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Bridging the gap in infant nutrition: Precision fermentation of human milk compounds

Laura Nyhan [a,2], Arianna Ressa [a,2], Anastasia V. Lilina [b], Stijn Mertens [b], Dustie N. Lombardi [b], Rosa Sanchez [b], Patrick O'Riordan [b], Nan Gai [c], André Brodkorb [c,1], Elke K. Arendt [a,d,*,1]

[a] School of Food and Nutritional Sciences, University College Cork, Cork, Ireland
[b] BioBrew Technologies Inc, New York, NY, 10036, USA
[c] Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland
[d] APC Microbiome Ireland, University College Cork, Cork, Ireland

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ABSTRACT

Background: Human milk (HM) is a unique nutritional source, tailored to meet infants' needs during early life. However, declining breastfeeding rates, influenced by lactation problems, health-related factors, psychological state, and work-related constraints, have increased reliance on infant milk formula (IMF). Most IMF is derived from bovine milk, which differs substantially from HM in protein composition, fat structure, carbohydrate profile, and bioactive components. These compositional gaps limit IMF's ability to replicate HM functionality with respect to digestibility, nutrient absorption, immune support, and microbiome maturation. Consequently, formula-fed infants may experience accelerated growth, increased susceptibility to allergic reactions, and gastrointestinal issues.

Scope and approach: This review examines the compositional and functional limitations of IMF relative to HM, focusing on proteins and human milk oligosaccharides (HMOs), and explores how precision fermentation (pFerm), an emerging biotechnology for large-scale production of bioidentical human milk proteins and oligosaccharides, can narrow the HM-IMF gap. We review the pFerm protein and HMO sectors, analysing case studies of both compounds and highlighting challenges and opportunities.

Key findings and conclusions: Market analysis shows that pFerm-derived bovine proteins and HMOs are well established, whereas human-derived proteins remain underdeveloped. Case studies of recombinant human lactoferrin and pFerm-derived HMOs demonstrate the ability of these innovations to narrow the HM-IMF gap. Key challenges to adoption include regulatory hurdles, economic scalability, and consumer apprehension regarding the perceived 'unnatural' nature of such ingredients. Nevertheless, the rapidly expanding pFerm sector - driven by biotechnological investment and growing consumer acceptance through effective communication - offers considerable opportunities to enhance IMF development through application of pFerm-derived HM compounds.

1. Introduction

Considered the gold standard for newborn infants, human milk (HM) is a uniquely tailored source of nutrition that meets the exclusive needs of infants during early life, with the WHO recommending exclusive breastfeeding until at least the age of 6 months (WHO, 2023). The high nutritional value of HM positively influences infant growth and development, cognitive function, and immune system maturation, while breastfeeding also confers benefits to the mother, including improved psychological well-being, and reduced risk of cardiovascular disease, hypertension, and diabetes (Purkiewicz et al., 2025). Although the percentage of exclusively breastfed infants globally has increased from 38% to 48% from 2012 to 2024, this still falls short of the 2030 target of 70% (UNICEF & WHO, 2025). Moreover, 29 countries have experienced declines in breastfeeding rates between 2017 and 2024, with 6 reporting decreases of >10% (UNICEF & WHO, 2025). Reductions in

* Corresponding author. School of Food and Nutritional Sciences, University College Cork, Cork, Ireland.
E-mail address: e.arendt@ucc.ie (E.K. Arendt).
[1] These authors contributed equally to this work and share last authorship.
[2] These authors contributed equally to this work and share first authorship.

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breastfeeding rates may be attributed to lactation issues (low milk supply, milk stasis, engorgement, pain and inflammation), infant-related health factors (incorrect latching, food allergies), psychological problems (anxiety, stress, fatigue, post-partum depression, perception of limited value or insufficient amount of breastmilk), or work-related issues (inadequate provision of suitable conditions or time for milk expression) (Purkiewicz et al., 2025).

Infant milk formula (IMF) is a powdered milk substitute for HM used when breastfeeding is not possible. While IMF in the US is regulated as a single category (birth to 12 months), EU legislation formally differentiates infant formula (0–6 months) and follow-on formula (6-12 months) under Commission Delegated Regulation (EU) 2016/127, supplementing Regulation (EU) 609/2013, a regulatory framework commonly reflected in industry staging terminology (e.g., stage 1 and stage 2). IMF is formulated with the aim of closely mimicking HM and is predominantly whey-based and formulated for ease of digestion. Despite this, IMF remains compositionally distinct due to constraints in ingredient sourcing and processing, resulting in differences in protein quantity and structure, lipid composition (lack of HM's native milk fat globule membrane (MFGM)), mineral balance and carbohydrate composition, particularly with respect to human milk oligosaccharides (HMOs).

The protein fraction of IMF is typically derived from animal-based sources, most commonly cow's milk (CM), which is naturally adapted to meet the nutritional needs of bovine calves (Guo, 2009). Consequently, bovine proteins cannot fully replicate the unique functions of human counterparts, including digestibility and nutrient absorption, immune protection, and support for optimal growth and development (Cronin et al., 2023; Roy et al., 2020). This can lead to various health concerns for the infant, with studies showing that formula-fed infants are more likely to experience gastrointestinal issues, accelerated weight gain, an increased risk of obesity, and heightened susceptibility to allergenic responses (Bellaiche et al., 2018; Kelly et al., 2019; Nilsson et al., 2024).

While incorporating HM proteins into IMF would be desirable, it remains impractical due to their low natural abundance and the considerable technical and economic challenges associated with their isolation. To address these limitations, recombinant expression of HM proteins such as lactoferrin (LF) and α-lactalbumin (α-lac) in transgenic cows has been explored since the early 2000s (Wang et al., 2008; Yang et al., 2008). However, most of this research has remained at the proof-of-concept stage, with limited commercialisation progress due to unresolved safety concerns (Vishwanath-Deutsch et al., 2024). More recently, precision fermentation (pFerm) has emerged as a promising technology capable of producing bioidentical HM-derived proteins at scale, offering a viable pathway for their incorporation into infant nutrition. This process involves the expression of target proteins in genetically engineered hosts, followed by purification through downstream processing (Nielsen et al., 2024). pFerm has already been successfully applied in the production of HMOs - the third most abundant solid component in HM - for incorporation into IMF, demonstrating both the technological feasibility and commercial potential of this approach. Emerging efforts also extend to other milk components, including fermentation-based approaches to produce HM fat analogues (Zhou et al., 2024), although lipids fall outside the scope of this review.

Accordingly, this review examines the differences between CM and HM, with a focus on protein and oligosaccharides as two of the most functionally significant components in infant nutrition and explores how these differences translate into functional consequences in bovine-derived IMF. Advancements in IMF formulation aimed at addressing these limitations will be discussed, along with the remaining gaps in current knowledge, with particular attention to the potential of pFerm as a biotechnology for producing human-derived milk compounds. An overview of the pFerm market for proteins and HMOs will be provided, including case studies on pFerm-derived recombinant human lactoferrin (rhLF) and HMOs. Finally, the challenges and opportunities associated with implementing pFerm-derived HM proteins, including the regulatory landscape, economic viability, and consumer perception, will be considered.

2. Compositional gaps between CM and HM

2.1. Protein

A critical difference between CM and HM is protein composition, with CM containing almost threefold more protein (~34 g/L) than HM (~10 g/L) (Guo, 2009). CM is casein-dominant with a whey-to-casein ratio of approximately 20:80, depending on breed and individual cow (Fox & McSweeney, 1998). In contrast, HM is whey-dominant in early lactation (70:30) and in mature milk (60:40), equilibrating to approximately 50:50 in late lactation (Guo, 2009; Lönnerdal, 2003) (Fig. 1). These variations in protein composition are fundamental to the nutritional complexity of both milks, underscoring the need to examine and elucidate their distinct roles in infant nutrition.

2.1.1. Caseins

Of the six major milk proteins, four are micellar caseins. As reviewed by Runthala et al. (2023), caseins are a group of phosphoproteins synthesised in the mammary gland, comprising α_{s1}-, α_{s2}-, β-, and κ-casein. Although both CM and HM contain these four casein families, they exhibit significant heterogeneity in terms of composition, amino acid (AA) sequence and length, structural features, and post-translational modifications.

The casein composition of HM and CM are shown in Fig. 1. HM caseins are dominated by β-casein (2.7 g/L) and κ-casein (0.9 g/L), with small amounts of α_{s1}-casein (0.6 g/L), and non-detectable levels of α_{s2}-casein. In contrast, CM contains substantially higher levels of α_{s1}-casein (12 g/L), followed by β-casein (9 g/L), κ-casein (3 g/L), and α_{s2}-casein (3 g/L) (Chatterton et al., 2013). Multiple sequence alignment has shown that β-casein and κ-casein are the most highly conserved forms, sharing 50-60% homology between human and bovine species, whereas bovine α_{s1}-casein exhibits only ~40% identity with its human counterpart (Runthala et al., 2023). Significant intraspecies variability also exists, particularly in CM, which exhibits extensive genetic polymorphism, with 53 known variants across the six major milk proteins, including 10 variants of α_{s1}-casein, 5 of α_{s2}-casein, 15 of β-casein, and 11 of κ-casein. HM caseins also (Soyyilmaz et al., 2021) display genetic variation, particularly in β-casein and κ-casein; however, they appear less diverse and are not as extensively characterised, with specific variant numbers remaining poorly documented (Runthala et al., 2023), largely due to more limited investigation and a lack of commercial interest.

Caseins in milk exist in the form of spherical, colloidal aggregates known as casein micelles, ranging from 50 to 500 nm in diameter, with average values of 150-180 nm and 64-80 nm in CM and HM, respectively (Fox & Brodkorb, 2008; Meng et al., 2021). Knowledge of the micelle structure is largely derived from CM due to its industrial relevance, with several theories proposed for bovine micelle organisation. According to the multivalent binding model (Holt & Carver, 2022), bovine casein micelles are structured as dynamic assemblies in which casein molecules interact throughout the micellar matrix via colloidal calcium phosphate (CCP) nanoclusters dispersed within the structure. These CCP nanoclusters cross-link highly phosphorylated regions of α- and β-caseins through calcium-mediated interactions, thereby maintaining micellar integrity and enabling reversible calcium and phosphate sequestration without precipitation. κ-casein permeates the entire micelle and participates in interactions with other caseins; at the same time, a proportion is enriched at the micellar surface, where its negatively charged, hydrophilic, and heavily glycosylated “hairy” layer extends into the aqueous phase, providing steric and electrostatic stabilization that prevents aggregation (Holt & Carver, 2022; Raynes et al., 2024; Waugh, 1971). Despite their relevance for IMF development and infant health, human casein micelles remain underexplored; however, some structural

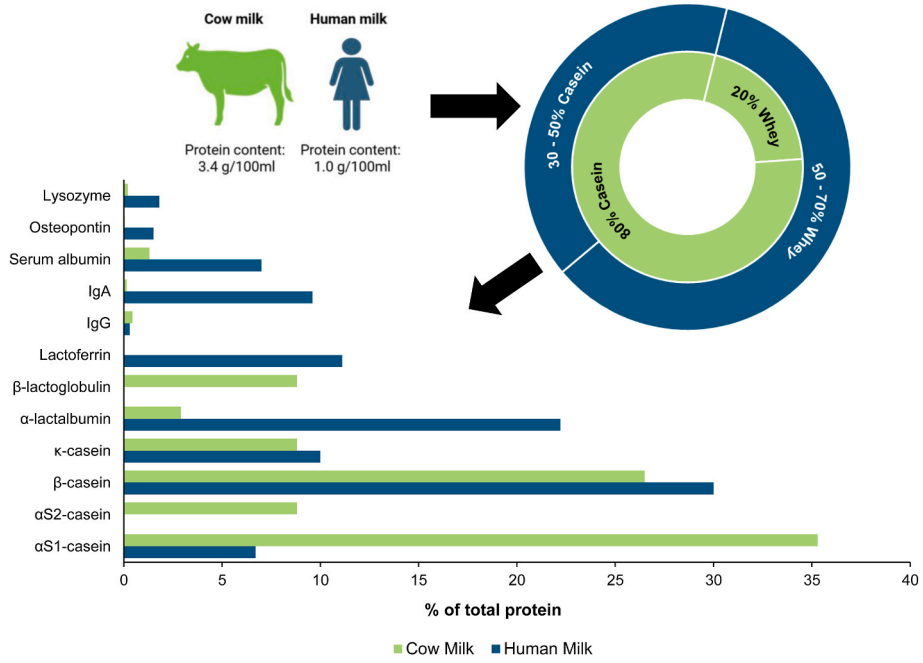

Fig. 1. Protein content, whey-to-casein ratio, and protein composition of cow milk and human milk. Values adapted from Chatterton et al. (2013), Claeys et al. (2014), and Guo (2009).

aspects have been identified. Although κ-casein is still required for micelle stabilization, its role in HM micelle formation appears less pronounced than in CM. Additionally, HM caseins are less phosphorylated than their CM counterparts (Neville et al., 1994), resulting in micelles with less associated CCP and reduced mineralisation (Runthala et al., 2023). Despite this, human micelles can form and maintain stable structures, likely due in part to the amphiphilic nature of β-casein, which supports self-assembly with reduced dependence on mineral cross-linking (Meng et al., 2021).

These compositional and structural differences between human and bovine caseins directly influence their physicochemical behaviour. The higher proportion of α_{s1}-casein and greater degree of phosphorylation in CM contribute to more strongly mineralised micelles with increased CCP content, resulting in larger, more rigid structures that are more prone to aggregation and coagulation (Fox & Brodkorb, 2008; Runthala et al., 2023). In contrast, the lower phosphorylation levels and reduced mineralisation observed in HM caseins produce smaller, less densely cross-linked micelles with greater structural flexibility and increased susceptibility to enzymatic digestion (Meng et al., 2021). Differences in κ-casein abundance and surface presentation further affect micellar stability, as variations in the steric and electrostatic "hairy layer" alter resistance to aggregation under changing pH and ionic conditions (Fox & Brodkorb, 2008; Runthala et al., 2023). Collectively, these physicochemical distinctions underpin key functional differences in digestibility, gastric curd formation, and nutrient bioavailability, which are further discussed in Section 3.

2.1.2. *Whey proteins*

Whey is the soluble fraction of milk obtained after casein separation and contains bioactive and functional proteins essential for infant growth and development, immune function, and nutrient provision. The whey protein composition of CM and HM differs considerably (Fig. 1). α-lac is the most abundant whey protein in HM (2 g/L, 22 % of total protein) and serves as a regulatory subunit of the lactose synthase complex (Brew, 1969). In CM, α-lac comprises only ~3% of total protein (1 g/L), while β-lactoglobulin (β-lac; ~3 g/L; ~9% of protein) predominates, a protein which is absent from HM, resistant to gastric proteolysis in its native state, and recognised as a potential allergen (Roy et al., 2020). LF, an iron-binding glycoprotein with antimicrobial and immunomodulatory activity, is present at markedly higher concentrations in HM (1 g/L) than in CM (0.01 g/L) (Chatterton et al., 2013). Osteopontin (OPN) similarly represents a greater proportion of HM (0.14 g/L, 1.5% of total protein) than CM (0.02 g/L, 0.1% of protein) and is associated with multiple functions, including immunological effects, intestinal development, cognitive behaviour, and gut microbiome modulation (Sørensen & Christensen, 2023). Immunoglobulins (Igs) account for 2% (0.6 g/L) of CM protein and 13% (1.2 g/L) of HM protein, although their class distribution differs significantly between species; secretory IgA (SIgA) is the predominant immunoglobulin in HM, while the dominant class in CM is immunoglobulin G (IgG) (Claeys et al., 2014). Minor whey proteins include serum albumin and lysozyme; human serum albumin (HSA) and bovine serum albumin (BSA) comprise ~8% and ~1% of total protein in HM and CM, respectively, while lysozyme contributes 1–3% of HM protein but is present only in trace amounts in CM (Chatterton et al., 2013).

Except for β-lac, whey proteins share around 60–80 % sequence identity between bovine and human species, highlighting a moderate degree of conservation (Demmelmair et al., 2017; Layman et al., 2018). However, post-translational modifications of homologous proteins differ markedly between species, particularly with respect to glycosylation. HM proteins generally exhibit greater glycan diversity, including fucosylated and sialylated structures, which support immune modulation and microbiome development in infants (Orczyk-Pawiłowicz & Lis-Kuberka, 2020; Wang et al., 2021). In contrast, CM proteins typically exhibit less diverse glycosylation patterns with a higher relative abundance of sialylated structures, reflecting species-specific adaptations in immune function (O'Riordan et al., 2014).

2.2. *Oligosaccharides*

HMOs are the third most abundant solid component in HM, present at concentrations of approximately 11 g/L and 18 g/L in mature milk and colostrum, respectively, although levels vary depending on the individual and the lactation duration (Soyyilmaz et al., 2021). Over 200 distinct HMO species have been identified to date (Ninonuevo et al., 2006), though ~10 abundant species (e.g. 2′-fucosyllactose (2′-FL),

3-fucosyllactose (3-FL), lacto-*N*-tetraose (LNT), lacto-*N*-neotetraose (LNnT), 3′-sialyllactose (3′-SL), 6′-sialyllactose (6′-SL)) comprise 80-90% of total HMO content and are strongly associated with key functions such as bifidogenesis and pathogen exclusion (Sprenger et al., 2022). HMOs are typically classified into three categories based on their core structure and modification types: 1) neutral fucosylated (e.g. 2′-FL and 3-FL), which contain fucose residues and are the most abundant HMOs in HM; 2) neutral non-fucosylated (e.g. LNT and LNnT) which are neutral but lack fucose residues; and 3) acidic sialylated HMOs (e.g. 3′-SL and 6′-SL), which are negatively charged due to the presence of sialic acid (Hobbs et al., 2021). Interestingly, HMOs share structural similarity with the glycans on HM proteins. Both glycan types play roles in modulating infant health with respect to immune function, microbiome maturation and modulation, and pathogen blocking. In contrast, bovine milk contains a markedly lower concentration of oligosaccharides (0.1-1 g/L, depending on lactation stage) with less diverse and complex structures (30 – 50 identified), most of which are sialylated (~70% in colostrum) and non-neutral fucosylated types (Hobbs et al., 2021), while fucosylated oligosaccharides have been reported only in trace amounts (~1%) and inconsistently across studies (Mehra et al., 2014; Tao et al., 2009). Due to the lower diversity and concentrations of oligosaccharides in CM, the full range of activities observed in HM cannot be fully replicated (O'Riordan et al., 2014).

3. Functional consequences of compositional gaps

3.1. Physical stability

HM and CM exhibit distinct physical behaviours, largely influenced by their protein composition and protein-protein interactions. CM exhibits remarkable heat stability, mainly due to the stability of the casein micelles, although heat-labile whey proteins ultimately determine its thermal behaviour. Heating affects the casein micelle structure by partially solubilising stabilising CCP, altering mineral equilibrium between the micelle and serum and promoting further protein-protein interactions that can lead to protein aggregation, and in extreme cases, gel formation. Simultaneously, heat-denatured whey proteins, particularly β-lac, covalently interact with other whey proteins and κ-casein through covalent disulfide bonds and hydrophobic interactions, forming both whey protein aggregates in the serum and complexes on the surface of the casein micelle, destabilising the micellar structure (Gaspard et al., 2017). These changes increase micellar size, viscosity, and physical instability, the extent of which is highly pH-dependent (Singh & Fox, 1986; Smits & Van Brouwershaven, 1980). In contrast, HM contains smaller, less mineralised micelles with lower κ-casein and lacks the heat-labile β-lac (Fox & Brodkorb, 2008; Neville et al., 1994). Consequently, CCP solubilisation during heating is less pronounced, and although α-lac denatures, it lacks free thiol groups and cannot readily form covalent disulfide bonds with κ-casein (Singh, 1995; Smits & Van Brouwershaven, 1980), contributing to the reduced tendency of HM to aggregate during thermal treatment.

These contrasting thermal behaviours have clear implications for IMF manufacturing. Increasing the α-lac to β-lac ratio in 'humanised' formulas enhances resistance to heat-induced coagulation, reduces viscosity, and maintains particle size, thereby improving physical stability (Crowley et al., 2016). Moreover, despite higher levels of the agglutinins IgA and IgM, HM is less prone to creaming and cold agglutination at 5 °C than CM, potentially due to weaker interactions between Igs, milk fat globules, and the MFGM (Meng et al., 2020). This enhanced stability during refrigeration minimises adhesion to containers and ensures consistent nutrient delivery. Consistent with these observations, Basdeki et al. (2021) reported superior stabilising activity of HM protein concentrate in oil-in-water emulsions compared with CM proteins. Collectively, these findings highlight HM's superior physical stability relative to CM.

3.2. Digestibility

The composition and structure of HM are uniquely tailored to the immature digestive system of the infant, with the lower protein content and higher whey-to-casein ratio serving as key features that support gentle and efficient gastric processing and nutrient delivery to the small intestine (Roy et al., 2020). Whey proteins are often referred to as 'fast proteins' due to their faster gastric emptying and postprandial AA release in the plasma (Boirie et al., 1997). In addition, the higher β-casein to α_s-casein ratio of HM, combined with the inherently smaller casein micelle size and lower degree of phosphorylation of HM, leads to the formation of softer, more porous curd structures during the gastric phase. This is seen to make HM more readily and "easier" digestible by the infant. In contrast, while the flexibility and structure of bovine casein micelles make them susceptible to enzymatic hydrolysis, their larger size, higher degree of mineralisation, and greater proportion of highly phosphorylated α_s-caseins promote the formation of denser coagula, which persist throughout gastric digestion, posing challenges for infant digestion (Dupont & Tomé, 2019; Yang et al., 2024). Recent work by Yang et al. (2024) highlighted improved *in vitro* infant gastrointestinal digestibility of CM formed from the fractionation of bovine caseins and the simulation of human casein composition (FM-MP), with gastric coagulation, casein degradation rate, and free amino group release comparable to those of human casein micelles. This study demonstrates the importance of phosphorylation patterns in modulating protein digestion and highlights the superiority of FM-MP for infant nutrition, owing to its similarity to human caseins. The impact of whey-to-casein ratios on gastric behaviour has been demonstrated in dynamic *in vitro* digestion models simulating infant conditions. Lin et al. (2023), using an infant human gastric simulator (iHGS) with milk-based systems at various whey-to-casein ratios (100:0, 60:40, 40:60, and 20:80), found that casein-rich formulations formed larger, more aggregated structures and exhibited slower proteolysis, whereas whey-rich mixtures formed smaller, looser aggregates and were digested more rapidly. Consistent findings were reported by Descallar et al. (2024), who observed stronger coagulation and slower gastric emptying in casein-dominant commercial IMF under similar dynamic infant-gastrointestinal conditions. Collectively, these results underscore the critical role of the gastric phase in modulating protein structural organisation and digestion kinetics, with whey-to-casein ratio influencing the formation of either firmer or softer coagulates. This has important implications for the design of bovine-based IMF, particularly in relation to replicating the dynamic digestive behaviour of HM.

3.3. Immune development, gut microbiome, and allergenicity

It is well-established that HM plays a crucial role in protecting the infant, providing not only passive immunity but also targeted modulation of immune development. Central to immune system maturation during early life is the gut microbiota, with evidence that infant feeding practices significantly affect both gut microbiome composition and immune development (Davis et al., 2022). Considerable differences exist between HM and CM in their ability to modulate infant immune development.

SIgA, the predominant immunoglobulin in HM, is a key regulator of non-inflammatory mucosal immunity in infants, binding to pathogens to prevent attachment to the gut epithelium while permitting the colonisation of beneficial microorganisms (Guo et al., 2021). Although IgG can also perform a similar function in bovines, it primarily provides systemic immune protection through enhancing phagocytosis and activation of the complement system – a mechanism which is highly functional in calves, but ineffective in human infants who demonstrate poor absorption of bovine IgG, limiting its contribution to systemic immunity (Gazi et al., 2023). The superior immune protection of HM for infants can also be attributed to the higher concentrations of other immune-active compounds, including LF, lysozyme, and cytokines. Moreover, the

beneficial effects of HMOs on the infant gut microbiota have been reviewed by Zhang et al. (2021), including supporting the proliferation of *Bifidobacterium*, inhibiting the colonisation of pathogens such as *Campylobacter jejuni* and *Escherichia coli,* and preventing viral infections, including rotavirus and norovirus. While CMOs have also been associated with some of these effects, their less diverse, less complex structure precludes them from fully replicating the wide-ranging benefits of HMOs in supporting infant development (Hobbs et al., 2021).

This immune developmental divergence is further reflected in differences in allergenic outcomes. A key manifestation of immune developmental imbalance is cow's milk protein allergy (CMPA), one of the most prevalent food allergies in infancy, reflecting the considerable compositional gap between CM and HM with respect to protein types and concentrations. CM contains over 20 potential allergens, β-lac, α-lac, and caseins being the most clinically relevant (Cronin et al., 2023). Most CMPA cases triggered by whey proteins, mainly β-lac, which is absent from HM. The allergenicity of β-lac is largely attributed to its resistance to gastric and intestinal proteolysis, which allows intact protein to be absorbed through the infant intestinal mucosa, thereby eliciting an allergic response (Jaiswal & Worku, 2022). This risk is considered to be higher in pre-term infants, whose immature immune system and increased intestinal permeability ('leaky gut') may facilitate the transfer of intact proteins (Ma et al., 2018). However, term infants appear to equally susceptible, as several studies have reported no significant difference in the incidence of CMPA between pre-term and term cohorts (Henderson et al., 2022; Sağır et al., 2025). α-lac and caseins can also trigger and further exacerbate CMPA, as can minor allergens such as LF and BSA, albeit less frequently. In more than 70% of cases, affected infants exhibit sensitivity to multiple CM allergens, underscoring the condition's multi-allergen nature. CMPA may be IgE-mediated, non--IgE-mediated, or a combination of both, with symptoms including diarrhoea, vomiting, hives, wheezing, and, in extreme cases, anaphylaxis (Cronin et al., 2023). Early-life CMPA also increases the risk of developing other allergies later in life; Reynolds et al. (2016) reported co-allergy development in 45% of children diagnosed with CMPA in an Irish cohort, most commonly to egg (70%) and soya (39%). Unlike CM, HM contains immunoprotective factors, such as SIgA, LF, and lysozyme, which contribute to immune exclusion and modulation of antigen exposure (Jackson et al., 2026). The absence of these components in CM-based products may partially underlie differences in allergenic outcomes between breastfed and formula-fed infants.

Altogether, these gaps indicate the unique and specialised role of HM during early-life immune development, further highlighting how compositional differences between CM and HM can translate into functional consequences for the infant, with HM being uniquely tailored to support immune maturation and reduce the risk of allergenic responses, critical attributes which CM lacks.

4. Current limitations of IMF and strategies for improvement

Considering the significant compositional and functional disparities between CM and HM, it is unsurprising that these differences are reflected in bovine-derived IMF. Studies have demonstrated slower *in vitro* and *in vivo* (in piglet models) gastric digestion of IMF (whey-to-casein ratio of 59:41) compared with HM, primarily due to the formation of smaller, denser, more persistent gastric curds (Charton et al., 2024),

Table 1
Summary of limitations of infant milk formula (IMF) compared to human milk (HM).

Parameter	HM	IMF	Limitation of IMF compared to HM
Protein content & source	• 0.9 – 1.2 g/100 mL • Human-derived protein	• EU: infant formula (0-6 months) and follow-on formula (6-12 months): 1.2 -1.5 g/100 ml. US: infant formula (0-12 months): 1.2 - 3.0 g/100 mL • Bovine-derived protein	• Excess protein; increased risk of accelerated growth, obesity, metabolic syndrome in later life • Higher renal load • Bovine proteins affect physical stability, digestibility, immune system modulation, gut microbiome
Whey-to casein ratio & protein composition	• Early lactation: 70:30; mature milk: 60:40; late lactation: 50:50 • Protein fraction (% of total protein): a_{s1}-casein (6.5%), β-casein (30 %), κ-casein (10 %), α-lactalbumin (22 %), lactoferrin (11 %), IgA (13 %), HSA (7 %), lysozyme (2 %), osteopontin • Lacks a_{s2}-casein and β-lactoglobulin	• Not regulated; typically reported in the range of ~40:60 to ~60:40 (variable) • Protein fraction (% of total protein): a_{s1}-casein (14.5 %), a_{s2}-casein (4 %), β-casein (13.5 %), κ-casein (5 %), α-lactalbumin (19 %), β-lactoglobulin (29 %), IgG (trace), BSA (1 %)	• Dominated by β-lactoglobulin (absent in HM) and higher a_{s1}- and a_{s2}-caseins; increased risk of cow's milk protein allergy (CMPA) • Lacks lysozyme • Lactoferrin and osteopontin only added in 'premium' IMF, at lower levels; reduced roles in immune development • Contains bovine IgG and BSA (more suited for calves than humans)
Processing	• Usually consumed fresh • Donor milk: minimally processed (.e.g. pasteurised)	• Thermal treatment (UHT, pasteurisation) • Mechanical treatment (homogenisation, spray-drying)	• Denaturation of proteins • Degradation of heat-sensitive vitamins and bioactives • Casein structural changes (peptide cleavage, dephosphorylation) • Reduced digestibility, bioavailability, functionality
Digestibility	• Smaller casein micelles, less phosphorylation • Softer curd, porous structure • Faster gastric emptying	• Larger casein micelles, higher degree of phosphorylation • Denser curd • Slower gastric emptying	• Reduced nutrient bioaccessibility • More gastrointestinal discomfort
Human milk oligosaccharides (HMOs)	• 20 - 25 g/L (colostrum), 5 - 15 g/L (mature milk) • > 200 structurally distinct HMOs	• 0 – 6 HMOs, at lower concentrations	• Lacks HMO diversity and complexity • Reduced prebiotic and immune-modulating effects
Microbiota influence	• HMOs act as prebiotics • Contains live maternal microbes • Promotes colonisation by beneficial microorganisms	• Sterile • Some IMF fortified with probiotic or prebiotics e.g. GOS, FOS	• Reduced microbiota diversity • Less effective immune system priming • Higher risk of dysbiosis
Immune function	• Synergy of bioactive proteins, HMOs, and live microbiota provide passive protection and guides immune maturation	• Immune-active proteins mostly absent • Lacks HMO diversity • Limited prebiotic/probiotic function • Lacks synergy	• Inferior immune protection • Increased risk of infections, allergies, immune dysregulation

behaviour consistent with CM. In addition, Camacho-Morales et al. (2021) reported that IMF fails to promote adaptive immune activation in newborns and alters the establishment of a healthy gut microbiome, indicating that IMF more closely mirrors the functional properties of CM rather than the unique immunological and microbiological benefits of HM. These functional limitations are closely linked to IMF composition and processing, including protein content and structure, the absence or low abundance of bioactive compounds, and the effects of thermal and mechanical treatments (Table 1). Recent efforts to address these divergences typically involve targeted modifications to both formulation and manufacturing processes, with the aim of developing next-generation products that more closely resemble HM.

4.1. Protein content and composition

IMF typically contains a higher protein content than HM to compensate for differences in AA profiles and the inferior protein quality of bovine-derived proteins. EU regulations require infant formula (0-6 months) and follow-on formula (6-12 months) manufactured from non-hydrolysed cow's milk proteins to contain 1.2-1.5g/100 mL of protein, while in the US, an upper limit of 3.0g/100 mL is permitted for all IMF intended for infants from birth to 12 months. This higher ceiling largely accommodates specialised formulas or exempt medical formulas rather than standard products. Unlike HM, where protein concentrations decrease dynamically from approximately 2.1 to 1.1g/100 mL during the first 90 days of lactation to match the infant's evolving growth requirements, IMF protein content remains fixed within each feeding stage (Ren et al., 2022). This lack of adaptation may lead to excess protein intake, which has been associated with accelerated growth, altered body composition, and distinct metabolic markers in formula-fed infants (Nilsson et al., 2024). A meta-analysis of 20 clinical trials found that feeding low-protein IMF can support adequate growth comparable to that of breastfed infants (Ren et al., 2022), while long-term follow-up has linked high-protein IMF intake to increased kidney volume and higher systolic blood pressure in later childhood (Parada-Ricart et al., 2023).

Beyond total protein content, protein composition plays a critical role in the nutritional and allergenic profile of IMF. Although the whey-to-casein ratio in IMF is often adjusted to more closely resemble HM, the overall protein profile remains closer to that of CM. IMF contains higher levels of α_{s1}-casein (15%) and lower proportions of β-casein (14.5%) and α-lac (12%) than HM. Both β-lac and α_{s2}-casein (present in IMF but absent from HM) are recognised allergens, contributing to the higher incidence of CMPA in formula-fed infants (~2-3%) compared with exclusively breastfed infants (Høst, 2002; Jakobsson & Lindeberg, 1979). Moreover, Kelly et al. (2019) found that breastfed infants who were supplemented with IMF within 24 h of birth were 16 times more likely to develop CMPA, aligning with the 'leaky gut' phenomenon of the infant gut during this initial postnatal period (Ma et al., 2018; Rouwet et al., 2002).

To address these limitations, ingredient suppliers and manufacturers have focused on optimising both protein content and composition. α-lac-enriched ingredients produced by suppliers such as Agropur, Actus Nutrition, Arla Food Ingredients, and Hilmar Ingredients enable lower-protein IMF formulations while maintaining AA adequacy. Clinical studies have demonstrated that low-protein IMF supports anthropometric outcomes (weight, length, head circumference) and biochemical parameters (serum insulin, IGF-1, C-peptide) that more closely resemble those of breastfed infants, while α-lac enrichment helped to offset the reduction in indispensable amino acids (IAA) that is typically associated with low-protein formulations (Nilsson et al., 2023, 2024). Several commercial products have adopted this approach; for example, ByHeart reported improved protein utilisation and reduced gastrointestinal discomfort in infants fed an α-lac- and LF-enriched IMF (Kuehn et al., 2022). Efforts are also ongoing at a regulatory level. Nestlé Australia and New Zealand sought amendments to the regulatory limits for low-protein IMF in their respective countries, proposing that the minimum protein requirement for follow-on formula should be reduced from 0.45g/100 kJ to 0.38g/100 kJ, with these revised regulations successfully implemented in Australia in 2024. In May 2025, U.S. Food and Drug Administration (FDA) announced the first nutrient review of IMF since 1998, as part of Operation Stork Speed, issuing a Request for Information to determine whether the existing nutrient requirements should be revised. This process could involve reconsidering protein requirements, potentially aligning more closely with HM and with the lower thresholds already adopted in regions such as Australia.

4.2. Bioactive compounds

The negligible levels of bioactive compounds in CM are reflected in bovine-derived IMF. Although some 'premium' IMF are supplemented with bovine LF (bLF) (e.g. ByHeart, Enfamil Optimum), concentrations remain much lower than those found in HM. Moreover, IMF contains only trace levels of Igs, and lysozyme supplementation is rare (Demmelmair et al., 2017). Considering the crucial functional roles that these bioactive proteins play, their scarcity in IMF remains a limitation for formula-fed infants.

Clinical trials by Lönnerdal et al. (2016) and West et al. (2017) investigated IMF supplementation with bovine OPN at 50% and 100% of HM concentrations. Supplementation at the full HM level resulted in plasma branched-chain AA concentrations and circulating T-cell profiles more comparable to those of breastfed infants, alongside significantly lower levels of the proinflammatory cytokine TNF-α, suggesting immune-modulatory effects. Similarly, bLF supplementation has been shown to be safe for infant consumption, with some studies reporting reduced duration of respiratory illness symptoms and lower prevalence of acute gastrointestinal symptoms (Motoki et al., 2020). However, evidence remains inconsistent, as other trials have not observed significant reductions in sepsis (Ochoa et al., 2020) or infection-related morbidity (Björmsjö et al., 2022). These discrepancies raise questions about whether bovine-derived proteins can replicate the functionality of their human counterparts, with such variability likely arising from a combination of factors, including study design, dose, population, and processing-related effects. Specifically, heat-induced denaturation and aggregation during IMF manufacturing can alter bLF's tertiary structure, promote interactions with other IMF components, and impair stability, antimicrobial properties, bioactivity, and gastrointestinal digestion kinetics (Goulding et al., 2021). Additionally, commercial bLF's high iron saturation limits iron sequestration and bacteriostatic function compared to the predominantly apo-form of hLF in HM, which optimises gut iron withholding (Huang et al., 2025). These processing-related modifications, together with trial-specific variables such as differences in bLF source (e.g., purity, glycosylation profile), formulation matrix, dosing regimen, and timing of administration, are likely to contribute to the inconsistent outcomes reported across clinical studies.

Beyond protein functionality, the oligosaccharide fraction further distinguishes HM from IMF (Wiciński et al., 2020). HMO supplementation has shown promising benefits; for example, IMF containing 2′-FL and LNnT was well-tolerated by infants and resulted in lower morbidity and medication use (Puccio et al., 2017). Elsewhere, consumption of IMF enriched with a blend of five distinct HMOs was shown to increase the abundance of *Bifidobacterium* and decrease the prevalence of *Clostridium difficile*, shifting the gut microbiota closer to that of breastfed infants (Bosheva et al., 2022). Nonetheless, many IMFs do not contain HMOs, and those that do typically include only a limited number at low concentrations, thereby falling short of the >200 distinct HMOs found in HM (Wiciński et al., 2020). Although a limited number of abundant HMOs dominate overall content, evidence suggests that biological activity is not solely determined by abundance but is instead highly structure-specific and influenced by interactions between multiple glycans. Consequently, the exclusion of lower-abundance HMOs and the simplification of overall HMO profiles in IMFs may limit the ability to

replicate the integrated functional effects observed in HM. Nevertheless, the area is advancing, with IMFs from brands such as Nestlé and Vinamilk incorporating up to 6 HMO variants to better align with the complexity of HM. However, a key limitation in bioactive supplementation is that many HM components are dynamically regulated across lactation, rather than static. In particular, HMO composition and concentration vary significantly over time (from colostrum to mature milk), within feeds, and between individuals (Soyyilmaz et al., 2021). This dynamic regulation extends beyond HMOs to other bioactive components, which also change with lactation stage and maternal-infant conditions. As a result, IMFs that rely on fixed, static formulations of a limited set of bioactives struggle to replicate the evolving functional profile of HM.

4.3. Processing

Although many biologically active compounds in HM are thermolabile, they are largely preserved because HM is typically consumed fresh, or in the case of donor milk, minimally processed. As a result, a considerable proportion of these compounds remains structurally intact and functionally active (Meng et al., 2021). In contrast, IMF undergoes a series of intensive thermal and mechanical processes during production, including ultra-high-temperature (UHT) treatment, pasteurisation, homogenisation, and spray-drying. While these treatments are essential for microbiological safety and product stability, they promote denaturation of whey proteins, protein aggregation, and physical instability, as previously discussed in Section 3.1 (Singh & Fox, 1986; Smits & Van Brouwershaven, 1980).

IMF are generally safe, nutritionally adequate, and well tolerated under regulatory standards, with mineral fortification designed to meet Codex/EU requirements; however, heat-induced differences in protein structure and matrix composition may still influence mineral bioaccessibility and absorption dynamics relative to HM (Fioravanti et al., 2020; Francisquini et al., 2020), highlighting functional rather than nutritional distinctions. Thermal processing can also contribute to poorer digestibility and reduced IAA bioaccessibility compared with HM (Charton et al., 2024), as well as to compromised functional properties, including emulsion instability, decreased solubility, and increased viscosity (Bakshi et al., 2023). Moreover, processing and formulation can alter the structure and functionality of supplemented bioactives, as exemplified by bLF.

Dry blending has been explored as one approach to retain heat-sensitive compounds in IMF, although this production method poses a risk of microbial contamination and is often associated with solubility, wettability, and homogeneity issues (Bakshi et al., 2023). Recent advancements in non-thermal processing technologies for IMF have been extensively reviewed and include high-pressure processing (HPP), pulsed electric field (PEF), cold plasma inactivation, and membrane filtration (Bakshi et al., 2023; Dold et al., 2025; Pasdar et al., 2024). These emerging technologies have demonstrated desirable outcomes, including accelerated protein hydrolysis during both *in vitro* and *in vivo* digestion, enhanced sensory properties, and improved microbial inactivation. However, most studies currently compare non-thermally treated IMF directly with its thermally treated counterpart, leaving its comparability with HM largely unexplored. Nevertheless, the improvements over traditional IMF serve as a positive step forward for infant health.

5. pFerm of HM molecules: a biotechnological opportunity

Despite recent advances, IMF remains limited in its ability to fully replicate the functionality of HM, primarily because of its reliance on bovine-derived protein, and the methods by which it is processed. These limitations are particularly evident when considering some of the studies discussed in sections 4.1 and 4.2, where adjustments to IMF resulted in outcomes consistently described as “more similar to”, “close to”, “closer to”, “approaching”, and “comparable with” HM. While such language highlights the positive impact of these improvements, it simultaneously underscores the continued inability of IMF to fully bridge the functional gap between bovine-derived formulations and HM. Moreover, most of these advancements have focused on the adjustment or supplementation of a single component - for example, modifying the α-lac content, reducing β-lac levels, adding LF, or applying an alternative processing technique - but rarely a combination of multiple factors. To date, no published studies, nor any commercial IMF, comprehensively replicate the complex and dynamic nature of HM.

The incorporation of HM-derived proteins and oligosaccharides is a promising strategy to better align the nutritional and functional aspects of IMF with those of HM. However, the direct isolation of these molecules from HM for large-scale application in IMF is constrained by ethical, logistical, and scalability limitations. While HM-derived milk fortifiers have been commercialised by Prolacta Bioscience, their use is primarily restricted to preterm infants in clinical settings due to reliance on donor milk and limited supply. Consequently, such approaches are not feasible for widespread incorporation into standard IMF. In contrast, recombinant biotechnologies offer a scalable platform for the industrial production of HM proteins and oligosaccharides, with potential for broad application across the general infant population. Accordingly, these technologies have received significant attention in recent years. Since the early 2000s, one approach has involved the expression of HM proteins such as LF, α-lac, and lysozyme in transgenic animals (Wang et al., 2008; Yang et al., 2008). However, most of this research has remained at the experimental stage, with limited advancement towards commercialisation due to economic, safety, and regulatory constraints (Vishwanath-Deutsch et al., 2024) In this context, pFerm has emerged as a particularly promising biotechnology for the scalable the production of bio-identical HM components, thereby enabling IMF formulations to more closely mimic the composition and biological activity of HM. Historically, pFerm was used in the pharmaceutical and food industries to produce human insulin and chymosin (Nielsen et al., 2024), although the focus has recently shifted towards the generation of various molecules, including proteins, oligosaccharides, lipids, and vitamins, for use in food products.

5.1. The pFerm process

Extensively reviewed by Knychala et al. (2024) and Nielsen et al. (2024), the pFerm process comprises several steps (Fig. 2). Briefly, a gene encoding the target protein is inserted into a bacterial, yeast, or filamentous fungal host, with microorganisms such as *Escherichia coli*, *Aspergillus oryzae, Saccharomyces cerevisiae, Komagataella phaffii,* and *Trichoderma reesei* commonly used due to their well-established roles in industrial biotechnology. For non-protein targets, the introduced genetic material may encode entire biosynthetic pathways or metabolic enzymes required for precursor synthesis and product conversion (Augustin et al., 2024). Following strain construction, lab-scale cultivation is used to optimise recombinant production through a combination of genetic engineering approaches (e.g. codon optimisation, signal peptides, CRISPR-cas) and process parameter control (e.g. pH, temperature, medium, dissolved oxygen) with the aim of improving yield and economic feasibility (Liu et al., 2025; Nielsen et al., 2024). The process is subsequently scaled up from laboratory to pilot and industrial production. Downstream processing involves recovery and purification of the target compound and may include cell lysis (for intra-cellular products), centrifugation, filtration, chromatographic separation, membrane filtration, and/or solvent extraction. The purified molecule is typically stabilised via freeze-dried or spray-dried, although liquid formulations may also be used. Prior to food application, pFerm-derived ingredients undergo extensive safety and functional testing, including compositional and molecular characterisation, toxicological and allergenicity assessment, microbiological and shelf-life testing, and technofunctional evaluation. Regulatory approval from relevant authorities is required

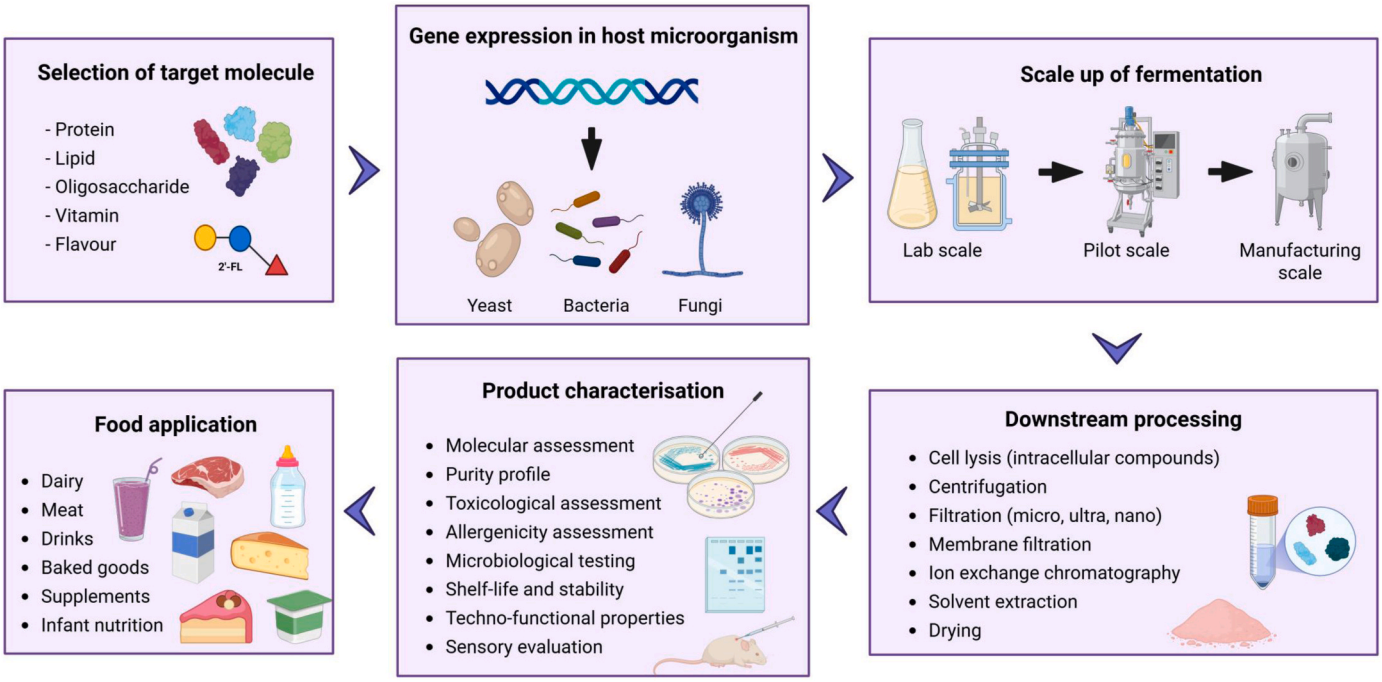

Fig. 2. Precision fermentation process. A gene for the expression of the target compound of interest is inserted into a host microorganism. Expression of the recombinant molecule is optimised at lab-scale, and the production is scaled up to pilot and industrial scale manufacture. The product is recovered and purified downstream and undergoes a range of safety testing and characterisation prior to food application. Created in BioRender. Nyhan, L. (2026) https://BioRender.com/4ylewwj.

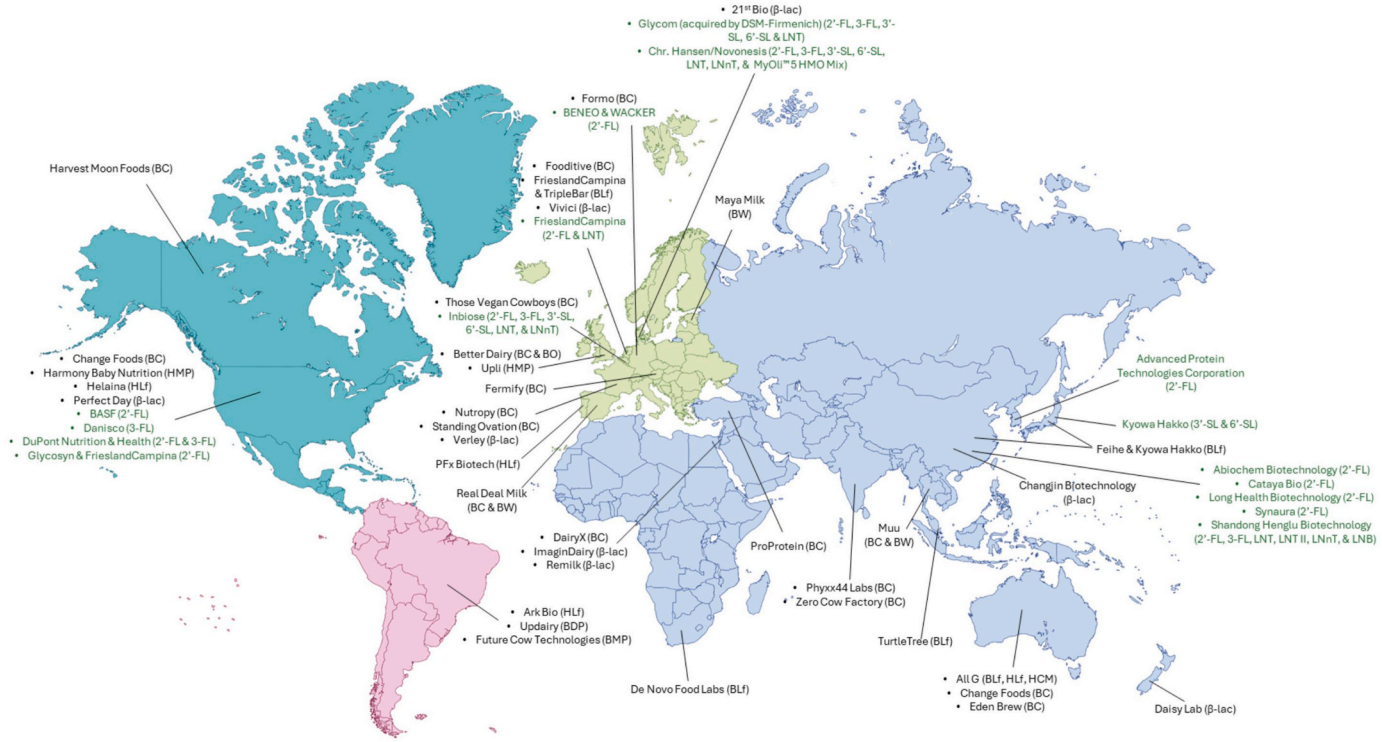

Fig. 3. Global location of companies publicly producing recombinant milk proteins (black text) or human milk oligosaccharides (HMOs) (green text) via precision fermentation. Abbreviations in brackets denote the type and source of protein or HMO produced. BLf: bovine lactoferrin, BC: bovine casein, BDP: bovine dairy proteins, BW: bovine whey, β-lac: β-lactoglobulin, HLf: human lactoferrin, HCM: human casein micelles, HMP: human milk proteins. Information correct at the time of writing. Created in BioRender. Nyhan, L. (2026) https://BioRender.com/4kl1ind. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

before commercialisation. The final ingredient can then be incorporated into a range of food matrices, including dairy and meat analogues, nutritional products, and IMF (Knychala et al., 2024; Nielsen et al., 2024).

5.2. Market overview of pFerm-derived milk proteins and HMOs

pFerm of milk molecules is a rapidly evolving area of research, as evidenced by the increasing number of companies entering the market. A market analysis was performed to identify companies producing recombinant milk proteins or HMOs via pFerm (Fig. 3). For this analysis, each independent company was counted separately, regardless of its role in production, technology development, or strategic partnerships (e.g. FrieslandCampina and Triplebar were counted as two distinct entities).

At the time of writing, 39 independent companies are actively involved in manufacturing pFerm-derived recombinant dairy proteins, distributed across Europe (40%), North America (15%), Latin America (8%), and the Asia-Pacific region (38%) (Fig. 3). Except for Perfect Day, founded in 2014, most of these firms were established or shifted their focus towards precision-fermented milk compounds from 2018 onwards, highlighting the relative novelty and growing momentum within the field. Notably, 87% of the identified manufacturers are producing only bovine proteins with a predominant emphasis on casein (41%), followed by LF (30.8%), β-lac (20.5%), OPN (2.6%), whey protein (5.1%), and unspecified dairy or milk proteins (3.5%). While most are commercialising isolated ingredients, several are incorporating recombinant proteins into finished food products; for example, Formo and New Culture are producing cheese using pFerm-derived casein molecules.

In contrast, few companies are focused on HM proteins, with only six identified in this analysis. Upli has broadly indicated interest in HM proteins, while Harmony Baby Nutrition is developing an IMF formulation incorporating breast milk proteins. Four companies (Ark Bio Solutions, Helaina Inc., PFx Biotech, and All G) are developing rhLF. In addition, All G is pursuing the production of human casein micelles and has filed a patent application describing a formulation comprising five recombinant HM proteins: α-lac, LF, β-casein, κ-casein and HSA. As the first reported effort to produce human casein micelles at scale via pFerm, this approach represents the closest attempt to date to replicate the compositional complexity of HM.

Compared with recombinant protein production, HMO production via pFerm is well established. The feasibility of this approach was first demonstrated in the late 1990s and early 2000s, with the first regulatory-approved product (2′-FL by Jennewein Biotechnologies Gmb (acquired by Chr. Hansen, now part of Novonesis) entering the market in 2015 (Cornall, 2019; Walsh et al., 2020). The HMO sector is currently dominated by companies based in Europe (44%), Asia-Pacific (31%), and North America (25%), including Glycom (now part of DSM-Firmenich), FrieslandCampina, and Chr. Hansen (now part of Novonesis), Shandong Henglu Biotechnology, and Inbiose, with a comprehensive overview provided in Fig. 3. Market activity is strongly centred on 2′-FL, produced by 88 % of the profiled companies manufacturing this compound, followed by 3-FL (44%), LNT (38%), LNnT (31%), 3′-SL (31%) and 6′-SL (31%). Notably, only one company, Glycom (DSM-Firmenich), currently produces LNFP-I, despite it being one of the most fucosylated oligosaccharides in HM after 2′-FL. Glycom (DSM-Firmenich) additionally offers LNFP-I in combination with either 2′-FL or difucosyllactose (DFL). To date, Chr. Hansen (Novonesis) remains the only company to commercialise a five-HMO blend.

Building on the overview of market activity and key players, the following case studies illustrate how pFerm-derived rhLF and HMOs are currently being applied and investigated within the context of IMF development. These two categories represent both an emerging, protein-based innovation (rhLF) and a mature, widely adopted class of ingredients (HMOs), reflecting different stages of technological and commercial maturity.

5.3. Case study 1: rhLF

bLF isolated from CM is globally approved for use in IMF and other food applications, including in the US and the EU. While bLF and human milk lactoferrin (hmLF) are similar in some respects, the human form is considered to be more functionally active, particularly with regard to binding affinity to the small intestinal LF receptor, digestibility and peptide release, and immunogenicity (Lönnerdal et al., 2011; Ma et al., 2021). Previous efforts to produce rhLF using expression systems such as transgenic plants, fungi, and animals have shown limited commercial success (Vishwanath-Deutsch et al., 2024). Moreover, although talactoferrin (an rhLF) has been investigated in infant clinical studies (Sherman, Adamkin, et al., 2016; Sherman, Sherman, et al., 2016), it was evaluated as a pharmaceutical agent/drug rather than as a food ingredient or dietary supplement, placing it outside the scope of this review.

As the use of pFerm to produce rhLF for nutritional applications remains an emerging field, published literature on food-grade pFerm-derived rhLF remains limited. To date, the most comprehensive body of work has been generated by Helaina, the first company to report industrial-scale production of rhLF (effera®) that is bioidentical to hmLF. Notably, Helaina has actively generated and disseminated data on the structural, functional, and safety characteristics of its rhLF, providing valuable insights into this evolving field. A key focus of these studies is the direct comparison of rhLF with hmLF from multiple sources (commercial samples and hmLF isolated in-house from HM) and/or bLF, further highlighting the potential of rhLF as a bioidentical and functionally relevant alternative for use in infant nutrition.

Lu et al. (2024) reported a comprehensive molecular characterisation of Helaina's rhLF, produced using a modified *Komagataella phaffii* expression system at industrial scale and purified to ≥98% protein purity. The AA sequence of rhLF was found to be 100% identical to that of hmLF, with comparable molecular weights (rhLF: ~76-80 kDa; hmLF: ~80-83 kDa) and antibody recognition, confirming equivalence in primary structure and molecular identity. Site-specific glycosylation was detected at the three N-linked asparagine sites previously reported for native hmLF, with high occupancy at Asn-156 and Asn-497 (~75–80%) and lower occupancy at Asn-642 (~18%), consistent with native hmLF. Some differences in glycan composition were observed, reflecting host-specific post-translational modifications. Secondary and tertiary structural analyses demonstrated a high degree of similarity between rhLF and hmLF, with both proteins adopting well-folded, globular conformations and exhibiting an overall structural similarity of approximately 96%. Differences in iron saturation profiles indicated that rhLF was present as a mixture of apo- and holo-forms, with an intermediate iron saturation level (50-55%) relative to apo- and holo-hmLF, a property also reflected in its thermal stability, which closely resembled that of native hmLF (Lu et al., 2024).

Kim et al. (2024) investigated whether the observed structural similarity between rhLF and hmLF translated into comparable functional behaviour during digestion. Using a static *in vitro* digestion model, the authors examined protein retention, peptide release, and the influence of iron saturation. Partial survival of both hmLF and rhLF was observed after the gastric phase, followed by extensive degradation during intestinal digestion, consistent with previous reports of hmLF behaviour. In contrast, intact bLF was not detected in any of the digestive samples, confirming its susceptibility to gastric digestion. Overall, rhLF exhibited digestion kinetics and peptide profiles more comparable to hmLF than bLF, reflecting their identical amino acid sequences. Identification of bioactive peptides further supported this similarity, with a greater overlap between hmLF and rhLF digests (14 peptides) than between hmLF and bLF (1 peptide).

Despite complete sequence identity, host-derived post-translational modifications raise theoretical concerns regarding the generation of new epitopes and potential immunogenicity (Lu et al., 2024). Addressing this, Anaya et al. (2024) conducted a literature search and bioinformatic

allergenicity analyses and reported no evidence of allergenic risk associated with Helaina's rhLF. Pre-clinical toxicology studies demonstrated that rhLF was well tolerated in rats at doses substantially exceeding intended commercial exposure levels in non-infant populations (Peterson et al., 2024, 2025b). These findings were supported by a 28-day randomised clinical trial in healthy adults (Peterson et al., 2025a)., which showed no change in anti-hLF antibody levels after consumption of any rhLF dose at either low or high doses during the study, or 28 days after the last exposure, highlighting the absence of an allergenic response. In contrast, increased anti-bLF antibody responses were observed in more than 50% of participants in the bLF group, although these responses were not associated with adverse clinical outcomes. Collectively, these data support a reasonable certainty that pFerm-derived rhLF does not pose an increased immunogenic or allergenic risk and highlight its suitability for further evaluation as a nutritional ingredient.

Within the same clinical trial, Peterson et al. (2025c) also investigated the impact of rhLF supplementation on gut microbiome diversity and short-chain fatty acid (SCFA) production. The study revealed that rhLF supplementation maintained a diverse microbiome and was associated with increases in potentially beneficial taxa, including *Eubacterium, Agathobacter, Lachnospira, Faecalibacterium,* and *Paraprevotella*. Moreover, acetate levels were significantly higher in the rhLF groups compared with bLF, indicating that rhLF may also promote a more health-associated, acetate-enriched fermentative profile rather than just increasing total SCFA production.

5.4. Case study 2: HMOs

Unlike recombinant HM proteins that require rigorous structural and functional characterisation to establish bioequivalence to native human counterparts, HMOs produced via pFerm are structurally and chemically identical to those found in HM. Consequently, the literature places less emphasis on direct comparison with breastmilk-derived HMOs and focuses primarily on functional outcomes. Although most studies do not explicitly state the production method, the scalability, efficiency, and regulatory success of microbial fermentation suggest that most HMOs evaluated in recent studies are pFerm-derived, reflecting a broader shift away from chemical or enzymatic synthesis (Walsh et al., 2020). Accordingly, this case study focuses on key investigations assessing the physiological impacts of individual and blended HMOs rather than comparative characterisation.

In contrast to rhLF, for which only a limited number of clinical studies have been completed or are ongoing to date, a substantial body of human intervention trials evaluating HMO supplementation have been conducted between 2010 and 2025 (Table 2). This is further supported by a recent review, which identified 26 HMO intervention studies published up to 2023 (Schönknecht et al., 2023). Encompassing adults, infants, and children. Of the 30 studies identified, 22 have been completed, with published results available for 18, while the remaining trials are ongoing. Most studies are industry-sponsored and involve HMOs already authorised for use in IMF, with 2′-FL - alone or in combination with LNnT - being the most frequently studied.

Across studies, HMO-fortified products were consistently reported to be safe and well-tolerated, with similar rates of adverse events between experimental and control groups. Improved or comparable gastrointestinal tolerance was observed, including reduction in regurgitation, flatulence, vomiting, crying, and fussiness groups (Alliet et al., 2022; Gold et al., 2022; Jochum et al., 2023; Lasekan et al., 2022; Marriage et al., 2015; Parschat et al., 2021; Puccio et al., 2017; Ramirez-Farias et al., 2021; Riechmann et al., 2020; Storm et al., 2019), while several studies reported stool characteristics more similar to those of breastfed infants (Lasekan et al., 2022; Parschat et al., 2021). Notably, trials evaluating blends of multiple HMOs reported more frequent and softer stools, suggesting that multi-HMO formulations, as investigated in studies using physiologically relevant concentrations (~3 g/L 2′-FL, ~0.8 g/L 3-FL, ~1.5 g/L LNT, ~0.2 g/L 3′-SL,~0.3 g/L 6′-SL) (Lasekan et al., 2022; Parschat et al., 2021), may better replicate aspects of HM functionality.

Most trials assessing anthropometric measurements reported comparable growth between HMO-supplemented and control groups, highlighting the ability of HMO-containing products to support age-appropriate growth in infants. Notably, Hascoët et al. (2022) observed a moderate but significant (and clinically meaningful) increase in the length and head circumference of pre-term infants by the time of discharge. This was the first interventional study evaluating HMO supplementation in a premature cohort, thereby advancing the use of these ingredients in pre-term infant nutrition.

HMO supplementation was also associated with modulation of the infant gut microbiome. In infants with CMPA, Gold et al. (2022) reported that an AA-based IMF supplemented with 2′-FL (1.0 g/L) and LNnT (0.5 g/L) increased the abundance of HMO-utilising bifidobacteria and reduced Proteobacteria, including *E. coli spp*. and *Klebsiella* spp., partially correcting baseline gut dysbiosis. Similiarly, Boulangé et al. (2023) reported comparable effects in CMPA-diagnosed infants fed an extensively hydrolysed IMF fortified with the same HMO combination, including enrichment of bifidobacteria and delayed maturation of the gut microbiome towards an adult-like profile. Comparable trends were also observed in healthy infants, with several studies reporting increased abundance of bifidobacteria and a shift towards a breastfed-like microbiome profile (Alliet et al., 2022; Berger et al., 2020; Bosheva et al., 2022). In contrast, Wallingford et al. (2022) did not observe any differences in microbiome richness or diversity between experimental and control IMF groups, potentially due to the use of a single HMO (2′-FL, 1.0 g/L), which the authors suggested may have been insufficient to elicit an effect. Notably, although Alliet et al. (2022) used the same concentration of 2′-FL, it was combined with *L. reuteri,* while other studies combined 2′-FL with additional HMOs such as LNnT (0.5 g/L) (Berger et al., 2020; Gold et al., 2022), or evaluated more complex HMO blends (1.5 or 2.5 g/L HMOs, comprising 0.87 or 1.45 g/L 2′-FL, 0.10 or 0.14 g/L DFL, 0.29 or 0.48 g/L LNT, 0.11 or 0.18 g/L 3′-SL, 0.14 or 0.24 g/L 6′-SL). (Bosheva et al., 2022). Collectively, these findings suggest that multi-HMO formulations, alone or in combination with probiotics, may more effectively modulate the infant gut microbiome and related functional outcomes; however, heterogeneity in study design, dosing, and HMO composition limits direct comparison across trials. It should also be acknowledged that donor-specific responses may influence observed outcomes. Differences in baseline microbiota composition and strain-level metabolic capacity, including the ability of distinct *Bifidobacterium* species and strains to utilise fucosylated HMOs such as 2′-FL (He et al., 2021), can affect the utilisation efficiency of specific HMOs and thus the magnitude and direction of microbiome and health-related effects. Host genetics, including fucosyltransferase2 (*FUT2)* gene secretor status, may also modulate infant responses to 2′-FL (Thorman et al., 2023), and differences in the proportion of secretor infants between cohorts could contribute to heterogeneity in microbiome outcomes observed in HMO-intervention trials. Overall, further studies exploring a wider range of HMO doses and compositional ratios are required to fully elucidate structure–function relationships and their physiological relevance, alongside further investigation into the role of infant host genetics and inter-individual variability in shaping responses to HMO supplementation.

5.5. Knowledge gaps

While research on pFerm-derived HM molecules is advancing, several critical gaps remain. For recombinant proteins, there is a substantial lack of data directly characterising and comparing the structural and functional bioequivalence of HM proteins with their pFerm-derived counterparts. Moreover, although it is promising that clinical studies investigating the safety and efficacy of recombinant HM proteins are underway or planned, they focus on adults rather than infants. This

Table 2
Clinical trials registered between 2010 and 2025 investigating supplementation with recombinant human lactoferrin (in adults) and human milk oligosaccharides (in infants) produced via precision fermentation. Information correct at the time of writing.

Study title and ID	Study population	Intervention group(s)	Comparison group(s)	Duration of intervention	Key findings	Reference
Effects of effera Human Lactoferrin at One Dose Compared to Placebo on Gut Permeability (NCT07035964; Recruiting)	Healthy adults aged 18-40 years	Supplement containing effera human lactoferrin	Control placebo supplement	4 weeks	Study ongoing	-
Effects of Lactoferrin at Two Doses vs. Active Control on Markers of Immune Function (NCT06012669; Completed)	Healthy adults aged 18-45 years	- Supplement containing 0.34g human recombinant lactoferrin (low dose) (n = 21) - Supplement containing 3.4g human recombinant lactoferrin (high dose) (n = 22)	- Supplement containing 3.4g bovine lactoferrin (n = 23)	4 weeks	- Safe and well tolerated - No changes in anti-hLF antibody levels - No evidence of alloimmunisation - Increased short chain fatty acid production (particularly acetate) - Maintained microbial diversity and supported potentially beneficial taxa	Peterson et al. (2025a); Peterson, Crawford, et al., 2025
Clinical Safety of a New Infant Starter Formula Containing 2 Human Milk Oligosaccharides (HMOs) (NCT01715246; Completed)	Healthy term, formula-fed infants aged ≤14 days	IMF fortified with 2′-FL (1.0 g/L) + LNnT (0.5 g/L) (n = 88)	- Control IMF without HMOs (n = 87) - Breastfed reference group (n = 38)	6 months (HMO intervention) + standard formula up to 12 months of age (no HMO intervention)	- Safe and well tolerated - Supported age-appropriate growth - Shifted gut microbiota composition closer to that of BF infants - Fewer parental reports of bronchitis, lower respiratory tract infection (LRTI), and antibiotic use - Identified HMO-modulated molecular pathways linked to the lower incidence of LRTI	Berger et al. (2020); Dogra et al. (2021); Martin et al. (2022); Puccio et al. (2017)
Growth and Tolerance of Young Infants Fed Infant Formulas (NCT01808105; Completed)	Healthy term infants aged 0-5 days	- IMF fortified with 2′-FL (0.2 g/L) + GOS (2.2 g/L) (n = 54) - IMF fortified with 2′-FL (1.0 g/L) + GOS (1.2 g/L) (n = 48)	- Control IMF fortified with GOS (2.4 g/L) (n = 48) - Breastfed reference group (n = 51)	4 months	- Safe and well tolerated - Supported age-appropriate growth (comparable to breastfed infants) - Modified innate and adaptive immune profiles to be more comparable to BF infants - Shifted circulating levels of secondary bile acids and other metabolites closer to that of the breastfed group	Goehring et al. (2016); Hill and Buck (2023); Marriage et al. (2015)
Growth of Infants Fed a New Term Infant Formula with a Prebiotic (NCT03090360; Completed)	Healthy term infants aged <14 days	IMF fortified with *L. reuteri* DSM 17938 (1×10^7CFU) + 2′-FL (1 g/L) (n = 144)	- Control IMF without HMO fortified with *L. reuteri* DSM 17938 (1×10^7 CFU) (n = 145) - Breastfed reference group (n = 60)	180 days	- Safe and well tolerated - Supported age-appropriate growth - Shifted β-diversity of gut microbiome closer to that of BF infants	Alliet et al. (2022)
Clinical Safety & Efficacy of a New Infant Formula with Specific Medical Purpose (FSMP) Containing 2 Human Milk Oligosaccharides (HMOs) (NCT03085134; Completed)	Full-term infants aged 0–6 months with physician-diagnosed CMPA	IMF fortified with 2′-FL (1.0 g/L) + LNnT (0.5 g/L) (n = 94)	- Control IMF without HMOs (n = 96)	4 months	- Safe and well tolerated - Significant reduction in CMPA-associated symptoms - Supported age-appropriate growth - Reduced incidence of upper respiratory tract infections	Boulangé et al. (2023); Vandenplas et al. (2022))

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Table 2 (*continued*)

Study title and ID	Study population	Intervention group(s)	Comparison group(s)	Duration of intervention	Key findings	Reference
					- Reduced incidence of gastrointestinal infections and medication use (not statistically significant) - Enriched bifidobacteria in gut microbiome and partially corrected dysbiosis in infants with CMPA	
Effects of Addition to Infant Formula of 2-fucosyllactose (2-FL) on Growth, Fecal Bacterial Populations and Safety (NCT03109223; Completed)	Healthy term infants aged <14 days	IMF fortified with 2′-FL (1 g/L) (n = 66)	- Control IMF without HMO (n = 66) - Breastfed refence group (n = 89)	16 weeks	- Safe - Supported age-appropriate growth - Moderately shifted the gut microbiome towards that of BF infants	Wallingford et al. (2022)
Hypoallergenicity Evaluation of a New Extensively Hydrolysed Formula (NCT03236207; Completed)	Term infants aged 2 months to 4 years with diagnosed CMPA (n = 67)	Extensively hydrolysed IMF fortified with 2′-FL (1.0 g/L) + LNnT (0.5 g/L)	Extensively hydrolysed IMF without HMOs	1 week	- Safe and well tolerated - Confirmed hypollergenicity	Nowak-Wegrzyn et al. (2019)
Infant Formula GI Tolerance Evaluation (NCT03307122; Completed) 21/09/2017	Healthy term infants aged 9-19 days	IMF fortified with *B. lactis* + 2′-FL (0.25 g/L) (n = 38)	Control IMF fortified with *B. lactis* (n = 40)	6 weeks	- Safe and well tolerated - Reduced incidence of infection (not statistically significant) - Supported age-appropriate growth	Storm et al. (2019)
Growth of Infants with Cow's Milk Allergy Fed an Amino Acid-based Formula Containing Two Human Milk Oligosaccharides: Comparison with World Health Organization (WHO) Growth Standards in an Observational, Single-arm Study (NCT03661736; Completed)	Term infants aged 1-8 months with physician-diagnosed moderate-to-severe CMPA	Amino acid-based IMF fortified with 2′-FL (1.0 g/L) and LNnT (0.5 g/L) (n = 32)	-	12 months	- Safe and well tolerated - Supported age-appropriate growth - Improved CMPA-associated symptoms - Enriched abundance of HMO-utilising bifidobacteria in the gut microbiome - Partial correction of dysbiosis	Gold et al. (2022)
Use of a Liquid Supplement Containing 2 Human Milk Oligosaccharides (HMOs) in Preterm Infants: a Double-blind, Randomized, Controlled Trial (NCT03607942; Completed)	Pre-term infants with low birth weight (<1700 g) aged ≤7 days	Liquid supplement containing 2′-FL and LNnT in a 10:1 ratio (0.340 and 0.034 g/kg body weight/day, respectively) (n = 43)	Liquid supplement containing only glucose (0.14 g/kg body weight/day) (n = 43)	-	- Safe and well tolerated - Significantly improved length and head circumference by time of hospital discharge	Hascoët et al. (2022)
Growth, Safety and Efficacy of a Starter Infant Formula, Follow-up Formula, and Growing-up Milk Supplemented with a Blend of Five Human Milk Oligosaccharides: a Double-blind, Randomized, Controlled Trial (NCT03722550; Completed)	Healthy term infants aged 7-21 days	- IMF fortified with 1.5 g/L of HMO blend: 2′FL (0.87 g/L), DFL (0.10 g/L), LNT (0.29 g/L, 3′-SL (0.11 g/L), and 6′-SL (0.14 g/L) (n = 158) - IMF fortified with 2.5 g/L of HMO blend: 2′FL (1.45 g/L, DFL (0.14 g/L), LNT (0.48 g/L, 3′-SL (0.18 g/L), and 6′-SL (0.24 g/L) (n = 153)	- Control IMF without HMOs (n = 155) - Breastfed refence group (n = 69)	6 months	- Safe and well tolerated - Shifted gut microbiome composition and diversity towards that of BF infants - Potentially contributed to the observed effect on intestinal immune response	Bosheva et al. (2022)
Growth and Feeding Tolerance of Infants Consuming a Formula Supplemented With Human Milk	Healthy term infants aged 7 days to 2 months	IMF fortified with 2′-FL (1.0 g/L) + LNnT (0.5 g/L) (n = 66)	- HMO formula + breastmilk (mixed group) (n = 48) - Breastfed refence group (n = 45)	8 weeks	- Safe and well tolerated - Supported age-appropriate growth	Riechmann et al. (2020)

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Table 2 (*continued*)

Study title and ID	Study population	Intervention group(s)	Comparison group(s)	Duration of intervention	Key findings	Reference
Oligosaccharides (HMOs): An Uncontrolled, Open-label, Prospective Study (NCT04055363; Completed)					- Shifted stool profile closer to that of BF infants	
Growth and Tolerance of Young Infants Fed Milk-Based Infant Formula with Oligosaccharides (NCT03967132; Completed)	Healthy term infants aged ≤14 days	IMF fortified with HMO	- Control IMF without HMO - Breastfed refence group	4 months	-	-
Evaluation of Nutritional Suitability and Tolerability of a Human Milk Oligosaccharide (HMO) Mix in Infant Formula for Term Infants (NCT03513744; Completed)	Healthy term infants aged ≤14 days	IMF fortified with 5.75 g/L of 5HMO-Mix: 2′-FL (2.99 g/L), 3-FL (0.75 g/L), LNT (1.5 g/L), 3′-SL (0.23 g/L), and 6′-SL (0.28 g/L) (n = 113)	- Control IMF without 5HMO-Mix (n = 112) - Breastfed refence group (n = 116)	4 months (HMO intervention) + 2 months voluntary follow-up (HMO intervention)	- Safe and well tolerated - Supported age-appropriate growth - More frequent, softer stools than control IMF group	Parschat et al. (2021)
Tolerance, Compliance and Growth of Infants Fed an Extensively Hydrolysed Infant Formula With Added Human Milk Oligosaccharides (NCT03884309; Completed)	Term infants aged <60 days already consuming a hypoallergenic extensively hydrolysed formula (eHF)	eHF fortified with 2′-FL (0.2 g/L) (n = 48)	-	60 days	- Safe and well tolerated - Supported age-appropriate growth - Improvement in parental reported symptoms	Ramirez-Farias et al. (2021)
Growth and Feeding Tolerance of Infants Consuming a Formula Supplemented With Human Milk Oligosaccharides (HMOs): An Uncontrolled, Open-label, Prospective Study (NCT05150288; Completed)	Healthy term infants aged 7 days to 2 months	IMF fortified with 2′-FL (1.0 g/L) + LNnT (0.5 g/L)	- Control IMF without HMO - Breastfed refence group	8 weeks	- Safe and well tolerated - Supported age-appropriate growth	Jochum et al. (2023)
Exclusively Formula-Fed Infants Effectively Colonizing with *B. Infantis* EVC001 From Lacto-N-Tetraose Supplementation (NCT03994315; Completed)	Healthy term infants aged 0-60 days who are exclusively formula-fed	- IMF with *B. infantis* EVC001 (8×10^9CFU) + LNT (3 g/L for 2 weeks, 8 g/L for following 2 weeks) - IMF with *B. infantis* EVC001 (8×10^9CFU) + LNT (6 g/L for 2 weeks, 12 g/L for following 2 weeks)	IMF with *B. infantis* EVC001 (8×10^9 CFU)	7 weeks	-	-
Growth and Tolerance of Young Infants Fed Milk-Based Infant Formula With Oligosaccharides (NCT04105686; Completed)	Healthy term infants aged ≤14 days	IMF fortified with 5 HMOs: 2′-FL (3.0 g/L, 3-FL (0.8 g/L), LNT (1.5 g/L), 3′-SL (0.2 g/L), and 6′-SL (0.3 g/L) (n = 130)	- Control IMF without HMOs (n = 129) - Breastfed refence group (n = 104)	4 months (extended to 6 months if required due to COVID restrictions)	- Safe and well tolerated - Comparable growth outcomes to the comparison groups - Softer, more frequent, and more yellow stools (more comparable to BF infants) - Less frequent visits to healthcare professionals	Lasekan et al. (2022)
The Effect of Human Milk Oligosaccharides (HMOs) (2′-Fucosyllactose (2′-FL) and Lacto-N-neotetraose (LNnT)) and Galacto-oligosaccharides (GOS) on Iron Absorption From a Maize-based Porridge in Kenyan Infants	Healthy term infants aged 8-12 months	Maize-based porridge fortified with iron (5 mg) in form of ferrous fumarate and HMOs (2′-FL (2g) + LNnT(1g))	- Maize-based porridge fortified with iron (5 mg) - Maize-based porridge fortified with iron (5 mg) + GOS (3g)	20 days	- Safe and well tolerated - Co-provision of HMO with iron did not affect iron absorption	Giorgetti et al. (2023)

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Table 2 (*continued*)

Study title and ID	Study population	Intervention group(s)	Comparison group(s)	Duration of intervention	Key findings	Reference
(NCT04163406; Completed)						
Iron Absorption from an Iron-fortified Follow-up Formula With and Without the Addition of a Synbiotic or Human Milk Oligosaccharides: a Stable Isotope Study in 10-14 Month-old Thai Children (NCT04774016; Completed)	Infants aged 8-14 months	Follow-up formula (FUF) fortified with iron (2.2 mg) and 2′-FL (100mg/100 ml)	- FUF with 2.2 mg iron and 400 mg/ml GOS +*L. reuteri* DSM 17938 - Control FUF with 2.2 mg iron and without GOS, *L. reuteri*, or 2′-FL	51 days	- Safe and well tolerated - Supplementation with 2′-FL did not affect iron absorption	Scheuchzer et al. (2024)
Infant Formula and Toddler Drink Feeding Intervention Through 24 Months of Age (NCT04957992; Completed)	Healthy term infants aged 0-14 days	- IMF fortified with oligosaccharides - Toddler drink fortified with oligosaccharides	- Control IMF without oligosaccharides - Control toddler drink without oligosaccharides - Breastmilk reference group (breastmilk, breastmilk supplemental formula, breastmilk toddler drink)	24 months	-	-
An Investigation of the Influence by Dietary Human Milk Oligosaccharide on Growth Factors and Cytokines in Blood, and Gut Microbiota in Low Birth Weight Infants (a Non-blinded Pilot Clinical Study) (NCT05203900; Completed)	Low birth weight (≥1500 g and <2500 g) infants aged up to 1 month	IMF fortified with HMO	Control IMF without HMO	-	-	-
Tolerance of Infants Fed a Hydrolysed Infant Formula (NCT05369494; Completed)	Formula-fed infants aged <90 days who are experiencing feeding intolerance, symptoms of suspected food protein allergy or currently consuming an extensively hydrolysed formula (EHF)	EHF fortified with 2′-FL (0.2 g/L) (n = 19)	-	1 month	- Safe and well tolerated - Significant improvement of weight-for-age z-scores in infants with suspected CMPA and/or persistent feeding difficulties.	Ramirez-Farias et al. (2024)
To Explore and Develop the Effective Human Milk Oligosaccharides and Microbiomes on Maternal and Infant Health and Neurodevelopment in Early Infancy (NCT05992493; Active, not recruiting)	Healthy term infants aged >1 day	IMF fortified with HMO	- Control IMF without HMO - Breastfed refence group	4 months	Study ongoing	-
Safety and Efficacy of Infant Formula Containing a Specific Blend of Six Human Milk Oligosaccharides: A Double-blind, Randomized, Controlled Trial (NCT06053112; Recruiting)	Healthy term infants aged 0-14 days	IMF fortified with a blend of 6 HMOs	- Control IMF without HMOs - Breastfed refence group	6 months	Study ongoing	-
A Randomized, Controlled Trial of Improved Immunology Outcomes Associated With Lactoferrin Fortified With HMO in Infant Formula	Healthy term infants aged 1-28 days	Stage 1 (up to 6 months) and stage 2 (6-12 months) IMF fortified with 2 types of HMOs	- Control stage 1 and stage 2 formulas without HMOs - Breastfed refence group	6 months (HMO intervention) + follow-up up to 12 months of age (no HMO intervention)	Study ongoing	-

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Table 2 (*continued*)

Study title and ID	Study population	Intervention group(s)	Comparison group(s)	Duration of intervention	Key findings	Reference
(NCT06158659; Recruiting)						
A Randomized, Controlled Trial of Improved Cognitive Outcomes Associated With Feihe HMO With DHA/ARA in Infant Formula (NCT06146387; Recruiting)	Healthy term infants aged 1-28 days	Stage 1 (up to 6 months) and stage 2 (6-12 months) IMF fortified with 2 types of HMOs	- Control stage 1 and stage 2 formulas without HMOs - Breastfed refence group	6 months (HMO intervention) + follow-up up to 12 months of age (no HMO intervention)	Study ongoing	-
Feeding Tolerance and Growth of Preterm Infants Consuming a Supplement Containing Two Human Milk Oligosaccharides (HMOs): A Post-Market Study (NCT06212427; Recruiting)	Pre-term infants with low birth weight (<2500 g) aged 0-14 days	Liquid supplement containing 2′-FL and LNnT	-	-	Study ongoing	-
Efficacy of a Ready to Feed (RTF) Starter Liquid Infant Formula Containing 2′FL and LNnT in Chinese Infants: A Double-blind, Randomized Controlled Trial Including a Breastfed Reference Group (NCT06361719; Recruiting)	Healthy term infants aged 3-14 days	Starter IMF fortified with 2 HMOs (up to 6 months) followed by classical follow-up formula not containing HMOs (6-12 months)	- Control IMFs not supplemented with HMOs - Breastfed refence group	6 months (HMO intervention) + follow-up up to 12 months of age (no HMO intervention)	Study ongoing	-
The Effect of Human Milk Oligosaccharides on Infant Gut Microbiota Modulation, Development of the Immune System and Health Later in Life: the BELIEF Study (NCT06631937; Recruiting)	Healthy term infants aged 0-28 days	IMF fortified with HMOs	- Control IMF without HMOs - Breastfed refence group	6 months	Study ongoing	-
Artemis: Evaluating the Impact of Synbiotic Supplementation on Infants and Toddlers (NCT06746285; Recruiting)	Healthy term infants and children aged 2-24 months	1 g of a synbiotic supplement containing a Bifidobacterium blend (1 billion CFU) and a mixture of 4 HMOs	1 g of lactose	1 month	Study ongoing	-

evidence gap is more extensively addressed for HMOs, with numerous completed and ongoing interventional studies evaluating the effects of HMO supplementation (many of which are likely conducted via pFerm) in both healthy and clinical infant cohorts. Despite increasing interest in multi-HMO blends, the diversity of HMOs currently used in formulations remains insufficient to fully capture the complexity of HM, underscoring the need for further research. Nevertheless, the clinical and regulatory success of pFerm-derived HMOs establishes a model for recombinant HM proteins. The growing number of market players and ongoing studies further indicate continued progress in this field. As research advances, remaining challenges can be broadly framed using the 3As framework proposed by Lurie-Luke (2024): (i) accessibility (regulatory landscape); (ii) affordability (economic viability); and (iii) acceptability (consumer perception). Each of these challenges also presents corresponding opportunities, which are discussed in the following sections.

6. Challenges and opportunities for pFerm-derived HM compounds

6.1. Regulatory challenges

Regulatory approval of recombinant milk proteins and oligosaccharides is imperative for ensuring safety, market entry, and consumer acceptance. Two key authorities exist within the global regulatory landscape for food ingredients: the FDA, which authorises the Generally Regarded as Safe (GRAS) pathway, and the European Food Safety Authority (EFSA), which conducts safety assessments under the EU Novel Foods Regulation (Eastham & Leman, 2024). Notably, unlike pharmaceutical compounds, regulatory approval of food ingredients by both the FDA and EFSA focuses on demonstrating safety rather than efficacy. While this is important for establishing a safety baseline for the consumption of recombinant milk proteins or HMOs at proposed inclusion levels, it has implications for functional claims and consumer perception, as it does not guarantee bioequivalence to HM compounds. This distinction is crucial for infant nutrition, where efficacy must be demonstrated through additional, independent clinical research.

6.1.1. US and EU regulatory pathways for pFerm-derived milk compounds

In the US, the GRAS framework is used to establish the safety of food ingredients under their intended conditions of use based on scientific evidence, rather than efficacy (Malinczak et al., 2024). pFerm-derived milk proteins may be authorised for food use through either the GRAS pathway or a Food Additive Petition. While GRAS determinations for general food use may be self-affirmed or FDA-reviewed, self-affirmed GRAS is not considered appropriate for IMF applications, which require FDA-reviewed authorisation supported by additional safety data specific

to infant exposure, followed by submission of an Infant Formula Notification. As a result, IMF applications represent a substantially higher regulatory threshold than general food use.

In contrast, the EU Novel Foods Regulation (EU 2015/2283) mandates a centralised, authority-driven safety assessment by EFSA for novel recombinant ingredients. This process requires a comprehensive dossier outlining ingredient identity, production process, nutritional data, toxicology, allergenicity, proposed conditions of use, and estimated intake (Fytsilis et al., 2024). While the EU Novel Foods process is widely regarded as less flexible and more time-intensive than US GRAS for general food use, IMF applications face comparably stringent and prolonged regulatory scrutiny in both jurisdictions, reflecting the higher safety bar applied to infant nutrition (Nielsen et al., 2024).

6.1.2. Current regulatory status of pFerm-derived milk compounds

The current regulatory status of pFerm-derived milk proteins and HMOs is summarised in Table 3. HMOs have a well-established regulatory history and generally face fewer regulatory challenges than recombinant proteins, due to their chemically defined structures, demonstrated safety, and non-immunogenic nature. The first HMO received FDA-reviewed GRAS notification and EU Novel Food Approval in 2015 and 2016, respectively (2′-FL by Glycom). Since then, numerous pFerm-derived HMOs have obtained regulatory clearance in both regions, with some also authorised for use in locations such as China, Canada, Australia, and Thailand. The field continues to evolve, with several GRAS and Novel Food applications currently pending from companies such as Zhuhai Long Health Biotechnology, Shandong Henlu Biotechnology, Cataya Bio and Chr. Hansen (Novonesis).

In contrast, the regulatory progression of pFerm-derived proteins has been comparatively limited, with approvals emerging only since 2020 and largely restricted to bovine-identical proteins approved for general food use in the US. Five manufacturers (New Culture, 21st.Bio, Fermify, All G, and Verley) have obtained self-affirmed GRAS status for pFerm bovine proteins, and five others (Perfect Day, Remilk, ImaginDairy, Vivici, and Turtle Tree) received no objection letters from the FDA for their pFerm-derived bovine proteins. The stringent and time-intensive nature of the European process is evidenced by the absence of approved pFerm-derived bovine milk proteins, with only two companies having submitted dossiers to date. The first was Perfect Day (2022) for β-lac, which remains paused in the validation phase, suggesting compliance or data availability issues. This was followed by Remilk (2023), also for β-lac; they have since received a non-suitability letter but have resubmitted corrections. To date, no recombinant HM proteins have received regulatory approval in either jurisdiction, and no active applications have been publicly disclosed.

6.1.3. Pathway to regulatory approval and remaining challenges

Considering the lack of regulatory approvals for recombinant HM proteins, and that prior FDA reviews of rhLF expressed in transgenic systems were withdrawn due to unresolved safety questions regarding immunotoxicity, potential for alloimmunisation, and allergenicity, it is essential to evaluate these aspects to demonstrate safety. To address this, Helaina designed a roadmap outlining the preclinical and clinical studies required to validate effera® rhLF for GRAS status for general food use (Fig. 4). This framework not only targets the specific safety concerns raised in past regulatory reviews but also generates data packages aligned with FDA and international guidance, making the approach broadly applicable to any recombinant HM protein seeking regulatory approval as a food ingredient (Malinczak et al., 2024).

To develop this pathway, Helaina convened a one-day virtual workshop in 2023 with a panel of leading scientific experts. The workshop included a review of existing rhLF safety evidence from transgenic systems (Vishwanath-Deutsch et al., 2024), which revealed a significant knowledge gap: most prior studies had focused on efficacy endpoints rather than exposure-related safety risks. The expert panel concluded that the initial rhLF characterisation data (Lu et al., 2024) were

Table 3
Global regulatory approvals of recombinant bovine milk proteins and human milk oligosaccharides. Information correct at the time of writing.

Recombinant bovine milk proteins		
Company	**Approved ingredient (s)**	**Regulatory approval(s)**
All G	Lactoferrin	- Approval to sell its recombinant bovine lactoferrin in China (Nov 2024) - Self-affirmed GRAS (Dec 2024)
Changing Bio	β-lactoglobulin (Kluvy®)	- GRN 1247, FDA no questions letter (Sep 2025)
Daisy Lab	Dairy-identical proteins	- Approval by the EPA for production of dairy-identical proteins using genetically modified organisms in a contained facility (May 2024)
Fermify	Casein	- Self-affirmed GRAS (Oct 2024)
ImaginDairy	β-lactoglobulin	- Approval by the Israeli Ministry of Health - GRN 1145, FDA no questions letter (Dec 2023)
New Culture	Casein	- Self-affirmed GRAS (Feb 2024) - Submitted product label and registration of animal-free mozzarella made with casein to the California Department of Food and Agriculture (CFDA) for review (Jan 2025)
Perfect Day	β-lactoglobulin (ProFerm™)	- GRN 863, FDA no questions letter (Mar 2020) - Approval by the Food Safety and Standards Authority of India (Nov 2022)
Remilk	β-lactoglobulin	- GRN 1056, FDA no questions letter (Feb 2023) - Approval by the Singapore Food Authority (Feb 2023)

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Table 3 (*continued*)

		- Approval by the Israeli Ministry of Health (Apr 2023)
		- Approval by Health Canada for the use of its cow-free milk protein in dairy products (Jan 2024)
Turtle Tree	Lactoferrin (LF+®)	- GRN 1219, FDA no questions letter (May 2025)
Verley	β-lactoglobulin (FermWhey™ Native, FermWhey™ Microstab, FermWhey™ Gel)	- Self-affirmed GRAS (Dec 2024)
Vivici	β-lactoglobulin (Vivitein™ BLG)	- GRN 1200, FDA no questions letter (Feb 2025)
21st.Bio	β-lactoglobulin (BLG Essential+)	- Self-affirmed GRAS (Sep 2024)
Human milk oligosaccharides		
Abiochem Biotechnology	2′-FL	- Approval by China's National Health Commission (Oct 2023)
Advanced Protein Technologies	2′-FL	- GRN 932, FDA no questions letter (Feb 2021)
		- EU Novel Food approval (Apr 2023)
BASF SE	2′-FL	- GRN 852, FDA no questions letter (Nov 2019)
Cataya Bio	2′-FL	- GRN 1238, FDA no questions letter (Jun 2025)
Danisco	3-FL	- GRN 951, FDA no questions letter (Aug 2021)
DuPont Nutrition & Health	- 2′-FL	- GRN 749, FDA no questions letter for 2′-FL (Apr 2018)
	- 3-FL	- GRN 897, FDA no questions letter for 2′-FL (Jun 2020)
		- EU Novel Food approval for 3-FL (Nov 2021)
		- Approval of 2′-FL by Health Canada (Nov 2021)
FrieslandCampina	- 2′-FL	- EU Novel Food approval for 2′-FL in partnership with Glycosyn (Dec 2017)
	- LNT	- GRN 735, FDA no questions letter for 2′-FL in partnership with Glycosyn (Apr 2018)
		- Approval of 2′-FL by the Food Standards Australia New Zealand (May 2022)
		- EU Novel Food approval for LNT (Jan 2023)
		- Approval of 2′-FL by the Chinese Ministry of Agriculture and Rural Affairs (Jan 2023)
		- Approval for use of 2′-FL in flavoured milk drinks (for children aged >3 years) and in products for infants and younger children in Thailand (Jan 2024)
		- Self-affirmed GRAS status for LNT (Jun 2024)
		- Approval of LNT by the Chinese Ministry of Agriculture and Rural Affairs (Jun 2024)
Glycosyn	2′-FL	- EU Novel Food approval in partnership with FrieslandCampina (Dec 2017)
		- GRN 735, FDA no questions letter in partnership with FrieslandCampina (Apr 2018)
Glycom (acquired by DSM-Firmenich in 2020)	- 2′-FL	- CELEX 32016D0375, EU Novel Food Approval for LNnT (Mar 2016)
	- 3-FL	- CELEX 32016D0376, EU Novel Food Approval for 2′-FL (Mar 2016)
	- 3′-SL	- GRN 650, FDA no questions letter for 2′-FL (Nov 2016)
	- 6′-SL	- GRN 659, FDA no
	- LNT	
	- LNnT	

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Table 3 (*continued*)

		questions letter for LNnT (Nov 2016)
	- LNFP-I	-
	- 2′-FL/DFL mix	GRN 815, FDA no questions letter for 2′-FL/DL mix (Aug 2019)
	- LNFP-I/2′-FL mix	- GRN 833, FDA no questions letter for LNT (Oct 2019)
		- CELEX 32019R1979, EU Novel Food Approval for 2′-FL/DL mix (Nov 2019)
		- Approval of 2′-FL and LNnT by the Food Standards Australia New Zealand (Dec 2019)
		- GRN 880, FDA no questions letter for 3′-SL (Feb 2020)
		- GRN 881, FDA no questions letter for 6′-SL (Feb 2020)
		- CELEX 32020R0484, EU ovel Food Approval for LNT (Apr 2020)
		- GRN 895, FDA no questions letter for LNnT (Dec 2020)
		- Approval of 2′-FL by Health Canada (Jul 2022)
		- GRN 1034, FDA no questions letter for 2′-FL (Oct 2022)
		- GRN 1037, FDA no questions letter for 3-FL (Nov 2022)
		- GRN 1059, FDA no questions letter for LNnT (Dec 2022)
		- GRN 1035, FDA no questions letter for LNFP-1/2′-FL mix (Jan 2023)
		- GRN 1060, FDA no questions letter for 2′-FL (Apr 2023)
		- CELEX 32023R0859, EU Novel Food Approval for 2′-FL (Apr 2023)
		- Approval of LNT, 3‘-SL, 6’-SL and 2′-FL/DFL by the Food Standards Australia New Zealand (Sep 2023)
		- CELEX 32023R2210, EU Novel Food Approval for 3-FL (Oct 2023)
Shandong Henglu Biotechnology	LNT II	- GRN 1208, FDA no questions letter (Apr 2025)
Inbiose		
	- 2′-FL	- GRN 1075, FDA no questions letter for 6′-SL (Mar 2023)
	- 3′-SL	
	- 6′-SL	- GRN 1067, FDA no questions letter for LNnT (Apr 2023)
	- LNT	
	- LNnT	- GRN 1074, FDA no questions letter for 3′-SL (Apr 2023)
		- GRN 1068, FDA no questions letter for LNT (Jun 2023)
		- GRN 1091, FDA no questions letter for 2′-FL (Dec 2023)
		- Approval of 2′-FL by the Chinese Ministry of Agriculture and Rural Affairs (Dec 2023)
		Approval of 2′-FL by Health Canada (May 2025)
Jennewein Biotechnologie (acquired by Chr. Hansen in 2020, now part of Novonesis)	- 2′-FL	- GRN 571, FDA no questions letter for 2′-FL (Nov 2015)
	- 3-FL	
	- 3′-SL	- EU Novel Food approval for 2′FL (Nov 2017)
	- 6′-SL	- Approval of 2′FL by Health Canada (Dec 2018)
	- LNT	
	- LNnT	- GRN 919, FDA no questions letter for LNnT (Oct 2020)
		- GRN 921, FDA no questions letter for 3′-SL (Oct 2020)
		- GRN 923, FDA no

(*continued on next page*)

Table 3 (*continued*)

		questions letter for LNT (Feb 2021)
		- GRN 925, FDA no questions letter for 3-FL (Feb 2021)
		- GRN 929, FDA no questions letter for 2′-FL (Feb 2021)
		- GRN 922, FDA no questions letter for 6′-SL (Apr 2021)
Kyowa Hakko Bio Co.		
	- 2′-FL	- GRN 1052, FDA no questions letter for 3′-SL (Apr 2023)
	- 3′-SL	
	- 6′-SL	- GRN 1053, FDA no questions letter for 6′-SL (May 2023)
		- CELEX 32023R2215, EU Novel Food Approval for 6′-SL (Oct 2023)
		- GRN 1051, FDA no questions letter for 2′-FL (Nov 2023)
		- CELEX 32024R1047, EU Novel Food Approval for 3′-SL (Apr 2024)
		- CELEX 32024R2036, EU Novel Food Approval for 2′-FL (Jul 2024)
		- Approval of 2′-FL by the Food Safety and Standards Authority of India (Sep 2024)
		- Approval for use of 2′-FL as a food additive by the National Health Commission of the People's Republic of China (Feb 2025)
Chr. Hansen (now part of Novonesis)		
	- 2′-FL	- Approval of 2′-FL by the Food Standards Australia New Zealand (Jun 2022)
	- 3-FL	
	- 3′-SL	- Approval of 2′-FL, 3-FL, LNT, 3′-SL, and 6′-SL by the Israeli Ministry of Health (2022)
	- 6′-SL	
	- LNT	- GRN 1014, FDA no questions letter for 2′-FL (Jul 2022)
	- LNnT	- GRN 1015, FDA no questions letter for 3′-SL (Jul 2022)
	- MyOli™ 5 HMO Mix	- GRN 1016, FDA no questions letter for 6′-SL (Jul 2022)
		- GRN 1017, FDA no questions letter for LNT (Aug 2022)
		- GRN 1040, FDA no questions letter for 2′-FL (Sep 2022)
		- CELEX 32023R0052, EU Novel Food Approval for 3-FL (Jan 2023)
		- CELEX 32023R0007, EU Novel Food Approval for LNT (Jan 2023)
		- CELEX 32023R0113, EU Novel Food Approval for 3′-SL (Jan 2023)
		- CELEX 32023R0948, EU Novel Food Approval for 6′-SL (May 2023_
		- GRN 1099, FDA no questions letter for 3-FL (Aug 2023)
		- GRN 1257, 2′-FL, pending
		- GRN 1258, LNT, pending
		- Approval of 2′-FL by the Chinese Ministry of Agriculture and Rural Affairs (Dec 2024)
Synaura	2′-FL	- GRN 1157, FDA no questions letter (Aug 2024)

sufficient and endorsed Helaina's proposed preclinical and clinical roadmap (Malinczak et al., 2024). The roadmap includes prediction of allergenicity (Anaya et al., 2024), assessment of digestive profile (Kim et al., 2024), a 14-day dose-range-finding toxicity rat study (Peterson et al., 2024), a 28-day immunotoxicity rat study (Peterson et al., 2025b), human cellular immunogenicity modelling (not yet published), and two adult clinical studies to evaluate immunogenicity/alloimmunisation potential (Peterson et al., 2025a). An additional clinical trial assessing the effect of rhLF on gut permeability, a safety-relevant marker of intestinal barrier function, is currently underway (trial ID: NCT07035964).

However, regulatory approval of pFerm-derived HM proteins

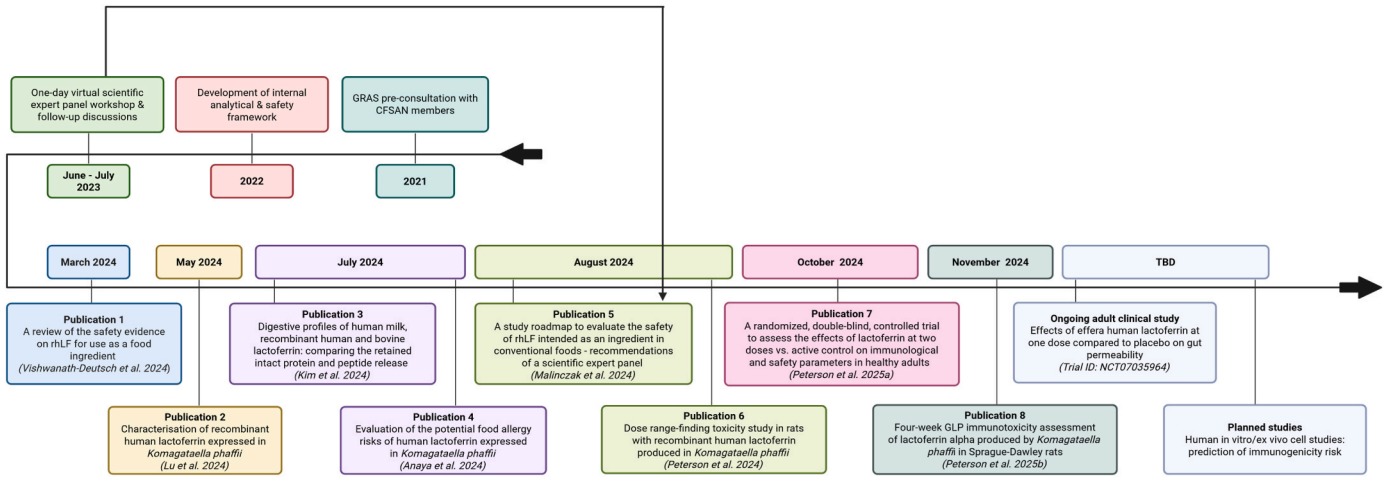

Fig. 4. Helaina's roadmap for safety validation of effera® recombinant human lactoferrin and other recombinant human milk proteins to achieve GRAS status as general food ingredients. Created in BioRender. Nyhan, L. (2026) https://BioRender.com/0ut1oai.

remains challenging due to several factors. The absence of a harmonised global definition for 'precision fermentation' contributes to variability in regulatory approaches across different jurisdictions, while ambiguity in the classification of pFerm-derived proteins as foods or food additives further complicates pathway selection (Sturme et al., 2025). Safety considerations remain imperative, particularly with respect to immunogenicity potential and alloimmunisation risk associated with bovine-identical and human-derived proteins, respectively (Fytsilis et al., 2024). These challenges are further amplified for IMF applications, where regulatory requirements are substantially more stringent than for general food use and necessitate additional exposure- and formula-specific safety evidence. More broadly, regulatory governance must continue to evolve alongside the field to enable faster commercialisation and market entry, with current regulatory approval processes (particularly in Europe) taking several years (Williams, 2021).

6.2. *Economic viability*

pFerm is poised to disrupt the conventional dairy protein market by enabling the production of animal-identical proteins without reliance on livestock farming, thereby addressing ethical concerns, reducing environmental impact, and potentially enhancing food production efficiency (Knychala et al., 2024; Nielsen et al., 2024). The global pFerm market was valued at USD 3.1 billion in 2025, and is forecasted to reach USD 34.9 billion by 2034, representing a compound annual growth rate of 27.9% (Custom Market Insights, 2025). This growth is driven by projected increases in global dairy consumption by 2050, rising consumer demand for sustainable and alternative food ingredients, and ongoing biotechnological advancements (Knychala et al., 2024).

At the biopharmaceutical scale, recombinant proteins are produced at low volumes with high purity but at high cost. In contrast, the production of food-grade pFerm-derived proteins requires substantial scale-up to achieve cost parity with traditional proteins. Although costs were initially high due to the novelty of the field, they have significantly decreased, from approximately USD 1 million/kg in 2000 to USD 100/kg in 2024, with forecasts projecting further reductions to below USD 10/kg by 2030 (Knychala et al., 2024). A recent report estimated the current cost of pFerm-derived dairy proteins at USD 30/kg, around three-fold than traditional dairy proteins; however, they predicted that this could reduce by 50-75% to approximately $8-$13/kg through optimisation of process design, strain selection, and scale-up (Mayers et al., 2025). These ongoing cost reductions are driven by significant investment in scale-up technologies, enabling economies of scale that minimise unit costs and enhance commercial viability (Ajayeoba & Ijabadeniyi, 2025). In 2024, several pFerm-focused companies received investments, including Perfect Day (USD 90 million), Formo (USD 61 million), and Future Cow (USD 246k) (The Good Food Institute, 2025). However, many start-ups still struggle with the 'scale-cost paradox': they find it difficult to finance large-scale production to demonstrate market demand, yet cannot demonstrate market viability without the economies of scale associated with large production capacity (Marcellin et al., 2024). The development of an industry roadmap for scaling would be invaluable, addressing critical factors including supply and demand dynamics, strategies to accelerate market entry, and requirements for large-scale production, such as feedstock availability and sustainable energy inputs (Mayers et al., 2025).

pFerm can stabilise supply chains by reducing reliance on volatile agricultural markets, which are influenced by factors such as climate, feed prices, and animal health (Marcellin et al., 2024). Beyond this, pFerm may also confer economic advantages through reductions in environmental footprint. A recent life cycle assessment compared the production of 1 L of conventional milk with an equivalent volume produced using pFerm-derived bovine proteins, reporting a 72% reduction in greenhouse gas emissions, an 81% decrease in water consumption, and a 99% reduction in arable land use (Hamelin & Cellier, 2024). Such improvements highlight the sustainability advantages associated with pFerm and may also translate into economic gains through tax incentives and subsidies (Marcellin et al., 2024).

While most economic and LCA studies compare pFerm dairy proteins with their animal-based counterparts, this framework does not fully reflect the case for pFerm HM proteins. As no agricultural pathway exists to produce these proteins at scale, pFerm is the only viable production route. This shifts the emphasis from cost substitution and sustainability comparisons to value creation, driven by the production of novel, high-value ingredients.

6.3. *Consumer perception*

Foods produced through fermentation are familiar to consumers, with products such as Quorn (mycoprotein) available on the market since 1985. However, pFerm represents a relatively new concept for most consumers. Recent surveys indicate low levels of familiarity with the terms 'precision fermentation' and 'precision-fermented dairy', with only 13-23% of respondents in the US and 14% in the United Kingdom reporting recognition of these terms. In Germany, 81% of surveyed individuals had never previously encountered the concept of 'animal-free dairy' (The Good Food Institute, 2025). Concerns also exist around the negative public attitudes towards GMO technology and its association with pFerm (Teng et al., 2021), even though GM microorganisms should not remain in the final pFerm-derived product. Education and public

engagement are key to shifting these perceptions, as they allow consumers to understand the pFerm process, the safety measures implemented, and the benefits of these products (Marcellin et al., 2024). Studies have shown that providing a simple explanation can substantially increase purchase likelihood, with 56% of individuals indicating they would likely purchase after receiving the information, expressing likelihood to purchase after being provided with a simple description, compared to 43% beforehand (Hartman Group, 2023). The way in which pFerm and its products are framed to consumers also influences perception, with Banovic and Grunert (2023) reporting that 'natural' framing evoked more positive consumer attitudes than 'sustainable'. This may be a beneficial approach, considering that many Danish consumers perceive pFerm as 'unnatural' and 'artificial' (The Good Food Institute, 2025). Consumers also want to be assured that pFerm products 'taste good', 'are safe', 'are good value' and 'are healthy', although the priority ranking of these attributes differs among generations (Hartman Group, 2023).

Currently, most studies focus on consumer acceptability of pFerm dairy compared with traditional bovine sources, and little information exists on public perceptions of acceptability and risk associated with pFerm-derived HM proteins, particularly in infant nutrition. Mankad and Carter (2025) sought to address this gap by assessing primary carers' readiness to incorporate pFerm-derived rhLF into IMF through focus groups. Overall, participants responded extremely favourably to its proposed inclusion, communicating comfort with pFerm technology due to their familiarity with fermentation as a 'well-known process in terms of making beer and bread'. Trust in the Australian regulatory system was also communicated, with participants expressing confidence that such technologies would undergo adequate testing and be deemed safe prior to market entry; however, this level of assurance may not be widely applicable, as some regulatory frameworks may be less transparent e.g. the FDA requires notification by manufacturers to ensure compliance with nutrition and labelling requirements. Interestingly, the digestibility of a rhLF-fortified IMF was important to parents, particularly for infants with gastrointestinal issues, whereas functional properties such as allergy-friendliness, immune-boosting, and improved iron absorption capacity were also considered desirable. These findings align closely with the previously discussed roadmap outlined by Helaina (Fig. 4). Although developed primarily as a GRAS pathway for rhLF, it also provides insights relevant to caregivers, particularly regarding the incorporation of rhLF and other recombinant HM proteins into IMF. This approach enables the simultaneous communication of functional benefits to parents while maintaining regulatory compliance.

7. Conclusion and future perspectives

Despite recent advances in IMF processing and formulation, significant compositional and functional gaps remain relative to HM. These disparities continue to affect growth and development, digestibility, immune function, and microbiome maturation in formula-fed infants. pFerm has emerged as a transformative approach to bridge the gaps between IMF and HM through the large-scale production of human-derived milk proteins and oligosaccharides. Case studies of pFerm-derived rhLF and HMOs demonstrate the feasibility and functional benefits of this strategy, although challenges remain regarding regulatory approval pathways, achieving economic viability, and increasing consumer awareness and acceptance. At the same time, opportunities are evident, as reflected in the rapidly expanding pFerm market and growing biotechnological investment.

Looking forward, future research should move beyond the production of isolated HM components toward the development of integrated, functionally biomimetic systems that more closely recapitulate the complexity of HM. Greater emphasis is needed on reconstructing protein-oligosaccharide interactions characteristic of HM, such as LF-HMO or casein-HMO complexes and human-like glycoproteins, where caseins, whey proteins, and HMOs act synergistically to influence nutrient bioavailability, immune modulation, and microbial colonisation. This will require advances in engineered glycosylation, molecular conjugation, and co-assembly strategies within pFerm platforms, enabling the controlled formation of HM-like complexes rather than merely supplying individual molecules. In parallel, microbiome-informed approaches may guide the selection of specific HMOs and bioactive proteins to better replicate key functional properties of human milk in infant formula, including the promotion of beneficial gut microbial taxa such as *Bifidobacterium* spp. and support of early-life gut barrier function. Integration of systems biology and computational modelling may further assist in linking engineered milk compositions with functional outcomes in infant gut and immune development. Overall, these advances support the continued refinement of infant milk formula to more closely align with the structure and function of HM, thereby improving infant nutrition outcomes.

CRediT author statement

Laura Nyhan: Writing – review & editing, Writing – original draft, Investigation. **Arianna Ressa**: Writing – review & editing, Investigation. **Anastasia Lilina**: Writing – review & editing, Resources, Investigation, Conceptualisation. **Stijn Mertens**: Writing – review & editing, Resources, Conceptualisation. **Dustie N. Lombardi**: Writing – review & editing, Resources, Conceptualisation. **Rosa Sanchez**: Writing – review & editing, Resources, Conceptualisation. **Patrick O'Riordan**: Writing – review & editing, Resources, Conceptualisation. **Nan Gai**: Writing – review & editing, Investigation **André Brodkorb**: Writing – review & editing, Supervision. **Elke K. Arendt**: Writing – review & editing, Funding acquisition, Supervision, Project administration.

Declaration of generative AI use

During the preparation of this work, the authors used Grammarly and ChatGPT to proofread the manuscript and enhance grammar and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Elke K. Arendt reports financial support was provided by BioBrew Technologies Inc.Anastasia Lilina reports a relationship with BioBrew Technologies Inc. that includes: employment.Stijn Mertens reports a relationship with BioBrew Technologies Inc. that includes: employment. Dustie N. Lombardi reports a relationship with BioBrew Technologies Inc. that includes: employment. Rosa Sanchez reports a relationship with BioBrew Technologies Inc. that includes: employment. Patrick O'Riordan reports a relationship with BioBrew Technologies Inc. that includes: employment.If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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