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MODULATION OF INTESTINAL BARRIER FUNCTION AND NUTRITIONAL STATUS USING A CHITOSAN–SKIM MILK POWDER COMPLEX: AN IN-SILICO FRAMEWORK AND MORPHOFUNCTIONAL CORRELATIONS

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ABSTRACT

The intestinal barrier is a key regulator of host homeostasis, balancing selective nutrient absorption with protection against endotoxins and inflammatory triggers. Chitosan is a cationic biopolymer with mucoadhesive and sorption properties and is widely considered a functional ingredient. However, depending on dose, molecular weight and protonation state, chitosan may bind micronutrients and alter lipid assimilation, potentially shifting the benefit–risk balance. Complexation of chitosan with a protein matrix, including skim milk powder (SMP) fractions, may provide controlled release, improved colloidal stability and a food-matrix effect that supports mucosal integrity [14–20]. This paper proposes an in silico framework and an experimentally testable design to evaluate the chitosan–SMP complex as a barrier- and nutrition-modulating system. The model assumes 60 male Wistar rats allocated into five groups and fed for 42 days with low-/high-molecular-weight chitosan, either alone or complexed with SMP. Barrier integrity is assessed using a multi-parameter panel: villus–crypt morphometry, ultrastructural evaluation, tight junction proteins (ZO-1, occludin, claudin-1), mucus marker MUC2, and functional absorption tests (D-xylose and paracellular permeability proxies). Based on mechanistic evidence, the SMP matrix is expected to mitigate excessive sorption-related drawbacks while enhancing epithelial recovery and stabilizing nutritional indices (absorption efficiency, antioxidant status, micronutrient balance). The framework may guide dose–molecular-weight–matrix optimization and supports future in vivo validation and translational applications in functional nutrition and enteral delivery systems.

Keywords: Intestinal barrier, epithelial permeability, chitosan, skim milk powder, polyelectrolyte complex, tight junction, mucoadhesion, D-xylose test, microbiota, functional nutrition

INTRODUCTION

The intestinal epithelium and its mucus layer constitute a dynamic barrier that regulates nutrient transport while limiting the translocation of microbial products. Barrier dysfunction and increased permeability are implicated in inflammatory cascades, endotoxemia and metabolic disturbances across a broad spectrum of gastrointestinal and systemic conditions [1–3]. Experimentally, permeability can be quantified by sugar probe tests, D-xylose absorption, and biomarker panels combined with morphological and immunohistochemical analyses [3–5]. Nutritional modulation is increasingly recognized as a practical lever to

support barrier homeostasis through dietary fibers, bioactive peptides, and microbiota-mediated metabolites [6,13].

MAIN PART

Chitosan, a partially deacetylated derivative of chitin, is a cationic polysaccharide with mucoadhesive, antimicrobial and antioxidant-related properties, and with notable sorption capacity [7–10]. Yet, its interaction with lipids and minerals may reduce bioavailability under certain physicochemical conditions, particularly when molecular weight and dose are not optimized [11,12]. Therefore, formulation strategies that control chitosan release and reduce non-specific binding are crucial for functional nutrition applications.

Protein–polysaccharide complexes are formed through electrostatic interactions, hydrogen bonding and hydrophobic forces. Their stability depends on pH and ionic strength, and they can be engineered as micro-/nano-carriers [9,10]. Milk proteins (casein and whey) represent a food-grade matrix and exemplify the 'food matrix effect', which can influence digestion kinetics and bioaccessibility of bioactives [14,15]. Chitosan–casein interactions have been shown to depend on chitosan molecular weight, altering complex structure and dispersion behavior [11]. Whey protein–chitosan hydrogels, fibril complexes and polyelectrolyte nanoparticles provide additional evidence for controllable release under simulated gastrointestinal conditions [18–21].

At the level of barrier biology, nutrition can modulate tight junction stability and mucus production via redox and inflammatory pathways (e.g., Nrf2/HO-1 and MAPK signaling), and chitosan oligosaccharides have shown barrier-protective effects in inflammatory models [21,22,24–27]. Dietary regulation of the mucus layer is an additional mechanism by which functional ingredients may reinforce barrier function [23]. Collectively, these data support the hypothesis that a chitosan–SMP polyelectrolyte complex may act as a 'soft' sorption system while promoting epithelial recovery and sustaining nutrient absorption.

METHODOLOGY

Design (in silico to in vivo translation). The framework proposes a 42-day feeding intervention in 60 male Wistar rats (180–220 g), randomized into five groups: (1) control; (2) low-molecular-weight chitosan; (3) high-molecular-weight chitosan; (4) low-MW chitosan–SMP complex; (5) high-MW chitosan–SMP complex. Doses are normalized to body weight and dietary energy to ensure comparability [3–5].

Complex preparation concept. Chitosan solution (pH < 6.5) is combined with an SMP protein dispersion (pH 6.8–7.2) to form a polyelectrolyte complex. Particle size distribution and ζ-potential are characterized; simulated gastric (pH 1.2) and intestinal (pH 6.8) media are used to model stability and release profiles [9,10,18–21].

Outcome measures. Barrier integrity: villus height, crypt depth, villus/crypt ratio; ultrastructure (microvilli, intercellular contacts); tight junction proteins ZO-1, occludin, claudin-1; mucus marker MUC2; functional assays including D-xylose absorption and paracellular permeability proxies [1–5,23]. Nutritional and metabolic indices include serum protein fractions, lipid profile, micronutrients (Fe, Zn, Ca), oxidative stress markers (MDA) and antioxidant enzymes (SOD, GPx), and inflammatory cytokines (TNF-α, IL-6) [13,21,22,24–

27]. Statistics: Shapiro–Wilk; ANOVA or Kruskal–Wallis; $p<0.05$. Ethics approval and humane endpoints are mandatory for in vivo validation.

ANALYSIS

The mechanistic synthesis suggests that uncomplexed chitosan may reduce endotoxin load through sorption and microbiota modulation, but could compromise micronutrient bioavailability if physicochemical parameters are suboptimal [6,11,12]. Complexation with an SMP protein matrix is expected to mitigate non-specific binding while improving dispersion and providing controlled release at intestinal pH [14,15,18–21].

From a signaling perspective, chitosan oligosaccharides have been linked to improved tight junction expression and reduced oxidative stress in inflammatory models, supporting a plausible pathway for barrier normalization [21,22,24–27]. In addition, matrix-controlled digestion may influence the temporal exposure of epithelial cells to the functional component, which is relevant for mucosal adaptation and microvillus integrity [14,15].

Previous publications by Rakhmonov and co-authors in broiler models indicate that combined chitosan with milk-derived ingredients can modulate mineral metabolism, immunobiochemical indices and digestive enzyme activity [30–32]. Although species and matrices differ, these findings strengthen the rationale for exploring the chitosan–SMP complex as a multi-target nutritional tool.

RESULTS

As the present work is an in silico framework, the 'results' are formulated as testable expectations. The complexed groups are predicted to show: (i) preservation of villus height and microvillus ultrastructure; (ii) higher or normalized ZO-1/occludin/claudin-1 expression; (iii) improved D-xylose absorption efficiency; (iv) lower oxidative stress (MDA↓) with enhanced antioxidant enzyme activity (SOD/GPx↑); (v) more stable micronutrient indices compared with uncomplexed chitosan [1–5,14,21,22,24–27]. These outcomes require rigorous experimental validation and dose–molecular-weight optimization.

CONCLUSION

A chitosan–skim milk powder polyelectrolyte complex is a promising food-grade strategy to co-modulate intestinal barrier function and nutritional status. The matrix may soften excessive sorption-related drawbacks while supporting controlled release and epithelial recovery. Future work should integrate tight-junction panels with microbiota profiling, metabolomic readouts and fraction-specific SMP comparisons (casein vs whey) to define the optimal formulation window.

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