetic)	pH reduction; metabolic disruption	Environmental acidification during fermentation	pH monitoring; growth suppression assays	Enteric pathogens	Sanam, 2022
	Bacteriocins (e.g. plantaricin)	Membrane pore formation; cell wall inhibition	Targeted microbial competition	Agar diffusion; co-culture inhibition tests	<i>Salmonella</i> , <i>Staphylococcus</i>	Sanam, 2022
Auxiliary lipid associated defense	Bioactive fatty acids	Membrane destabilization; increased permeability	Enhancement of pathogen susceptibility	<i>In vitro</i> membrane disruption assays	Gram-positive and Gram-negative bacteria	Brumini <i>et al.</i> , 2016
System level interaction	Protein-probiotic synergy	Combined effects of proteins and LAB metabolites	Reinforced multi-layer antimicrobial architecture	Integrative interpretation of multiple assays	Gastrointestinal pathogens	Sanam, 2022; Khan <i>et al.</i> , 2024

Note: The components summarized represent mechanistic contributions reported across multiple studies. Direct quantitative synergy testing (e.g., checkerboard or fractional inhibitory concentration assays) remains limited, and interactions are therefore described at a conceptual or functional level.

Within the Sumbawa context, this intrinsic milk composition supports natural microbial succession. Metagenomic studies have reported LAB enriched microbial communities dominated by Firmicutes, particularly *Lactobacillus* and *Streptococcus*, together with functionally relevant yeasts such as *Kazachstania* (Jatmiko *et al.*, 2019). Similar community structures have been observed across multiple production areas within Sumbawa, suggesting ecological consistency under comparable low input practices, while acknowledging variability related to sampling, handling, and analytical approaches.

From a technological perspective, the compositional characteristics of SMH impose both opportunities and constraints. The lactose whey matrix readily supports spontaneous fermentation without exogenous starter cultures, whereas the low casein content limits rennet based coagulation and hard cheese production. As a result, fermented beverages predominate, reflecting inherent properties of the milk matrix rather than technological limitations.

MILKING PRACTICES AND SPONTANEOUS FERMENTATION AS AN OBSERVATIONAL SYSTEM

Traditional SMH milking practices are manual, small scale, and involve minimal mechanical processing or sterilization. Rather than being viewed solely as a limitation, these practices create an observational system in which interactions among milk composition, indigenous microbiota, and environmental inocula can be examined with limited external interference. Under such conditions, raw SMH typically undergoes spontaneous fermentation shortly after milking, driven by endogenous and environmental LAB populations.

Studies have reported that naturally fermented SMH maintains acceptable sensory properties, with pH values remaining moderately acidic during early fermentation stages (Ardiansyah *et al.*, 2021). Compared with ruminant milk fermentations, the pH decrease is often less pronounced, which may be attributed to the whey-dominant protein system, buffering capacity, and relatively short fermentation periods typical of traditional mare's milk practices. However, fermentation trajectories vary depending on time, temperature, and handling conditions, and should not be interpreted as fixed system characteristics. These fermentation patterns are broadly consistent with those observed in classical equid systems such as koumiss and qymyz. High lactose concentrations (approximately 6–7%) promote LAB proliferation, while the near equivalent whey casein ratio prevents firm gel formation, favoring liquid or semi liquid fermented products. The recurrence of these features across temperate and tropical systems suggests that mare's milk fermentation

is largely species driven, while being modulated by local environmental and cultural factors (Shokrollahi *et al.*, 2025).

Indonesian studies further indicate that spontaneous fermentation of SMH is associated with antimicrobial activity. Inhibition of major foodborne pathogens has been reported in laboratory based assays (Hermawati *et al.*, 2004), and probiotic beverages prepared from SMH using *Lactobacillus rhamnosus* have demonstrated inhibitory effects against enteric pathogens (Fajriani *et al.*, 2023). While these findings support the functional potential of SMH, the available evidence is predominantly derived from *in vitro* or laboratory-scale studies.

ETHNOMEDICINAL CONTEXT AND TRANSLATIONAL POTENTIAL

Sumbawa mare's milk has long been associated with ethnomedicinal practices related to digestive health, immune support, and general well being. Increasingly, these traditional claims are being examined through biochemical and microbiological analyses, positioning SMH as an interface between indigenous knowledge and modern functional food research.

Analytical studies have confirmed that SMH contains bioactive proteins such as lysozyme, lactoferrin, immunoglobulins, and antimicrobial peptides components that align with the multilayered antimicrobial framework described in Section 3. Together with metabolites produced during LAB mediated fermentation, these factors may contribute to antibacterial, anti inflammatory, and gut modulating effects. Recent processing studies further indicate that selected functionalities can be retained beyond the fresh state. For example, microencapsulation of SMH using gum arabic has been reported to preserve antimicrobial activity while improving the stability of protein bioactives (Amara *et al.*, 2016), highlighting translational opportunities alongside the need for further validation.

CONCEPTUAL INTEGRATION, LIMITATIONS, AND RESEARCH OUTLOOK

When considered collectively, the compositional characteristics of mare's milk high lactose availability, whey-dominant protein fractions, bioactive peptides, and distinctive lipid profiles support the conceptualization of SMH as a tropical, low input model of an intrinsic probiotic antimicrobial dairy ecosystem. In this framework, spontaneous fermentation is interpreted as an emergent outcome shaped by interactions among milk biochemistry, indigenous microbiota, ecological drivers (Rifa'i *et al.*, 2025), and traditional management practices (Surjowardojo *et al.*, 2025), rather than as an uncontrolled

or incidental process.

From a systems perspective, SMH may be viewed as a self organizing fermentation matrix in which ecological conditions influence milk composition, which in turn imposes selective pressures on microbial communities. These communities may subsequently reinforce antimicrobial functionality through the production of organic acids, bacteriocins, and other metabolites that act synergistically with host-derived antimicrobial proteins such as lysozyme and lactoferrin. Nevertheless, variability related to animal factors, environmental conditions, hygiene practices, and analytical methodologies represents an important limitation that must be addressed when extrapolating findings across systems.

Future research should therefore move beyond descriptive characterization and prioritize:

- Longitudinal and seasonal analyses of microbial succession during spontaneous SMH fermentation;
- Establishment of safety, quality, and functional benchmarks for SMH derived fermented products;
- Validation of ethnomedicinal claims using clinically relevant antimicrobial, anti-inflammatory, and gut microbiome models
- Translation of traditional practices into scalable, clean-label functional dairy innovations.

Together, these considerations support the positioning of Sumbawa mare's milk not merely as a regional specialty, but as a contextually informative model system for studying biologically integrated dairy fermentation under tropical low input conditions. In addition to milk composition acting as an ecological filter, it is also plausible that the maternal gut and mammary microbiota contribute to the initial microbial inoculum of mare's milk, suggesting a bidirectional interaction between milk composition and microbial community assembly.

CONCLUSIONS AND PERSPECTIVES

This review synthesizes previously fragmented research on mare's milk into an integrative framework linking ecological context, milk biochemical composition, microbial dynamics, and antimicrobial functionality. Collectively, available evidence suggests that mare's milk can be understood not merely as a vehicle for probiotic microorganisms or isolated bioactive compounds, but as an intrinsically organized probiotic antimicrobial system shaped by species specific physiology and predominantly low input production conditions.

Across diverse geographic regions, mare's milk is frequently characterized by high lactose availability, whey-dominant protein architecture, and low casein content. These compositional features are repeatedly associated with LAB

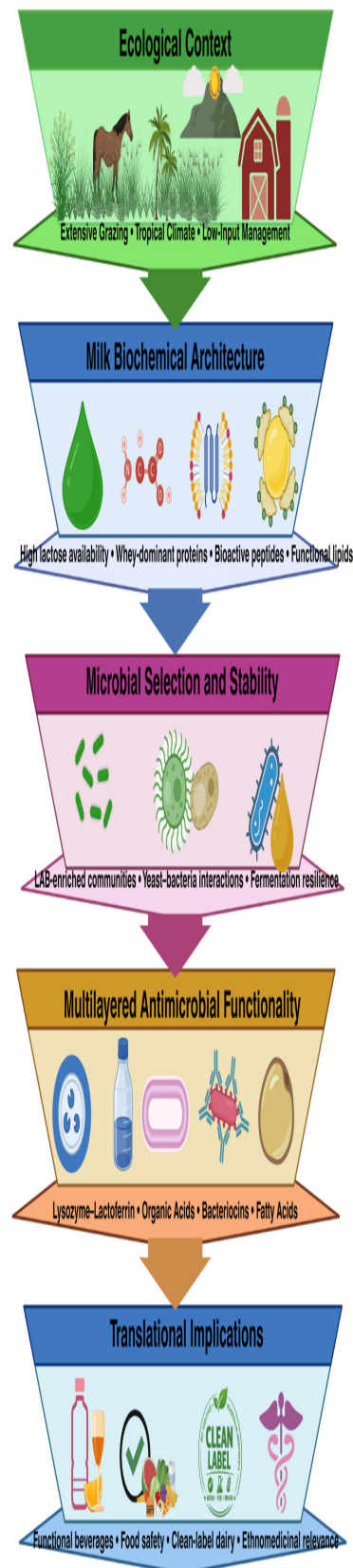


Figure 1: Conceptual framework illustrating Sumbawa mare's milk as a tropical probiotic-antimicrobial system. The model integrates low-input ecological context, milk biochemical architecture, microbial selection, and multilayered antimicrobial functionality. Boxes represent measurable or experimentally addressable variables derived from published evidence. Created with BioRender.com.

enriched fermentation systems and antimicrobial activity. However, the extent to which such traits consistently act as ecological filters favoring LAB dominance remains dependent on environmental conditions, management practices, and methodological approaches, and should therefore be interpreted with appropriate caution.

The antimicrobial functionality of mare's milk appears to arise from the combined action of host derived proteins, such as lysozyme and lactoferrin, and metabolites produced during LAB mediated fermentation, including organic acids and bacteriocins. Importantly, much of the current evidence supporting these effects is derived from *in vitro* assays, culture based studies, and observational analyses. Consequently, antimicrobial activity should be regarded as inhibitory potential rather than definitive pathogen exclusion, and probiotic potential should be clearly distinguished from clinically validated probiotic effects.

Within this broader framework, Sumbawa mare's milk provides a valuable tropical case study that illustrates how species specific milk architecture may support LAB enriched fermentation and antimicrobial functionality under low input conditions. While reported microbial patterns and functional outcomes show similarities to classical Central Asian koumiss systems, variability related to animal factors, hygiene practices, fermentation conditions, and analytical methodologies highlights the need for cautious generalization beyond specific contexts.

From a translational perspective, this integrative understanding offers a scientific basis for the development of clean-label fermented beverages and non thermal dairy products that leverage intrinsic milk functionality. Future research should prioritize longitudinal and seasonal studies of microbial succession, standardized safety and quality benchmarks, quantitative antimicrobial assessment, and *in vivo* or clinically relevant validation of functional and ethnomedicinal claims. Addressing these gaps will be essential for translating traditional mare's milk systems into safe, scalable, and sustainable functional dairy innovations across both temperate and tropical settings.

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NOVELTY STATEMENT

This review introduces a system level framework that integrates milk composition, microbial ecology, and antimicrobial functionality to conceptualize mare's milk as an intrinsic probiotic antimicrobial system, with Sumbawa mare's milk proposed as a tropical low-input model analogous to Central Asian koumiss fermentation.

AUTHOR'S CONTRIBUTION

RF: Conceptualization, investigation, writing-original draft, writing-review and editing, project administration. TES and LER: Conceptualization. PS, KUA and AS: Supervision. R: Writing-original draft, writing-review and editing, project administration. HE: Validation.

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GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The authors declare that generative AI tools were not used to generate scientific content, data interpretation, or conclusions in this manuscript. AI-assisted tools were used only for language editing and clarity improvement, and all content was carefully reviewed and validated by the authors. The authors take full responsibility for the integrity and originality of the work.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest with stakeholders related to the material written in this manuscript.

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