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MILK GLYCANS, MANUFACTURED

Human-Identical Oligosaccharides and the Emerging Architecture of Precision Nutrition

From Glycan Structure and Biomanufacturing to Human Intervention, Ingredient Specifications and European Novel-Food Law

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Abstract

Human milk oligosaccharides (HMOs) have moved from being described principally as constituents of breast milk to becoming a family of structurally defined carbohydrates manufactured by microbial or chemoenzymatic routes. This transformation expands the technological repertoire of food developers but also creates new scientific and legal questions. A manufactured molecule can be structurally identical to one present in human milk without reproducing the biological diversity of milk, the food matrix in which it naturally occurs, or the physiological effects of breastfeeding. Nor are different oligosaccharide structures clinically or legally interchangeable. This interdisciplinary critical review examines fucosylated, neutral-core and sialylated glycans, their biosynthesis and purification, microbial utilisation, potential host interactions, processing stability, human intervention evidence and the European novel-food authorisation framework. Particular attention is given to a 2026 double-blind crossover trial of 2'-fucosyllactose in adults with ulcerative colitis: the intervention modified bifidobacterial abundance and systemic metabolic profiles but did not significantly improve the prespecified primary clinical response. A 2025 trial in older adults likewise documented microbiome and metabolic signals while failing its principal immune-response endpoint. In parallel, Commission Implementing Regulation (EU) 2026/397 authorised a specified lacto-N-tetraose preparation produced by a defined *Escherichia coli* strain, subject to composition, use, labelling and protected-data conditions. The paper proposes an evidence-gated industrial platform integrating molecular identity, manufacturing provenance, analytical release, matrix performance, product-specific exposure, clinical substantiation and legal eligibility. The opportunity is substantial: precisely manufactured glycans offer an unusually well-characterised way to test structure–microbiome relationships. Their responsible commercialisation, however, depends on resisting the substitution of a convincing mechanistic story for a demonstrated human benefit or an exact EU authorisation.

Keywords: *human milk oligosaccharides; human-identical milk oligosaccharides; 2'-fucosyllactose; 3-fucosyllactose; lacto-N-tetraose; lacto-N-neotetraose; sialyllactose; glycosyltransferases; precision fermentation; microbiome; bifidobacteria; clinical trials; ulcerative colitis; novel foods; European Union; food formulation.*

1. From a biological ensemble to a library of manufactured molecules

Human milk is not a solution of a single immunologically interesting sugar. Its carbohydrate fraction contains lactose and a complex population of oligosaccharides with different monosaccharide sequences, linkage positions, branching patterns and terminal fucose or sialic-acid residues. Their distribution changes with stage of lactation and maternal genetic characteristics, including secretor status, as well as other biological influences. The descriptive term *HMO* therefore refers to a family, not a chemically uniform substance.

Commercial manufacture changes the situation. A developer can specify a particular structure, as 2'-fucosyllactose (2'-FL), 3-fucosyllactose (3-FL), lacto-N-tetraose (LNT), lacto-N-neotetraose (LNnT), 3'-sialyllactose (3'-SL) or 6'-sialyllactose (6'-SL), and manufacture it at defined purity using controlled biosynthetic pathways. This is genuine molecular precision. It does not follow that the isolated glycan is a substitute for the total HMO profile of human milk, nor that adding it to an adult beverage recreates the nutrition of an infant.

The transition has three industrial consequences. First, the chemical specification can be substantially tighter than that of many natural polysaccharide extracts. Second, the functionality becomes experimentally tractable: different linkages can be compared under defined conditions. Third, legal identity becomes unusually granular. The same named molecule can be the subject of distinct authorised novel-food entries because its manufacturing microorganism, processing route, impurity pattern and data-protection conditions differ. For companies accustomed to treating a generic carbohydrate name as sufficient for procurement, this is an important change of discipline.

The editorial premise of this review is not that every HMO will become an adult functional ingredient. It is that the ability to manufacture selected glycans creates a new experimental platform, provided chemical identity, microbial ecology, human outcomes and legal permission are evaluated as separate propositions. The evidence and EU legal cut-off used here is 19 November 2026.

2. Molecular architecture: why the location of a bond matter

HMOs are assembled principally from five monosaccharide building blocks: glucose, galactose, N-acetylglucosamine, fucose and N-acetylneuraminic acid, commonly called sialic acid. Many share a lactose

reducing end, but the extension and terminal substitutions define different substrates. Minor changes in linkage position can change recognition by microbial transporters, glycosidases, lectins and other binding proteins.

The distinction between LNT and LNnT illustrates this precision. Both are tetra-saccharides composed of related constituent sugars, yet their internal galactosyl linkage architecture differs. The resemblance in analytical mass or broad compositional terminology does not make their behaviour identical. Equally, 2'-FL and 3-FL differ in where fucose is attached to lactose. Sialylated species have an additional acidic group, introducing charge, different ionic interactions and potentially distinct stability behaviour. A supplier's description of material as 'HMO 95%' or 'prebiotic oligosaccharide' is therefore inadequate for development or comparative clinical interpretation.

Function may depend on several structural variables simultaneously: chain length, fucosylation site, sialylation, branching, reducing-end chemistry, the distribution of related saccharides, and the presence of other residual carbohydrates. These variables affect more than a laboratory spectrum. They influence which bacterial strains can import or hydrolyse a glycan, whether breakdown products support cross-feeding, and how the ingredient behaves in a protein-rich, mineral-rich or acidic food matrix. Manufacturing standards should reflect the specificity of this structure–function relationship.

Table 1. Selected human-identical milk glycans and the questions they create

Glycan class / example	Structural distinction	Research implication	Industrial or legal checkpoint
Fucosylated: 2'-FL	Fucose linked to lactose in the 2' configuration	Utilisation depends on relevant fucosidases and microbial populations	Exact manufacturing strain, purity, approved uses and dose
Fucosylated: 3-FL	Different fucose-linkage position	Do not transfer 2'-FL study effects automatically	Separate novel-food entry and ingredient specification
Neutral core: LNT	Type-I chain linkage	Structure-specific microbial metabolism	Compare the exact source and authorised LNT preparation
Neutral core: LNnT	Type-II linkage	Not automatically functionally equivalent to LNT	Distinguish analytical methods and legal entry
Sialylated: 3'-SL	Acidic terminal sialic acid in 3' position	Charge-sensitive interactions and different enzymology	Sodium-salt specifications and use conditions
Sialylated: 6'-SL	Different sialic-acid linkage	Separate degradation and binding hypotheses	Distinct authorised preparation and labelling rules

This table identifies research and regulatory distinctions; it does not establish clinical superiority or authorisation for a generic ingredient family.

3. Biosynthesis: turning glycobiology into industrial chemistry

Human-identical oligosaccharides can be obtained through biosynthetic routes that include engineered microbial fermentation and chemoenzymatic synthesis. In microbial systems, the manufacturing organism is selected or modified to produce the necessary activated sugar donors and glycosyltransferase activities. Carbon-source metabolism, nucleotide-sugar supply, competing reactions and transport of the target oligosaccharide become elements of production engineering. Chemoenzymatic systems may use purified enzymes or controlled cascades to extend or modify a carbohydrate scaffold.

In principle, biological catalysis gives producers high regioselectivity and stereochemical control. In practice, productivity is constrained by donor-substrate supply, by-product formation, enzyme specificity, strain stability, fermentation oxygen transfer and the capacity of downstream operations. A high concentration of the target molecule in broth is not the same as a high kilogram yield of compliant product: separation losses, impurity rejection and batch failures can dominate actual costs. The relevant economic denominator is the mass of released ingredient meeting its full specification.

A central engineering challenge is metabolic flux. Directing more precursor towards a desired glycan can increase intracellular stress or the accumulation of competing intermediates. Systems engineering may improve yield by changing pathway balance, transport, enzyme expression or substrate feeding, but each manufacturing modification must be reviewed for its effect on the product's declared identity. The EU approval of one fermentation-derived glycan manufactured by a specified derivative strain is not a general licence for any other engineered organism to supply the same nominal product without further assessment.

A second challenge lies downstream. Fermentation broth contains salts, biomass-derived constituents, unreacted carbon sources, structurally related carbohydrates and potentially residual nucleic acids or proteins from the producing organism. Typical purification approaches can involve cell separation, membrane operations, ion exchange, chromatography, concentration, crystallisation or drying; the sequence is product-specific. The fact that the final target molecule is identical to a natural milk oligosaccharide does not prove that all possible impurities are harmless or that two manufacturers' preparations are compositionally equivalent. Process design and analytical release must be developed together.

Recent production reviews emphasise the importance of integrating pathway design, purification and scale-up rather than celebrating fermentation titre alone (Human Milk Oligosaccharides: Overcoming Production Bottlenecks, 2026). For an ingredient company, the preferred supplier is not necessarily the company reporting the highest experimental yield. It is the operator able to demonstrate repeatable specifications, lawful supply, traceability, stable quality and commercially sustainable output.

4. The microbial pathway is selective, not universal

Much interest in HMOs originates in the specialised capacity of some bifidobacteria to access these glycans. Certain organisms possess the transport systems and glycoside hydrolases needed to use defined HMO structures; other organisms do not. Some release monosaccharides or oligosaccharide fragments that can be consumed by other community members. The resulting ecosystem behaviour depends on the starting microbiota, available alternative substrates and metabolic interactions. A glycan that strongly stimulates one well-equipped isolate in vitro may cause only a small community change in an adult whose resident microbiota lacks that capability.

This variability has special relevance to the idea of 'precision prebiotics'. The ISAPP consensus definition requires selective utilisation by host microorganisms that confers a health benefit; taxonomic change alone is not the full demonstration. It is legitimate to investigate specific HMOs as candidate prebiotics, but evidence of bifidobacterial enrichment should not be silently translated into a claim of improved immunity, prevention of disease or guaranteed gastrointestinal health. An endpoint based on microbial abundance and one based on symptom control or human immune function are different experiments (Gibson et al., 2017).

Fermentation metabolites also need discipline. Acetate and other short-chain fatty acids can be produced directly or through cross-feeding, and changes in their concentrations may inform a mechanistic hypothesis. Yet faecal concentrations are affected by production, absorption, intestinal transit and sampling; they are not transparent measures of total colonic production. Metagenomic prediction of a metabolic pathway is less direct still. In a product programme, microbial, metabolomic and host-level outcomes should be analysed jointly without allowing a favourable biomarker to replace a prespecified clinical endpoint.

A 2026 pH-controlled faecal-fermentation study examining 2'-FL with *Bifidobacterium longum* subsp. *infantis* M-63 provides a useful mechanistic illustration. The experiment evaluated whether adding a strain able to utilise HMOs could alter responses in donor communities with different starting capacities. Its value is in showing how the availability of appropriate microbial partners can constrain substrate utilisation. As an ex-vivo system, however, it cannot independently prove clinical benefit, long-term colonisation or permission to market a symbiotic claim in the Union (Synbiotic Effects of 2'-Fucosyllactose, 2026).

5. Adult intervention evidence: where response and benefit diverge

The principal development in 2026 was a randomised double-blind crossover trial in adults with mild-to-moderate ulcerative colitis. Thirty-eight participants were recruited; the intervention consisted of 5 g/day of 2'-FL for 28 days and a maltodextrin-placebo period, separated by washout. The study deliberately combined symptoms and clinical activity with microbial and metabolomic measurements. In the per-protocol analysis, the primary clinical response, at least a two-point improvement on the Simple Clinical Colitis Activity Index, did not significantly differ between intervention and placebo (18.5% versus 29.6%; P = .11). Clinical remission was likewise not significantly different (25.9% versus 18.5%; P = .32). These results must occupy the centre of any fair interpretation, because they answer the main clinical question more directly than secondary microbial signatures.

The same trial reported a significant increase in *Bifidobacterium* abundance, with an effect size of 4.4 percentage points and a 95% confidence interval of 2.33–6.47, together with changes in systemic metabolic patterns. Those

observations demonstrate biological activity under the studied conditions; they do not establish that 2'-FL is a treatment for ulcerative colitis. The trial is scientifically valuable precisely because it separates pathway engagement from failure to achieve the principal clinical response. A product supplier describing the trial only as a 'clinically proven gut-health benefit' would omit its most consequential limitation (PRInCE-UC trial, 2026).

A 2025 randomised controlled trial in 89 generally healthy older adults administered 1 or 5 g/day 2'-FL or placebo over six weeks. It observed bifidobacterial changes and shifts in circulating metabolic or protein markers. Yet the prespecified primary Cytokine Response Score was not improved. Elevated circulating insulin and other secondary changes must be interpreted cautiously: they are not necessarily beneficial endpoints and do not demonstrate an immune or cardiometabolic advantage. The trial expands the range of plausible hypotheses, while underlining the limited relationship between microbiome enrichment and clinical success (Carter et al., 2025).

A different 2026 line of evidence concerns the use of 2'-FL after antibiotic-induced microbiome disturbance. A randomised placebo-controlled study in adults with overweight or obesity examined supplementation following vancomycin treatment. Results suggested transient shifts in recovery patterns and a change in fasting plasma IL-6 over the intervention, but did not establish a general, sustained restoration of all microbial and metabolic endpoints. This experiment should not be converted into advice to treat antibiotic-associated illness with an HMO, nor generalised to other antibiotics or healthy populations (2'-Fucosyllactose Supplementation After Vancomycin, 2026).

Table 2. Evidence interpretation in selected HMO intervention studies

Study and population	Exposure and design	Finding supporting further research	Major limitation for claims
Ulcerative colitis, 2026	38 recruited; crossover; 5 g/day 2'-FL; 28-day periods	Greater bifidobacterial abundance and metabolic changes	Primary clinical response and remission not significantly improved
Healthy older adults, 2025	89 adults; 1 or 5 g/day 2'-FL; 6 weeks	Microbial and selected circulating-marker changes	Primary cytokine-response endpoint not met
Adults after vancomycin, 2026	Randomised placebo-controlled post-antibiotic supplementation	Transient resilience-related or inflammatory-marker signals	No proof of broad, durable microbiome or clinical recovery
Infant formula, Puccio et al., 2017	Formula with 2'-FL and LNnT versus matched formula	Adequate growth; differences in some parent-reported morbidity outcomes	Infant formula evidence cannot be applied directly to adult HMO products

A further qualification concerns trial dose. A published human intervention using several grams per day is not evidence that the same dose is legal for every HMO structure or product format in Europe. Such comparisons require the specific novel-food authorisation, not extrapolation from the study protocol.

6. Evidence from infant feeding must remain in its population

There is a legitimate history of HMO research in infant nutrition, where the biological context differs from adult supplementation. The neonatal microbiome, intestinal maturation, milk matrix, feeding frequency, immune development and underlying nutritional requirements are not those of a healthy middle-aged adult. Clinical results obtained using infant formulas with 2'-FL and LNnT should be interpreted within that composition, population and comparator.

In the multicentre randomised trial reported by Puccio and colleagues, formula containing 1.0 g/L 2'-FL and 0.5 g/L LNnT was compared with a corresponding control formula in healthy infants. Growth and tolerance were examined, with certain secondary parent-reported morbidity and medication-use measures differing between groups. These findings were informative for a particular infant-formula research programme. They neither demonstrate equivalence to human milk nor provide a transferable preventive-health claim for adult beverages.

The ethical and legal stakes are also distinct. Food for infants and young children is subject to specialised requirements, including Regulation (EU) No 609/2013 and relevant delegated rules for formula composition and information. Appropriate use of an authorised novel ingredient is only one layer of compliance; it does not relieve the manufacturer of obligations governing the finished infant food. This distinction should govern any supplier dossier proposed for maternal-and-child nutrition.

7. Manufacturing purity is a functional and legal attribute

For a fermentation-derived HMO, chemical purity is not satisfactorily expressed by a single percentage without its analytical basis. Specifications may need to distinguish target glycan, related carbohydrate isomers, residual lactose, glucose or galactose, salts, moisture, microbiological status, residual protein, absence of viable production organisms and manufacturing-process impurities. The authorised Union-list entry, not a generic laboratory convention, determines the mandatory specifications for the exact novel food.

High-resolution chromatography, chromatographic mass spectrometry, NMR and appropriately validated standards can each contribute to identity. HPAEC-PAD may resolve certain carbohydrates; LC-MS can strengthen structural characterisation and impurity profiling; NMR is valuable for linkage confirmation in reference materials. The suitable method depends on the question. A total-carbohydrate assay cannot establish 2'-FL identity; a peak at the expected mass does not always distinguish structural isomers; and a supplier's declaration of 'bio-identical' does not replace proof of manufacturing equivalence and permitted use.

Microbiological and molecular controls matter because the production organism may be engineered. A risk assessment should consider the complete manufacturing system, separation and purification, and the final product's defined properties. The presence of DNA or protein at a particular detectable level, however, should not be discussed without reference to the method, regulatory specification and exposure relevance. The evaluation of novel foods by EFSA typically brings these elements together rather than assuming that every derivative of an approved host organism is safe without evidence. The EFSA opinion preceding Regulation (EU) 2026/397 illustrates this preparation-specific approach (EFSA NDA Panel, 2025).

Industrial qualification needs at least three batches representative of normal production conditions, with more extensive sampling where process variability justifies it. Measurements should cover critical in-process stages, finished dry ingredient, packed material and realistic shelf life. A strong supplier qualification system also records strain and process change control, analytical method transfers and traceability from raw materials through finished lots.

8. Food-matrix engineering: what happens outside the capsule

The relative simplicity of dosing a purified powder can conceal complexity at the finished-food stage. HMOs may be water-soluble, but water solubility is only the starting point for matrix suitability. Acid concentration, water activity, heat exposure, neighbouring sugars, metal ions, proteins, other hydrocolloids, packaging conditions and microbiological stability can affect the final product. Their chemical structures must remain sufficiently intact for the formulation to reproduce the exposure of interest.

In an acidified beverage, process engineering should track the specific oligosaccharide through mixing, pasteurisation, cooling and storage, while monitoring pH, haze, colour and taste. In a protein drink, interaction with protein hydrolysates or mineral fortification must be studied empirically. In a dry stick-pack or supplement, the main risks may shift towards hygroscopicity, caking, segregation, blend homogeneity and reconstitution. In a cereal bar, moisture gradients, processing temperature and shelf-life texture create other controls. None of these problems is resolved by displaying a favourable chromatogram of the incoming ingredient alone.

The EU legal category matters from the first formulation sketch. A glycan authorised in specific dairy-based beverages, flavoured drinks or food supplements may have different maximum levels and population restrictions for each category. A concentration that is lawful in one food cannot be carried into another by calling both 'functional nutrition'. If the ingredient is used in an infant preparation, specific infant-food rules also apply. Even when a preparation is authorised, an added HMO does not automatically permit a nutrition or health claim.

There is a second, often neglected, sensory issue. HMOs are not necessarily sweetness replacements, despite being carbohydrates. Their sweetness, solubility, osmotic contribution and rheological effect differ from sucrose and from conventional bulking fibres. An adult beverage that supplies several grams per serving still requires an independent taste, texture, stability and nutritional review. Treating HMOs as general prebiotic fibres may also create inaccurate nutrition-labelling assumptions unless the relevant analytical and regulatory criteria are satisfied.

9. European law: molecular identity meets manufacturing history

The regulatory framework starts with Regulation (EU) 2015/2283 on novel foods and Commission Implementing Regulation (EU) 2017/2470, which establishes the Union list. The question is not whether a molecule occurs naturally in human milk; the marketed ingredient must correspond to an authorised novel food or otherwise possess a demonstrable lawful food status. Source strain, process, composition, specifications, permitted categories, maximum use levels, population restrictions, labelling and, where applicable, protected-data provisions all matter.

Commission Implementing Regulation (EU) 2026/397, adopted on 23 February 2026, authorised LNT manufactured using a derivative of *E. coli* K-12 MG1655 (ATCC 700926). The act is particularly instructive because authorisation of the molecule was accompanied by a defined production identity and a five-year data-protection arrangement. The relevant provision identifies Inbiose N.V. as the beneficiary of the protected authorisation period starting 16 March 2026, while allowing independent approvals under the conditions stated in the law. The act also sets product-category maximum levels and specifications. For this particular LNT entry, the annex includes, among other categories, flavoured drinks at 1.0 g/L, cereal bars at 10 g/kg and food supplements for the general population over three years at 2.0 g/day. These figures are entry-specific; they are not universal HMO limits.

Other structures have different legal histories. The Commission has separately authorised 2'-FL manufactured by defined production systems, including Regulation (EU) 2024/2036 for a derivative strain of *E. coli* W (ATCC 9637). Separate entries also exist for 3-FL and sialylated structures, such as those addressed in Regulations (EU) 2023/2210, 2023/2215 and 2024/1047. The presence of several entries is precisely why a purchasing specification stating 'HMO powder' cannot support a legal sign-off. Even two preparations with the same target-glycan name may have different protected-data or manufacturing specifications.

The positive EFSA safety opinion underlying an authorisation must also be distinguished from the Commission's legal act. EFSA evaluates safety under proposed conditions; the Union-list entry determines the commercial scope. Conversely, an authorisation of LNT at a specified concentration does not demonstrate efficacy for any marketed health benefit. Regulation (EC) No 1924/2006 governs claims, and Regulation (EU) No 1169/2011 continues to govern truthful, non-misleading food information. Medical claims relating to inflammatory bowel disease are not permitted simply because a clinical paper measures colitis outcomes.

Table 3. A regulatory decision matrix for EU commercialisation of human-identical glycans

Question	Principal legal source	Evidence that must be checked	Invalid inference
Is the exact glycan preparation authorised?	Regulation 2015/2283; Union list 2017/2470	Structure, manufacturing organism and process, specifications	'The molecule occurs in milk, so it is automatically traditional'
Is the new 2026 LNT preparation covered?	Implementing Regulation 2026/397	Exact strain-derived product, category, level, labels, data protection	'Every manufacturer's LNT has the same permission'
Is a 2'-FL source covered?	Applicable 2'-FL-specific entry, e.g. 2024/2036	Exact strain and process, final food, age group, concentration	'An LNT authorisation covers 2'-FL'
Can a selected food contain it at the proposed dose?	Current Union-list conditions	Category-specific levels and ready-to-use basis where relevant	'A clinical dose is automatically a lawful food dose'
Can the label promise microbiome or immune benefit?	Regulation 1924/2006; EU claims register	Authorised claim, exact wording, conditions and finished product	'A significant bifidobacterial result is a health-claim approval'
Are infant products compliant?	Regulation 609/2013 and applicable delegated legislation	Formula specifications, populations, ingredients and information	'Novel-food permission alone makes the infant formula compliant'
Is the material traceable and safe?	General food law 178/2002; hygiene 852/2004	Batch integrity, impurities, contaminant and microbiological controls	'Chemical identity guarantees process safety'

Commercial teams should consult the consolidated law and exact entry immediately before placing a product on the market; future amendments and protected-data expiries can change the practical supply position.

10. The dose–law disconnect: a practical illustration

Consider two separate observations. A clinical investigator reports biological activity after administering 5 g/day of 2'-FL to adults with ulcerative colitis. A different European novel-food entry, the 2026 LNT authorisation for a specified microorganism-derived preparation, sets 2 g/day as the maximum for food supplements supplied to consumers older than three years. The first number cannot be imported into the second ingredient's permitted conditions, nor can the second number be treated as an HMO-wide ceiling. They concern different molecules, legal entries and purposes. This simple comparison captures a recurring translational error in the functional-ingredient sector.

The same reasoning applies within a given molecular class. A human trial may use an HMO at a dosage appropriate to its approved investigational protocol. Food law may nevertheless restrict a particular commercial use. A further study might demonstrate a response in a population that the marketed supplement is not authorised to target. Product viability therefore requires a matrix-specific exposure calculation, a defined serving size, anticipated daily consumption, cumulative sources and a check against the actual authorised category.

There is also a scientific reason not to maximise dose reflexively. Different organisms may show saturable substrate utilisation, shifts in cross-feeding and altered tolerance at higher concentrations. The 2025 older-adult study comparing 1 and 5 g/day 2'-FL did not establish that higher doses translate reliably into better human health outcomes. Increasing the target percentage in a powder specification may improve a sales narrative while weakening the economic and regulatory position of the finished product.

11. Safety, vulnerable populations and the limits of 'naturally present'

The argument that an oligosaccharide occurs in human milk is biologically relevant but insufficient as a complete safety assessment. Purified preparations may contain different impurity profiles and are consumed by populations with different gastrointestinal function, metabolic state and total dietary exposure. Formula-fed infants and young children present specific considerations because their intake per kilogram of body weight and nutritional dependence on the product differ sharply from those of adults.

Tolerability deserves prospective evaluation. Non-digestible carbohydrates can alter fermentation and intestinal water balance, and some individuals may experience bloating, gas or altered stool characteristics, especially as doses rise. A development programme should establish tolerability in the intended matrix and population rather than borrow a general statement from unrelated fibre studies. Where a specific ingredient has a documented authorised level, that level is a necessary regulatory boundary but not proof of a health benefit or universal individual tolerance.

Residual manufacturing materials and microbial safety must be addressed proportionately. The dossier should document production-strain controls, downstream separation, appropriate microbiological specifications, allergen considerations where relevant, purity and potential process impurities. For highly purified carbohydrates, adverse effects may arise from co-formulated ingredients or excessive consumption rather than from the target glycan alone; this is a reason to investigate finished-product exposure, not to omit source-specific qualification.

12. Industrial case study: an adult fermented-milk beverage

A hypothetical client asks for a drinkable cultured product with a human-identical milk oligosaccharide, marketed for adults interested in digestive health. The client proposes 2'-FL, LNT or a combination at a concentration selected primarily from the published trial literature. An evidence-led project would reverse that order. Before the formulation phase, the company should identify an exact ingredient supplier, its manufacturing process, the applicable Union-list entry, whether the planned fermented-milk category is authorised, and the maximum permitted level. If the category or preparation is not covered, the development should not progress under an assumption of generic HMO permission.

Subject to an affirmative legal screen, the first pilot would compare two suitably documented individual glycan candidates rather than immediately adopting a complex mixture. Each trial would use an otherwise standardised beverage matrix with fixed protein, fat, acidification culture, mineral and sweetener specifications. The initial questions would be straightforward: Can the target glycan be measured accurately after fermentation? Does it

change culture kinetics, acidity, sedimentation, flavour or shelf-life stability? Is the final serving nutritionally and sensorially acceptable?

Because some microbial starters or adjuncts may metabolise the target glycan, recovery measured at production may differ from the amount dosed into the tank. The assay must track free target glycan and relevant transformation products as required by the hypothesis. A change in viable culture count is not proof of benefit to the consumer, and a favourable in-vitro fermentation signature is not a lawful health claim. Independent batches should be prepared across realistic manufacturing and storage conditions, including distribution-temperature challenges and sensory testing of aged rather than only fresh product.

If an authentic physiological proposition remains after these steps, a controlled human trial could be considered. Its endpoint should be chosen from an identifiable consumer benefit and supported by the permitted marketing strategy. A trial intended to assess bowel-function outcomes would need different measures, duration and comparator from one designed to investigate host immune responses. At every stage, results would be reported as those of a proposed industrial programme, not as proprietary Ifindings already obtained.

13. Analytical validation and shelf-life design

An HMO technical dossier should permit another laboratory to verify what the ingredient is, how much is present and whether it remains in the specified form through use. A suitable testing strategy may combine identity by chromatographic retention and structural confirmation with quantitative assays using traceable reference standards. Where isomer separation matters, orthogonal methods should be considered. Analytical validation should cover specificity, calibration, precision, accuracy or recovery, limit of quantification where relevant, uncertainty, and stability of samples during transport and preparation.

Finished-food sample preparation can alter measurement. In a fermented dairy matrix, proteins, salts and residual lactose may require removal or adequate chromatographic resolution; in high-protein clear beverages, the analytical matrix differs; in a powdered premix, blend heterogeneity can dominate. A method validated for a purified ingredient solution does not automatically quantify the same oligosaccharide reliably in a complex finished food. Method-transfer studies should include recovery experiments in the exact formulation and independently prepared production lots.

Shelf-life verification should separate chemical loss, microbial transformation and changes in product quality. Multiple time points are preferable to a single accelerated endpoint. It is also necessary to distinguish total detected carbohydrate from the specific HMO structure remaining: a breakdown product may preserve much of the carbohydrate mass while changing the hypothesis on which the ingredient was selected. This issue becomes particularly important when the commercial proposition is structure-specific rather than merely nutritional bulking.

14. Evidence architecture for a supplier-to-client platform

The most valuable service offered by an advanced ingredient supplier may be the integration of molecular, industrial and legal evidence. The supplier should know not only the nominal structure and purity of its powder but also its likely performance under client manufacturing conditions, its authorised categories, its source restrictions and the human outcomes that can genuinely be discussed. This knowledge is difficult to replace with an attractive ingredient catalogue.

Table 4. Minimum evidence package proposed for industrial qualification

Domain	Essential evidence	Decision enabled
Structural identity	Nomenclature, linkage confirmation, assay and related-saccharide profile	Is the supplied material the intended glycan?
Manufacturing provenance	Authenticated producing strain, production process, change control and traceability	Is this the legally and technologically described ingredient?
Purity and safety	Residual sugars, moisture, salts, microbes, proteins or process impurities as applicable	Is the batch compliant and appropriately safe?
Matrix performance	Product-specific recovery, processing response, taste, texture and stability	Does the ingredient survive the real food process?

Domain	Essential evidence	Decision enabled
Microbial function	Strain/community utilisation, metabolites and appropriate controls	Is the mechanism plausible for the intended population?
Human evidence	Prespecified clinical endpoints, target group, dose, placebo, uncertainty and adverse events	What benefit, if any, is demonstrated?
EU legal position	Exact Union-list entry, category, dose, labels, protections and claims	Is the intended finished product lawfully marketable?
Scale and economics	Independent batches, recovery, processing cost, usable dose and supply security	Is the platform commercially sustainable?

Such a package allows clients to reject unsuitable proposals early. A product that performs in a laboratory but cannot be sourced lawfully at the intended dose is not a viable commercial lead. A lawful ingredient with no evidence of a marketed benefit can still have research value but should not be presented as a finished health proposition.

15. Predictive design and the role of computational methods

The combinatorial nature of glycan structure suggests opportunities for data-driven discovery. Chemical descriptors, (monosaccharide sequence, glycosidic linkages, fucosylation, sialylation and molecular size), can be connected to bacterial utilisation assays, enzyme specificities and observed fermentation patterns. Mechanistic models may identify plausible interactions between substrate supply and microbial capabilities. Machine learning may assist in prioritising experiments, but reliable prediction demands standardised and independently validated data.

A major danger is data leakage. If spectra, microbial isolates or fermentation wells derived from the same source material are divided indiscriminately between training and testing, apparent predictive accuracy may reflect source identity instead of a transferable structure–function relationship. Better designs separate validation by producing strain, manufacturing batch, donor microbiome, study site or glycan structure, depending on the intended claim. The model should disclose uncertainty and be tested on conditions it has not seen.

Computational attractiveness does not excuse experimental or legal deficiencies. A predicted highly bifidogenic molecule cannot enter the EU market merely because its model score is high; it must have a lawful food status. Nor can an observed bacterial increase be described as clinical immune enhancement without appropriate human evidence. The most productive application of AI is to reduce the number of poorly chosen experiments and to identify where present evidence is insufficient for the next development decision.

16. Economic and strategic implications for ingredient developers

Precision glycan manufacture combines the cost structure of bioprocessing with that of a high-specification ingredient. Major cost drivers include fermentation substrates, yield and concentration, utilities, purification losses, analytical release, batch rejection, drying, packaging and intellectual-property or licence conditions. The cost of a kilogram of fermentation broth is immaterial unless it can be translated into a kilogram of lawful, released ingredient. A business case should use cost per compliant serving of the final food, allowing for the permitted dose and realistic inclusion losses.

The 2026 LNT authorisation shows why regulation can also become an economic variable. Protected-data arrangements may shape short-term supplier availability, competition and licensing. These mechanisms should be examined legally and commercially rather than treated as an implicit monopoly over an entire molecular category. A different lawful authorisation may exist for another production route, and the Union list changes as additional applicants enter. Procurement must therefore follow the exact material specification and the latest regulatory position.

For INTABIOTECH, the opportunity is not a claim of currently manufacturing these molecules or proving their effects clinically. A credible role would be to identify qualified sources, evaluate glycan structures for specific finished-food matrices, coordinate analytical method validation and supplier qualification, design-controlled trials with appropriate partners, and provide evidence-based EU regulatory mapping. This proposition is especially relevant to manufacturers trying to distinguish a truly novel ingredient platform from an undifferentiated blend of common soluble carbohydrates.

Commercial strategy must also account for the limits of the adult market. Evidence that an HMO changes the gut microbiome may encourage preliminary research, but consumers and regulators require more than a microbial taxonomy chart. Development resources should favour applications with technically realistic dosing, lawful use categories, reproducible supply and a demonstrable consumer benefit. The less mature the clinical evidence, the more carefully research expenditure and communications should be separated from immediate sales projections.

17. Discussion: structural precision is not physiological certainty

The case for human-identical oligosaccharides is intellectually strong because they connect a detailed molecular structure to measurable microbial substrate utilisation. Few functional-food ingredient families offer so many defined linkage variants with such a direct enzymatic rationale. The technology of modern fermentation makes some of these structures accessible without extraction from human milk. Yet these advantages can create a false sense of biological inevitability: if the molecule is natural to milk, is manufactured precisely and is utilised by a desirable bacterium, it must improve human health. Each part of that sentence needs a separate evidential test.

The 2026 colitis trial provides a particularly important corrective. A coherent microbiome and metabolic response was detected, but the primary clinical endpoint was not achieved. The older-adult intervention yielded a similar interpretive distinction between microbial change and an unachieved principal immune outcome. These studies do not invalidate HMO research; they define its present limits. A responsible review should neither dismiss significant biological observations nor promote them as outcomes they were not designed or able to demonstrate.

The European framework reinforces scientific precision. LNT, 2'-FL, 3-FL and sialylated glycans may each have separately defined authorised sources, uses and restrictions. Approval of an exact composition at an exact level is compatible with substantial remaining uncertainty about efficacy. These are two parallel forms of evidence: safety and lawful market access on one side, proof of a claimed human benefit on the other. Combining them rhetorically is a source of avoidable regulatory exposure.

The strategic development question is accordingly narrower and more useful than whether HMOs will become 'the next great prebiotic'. It is whether an identified glycan, produced through a controlled route and incorporated into a specified food, creates a reproducible, relevant effect in the population to whom that finished food may lawfully be sold. The answer may be positive for some structure–matrix–population combinations and negative for others. That selectivity should be viewed as a scientific result rather than a defect of the platform.

18. Conclusions

The industrialisation of human-identical milk oligosaccharides marks a substantive advance in food biotechnology. Modern manufacturing can produce chemically specific glycans that permit rigorous testing of host–microbe interactions. Their utility depends on more than chemical resemblance to constituents of human milk. Manufacturing impurities, molecular linkage, microbiome capacity, finished-food processing, individual exposure and human clinical endpoints all govern interpretation.

Recent adult intervention trials demonstrate measurable microbial or metabolic effects but do not establish a general therapeutic or immune-health benefit. The 2026 ulcerative-colitis trial is especially informative because its principal clinical result was negative despite a significant bifidobacterial signal. The new LNT authorisation under Regulation (EU) 2026/397 likewise creates a defined commercial opportunity rather than an unrestricted right to market every HMO, use any dose or claim improved gut health.

For food-ingredient developers, the next advance will be an integrated programme in which a molecule's chemical identity, lawful source, measured stability, microbiological mechanism and independently demonstrated consumer-relevant outcome are maintained within one traceable evidence chain. Precision should describe the quality of those decisions, not merely the exactness of the glycan's name.

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