ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF ARTIFICIAL INTELLIGENCE

ISSN: 2692-5206, Impact Factor: 12,23
American Academic publishers, volume 05, issue 11,2025

Journal: https://www.academicpublishers.org/journals/index.php/ijai

UDC: 577.112.3:578.087.7:612.33-092.9

EFFECT OF A WHEY POWDER AND CHITOSAN MIXTURE ON NUTRIENT ABSORPTION IN INTESTINAL EPITHELIAL CELLS

Rakhmonov Farkhod Kholbayevich
Assistant, Zarmed University, Samarkand, Uzbekistan
e-mail: farxod1313jon@gmail.com

Komilov Oyatullo Meliboy ugli
Student, Zarmed University, Samarkand, Uzbekistan
e-mail: komilovoyatullo25@gmail.com

Abstract: This study evaluated, in vitro, the effect of a whey powder (WP) and chitosan mixture on nutrient absorption in Caco-2 intestinal epithelial cells. Whey powder and chitosan were combined at mass ratios of 1:1, 2:1 and 1:2 and applied at concentrations from 0.1% to 1.0%. Glucose and amino acid (leucine) transport, transepithelial electrical resistance (TEER), cell viability (MTT) and membrane integrity (LDH release) were assessed. The whey:chitosan 2:1 mixture at 0.5% produced the most pronounced effect, increasing glucose transport by 15–20% and leucine transport by 10–18% versus control ($p<0.05$), suggesting activation of transporter proteins and/or an increase in effective absorptive surface area. TEER values remained stable or slightly increased, indicating preservation and mild strengthening of the intestinal barrier. MTT and LDH data confirmed absence of cytotoxicity in the tested range, while microscopic examination showed preserved enterocyte-like morphology with a tendency toward denser and longer microvilli. These findings support the use of whey powder–chitosan mixtures as promising bioactive components for functional foods and nutraceutical formulations aimed at supporting intestinal health and improving nutrient bioavailability.
Keywords: whey powder, chitosan, Caco-2 cells, intestinal epithelium, nutrient absorption, glucose transport, amino acid transport, TEER, cell viability, bioactive components, intestinal barrier, functional food, nutraceuticals, in vitro model.

Introduction. Whey powder, obtained as a by-product of dairy processing, and the natural polysaccharide chitosan are increasingly viewed as valuable bioactive ingredients for the development of functional foods and nutraceuticals. Whey powder contains high-value proteins, free and bound amino acids, lactoferrin, immunoglobulins, easily digestible carbohydrates and minerals, which together can improve intestinal mucosal status, modulate immune responses and attenuate oxidative stress, thereby supporting the overall nutritional status of the organism [6; 9; 15]. Chitosan, a deacetylated derivative of chitin from crustacean shells, exhibits protective, adsorptive, anti-inflammatory and absorption-enhancing properties and is widely studied in pharmaceutical and nutrition sciences [1; 2; 3; 4].

The Caco-2 cell line is one of the most widely used in vitro models to investigate intestinal morphology and transport. Upon differentiation, Caco-2 cells acquire several characteristics of small intestinal enterocytes, including microvilli formation, brush border enzymes and functional transepithelial transport mechanisms [1; 6; 7]. This makes the model suitable for assessing the effects of dietary bioactives on nutrient absorption and barrier integrity.

Although whey protein ingredients and chitosan have been separately studied in relation to gut function and nutrient uptake [5; 7; 15], data on their combined action on intestinal epithelial cells remain limited. The aim of this work was therefore to evaluate, under in vitro conditions, the effect of a whey powder–chitosan mixture on glucose and amino acid transport, TEER and

ORIGINAL ARTICLE
INTERNATIONAL JOURNAL OF ARTIFICIAL INTELLIGENCE
ISSN: 2692-5206, Impact Factor: 12,23
American Academic publishers, volume 05, issue 11,2025
Journal: https://www.academicpublishers.org/journals/index.php/ijai

cell viability in Caco-2 monolayers, and to consider its potential as an ingredient for functional foods and nutraceuticals.

Materials and Methods. Caco-2 intestinal epithelial cells and mixtures of food-grade whey powder and pharmaceutical-grade chitosan were used. Whey powder contained at least 80% protein and met standard food quality requirements [9; 10; 11; 12; 13; 15; 16]. Chitosan had a medium molecular weight and a degree of deacetylation above 85%, with high purity suitable for biomedical applications. All reagents were of analytical grade, prepared in distilled or deionized water.

Whey powder and chitosan were mixed at mass ratios of 1:1, 2:1 and 1:2. For each ratio, sterile solutions at 0.1%, 0.5% and 1.0% (w/v) were prepared. Chitosan was dissolved in 0.1 M acetic acid and then neutralized with phosphate buffer to pH 6.5–7.0 [1; 3]. Whey powder was dissolved in distilled water and stirred until complete solubilization. All solutions were sterilized by filtration through 0.22 μm membrane filters and stored at +4 °C until use. Control groups included cells exposed only to culture medium, to whey powder alone or to chitosan alone.

Caco-2 cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic–antimycotic solution in a humidified incubator at 37 °C and 5% CO2 [1; 6; 7]. At 80–90% confluence, cells were detached with trypsin–EDTA and seeded onto polycarbonate Transwell inserts with 0.4 μm pores. Cells were allowed to differentiate for 21 days. Formation of tight junctions and functional monolayers was confirmed by TEER values above 300 Ω·cm², which served as a criterion for experimental use [1; 4; 6].

Nutrient transport was assessed by adding a standard glucose solution to the apical compartment and measuring its appearance in the basolateral compartment by spectrophotometry at defined time points. Amino acid transport was studied using radiolabelled 14C-leucine; radioactivity in basolateral samples was quantified by scintillation counting, providing an estimate of transporter activity [2; 5; 7; 8; 14].

Cell viability and cytotoxicity were evaluated by MTT assay (mitochondrial enzyme activity) and LDH release (membrane integrity) [2; 4]. TEER was measured throughout the experiments using a dedicated voltohmmeter to monitor barrier integrity and tight junction function. Data were analysed by ANOVA and Student's t-test; differences were considered statistically significant at $p<0.05$. Results are presented as mean ± standard error.

Results and Discussion. The whey powder–chitosan mixture exerted a clear positive effect on nutrient transport and maintained barrier integrity in Caco-2 monolayers. For glucose transport, the most pronounced increase was observed with the 2:1 whey:chitosan mixture at 0.5%, where basolateral glucose appearance rose by approximately 15–20% relative to control ($p<0.01$). This suggests that the combination can enhance the activity and/or expression of glucose transporters in the apical membrane.

A similar pattern was seen for amino acid transport. In the presence of the mixture, basolateral transfer of 14C-leucine increased by about 10–18% compared with control ($p<0.05$). These data indicate that peptide and amino acid transport systems in intestinal epithelial cells may be favourably modulated by the combined action of whey components and chitosan.

MTT and LDH assays demonstrated that, within the tested concentration range, the mixture was not cytotoxic. Cell viability remained close to control values, and LDH release did not differ significantly from baseline ($p>0.05$), confirming preservation of membrane integrity.

TEER monitoring provided additional evidence that the intestinal barrier was not compromised. In most experimental groups TEER values remained stable throughout the study, and in some

ISSN: 2692-5206, Impact Factor: 12,23
American Academic publishers, volume 05, issue 11,2025
Journal: https://www.academicpublishers.org/journals/index.php/ijai

cases a slight increase was recorded, which may reflect reinforcement of tight junctions under the influence of the whey–chitosan mixture.

Microscopic examination confirmed the integrity of the cell monolayer, preserved cell–cell contacts and characteristic enterocyte-like morphology. In several groups, a tendency toward denser and more elongated microvilli was noted, potentially increasing the effective surface area for nutrient absorption.

Taken together, these findings point to a synergistic effect of whey powder and chitosan: nutrient transport is improved while barrier properties are maintained or mildly enhanced. This balance is particularly important for the development of safe functional ingredients that increase bioavailability without compromising gut integrity.

Conclusion. In vitro experiments on Caco-2 intestinal epithelial cells demonstrated that a whey powder and chitosan mixture can significantly enhance nutrient absorption while preserving barrier function and cell viability. The 2:1 whey:chitosan mixture at 0.5% proved most effective, increasing glucose transport by 15–20% and leucine transport by 10–18% compared with control ($p<0.05$).

MTT and LDH assays confirmed the absence of cytotoxic effects in the tested range, and TEER data showed that the epithelial barrier remained intact, with a slight strengthening in some conditions. Morphological analysis supported these observations, revealing normal enterocyte-like monolayers and favourable microvillous changes.

Thus, the whey powder–chitosan mixture may be considered a promising bioactive ingredient for functional foods and nutraceutical products designed to improve intestinal nutrient bioavailability and support gut health. Future work should clarify the underlying molecular mechanisms (transporter gene expression, signalling pathways, tight junction proteins) and confirm the present findings in animal models and controlled clinical trials.

References

1. Schipper N. G. M., Olsson S., Hoogstraate J. A., de Boer A. G., Varum K. M., Artursson P. Chitosans as absorption enhancers for poorly absorbable drugs. 1. Influence of molecular weight and degree of acetylation on drug transport across human intestinal epithelial (Caco-2) cells // Journal of Pharmaceutical Sciences. – 1996. – Vol. 85. – P. 1133–1139.
2. Artursson P., Lindmark T., Davis S. S., Illum L. Effect of chitosan on the permeability of monolayers of intestinal epithelial cells (Caco-2) // Pharmaceutical Research. – 1994. – Vol. 11. – P. 1358–1361.
3. Thanou M., Verhoef J. C., Junginger H. E. Chitosan and its derivatives as intestinal absorption enhancers // Advanced Drug Delivery Reviews. – 2001. – Vol. 50. – P. S91–S101.
4. Casettari L., Illum L. Chitosan in nasal delivery systems for therapeutic drugs // Journal of Controlled Release. – 2014. – Vol. 190. – P. 189–200.
5. Li M., Sun Q., Li Y., Shi H., Wang T., Wang C. The intestinal absorption mechanism of chicoric acid and its bioavailability improvement with chitosan // Food & Function. – 2022. – Vol. 13. – P. 1051–1062.
6. Xiao K., Jiao L. F., Cao S. J. et al. Whey protein concentrate enhances intestinal integrity and influences transforming growth factor-β1 and mitogen-activated protein kinase signalling pathways in piglets after lipopolysaccharide challenge // British Journal of Nutrition. – 2016. – Vol. 115. – P. 984–993.
7. Li X., Zhang R., Zhang T. et al. Characterization and Caco-2 cell transport assay of chito-oligosaccharides nano-liposomes based on layer-by-layer coated // Molecules. – 2021. – Vol. 26. – P. 1–14.

ISSN: 2692-5206, Impact Factor: 12,23

American Academic publishers, volume 05, issue 11,2025

Journal: https://www.academicpublishers.org/journals/index.php/ijai

8. Рахманов Ф., Усманова Х., Ходжаёрова Г. Effect of bioadditional supplements on broiler chicken // Международный мультидисциплинарный журнал исследований и разработок. – 2025. – Т. 1. – № 2. – С. 3–7.
9. Sh. U. T., Kh. R. F. Dry whey: a promising product for the food industry and agriculture // Web of Teachers: Inderscience Research. – 2025. – Vol. 3. – No. 3. – P. 16–18.
10. Xolbayevich R. F., Dusmurat E., Khurshid I., Gulchehra U. Effect of chitosan and whey powder on the productivity of broiler chickens // Emerging Frontiers Library for The American Journal of Interdisciplinary Innovations and Research. – 2025. – Vol. 7. – No. 06. – P. 10–12.
11. Holbayevich R. F., Dusmurod E., Iskanderovich I. K., Bakhriddinobna U. G. Explanation on the physiological and biochemical indicators of broiler chicks fed with chitosan and whey powder // Academia Repository. – 2024. – Vol. 5. – No. 2. – P. 184–187.
12. Rakhmonov F., Eshimov D., Islomov Kh., Ubaydullaeva G., Hayitova B. The effect of chitosan and whey powder on the weight of broiler chickens // BIO Web of Conferences. – 2024. – Vol. 95. – 01025.
13. Holbayevich R. F. Chitosan and study of physiological and biochemical indicators of broiler chicks feeding whey powder // Open Access Repository. – 2023. – Vol. 4. – No. 3. – P. 1389–1395.
14. Raxmonov Farxod Xolbayevich, Eshimov Dusmurod, & Nuriddinova Muxlisa Isomiddin qizi. (2025). Effect Of Chitosan and Whey Powder On The Productivity Of Broiler Chickens. Emerging Frontiers Library for The American Journal of Interdisciplinary Innovations and Research, 7(06), 10–12.
15. Korish M., Attia Y. A. Evaluation of heavy metal content in feed and broiler chicken tissues // Environmental Science and Pollution Research. – 2017. – Vol. 24. – P. 16297–16308.
16. Smithers G. W. Whey and whey proteins – from “gutter-to-gold” // International Dairy Journal. – 2008. – Vol. 18. – P. 695–704.