

UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II



PHD IN FOOD SCIENCE

XXXVIII CYCLE

From legumes to postbiotics: exploring plant-based strategies to modulate the gut microbiome and promote human health

Tutor:

Prof.

Danilo Ercolini

PhD Candidate:

Roberto Marotta

Co-Tutor:

Andrea Luigi Budelli

Coordinator:

Prof.

Amalia Barone 

Academic year 2024-2025

Table of Contents

Table of Contents	2
List of figures	4
List of tables	6
List of abbreviations	7
CHAPTER 1	11
Brief thesis presentation	
1.1 Overview	11
1.2 Aims and outline	13
1.1 Panoramica	15
1.2 Obiettivi e struttura della tesi	17
CHAPTER 2	19
Literature Review	
2.1 Introduction	19
2.2 Microbiota, Dysbiosis and Diseases	23
2.3 Plant-based diet and gut microbiome	25
2.4 Plant proteins, health and the gut microbiome	27
2.5 Pulses: a key protein source in plant-based diets	31
2.6 Influence of pulses on gut microbiome and health outcomes	33
2.7 Traditional fermented legume foods	41
2.8 Fermentation of legumes	44
2.8.1 Impact of fermentation on macronutrient composition and digestibility	45
2.8.2 Techno-functional properties of fermented legume products	47
2.8.3 Sensory quality and consumer acceptability	48
2.8.4 Enhancement of antioxidant capacity, micronutrient bioavailability and bioactive compounds production	49
2.9 Effects of fermented legumes on gut microbiome and host health	50
2.10 Final remarks and future perspectives	61
2.11 References	63

CHAPTER 3	81
Impact of dietary substitution of red meat proteins with pulse proteins on the human gut microbiome and related health outcomes	
3.1	Introduction 81
3.2	Materials and Methods 82
3.3	Results 88
3.4	Discussion 106
3.5	References 110
CHAPTER 4	116
Modulation of the gut microbiome by a postbiotic derived from navy bean flour fermented with <i>Lacticaseibacillus paracasei</i> CBA L74: A SHIME study	
4.1	Introduction 116
4.2	Materials and Methods 117
4.2.1	Laboratory scale experimental procedures 117
4.2.2	Pilot-scale experimental procedures 119
4.2.3	Pre-digestion phase 121
4.2.4	Multi mSHIME experiment 122
4.2.5	DNA extraction and microbiome analysis of mSHIME samples 124
4.2.6	SCFA analysis in mSHIME samples 125
4.2.7	Statistical analysis 125
4.3	Results 125
4.3.1	Impact on gut microbiome and metabolome in a 4-arm mSHIME model 125
4.4	Discussion 131
4.5	References 134
CHAPTER 5	138
General discussion and conclusions	

List of figures

Figure 2.1 Phylogenetic differences between enterotypes.	20
Figure 2.2 Interaction between gut microbiota and host health.	22
Figure 2.3 Impact of western and plant-based diets on gut microbiota composition and related health outcomes.	26
Figure 2.4 The metabolic journey of dietary proteins throughout the gastrointestinal tract.	30
Figure 3.1 Study design.	83
Figure 3.2 Participant flow diagram.	88
Figure 3.3 Baseline distribution of sex and BMI among study participants.	89
Figure 3.4 Energy intake, macronutrient distribution, and vegetable-to-animal protein ratio across the intervention.	90
Figure 3.5 Correlative and longitudinal analyses of gut microbiota in relation to dietary fiber intake.	92
Figure 3.6 Spearman correlation analyses assessing the relationships between gut microbial taxa relative abundance and SCFA concentrations.	94
Figure 3.7 Relative abundance of selected bacterial taxa in the PulD group.	96
Figure 3.8 GMW12 index across dietary interventions and timepoints.	98
Figure 3.9 Principal Coordinates Analysis of gut microbiome profiles across treatment groups and responder status.	99
Figure 3.10 Temporal dynamics of faecal short-chain fatty acids among dietary groups.	101
Figure 3.11 Longitudinal changes in glutarate-pathway gene abundance (RPKM) in response to dietary interventions.	103
Figure 3.12 Completeness and temporal distribution of the glutarate pathway in MAGs.	105
Figure 4.1 PCoA of gut microbiome profiles of samples collected during the SHIME experiment.	127

Figure 4.2 PCoA plots of gut microbiome composition across sampling sites, colonic regions, and experimental conditions.	128
Figure 4.3 Relative abundance of selected bacterial taxa in control and treatment samples.	129
Figure 4.4 SCFA levels in lumen samples across experimental phases, treatment doses, and intestinal regions.	130

List of tables

Table 2.1 Intervention studies investigating the effects of legumes and PBMA consumption on gut microbiome and human health.	34
Table 2.2 In vitro, animal, and human studies investigating the effects of fermented legume products on gut microbiome and host health.	51
Table 3.1 Macronutrient composition of canned pulses provided to participants in the PulD group.	85
Table 3.2 Macronutrient composition of pulse-based plant products included in the PPD dietary intervention.	85
Table 3.3 Spearman correlation analyses between the relative abundance of gut microbial taxa and SCFA concentrations.	93
Table 4.1 Formulation used for laboratory fermentation trials on navy bean flour.	118
Table 4.2 Formulation used for fermentation trials on navy bean flour at pilot scale.	121
Table 4.3 Nutrient composition of raw navy bean flour and navy bean-based postbiotic produced at pilot scale (provided by KH Company).	121
Table 4.4 Number of lumen and mucosa samples collected and analyzed across the entire mSHIME experiment.	124

List of abbreviations

ACE – Angiotensin-Converting Enzyme

ALT – Alanine Aminotransferase

ANFs – Anti-Nutritional Factors

AST – Aspartate Aminotransferase

BAPs – Bioactive Peptides

BCFAs – Branched-Chain Fatty Acids

BMI – Body Mass Index

BSHs – Bile Salt Hydrolases

CAZy – Carbohydrate-Active Enzymes

CD8A – T-cell Surface Glycoprotein CD8 Alpha Chain

CRC – Colorectal Cancer

CVD – Cardiovascular Diseases

DC – Distal Colon

DIAAS – Digestible Indispensable Amino Acid Score

DPP-IV – Dipeptidyl Peptidase-IV

EAA – Essential Amino Acid

EPS – Exopolysaccharides

FAAs – Free Amino Acids

FGF – Fibroblast Growth Factor

FFQ – Food Frequency Questionnaire

FODMAPs – Fermentable Oligo-, Di-, Monosaccharides and Polyols

GMMs – Gut Microbiota-Derived Metabolites

GMWI2 – Gut Microbiome Wellness Index 2

HABD – Habitual Diet

HDL-C – High-Density Lipoprotein Cholesterol

HFD – High-Fat Diet

HFHSD – High-Fat/High-Sucrose Diet

IBD – Inflammatory Bowel Diseases

IBS – Irritable Bowel Syndrome

IHMS – International Human Microbiome Standards

IL10RA – Interleukin 10 Receptor Alpha

IPAQ – International Physical Activity Questionnaire

KBF – Fermented Kidney Bean Broth

KCS – King’s Stool Chart

KH – Kraft Heinz Company

LAB – Lactic Acid Bacteria

LDL-C – Low-Density Lipoprotein Cholesterol

LPC – Lysophosphatidylcholine

LPS – Lipopolysaccharides

MAGs – Metagenome-Assembled Genomes

mSHIME – Mucosal-Simulator of the Human Intestinal Microbial Ecosystem

PBDs – Plant-Based Diets

PBMA – Plant-Based Meat Alternative

PC – Proximal Colon

PCoA – Principal Coordinates Analysis

PDCAAS – Protein Digestibility-Corrected Amino Acid Score

PPD – Plant Protein Diet

PSQI – Pittsburgh Sleep Quality Index

PulD – Pulses Diet

QoL – Quality of Life Questionnaires

RCT – Randomized Controlled Trial

RDA – Recommended Daily Allowance

RPKM – Reads per Kilobase per Million Mapped Reads

SCFAs – Short-Chain Fatty Acids

S-FB – Soybean Fermentation Broth

SHIME – Simulator of Human Intestinal Microbial Ecosystem

ST-SI – Stomach and Small Intestine

TC – Total Cholesterol

TG – Triglycerides

T1D – Type 1 Diabetes

T2D – Type 2 Diabetes

TMA – Trimethylamine

TMAO – Trimethylamine-N-oxide

TNF- α – Tumor Necrosis Factor Alpha

TRACE – Tumor Necrosis Factor-Related Activation-Induced Cytokine

WCB – Working Cell Bank

CHAPTER 1

Brief thesis presentation

1.1 Overview

The human gut microbiome represents a complex ecosystem including of Bacteria, Archaea, Fungi, and viruses, with their collective genetic content and functional potential, that influence host physiology, metabolism, and immune function. This intricate microbial community performs key metabolic activities, including the metabolization of dietary substrates, the production of bioactive compounds, and the modulation of the intestinal and systemic immune responses. Each individual harbours a unique gut microbial composition that act as a distinct fingerprint, and it is widely recognized that alterations in this microbial equilibrium, known as dysbiosis, are linked to the development of several human diseases. The structure and functionality of the gut microbiota are strongly influenced by both genetic and environmental factors, among which diet is regarded as a key determinant. Indeed, long-term dietary patterns have been shown to shape microbial diversity, metabolic activity, and host-microbiome interactions, establishing the fundamental axis linking diet, microbiome, and health.

In the last decade, increasing evidence has highlighted that Westernized dietary habits, typically characterized by high intake of red and processed meat, refined carbohydrates, and saturated fats, are associated with dysbiosis and with an increased risk of several non-communicable diseases, including obesity, type 2 diabetes (T2D), inflammatory bowel disease (IBD), and cardiovascular disorders. Conversely, plant-based and fiber-rich diets, such as the Mediterranean diet, are consistently associated with beneficial microbiome composition, greater short-chain fatty acid (SCFA) production, and improved intestinal barrier function. Within this framework, legumes stand out as a nutritionally dense food group, rich in fermentable fibers, plant proteins, and bioactive compounds. Their regular consumption has been associated with improved lipid and glucose metabolism, reduced systemic inflammation, and beneficial modulation of gut microbiota composition. Beyond their intrinsic nutritional and functional properties, legumes also represent promising substrates for the development of fermented and postbiotic products. Through controlled fermentation process, legume matrices can be transformed into novel functionalized foods with enhanced nutritional value and enriched concentration of beneficial metabolites. These legume-derived fermented and postbiotic preparations are gaining increasing scientific attention as sustainable, plant-based

dietary approaches to beneficially modulate the gut microbiome and promote host health across different physiological and pathological context.

Despite these advances, many challenges remain in establishing causal relationships and mechanistic insights into the diet-microbiome-health axis. Most existing studies focusing on legumes have relied on observational or short-term interventions, often limiting our understanding of how these specific dietary components can sustainably shape microbial ecology and host physiology. Therefore, further research is required to clarify the effect of this dietary modulation on the gut microbiome and to design nutritional strategies capable of fostering a beneficial gut microbial ecosystem and improving health outcomes. In this context, legumes and legume-derived postbiotic products emerge as promising and sustainable dietary approaches to promote host health through targeted modulation of the gut microbiome.

1.2 Aims and outline

The overall aim of this doctoral thesis is to contribute to the understanding of how legume-based dietary strategies and legume-derived postbiotic formulations can modulate the human gut microbiome and promote host health. This general objective was pursued through **i)** the implementation of a randomized controlled trial (RCT) evaluating the effects of an isoproteic and isocaloric dietary intervention, in which red meat proteins were replaced with pulse ones, on the gut microbiome of habitual high red meat consumers; **ii)** the development of a postbiotic formulation obtained from the fermentation of navy bean flour with *Lactocaseibacillus paracasei* CBA L74 and the subsequent assessment of its microbiome-modulating potential through the in vitro dynamic model simulating the human intestinal environment. These objectives were addressed through a research plan articulated into the following chapters.

To provide the theoretical framework for the experimental research, **Chapter 2** presents an extensive literature review addressing the complex interplay between diet, gut microbiome, and human health. The review first discusses the structure and functions of the gut microbial ecosystem, emphasizing the crucial role of diet as a major determinant of microbial diversity and metabolic capacity. Particular attention is given to the impact of Westernized versus plant-based dietary patterns, highlighting how the latter, characterized by high fiber and vegetable protein intake supports microbial eubiosis and SCFA production. The chapter then focuses on legumes as a fundamental component of sustainable dietary patterns, highlighting their nutritional, biochemical, and functional attributes, including fermentable fibers, plant proteins, bioactive compounds, and their suitability for microbial fermentation. Furthermore, the review examines current evidence on fermented and postbiotic products derived from legume matrices, showing how fermentation processes can enhance nutritional quality, generate bioactive metabolites, and open new perspectives for microbiome-targeted nutritional interventions.

Building upon this conceptual foundation, **Chapter 3** presents the results of an 8-weeks randomized controlled trial conducted in subjects with unhealthy lifestyle habits at high risk for cardiovascular diseases. The study investigated the effect of an isocaloric and isoproteic substitution of red meat proteins with proteins derived from whole pulses (PulD group) or from a combination of pulses and pulse-based meat alternatives (PPD group). Dietary adherence, metabolic parameters, gut microbiome composition, function, and SCFA profiles were monitored throughout the intervention. In particular, the RCT aimed to identify gut microbiome

changes responsive to legume intake and to explore potential links between these microbial adaptations and improvements in host health.

Chapter 4 describes the development of a legume-derived postbiotic obtained from the fermentation of navy bean flour by *Lacticaseibacillus paracasei* CBA L74. The impact on the gut microbiome was evaluated by using an in vitro Simulator of Human Intestinal Microbial Ecosystem (SHIME) model. The administration of two different doses (high and low dose) was assessed. This experiment enabled a detailed characterization of the dose-dependent microbial response to the postbiotic product by comparing the highest and lowest administered doses with their respective controls (unfermented navy bean flour). In addition, changes in luminal SCFA production were assessed to further elucidate the functional impact of the nutritional intervention on microbial metabolism.

Finally, **Chapter 5** provides a general discussion integrating the findings from both in vitro and in vivo studies, providing a comprehensive interpretation of the outcomes in the broader context of microbiome-targeted nutrition. The discussion also considers the implications of these results for sustainable dietary design and health promotion and outlines future research perspectives aimed at optimizing legume-based strategies to support gut and metabolic health.

All the studies presented in this thesis were conducted within a multidisciplinary framework and involved collaboration with researchers from diverse scientific backgrounds. My specific contributions across all studies included the evaluation of gut microbiota structure, bioinformatics analyses, and statistical analyses of microbiological and complementary datasets.

CHAPTER 1

Breve presentazione della tesi

1.1 Panoramica

Il microbioma intestinale umano rappresenta un ecosistema complesso costituito da batteri, archea, funghi e virus che, attraverso il loro patrimonio genetico e il loro potenziale funzionale, contribuiscono a regolare la fisiologia, il metabolismo e la funzione immunitaria dell'organismo ospite. Questa intricata comunità microbica svolge un ruolo cruciale in numerose attività metaboliche, tra cui la metabolizzazione dei substrati dietetici, la produzione di composti bioattivi e la modulazione delle risposte immunitarie a livello intestinale e sistemico.

Ogni individuo possiede una composizione microbica unica, assimilabile a una sorta di "impronta digitale", e alterazioni di questo equilibrio, note come disbiosi, sono oggi ampiamente riconosciute come fattori associati allo sviluppo di numerose patologie umane. La struttura e la funzionalità del microbiota intestinale sono fortemente influenzate da fattori genetici ed ambientali, tra i quali la dieta è considerata uno dei determinanti principali. È ormai ampiamente dimostrato che i modelli alimentari seguiti nel lungo periodo influenzano la diversità microbica, l'attività metabolica e le interazioni tra microbioma e ospite, delineando il fondamentale asse che collega dieta, microbioma e salute. Negli ultimi anni, numerosi studi hanno dimostrato come le abitudini alimentari tipiche dei Paesi occidentali, caratterizzate da un elevato consumo di carne rossa e trasformata, carboidrati raffinati e grassi saturi, siano associate a disbiosi e a un aumentato rischio di malattie croniche non trasmissibili, tra cui obesità, diabete di tipo 2 (T2D), malattie infiammatorie intestinali (IBD) e patologie cardiovascolari. Al contrario, i regimi alimentari a base vegetale e ricchi in fibre, come la dieta mediterranea, sono costantemente associati a una composizione microbica più equilibrata, a una maggiore produzione di acidi grassi a catena corta (SCFA) e a un miglioramento della funzionalità della barriera intestinale. In questo contesto, i legumi si distinguono come gruppo alimentare ad alta densità nutrizionale, ricchi di fibre fermentescibili, proteine vegetali e composti bioattivi. Il loro consumo regolare è stato associato a un miglior metabolismo lipidico e glucidico, a una riduzione dell'infiammazione sistemica e a una modulazione benefica della composizione del microbiota intestinale. Oltre alle loro intrinseche proprietà nutrizionali e funzionali, i legumi rappresentano anche substrati promettenti per la produzione di alimenti fermentati e postbiotici. Attraverso processi di fermentazione controllata, le matrici alimentari a base di legumi possono

essere trasformate in nuovi alimenti funzionali, caratterizzati da un migliore valore nutrizionale e una maggiore presenza di metaboliti benefici. Questi prodotti fermentati e postbiotici a base di legumi stanno suscitando un crescente interesse scientifico come soluzioni alimentari sostenibili e di origine vegetale, in grado di modulare positivamente il microbioma intestinale e di promuovere la salute umana in diversi contesti fisiologici e patologici. Nonostante tali progressi, rimangono aperte diverse sfide nel comprendere le relazioni causali e i meccanismi alla base che collegano dieta, microbioma e salute. La maggior parte degli studi condotti sui legumi si basa infatti su approcci osservazionali o interventi di breve durata, limitando la comprensione di come questi specifici componenti alimentari possano modellare in modo duraturo l'ecologia microbica e la fisiologia dell'ospite. È pertanto necessario condurre ulteriori ricerche per chiarire gli effetti di questa modulazione dietetica sul microbioma intestinale e per sviluppare strategie nutrizionali mirate a promuovere un ecosistema microbico equilibrato e a favorire benefici per la salute. In questo scenario, i legumi e i prodotti postbiotici a base di legumi rappresentano soluzioni alimentari sostenibili e promettenti per promuovere la salute dell'ospite attraverso una modulazione mirata del microbioma intestinale.

1.2 Obiettivi e struttura della tesi

L'obiettivo generale di questa tesi di dottorato è contribuire alla comprensione di come strategie dietetiche a base di legumi e formulazioni postbiotiche derivate da legumi possano modulare il microbioma intestinale umano e promuovere la salute dell'ospite. Questo obiettivo generale è stato perseguito attraverso: **i)** la realizzazione di uno studio clinico randomizzato controllato (RCT) volto a valutare gli effetti di un intervento dietetico isoproteico e isocalorico, in cui le proteine della carne rossa sono state sostituite con proteine derivate dai legumi, sul microbioma intestinale di soggetti abituali consumatori di carne rossa; **ii)** lo sviluppo di una formulazione postbiotica ottenuta dalla fermentazione della farina di fagioli bianchi con *Lactocaseibacillus paracasei* CBA L74 e la successiva valutazione del suo potenziale di modulazione del microbioma intestinale mediante un modello dinamico in vitro che simula l'ambiente intestinale umano.

Per fornire il quadro teorico di riferimento alle attività sperimentali, il **Capitolo 2** presenta un'ampia revisione della letteratura scientifica dedicata alla complessa interazione tra dieta, microbioma intestinale e salute umana. La revisione descrive inizialmente la struttura e le funzioni dell'ecosistema microbico intestinale, sottolineando il ruolo cruciale della dieta quale principale determinante della diversità microbica e della capacità metabolica. Particolare attenzione è dedicata al confronto tra i modelli alimentari di tipo occidentale e quelli a base vegetale, evidenziando come questi ultimi, caratterizzati da un elevato apporto di fibre e proteine vegetali, favoriscano l'eubiosi microbica e la produzione di acidi grassi a corta catena (SCFA). Il capitolo si concentra poi sui legumi come componente fondamentale dei modelli alimentari sostenibili, illustrandone le caratteristiche nutrizionali, biochimiche e funzionali, tra cui la presenza di fibre fermentescibili, proteine vegetali, composti bioattivi e la loro idoneità ai processi di fermentazione microbica. Inoltre, viene presentata un'analisi delle evidenze attuali riguardanti i prodotti fermentati e postbiotici a base di legumi, mostrando come i processi fermentativi possano migliorare la qualità nutrizionale, generare metaboliti bioattivi e aprire nuove prospettive per interventi nutrizionali mirati al microbioma.

Sulla base di questo quadro concettuale, il **Capitolo 3** presenta i risultati di uno studio clinico randomizzato controllato della durata di otto settimane, condotto su soggetti con abitudini di vita non salutari e ad alto rischio cardiovascolare. Lo studio ha valutato l'effetto della sostituzione isocalorica e isoproteica delle proteine della carne rossa con proteine provenienti da legumi (gruppo PulD) o da una combinazione di legumi e sostituti vegetali della carne a base

di legumi (gruppo PPD). Durante l'intervento sono stati monitorati l'aderenza dietetica, i parametri metabolici, la composizione e la funzione del microbioma intestinale, nonché i profili degli acidi grassi a corta catena. In particolare, lo studio aveva l'obiettivo di identificare le modificazioni del microbioma intestinale indotte dal consumo di legumi e ad esplorare i potenziali collegamenti tra tali adattamenti microbici e gli effetti benefici sulla salute dell'ospite.

Il **Capitolo 4** descrive lo sviluppo di un postbiotico derivato da legumi ottenuto mediante la fermentazione di farina di fagioli bianchi con *Lacticaseibacillus paracasei* CBA L74. L'impatto sul microbioma intestinale è stato valutato utilizzando il modello in vitro SHIME (Simulator of Human Intestinal Microbial Ecosystem). Sono state testate due diverse dosi (alta e bassa), consentendo una caratterizzazione dettagliata della risposta microbica dose-dipendente al prodotto postbiotico, attraverso il confronto con i rispettivi controlli (farina di fagioli bianchi non fermentata). Inoltre, è stata analizzata la produzione di SCFA a livello del lume intestinale per approfondire l'impatto funzionale dell'intervento nutrizionale sul metabolismo microbico.

Infine, il **Capitolo 5** presenta una discussione generale che integra i risultati ottenuti negli studi in vitro e in vivo, fornendo un'interpretazione complessiva degli esiti nel più ampio contesto della nutrizione mirata al microbioma. La discussione affronta inoltre le implicazioni di tali risultati per la progettazione di modelli alimentari sostenibili e per la promozione della salute, con uno sguardo alle prospettive di ricerca future volte a valorizzare il ruolo dei legumi nella salute intestinale e metabolica.

Tutti gli studi presentati in questa tesi sono stati condotti in un contesto multidisciplinare e in collaborazione con ricercatori appartenenti a diversi ambiti scientifici. Il mio contributo specifico, comune a tutte le fasi della ricerca, ha riguardato la valutazione della struttura del microbiota intestinale, le analisi bioinformatiche e le analisi statistiche dei dati microbiologici e dei dataset complementari.

CHAPTER 2

Literature Review

CHAPTER 3

Impact of dietary substitution of red meat proteins with pulse proteins on the human gut microbiome and related health outcomes

CHAPTER 4

Modulation of the gut microbiome by a postbiotic derived from navy bean flour fermented with *Lacticaseibacillus paracasei* CBA L74: A SHIME study

CHAPTER 5

General discussion and conclusions

Over the past decade, the human gut microbiome has emerged as central regulator of host physiology, influencing metabolism, immune function, and disease susceptibility (Bull and Plummer 2014). Perturbation of its ecological balance, a condition commonly referred to as dysbiosis, have been extensively associated with a broad spectrum of chronic diseases, including metabolic and inflammatory disorders (Shen et al. 2025). Consequently, the modulation of the gut microbiome through diet-based strategies has gained remarkable attention as a promising avenue for health promotion and disease prevention (De Filippis et al. 2018). In this context, evidence-based nutritional interventions, particularly randomized controlled trials targeting diet-microbiome interactions, are essential to elucidate the casual links between specific dietary components, microbial functions, and host health outcomes (Diacova et al. 2025). Reaching this objective requires a multidisciplinary approach integrating nutritional sciences, microbiology, biology, with ultimate goal of translating microbiome research into effective, personalized, and sustainable dietary applications.

Within this framework, the present doctoral thesis explored the potential of legume-based dietary strategies and legume-derive postbiotic formulations to beneficially modulate the gut microbiome and support host health. Together, these studies advance our understanding of how plant-based proteins and bioactive compounds from legumes, either directly consumed or subjected to food technological processing and microbial fermentation, can shape gut microbiome ecology and metabolic activity. Importantly, both *in vivo* and *in vitro* investigations converged in showing that legume-derived substrates foster a health-associated microbial profile, characterized by the enrichment of SCFA producers and the reduction of potentially detrimental taxa.

In particular, the results from the *in vivo* randomized controlled trial (**Chapter 3**), conducted in high red meat consumers, revealed that the isocaloric and isoproteic replacement of red meat with pulse-derived proteins resulted in a significant reshaping of the gut microbial community. This dietary shift promoted the enrichment of beneficial microbial taxa and the reduction of mucin-degrading and pro-inflammatory species, thereby suggesting improved gut barrier functions and microbial metabolism. Although fecal SCFA concentration did not significantly change, the concurrent microbial and functional trends pointed toward increased SCFA utilization and epithelial absorption, consistent with a more efficient host-microbiome symbiosis.

Complementary insights were provided by the in vitro study conducted using the SHIME model, which evaluated a legume-derived postbiotic obtained from the fermentation of navy bean flour with *Lc. paracasei* CBA L74, as described in **Chapter 4**. This study demonstrated a dose-dependent modulation of the gut microbiome, driven by the selective enrichment of butyrate-producing bacteria associated with gut and metabolic health. The persistence of several microbial effects beyond the intervention period further supports the potential of legume-based postbiotic supplementation to promote stable and beneficial changes in gut microbiome composition. To our knowledge, this was the first study to show such effects using a plant-based postbiotic formulation, highlighting the promising application of legume matrices as sustainable sources of novel functional foods.

Altogether, the evidence gathered throughout this thesis contributes to advancing the concept that targeted legume-based nutritional strategies can beneficially modulate the gut microbiome and contribute to metabolic health. However, despite these encouraging results, several challenges remain. The complexity and interindividual variability of gut microbiome responses make it difficult to establish direct causal relationships between specific dietary components and clinical outcomes. Therefore, future investigations should aim to conduct longer-term and larger-scale interventions to elucidate the mechanisms underlying host–microbe interactions and to validate the clinical relevance of the observed microbial shifts. Furthermore, integrating metagenomic, metabolomic, and immunologic data will be crucial to identify microbial and metabolic signatures predictive of individual responsiveness to legume-based diets and postbiotic treatments.

The stratification of individuals into responsive phenotypes or metabolic profiles may pave the way for the development of ad hoc personalized nutrition approaches, where dietary interventions are tailored according to the baseline microbiome configuration and metabolic profile (Zeevi et al. 2015). Such an approach aligns with the emerging paradigm of precision nutrition, which positions diet not only as a means of nourishment but as a tool for modulating the microbiome to prevent or manage disease (Mills et al. 2019). In this perspective, legume-based foods and postbiotic formulations represent promising, safe, and sustainable strategies for microbiome-centered health promotion.

In conclusion, this doctoral work contributes to the growing body of evidence supporting diet as a powerful determinant of gut microbial balance and human health. As Hippocrates suggested more than two millennia ago, “*Let food be thy medicine, and medicine be thy food*”. The findings presented herein underscore that the thoughtful use of food, particularly plant-based sources such as legumes, can serve as a rational and effective mean to promote host well-being through the modulation of our microbial ecosystem.

References

- Bull, Matthew J., and Nigel T. Plummer. 2014. "Part 1: The Human Gut Microbiome in Health and Disease." *Integrative Medicine (Encinitas, Calif.)* 13 (6): 17–22.
- De Filippis, Francesca, Paola Vitaglione, Rosario Cuomo, Roberto Berni Canani, and Danilo Ercolini. 2018. "Dietary Interventions to Modulate the Gut Microbiome-How Far Away Are We from Precision Medicine." *Inflammatory Bowel Diseases* 24 (10): 2142–54.
- Diacova, Tatiana, Christopher J. Cifelli, Cindy D. Davis, Hannah D. Holscher, Mary E. Kable, Johanna W. Lampe, Marie E. Latulippe, Kelly S. Swanson, and J. Philip Karl. 2025. "Best Practices and Considerations for Conducting Research on Diet-Gut Microbiome Interactions and Their Impact on Health in Adult Populations: An Umbrella Review." *Advances in Nutrition (Bethesda, Md.)* 16 (5): 100419.
- Mills, Susan, Catherine Stanton, Jonathan A. Lane, Graeme J. Smith, and R. Paul Ross. 2019. "Precision Nutrition and the Microbiome, Part I: Current State of the Science." *Nutrients* 11 (4): 923.
- Shen, Yao, Nairui Fan, Shu-Xia Ma, Xin Cheng, Xuesong Yang, and Guang Wang. 2025. "Gut Microbiota Dysbiosis: Pathogenesis, Diseases, Prevention, and Therapy." *MedComm* 6 (5): e70168.
- Zeevi, David, Tal Korem, Niv Zmora, David Israeli, Daphna Rothschild, Adina Weinberger, and Orly Ben-Yacov, et al. 2015. "Personalized Nutrition by Prediction of Glycemic Responses." *Cell* 163 (5): 1079–94.