Probiotic supplementation: a universal solution or a case of limited efficacy?

Ph.D. thesis

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1. Introduction

Probiotics are live microorganisms that confer health benefits when consumed in adequate amounts, commonly including species from *Lactobacillus*, *Bifidobacterium*, and *Bacillus*. They may modulate immunity, compete with pathogens, produce short-chain fatty acids, and interact with host cells. Despite market growth, evidence for their broad efficacy, especially in healthy individuals, remains limited and strain-specific. This has driven interest in next-generation and personalized probiotics.

The gut microbiota, dominated by *Firmicutes* and *Bacteroidetes* phyla, supports digestion, vitamin synthesis, immunity, and gut barrier integrity. Dysbiosis, often antibiotic-induced, is linked to disorders like IBD and obesity. Recovery varies by host and treatment factors. Microbiome profiling uses both traditional cultures and molecular methods. 16S rRNA sequencing allows genus-level analysis, while shotgun metagenomics enables species-level resolution and functional insights, though at a higher cost and complexity. Microbial diversity reflects ecosystem health. α-diversity (within-sample richness, evenness) and β-diversity (between-sample compositional differences) are assessed using indices like Shannon, Chao1, and UniFrac. Greater diversity is linked to resilience and metabolic flexibility. Antibiotics can reduce diversity and promote pathogen overgrowth. Although probiotics are often used to counteract this, their effectiveness on microbiota composition during treatment remains unclear, and clinical guidelines are lacking in this regard. Zonulin regulates intestinal permeability via tight junctions. Elevated levels may lead to a “leaky gut,” contributing to inflammation and disease. Probiotics may influence zonulin, but current evidence is inconclusive.

Systematic reviews and meta-analyses synthesize scientific evidence, improving precision and guiding practice. Their reliability depends on study quality and consistency, but remains essential in evidence-based health sciences.

2. Objectives

I. The effect of probiotic supplementation on the gut microbiome during antibiotic treatment: To evaluate the effect of probiotic supplementation on gut microbiome diversity and composition during antibiotic treatment by systematically reviewing and synthesizing available evidence based on randomized controlled human trials.

II. The effect of probiotic supplementation in healthy populations: To assess the impact of probiotic supplementation in healthy populations by systematically reviewing and synthesizing available evidence based on randomized controlled human trials. In this thesis, I intend to report results on the following specific outcomes:

a) gut microbiome diversity
b) zonulin levels

2.1. Hypotheses

It is hypothesized that probiotic supplementation does not have a statistically significant or clinically relevant overall effect on gut microbiome diversity or zonulin levels, both during antibiotic treatment and in healthy populations. This assumption is based on the variability in individual microbiome responses, strain-specific probiotic effects, and inconsistencies in current literature.

3. Methods

All investigations followed Cochrane guidelines and PRISMA 2020 reporting standards. The protocols were pre-registered in PROSPERO under the following IDs:

1. CRD42021282983 – The effect of probiotic supplementation on the gut microbiome during antibiotic treatment
2. CRD42022286137 – The effect of probiotic supplementation in healthy populations

3.1. Search strategy and study selection

Using the PICO-S framework, eligibility was defined as follows:

1. *Population (P)* — people treated with antibiotics regardless of indication; *Intervention (I)* — probiotic supplementation along with antibiotic treatment; *Comparison (C)* — no probiotic supplementation (placebo or no intervention); *Outcome (O)* — gut microbial diversity (any reported diversity indices) and composition at the end of the intervention (and after a follow-up period)
2. *Population (P)* — healthy individuals as specified in the articles; *Intervention (I)* — probiotic supplementation; *Comparison (C)* — no probiotic supplementation (placebo or no intervention); *Outcome (O)* — the primary outcome was gut microbial diversity (any reported diversity indices) at the end of the intervention (and after a follow-up period). Additionally, we aimed to assess any other outcomes as reported in the identified studies. In this thesis, I report results on zonulin levels

Only randomized controlled trials (RCTs) were included, with no restrictions on age, sex, or ethnicity. Searches were conducted in MEDLINE (PubMed), Embase, and CENTRAL without filters. The systematic search was conducted on:

1. 15/10/2021 - The effect of probiotic supplementation on the gut microbiome during antibiotic treatment
2. 12/04/2024 - The effect of probiotic supplementation in healthy populations

Rayyan was used for study screening, and EndNote X9 for reference management. Two reviewers independently conducted screening and selection, with discrepancies resolved by consensus. Cohen's kappa quantified inter-rater reliability. Selection included title/abstract screening followed by full-text evaluation.

3.2. Data collection

Data were extracted independently by two reviewers using a standardized form and cross-checked. Extracted information included study characteristics, participant demographics, intervention details (strain, dose, duration), and outcomes. Where only graphical data were available, values were extracted using GetData Graph Digitizer and PlotDigitizer.

3.3. Synthesis methods

3.3.1. The effect of probiotic supplementation on the gut microbiome during antibiotic treatment

Meta-analysis was conducted in *R* (v4.2.1) using *meta* and *dmetar*. Mean differences (MD) with 95% confidence intervals (CIs) were calculated for Shannon, Chao1, and observed OTU indices. If only quartile data were available, means and SDs were estimated using the methods by Luo and Shi. Assumptions of normal distribution were supported by raw data from Oh et al. A random-effects model with inverse variance weighting was used due to expected heterogeneity. Sensitivity analyses included before-treatment comparisons and before–after changes using a correlation coefficient derived from Oh et al. Heterogeneity was assessed using Cochran's Q and I^2 statistics; τ^2 was estimated using the Q profile method with a maximum-likelihood estimator. Publication bias was not assessed due to the limited number of studies (<10). Forest plots illustrated the pooled effects. The findings are reported as (MD [95% CI lower limit – 95% CI upper limit]).

3.3.2. The effect of probiotic supplementation in healthy populations – Diversity indices

Due to the frequent reliance on box plots, effect sizes were expressed as median differences (MedD) with corresponding 95% CIs. The pooled estimate of MedDs was calculated using a

random-effects model with inverse variance weighting. Heterogeneity variance (τ^2) was estimated with the restricted maximum-likelihood method and Q profile confidence intervals. Potential outliers were assessed using leave-one-out influence measures as recommended by Harrer et al. Subgroup and sensitivity analyses were performed based on probiotic type, intervention duration, and risk of bias. Publication bias was assessed using funnel plots and Egger's test. We assumed a possible small study bias if the p-value was less than 10%. All analyses were performed in *R* (v4.4.1) with *meta*, *metamedian*, *metafor*, and *dmetar*. The findings are reported as (MedD [95% CI lower limit – 95% CI upper limit]).

3.3.3. The effect of probiotic supplementation in healthy populations – Zonulin levels

A random-effects model was used to calculate standardized mean differences (SMD, Hedges's g) between probiotic and control groups. Post-intervention values were analyzed assuming comparable baselines due to the RCT design. Heterogeneity was quantified using τ^2 and I^2. Results were significant if the 95% CI excluded zero. Analyses were performed in *R* (v4.4.1) using the *meta* package. The findings are reported as (SMD [95% CI lower limit – 95% CI upper limit]).

3.4. Risk of Bias assessment

Two reviewers independently assessed the risk of bias using the Cochrane RoB2 tool, resolving disagreements by consensus. Domains included randomization, deviations from intervention, missing outcome data, measurement of outcomes, and selective reporting. Overall risk was categorized as low, some concerns, or high.

3.5. Certainty of evidence

Certainty was evaluated using the GRADE framework, considering risk of bias, inconsistency, indirectness, imprecision, and publication bias. The evidence was classified as high, moderate, low, or very low. Two reviewers performed the assessments independently, with consensus reached in all cases.

4. Results

4.1. The effect of probiotic supplementation on the gut microbiome during antibiotic treatment

4.1.1. Study selection

The search identified 19,596 records, with Cohen's kappa values of 0.86 (title/abstract) and 0.95 (full text), indicating high inter-rater agreement. Fifteen studies (877 patients) were included in the qualitative synthesis, of which five (335 patients) contributed data on Shannon diversity index and three (236 patients) on Chao1 index and observed OTUs. No additional records were found via reference screening, and overlapping populations were excluded. Most studies involved adults; one included neonates, and another adolescents.

Eight studies focused on *Helicobacter pylori* eradication, one on *Clostridioides difficile* infection, two on non-gastrointestinal infections, and four on healthy participants without clinical indication for antibiotics. Microbiome analysis methods included 16S rRNA sequencing (9 studies), standard culturing (3), terminal restriction fragment length polymorphism (TRFLP) plus culturing (1), and other PCR-based techniques (2). All studies were peer-reviewed and available in full text, except for one protocol-based publication.

4.1.2. Quantitative synthesis

Five studies (335 patients) were included in the meta-analysis of the Shannon diversity index, showing no significant difference

between probiotic and control groups at the end of antibiotic treatment (MD = 0.23; 95% CI: –0.06 to 0.51). Three studies (236 patients) were analyzed for Observed OTUs (MD = 17.15; 95% CI: –9.43 to 43.73) and Chao1 index (MD = 11.59; 95% CI: –18.42 to 41.60), also with no significant differences (**Figure 1).** Neonate data and studies with unclear time points were excluded to reduce indirectness and heterogeneity.

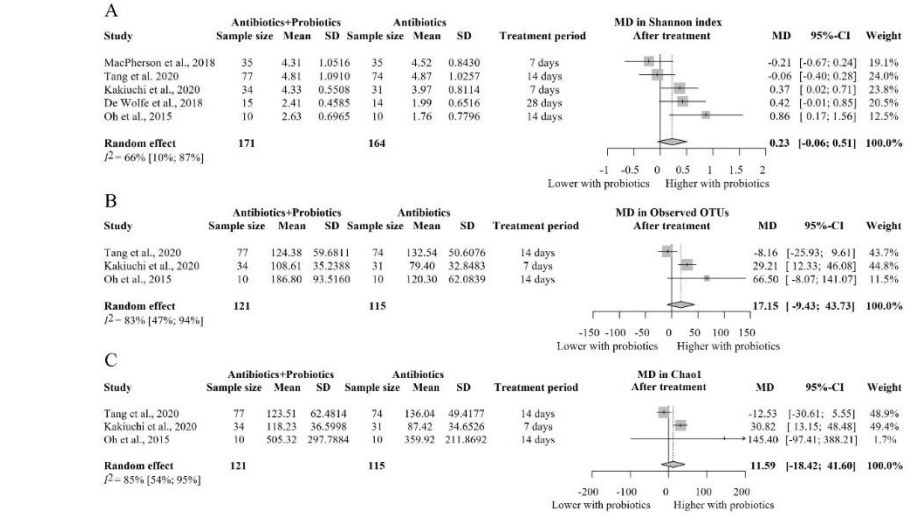

Figure 1. After antibiotic treatment, the Shannon diversity index (A), Observed OTUs (B) and Chao1 index (C) are not significantly higher in patients receiving concurrent probiotic supplementation than in those treated with antibiotics alone, as measured immediately after antibiotic treatment. CI: confidence interval; MD: mean difference

As a sensitivity analysis, we calculated pre-treatment values separately and analyzed the magnitude of change ("before-after" differences) within each study. No significant differences were found between groups for any diversity index (Shannon: MD = 0.07; 95% CI: –0.19 to 0.32; Observed OTUs: MD = 8.09; 95% CI: –3.87 to 20.05; Chao1: MD = 3.77; 95% CI: –10.17 to 17.71) **(Figure 2)**.

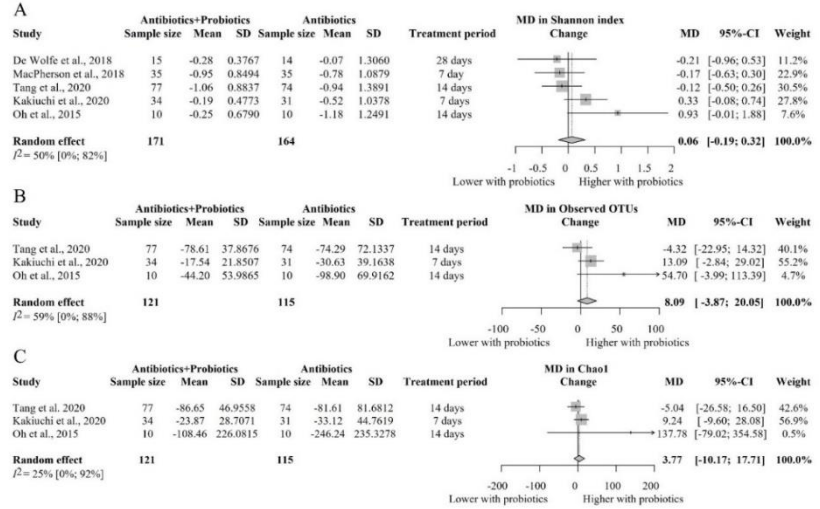

Figure 2. The magnitude of change compared to pre-treatment values of the Shannon diversity index, Observed OTUs, and Chao1 indices is not significantly different in the group receiving concurrent probiotic supplementation than in the group treated with antibiotics alone. CI: confidence interval; MD: mean difference

4.1.3. Qualitative synthesis

Most studies not included in meta-analysis showed no significant differences between probiotic and control groups in α-diversity indices. β-diversity measures also generally showed no group differences, except one study reporting improved stability with probiotics. Antibiotics reduced *Firmicutes* and *Bacteroidetes*, increased *Proteobacteria*, and altered the *Bacteroidetes*:*Firmicutes* ratio in both groups. Some studies showed probiotics helped maintain *Bifidobacterium* levels and modulate *Escherichia*, *Enterococcus*, *Roseburia*, and *Blautia*, but findings were inconsistent and often transient.

4.1.4. Risk of Bias

The overall risk of bias ranged from low to high, mainly due to baseline differences between groups in some studies.

4.1.5. GRADE Assessment

According to the GRADE assessment, the quality of evidence was rated as low.

4.2. Probiotics for healthy populations – Diversity indices

4.2.1. Study selection

From 13,625 records, 47 randomized, placebo-controlled studies were included in the qualitative synthesis of gut microbiome diversity. Inter-rater agreement was high, with Cohen's kappa values of 0.94 and 0.81 (title/abstract screening) and 0.94 and 0.98 (full-text screening). Most studies involved adults; some focused on infants, children, or the elderly. Pediatric studies were excluded from meta-analysis to avoid indirectness. Usable data were available in 22 studies for the Shannon diversity index, 7 for observed OTUs, 9 for the Chao1 index, and 10 for the Simpson's Index of Diversity.

4.2.2. Quantitative synthesis

The meta-analysis of 22 studies (1,068 participants) showed no significant effect of probiotics on Shannon diversity index at the end of treatment (MedD = –0.08; 95% CI: –0.16 to 0.01) **(Figure 3)**. Subgroup analysis by probiotic composition (*Lactobacillaceae*, *Bifidobacteriaceae*, *Bacillaceae*, or mixtures) also revealed no significant differences. Seven studies (447 participants) were included in the meta-analysis of observed OTUs. No significant difference was found between probiotic and control groups (MedD = 2.19; 95% CI: –2.20 to 6.57) **(Figure 4)**. Subgroup analyses by probiotic composition also showed no significant or clinically relevant effects. Nine studies (456 participants) were included in the meta-analysis of the Chao1 index. No significant difference was found between probiotic and control groups (MedD = –3.19; 95% CI: –27.28 to 20.89) **(Figure 5)**. Subgroup analysis by probiotic composition confirmed the absence of significant effects. Ten studies (455 participants) were included in the meta-analysis. Data were

standardized to ensure higher values reflected greater diversity, assuming the use of Simpson's Index of Diversity (1–D). No significant difference was found between probiotic and control groups (MedD = –0.01; 95% CI: –0.02 to 0.00) **(Figure 6.)**, and subgroup analysis by probiotic composition showed no significant effects.

Sensitivity, subgroup, and meta-regression analyses (by risk of bias and intervention duration) showed no significant effects. Publication bias and leave-one-out analyses did not reveal major concerns or influential studies affecting the meta-analysis outcomes.

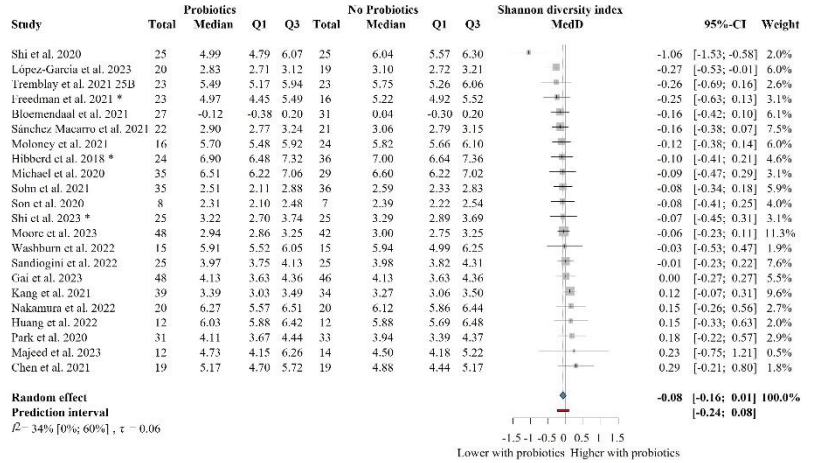

Figure 3. Shannon diversity index is not significantly different in healthy people receiving probiotic supplementation than in those in the control group, as measured immediately after the treatment period; CI: confidence interval; MedD: mean of median differences. Q1: first quartile; Q3: third quartile.

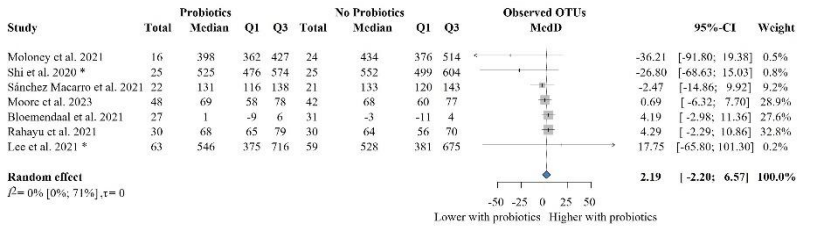

Figure 4. The number of Observed OTUs is not significantly different in healthy people receiving probiotic supplementation than in those in the control group, as measured immediately after the treatment period; OTU: operational taxonomic unit; CI: confidence interval; MedD: mean of median differences. Q1: first quartile; Q3: third quartile.

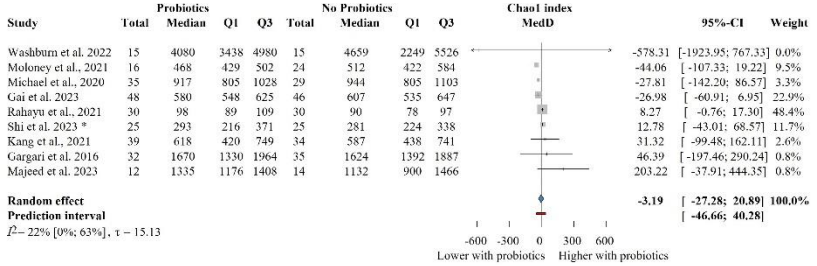

Figure 5. Chao1 index is not significantly different in healthy people receiving probiotic supplementation than in those in the control group, as measured immediately after the treatment period; MedD: mean of median differences. Q1: first quartile; Q3: third quartile.

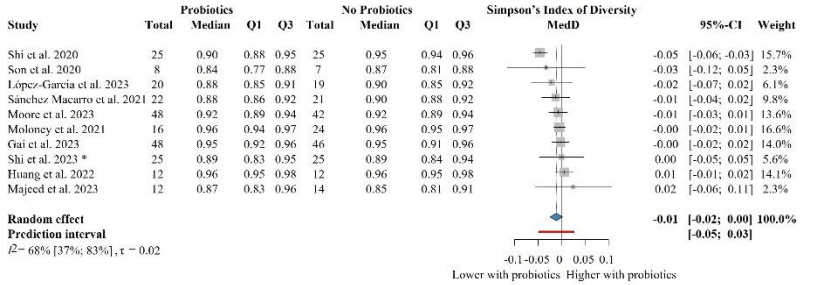

Figure 6. Simpson's Index of Diversity is not significantly different in healthy people receiving probiotic supplementation than in those in the control group, as measured immediately after the treatment period; CI: confidence interval; MedD: mean of median differences. Q1: first quartile; Q3: third quartile.

4.2.3. Qualitative synthesis

Most studies reported no significant differences in α-diversity between probiotic and control groups. A few studies showed within-group changes, but many lacked placebo comparisons. Results for specific bacterial families were inconsistent, and overall findings from the meta-analysis confirmed no significant effect. β-diversity analyses also largely showed no relevant differences, though isolated studies reported significant changes observed after the supplementation with certain strains (e.g. *L. paracasei*, *L. rhamnosus*, *L. plantarum, H. coagulans*).

4.2.4. Risk of Bias

The risk of bias ranged from low to high across included studies.

4.2.5. GRADE Assessment

The overall quality of evidence was rated as moderate.

4.3. Probiotics for healthy populations – Zonulin levels

4.3.1. Study selection

From 13,625 records, five studies met the criteria for qualitative synthesis of blood zonulin levels, with high inter-rater agreement (Cohen's kappa: 0.90–0.98). One additional study measuring stool zonulin was included in the review but not pooled with blood data. All studies involved generally healthy adults, including male athletes, pregnant women, and individuals with minor gastrointestinal symptoms or varying BMI.

4.3.2. Quantitative Synthesis

The meta-analysis of five studies (307 participants) showed no significant or clinically relevant difference in blood zonulin levels between probiotic and control groups at the end of treatment (SMD = –0.01; 95% CI: –0.39 to 0.37) **(Figure 7)**.

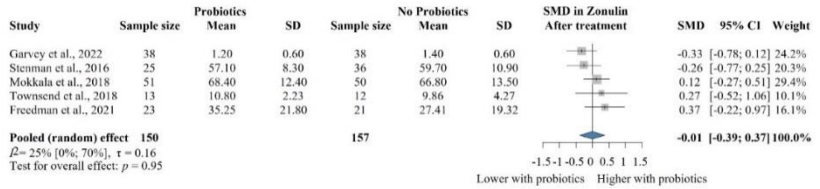

Figure 7. Zonulin levels are not significantly different in healthy people receiving probiotic supplementation than in those in the control group, as measured immediately after the treatment period. Concentrations are expressed as ng/ml. CI: confidence interval; SD: standard deviation; SMD: standardized mean difference

4.3.3. Qualitative synthesis

Consistent with the meta-analysis, individual studies reported no significant differences in blood zonulin levels between probiotic and placebo groups. One study measuring stool zonulin (not included in the meta-analysis) showed a significant reduction following probiotic use.

4.3.4. Risk of Bias

Most included studies had a low risk of bias. One study was rated high risk due to a high dropout rate.

4.3.5. GRADE Assessment

The overall quality of evidence for the meta-analysis was rated as very low

5. Conclusions

The summarized results from the currently available randomized controlled trials do not support probiotic supplementation as an effective strategy to modify gut microbiome diversity during antibiotic treatment or in healthy populations. In these contexts, the meta-analyses of the most common diversity indices, including Shannon, Chao1, Observed OTUs, and Simpson's Index of Diversity, revealed no significant effect of probiotics on modulating or increasing microbial diversity. While not all reported outcomes could be analyzed quantitatively, the strong overall trend across studies suggests a lack of influencing effect on both α- and β-diversity metrics. Furthermore, our meta-analysis of five studies with 307 healthy individuals revealed no significant effect of probiotics on circulating zonulin levels.

There is a strong need for standardized normal ranges and consistent reporting of diversity metrics to support more robust and comparable analyses. A consensus for appropriate methods and clinically important outcomes is critical for further research. Studies should focus on the potential clinical relevance of probiotics in specific populations and on understanding the functional impacts of microbiota modulation.

6. Bibliography of the candidate's publications

Publications related to the thesis (∑IF: 7.1):

1. **Éliás AJ,** Barna V, Patoni C, Demeter D, Veres DS, Bunduc S, et al. Probiotic supplementation during antibiotic treatment is unjustified in maintaining the gut microbiome diversity: a systematic review and meta-analysis. *BMC Med*. 2023;21(1):262. doi: 10.1186/s12916-023-02961-0

2. Földvári-Nagy KCs, Simon V, Csősz Cs, Schnabel T, Veres DS,…, **Éliás AJ**. The effect of probiotic supplementation on zonulin levels in healthy individuals – A systematic review and meta-analysis. *Developments in Health Sciences*. in press Paper: in press (2025) doi: 10.1556/2066.2025.00081

Publications not related to the thesis (∑IF: 8.6):

1. Gul L, **Elias AJ**, Tambaku T, Olbei M, Watters E, Bohar B, Modos D, Madgwick M, Korcsmaros T. Protocol for predicting host-microbe interactions and their downstream effect on host cells using MicrobioLink. *STAR Protoc*. 2025;6(1):103570. doi: 10.1016/j.xpro.2024.103570

2. Pokhilenko I, Afentou N, Fu L, Hiligsmann M, Witthoft C, Hefni M, Nzefa LD, Randelli F, **Elias AJ**,…, Frew E. What's for lunch? Eliciting preferences for food on university campus: discrete choice experiment protocol [Preprint, not peer reviewed]. *Research Square*. 2024 Jun 7. Available from: https://doi.org/10.21203/rs.3.rs-4436883/v1.

3. **Éliás AJ**, Bodor Z, Benedek C. Xanthohumol (*Humulus lupulus* L.): Potential of bioactive polyphenols in beer. In: Goyal MR, Nath A, Kovács Z, editors. *Sustainable and Functional Foods from Plants: Health Impact, Bioactive Compounds, and Production Technologies*. 1st ed. Boca Raton: CRC Press; 2024. p. 253–90.

4. **Éliás AJ,** Balogh L, Brányik T, Mák E, Csajbókné Csobod É, Veresné Bálint M, et al. Xanthohumol-Enriched Beer Does Not Exert Antitumorigenic Effects on HeLa Cell Line *In Vivo*. *Molecules*. 2023;28(3):1070. doi: 10.3390/molecules28031070.
5. Patoni C, Bunduc S, Frim L, Veres DS, Dembrovszky F, **Éliás AJ**, et al. Low molecular weight heparin decreases mortality and major complication rates in moderately severe and severe acute pancreatitis-a systematic review and meta-analysis. *Front Med (Lausanne).* 2023;10:1241301.
6. **Éliás AJ**, Juhász R, Hajas L, Benedek C. *In vitro* study of the protein digestibility of various lentil-based gluten-free biscuits. *Új Diéta: A Magyar Dietetikusok Lapja* **(2001-)**. 2022;31. Hungarian.
7. **Éliás AJ**. Article review: Nutri-Score nutrition labeling system clarity and impact on food choice. *Egészségfejlesztés*. 2021;62. Hungarian.

∑IF: 15.7