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Review

A Comprehensive Review on Equine Milk: Composition, Functional Properties, Technological Applications, and Future Perspectives

Claudia Pânzaru, Mădălina Alexandra Davidescu, Cristina Simeanu, Constantin Pascal, Alexandru Usturoi * and Marius Gheorghe Doliș

Faculty of Food and Animal Sciences, "Ion Ionescu de la Brad" Iasi University of Life Sciences, 700490 Iasi, Romania; claudia.panzaru@iuls.ro (C.P.); madalina.davidescu@iuls.ro (M.A.D.); cristina.simeanu@iuls.ro (C.S.); constantin.pascal@iuls.ro (C.P.); marius.dolis@iuls.ro (M.G.D.)

* Correspondence: alexandru.usturoi@iuls.ro

Abstract

Mare and donkey milk have attracted scientific and commercial interest due to their distinctive biochemical composition and functional potential as nutritional sources. Their compositional similarity to human milk—particularly regarding lactose content, protein profile, and mineral balance—makes them relevant alternatives for individuals with bovine milk intolerance and suitable candidates for nutraceutical and biomedical research. This systematic review provides an overview of the physicochemical properties of the milk of these species, examining the influence of breed, diet, and lactation stage on yield and composition. Particular attention is given to bioactive compounds, including lysozyme, lactoferrin, and immunoglobulins, which have been associated with antimicrobial, anti-inflammatory, and immunomodulatory activities. The review also discusses technological processing methods, such as fermentation, pasteurization, and lyophilization, and their effects on functional and therapeutic properties. Finally, current challenges in production and research are addressed, including the need for standardized protocols, sustainable management strategies, and further investigation into the health-related properties of mare and donkey milk.

Keywords: mare milk; donkey milk; bioactive proteins; lactation stage; dairy processing; nutritional variability

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1. Introduction

Mare and donkey milk has attracted considerable scientific and commercial scrutiny, primarily due to its distinctive nutritional and functional attributes, which differentiate it from widely consumed dairy alternatives [1–3]. The biochemical composition of this animal product, characterized by a judicious balance of proteins, lipids, lactose, and minerals, exhibits a close analogy to human milk. This compositional profile renders mare and donkey milk appropriate for populations with specialized dietary requirements, notably including infants, geriatric subjects, and those displaying intolerance to lactose [4–8].

Furthermore, the endogenous presence of bioactive constituents such as lysozyme, lactoferrin, and immunoglobulins underscores the substantial therapeutic potential of mare and donkey milk, particularly in relation to antimicrobial, anti-inflammatory, and immunomodulatory activities [9,10]. These compounds exert essential physiological functions, including inhibition of pathogenic microorganisms, regulation of immune responses,

and maintenance of gut microbiota homeostasis. Lysozyme provides potent antibacterial activity, lactoferrin acts as a multifunctional glycoprotein with antimicrobial and anti-inflammatory properties while facilitating the absorption and regulation of essential minerals such as iron, and immunoglobulins contribute to passive immune protection, especially in vulnerable populations such as infants and older adults. Collectively, the synergistic action of these bioactive components enhances the nutritional and health-promoting value of mare and donkey milk, supporting its use not only as a dietary alternative for individuals with specific nutritional requirements but also as a versatile substrate for the development of functional foods, dietary supplements, therapeutic formulations, and novel biomedical applications. Moreover, the concentration and biological activity of these compounds are influenced by factors such as breed, diet, and stage of lactation, highlighting the importance of optimized production and management practices to maximize the compositional and functional quality of mare and donkey milk [11,12]. Beyond its nutritional and health attributes, the milk of these species has generated commercial appeal as a fundamental raw material for functional foods, nutraceuticals, cosmeceuticals, and medical preparations, thereby demonstrating its increasing relevance across the food processing and biomedical research sectors [12–14]. This confluence of nutritional, functional, and economic determinants collectively substantiates the amplified research focus directed towards mare and donkey milk in recent decades [1,2,5–8,11,14].

Due to its marked similarity to human milk regarding its lactose content, protein fractionation, and mineral homeostasis, mare and donkey milk constitutes a significant substitute for individuals exhibiting lactose intolerance, simultaneously functioning as a highly prospective source for nutraceutical and biomedical utilization [15,16]. The comparatively lower casein concentration coupled with a higher ratio of whey proteins in the milk of these species ameliorates its digestibility, consequently mitigating the gastrointestinal distress frequently correlated with bovine milk intake [17,18].

Technological processing of mare and donkey milk, including fermentation, pasteurization, and lyophilization, is essential for ensuring safety, preserving bioactive and nutritional components, and enabling its sustainable application across human nutrition, nutraceutical, cosmetic, and biomedical sectors [13]. Importantly, integrating contemporary technologies with sustainable production practices can enhance the environmental performance of mare and donkey milk production. Equally critical is the management of animal welfare, which directly influences milk quality. Optimal nutrition ensures the availability of essential amino acids, fatty acids, and micronutrients that support both milk synthesis and bioactive compound content. Proper housing and environmental enrichment reduce stress, thereby stabilizing hormone levels such as cortisol and prolactin, which are key regulators of lactation and milk composition. Health monitoring and disease prevention further safeguard milk safety and consistency. Together, these welfare measures not only uphold ethical standards but also enhance milk yield, nutrient density, and the functional properties of mare and donkey milk, linking husbandry practices mechanistically to compositional outcomes [16,19,20]. This review aims to provide a comprehensive overview of mare and donkey milk research, addressing factors that influence its composition and production (breed, diet, and lactation stage), its functional characteristics, modern processing and preservation technologies, and its applications in human nutrition, cosmetics, and therapeutic fields. Additionally, current challenges and future research directions are discussed, highlighting the need for standardized production systems and the sustainable utilization of this emerging product in global markets.

2. Materials and Methods: Review Strategy

A systematic literature search was conducted to identify studies on mare and donkey milk, including mare and jennies (donkey) milk, with a focus on their biochemical composition, bioactive properties, and potential nutritional or therapeutic applications. The databases searched included MDPI, PubMed, Scopus, Web of Science, and Google Scholar, covering recent publications (up to 2025); also, several official websites were accessed to sustain ideas regarding the European regulations and worldwide equine populations. Studies were considered eligible if they were original research articles, clinical studies, or review papers published in English that reported on the biochemical composition, bioactive properties, or nutritional and therapeutic potential of mare and donkey milk. Publications were excluded if they lacked sufficient methodological detail, were non-peer-reviewed, consisted solely of conference abstracts without full text, or were published in languages other than English.

All identified articles were screened through a two-step process. Initially, titles and abstracts were reviewed to assess relevance, followed by a full-text review to confirm eligibility. Data extracted from each study included the study design, population or experimental model, equine species, intervention characteristics, and reported outcomes. Any discrepancies encountered during screening or data extraction were resolved through discussion and consensus among the authors. This structured approach was implemented to ensure transparency, reproducibility, and a clear rationale for study selection.

The search strategy employed keywords such as mares, jennies, milk, bioactive compounds, therapy, nutrition, thereby indicating the main aspects of this review.

3. Equine Population Dynamics and Production Across the Globe

3.1. Worldwide Equine Demographics

It is estimated that the total number of equines—including horses, donkeys, and mules—reaches approximately 116 million, of which 36 million are concentrated in 38 of the world's lowest-income countries [21]. Reliable population data are crucial not only for disease control and epidemiological research, but also for informing policy decisions on broader challenges such as climate change adaptation, water management, and global food security. While a recent report covering 36 countries highlighted key governance challenges and proposed potential solutions, the limited availability and quality of data restrict the capacity to assess equid populations accurately. Specific studies from countries including the United Kingdom, China, India, Mexico, Kenya, and Senegal illustrate that population distributions vary widely, reflecting differences in socio-economic reliance on equids, land use patterns, and cultural practices. From the estimated global population, 57 million are horses, 50.5 million are donkeys—with 99% concentrated in low- to middle-income countries (LMICs)—and 7.9 million are mules. However, the fact that 22 out of the 36 surveyed countries have not reported their equid populations over the past decade points to a systemic data gap that disproportionately affects donkeys, many of which are vital for subsistence farming and transport in LMICs yet are experiencing localized declines. This absence of reliable statistics undermines the ability to evaluate socio-economic contributions, anticipate emerging threats such as disease outbreaks or environmental pressures, and monitor cross-border movements that have implications for trade, biosecurity, and conservation. Consequently, the data deficiency is not merely an administrative challenge but a critical bottleneck that limits evidence-based policy formulation, targeted interventions, and the sustainable management of equid populations, particularly in regions where these animals are essential for livelihoods [3].

Evaluating the equine populations over a forty-year interval, it can be observed that in 1974 (Figure 1) the total number of registered horses was approximately 61.54 million, while

donkeys accounted for 39.68 million. By 2014 (Figure 2), these figures had shifted slightly, with 58.83 million horses and 42.76 million donkeys recorded globally. This comparison highlights a relative stability in horse populations over the four decades, accompanied by a modest increase in the number of donkeys. These trends may reflect changes in agricultural practices, mechanization, socio-economic factors, and the evolving roles of equids in transport, labor, and subsistence economies. The data underscore the importance of continuous monitoring of equine populations to inform sustainable management strategies and support the conservation and welfare of these animals worldwide [21].

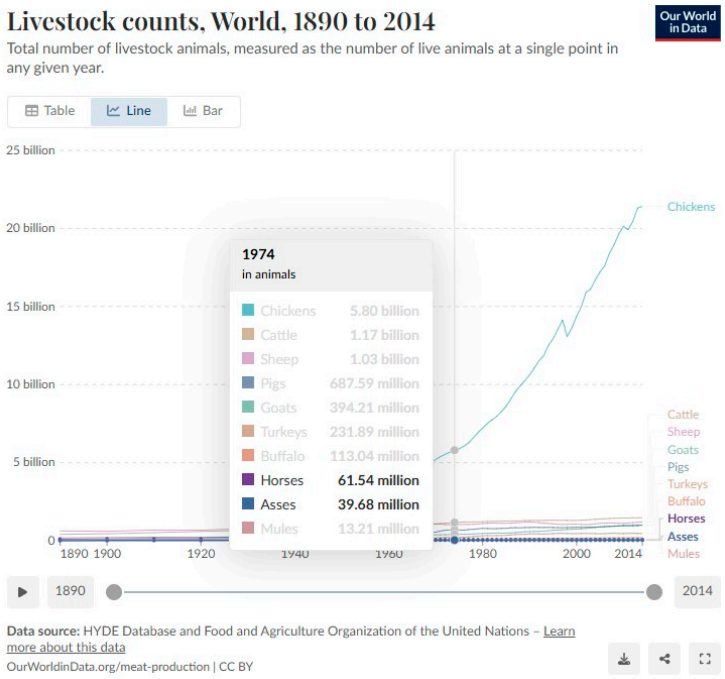


Figure 1. The population dynamics of horses and donkeys (1974) [21].

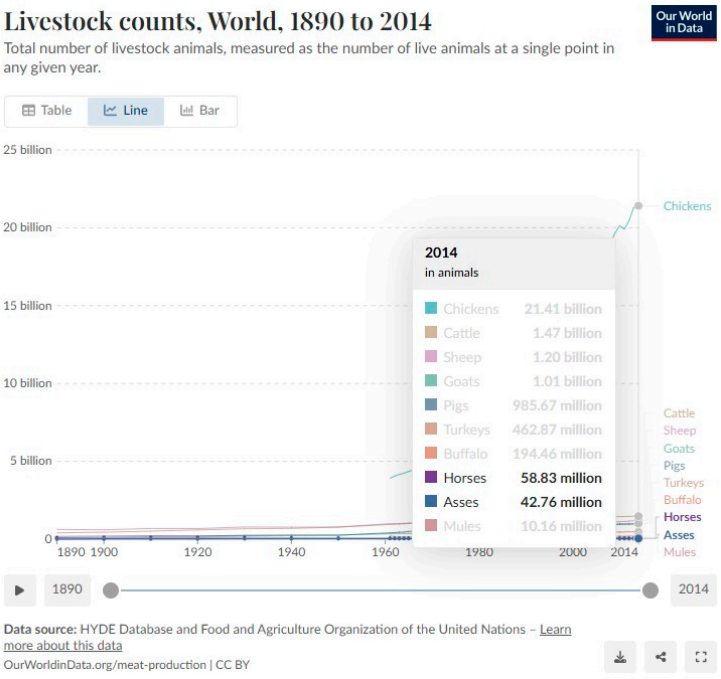


Figure 2. The population dynamics of horses and donkeys (2014) [21].

3.2. Equine Populations Utilized for Milk Production

While the global dairy sector remains predominantly dominated by bovine species, with buffaloes, goats, and sheep contributing the majority of the residual milk volume, equine species—specifically horses (*Equus caballus*) and donkeys (*Equus asinus*)—represent an emerging domain of nutritional, socio-economic, and scientific interest. Although mare and donkey milk production is currently considered a niche market, it contributes to diversifying global dairy resources, particularly in regions facing environmental constraints such as climate variability, water scarcity, and land degradation. The resilience of equines in marginal ecosystems arises from their adaptive foraging behavior, tolerance to suboptimal feed and water availability, and robust reproductive and health traits. These characteristics not only enhance the stability of milk production under variable environmental conditions but also support sustainable rural livelihoods by providing a reliable source of nutrition and income. Consequently, equine dairy systems offer quantifiable benefits for local food security, resource efficiency, and ecosystem-based management strategies, linking resilience and sustainability to measurable ecological and socio-economic outcomes [20].

Globally, the availability of precise census data concerning the population of lactating mares and jennies is constrained by heterogeneous national livestock reporting frameworks and the inherently marginal scale of production operations [22]. Notwithstanding, while only a small fraction of the estimated equids worldwide undergoes milking, their regional socio-economic and nutritional contribution remains demonstrably substantial. The estimated total production presented in Table 1 was calculated using the formula: Estimated total production = Livestock (N) × Lactating individuals (%) × Annual production per individual (liters), based on available national and regional livestock data. Table 1 presents the estimated milk yield per female and total annual production for mares and jennies for the period 2019–2022 [23].

Table 1. Worldwide milk production of mares and jennies (female donkeys).

Year	Species	Livestock (N)	Lactating Individuals (%)	Annual Production/Individual (Liters)	Estimated Total Production (Liters/Year) [c]
2019	Mares [a]	58,819,179	20	2160	~25.4 billion
	Jennies [b]	51,603,917	15	336	~2.6 billion
2020	Mares [a]	59,592,065	20	2160	~25.7 billion
	Jennies [b]	52,780,596	15	336	~2.65 billion
2021	Mares [a]	59,780,596	20	2160	~25.8 billion
	Jennies [b]	52,075,158	15	336	~2.63 billion
2022	Mares [a]	60,581,289	20	2160	~26.2 billion
	Jennies [b]	51,687,273	15	336	~2.61 billion

Notes: [a] [13], [b] [21], [c] [24].

Central Asia, particularly Mongolia and Kazakhstan, remains the primary hub for mare's milk production, where it is deeply embedded in pastoral heritage and local food systems. In these regions, mare's milk is consumed fresh or traditionally fermented into kumîs, a beverage resulting from lactic acid and alcoholic fermentation. China has become a major global producer of donkey milk, with annual production reaching approximately 270,000 metric tons, driven by growing demand for hypoallergenic milk alternatives and nutraceutical applications [24–28]. Donkey milk is prized for its digestibility and low allergenicity, making it suitable for pediatric nutrition and individuals with bovine milk protein sensitivity. In Mediterranean countries such as Italy, donkey milk is increasingly valorized

in specialized cosmetic products and as a high-value nutritional option, highlighting its expanding importance beyond traditional Asian production centers [14,27–29].

In Europe, mare and donkey milk production remains limited in scale but is increasingly emerging as a structured, high-value niche within the dairy sector. In countries such as France, Italy, and parts of Central Europe, dedicated equine dairies operate at semi-industrial levels, transitioning from artisanal practices toward controlled, traceable, and economically viable systems. These enterprises often involve indigenous horse breeds, integrating dairy production with breed conservation, welfare-focused management, and multifunctional agriculture. The adaptability of equines allows efficient use of marginal areas where conventional cattle farming is constrained, supporting extensive grazing, landscape maintenance, and reduced environmental impact. European equine dairy systems align with EU policy objectives, promoting rural diversification, sustainability, and resilience, while occupying a premium market niche that emphasizes ethical and nature-friendly production. Realizing the full socio-economic and environmental potential of this sector requires coordinated policy frameworks, targeted research, and further standardization in traceability, food safety, and labeling. Consequently, mare and donkey milk production represents both a continuation of pastoral heritage and a model of sustainable innovation, delivering economic, social, and ecological benefits in response to climate change and evolving consumer demand for functional and ethically produced foods [30–32].

Equine dairying requires distinct management compared with conventional bovine systems. Milk production in mares occurs only in the presence of their foals, necessitating multiple daily milking sessions and close human–animal interaction. These operational constraints limit large-scale productivity but inherently promote welfare-centered practices, which are critical for maintaining milk quality, yield, and bioactive composition, including compounds such as lactoferrin and lysozyme. Stress mitigation is essential, as acute stress can inhibit oxytocin release and reduce milk output. These management requirements also reinforce consumer perceptions of naturalness and authenticity, strengthening the niche market positioning of mare and donkey milk as a high-quality, ethically produced, and functionally beneficial product mare and donkey milk [3,33–35].

The European sector for mare and donkey milk exemplifies the crucial intersection between nutritional science and strategic market differentiation. Owing to its intrinsic compositional analogy to human milk and its hypoallergenic properties, mare and donkey milk has garnered significant interest for both pediatric feeding and specialized dermatological applications, thereby offering potential alternative products for infants and individuals facing specific dietary sensitivities. However, stringent regulations enforced by the European Food Safety Authority (EFSA) severely constrain the scope for making explicit health-related claims (health claims) on product labeling. This regulatory environment underscores the critical necessity for coordinated scientific research and product standardization across the industry. In practical terms, sustainable market expansion requires a dual and clearly differentiated effort. On the scientific level, significant investment is needed to address existing evidence gaps through well-designed, placebo-controlled clinical trials and mechanistic studies capable of substantiating proposed health-related effects. In parallel, from a regulatory perspective, the absence of harmonized quality standards, traceability systems, and product specifications represents a key constraint that must be addressed to ensure compliance with food safety and health-claim regulations. Strengthening this scientific and regulatory framework is essential for building a robust evidence base that can support commercial positioning and withstand scrutiny by regulatory authorities [33,36–38].

In Germany, small-scale operations such as Lindenhof Stud in Brandenburg integrate mare milking with horse breeding, agritourism, and direct-to-consumer sales. Each mare

produces approximately one liter of surplus milk per day, which is frozen to preserve quality. Rich in vitamin C and iron but low in fat and casein, the milk retails at up to 10 EUR per liter, reflecting its artisanal and health-oriented character. Across Europe, fewer than 30 mare's milk producers operate—mainly in Germany, France, Belgium, and the Netherlands—under strict food safety and labeling regulations [38]. Table 2 contains data regarding the qualitative estimates of mare and donkey milk production in Europe (data collected during the interval 2019–2022).

Table 2. Milk production of mares and jennies (female donkeys) in Europe.

Year	Species *	Annual Production (Tonnes)	Principal European Producers
2019–2022	Mares	200–300	France, Belgium, Netherlands, Italy
	Jennies	Several hundred	Italy, Greece, Spain

Notes: * [21].

Despite its limited scale, consumer interest in mare and donkey milk is gradually increasing, particularly among individuals seeking alternative nutritional options in the context of dermatological or gastrointestinal conditions. However, robust clinical evidence supporting specific therapeutic effects remains limited, and any potential anti-inflammatory or immunomodulatory properties are currently under investigation. However, under current EU regulations, producers are prohibited from making curative or therapeutic claims without approval from the European Food Safety Authority (EFSA), which is still evaluating the available scientific data [38,39].

Milk obtained from mares and donkeys offers distinctive nutritional, functional, and socio-economic benefits, supporting rural livelihoods, breed conservation, and sustainable management of marginal landscapes. However, the literature demonstrates considerable variability in protein quantification methods, a lack of standardized clinical trials—particularly for Cow's Milk Protein Allergy (CMPA)—and inconsistent reporting on differences between raw and processed milk. These methodological limitations hinder direct comparisons and robust conclusions. Addressing these gaps through rigorous, harmonized research is essential to substantiate health claims, optimize production, and fully leverage mare and donkey milk's potential in contemporary and future dairy systems.

4. Mare and Donkey Milk: Therapeutic Potential and Role in Human Nutrition

4.1. Historical Overview

The medicinal and nutritional properties of mare and donkey milk have been recognized since antiquity, with early insights shaping contemporary understanding [40–43]. Herodotus (c. 484–c. 425 BCE) provides one of the earliest accounts of mare's milk use among nomadic steppe peoples, describing specialized milking practices and the centrality of fermented mare's milk, notably kumîs, to their diet [40,41]. Hippocrates (c. 460–c. 370 BCE) discussed mare and donkey milk therapeutically, recommending it for digestive, respiratory, and convalescent support [41]. Later authors, including Pliny the Elder (c. 23–79 CE) and Galen (c. 129–c. 216 CE), emphasized its restorative and nutritive qualities, prescribing it for pulmonary weakness, chronic digestive disorders, and systemic debilitation [42,44].

During the Middle Ages, mare and donkey milk continued to be valued across Eurasia. Marco Polo (1254–1324 CE) noted that kumîs provided hydration and substantial caloric value for Mongol populations [45]. In the Islamic Golden Age, Avicenna (c. 980–1037 CE) described mare's milk as light, easily digestible, anti-inflammatory, and suitable for chronic

conditions, particularly weakness or malabsorption [46]. Ethnographic records further highlight the cultural significance of mare's milk among Turkic, Kazakh, Kyrgyz, and Bashkir communities, where fermentation and kumîs consumption were linked to food, medicinal use, and social rituals [46–50]. Current research substantiates historical observations by characterizing mare and donkey milk's unique biochemical profile. Compared with bovine milk, mare and donkey milk has higher lactose content, enhancing palatability and supporting probiotic fermentation, and lower casein, particularly α-casein, conferring superior digestibility and reduced allergenicity. Elevated levels of lysozyme and lactoferrin provide antimicrobial, immunomodulatory, and anti-inflammatory effects, while the whey-to-casein ratio closely mirrors that of human milk, facilitating assimilation in pediatric populations and individuals with gastric sensitivities. The lipid fraction is rich in polyunsaturated fatty acids, including linoleic and α-linolenic acids, essential for cardiovascular and immune health. Bioactive peptides may contribute to antioxidant defense and metabolic regulation. These properties have driven interest in clinical applications, including Cow's Milk Protein Allergy, atopic dermatitis, and gastrointestinal or metabolic dysfunctions, with ongoing research investigating effects on mucosal immunity, gut microbiota, and dermal barrier function [51–58].

Mare and donkey milk occupies a nexus of ethnocultural heritage and scientific scrutiny. Historically central to the lifeways of nomadic Eurasian societies, mare's milk—often fermented into kumîs—served as both a vital nutritional and ethnomedicinal resource across Kazakhstan, Kyrgyzstan, Mongolia, and Siberia. Its production and consumption were deeply intertwined with pastoral mobility, seasonal rhythms, and communal identity, reflecting sophisticated indigenous knowledge of herd management and fermentation. Today, mare and donkey milk is the subject of multidisciplinary investigation into its nutritional, immunological, therapeutic, and socio-economic value, bridging traditional practice with modern scientific validation [59].

The revitalization of mare and donkey milk production illustrates the revalorization of traditional foods in response to modern nutritional demands, including hypoallergenic alternatives and interest in naturally fermented products. In Kazakhstan, kumîs continues as both a heritage food and emerging economic sector, supported by smallholders and commercial dairies that integrate traditional knowledge with contemporary hygiene and processing standards. Mongolia maintains a large seasonal kumîs system embedded in nomadic mobility, with recent studies exploring microbial biodiversity in traditional fermentation vessels and potential health benefits. In China, particularly Inner Mongolia and Xinjiang, mare's milk and kumîs production have expanded under mixed models of cultural preservation, rural development, and functional-food marketing [59–61].

Similarly, the intrinsic properties of mare and donkey milk strategically position this resource at the crucial intersection of established ethnocultural heritage and contemporary scientific scrutiny. The milk obtained from these species, historically integral to the subsistence strategies and lifeways of nomadic Eurasian cultures, exemplifies a compelling continuum, bridging ancestral practice with modern scientific inquiry. Its utilization is evidenced by a profound historical footprint, having functioned for millennia as an essential nutritional, ethnomedicinal, and ceremonial commodity within the pastoral societies of Central Asia (e.g., Kazakhstan, Kyrgyzstan, Mongolia, and parts of Siberia) [62].

Mare and donkey milk systems exemplify how traditional animal-derived products are integrated into contemporary scientific and policy frameworks, addressing biodiversity conservation, sustainable pastoralism, niche dairy markets, and resilient food systems. With growing demand for functional and hypoallergenic dairy products, mare and donkey milk bridges centuries of pastoral practice with modern advancements in dairy technology, microbiology, clinical nutrition, and socio-economic research. Its combination of cultural

resilience and emerging scientific validation positions it not only as a heritage food but also as a contributor to diversified dairy production, rural development, and sustainable animal-derived nutrition [31,36–39,63,64].

In summary, historical sources document the use of mare and donkey milk for nutritional and medicinal purposes since antiquity, particularly among nomadic Eurasian populations, where fermented products such as kumîs were integral to diet and culture. Classical and medieval medical authors described equine milk as easily digestible and restorative. Modern research has revisited these observations by characterizing its biochemical composition, including lactose content and bioactive proteins, while highlighting substantial variability across breeds, production systems, and processing practices.

4.2. Mechanistic Basis of the Functional and Health-Related Properties of Mare and Donkey Milk

While numerous nutritional and health-related benefits of mare and jenny milk have been reported, a mechanistic understanding of the biological pathways underpinning these effects remains comparatively underdeveloped. Current data indicate that the functional properties of mare and donkey milk arise primarily from its distinctive whey protein profile, antimicrobial enzymes, and bioactive peptides generated during digestion or processing.

Lactoferrin represents one of the most biologically active components of mare and donkey milk and plays a central role in immune modulation, antimicrobial defense, and iron homeostasis. Mechanistically, lactoferrin exerts antimicrobial effects through iron sequestration, depriving pathogenic microorganisms of an essential growth factor [65]. Beyond this bacteriostatic function, lactoferrin can directly interact with microbial membranes, increasing permeability and inducing cell damage. At the host level, lactoferrin has been shown to modulate inflammatory signaling pathways, notably through the regulation of nuclear factor kappa B (NF-κB). By inhibiting NF-κB activation, lactoferrin can downregulate the expression of pro-inflammatory cytokines, thereby contributing to anti-inflammatory and immunomodulatory effects [66,67]. These mechanisms are particularly relevant in the context of gastrointestinal inflammation, allergy-related immune responses, and mucosal immunity, which are frequently cited targets for mare and donkey milk consumption [68].

Lysozyme, present at substantially higher concentrations in mare and donkey milk compared with bovine milk, provides a second key mechanistic pathway. Lysozyme catalyzes the hydrolysis of β-(1,4)-glycosidic bonds in the peptidoglycan layer of Gram-positive bacterial cell walls, leading to osmotic lysis and microbial death. This enzymatic activity contributes directly to the antimicrobial spectrum of mare and donkey milk and supports gut microbial balance by suppressing pathogenic bacteria while allowing commensal populations to persist. Additionally, lysozyme may indirectly modulate immune responses by reducing pathogen-associated molecular patterns (PAMPs) that trigger inflammatory signaling cascades in the intestinal epithelium [69].

Bioactive peptides generated from mare and donkey milk proteins during gastrointestinal digestion or fermentation represent another major mechanistic link between composition and physiological outcomes. Enzymatic hydrolysis of whey proteins and, to a lesser extent, caseins can release peptides with antioxidant, antihypertensive, and immunomodulatory properties. Antioxidant peptides exert their effects through free radical scavenging, metal chelation, and inhibition of lipid peroxidation, thereby contributing to cellular protection against oxidative stress. Other peptides exhibit angiotensin-converting enzyme (ACE) inhibitory activity, providing a plausible mechanistic basis for the modest antihypertensive effects reported in some experimental models. These mechanisms align with broader evidence from dairy peptide research, although equine-specific peptide sequences and their bioavailability remain insufficiently characterized [70–73].

Processing methods exert a critical influence on these mechanistic pathways. Pasteurization, when carefully controlled (LTLT or HTST), generally preserves lactoferrin and lysozyme activity, whereas ultra-high-temperature treatments may induce protein denaturation, reduce enzymatic activity, and alter peptide release patterns. Fermentation can further modulate bioactivity by promoting proteolysis, increasing peptide diversity, and enhancing mineral and vitamin bioavailability. Consequently, differences in reported bioactivity across studies may reflect not only biological variability but also methodological heterogeneity in processing conditions [74–77].

In summary, the functional effects attributed to mare and donkey milk can be mechanistically linked to well-defined biological pathways involving antimicrobial defense, immune modulation, oxidative stress regulation, and cardiovascular signaling. However, the integration of these mechanisms remains fragmented across the literature [14,16–18,63,66,76–78]. Future research should prioritize mechanistic studies that systematically link milk composition, processing-induced modifications, and bioactive compound stability with measurable physiological outcomes. The inclusion of integrative schematic models connecting mare and donkey milk components, processing pathways, and target biological mechanisms would substantially enhance conceptual clarity and translational relevance.

4.3. Contemporary Trends and Market Integration of Mare and Donkey Milk Within Global Dairy Systems

4.3.1. Transition from Traditional Use to Global Consumption

In the contemporary era, mare and donkey milk has evolved from a regionally traditional product into a globally recognized commodity. It now features in functional nutrition, therapeutic diets, artisanal food production, and sustainable rural development. This transition reflects the convergence of scientific validation, consumer health trends, cultural valorization, and the diversification of dairy systems in both high-income and emerging economies. While its use remains rooted in Eurasian pastoral traditions, mare and donkey milk's modern role highlights the growing importance of alternative dairy species and biologically active, minimally processed products. Understanding its consumption today requires a multidimensional framework integrating nutritional biochemistry, clinical applications, socio-economic dynamics, and evolving food culture mare and donkey milk [3,65].

4.3.2. Nutritional and Biomedical Context of Contemporary Consumption

Renewed interest in mare and donkey milk stems from its distinctive composition and suitability for individuals with specific dietary needs. Donkey and mare milk are highly digestible, low in casein—particularly αs1-casein—and possess a whey-to-casein ratio similar to human milk, making them appropriate for infants with Cow's Milk Protein Allergy (CMPA) or gastrointestinal intolerance [66]. Elevated levels of immunomodulatory proteins, including lysozyme, lactoferrin, and serum albumin, along with oligosaccharides, confer antimicrobial and anti-inflammatory effects, supporting gastrointestinal homeostasis and favorable modulation of the intestinal microbiota. These properties have prompted clinical investigation into mare and donkey milk's potential for managing atopic dermatitis, mild gastrointestinal disorders, post-antibiotic recovery, and metabolic dysregulation. Its lipid profile, characterized by moderate fat content, a high proportion of polyunsaturated fatty acids, and a balanced omega-6 to omega-3 ratio, aligns with contemporary nutritional priorities emphasizing cardiovascular health and anti-inflammatory diets. As a result, mare and donkey milk is increasingly incorporated into functional foods, preventive health strategies, sports nutrition, and anti-aging dietary protocols [8,11,14–17,66].

4.3.3. Applications in Cosmetics and Dermatology

Mare and donkey milk has emerged as a valuable bioactive ingredient in cosmetics and dermatology. Topical products such as creams, soaps, and emulsions leverage its biochemical profile—including lysozyme, lactoferrin, and vitamins A, C, and E—supporting antimicrobial, antioxidant, and regenerative effects. This positions mare and donkey milk within premium cosmetic lines, spa products, and therapeutic skincare for sensitive or immunocompromised skin. European producers, particularly in Italy and Spain, have developed branded cosmetic lines that integrate traditional knowledge, maintain high animal welfare standards, and use artisanal processing techniques. These initiatives support rural entrepreneurship and contribute to the conservation of local equine breeds, including the Martina Franca, Ragusana, and Zamorano-Leonesa, whose preservation is now linked to the economic viability of milk production. This model demonstrates the synergy between socio-economic development and biodiversity conservation [67,72].

4.3.4. Socio-Economic Dimensions and Rural Development

The socio-economic dimensions of modern mare and donkey milk consumption are equally significant. In Europe, the expansion of regulated donkey and mare dairies has occurred primarily in Italy, France, Belgium, Greece, and parts of Eastern Europe, where producers have implemented short supply chains that emphasize freshness, traceability, and controlled hygienic conditions. Italy stands out as a leader in donkey milk production, with regions such as Sardinia, Emilia-Romagna, and Tuscany developing integrated systems that combine dairy production with agritourism, on-farm education, and biodiversity conservation. These activities contribute not only to local economies but also to the preservation of endangered donkey breeds such as Amiata, Ragusano, and Martina Franca, positioning dairy production as a tool for genetic conservation and rural sustainability [68,69].

4.3.5. Regional Case Studies Beyond Europe

The global trend extends beyond Europe to regions with strong equine traditions. In Kazakhstan, Kyrgyzstan, and Mongolia, modernization of kumîs production has enabled traditional fermented mare's milk to reach urban and international markets, supported by state programs that reinforce national food heritage and enhance the economic resilience of nomadic households. In China, rising consumer demand for functional and premium dairy products has driven rapid expansion of mare and donkey milk production. In Inner Mongolia and Xinjiang, mare and donkey dairies employ modern equipment to produce pasteurized milk, freeze-dried powders, and fermented beverages targeted at urban middle-class consumers. By combining traditional heritage with contemporary quality standards, Chinese companies are establishing a growing niche for mare and donkey milk. This global expansion highlights the sector's multifaceted significance and its links to socio-economic development and biodiversity conservation [72].

Modern consumption of mare and donkey milk reflects a global preference for authenticity, naturalness, and tradition-based nutrition. Consumers increasingly view it as a product of cultural continuity, artisanal craftsmanship, and high animal welfare standards. Marketing emphasizes nomadic heritage, traditional fermentation practices, and the symbolic importance of horses and donkeys in pastoral societies, enhancing its value as a specialty food within the broader dairy economy [3,68,69,72]. The sector is also characterized by environmental and sustainability considerations: mare and donkey dairies typically operate under extensive or semi-extensive systems with low inputs, supporting landscape preservation, low stocking densities, and sustainable use of marginal or mountainous terrains. The foal-at-foot milking system further ensures high animal welfare, aligning with

modern ethical expectations. Collectively, these factors underscore the multifaceted significance of the equine dairy sector, linking efficacy, socio-economic development, cultural continuity, and biodiversity [3,69,72].

4.4. Composition and Bioactivity of Mare and Donkey Milk

4.4.1. Protein Composition and Digestibility

While functional compounds are present across all domestic milks, mare and donkey milk is distinguished by compositional and functional differences that influence digestibility, immunological activity, and potential health effects in humans [73]. Proteins are the primary bioactive macromolecules in all mammalian milks, providing nutrition and contributing to antimicrobial defense, immune modulation, and gastrointestinal development. In ruminant milks—such as cow, goat, and sheep—caseins dominate (approximately 60–80% of total proteins), forming firm curds under gastric conditions that slow nutrient release and absorption [74]. Caseins also provide milk opacity and act as carriers for calcium and phosphorus, supporting neonatal bone growth. Whey proteins, including β-lactoglobulin, α-lactalbumin, serum albumin, lactoferrin, and immunoglobulins, constitute the remaining fraction, are more rapidly digested, and deliver bioactive peptides with high biological value [79]. Minor but functionally relevant enzymes, such as lipases and lactoperoxidase, along with antimicrobial proteins like lactoferrin and lysozyme, contribute to innate immune protection and influence microbiological stability and fermentation dynamics. Compared with ruminant milks, mare and donkey milk's higher whey-to-casein ratio and lower αs1-casein content support faster digestion, enhanced nutrient assimilation, and reduced allergenicity, underlining its potential functional and therapeutic applications [75,76].

In contrast to ruminant milks, mare and donkey milk features a casein-to-whey ratio closely resembling human milk, with caseins comprising only 40–60% of total protein and whey proteins making up the remainder, though proportions vary with lactation stage and individual differences. Notably, mare and donkey milk contains very low or negligible αs1-casein, the main protein linked to allergenicity in bovine milk, conferring reduced allergenic potential. Clinical studies support its suitability as a nutritional substitute for infants and individuals with Cow's Milk Protein Allergy (CMPA) or digestive sensitivities.

Among whey proteins, α-lactalbumin plays a critical functional role beyond nutrition. Its high tryptophan content supports serotonin production and neurodevelopment in infants and contributes to mood regulation and cognitive function in adults and elderly individuals. Additionally, bioactive peptides released during digestion exhibit antimicrobial and immunomodulatory effects, enhancing gut barrier integrity and supporting host defense. These mechanistic features underlie mare and donkey milk's suitability as a dietary alternative for infants and individuals with Cow's Milk Protein Allergy (CMPA), as well as its potential benefits in elderly nutrition [74].

4.4.2. Bioactive Proteins and Immunological Activity

Functionally, mare and donkey milk is distinguished by high concentrations of lysozyme—particularly in donkey milk, where levels can exceed those in bovine milk by an order of magnitude—providing broad-spectrum antimicrobial defense. Lactoferrin, while lower than in human milk, remains biologically relevant, supporting iron homeostasis and mucosal immunity [51–54,59–61].

4.4.3. Bioactive Peptides and Fermentation-Derived Functionality

Beyond its protein fraction, mare and donkey milk provides bioactive peptides formed during digestion or fermentation, exhibiting antioxidant, anti-inflammatory, antihypertensive, and microbiota-modulating activities. Its higher lactose content supports ben-

eficial lactic fermentations, while lower total fat and saturated fatty acid levels make it a "lighter" dairy option, suitable for individuals with metabolic or cardiovascular concerns [3,53,60,61,80–84].

4.4.4. Comparative Protein Profiles Across Mammalian Species

Compared with ruminant milks, mare and donkey milk's high whey content, minimal α-casein, and elevated antimicrobial components—particularly lysozyme—confer superior digestibility, immunological support, and therapeutic potential. These features underpin its growing use as a complementary or alternative resource in specialized diets, clinical nutrition, and functional dairy products for sensitive or health-conscious consumers [85–89]. The comparison of these compositional and functional aspects across species is further detailed in Table 3.

Table 3. Variations in Bioactive and Functional Milk Constituents among Animal Species and Breeds.

	Species						
Item	**Mare** [a]	**Jenny** [b]	**Cow** [c]	**Sheep** [d]	**Goat** [e]	**Buffalo** [f]	**Camel** [g]
Casein (%)	1.1–1.4	0.7–1.0	2.6–2.8	3.8–4.2	2.4–2.7	3.2–4.0	1.6–2.8
Bioactive proteins/peptides	Lysozyme, lactoperoxidase, cytokines, anti-inflammatory peptides	Lysozyme, lactoferrin, immunoglobulins, growth factors	Beta-lactoglobulin, casein-derived peptides, immune-active peptides	Antioxidant peptides, lactoperoxidase	Gut-health peptides	ACE-inhibitory peptides, lactoferrin, lysozyme	Lactoferrin, lysozyme, immunoglobulins, lactoperoxidase, peptidoglycan recognition protein
Bioactive peptides (mg/mL)	1.5–2.5	1.0–1.8	2.0–4.0	3.5–5.0	2.5–3.5	-	0.9–2.9
Conjugated linoleic acid (CLA, mg/g fat)	1.0–1.5	1.0–1.2	3.5–7.0	6.0–11.0	4.0–8.0	-	-
Omega-3 fatty acids (mg/100 g fat)	70–100	60–90	30–50	40–60	20–40	-	-
Unsaturated fats (%)	60–70	55–65	25–35	30–40	27–35	-	-
Vitamins (general)	A, B-complex, C, D, E	A, B-complex, C, D, E	A, B-complex, C, D, E	A, B-complex, C, D, E	A, B-complex, C, D, E	A, B-complex, C, D, E	A, B-complex, C, D, E
Carotenoids (µg/100 mL)	0.3–0.5	0.2–0.4	15–30	20–40	8–15	12–25	0.1–0.3
Minerals (mg/100 mL)	Ca 110–130, P 85–100, Mg 10–15, K 150–200, Na 35–40	Ca 35–45, P 20–30, Mg 6–10, K 150–200, Na 15–20	Ca 120–130, P 90–100, Mg 10–15, K 140–150, Na 40–50	Ca 160–180, P 110–130, Mg 20–25, K 150–200, Na 45–55	Ca 120–140, P 90–100, Mg 10–15, K 180–200, Na 40–50	Ca 120–140, P 90–110, Mg 12–18, K 150–180, Na 40–50	Ca 90–110, P 60–90, Mg 8–12, K 120–160, Na 30–40
Digestibility	High	Very high	Moderate	Low–moderate	High	Moderate–high	High

Notes: [a] [63], [b] [68], [c] [75], [d,e] [73], [f] [74], [g] [75].

Bioactive peptides, released from precursor proteins during digestion or fermentation, are central to the functional value of mammalian milks. Mare and donkey milk offers

distinct advantages over ruminant (cow, sheep, goat, buffalo) and camel milks due to its unique protein composition and superior digestibility. Its high whey-to-casein ratio results in soft, flocculent curds that are rapidly broken down by digestive enzymes, allowing efficient release of bioactive peptides with antihypertensive, antioxidant, anti-inflammatory, and microbiota-modulating activities. Additionally, the high lactose content acts as a prebiotic substrate, promoting lactic acid bacterial growth during fermentation (e.g., kumîs) and generating further microbiota-modulating peptides [74].

Mare and donkey milk contain approximately 1.1–1.6% protein, of which 50–60% is whey, whereas cow, sheep, and buffalo milks are dominated by caseins (>75–80%), producing firmer curds, slower digestibility, and higher allergenicity. Goat milk, while somewhat more digestible than cow milk, does not match mare and donkey milk's digestibility [76–80]. Equine whey proteins—α-lactalbumin, β-lactoglobulin, serum albumin, lactoferrin, lysozyme, and immunoglobulins—contribute substantially to nutritional and immunological value. α-Lactalbumin provides essential amino acids, including tryptophan, supporting infants, the elderly, and convalescent patients. β-Lactoglobulin, structurally distinct from the bovine isoform, exhibits reduced allergenicity. High levels of lactoferrin and lysozyme confer antimicrobial, immunomodulatory, and iron-binding activities, enhancing biochemical stability and health potential. While camel milk shares some properties, mare and donkey milk's overall bioactive profile more closely resembles human [81–83].

Bioactive peptides released during digestion or fermentation enhance the trophic and health-promoting properties of mare and donkey milks, exhibiting antioxidant, antimicrobial, antihypertensive, and immunomodulatory effects. These attributes contribute to donkey milk being classified in some literature as a nutraceutical or "pharmafood," and its peptide activity generally more closely resembles that of human milk compared with ruminant milks [84–86].

4.4.5. Lipid Content and Fatty Acid Composition

Mare and donkey milk is distinguished among dairy species by low lipid content, favorable fatty-acid composition, low atherogenic and thrombogenic indices, high whey-protein proportion, rich bioactive-protein profile, and low allergenicity. Camel milk offers intermediate benefits, with improved fatty-acid balance and bioactive compounds relative to ruminant milks, whereas bovine, ovine, and caprine milks, despite higher nutrient density, have higher saturated-fat levels, stronger casein dominance, and different digestibility and biological functionality. These compositional distinctions underscore the unique nutritional and health-promoting value of mare and donkey milks for consumers requiring easily digestible, hypoallergenic, or functionally bioactive dairy alternatives [87].

Table 4 illustrates the differences between species concerning the key compositional and processing factors that determine the functional value of milk.

The fat content and composition of mammalian milks vary widely among species, influencing nutritional quality, digestibility, functional properties, and health-related indices. Mare and donkey milks are among the lowest in fat, with mare milk containing approximately 1.0–1.3% and donkey milk often below 1%, closely resembling human milk. This low lipid content, combined with high lactose and low casein levels, supports rapid gastric emptying, easy digestibility, and functional similarity to human milk. These properties make mare and donkey milk a suitable alternative for sensitive populations, including infants with Cow's Milk Protein Allergy, although clinical tolerance should be assessed individually [89–94]. Comparison between various species regarding the fat content is illustrated in Table 5.

Table 4. Compositional and processing-related bio-functionality parameters.

Milk Type	Dominant Precursor Proteins	Primary Peptide Release Mechanism	Major Functional Proteins	Functional Advantage [d]
Equine (mares and jennies) [a]	whey-dominant (40–60%), casein-low (αs1-casein minimal)	enzymatic hydrolysis during consumption; fermentation (e.g., kumîs)	mares: high α-La, lysozyme, lactoferrin female donkeys: lysozyme-rich, lactoferrin, Ig	easy and rapid release of small peptides due to soft curd formation and low αs1-casein content.
Ruminants (cow, sheep, goat) [b]	casein-dominant (80%+) (αs1 and β-caseins)	enzymatic hydrolysis during digestion (slow); specific technological processing (cheese-making)	bovine: low lysozyme, moderate LF ovine: high total protein caprine: some bioactivity	high yield of specific peptides (e.g., β-casomorphins) but slower release due to firm gastric curds
Camel [c]	casein-to-whey ratio is closer to ruminants, but lacks β-lactoglobulin; rich in immunoglobulins	enzymatic hydrolysis; fermentation (traditional Shubat)	high lactoferrin	unique peptide profile due to the absence of β-lactoglobulin and different casein micelle structure, offering high stability

Notes: [a] [76], [b] [75], [c] [48]; [d] Functional Advantage = species-characteristic differences in milk protein digestion and peptide release.

Table 5. The fat content in various species' milk.

	Species						
Item	**Mare [a]**	**Jenny [b]**	**Cow [c]**	**Sheep [d]**	**Goat [e]**	**Buffalo [f]**	**Camel [g]**
Average fat content (%)	1.2–4.2	0.4–1.2	3.5–4.6	≥7.1	4.1–6.7	6.0–15.0	3.8–5.4
Key fatty acid/ compound notes	Low total fat High lactose content	Low total fat High lactose content	Mix of unsaturated and saturated fatty acids, CLA	Mix of unsaturated fatty acids (including Omega-3)	High short-chain and medium-chain fatty acids	High mono-unsaturated and saturated fatty acids	Rich in mono- and polyunsat-urated fatty acids
Digestibility (Health Benefits)	Therapeutic uses Similar to human milk	Hypoallergenic alternative, easy on renal load (closest to human milk)	General energy and fat-soluble vitamin absorption	Highest energy, car-diovascular, anti-inflammatory	Easiest to digest due to smaller fat globules	High energy density, excellent for rich dairy products	High in Vitamin C (similar to human milk content)

Notes: [a] [76], [b] [79], [c] [65], [d,e] [73], [f] [74], [g] [75].

4.4.6. Cardiovascular Health Indices (AI and TI)

From a health perspective, mare and donkey milk exhibits favorable lipid profiles; mare milk contains higher proportions of mono- and polyunsaturated fatty acids and relatively low levels of short- and medium-chain saturated fatty acids, such as lauric (C12:0), myristic (C14:0), and palmitic (C16:0), which contribute to elevated Atherogenic (AI) and Thrombogenic (TI) Indices. Reported values for mare milk approximate AI 1.06 and TI 0.68, markedly lower than those of bovine milk [88]. Donkey milk, even lower in total fat, shows similarly favorable unsaturated fatty-acid patterns. These characteristics position mare and donkey milks closer to human milk than to ruminant milks in

lipid composition. By contrast, cow, goat, sheep, and buffalo milks contain higher fat levels (3.5–7.5%) and greater saturated- and medium-chain fatty-acid content, elevating AI and TI. Among ruminants, sheep and buffalo milk have the highest atherogenic potential, goat milk is intermediate, and camel milk, while richer in unsaturated long-chain fatty acids than bovine milk, still has higher AI and TI than mare or donkey milk [95,96].

Variability in fat content strongly affects the energy density of different types of milk; sheep and buffalo milks are the most energy-dense, whereas mare and donkey milks are the lowest. Cow and camel milks are intermediate, providing approximately 3.5–5.4% fat depending on breed, diet, and lactation stage. Cow milk also contains conjugated linoleic acid (CLA), a bioactive fatty acid with potential anti-inflammatory and immunomodulatory effects, while camel milk is characterized by a higher proportion of mono- and polyunsaturated fatty acids, elevated vitamin C, and a distinctive protein structure that enhances tolerability for some individuals with dairy allergies [97]. A comparative perspective over these characteristics across different species is presented in Table 6.

Table 6. Comparative fat content and sanogenic lipid indices (AI and TI) of milk from various species.

	Milk origin						
Item	**Mare** [a]	**Jenny** [b]	**Cow** [c]	**Sheep** [d]	**Goat** [e]	**Buffalo** [f]	**Camel** [g]
AI/TI trend	Very low AI/TI (AI ≈ 1.06; TI ≈ 0.68)	Very low AI/TI (few standardized values)	Higher AI/TI	High AI/TI	Moderate AI/TI	High AI/TI	Lower than cow, higher than equines
PUFA:SFA	High	High	Low	Lower PUFA	Moderate	Low PUFA	Moderate-high
UFA:SFA	High	High	Low-moderate	Low	Moderate-high	Low	Moderate
n-6:n-3 ratio	Low	Low	High	High	Low-moderate	High	Moderate
LA:ALA ratio	Low	Low	High	High	Moderate	High	Moderate
H/H ratio	High	High	Low	Low	High	Low	Moderate
HPI	High	High	Low	Low	Moderate-high	Low	Moderate

Notes: [a] [84], [b] [72], [c] [7], [d,e] [92,93], [f] [74], [g] [75]. AI—Atherogenic Index (AI = (C12:0 + 4 × C14:0 + C16:0)/(Σ MUFA + Σ PUFA), where C12 = lauric acid, C14 = myristic acid, C16 = palmitic acid, MUFA = Monounsaturated Fatty Acids. TI—Thrombogenic Index (TI = (C14:0 + C16:0 + C18:0)/[(0.5 × Σ MUFA) + (0.5 × Σ n-6 PUFA) + (3 × Σ n-3 PUFA) + (Σ n-3/Σ n-6)], where C18 = stearic acid. PUFA—Polyunsaturated Fatty Acids; UFA—Unsaturated Fatty Acids; SFA—Saturated Fatty Acids; n-6:n-3 ratio = the ratio of omega-6 (n-6) to omega-3 (n-3) polyunsaturated fatty acids (PUFAs); LA—linoleic acid (C18:2 n-6); ALA—α-Linolenic acid (C18:3 n-3); LA:ALA ratio—the balance between omega-6 and omega-3 precursors; H—hypocholesterolemic fatty acids (oleic acid, linoleic acid, α-linolenic acid, other polyunsaturated fatty acids (PUFAs); H—hypercholesterolemic fatty acids (lauric acid, myristic acid, palmitic acid); HPI—Health-Promoting Index.

4.4.7. Lactose Content and Gastrointestinal Tolerance

Comparisons of lactose content and digestibility across milk species reveal important differences. While lactose-free products exist, residual proteins, peptides, or processing-derived compounds can still cause discomfort in sensitive individuals. Sheep milk contains more lactose than cow milk, yet its protein and fat composition slows gastric emptying, allowing gradual digestion and reducing lactose-related symptoms. Goat milk, with slightly lower lactose levels, forms softer curds and contains higher proportions of medium-chain fatty acids, which are absorbed efficiently and support smoother digestion. These

characteristics make goat milk a suitable option for individuals with mild lactose intolerance or generally sensitive digestive systems [92–108].

Donkey milk, despite having lactose levels comparable to cow milk, is frequently better tolerated because of its higher whey protein content. Whey proteins form fine, soft curds that pass more easily through the gastrointestinal tract, resulting in faster digestion. This makes donkey milk a viable option for some individuals who experience discomfort with cow milk. Mare milk shows similar characteristics: although its lactose concentration is close to that of cow milk, its whey-dominant protein profile leads to the formation of highly digestible curds. This contributes to improved tolerance and makes mare milk an alternative worth considering for people with mild lactose intolerance [108–111]. Table 7 reflects the comparison between species regarding the lactose content and digestibility characteristics of milk across various mammalian species.

Table 7. The lactose content and digestibility features of milk from various species.

	Species						
Item	**Mare** [a]	**Jenny** [b]	**Cow** [c]	**Sheep** [d]	**Goat** [e]	**Buffalo** [f]	**Camel** [g]
Lactose content (%)	approx. 6.0–6.8	approx. 6.0–6.5	approx. 4.8–5.0	approx. 4.8–5.2	approx. 4.5–4.8	approx. 4.8–5.0	approx. 4.8–5.0
Observations (tolerance)	Generally well tolerated; suitable for mild lactose intolerance	Often better tolerated than cow milk; suitable for sensitive individuals	May cause discomfort in lactose-intolerant individuals	Often tolerated by people with mild lactose intolerance	Often tolerated by people with mild lactose intolerance	Less suitable for lactose-intolerant individuals	Often better tolerated than cow milk; some hypoallergenic properties

Notes: [a] [26], [b] [28], [c] [8], [d,e] [65], [f] [7], [g] [75].

4.5. Mare and Donkey Milk Technological Processing

Nutritional Suitability for Sensitive Populations

Mare and donkey milk is increasingly recognized not only for their nutritional and sanogenic properties but also for their potential in functional foods, clinical nutrition, and cosmeceutical applications. The processing of mare and donkey milk requires careful consideration due to its low-fat content, high lactose concentration, and whey-dominant protein profile, which may play a potential supportive role in maintaining its nutritional and bioactive properties compared with ruminant milk. The main technological processes applied to mare and donkey milk include pasteurization, fermentation, and lyophilization, each affecting microbial safety, shelf-life, nutritional integrity, and bioactive components [88–91].

Pasteurization is essential for ensuring the microbial safety of mare and donkey milk. Low-temperature, long-time (LTLT; 63 °C for 30 min) and high-temperature, short-time (HTST; 72 °C for 15 s) protocols are most commonly used, effectively reducing pathogens such as *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* while preserving key bioactive proteins, including immunoglobulins, lactoferrin, and lysozyme, when temperature control is precise [92,93]. In contrast, ultra-high-temperature (UHT) treatments, although extending shelf-life, can compromise whey protein structure, reduce digestibility, and diminish bioactive peptide formation. Consequently, LTLT and HTST methods are preferred for products where functional bioactivity is critical. Pasteurized mare and donkey milk can be consumed fresh or used as a base for fermented, powdered, or specialized dairy products, including clinical nutrition and infant formulas [94]. Table 8 presents the products obtained through mare and donkey milk exposed to pasteurization process.

Table 8. The chemical and functional characterization of pasteurized mare and donkey milk products.

Type of Product	Species	Fat (%)	Protein (%)	Lactose (%)	Bioactive Proteins and Peptides	Observations
Fresh pasteurized milk	Mares [a]	1.0–1.3	1.5–1.8	6.0–6.8	α-lactalbumin, β-lactoglobulin, lactoferrin, lysozyme, immunoglobulins	Mild pasteurization (63 °C, for 30 min) preserves most bioactive proteins; easily digestible
	Jennies [b]	0.2–1.2	1.1–1.6	6.0–6.5	α-lactalbumin, β-lactoglobulin, lactoferrin, lysozyme, immunoglobulins	Very low fat; lactose-rich; mild pasteurization maintains protein functionality
Yogurt	Mares [a]	1.0–1.3	1.5–1.8	5.0–6.0	Whey proteins + fermentation-derived peptides; α-lactalbumin, β-lactoglobulin partially preserved	Lactose partially metabolized; improved digestibility; mild acidity; probiotic potential
	Jennies [b]	0.2–1.2	1.1–1.6	5.0–6.0	Whey proteins + fermentation-derived peptides	Lactose partially metabolized; soft texture; probiotic and functional food potential
Kefir	Mares [a]	1.0–1.3	1.5–1.8	4.8–5.8	Whey proteins + bioactive peptides; probiotics (*Lactobacillus*, *Leuconostoc*, yeasts)	Slight effervescence; improved digestibility; immunomodulatory effects
	Jennies [b]	0.2–1.2	1.1–1.6	4.8–5.8	Whey proteins + bioactive peptides; probiotics	Soft, drinkable texture; probiotic-rich; suitable for lactose-sensitive individuals
Probiotic drink	Mares [a]	1.0–1.3	1.5–1.8	5.0–6.5	Whey proteins + fermentation peptides; live cultures	Liquid form, high digestibility, functional beverage
	Jennies [b]	0.2–1.2	1.1–1.6	5.0–6.5	Whey proteins + fermentation peptides; live cultures	Liquid form, soft texture; probiotic activity; suitable for sensitive populations

Notes: [a] [101], [b] [112].

Overall, LTLT and HTST pasteurization are preferred when bioactivity must be retained, fermentation enhances functional and probiotic potential, and lyophilization offers long-term preservation with minimal impact on bioactive components. Comparisons between mare and donkey milk indicate that both species respond similarly to these processes, with minor differences in fat and protein content that can influence final product composition and digestibility. Table 9 presents the key differences between processing techniques (pasteurization, fermentation, lyophilization/UHT) and their effects on bioactive components (Table 9).

Fermentation of mare and donkey milk produces products with extended shelf-life, improved digestibility, and enhanced functional properties. Common fermented forms include yogurt, kefir, and probiotic drinks, prepared using lactic acid bacteria such as *Lactobacillus* spp., *Bifidobacterium* spp., or traditional kefir grains. During fermentation, lactose is partially converted to lactic acid, reducing osmotic load and improving tolerance in individuals with mild lactose intolerance. Fermentation preserves much of the bioactive whey protein content and generates peptides with antimicrobial, immunomodulatory, and

antioxidant activities, while also enhancing vitamin bioavailability—particularly B-group vitamins—and sensory qualities such as flavor, aroma, and texture. These products are well suited for functional foods, clinical nutrition, and nutraceutical applications [95,108–113]. In Table 10, the characteristics of various products are illustrated.

Table 9. Comparison of technological processing methods of mare and donkey milk and their effects on bioactive components.

Processing Method	Temperature/Time	Key Effects on Microbial Safety	Impact on Bioactive Proteins/Peptides	Nutritional/Functional Notes
LTLT Pasteurization	63 °C, 30 min	Reduces pathogens (*Listeria*, *Salmonella*, *E. coli*)	Preserves most bioactive proteins: α-lactalbumin, β-lactoglobulin, lactoferrin, lysozyme, immunoglobulins	Mild processing; easy digestibility; functional activity maintained
HTST Pasteurization	72 °C, 15 s	Reduces pathogens	Most bioactive proteins preserved; slight loss of sensitive proteins	Preferred for functional dairy products; maintains nutritional and bioactive properties
Ultra-High Temperature (UHT)	≥135 °C, 2–5 s	Sterilizes milk; extended shelf-life	Denatures whey proteins; reduces bioactive peptide formation; may decrease digestibility	Suitable for long shelf-life products, but lower functional activity
Fermentation (Yogurt/Kefir/Probiotic drinks)	25–42 °C (varies by culture)	Improves microbial safety via acidification	Whey proteins partially preserved; bioactive peptides generated	Lactose partially metabolized; improved digestibility; probiotic effects; immunomodulatory benefits
Lyophilization/Freeze-drying	Low temperature, vacuum drying	Long shelf-life; microbial load minimal	Bioactive proteins largely preserved	Powder form; functional properties maintained; easy storage and transport

Notes: LTLT Pasteurization = Low-Temperature, Long-Time Pasteurization; HTST Pasteurization = High-Temperature, Short-Time [29,35,49].

Lyophilization is widely applied to mare and donkey milk to produce powdered formulations. Freeze-drying removes water under low temperature and vacuum conditions, preserving heat-sensitive components such as α-lactalbumin, β-lactoglobulin, lactoferrin, lysozyme, and immunoglobulins. The resulting powders can be reconstituted into liquid milk or incorporated as ingredients in infant formulas, nutraceuticals, functional foods, or cosmeceuticals. Lyophilized mare and donkey milk offers several advantages: it extends shelf-life without refrigeration, facilitates transport and storage, and allows precise dosing in clinical or nutraceutical applications. However, care must be taken during reconstitution to prevent clumping and ensure full solubility [114]. In Table 11, the key characteristics, applications, and technological processing of lyophilized products are registered.

Milk from mares and donkeys offers distinctive nutritional, functional, and socio-economic benefits, supporting rural livelihoods, breed conservation, and sustainable use of marginal landscapes. However, variability in protein analyses, limited standardized clinical trials for Cow's Milk Protein Allergy (CMPA), and differences between raw and processed milk highlight critical knowledge gaps. Addressing these limitations through rigorous, harmonized research is essential to substantiate health claims, optimize production, and realize the full potential of mare and donkey milk as a functional, culturally significant, and sustainable dairy resource [115].

Table 10. The features of various fermented products obtained from mare and donkey milk.

Species	Product	Key Features	Technological Features	Therapeutic Applications	Starter Culture
Mares [a]	Yogurt	Mildly acidic, fluid to semi-fluid texture, high retention of bioactive whey proteins, good digestibility	Weak curd formation due to low casein; rapid acidification supported by high lactose; requires controlled incubation	Clinical nutrition, CMPA (Cow's Milk Protein Allergy) pediatric use, functional dairy	Selected LAB (e.g., *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus rhamnosus*)
	Kefir	Lightly effervescent, aromatic, rich in probiotics and bioactive peptides	Efficient fermentation due to lactose richness; CO_2 and mild ethanol production; good foaming	Functional beverages, gut microbiota modulation, immune support	Kefir grains (LAB + yeasts)
	Probiotic drink	Smooth, fluid product; partially hydrolyzed lactose; excellent digestibility; stable probiotic counts	Remains liquid because of low casein; starter grows well; minimal syneresis	Supplements, lactose-sensitive consumers, therapeutic diets	*Lactobacillus* spp. + *Bifidobacterium* spp.
Jennies [b]	Yogurt	Fluid or very soft texture; delicate flavor; high digestibility; preserved lysozyme and bioactive proteins	Very weak gel formation because of low casein; high lysozyme inhibits some LAB; long fermentation time	Hypoallergenic nutrition, pediatric diets, functional dairy	Enriched LAB cultures (often *Lactobacillus* + *Streptococcus*, sometimes with texture-enhancing adjuncts)
	Kefir	Mildly effervescent but less CO_2 than mare kefir; probiotic-rich; antimicrobial profile influenced by lysozyme	Slower fermentation; reduced yeast activity; low ethanol formation	Probiotic beverages, geriatric diets, immune modulation	Adapted kefir grains (LAB + yeasts capable of tolerating lysozyme)
	Probiotic drink	Very smooth drink, mild taste, partially hydrolyzed lactose, very high digestibility	Remains liquid; excellent stability; minimal curd	Therapeutic nutrition, post-illness recovery, products for sensitive consumers	LAB + bifidobacteria blends selected for lysozyme tolerance

Notes: [a] [101], [b] [112].

Table 11. The features of various lyophilized products obtained from mare and donkey milk.

Species	Product	Key Features	Applications	Technological Processing
Mare [a]	Freeze-dried powder	Excellent preservation of α-lactalbumin, β-lactoglobulin, immunoglobulins; good solubility; low microbial load; extended shelf-life	Clinical nutrition, dietary supplements, maternal/infant nutrition, therapeutic formulations	Full lyophilization under vacuum (low temperature dehydration)
	Milk–based infant formula	Whey-rich profile similar to human milk; hypoallergenic potential; high digestibility; suitable osmolarity	Infants with CMPA, pediatric clinical diets, transitional feeding	Reconstitution + fortification + freeze-drying
	Nutraceutical powder	High in bioactive peptides (antioxidant, antimicrobial); retains lactoferrin and lysozyme; functional amino acid profile	Functional foods, immune-support supplements, anti-inflammatory formulations	Freeze-dried and incorporated into functional blends

Table 11. *Cont.*

Species	Product	Key Features	Applications	Technological Processing
Mare [a]	Cosmetic ingredient	Rich in vitamins C and B-complex, whey proteins with antioxidant effects; moisturizer, skin barrier support	Skincare creams, serums, masks, dermocosmetic formulations	Extraction or freeze-drying followed by micronization
	Colostrum freeze-dried powder	High immunoglobulin content, antimicrobial peptides, growth factors; sensitive to heat	Immune-boosting supplements, post-illness recovery formulas	Gentle lyophilization of colostrum
Jennies [b]	Freeze-dried donkey milk powder	High lysozyme stability, preserved lactoferrin, immunoglobulins; excellent microbial safety due to lysozyme; long shelf-life	Infant nutrition, hypoallergenic clinical diets, dietary supplements	Complete freeze-drying under vacuum
	Milk–based infant formula	Protein profile closest to human milk among domestic species; very low allergenicity; high whey fraction	CMPA (Cow's Milk Protein Allergy) infants, pediatric hypoallergenic formulas	Reconstitution + fortification + freeze-drying
	Nutraceutical ingredient	Strong antimicrobial activity (lysozyme), balanced amino acid profile, antioxidant peptides	Supplements, functional drinks, immune-support and gut-health products	Freeze-dried and added to functional blends
	Cosmetic ingredient	Hydrating, anti-aging properties; lysozyme and lactoferrin contribute to antimicrobial and skin-calming effects	Moisturizers, soaps, creams, serums, therapeutic skincare	Extracted or freeze-dried + cosmetic processing
	Freeze-dried fermented donkey milk powder	Probiotic preservation, enhanced peptide release, reduced lactose, stable microbiota counts	Probiotic powders, sensitive-digestive supplements, functional foods	Fermentation → lyophilization

Notes: [a] [109], [b] [112].

5. Therapeutic and Functional Properties of Fresh Mare and Donkey Milk and Processed Products

5.1. Therapeutic Uses of Equine Fresh Milk

Fresh mare and donkey milk have attracted growing scientific and clinical interest due to their distinctive biochemical composition, high digestibility, and the presence of multiple bioactive constituents with potential immunological, antimicrobial, anti-inflammatory, and metabolic relevance. Their whey-dominant protein profile, low fat content, favorable fatty acid composition, and rich enzymatic and immunological fractions differentiate them markedly from ruminant milks and position them closer to human milk in terms of certain functional characteristics [116]. Donkey milk, in particular, contains lysozyme levels comparable to human milk, potentially providing protective effects against enteropathogens such as *Escherichia coli* and *Staphylococcus aureus*. These properties suggest that fresh mare and donkey milk may serve as a supportive nutritional strategy for individuals with mild gastrointestinal disturbances, including post-infectious dysbiosis, irritable bowel syndrome with inflammatory components, or mild malabsorption, although evidence is primarily derived from in vitro studies and animal models, with limited data from human trials [117]. Mare and donkey milk has been explored across a broad spectrum of nutritional and health-related contexts, but it should be regarded as a complementary strategy rather than a universal therapeutic substitute. Its use—particularly in pediatric populations—requires careful clinical oversight due to potential residual allergenicity [97]. Among the most substantiated clinical applications is the management of Cow's Milk Protein Allergy (CMPA) in infants and young children, where mare and donkey milk have been evaluated as al-

ternative nutritional options in carefully selected cases. Their high whey-to-casein ratio, relatively low total casein content, and the presence of α-lactalbumin and β-lactoglobulin variants with reduced allergenicity compared to bovine milk contribute to improved tolerability. Donkey milk has demonstrated favorable tolerance in some CMPA patients under medical supervision, including cases unresponsive to extensively hydrolyzed formulas, while mare milk, slightly higher in protein and fat, exhibits a similar whey-rich profile and forms soft gastric curds that may enhance digestibility. Beyond CMPA, additional areas such as gastroenterology, immunology, dermatology, metabolic health, and geriatric nutrition remain largely investigational or adjunctive, supported mainly by preliminary clinical observations, mechanistic insights, or traditional use rather than robust clinical trials [80–82].

The gastrointestinal benefits of mare and donkey milk extend beyond allergenicity. Their easily digestible whey proteins, low curd tension, and high lactose content promote gentle digestive processing and act as a prebiotic substrate for beneficial gut microbiota [100]. The lipid fraction, characterized by low saturated fat and elevated monounsaturated and polyunsaturated fatty acids, reduces metabolic stress on the gastrointestinal tract. Bioactive proteins, including lysozyme, lactoferrin, immunoglobulins, and antimicrobial peptides, contribute to a balanced intestinal microbiome by inhibiting pathogenic bacteria while supporting commensal populations [118]. Donkey milk, in particular, contains lysozyme levels comparable to human milk, providing additional protection against entero-pathogens such as *Escherichia coli* and *Staphylococcus aureus* [111]. These properties make fresh mare and donkey milk a promising nutritional adjunct for mild to moderate gastrointestinal disorders, such as post-infectious dysbiosis, IBS with inflammation, and mild malabsorption. Fresh mare and donkey milk is also associated with immunomodulatory and anti-inflammatory effects [106]. The presence of lactoferrin, lysozyme, immunoglobulins, and bioactive peptides released during digestion supports both innate and adaptive immune responses. Lactoferrin, a multifunctional glycoprotein capable of binding iron, inhibiting microbial growth, and modulating inflammatory pathways, is abundant in both mare and donkey milk. Lysozyme adds further antimicrobial and anti-inflammatory effects, promoting intestinal homeostasis. Collectively, these components may aid in the recovery of infants and adults from infections, mitigate low-grade inflammation linked to metabolic syndrome or chronic stress, and support immunity in vulnerable populations, including the elderly and convalescent patients [115].

The dermatological and skin-health properties of fresh mare and donkey milk have long been noted in traditional medicine and ethnopharmacological observations. Its whey-rich protein profile, essential fatty acids, and vitamins—particularly C and B-complex—may support skin repair, hydration, and antioxidant defenses. Donkey milk has been traditionally applied to conditions such as eczema, psoriasis, dermatitis, and acne-prone skin, likely benefiting from its natural antimicrobial enzymes and gentle lipid profile. Mare milk, with a slightly higher proportion of long-chain polyunsaturated fatty acids, may contribute additional anti-inflammatory effects. Both milks have shown potential wound-healing properties in preliminary studies and could be incorporated into dietary regimens that influence skin structure, collagen synthesis, and oxidative balance. Even without processing into topical formulations, fresh mare and donkey milk may hold relevance for nutritional dermatology, though controlled clinical evidence remains limited [119].

From a metabolic and cardiovascular perspective, mare and donkey milk possess lipid profiles with very low saturated fatty acids, elevated monounsaturated and polyunsaturated fatty acids, favorable PUFA:SFA ratios, and low atherogenic and thrombogenic indices. These properties support cardiovascular health by potentially improving lipid profiles, insulin sensitivity, and reducing inflammation. With low calories and fat, alongside

high-quality whey proteins, mare and donkey milk is suitable for diets targeting metabolic stability, weight management, and cardiometabolic support. It is particularly beneficial for elderly, malnourished, or convalescent individuals, offering easily digestible proteins, bioactive compounds, and gentle lipids. Its high digestibility is advantageous for patients with reduced gastric or pancreatic function, while the palatable sweetness and favorable nutrient composition encourage intake in individuals with diminished appetite. The combination of whey-rich proteins and bioactive factors supports muscle mass maintenance, immune function, and general recovery in clinical or geriatric contexts [111–120].

The antioxidant properties of fresh mare and donkey milk—stemming from bioactive peptides, vitamins C and E, lactoferrin, and enzymatic antioxidants—may help reduce oxidative stress, a factor in chronic respiratory and cardiovascular conditions. Traditional uses for respiratory health, such as in chronic bronchitis or asthma, likely reflect these combined antioxidant and anti-inflammatory effects. While not a substitute for medications, mare and donkey milk can serve as a complementary dietary measure to support antioxidant defenses, reduce inflammation, and enhance overall physiological resilience [121].

In summary, fresh mare and donkey milk exhibit a broad range of therapeutic properties, underpinned by their whey-rich protein composition, bioactive components, favorable lipid profiles, and high digestibility. They offer clinical and dietary benefits for infants with Cow's Milk Protein Allergy (CMPA), individuals with gastrointestinal or inflammatory conditions, patients requiring nutritional support during convalescence, elderly populations, and those seeking complementary approaches to skin health, metabolic balance, and cardiovascular protection. These multifaceted properties underscore their growing relevance as functional foods and support their inclusion in targeted nutritional and therapeutic interventions under medical guidance.

5.2. Therapeutic Uses of Processed Products from Mare and Donkey Milk

Processed products derived from mare and donkey milk, including pasteurized milk, fermented beverages such as yogurt, kefir, and probiotic drinks, as well as lyophilized (freeze-dried) formulations, have received increasing attention for their nutritional and therapeutic properties. Processing extends shelf-life, ensures microbial safety, and supports functional food development, while preserving or enhancing mare and donkey milk's bioactive components. These products offer versatile options for sensitive populations, including infants with CMPA, the elderly, convalescent patients, and health-conscious consumers mare and donkey milkCMPA [110].

Pasteurized mare and donkey milk is typically subjected to low-temperature, long-time (LTLT; e.g., 63 °C for 30 min) or high-temperature, short-time (HTST; e.g., 72 °C for 15 s) treatments to reduce pathogenic microorganisms while minimizing the denaturation of heat-sensitive proteins such as α-lactalbumin, β-lactoglobulin, lactoferrin, lysozyme, and immunoglobulins. Pasteurization ensures microbiological safety, making fresh mare and donkey milk suitable for general consumption or as a base for further processing. The therapeutic potential of pasteurized mare and donkey milk is maintained due to the preservation of these bioactive components, which contribute to antimicrobial, immunomodulatory, antioxidant, and digestive-supportive properties. Pasteurized mare and donkey milk can serve as a dietary supplement for infants with mild CMPA, individuals with digestive sensitivities, and elderly patients requiring high-quality, easily digestible protein [122].

Fermented mare and donkey milk products, including yogurt, kefir, and probiotic drinks, enhance digestibility, prolong shelf-life, and generate additional bioactive peptides through microbial activity. Fermentation partially metabolizes lactose, reducing osmotic load and improving tolerance for individuals with mild lactose intolerance. Fermentation enhances vitamin bioavailability, adds probiotics, and generates antioxidant, antimicrobial,

and immunomodulatory compounds. Yogurt made from mare or donkey milk typically exhibits soft curd formation, mild acidity, and retention of whey proteins, making it suitable for clinical nutrition, infant support, and functional dietary applications. Kefir, prepared using kefir grains, is slightly effervescent and rich in probiotics, conferring additional antimicrobial and immunomodulatory potential. Probiotic drinks made with Lactobacillus and *Bifidobacterium* strains are easily digestible and suitable for lactose-sensitive individuals, extending mare and donkey milk's role from basic nutrition to functional and therapeutic applications [76,104,112–116].

Lyophilization or freeze-drying is employed to produce stable powdered formulations of mare and donkey milk. This process removes water under low-temperature vacuum conditions, preserving heat-sensitive proteins, bioactive peptides, enzymes, immunoglobulins, and micronutrients. Lyophilized milk can be reconstituted for direct consumption or incorporated into infant formulas, nutraceuticals, dietary supplements, or cosmeceutical products. Powdered mare and donkey milk retains its antimicrobial, immunomodulatory, and antioxidant properties, while offering the convenience of extended shelf-life, microbiological stability, and transportability. Reconstituted powdered milk serves as a hypoallergenic, whey-rich source of high-quality proteins for infants with CMPA or for adults and elderly individuals requiring easy-to-digest nutritional support. Additionally, freeze-dried milk can be used as a nutraceutical ingredient in functional foods, providing bioactive peptides with antioxidant and immunomodulatory effects, or as a component in cosmeceutical formulations for moisturizing, anti-inflammatory, and antioxidant benefits [123].

Processed mare and donkey milk products also have significant metabolic and cardiovascular implications. The low-fat content and favorable fatty-acid composition are largely preserved during pasteurization and fermentation, providing products with low saturated fat, elevated monounsaturated and polyunsaturated fatty acids, and favorable PUFA:SFA ratios. The atherogenic and thrombogenic indices remain low compared to ruminant milks, supporting cardiovascular health and metabolic regulation. The whey-rich protein fraction continues to contribute to improved insulin sensitivity, reduction of postprandial glycemic response, and support of muscle mass maintenance, particularly in elderly or convalescent patients. The probiotic content of fermented products may additionally modulate gut microbiota, reduce low-grade inflammation, and enhance gastrointestinal barrier function, providing indirect metabolic and immunological benefits [112–116].

Processed mare and donkey milk products enhance the functional and practical value of fresh milk by providing safe, convenient, and enriched forms. Pasteurization ensures microbiological safety while largely preserving bioactive compounds; fermentation produces probiotic-rich functional foods with improved digestibility and bioactive peptide profiles; and lyophilization yields stable powders suitable for infant nutrition, clinical support, nutraceuticals, and cosmeceuticals. Together, these products enable applications in pediatric nutrition, gastrointestinal support, immune modulation, dermatological care, cardiovascular and metabolic health, and geriatric nutrition, highlighting mare and donkey milk as a versatile and functional component of specialized diets [124].

6. Animal Welfare Practices in Mare and Donkey Milk Production

6.1. Housing and Environmental Conditions

Animal welfare is a central component of sustainable and high-value mare and donkey milk production, influencing not only the ethical standing of dairy operations but also the quality, safety, and functional properties of the milk itself. Mares and jennies, like all dairy animals, exhibit physiological and behavioral responses that directly affect lactation performance, milk composition, and bioactive compound concentrations. Ensuring optimal

welfare is therefore a critical consideration for producers seeking to maintain the nutritional, therapeutic, and commercial value of mare and donkey milk [3].

Adequate housing is essential for equine welfare and milk quality. Stables should provide ample space for movement, rest, and social interaction, as overcrowding or confinement can increase stress and negatively impact lactation and milk composition. Stress elevates cortisol levels, reducing milk yield and altering the concentration of proteins, lipids, and bioactive compounds such as lysozyme, lactoferrin, and immunoglobulins. Clean, dry, and well-ventilated stables also minimize pathogen exposure and the risk of mastitis or other infections that compromise milk safety [117,118].

Bedding quality, stall layout, and ventilation are also crucial. Soft, absorbent bedding supports comfort and hoof health, while proper ventilation ensures air quality and prevents respiratory issues. Regular cleaning and maintenance of stalls are essential to minimize microbial contamination, not only safeguarding animal health but also enhancing the hygienic quality of milk. Environmental enrichment within the housing environment further supports welfare. Providing social interaction, visual or tactile stimuli, and opportunities for natural behaviors reduces stress, prevents stereotypic behaviors, and contributes to calmer, more productive animals. This, in turn, translates to milk with more stable biochemical profiles and higher functional quality, reinforcing the link between welfare and product excellence [3].

6.2. Access to Pasture and Exercise

Free access to pasture and regular exercise are key determinants of both physical and psychological well-being in lactating mares and jennies. Pasture access promotes natural feeding behaviors, encourages movement, and provides a diet with naturally occurring micronutrients and bioactive plant compounds, which can subtly enhance milk composition, particularly fatty-acid profiles and vitamin content. Exercise maintains muscle tone, supports joint health, and promotes efficient metabolic function, which is especially important during the demanding lactation period [125].

Environmental enrichment outdoors—such as shaded areas, social groupings, or varied terrain—encourages exploration and reduces boredom or stress-related behaviors. Horses that are able to express natural behaviors demonstrate improved physiological stability, resulting in more consistent milk yield and higher concentrations of bioactive proteins. Seasonal and rotational grazing strategies also influence milk quality. Grazing on nutrient-rich pastures or in rotation can increase polyunsaturated fatty acids in milk, improve antioxidant content, and reduce inflammatory fatty-acid fractions. These benefits illustrate how outdoor management practices directly translate into functional and therapeutic properties in mare and donkey milk [126].

6.3. Feeding and Nutritional Management

Balanced nutrition is essential for equine welfare and the functional quality of milk. Lactating mares and jennies require diets that adequately supply energy, protein, vitamins, and minerals, which vary according to lactation stage, age, and physiological status. Nutritional deficiencies or sudden diet changes can decrease milk yield, alter protein composition, and reduce bioactive compound levels. Appropriate feeding enhances digestibility, amino acid profiles, and immunological activity. High-quality forage should form the dietary foundation, complemented by concentrates or supplements to meet energy and protein needs. Minerals such as calcium, phosphorus, and magnesium are crucial for lactation performance and milk quality, and water must always be available, as even mild dehydration can reduce milk volume and affect nutrient content and viscosity [3,117,127].

Monitoring body condition and milk output allows timely dietary adjustments, ensuring that mares and jennies maintain optimal health and lactational efficiency. Nutritional management, when integrated with overall welfare practices, has a direct impact on the bioactive, therapeutic, and functional properties of mare and donkey milk.

6.4. Stress Reduction and Handling Practices

Stress during milking or daily handling can negatively affect cortisol levels, reduce milk yield, and alter biochemical composition. Gentle, consistent handling, positive reinforcement, and predictable routines reduce fear and agitation in mares and jennies, facilitating smoother milking and higher milk quality. Proper milking posture, calm handling, and hygiene measures also minimize physical strain and prevent injury [67,112].

Minimizing environmental stressors, such as sudden noises, overcrowding, or aggressive interactions, is equally important. Stress management contributes to the stability of heat-sensitive bioactive compounds such as lactoferrin, lysozyme, and immunoglobulins. Animals under lower stress demonstrate improved milk digestibility, higher bioactive content, and more consistent production, which are essential for therapeutic and functional applications. Training staff in low-stress handling techniques is therefore a key welfare measure with direct implications for product quality and animal health [3].

6.5. Links Between Welfare and Product Quality

Optimal welfare practices are directly linked to milk quality, bioactive content, and therapeutic potential. Healthy, low-stress mares and jennies produce milk richer in bioactive proteins, immunoglobulins, lysozyme, and lactoferrin, enhancing its antimicrobial, immunomodulatory, and antioxidant properties. Poor welfare, conversely, can reduce milk yield, alter fatty-acid profiles, and increase microbial contamination risk [121]. The integration of housing, nutrition, health management, and stress-reduction practices ensures that mare and donkey milk is both safe and functionally effective, supporting its use in infant nutrition, clinical support, nutraceuticals, and cosmeceuticals. Welfare-centered management is therefore both an ethical responsibility and a practical strategy for producing high-value dairy products [117].

6.6. Regulatory Compliance and Certification

Compliance with EU and national animal welfare regulations provides a framework for ethical mare and donkey milk production. Certification schemes for high-welfare products require demonstrable adherence to housing, feeding, health care, and handling standards [121]. These certifications not only reinforce ethical practices but also enhance product marketability by signaling traceability, safety, and high quality to consumers. Adopting welfare-oriented certification standards can also stimulate research and adoption of best practices, further integrating sustainability, ethical management, and functional milk production. High-welfare certification has been associated with consumer willingness to pay premium prices, supporting the economic viability of niche equine dairy operations [119,120].

6.7. Sustainability and Economic Implications

Healthy, well-managed equines show improved lactation and reproductive performance, reducing replacement costs and supporting stable milk production. Ethical husbandry meets consumer expectations for traceable, responsibly produced products and enhances the viability of niche dairies. By integrating welfare with sustainable practices, mare and donkey milk production can promote rural development, preserve agrobiodiversity, support eco-friendly farming, and maximize the milk's functional and therapeutic potential. In addition, welfare-oriented management practices contribute to long-term herd health

by reducing the incidence of mastitis, metabolic disorders, and reproductive inefficiencies, which directly impact milk yield and quality. Implementing regular health monitoring, appropriate nutrition, and stress-minimizing handling not only supports the physiological well-being of mares and donkeys but also ensures the preservation of bioactive compounds in milk, such as immunoglobulins, lysozyme, and lactoferrin. Beyond production benefits, ethical husbandry strengthens consumer confidence in niche dairy products by demonstrating transparency, traceability, and adherence to animal welfare standards. Moreover, integrating equine milk production with agroecological approaches—such as rotational grazing, use of local forage, and maintenance of pasture biodiversity—enhances ecosystem services, mitigates greenhouse gas emissions, and fosters climate resilience in rural landscapes. Collectively, these practices help position mare and donkey milk as a sustainable, high-value functional food, supporting both local economies and broader public health goals [122].

7. Industry Challenges, Sustainable Valorization, and Standardization in Mare and Donkey Milk Production

While mare and donkey milk presents unique nutritional, functional, and socioeconomic potential, advancing the sector requires a coordinated and strategic research agenda. Future investigations should focus on several key priorities:

- standardization of milking protocols—research should define optimal milking frequency, timing relative to foal suckling, and handling procedures to minimize stress and preserve bioactive components; comparative studies across small- and medium-scale farms can identify best practices that balance yield, milk quality, and animal welfare.
- breed-specific compositional mapping—systematic characterization of mare and donkey milk across breeds can clarify variations in protein fractions (casein/whey ratio), lipid profiles, lactose content, and concentrations of lysozyme and lactoferrin; this data will support traceability, breed-specific functional product development, and conservation of heritage breeds.
- controlled clinical trials for CMPA and sensitive populations—randomized controlled trials should evaluate hypoallergenic potential, digestive tolerance, and immunomodulatory effects in infants, children, and adults with Cow's Milk Protein Allergy; dose–response studies and longitudinal follow-ups will strengthen evidence for health claims while identifying potential limitations.
- impact of processing on bioactive compounds—studies should assess how pasteurization, fermentation, and lyophilization affect protein integrity, peptide bioactivity, and lipid oxidation. Experimental comparisons between raw, thermally treated, and fermented products can guide processing choices that maximize functional benefits without compromising safety.
- microbiome–mare and donkey milk interactions—investigation of gut microbiota responses to fresh and fermented mare and donkey milk can elucidate mechanisms behind observed health effects, including modulation of beneficial bacterial populations and production of bioactive metabolites; integrating metabolomics and metagenomics approaches may link specific milk components to microbial and host outcomes.
- sustainability and Life-Cycle Assessment (LCA)—quantifying environmental impacts, including greenhouse gas emissions, water footprint, land-use efficiency, and energy inputs, will provide evidence for low-input, resilient dairy systems; assessments should consider pasture management, supplementation practices, and integration with agroecological landscapes to optimize both productivity and ecosystem services.

By addressing these priorities with rigorous, interdisciplinary approaches integrating animal science, food technology, clinical nutrition, and socio-economic analysis, the mare and donkey milk sector can bridge current knowledge gaps, substantiate health-related claims, and reinforce its economic, ecological, and cultural value. This framework can help transition mare and donkey milk from a niche artisanal product into a sustainable, high-value component of diversified global dairy systems.

In addition, future research and development should consider regulatory compliance and certification frameworks to ensure product safety, quality, and consumer trust. Examples include adherence to European Union regulations on novel foods and dairy hygiene standards (Regulation (EC) No 178/2002, Regulation (EC) No 852/2004), implementation of Good Manufacturing Practices (GMP), and certification under Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI) schemes for heritage breeds. Integration of such regulatory measures, coupled with voluntary quality certifications such as organic, bioactive milk content, or probiotic claims, will facilitate market access, support traceability, and enhance the credibility of mare and donkey milk as functional and therapeutic products [125].

8. Conclusions

Mare and donkey milk represents distinctive dairy matrices whose compositional characteristics partially resemble those of human milk, including specific lactose levels, protein fractions, and mineral profiles. The presence of bioactive components such as lysozyme, lactoferrin, and immunoglobulins has been widely reported and has prompted investigation into their biological activities, including antimicrobial, anti-inflammatory, and immunomodulatory effects. However, the nutritional and functional attributes of equine milk are not uniform and are strongly influenced by breed, diet, and stage of lactation, highlighting the need for controlled production conditions when interpreting compositional data.

Technological processing approaches, including fermentation, pasteurization, and lyophilization, have been examined primarily in relation to safety, stability, and retention of bioactive compounds. While these methods expand the range of potential applications for equine milk-derived products, their effects on nutritional quality and bioactivity remain variable and context-dependent. Existing studies also reflect diverse methodological approaches, which complicates direct comparison across production systems and processing conditions.

Despite growing scientific interest, the equine dairy sector continues to face significant constraints, including low and variable milk yields, heterogeneous production and processing practices, regulatory inconsistencies, and limited high-quality clinical evidence regarding health-related outcomes. Addressing these challenges requires the development of standardized production protocols, harmonized quality control frameworks, and welfare-oriented management systems to ensure reproducibility, safety, and ethical compliance.

Overall, the current body of evidence indicates that mare and donkey milk warrant further investigation rather than definitive conclusions regarding their nutritional or therapeutic relevance. Future interdisciplinary research integrating animal science, food technology, clinical nutrition, and socio-economic analysis is essential to clarify compositional variability, assess health-related effects through well-designed clinical studies, and evaluate the sustainability and feasibility of equine milk production within broader food systems.

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