PhD DISSERTATION PROJECTS

Impact of vegetable proteins on gut microbiome modulation and health promotion

Roberto Marotta (roberto.marotta@unina.it)

Dept. of Agricultural Sciences, University of Naples Federico II, Portici, Italy

Tutor: Prof. Danilo Ercolini

This PhD thesis research project is aimed to investigate the impacts of plant-based diet, with an emphasis on legumes consumption, on gut microbiome and health outcomes in healthy individuals. The main focus is to understand how replacing animal-derived with plant-based proteins may affect the composition and potential functionality of the gut microbiome, potentially influencing host health, inflammatory response, and immune function. By demonstrating the benefits of this dietary pattern on gut health and overall well-being, this study has the potential to bring significant innovation to the plant-based food industry by adding value and differentiation to new food and dietary proposal.

Impatto delle proteine vegetali sulla modulazione del microbiota intestinale e sulla promozione della salute

Questo progetto di tesi di dottorato mira ad indagare gli effetti di una dieta a base vegetale, con un’enfasi sul consumo di legumi, sul microbiota intestinale e sulla salute di individui sani. L’obiettivo principale è comprendere come la sostituzione di proteine animali con quelle vegetali influenzi la composizione e la potenziale funzionalità del microbiota intestinale, incidendo sulla salute dell’ospite, sull’infiammazione e sulla funzione immunitaria. Dimostrando i benefici di questo regime alimentare sulla salute intestinale e il benessere generale, questo studio ha il potenziale per apportare un’innovazione significativa al settore dei prodotti vegetali, aggiungendo valore e differenziazione alle nuove proposte alimentari.

# **1. State-of-the-Art**

The gut microbiome, the complex community of microorganisms residing in our gastrointestinal tract, has significant influence on crucial human functions such as immunomodulation, behaviour, dietary nutrient and drug metabolism. The composition of this intricate community can be heavily influenced by long-term dietary habits (De Filippis et al. 2018). In recent years, plant-based diets (PBDs), characterized by a high intake of fruits, vegetables, legumes, and whole grains, and limited or absent consumption of animal-derived products, have gained global traction due to their benefits for individuals’ health and the environment sustainability. Research suggests that long-term PBDs notably modify the gut microbiome, fostering fiber-degrading bacteria and enhancing beneficial short-chain fatty acid production (Sidhu et al. 2023). Conversely, diets high in animal-based foods tend to promote bacteria specialized in fat/protein metabolism, thus resulting in an increase in potentially harmful microbial by-products (David et al. 2014).

Table 1 outlines the main dietary patterns along with their sources of protein ordered by frequency of use and highlights how these dietary patterns have distinct influences on gut microbiota composition.

***Table 1***Influences of dietary patterns on gut microbiota composition.

|  |  |  |
| --- | --- | --- |
| **Dietary pattern** | **Source of proteins (ranked by frequency of use)** | **Effects on gut microbiota composition** |
| Vegan / Vegetarian diet | Legumes, grains, nuts, seeds, plant-based meat alternatives | ↑ *Faecalibacterium prausnitzii, Bacteroides, Prevotella, Bacteroides thetaiotaomicron, Bacteroidetes, K. pneumoniae, Clostridium clostridioforme*↓ *Bifidobacteria, Clostridium cluster XIV, Bilophila* |
| Mediterranean diet | Dairy products, legumes, grains, nuts, seeds, fish, seafood, poultry, eggs, meat | ↑ *Bifidobacteria, Lactobacillus, Lachnospiraceae, Bacteroidetes*↓ *Clostridium, Enterobacteria* |
| Western diet | Meat, diary products, eggs, processed meats, poultry, fish, legumes, grains | ↑ *Ruminococcus torques, Enterobacteria, Bilophila, Alistipes, Bacteroides, Akkermansia*↓ *Bifidobacteria, Roseburia, Eubacterium rectale, Ruminococcus bromii, Lactobacillus, Prevotella* |

↑: Increase in the abundance; ↓: decrease in the abundance.

The protein source is a key difference between plant and animal-based diets, and it can significantly impact gut microbiota composition (Christudas et al. 2020). For instance, animal proteins may increase bile-tolerant anaerobic bacteria, while plant proteins (e.g., pea proteins) can increase gut-commensal bacteria and decrease pathogenic ones (Rinninella et al. 2019). In PBDs, legumes provide a protein source with a comprehensive amino acid profile similar to animal proteins when combined with other vegetable proteins from cereals. Moreover, research has highlighted their role in lowering heart disease risk and improving various health biomarkers related to lipid and glucose metabolism and brain function (Martini et al. 2021). However, gaps remain in our understanding of the physiological impacts of legumes, particularly during a shift from a meat-rich to a plant-based, legume-rich diet. The interactions of this food with gut microbiome and the bioavailability of their vegetable nutrients are underexplored in vivo. Thus, this PhD thesis aims to explore the impact of a plant-based diet, that substitute animal-origin with plant-based proteins from legumes, on the gut microbiome of individuals prone to lifestyle-induced cardiovascular diseases. The research will investigate the interplay between diet, bioactive plant compounds, and the gut microbiome, and how these factors influence health outcomes.

# **2. PhD Thesis Objectives and Milestones**

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) **Randomized controlled trial (RCT)** targeting high meat eaters with low physical activity (A1.1) will span over a period of 10 weeks. This includes a 2-week run-in and 8-week intervention period with regular anthropometric measurements (A1.2). Dietary and activity level questionnaires (A1.3) will monitor the compliance.

A2) **Biomarker collection and analysis** will be divided into two parts. The first one includes a metabolome and health marker analysis (A2.1) evaluating blood markers related to metabolism, inflammation, brain function, and oxidative stress. The levels of urinary, blood and fecal polyphenols and microbial metabolites associated with positive or negative effects (e.g., urolithins, equol, Trimethylamine N-oxide (TMAO)) will be also assessed. The second part will focus on gut microbiome analysis and data integrations (A2.2).

A3) **In-vitro** **SHIME (Simulator of the Human Intestinal Microbial Ecosystem) experiment** will be divided into two steps. Firstly, the fecal donor (A3.1) will be selected among the study participants. Secondly, the shotgun metagenomics analysis (A3.2) at different treatment times.

A4) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

***Table 2***Gantt diagram for this PhD thesis project.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity  | Months | **01** | **02** | **03** | **04** | **05** | **06** | **07** | **08** | **09** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***Randomized Controlled Trial***  |  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 1) Subject recruitment |  |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 2) RCT phases  |  |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 3) Dietary and activity levels assessment |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | **Biomarker collection and analysis** |  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |   |  |   |   |   |  |  |  |  |
|  | 1) Metabolome and health marker analysis |  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |  |  |  |   |   |  |  |  |  |
|  | 2) Microbiome analysis and data integration |  |   |  |   |  |  |  |  |  |  |  |  |  |  |   |  |   |  |   |   |   |  |  |  |  |
| A3) | ***SHIME experiment*** |  |   |  |   |   |   |   |  |   |   |   |   |   |  |   |  |  |  |  |   |   |   |   |   |  |
|  | 1) Fecal inoculum selection |  |   |  |   |   |   |  |  |   |   |   |   |  |  |   |  |  |  |  |   |   |   |   |  |  |
|  | 2) Shotgun metagenomic analysis  |  |   |  |   |  |  |   |  |   |   |  |  |   |  |   |  |  |  |  |   |   |  |  |   |  |
| A4) | ***Thesis and Paper Preparation*** |  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |   |   |   |   |   |   |

# **3. Selected References**

Christudas S, Devaraj RD, Xu B (2020). Different impacts of plant proteins and animal proteins on human health through altering gut microbiota, *Functional Foods in Health and Disease*. **10**:228–241.

David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ (2014). Diet rapidly and reproducibly alters the human gut microbiome, *Nature*. **505**:559–563.

De Filippis F, Vitaglione P, Cuomo R, Canani RB, Ercolini D (2018). Dietary interventions to modulate the gut microbiome-how far away are we from precision medicine, *Inflammatory Bowel Diseases*. **24**:2142–2154.

Martini D, Godos J, Marventano S, Tieri M, Ghelfi F, Titta L, Lafranconi A, Trigueiro H, Gambera A, Alonzo E, Sciacca S, Buscemi S, Ray S, Galvano F, Del Rio D, Grosso G (2021). Nut and legume consumption and human health: an umbrella review of observational studies, *International Journal of Food Sciences and Nutrition*. **72**:871–878.

Rinninella E, Cintoni M, Raoul P, Lopetuso LR, Scaldaferri F, Pulcini G, Miggiano GAD, Gasbarrini A, Mele MC (2019). Food components and dietary habits: Keys for a healthy gut microbiota composition, *Nutrients*. **11**(10).

Sidhu SRK, Kok CW, Kunasegaran T, Ramadas A. (2023). Effect of Plant-Based Diets on Gut Microbiota: A Systematic Review of Interventional Studies, *Nutrients*. **15**(6).