Protein quality in the New Zealand vegan diet:

Challenges and solutions

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Abstract

The global shift toward plant-based dietary patterns has intensified interest in the nutritional adequacy of vegan diets, particularly regarding protein quality. Plant proteins are often considered poorer sources of several indispensable amino acids (IAAs), which are essential for metabolic function in the human body, and must be obtained from the diet. The lower digestibility and less balanced amino acid profiles of many plant-based foods compared to animal-sourced foods raise concerns regarding the adequacy of utilisable protein in individuals consuming fully plant-based diets.

Although total protein intake in vegan populations is frequently reported to be sufficient, protein intake and protein quality are rarely assessed using digestibility-adjusted measures. Consequently, uncertainty remains regarding the capacity of vegan diets to meet protein requirements. Overall, this thesis aimed to 1) evaluate protein intake and protein quality in habitual vegan diets in New Zealand (NZ), and 2) develop practical and data-driven strategies to improve protein and IAA adequacy within realistic dietary constraints.

Baseline protein intake and protein quality were assessed with four-day food diaries collected from 193 adult vegans in NZ. Protein content of each food was derived from the NZ FOODFiles™ food composition database, while IAA content for each food was obtained from the most appropriately matched food in the USDA food composition database. To reflect the portion of food that provides utilisable protein and IAA, the true-ileal digestibility coefficients for available foods and available IAA were applied. Although total protein intake met minimum daily requirements for most participants, lysine and leucine were identified as the most limiting IAAs, with nearly half of the cohort at risk of inadequate intake when digestibility was considered.

To further characterise dietary patterns, time-series and clustering analyses were applied to examine meal-level protein intake. Uneven protein distribution was observed across the day, with the emergence of distinct dietary clusters stratified by protein quantity and protein quality. Further meal composition analysis was undertaken to examine how the combination of different plant food groups influences protein quality, as measured by the Digestible Indispensable Amino Acid Score (DIAAS). Eight distinct meal clusters with varying protein quality were observed.

Overall, the baseline diets of vegans indicate risks of deficiency in protein quality, particularly for lysine, leucine and the sulphur-amino acids. Dietary interventions will require the use of

diverse plant foods and ingredients in complementary proportions to meet individual IAA requirements, while balancing energy consumption and dietary acceptability.

These findings informed the development of diet optimisation frameworks designed to explore food combinations at both the daily and meal level to address individual shortfalls in protein and IAA intake. Most plant foods contain all IAAs in varied amounts. However, legumes and pulses (including legume-based protein isolates), nuts and seeds were particularly important contributors to protein quality, with many foods from these groups providing higher IAAs at a lower caloric cost than other food groups, such as grain-based foods, fruits and starchy vegetables. When plant protein sources were combined to achieve high protein quality, as assessed by the DIAAS, the optimisation models prove that vegan diets can theoretically meet protein quality requirements without necessitating excessive food intake or energy consumption, thus countering the assumption that plant-based diets are intrinsically limited in protein quality.

Dietary patterns influencing protein quality may also affect the overall nutritional adequacy of vegan diets. Considering that some micronutrient requirements may be even more difficult to achieve in vegan diets without the use of fortified foods and supplementation, future research in plant-based diets should examine strategies that jointly optimise protein quality and micronutrient adequacy.

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Abbreviations

AA	Amino Acid
AAA	Aromatic Amino Acid
ALA	Alpha-Linolenic Acid
AI	Adequate Intake
AMDR	Acceptable Macronutrient Distribution Range
ANF	Anti-Nutritional Factor
ASF	Animal-Sourced Food
ASP	Animal-Sourced Protein
BCAA	Branched-Chain Amino Acid
BMR	Basal Metabolic Rate
DAA	Dispensable Amino Acid
DGA	Dietary Guidelines of America
DHA	Docosahexaenoic Acid
DIAAS	Digestible Indispensable Amino Acid Score
DTW	Dynamic Time Warping
EAR	Estimated Average Requirement
EER	Estimated Energy Requirement
EO	Eating occasion
EPA	Eicosapentaenoic acid
EPIC	European Prospective Investigation into Cancer and Nutrition
FAO	Food and Agriculture Organisation of the United States
FBDG	Food-Based Dietary Guidelines
FG	Food Group
GHGE	Greenhouse Gas Emissions
GIT	Gastrointestinal Tract
HC	Hierarchical Clustering
HEI	Healthy Eating Index
IAA	Indispensable Amino Acid
IAAO	Indicator Amino Acid Oxidation
IF	Intermittent Fasting
IPAQ	International Physical Activity Questionnaire
IQR	Interquartile Range

KW	Kruskal-Wallis
LNS	Legumes, nuts and seeds
LOAEL	Lowest-Observed-Adverse-Effect Level
LP	Linear Programming
MDS	Multidimensional Scaling
MET	Metabolic Equivalent Task
MPB	Muscle Protein Breakdown
MPS	Muscle Protein Synthesis
mTORC1	Mammalian Target of Rapamycin Complex 1
NB	Nitrogen Balance
NHANES	National Health and Nutrition Examination Survey
NOAEL	No-Observed-Adverse-Effect-Level
NRF	Nutrient Rich Food Index
NRV	Nutrient Reference Value
NZ	New Zealand
PA	Physical Activity
PAL	Physical Activity Level
PB	Plant-Based
PBA	Plant-Based Alternative
PBB	Plant-Based Beverage
PBMA	Plant-Based Meat Alternative
PC	Principal Component
PCA	Principal Component Analysis
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PSF	Plant-Sourced Food
PSP	Plant-Sourced Protein
RCT	Randomised Controlled Trial
RDA	Recommended Dietary Allowance
RDI	Recommended Dietary Intake
SD	Standard Deviation
SFA	Saturated Fatty Acid
SSB	Sugar-Sweetened Beverage
TID	True Ileal Digestibility

TSAA	Total Sulphur Amino Acid
UPF	Ultra-Processed Food
USDA	United States Department of Agriculture
VPQ	Vegan Protein Quality
WHO	World Health Organisation

Publications, awards and conferences

Publications

1. Soh, B. X. P., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2023). Evaluation of protein adequacy from plant-based dietary scenarios in simulation studies: A narrative review. *The Journal of Nutrition*, *154*(2), 300-313.https://doi.org/10.1016/j.tjnut.2023.11.018

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7. Soh, B. X. P., Vignes, M., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2026). Development of the Vegan Protein Quality (VPQ) tool to derive smarter vegan meals with high protein quality. [Under revision in *Scientific Reports*]

Awards

1. Third-place student animation at the Riddet Institute Colloquium in 2023
2. Second-place student oral presentation at the Nutrition Society NZ conference in 2023
3. Second-place student oral presentation at the Riddet Institute Colloquium in 2025

Conference Presentations

1. Soh, B. X. P., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2023). Poster presentation titled, *Determining Protein Adequacy in New Zealand vegans* at The Riddet Institute Conference and Student Colloquium, Queenstown, NZ

2. Soh, B. X. P., Vignes, M., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2024*).* Oral Presentation titled, *Unsupervised machine learning to determine dietary protein distribution among New Zealand vegans across time periods of the day* at the Nutrition Society Congress, Belfast, Northern Ireland https://doi.org/10.1017/S0029665124005731

3. Soh, B. X. P., Vignes, M., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2024). Oral presentation titled, *Assessment of protein intake and protein quality among New Zealand vegans* at The Nutrition Society of New Zealand, Christchurch, NZ https://doi.org/10.1017/S0029665125100359

4. Soh, B. X. P., Vignes, M., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2024) 3-minute thesis titled, *Breaking the wall of unbalanced vegan diets* at Falling Walls, Wellington, NZ

5. Soh, B. X. P., Vignes, M., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2024) 3-minute thesis titled, *Protein intake and protein distribution within a cohort of New Zealand vegans* at The Riddet Institute Conference and Student Colloquium, Palmerston North, NZ

6. Soh, B. X. P., Vignes, M., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2025). Oral presentation titled, *Evaluating protein intake and quality in New Zealand vegans: temporal patterns and dietary clusters* at The International Congress of Nutrition, Paris, NZ

7. Soh, B. X. P., Vignes, M., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2025). Oral presentation titled, Improving protein quality in the daily diets of New Zealand vegans, at The Riddet Institute Conference and Student Colloquium, Wellington, NZ

8. Soh, B. X. P., Vignes, M., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2025). Oral presentation titled, *Going plant-based? Motivations, Nutrition, Innovation* at the Plant-Based Food Ingredients workshop, Bioeconomy Science Institute, Palmerston North, NZ

Chapter 1 General introduction

This Chapter provides an introduction and justification for this thesis, including its overall aim and specific objectives. The Chapter concludes with an outline of the thesis structure.

1.1 Plant-based dietary shifts and nutritional considerations

What humans are currently eating and how the human diet is expected to evolve have become central concerns in global health, environmental sustainability, and public debate. This growing attention is largely driven by the increasing challenge of successfully nourishing the world's expanding population, of up to 10 billion people by 2050, with nutrient-adequate diets that promote health (1, 2). At the same time, human diets need to remain within the planetary boundaries and satisfy global commitments such as the Sustainable Development Goals (3, 4).

Within this context, plant-based (PB) diets have emerged as one potential strategy to mitigate the health and environmental burdens associated with current food systems (5, 6). PB diets are predominantly rich in vegetables, fruits, legumes, grains, nuts, seeds, herbs and spices, and may include small to moderate quantities of animal-sourced foods (ASFs). Among some consumers, ethical concerns about animal well-being in modern food systems motivate vegan diet adoption (7). A vegan diet is the most restrictive form of a PB diet because it omits all foods derived from or produced by animals. All meat, poultry, seafood, fish, dairy and bee products (honey, bee pollen) are strictly excluded. Distinguishing between different PB dietary patterns is essential when evaluating nutritional adequacy because these diets differ in the degree of ASFs inclusion. Vegan diets generally present greater challenges in meeting requirements for protein quality and several micronutrients than less restrictive PB diets (e.g., vegetarian where dairy and eggs are included).

In terms of health benefits, a PB diet appears to confer some protection against diabetes (35 to 47 % lower than meat-eaters), ischemic heart disease (22 % lower than meat-eaters) and some cancers (10 to 18 % lower than meat-eaters), but much higher risks of hip fractures (up to 50%) as compared to meat-eaters (8). While PB diets may confer some environmental benefits, such as reductions in greenhouse gas emissions (GHGEs), land, water and energy use as compared to diets containing ASFs, these benefits are not uniform across all foods, with several trade-offs emerging depending on the metric used (9-11). For example, adjustments for energy and nutrient bioavailability could change the relative environmental impact of foods, and narrow the discrepancy between PB foods and ASFs (12, 13).

Perhaps the most influential contribution to the promotion of PB diet adoption came from the first EAT-*Lancet* Commission report in 2019 (14) and its updated version in 2025, with the proposed Planetary Health Diet - a predominantly PB diet allocating 12 to 13 % of daily caloric consumption from ASFs (3). Given that PB foods provide reduced quantities of bioavailable micronutrients, concerns have been raised about nutritional adequacy when ASFs are reduced or excluded (1, 15, 16). Consequently, the EAT-*Lancet* Commission report and the PHD were criticised for overlooking micronutrient deficiencies, especially in vulnerable populations (2). Nutrients such as vitamin B12, vitamin D, calcium, iron, zinc, iodine, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and the indispensable amino acids (IAAs) are recognised as being at higher risk of inadequacy in PB diets (8, 17). By eliminating all forms of ASFs, achieving adequacy for these highlighted nutrients is a further challenge in vegan diets. Nutritional literacy, practical experience, and strategic food selection, supported by health professionals, are strategies that enable vegans to follow a nutrient adequate and health-promoting diet (18).

Past reviews noted an increased popularity of PB diets among consumers, particularly in higher-income countries (19). This is also reflected in the market growth of novel PB alternatives (PBAs), especially for PB yoghurt, sausages, burger patties and cheese alternatives (11). Although unofficial surveys indicated a growing interest in PB and vegan diets in NZ, nationally representative data indicated that the prevalence of self-identified vegans remained low. In 2021, 1.1 % in a sample of 47,000 NZ adults reported following a vegan diet (20). In a more recent assessment of 23,292 adults, the prevalence was only 0.74 % (21).

The number of vegans may however increase in future alongside public interest in sustainability. It remains essential to determine if such restrictive diets can reliably meet the nutrient requirements of all population groups, including those considered more vulnerable, amid the increasing discourse on the animal-to-plant protein transition. Research on vegan diets must not only highlight the benefits of PB foods but also address common misconceptions and the risks of nutrient deficiencies if these diets are inappropriately planned. Nutrition information is frequently disseminated through informal channels and can sometimes be misleading. Hence, evidence-based studies are essential to inform public health recommendations so that individuals adopting vegan diets can do so without compromising their nutrition.

1.2 Protein quality in vegan diets

Dietary protein supplies the nine IAAs (histidine, leucine, lysine, isoleucine, methionine, phenylalanine, threonine, tryptophan, valine) - which play important metabolic roles but cannot be synthesised by the body - to meet physiological requirements such as muscle protein synthesis (MPS) (22-24). Accordingly, the concept of protein quality is a measure of how effectively a dietary protein provides bioavailable or utilisable IAAs and nitrogen needed for growth and maintenance (25, 26). Plant proteins are often considered to have lower protein quality than ASFs as they tend to be less digestible, and their IAA profiles align less optimally with human requirements (27). To get a more accurate assessment of protein adequacy, it is crucial to account for the proportion of protein that is utilisable to the body, especially in predominantly PB diets (26), and comparing the utilisable intake to the relevant protein reference values – the Estimated Average Requirement (EAR) of 0.66 g/kg/day, which is estimated to meet the requirements of 50 % of the population or the Recommended Dietary Intake (RDI) of 0.83 g/kg/day, which is estimated to meet the requirements of 98 % of the population (25).

Total protein intake is generally not a major concern among vegans in higher-income regions, although most estimates were based on crude, rather than digestibility-adjusted protein. For example, two large prospective studies with substantial representation of western PB consumers - the European Prospective Investigation into Cancer and Nutrition (EPIC) -– Oxford and the Adventist Health Study - found protein intake to be at approximately 13 % of total energy intake, which is comfortably above the lower boundary of the Acceptable Macronutrient Distribution Range (AMDR) for protein (10 %) (28).

Systematic reviews which examined nutrient intake in vegan populations also found protein intake to be within the AMDR range, albeit lower than that of meat consumers (16, 17). Beans, peas, lentils, soy products, nuts, seeds and whole grains are major protein sources for vegans (29). When these foods are consumed within a diverse and energy-adequate diet, total protein intake is usually not limiting (30). However, many plant foods have relatively higher energy-to-total protein ratios (26), meaning that choosing lower-quality plant protein sources necessitates greater energy intake or larger serving sizes (22). This has challenging implications for populations with higher protein-to-energy requirements (e.g., sedentary older adults) (31), unless careful food selection and diet quality are considered.

Instances of inadequate protein intake have been documented in subgroups of vegan populations (32, 33), showing that adequacy is not guaranteed for all individuals. Protein intake is potentially overestimated if digestibility assessments are not applied. Modelling studies show that when protein is adjusted for digestibility, utilisable protein intake falls below the Recommended Dietary Allowance (RDA), especially in diets of poorer protein quality (26).

Certain IAAs, such as methionine, lysine, leucine, tryptophan and tyrosine, were observed to be more limiting than other amino acids (AAs) in the diet and plasma concentrations of long-term vegans (34, 35). Diets composed exclusively of PB proteins are constrained by suboptimal IAA profiles and lower digestibility, potentially impairing net protein balance in the body. To minimise compensatory increases in muscle protein breakdown (MPB) required to maintain plasma IAA concentrations, dietary protein must therefore provide adequate quantities of these nutrients to support protein synthesis and preserve whole-body protein balance (24). This highlights opportunities to identify effective combinations and quantities of PB foods to ensure that the intake of all IAAs meets the body's physiological requirements.

1.3 Scope of the thesis

Given the available evidence, it remains unclear if vegan diets can adequately meet protein quality requirements. If this represents a potential nutritional challenge, then strategies must be developed to support consumers who choose a vegan diet, so that the positive health outcomes associated with this dietary pattern can be realised without compromising protein or IAA adequacy. This thesis first aims to evaluate the protein adequacy and protein quality within the context of the modern-day vegan diet by leveraging the dietary intake data from a NZ vegan cohort. Establishing the presence and extent of inadequacies is a necessary step before implementing strategies to improve the protein quality in the diet of the target population.

The research portion of this thesis is presented in two major parts. Part I describes the protein adequacy and protein quality of vegan diets in NZ. Part II focuses on solution-driven approaches to optimise baseline meals and daily diets of NZ vegans to achieve protein adequacy and protein quality. The specific aims of each Chapter are provided below:

Part I. Investigating the intake of total protein and protein quality in current NZ vegan diets

Chapter	Overall aims
3	• Assess how the daily intake of protein and IAA among NZ vegans compares to daily protein and IAA requirements. • Explore how the vegan dietary pattern, in terms of food group (FG) contribution, impacts protein intake and protein quality
4	• Evaluate the temporal variation in total protein and IAA distribution across eating occasions over 24 hours • Assess and classify distinct protein and IAA consumption patterns with unsupervised data analytics • Investigate FG combinations that emerged from the categorised dietary patterns

Part II. Deriving solutions to improve protein intake and protein quality of vegan diets

Chapter	Overall aim
5	• Generate dietary modifications based on each individual's existing food intake, to rectify daily shortfalls in total protein and IAA intakes • Quantify the deviation of food intake and the contribution to total protein and IAA between baseline and modified diets across dietary clusters
6	• Evaluate the ideal proportions of PB food sources needed to derive meals of higher protein quality • Quantify the association between meal-level protein quality and daily protein adequacy by assessing the probability that meals with higher protein quality are found in clusters nearing daily targets
7	• Formulate meal pairings of varied PB foods that, when combined, provide high protein quality • Integration of these meal combinations into an interactive digital tool, the Vegan Protein Quality (VPQ) tool, to optimise for the ideal quantity of each food within a meal to meet user-specified protein, energy and IAA requirements

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certifythat all co-authors have consented to their work being included in the thesis and they have accepted the student'scontribution as indicated below in the Statement of Originality.

Student name:	Bi Xue Patricia Soh
Name and title of main supervisor:	Professor Warren C. McNabb
In which chapter is the manuscript/published work?	Chapter 2

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Bi Xue Patricia Soh conducted the literature review, analysed the results and wrote the manuscript. All supervisors, Professor Warren C. McNabb, Dr Nick W Smith and Professor Pamela von Hurst reviewed the manuscript and provided feedback.

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DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certifythat all co-authors have consented to their work being included in the thesis and they have accepted the student'scontribution as indicated below in the Statement of Originality.

Student name:	Bi Xue Patricia Soh
Name and title of main supervisor:	Professor Warren C. McNabb
In which chapter is the manuscript/published work?	Chapter 2

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Bi Xue Patricia Soh conducted the literature review, analysed the results and wrote the manuscript. All supervisors, Professor Warren C. McNabb, Dr Nick W Smith and Professor Pamela von Hurst reviewed the manuscript and provided feedback.

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Soh, B.X.P., Smith, N.W., von Hurst, P. R., & McNabb, W. C. (2024). Evaluation of protein adequacy from plant-based dietary scenarios in simulation studies: a narrative review. The Journal of Nutrition, 154(2), 300-313.

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Chapter 2 Literature Review

This first section of the Chapter presents an overview of the current literature on the key considerations that arise when protein is supplied exclusively from PB foods. The Chapter continues in the second section, with a review of evidence from diet modelling studies evaluating the effects of substituting animal-sourced proteins with plant-sourced proteins on total protein intake and protein quality.

The following Chapter contains material from published manuscripts with the full references below:

1. Soh, B. X. P., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2025). Achieving high protein quality is a challenge in vegan diets: A narrative review. Nutrition Reviews, *83*(7), e2063-e2081. https://doi.org/10.1093/nutrit/nuae176

2. Soh, B. X. P., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2024). Evaluation of protein adequacy from plant-based dietary scenarios in simulation studies: A narrative review. The Journal of Nutrition, *154*(2), 300-313. https://doi.org/10.1016/j.tjnut.2023.11.018

At the time of writing this thesis, a search for new studies was conducted, and the literature review has been updated to include relevant studies that were more recently published.

Abstract

A PB diet can confer nutritional and health benefits while being a plausible solution to promote environmental sustainability. However, unbalanced and restrictive PB diets, including vegan diets, may incur nutritional ramifications that warrant close examination. One such concern is the adequacy and bioavailability of IAAs from plant-sourced foods (PSFs) and the consequences on certain populations with elevated IAA requirements. Protein intake from these diets is widely discussed, but comparisons of animal and plant proteins often disregard IAA composition and digestibility as measures of protein quality.

Section I: Complexities and challenges in achieving high protein quality in vegan diets

The first part of this literature review aims to address the complexities and challenges posed by varied plant protein sources in achieving protein adequacy and quality. This review contributes to the emerging discussions of nutritional risks associated with vegan diets and adds perspective to the current dietary shifts towards PB diets. This section also outlines why the inclusion of novel PBAs introduces uncertainty in achieving protein adequacy. Meal distribution patterns of protein and the ensuing physiological impacts in certain populations, such as older adults, warrant further research and are highlighted in this review.

Section II: Protein adequacy of PB dietary scenarios

By leveraging dietary data from varied population groups, modelling studies provide a time-efficient and more ethically appropriate means of investigating the nutritional implications of the diet transition from animal- to PB protein sources. Studies typically adopt substitution frameworks by progressively replacing ASFs within existing dietary patterns with varying quantities and types of PSFs. The second part of this literature review thus aims to summarise the approaches to modelling protein intake and protein quality when ASFs were replaced with PB foods in diet simulations, and to examine how these factors vary across different population groups.

A total of 31 studies using dietary models to predict protein contribution from plant proteins were consolidated and categorised into the following themes: assessment of protein intake, assessment of protein quality and diet quality, the use of novel PBAs, and assessment of protein adequacy in special populations.

While protein intake from the PB diet simulations was lower than that from omnivorous diets, it still met country-specific nutrient requirements. However, protein adequacy from some PB foods was not met for simulated diets of children and older adults. Reduced AA adequacy was also observed with increasing PB food intake in some scenarios. Protein adequacy was

generally dependent on the choice of food substitution. Legumes, nuts and seeds (LNS) provided greater protein intake and quality than cereals or grain foods.

Summary

Taken together, both parts of the literature review consolidated the key challenges and observed results associated with transitioning towards an increasingly PB diet, as shown across human studies and dietary simulations. These insights highlight the need for careful dietary planning and continued innovation in food formulation to ensure adequate, high-quality protein in PB diets, especially in the more restrictive vegan diet.

2.1 Introduction

Due to the exclusion of all ASFs (1-3), micronutrient deficiencies are well-recognised nutrition problems in vegan diets (1, 4-6), particularly in more vulnerable population groups - infants, children, adolescents, pregnant and lactating females, and the elderly (7, 8). The adequacy of utilisable protein in these diets also remains insufficiently explored. While gross protein intake in high-income western countries often meets and/or exceeds daily requirements, this may be attributed to the predominantly omnivorous dietary patterns, and utilisable protein intake may fall below daily requirements in a completely PB diet (9). Protein adequacy in vegan populations thus requires detailed research, and this analysis must consider protein quality and total protein intake.

Dietary proteins provide AAs that perform structural, physiological and regulatory roles in the human body (10, 11). Of importance are the nine IAAs that cannot be endogenously synthesised and must be provided by food intake (12). Dietary choice is an important factor in achieving adequate overall protein intake and IAA adequacy to meet metabolic needs. However, foods differ in the distribution of fully utilisable AAs to meet each individual's unique metabolic requirements. This is understood as the protein quality aspect of food, which varies among sources (10), with notable differences between plant and animal-derived foods (13). While PSFs provide all the IAAs, the overall quality is generally less favourable as compared to ASFs due to factors such as poorer IAA composition compared to bodily requirement, reduced digestion, delivery, and bioavailability of IAAs to peripheral tissues – issues related to the inherent structural properties of plant proteins and the overall food matrix (10, 14, 15).

Given the described limitations in the protein quality of PB foods, the global interest in the shift towards PB diets warrants careful consideration as IAA inadequacy to body tissues leads to nutritional and health risks, especially during certain stages of the life cycle – pregnancy, lactation, infancy, adolescence and ageing (16, 17). Ensuring adequate and balanced consumption of all IAAs through a diet consisting solely of PSFs is challenging, though not impossible. Importantly, the strategic inclusion of varied and complementary protein sources is critical to achieve optimal intake of all utilisable AAs while maintaining a moderate food volume and caloric intake (18). This often depends on sufficient diet planning, consumer knowledge and intention. Support from the food industry to help vegan consumers effectively meet protein quality requirements could be beneficial. Against this backdrop, more research is needed on the nutritional adequacy and long-term health impacts of novel PB meat and dairy alternatives, particularly if these foods are used as direct replacements for ASFs or traditional PB staples. The impact of these substitutions on protein quality will depend critically on AA composition and digestibility of the replacement foods.

In **section I** of the literature review, the aim was to consolidate findings from relevant scientific publications to identify the necessary considerations for achieving high protein quality in modern-day vegan diets. Empirical evidence provided from human studies was analysed to identify the nutritional and physiological challenges associated with vegan diets, focusing on the following aspects: 1) why plant foods contribute poorer protein quality, 2) the implications of uneven protein consumption throughout the day, 3) current findings of protein quality in novel PSFs and 4) the problems of a vegan diet in special populations, specifically, the older adults.

Due to ethical, logistic and financial constraints with randomised-controlled trials (RCTs) or long-term cohort studies on the more restrictive PB diets, simulation studies using computational modelling have become reliable alternatives for predicting the nutritional impacts of shifting towards PB diets (19, 20). Computational models allow controlled manipulation of dietary variables to assess nutrient adequacy. This methodological approach informs subsequent research chapters, in which modelling is considered a strategy for assessing protein quality and examining potential dietary modifications in vegan diets.

While there have been several systematic reviews conducted on the outcomes of PB diets in human studies (21, 22), there are no reviews outlining the hypothesised outcomes of protein adequacy in PB dietary models. Therefore, **section II** of the literature review examined how protein intake and protein quality are affected in diet simulations when ASFs are replaced, either partially (graded substitution) or completely (full replacement consistent with vegan diets), with PB foods. While the literature in Chapter 2 includes studies of vegetarian and other PB diets to provide broader context and evidence, the empirical evidence generated in this thesis (from Chapters 3 to 7) is derived solely from a vegan cohort residing in NZ. Preserving this distinction ensures that the interpretations and implications of the findings remain specific to the dietary population from which the evidence is obtained.

2.2 Methods

2.2.1 Overview of the review process

Relevant articles were searched through recognised electronic databases – PubMed, Scopus, Google Scholar and Connected Papers. To attain saturation, additional references were identified through a manual search of the reference lists of the selected articles, and duplicates were removed. The search strategy was informed by keywords from past systematic and narrative reviews (23-26). **Table 2.1** lists the search terms used.

Table 2.1 Search summary table

Concept	Search terms
General background of protein adequacy in PB/ vegan diets	“protein-quality” OR “protein intake” OR “protein consumption” AND “vegan diets” OR “plant-based diets” OR “plant-sourced foods” OR “plant forward” OR “vegetarian diets”
Plant protein utilisation in the body	“digestibility and utilisation of amino acids in plant foods”, OR “anti-nutritional factors in plant foods” OR “absorption of plant-based proteins”.
Nutritional impacts of novel PBAs	“protein in plant-based alternatives” OR “plant-based meat analogues” OR “plant-based meat alternatives”, OR “plant-based-beverages”, OR “plant-based milk alternatives”, OR “plant-based dairy alternatives” OR “PBMA” OR “plant-based proteins” OR “plant-based meat” OR “plant-based meat analogue” OR “plant-based substitute” OR “alternative proteins” OR “dairy alternatives” OR “plant-based milk”
Protein quality (assessments and protein distribution)	“vegan diet” AND “amino acids” OR “amino acid composition” “essential amino acids” OR “indispensable amino acids”, “protein distribution across a day” OR “meal protein distribution”
Vegan diets in the elderly	“vegan” AND (“elderly” OR “ageing population”)
Simulation studies	“PB diet simulation” OR “PB diet modelling” OR “Vegan / vegetarian dietary model”

PB, Plant-Based; PBAs, Plant-Based Alternatives; PBMAs, Plant-Based Meat Alternatives

2.2.2 Inclusion and exclusion criteria

All articles in the literature review were published in English. In **section I**, RCTs, observational studies, meta-analysis, systematic reviews, government and organisational reports were included. Open-access full-text articles on vegan diet adoption in adults and older adults (> 18 years of age) from 1981 to 2025 were included. Articles studying other types of PB diets (e.g., vegetarian) were excluded.

In **section II**, simulation studies of PB dietary models that replace ASFs with PB foods in varied quantities were included. Few simulations model the nutritional impacts of a complete PB food transition (i.e., vegan diets). Hence, to assess all nutritional outcomes arising from varying levels of animal-to-plant protein substitution, studies that examined 'flexitarian' or 'pescatarian' diets were also analysed as types of PB diets. Studies that have not utilised simulated or computational dietary models were excluded. Studies that did not examine protein intake were also excluded. **Figure 2.1** illustrates the proposed framework of the **section II** review, as guided by the literature (23, 27, 28).

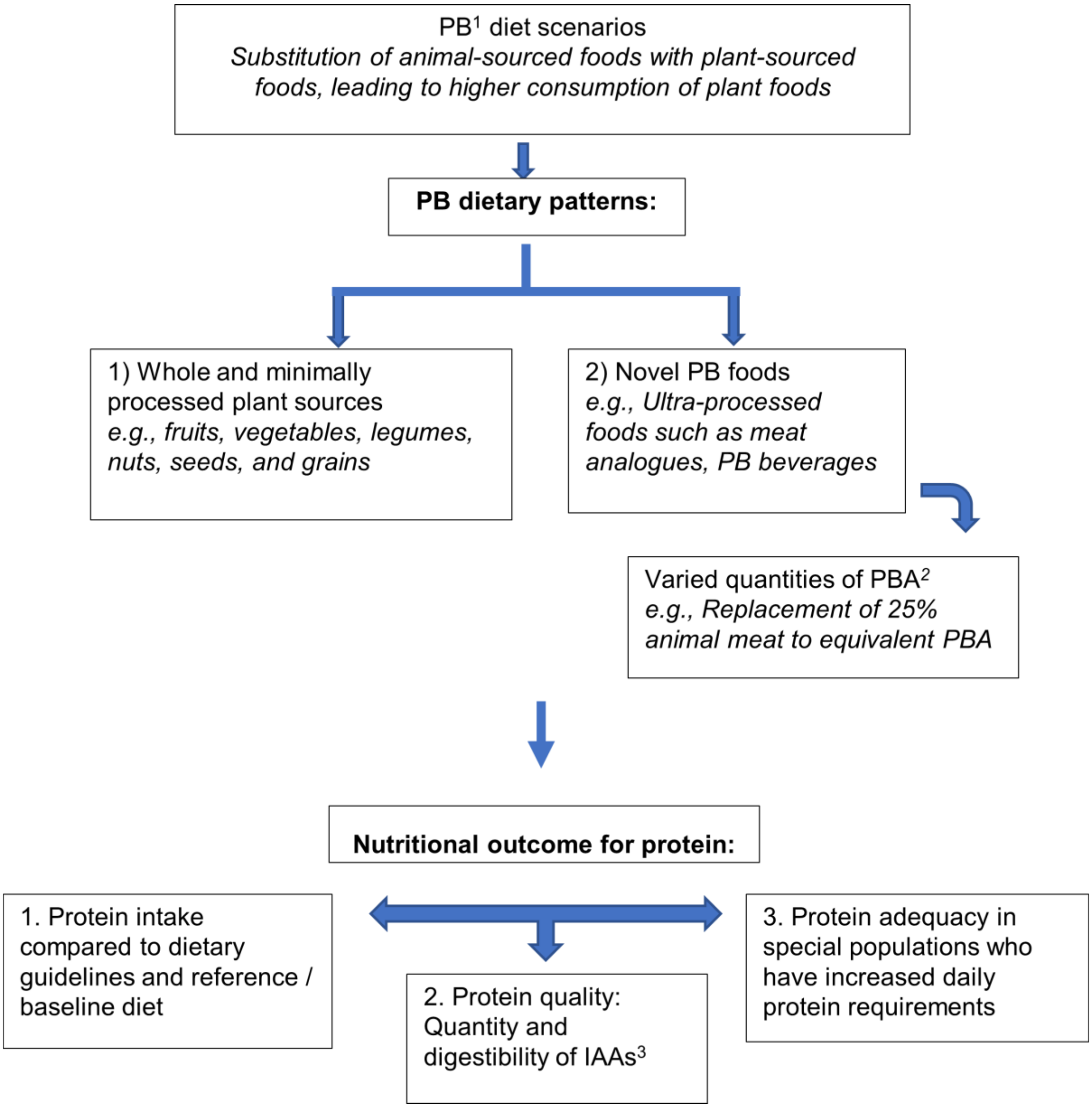

Figure 2.1 Framework of analysis
Abbreviations: [1]PB, Plant-Based; [2]PBA, Plant-Based Alternative; [3]IAA, Indispensable Amino Acids

2.3 Section I: Complexities and challenges in achieving high protein quality in vegan diets

2.3.1 Overview of nutrient intake in vegan diets

Nutritional deficiencies are a concern in unbalanced vegan diets (29). Intake of micronutrients such as vitamin B12, bioavailable iron, zinc, calcium, and iodine may be generally lower in vegan populations as compared to vegetarians and omnivores (26, 30-35). Even sufficient consumption of the long-chain alpha-linolenic acid (ALA) among young and healthy vegan men (36) showed low levels of plasma long-chain polyunsaturated fatty acids (PUFAs), especially for DHA (37). This is partly attributed to the limited efficiency of enzymatic conversion from ALA through the desaturation-chain elongation system in humans (38). Collectively, these findings highlight that several nutrients in vegan dietary patterns may either be consumed in insufficient quantities or provided in forms with reduced metabolic activity.

Protein deficiency is another relevant concern in vegan diets. While protein intake may be lower in vegan groups as compared to other diet groups, it is not necessarily below the daily requirements (15, 25). However, among 26 studies reviewed by Bakaloudi et al. (2021), two studies found protein consumption to be below the recommended daily intake (39, 40). The EPIC-Oxford study, a long-term prospective cohort study that examined health impacts of different dietary patterns (including vegans, vegetarians and omnivores), found higher incidences of inadequate protein intake with increasing intake of PB foods, with the highest prevalence observed among vegans (41). In contrast, a higher protein intake among 83 Norwegian vegans than vegetarians and pescatarians was observed and attributed to the inclusion of soy-based meat substitutes (42). The analysis must shift from simple measures of crude protein intake to measures in protein quality, which incorporate considerations of plant protein combination and digestibility compared against the requirements of specific populations (9, 15). This section focuses on addressing the complications in achieving adequate utilisable protein within the scope of a vegan diet.

2.3.2 Overview of protein structure, digestion and utilisation

While carbohydrates, fats and protein are macronutrients that can supply a source of energy for the body's needs, protein is distinguished by its provision of nitrogen (43, 44) in its building blocks - the AAs (45, 46). Protein digestion within the gastrointestinal tract (GIT) requires the successive cleavage of peptide bonds within the tertiary structure by hydrolytic enzymes, proteases and peptidases to yield smaller peptides and free AAs, which can then be absorbed through the enterocytes of the small intestine (11, 43, 47). Within the enterocytes, further hydrolysis of peptides is completed by pancreatic enzymes, yielding smaller molecules of 6-8

AA (oligopeptides), which are then completely hydrolysed at the apical brush border, to yield free AAs (43, 47, 48).

The highest absorption of free AAs occurs in the distal small intestine as a result of the higher activity of peptide hydrolysis by brush border enzymes in the ileal membrane (47). AAs derived from exogenous protein sources are extracted from the splanchnic tissues and directed towards metabolic functions within peripheral tissues such as muscles, organs, bone and skin (10). AAs that are absorbed in the large intestine and represented in faecal measurements do not have significant nutritional implications (47, 48). Hence, the region of the GIT in which digestibility scores are obtained is relevant for assessing protein quality.

AA turnover is represented by the continuous provision and utilisation of AAs, which creates variations in the body's nitrogen pool and overall nitrogen balance (NB) (11, 49-51). Degraded proteins need to be replaced primarily through dietary intake of IAAs, which serve as precursors of protein synthesis, which maintains the positive protein balance in the body (11, 51, 52). Removal of the amino group allows reutilization of AAs for protein synthesis or conversion to other nitrogenous compounds (e.g. purine and pyrimidine) (11, 43, 50). Lysine and threonine have side chains that contain an amino and hydroxyl group, respectively, which prevent enzymatic transamination (53). Other AAs could, however, be diverted to transamination pathways to synthesise dispensable AAs (DAAs) (50). Meeting the DAA requirements is therefore less reliant on dietary protein intake. Nitrogen is converted to urea via the urea synthesis in the liver and subsequently excreted in the urine, faeces, sweat and integument (49, 54). This process is significant in removing toxic ammonia from the catabolism of AAs (11, 51).

2.3.3 Challenges in the digestion and utilization of plant-sourced proteins

Poorer digestion of plant proteins as compared to animal proteins may be related to the inherent microstructural arrangement of the protein, such as protein cross-linking and protein chain rigidity (55). Secondary configurations, such as **β**-conformations, are hydrophobic and promote protein aggregation (56). This reduces the accessibility to proteases, thus inhibiting digestibility. (57) As compared to meat and milk proteins (56, 57), high levels (above 40 %) of **β**-pleated sheet are found in plant proteins such as soy (57, 58), peas (57, 59), rice (57, 60), wheat (57, 61) and oat (57, 62). The intercalation of plant proteins with fibre also creates steric hindrance, which reduces the access points for enzyme catalysis (63).

Anti-nutritional factors (ANFs) in PSFs also reduce enzyme proteolytic activity and hinder plant protein digestion (14, 43, 64). For instance, legumes, cereals, potatoes and tomatoes contain inhibitors of trypsin, chymotrypsin, carboxypeptidases, elastase and α-amylase (64, 65). Some processing techniques can reduce the concentration of these compounds, whereas in others, trypsin inhibitors stored in the cotyledons of foods such as soybeans may fractionate with the storage proteins and reach elevated concentrations in processed foods such as raw soybean flour (64, 66). Heat treatment may inactivate trypsin inhibitors in foods such as soy protein isolates, soy milk and miso (64, 67), but extensive heating may also reduce the protein digestibility of soy products (64). Animal models found a redirection of sulphur-containing AAs from tissue synthesis to trypsin and chymotrypsin synthesis as a mechanism to compensate for enzyme inhibition (64, 65).

Phytate is another ANF that is typically found in pulses, corn, wheat and rice (64). Its negatively charged phosphate group either chelates directly with carboxypeptidases and aminopeptidases, the mineral cofactor, or the protein substrate to inhibit enzyme activity (64). Chelation processes could depend on pH variation in the GIT (68). Varied processing treatments could either reduce or concentrate the quantity of phytates in plant foods. For example, extraction and purification processes convert some plant-sourced proteins (PSPs) to isolates or concentrates with digestibility that is comparable to animal-sourced proteins (ASPs) (14, 69, 70). Contrastingly, phytates are relatively resistant to heat processing, which instead destroys the heat-labile phytase enzyme (64).

After completing protein hydrolysis, 90% of free AA is transported into the portal circulation (47) However, because of the poorer digestibility of PSPs, splanchnic retention of AAs from these foods is higher (10, 71), leading to reduced availability and perfusion of AAs into the peripheral tissues for MPS. This may have greater implications for susceptible populations (e.g., elderly) who are more challenged in maintaining lean muscle mass (18).

Urea nitrogen salvage at the loops of Henle permits the retention of approximately two-thirds of the nitrogen pool in the body that can be incorporated into AAs (51). For example, the conditionally essential AA, arginine, may be synthesised from the incomplete urea cycle in the kidneys (50). Another one-third of urea is hydrolysed by colonic microbes, which can contribute to nitrogen cycling and urea salvage during periods of high growth or low dietary protein intake (51). These systems may still fail in prolonged periods of protein or energy restriction when the net loss of nitrogen is not balanced by sufficient AA intake (51).

The increased likelihood of urea conversion for some PSPs could reduce the available AAs for metabolic function in body tissues. Soy has superior digestibility as compared to other PSPs (70). However, a higher proportion of AAs from soy proteins are degraded to urea than

AAs from casein or whey, resulting in lower amounts incorporated into the protein structure of humans (71-74). This is attributed to differences in gastric emptying and the absorption rate of these protein sources, with higher splanchnic catabolism for soy (73). Wheat protein, which is of poorer quality, is even less incorporated into peripheral protein as compared to soy, as evidenced by examination of postprandial 8h-metabolic use of dietary nitrogen from wheat (73, 75). A summary of the discussed challenges is presented in **Table A1 of Appendix A**.

Unlike carbohydrates and lipids, the human body is incapable of storing reserves for protein, which must be consumed through the diet to provide an exogenous supply of IAAs, which are essential nutrients for growth and the maintenance of physiological functions (76). Protein synthesis is halted or less optimal if there is even one limiting IAA (77), with the excess oxidised or stored as other macromolecules (glycogen and triglyceride) (78). The slow turnover of lysine may attribute a conservative effect from meal to meal, but these conclusions were derived from animal studies (79). Moreover, post-translational modifications of lysine (e.g., methylation) are irreversible, rendering it unusable for protein synthesis (11, 51). Protein synthesis and utilisation can also be reduced in circumstances of limiting DAAs (76, 80). However, inadequate IAAs may surface as a bigger problem in vegan diets because DAAs can be contributed in substantial quantities by PSPs (81). Contrastingly, the provision of IAAs from vegetal proteins is poorer (82).

Three of the nine IAAs, leucine, isoleucine and valine, are classified as the branched-chain amino acids (BCAAs) due to their aromatic branched functional groups (78). Leucine is potentially most important for cell signalling, due to its role in activating the signal transduction pathway that stimulates the mammalian target of rapamycin complex 1 (mTORC1) signal cascade (83-86). This activation is necessary to increase in-vitro MPS in skeletal muscle (78). MPS begins at the gene transcription phase and ends with mRNA translation (86). As reviewed in literature, the activated mTORC1 and mTORC1-associated protein phosphatase activity upregulates several phosphorylation pathways of ribosomal S6 Kinases, eukaryotic initiation factor 4E binding protein-1 (eIF4G) and $p70^{s6k}$, which all positively influence the initiation phase of protein synthesis (45, 87, 88). Assemblage of the eukaryotic initiation factor 4F (eIF4F) congregates ribosomes on available mRNAs while activation of ribosomal protein S6 targets mRNAs and accelerates protein synthesis (86).

Insufficient leucine results in the binding and inhibition of GATOR2, which positively regulates mTORC1 activity (89). Leucine alone exerts the same potent effect as a mixture of BCAAs, but this is not observed with the sole provision of isoleucine or valine (90). Reduced intake of BCAAs was observed in vegan diets (91, 92) or PB diets where protein from meat, eggs and dairy were replaced with fish (93). A comparison between vegans and omnivores, however, only found significantly lower plasma concentrations of lysine ($P < 0.0001$) but not leucine among vegans (94). Leucine and lysine could be important IAAs contributing to normal bone health, due to effects on insulin secretion and associated osteoblast growth (94-96), so further verification on plasma concentrations of these IAAs in vegan diets is necessary.

Methionine, a sulphur AA, has an elevated role in protein synthesis due to its hydrophobic nature that promotes the binding of initiator tRNA to eIF-2 and the 40S ribosomal unit to initiate the process of translation (97, 98). Methionine is the major donor of the methyl group, which influences cellular DNA and protein methylation (99). Its levels are directly controlled by 1-carbon metabolism (100). Through transsulfuration mechanisms, methionine facilitates the production of cysteine, another sulphur AA, which in turn regulates the concentration of methionine (101). Meeting total sulphur amino acid (TSAA) requirements can be challenging with plant proteins. Soy and pea provide lower quantities, while cereals provide variable ratios (101). In an analysis of AA intake in German vegans, significantly lower intakes of methionine (51.9%) but also threonine (32.6%), tyrosine (37.2%) and histidine (37.5%) were observed in vegans as compared to omnivores (102). However, when compared to the EAR of the World Health Organisation (WHO), the median dietary intake of all IAAs was sufficient in the surveyed vegans (102).

As reviewed in Fernstrom et al (103), the brain concentrations of tryptophan, phenylalanine and tyrosine are important precursors of the neurotransmitters serotonin (from tryptophan) and catecholamines (from tyrosine and phenylalanine which serve as co-substrates). The consequence of poor provision could reduce the conversion rate of these precursors to neurotransmitters, and poorer physiological or health outcomes (103).

While foods from animal (meat, seafood and dairy) and vegetal (potatoes, chickpeas, soybeans and nuts) sources provide similarly high quantities of tryptophan, lower amounts are present in cereals and maize (103, 104). According to Palego et al. (104), although tryptophan requirements are higher in infants and children than in adults, the lower affinity of the AA for the apical carrier may place greater importance on a protein-rich meal to increase its gut absorption into the plasma. Tryptophan competes with other large neutral amino acids for transport across the blood-brain barrier, but this competition is interestingly mitigated in a low-protein diet. Hence, the role of diet in the metabolic fate of tryptophan is complicated and requires further exploration (104). **Figure 2.2** outlines the main challenges of achieving high protein quality in a vegan diet.

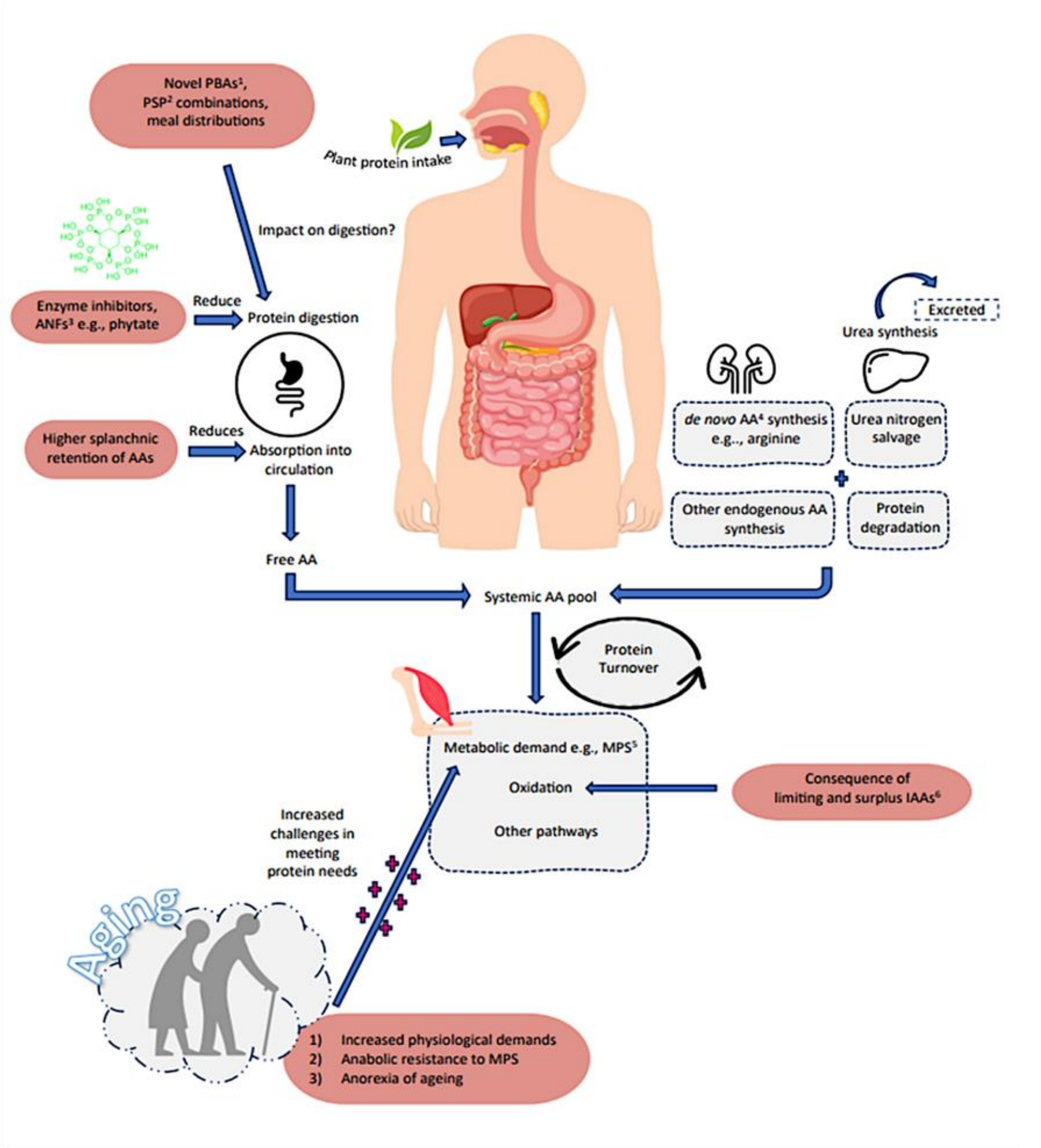

Figure 2.2 Plant protein utilisation in the human body and the associated challenges
[1]MPS, Muscle Protein Synthesis; [2]AAs, Amino Acids; [3]PSPs, Plant-Sourced Proteins. Red boxes indicate potential challenges and considerations associated with achieving NB and protein adequacy. These involve the contexts of plant protein intake and the impact of ageing. The figure is created with information adapted from the literature (51, 105). Design created with images from Canva Pro and PowerPoint.

2.3.4 Protein quality

Protein adequacy from the diet involves the choice of protein food(s), the quantity that can be consumed in one meal, and finally, the quality of the food source in its composition of digestible AAs. The overall food matrix (106), as well as an individual's physiological state, are additional factors that establish the ability of the food or meal (76, 105, 107) to provide sufficient and biologically available AAs that can be utilised for the individual's metabolic demands (11, 76). This defines protein quality.

2.3.5 Assessment of IAA intake in vegan populations

Analysis of protein intake is common in past observational studies involving vegan populations (110, 111). AA analysis is often overlooked, potentially due to the paucity of AA compositions for many foods in food composition databases. However, given the important metabolic roles of each AA, understanding the contribution of AA from PB foods provides clearer insight into their impact on nutrient adequacy and overall health outcomes. Several studies have investigated AA intake and plasma concentrations of individuals on vegan diets or simulated vegan diets, and findings from 16 articles are summarised in **Table 2.1**.

Table 2.1 Summary of studies on IAA intake in vegan populations

Reference	Study design	Study objective	Amino acid intake / status
1. Abdullah et al. (1981) (110)	Cross-sectional study of 3 adult males and 3 adult females	Compare the chemical analyses of vegan diets and Swedish-mixed diets to determine nutrient intake and health status	Lower intake (mg/kg BW) of isoleucine, leucine, lysine, threonine and valine in vegans compared to non-vegans
2. Anderson et al. (1981) (111)	Cross-sectional analysis of 32 Canadian adults	Determine the relationship between habitual food intake and plasma IAA ratios	Plasma tyrosine, phenylalanine, valine and leucine were lowest in the vegan group
3. Rana and Sanders (1986) (112)	Cross-sectional study of 48 adults in the UK	Investigate the effects of vegan and omnivore diet on plasma AAs	Mean values of plasma threonine, valine, leucine and lysine were lower in vegan subjects than omnivores
4. Schmidt et al. (2015) (113)	Cross-sectional study; Oxford arm of EPIC in 379 adult men	Compare the differences in plasma metabolites in relation to metabolism between self-declared omnivores, vegetarians and vegans	Lowest plasma concentrations of leucine, valine, lysine, methionine, tryptophan and tyrosine in vegans

5. López et al. (2016) (92)	Crossover design; consume single meal of each diet in 15 adult women	Compare the plasma AA profile of rural (PB protein sources) and urban Mexican diet (ASPs regularly consumed)	Lower plasma concentrations of leucine, isoleucine, valine, phenylalanine, tyrosine and proline in the rural than the urban diet
6. Schmidt et al. (2016) (91)	Cross-sectional study; Oxford arm of EPIC in 392 adult men	Compare the differences in intakes and plasma concentrations of AAs between self-declared omnivores, vegetarians and vegans	Lower plasma concentrations of BCAAs, lysine and tryptophan in vegans 50 % lower lysine and methionine intake in vegans compared to meat-eaters Significant correlations of lysine and tyrosine with non-soya plant intakes; inverse correlations of valine, lysine, methionine and tyrosine with soya products
7. Draper et al. (2018) (114)	Randomised, cross-over, controlled trial in 21 adults in Switzerland	Compare a 48-hour vegan diet with omnivorous diet in macronutrient composition and metabolic measurements	Reduced intake of cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine in vegan diets compared to omnivorous diets, for both sexes Decreased plasma BCAAs and other EAAs in the vegan diet
8. Kahleova et al. (2018) (115)	Randomised trial on 75 overweight participants in the US	Investigate the role of plant protein on body composition and insulin resistance	Reduction in leucine and histidine intake in the vegan diet as compared to the control diet
9. Lindqvist et al. (2019) (116)	Cross-sectional study of 120 adults of varied diet groups in Sweden	Assess if the analysis of serum samples with magnetic resonance can objectively distinguish diet patterns	BCAAs and lysine were among the most important metabolites to distinguish between vegan/non-meat eaters and omnivores.
10. Wang et al. (2019) (117)	Cross-sectional study of 36 adults of varied diet groups	Explore the links between gut microbiota and circulating metabolites	Lower circulating BCAAs in vegetarian and vegan diets as compared to the omnivorous diet.
11. MacArthur et al. (2021) (118)	Simulation models of diet patterns using NHANES database, consisting of 2	Analyse the total protein and AA composition of food items and dietary patterns (omnivorous versus vegetarians/ vegans)	AA intake ratios showed higher intake of histidine, BCAAs, lysine, methionine, threonine and valine in omnivorous compared to PB diets.

	24-hour dietary recalls		Small magnitude of difference (< 0.5 %) for AAs, apart from lysine.
12. Dietrich et al. (2022) (102)	Cross-sectional study on 36 adult vegans and 36 adult omnivores in Germany	Compare dietary intake and plasma concentrations of AAs between vegans and omnivores using 3-day food diaries.	For the same energy intake, vegans had lower median intake of the 9 IAAs than omnivores but were above the EAR[8] (mg/kg/day). Out of the 9 IAAs, only plasma lysine was significantly different between vegans and omnivores.
13. Conzuelo et al. (2022) (119)	Scenario-based analysis of 2-day vegan menu days of the EAT-diet for adults, using Swiss Food Composition data	Evaluate changes to protein quality when combining different protein sources in a vegan diet	Fulfilment of lysine, valine, threonine, leucine, isoleucine and histidine occurs with a combination of legumes, cereals, vegetables and fruits. Lysine was most limiting in all scenarios when the DIAAS method was incorporated.
14. Tallman et al. (2023) (120)	Modelling analysis of 3 different US diets with varied protein intake (g/kg/day)	Analyse the nutrient and EAA content of different dietary patterns for chronic kidney disease adult patients	Lysine was below the reference value in the vegan diet, at 0.6 and 0.8 g of protein/kg/day.
15. Aaslyng et al. (2023) (121)	Cross-sectional study of 40 Danish adult vegans	Analyse the intake of energy, protein and EAA composition of the vegan diet	Sufficient protein intake despite lower intake of energy, as compared to requirements. Lysine was the most limiting AA, followed by sulphur-containing AAs, leucine and valine.
16. Leitão et al. (2024) (122)	Cross-sectional study of 774 Brazilian adult vegans	Analyse protein and IAA intake by processing level	The median intake for all IAAs exceeded the recommended levels. Lysine had the lowest adequacy ratio, while phenylalanine and tyrosine had the highest.

BW, Body Weight; IAA, Indispensable Amino Acid; AA, Amino Acid; EPIC, European Prospective Investigation into Cancer and Nutrition; BCAA, Branched-Chain Amino Acid; EAA, Essential Amino Acid; NHANES, National Health and Nutrition Examination Survey; EAR, Estimated Average Requirements; DIAAS, Digestible Indispensable Amino Acid Score

Generally, lysine and other BCAAs were found to be the lowest in dietary intake and plasma circulation in vegans compared to omnivores. Lysine was the most limiting IAA and found to be most different between vegan and other diet groups (111, 113, 116, 119-121). This is not unexpected since many PB foods are known to contribute low quantities of lysine. Apart from one study (119), none accounted for the digestibility values of the AAs in PSPs. This can be attributed to insufficient True Ileal Digestibility (TID) data for many fruits and vegetables (119). Most studies were also limited by the duration of the study and a small sample size. Few studies examined the nutritional impact of vegan diets in special populations, including pregnancy, early life and older adulthood (123-127), with findings commonly consolidated with broader vegetarian dietary patterns. Results derived from healthy vegan adults should not be extrapolated to these special groups with higher nutrient demands. Given that vegan diets are more commonly observed in adult populations and may be maintained into older age or continued during pregnancy and lactation, a closer examination of the nutritional implications across the life course is needed.

2.3.6 Protein and IAA requirements in PB diets

NB reflects the balance of protein intake, synthesis and losses (128, 129). NB quantifies daily nitrogen utilisation in the body and is used to derive the EAR (the quantity which meets the needs of 50 % of healthy adults) and the Recommended Dietary Allowance (RDA; the 'safe' intake to prevent nutritional inadequacies in 97.5 % of healthy adults) (9, 86, 130, 131). The NB method only quantifies nitrogen intake and excretion and has often been critiqued for underestimating protein requirements, as it does not capture metabolic utilisation of IAAs. This limitation is overcome by the Indicator Amino Acid Oxidation (IAAO) method, which determines IAA requirements through breakpoint analysis by measuring the point at which oxidation of a labelled indicator AA decreases (80). At this point, the previously limiting AA is now provided in quantities that meet physiological requirements.

Questionable reliability of NB studies to ascertain protein requirements can be related to variability among individuals and difficulty in determining if nitrogen sources were from endogenous synthesis or only through food (131). A meta-analysis found that protein requirements were estimated to be significantly higher when measured using the IAAO method (mean of 0.88 g/kg/day), as compared to the NB method (0.64 g/kg/day) (132). Other studies have found IAA intake above EAR and RDA to be beneficial, especially for more vulnerable populations (133, 134). These outcomes indicate that true protein requirements of adults may be higher than NB-based estimates suggest. Additionally, the validity of the NB technique is uncertain under conditions of long-term low-protein intake, such as restrictive vegan diets, and physiological adaptations may mask inadequate intake. A zero balance may simply be a reflection of lower nitrogen excretion rather than poorer intake (10, 135).

A negative NB of 1.38 g/day, indicating a catabolic state, was observed among 18 minimally active male vegan adults of 20 to 45 years (129). To re-establish NB among vegans, the recommendation of 0.8 g/kg/day of protein based on NB was insufficient, and an additional 12 g of protein, leading to a 20 % increase in daily protein requirement (0.96 g/kg/day), was proposed by the authors. Recent evidence from IAAO and NB assessments has also suggested an increase of 16 % and 55 %, respectively, in requirements from the current RDA of 0.8 g/kg/day (136).

These increments of protein requirement may not always be realistic for older adults who cannot tolerate larger volumes of food, or individuals concerned with higher caloric intake (119, 137). As the measurements only consider overall protein intake rather that relate to the unique metabolic roles of each AA (85), even if protein intake is increased, inefficient utilisation of IAAs as a result of at least one limiting IAA from a poor-quality protein, or protein mixtures with the same limiting IAA would lead to nitrogen losses through increased AA oxidation and deamination (71, 128). Achieving nitrogen accretion requires adequate protein, but also a balanced profile of utilisable AAs, which is often the more challenging aspect in a diet consisting only of PSPs.

Both the EAR and RDA were established with the assumption that high-quality protein is consumed (85, 138, 139). Consequently, when diets rely mostly on plant proteins, which are generally lower in protein quality, protein requirements may likely exceed the EAR or the RDA if protein quality is not examined (9, 140). This demonstrates the importance of accounting for protein quality when evaluating predominantly PB diets, as the current crude protein recommendations become less applicable, particularly for vegan diets.

Protein utilisation from a food source can be more accurately determined by calculating the Protein Digestibility Corrected Amino Acid Score (PDCAAS) or Digestible Indispensable Amino Acid Score (DIAAS) for the food, both of which compare the quantity of digestible IAA in the food with the human requirement. These protein quality scores depend on the IAA reference patterns (mg IAA/g protein), which are derived from IAA requirements (mg IAA/kg BW) normalised by the EAR (g protein/kg BW) (52, 140).

An earlier review highlighted that the PDCAAS, which is intended to measure utilisable protein, was based on age-specific requirement patterns (49) and had not considered additional roles of IAAs beyond meeting NB (107). The application of the PDCAAS is especially limited in assessing protein quality from a diet high in plant foods. Faecal digestibility, which is the measurement utilised in PDCAAS, is usually an overestimation of AA digestibility. Nitrogenous compounds, which are synthesised by colonic bacteria, as well as AAs that are absorbed past the terminal ileum (141, 142), are both considered in the calculation, but neither is nutritionally significant to human physiological requirements (44). Higher fibre present in plant protein sources (105) not only leads to elevated faecal nitrogen loss (51), but also increases the fermentation and release of nitrogen by colonic bacteria, which will alter the measurement of AAs (141). Additionally, the truncation of scores above 100 % means that the benefits of complementing protein sources deficient in some IAAs within a mixed meal are not fully captured (76).

The DIAAS is the current protein quality measure, considered superior to the PDCAAS (105). However, it retains certain methodological limitations from PDCAAS, such as the use of a single nitrogen-to-protein conversion factor (typically 6.25). This factor assumes that all proteins contain 16 % nitrogen and that AA composition is uniform across foods, when in reality, the nitrogen content varies across protein sources, with larger differences in PSPs than ASPs (139). Consequently, the universal conversion factor may overestimate the protein content of many plant foods, thereby underestimating their IAA density and in turn, their DIAAS. In the context of a PB diet, the DIAAS may however confer advantages such as incorporating the more accurate TID values of AAs from PSPs, as tannins, phytates, trypsin inhibitors and reactive lysine in processed foods are accounted for (9, 64, 105). An exception would be foods that have undergone industrial or home-based heat treatments, in which the true TID values for some AAs, like methionine and tryptophan, are not well-reflected (18, 143). The use of the TID for each IAA in the calculation of DIAAS can also estimate and reflect the proportion of utilisable protein that can be obtained from the food source.

In circumstances of adequate caloric intake, PB diets can usually meet and even exceed protein recommendations with the provision of all AAs, including the nine IAA (139). A calorie-restrictive vegan diet may increase the likelihood of low glucose intake, which drives the catabolism of protein for gluconeogenesis rather than MPS (144-146). Vegans in high-income countries who have greater opportunities to consume diverse foods in a mixed diet are likely to achieve protein and IAA requirements through plant protein complementation, but those whose main protein source is cereals risk deficient intakes of IAAs, such as lysine, often the first limiting AA in PB diets (15, 147).

The protein quality of a vegan meal could be improved by complementing a low-quality protein source with a high-quality source (e.g. soy) by adding the TID scores of PSPs with different limiting IAAs (140). For PSPs with low DIAAS values (< 75 %), protein intakes higher than the RDA may be necessary to meet the higher metabolic demands of certain populations heavily reliant on these PSPs (140). Cereal-dominant diets that are not complemented by other protein sources will lead to the presence of at least one limiting IAA, and higher quantities of oxidised and non-absorbed AAs, as compared to diets containing soy, pea and animal-derived proteins (53). Blending protein sources with different limiting IAAs (e.g., rice and pea) in the correct ratios increases the overall DIAAS score and level of utilisable AAs (53). While a vegetarian can include some sources of ASPs (e.g. milk and eggs) that provide higher concentrations of IAAs, such as leucine (105, 148), vegans have more limited choices that can be added in a complementary manner to achieve meals of high protein quality.

2.3.7 Metabolic characteristics of plant- and animal-sourced proteins

The AA profile, rather than the consumed quantity of the protein, is the most significant attribute of the food's functional properties (149). A highly digestible protein provides a larger quantity of absorbable AAs (148) for a smaller consumed amount as compared to poorly digestible protein sources (150). The ratio of protein mass to energy is generally higher in ASPs, and even soybeans, which have a higher protein quality score than most PSPs, contribute more calories for an equivalent quantity of protein than do ASPs (55, 140). Cereal grains, the dominating source of plant protein globally (55, 151) have a relatively low protein-to-energy ratio (140) and are limited in lysine. A high consumption of plant foods is required to meet the daily requirements for IAA, but this strategy inevitably increases intake of carbohydrate, calories and dietary fibre, potentially resulting in impractical and unrealistic intakes, as well as some unfavourable health outcomes (55, 140). Higher proportions of these high-fibre foods also contribute to satiety, placing limits on consumption quantity (55, 57).

The ability to stimulate MPS may generally be higher in ASPs than in PSPs. In a comparison of ASPs and PSPs in healthy older men (n = 60; > 71 years old), the consumption of casein and whey protein resulted in a greater postprandial increase in plasma AA concentrations, particularly leucine, and a greater rate of myofibrillar protein synthesis as compared to an equivalent ingestion of wheat protein (152). Yet another study on 13 older men (60 to 75 years old) found prolonged phosphorylation of p70S6K, an indication of the MPS response, 4 hours post-exercise, only in whey protein ingestion, which was unobserved with an equivalent quantity of soy (153).

Overall protein accretion in the body may depend on differing mechanisms attributed to 'fast' and 'slow' proteins, which then impacts the acute response of MPS (154). One study found that isolated whey and soy protein, which were classified as 'fast' protein sources, significantly increased mixed muscle protein fractional synthetic rate in three small sample groups of healthy young men (n = 6 in each group), as compared to casein, a 'slow' protein with a slower rate of gastric emptying (154). However, a significant difference between whey and soy was observed only after resistance exercise (155), which may provide a synergistic effect in anabolic signalling (106). This disparity can also be explained by the small differences in digestion rates between these two protein sources, which impact blood leucine concentrations and result in different oxidation rates of AAs in post-exercise conditions (156).

Trials assessing associations between PSP intake and outcomes on skeletal muscle mass found poorer outcomes for muscle mass retention in adults consuming higher proportions of PSPs than in those with higher intake of ASPs (157-159). However, an RCT on 19 healthy older adults found contrasting evidence that a strict vegan diet, based on a 57 % daily protein intake from a mycoprotein-based protein source (mycoprotein obtained from Quorn Foods ™), provided an equivalent daily myofibrillar protein synthesis rate when compared to an isonitrogenous omnivorous diet (160). However, apart from three individuals who were vegetarians and thus assigned to the vegan group, none of the participants habitually consumed a vegan diet, so analysis from long-term vegan consumers is a research priority.

While most of these trials provide evidence that isolated ASPs may stimulate greater MPS as compared to PSPs, isolated soy protein, with its high protein quality, has the highest likelihood to provide a source of complete and digestible AA (161) that is of near comparison to whey protein. However, most meals throughout the day consist of mixed ingredients, rather than isolated proteins. Further research is required to understand how mixed vegan diets can compare against omnivorous diets in stimulating postprandial MPS, and is likely essential for older adults who face greater anabolic resistance of MPS and require higher doses of bioavailable AAs (162).

At least one study has found that when protein sources are appropriately complemented in a vegan diet, the daily MPS rates of vegan older adults do not differ from those consuming an isocaloric, isonitrogenous omnivorous diet (163). The provision of sufficient IAA through dietary protein is crucial for muscle protein accretion and avoid MPB – a process to endogenously compensate for deficient IAA intake (10, 144). However, resistance exercise also appears to modulate the impact of food on MPS and enhance muscle sensitivity to the dietary influx of AAs (106).

2.3.8 Protein intake and distribution throughout the day

While two vegan individuals may consume the same quantity of protein in a day from the same sources, meal distribution patterns may be different, and the frequency of consumption could influence the sensitivity of signalling pathways to postprandial AA concentration, MPS and net protein balance, with consequences for overall dietary protein adequacy (106). An even distribution of protein in temporal patterns of 3 to 5 hours throughout the day could increase

MPS and support muscle protein accretion (70, 85, 164, 165). The meal threshold for protein intake is proposed to be a 2-to-3-fold increase in plasma leucine to achieve maximum activation of mTORC1 (85, 166). Approximately 20 to 30 g of protein, to supply 10 g of IAA (of which 5 g is BCAA), may be needed to maximise MPS for 2 to 5 hours for healthy young adults (10).

Contrastingly, time-restricted feeding or intermittent fasting (IF) is proposed to coincide with the body's circadian rhythm by regulating the balance between mRNA and proteins and hence control aspects of protein metabolism (167, 168). In such infrequent meal patterns, perhaps ensuring at least one meal of sufficient protein intake with adequate leucine is crucial, with greater importance imposed for the first meal of the day (breakfast) to overcome the catabolic state of an individual after an overnight fast (10, 169). However, energy restriction and low insulin states in IF compromise MPS and net muscle balance with higher protein oxidation in longer periods of starvation over 24 hours (170, 171). As IAAs are not produced or stored in the body, periods of such prolonged meal abstinence are disadvantageous for lean muscle mass promotion, especially within the context of vegan diets characterised by low protein quality. A combination of plant protein sources is crucial to achieve sufficient AAs in vegan diets. This is easier to achieve with frequent meals than with smaller eating windows in IF.

Simply accounting for protein intake per day without considering protein distribution across meals can give false impressions of protein adequacy. Furthermore, the recommendation of 0.8 g/kg/day is based on assumptions of high-quality protein intake spread across 3 meals (172). Even if daily protein intake can be met by a skewed intake pattern (as commonly observed with high-protein evening meals (173, 174), the meal threshold may only be achieved once a day and no additional signal for MPS is stimulated in saturation (the refractory period), leading to oxidation rather than utilisation of surplus IAA (85, 174).

Redistributing protein to meals throughout the day is contrastingly more efficient, with improved stimulation of MPS, as observed by a 25 % increase in 24-h fractional mixed MPS (165). For older adults who are less tolerant of large food volumes in one meal moment (e.g., in pulse feeding), even protein distribution is beneficial to promote anabolism (130). Uneven patterns are associated with increased frailty and possibly reduced MPS activation (175). Clearly, increased empirical data is necessary to confirm the impact of meal distribution patterns on physiological impacts, especially for the vegan population and older adults, where frequent food intake could attenuate challenges in obtaining diets of high protein quality.

2.3.9 Protein quality of novel PBAs

Novel alternative protein sources are becoming ubiquitous in modern vegan diets, partly due to marketing messages emphasising environmental sustainability, animal welfare, and health benefits (176). In contrast to traditional PB sources, which are largely unprocessed, novel meat and bovine milk alternatives undergo varying levels of processing to mimic the sensory characteristics of meat and milk and could offer convenient and supportive foods to individuals newly transitioning to a PB diet (177). Commonly, the production of PB meat alternatives (PBMAs) isolate proteins from legumes, cereals and oilseeds, in combination with fats from coconuts and seeds (178). Many studies have investigated the nutrient profiles of novel foods, but further research is still required to determine how the nutritional compositions of these foods align with those of traditional plant- and animal-based foods, especially in terms of nutrient bioavailability (178). A summary describing the challenges in protein quality for novel PBAs is found in **Table A2 of Appendix A.**

Less purified plant proteins, such as wheat cereals, have poorer digestibility (< 80 %, as measured with PDCAAS), and this value is further decreased with the inclusion of plant cell walls, ANFs, levels of processing and heat treatment (141). Contrastingly, the removal of cell wall constituents in purified plant proteins results in products such as wheat gluten, wheat flour and soy protein isolates that have comparable digestibility to ASPs (141). Extrusion processes used in the manufacture of PBMAs similarly raise protein digestibility (179, 180). Hence, consumption of isolated plant proteins in these foods may exceed the protein requirements of an individual (181). However, because phytic acid is stored in the protein bodies of the endosperm in legumes, phytates may attach to proteins when they are extracted to form an isolate (180, 182). Hence, the bioavailability of AAs from these foods needs further confirmation.

Food formulations comprising mixed sources of PSPs can overcome limitations in single-protein formulations to improve overall functional properties and narrow the compositional difference with animal proteins (183). Efficiency, cost and sustainability are important considerations in extracting plant protein biomass, which is entrapped by cellulose and hemicellulose fibres (183). Usage of high quantities of fat as a lubricant may inhibit the protein denaturation process (184) and the nutritional implications of these procedures warrant attention.

Food preparation techniques impact AA digestibility. While germination, fermentation, and soaking could reduce ANFs (76, 185, 186), and improve the accessibility of proteases to peptides (64), heat processing, even at home, may chemically alter AA function (76, 185). High temperatures could affect an AA's nutritional viability, such as threonine, which undergoes uncontrolled phosphorylation and the resulting phosphothreonine cannot be utilised directly for protein synthesis (143). Racemisation of L-amino acids and the development of crosslinked peptide chains through alkaline and heat processing result in underutilisation for lysine and cysteine (187). Reactive side chains of lysine and cysteine may undergo Maillard reactions with reducing sugars or aldehyde compounds (143, 187), thereby reducing overall absorbability and utilisation for protein synthesis (76). SAAs (cysteine and methionine), tryptophan and threonine are also susceptible to oxidation (105, 188). Products from these reactions may not be accounted for accurately in calculations for TID (143), although DIAAS calculations can account for the true digestibility of 'reactive' lysine (43).

Protein content and protein quality from novel PBMAs

Supermarket audits in several regions reported varied protein content in PBMAs. While a large percentage of products in Australia and Sweden were eligible to make a "high-protein" claim (180, 189), a survey in the United Kingdom found the protein content of PBMAs to be lower than that of meat-derived products in four out of six categories, with burger patties having the largest difference (190). Similar observations of this variation between products were found in Sweden and Spain (180, 191). Details of AA composition are usually absent in the back-of-pack nutrition labels (192). Consumers thus need to be well-informed and discerning when selecting these PBMAs.

Analysis of AA composition and bioavailability of PBMAs are limited. One study compared metabolite classes between beef and PBMAs and found that AAs were among several metabolites that had the most pronounced differences (192). Owing to the important physiological roles of various food metabolites, van Vliet et al. (2021) argued that the nutritional inequivalence between ASPs and novel PBMAs is of crucial importance, as under- or oversupply of certain nutrients can translate to negative implications for human health (192). Hence, the authors assert that these products should fulfil a more complementary role in the diet, rather than as a complete replacement of ASPs.

An analysis in the United States, which compared three brands of PBMA burger patties (made with pea, pea protein isolate, wheat, soy protein concentrate, and black beans) with ground pork and beef, found comparable crude protein content between the PBMAs and animal-derived products (178). Total IAA content was similar to raw PBMAs, ranging between 70 and 76 mg/g, compared to 66 to 68 mg/g for raw animal products, but specific IAAs such as histidine, methionine and lysine, were highest in beef, while isoleucine and phenylalanine were higher in the PBMA patties than ground pork (178). A similar conclusion was reached by Andreani et al. (2023), who also stressed the presence of large nutrient variability among all PBMAs analysed (184). The overall balance of all IAAs in these products is also crucial. As mentioned in earlier sections, since surplus IAAs cannot be stored within the body, the presence of one deficient IAA in the excess of others can promote oxidisation in catabolic pathways (17, 46). As acknowledged by the authors, ANFs and fibrous compounds in these plant products must also be considered as they inhibit protein digestibility (193).

Challenges in achieving high protein adequacy in PB beverages (PBBs)

Several analyses found that PBBs had poorer nutritional composition and quality than bovine milk. In reviewing the importance of setting nutrition standards of PBBs, inferior protein content was observed in a wide range of PBBs, with a large number of PBBs surveyed falling below 1 g/100g of plant protein, as compared to 1 % bovine milk, which contains 3.3 g/100g protein (194, 195). Findings of poorer protein content in PBBs were corroborated by other similar studies, with the only exception being soy milk (196-199). One study found values ranging from 2.50 to 3.16 g/100 mL (200), comparable to the protein content of bovine milk (197, 199, 201).

A few studies evaluated the protein quality of PBBs by measuring the AA content or comparing the PDCAAS or DIAAS of isolated ingredients, such as soy and pea, which may be from raw food commodities rather than within the formulation of a PBB (195, 197, 198, 202). Generally, the authors of these studies concluded that the contribution of adequate IAAs was lower from PBBs as compared to bovine milk, which, in contrast, supplied all IAAs (200, 202). Measurements of AA bioavailability are uncommon, but when adjusting for reactive lysine, soy beverages were found to have the highest reactive lysine percentage out of all PBBs, but absolute reactive lysine content was still lower than that of bovine milk (198).

While PBBs provide palatable and reliable alternatives for consumers who cannot tolerate dairy, the absence of bovine milk in a vegan diet could be disadvantageous from a protein quality perspective, especially for individuals (young children and the elderly) who may require efficient sources of digestible proteins and micronutrients. The blending of plant proteins to achieve a formulated beverage that overcomes limiting AAs could be proposed. This is, however, limited by the paucity of digestibility values for mixed protein sources, palatability (194), and the difficulty in overcoming commonly limiting AAs, chiefly lysine and methionine, from varied plant foods (76, 200).

Further considerations of PBAs in vegan diets

Under the NOVA classification system, most novel PB foods and beverages fall within the category of ultra-processed foods (UPFs) (203, 204). Concerns were raised, most recently in the three-part Lancet Series on UPFs (204), on the use of these foods as replacements for ASFs and traditional, whole PB foods. PBA formulations are further scrutinised for their elevated levels of saturated fat, sodium, sugar and additives (177).

However, not all PBAs are nutritionally equivalent, and some may have favourable nutritional attributes that benefit certain populations (205). For example, mycoprotein-based analogues (e.g., Quorn ™) contain lower levels of sodium, saturated fat and energy as compared with other alternatives (206). In the context of protein supplementation, novel PBAs may add convenience for vegan consumers as a complementary food to meet protein and digestible IAA targets for the day. One study found that the consumption of textured soy protein and other UPFs was associated with substantially lower odds of inadequate protein intake, while individuals consuming higher proportions of unprocessed or minimally processed plant proteins exhibited greater odds of protein inadequacy (122). However, this evidence pertains to one aspect of nutrient adequacy, leaving other nutritional and long-term health impacts unclear. The current understanding of how such nutritional profiles translate to overall health is limited and potentially hindered by heterogeneous survey methods and product variations (177, 198).

Indices like “Healthy PB Dietary Index” and “Unhealthy PB Dietary Index” attempt to separate “healthy” and “unhealthy” PB foods based on the level of processing and association with health outcomes (207, 208). However, this does not accurately account for the discrepancies in specific nutrients, and foods grouped in the “healthy” category may not sufficiently address protein adequacy (203), especially for bioavailable AAs. Subject to ethical considerations, RCTs investigating the nutritional impact when replacing ASPs with PBMAs and PBBs would provide more conclusive evidence of the benefits or risks of these alternatives. A more recent approach undertaken by the International Union of Food Science and Technology was used to investigate formulation and processing as separate influencing factors on the nutritional value of food products (209), as measured by the Nutrient Rich Food Index (NRF) (210). These are encouraging steps that provide a more objective framework for discerning the nutritional and health impacts of processed foods across different product categories.

Irrespective of their UPF classification, vegan diets may beneficially incorporate some of these foods (203) especially for consumers newly transitioning to the diet. These foods may have a practical benefit, especially if they are micronutrient-fortified, hence supplying some of the nutrients that are limiting in a vegan diet. However, it is worth noting that reliance on novel alternatives is not inherently necessary to achieve high protein quality in vegan diets. Traditional PB foods such as LNS, and whole grains can meet both protein and IAA requirements when consumed in diverse and intentionally combined forms to complement different limiting IAAs. This requires knowledge, practice and sustained effort. Improved product formulation is the next step in providing nutrient dense foods while minimising negative outcomes.

2.3.10 Increasing risks of protein deficiency in special populations

Protein and AA requirements are elevated relative to total energy requirements in certain life stages such as pregnancy, lactation, early life, certain disease states and during older adulthood (16, 130, 211). Protein deficiency and protein quality are relevant concerns in these populations and may be more challenged if a vegan diet is adopted. Only a limited number of studies have investigated protein intake alongside metabolomic analysis of plasma AAs in young vegan subjects (126, 212). These studies generally report lower total protein intake and lower circulating IAAs in vegan children than in children in other dietary groups. However, the primary emphasis was placed on micronutrient status, particularly for vitamin B12. Similarly, Heniková et al. (2025) reported lower vitamin B12 concentrations, reduced urinary iodine and indicators suggestive of compromised bone health and growth status in vegan children as compared to the other vegetarians and omnivores (213).

Measures of protein adequacy among pregnant and lactating vegan women also remain under-investigated, despite elevated physiological requirements during this life stage where the RDI is above 1.0 g/kg/day (16). This gap may partly reflect the relatively low prevalence of vegan diets during pregnancy. For example, a previous review identified only six studies comparing pregnancy outcomes between vegans and other habitual diets (2). A more recent evaluation reported slightly lower total protein intake among vegan women compared with other dietary groups, although intakes were generally within daily requirements (123). Some evidence suggests that pregnancy may involve adaptive mechanisms, such as elevated circulating AA concentrations, to meet higher protein demands (214).

Importantly, trends show a growing number of current adults on vegan diets in Western countries, with the potential to increase in the coming decades (215). Apart from reviews and short-term trials (163, 216), long-term empirical studies on older adults are scarce. This is a research priority in the context of an ageing global population and the associated physiological changes in ageing that complicate the efficiency of protein utilisation (217). This thesis surveys dietary patterns among current adult vegans, recognising that these patterns may persist into older age, where challenges associated with anabolic resistance, reduced digestive efficiency, and altered protein metabolism can compromise protein adequacy. For this reason, a dedicated section examining protein nutrition in older adulthood is presented.

Challenges and solutions in achieving protein adequacy in vegan older adults

Old age is characterised by reduced anabolic response to protein, increased insulin resistance, and elevated inflammatory responses from emerging age-associated diseases (130, 218, 219). Ageing is a risk factor for protein misfolding diseases, in which amyloids form and are commonly observed in various neurodegenerative disorders (217). The unfolded protein response in the endoplasmic reticulum reduces protein synthesis as a stress response (220). This is an added problem to ageing muscle, which already faces increased anabolic resistance to protein synthesis due to reduced mTOR activation (162).

Overall, higher splanchnic AA retention is observed in older as compared to younger populations, decreasing distribution of plasma AA levels and perfusion to skeletal muscle, further inhibiting MPS (221). An added burden in ageing is the rise in inflammatory conditions, which could be linked to increased splanchnic extraction and subsequent diversion of leucine to the immune system, liver, spleen, inflammatory areas, and other core body functions, rather than to skeletal muscle (137, 145). Increased chronic conditions among the elderly also reduce nitrogen accretion and utilisation (222).

Over the age of 65, low protein intakes are associated with increased risks of mortality, while higher intakes reduce the risks of lean mass and bone density loss, and provide important AAs (223). Older epidemiological studies have found associations between poorer outcomes for body and bone mass with suboptimal MPS response and net protein balance (130). These outcomes exacerbate occurrences of sarcopenia and osteoporosis, two debilitating health conditions in old age that increase the risk of falls and fractures (130, 224) reduce the quality of life and impose substantial healthcare costs.

Recent evidence by Paoletti et al. (2023) found increased requirement of TSAAs for older adults, with an RDA of 32.1 mg/kg/day for men and 23.7 mg/kg/day for women of 60 years and above to cater for increased synthesis of the antioxidant glutathione or meet the requirement of methyl substrates for methylation (225). A higher requirement of TSAAs may be needed for elderly men than elderly women, due to the positive association of elevated oxidative stress and insulin resistance in this group (225).

Frailty is associated with lower blood levels of BCAAs (83). Leucine requirement as measured by the IAAO method was determined to be 2.2 times higher in older adults than in younger adults (226). A recent study using the IAAO method found that lysine requirements were higher than the current recommendation of 30 mg/kg/day for older women but not older men. These findings from recent bioassay methods convey the importance of revisiting the IAA reference patterns of the Food and Agriculture Organisation of the United States (FAO) (105) which currently combine the requirements of older adults with those of younger adults. Further considerations of stratifying requirement patterns by sex may also be necessary.

The consumption of 1.2 to 1.6 g/kg of BW/day is proposed for older adults, to overcome anabolic resistance, mitigate increased insulin resistance, prevent loss of muscle mass, and reduce risks of frailty (17, 130, 227, 228). In a review (229), IAAO measurements have shown higher protein requirements of 0.94 g/kg/day for older men (230) and 0.96 g/kg/day for older women (231) as compared to results from NB measurements. Similarly, a further study proposed the provision of approximately 25 to 30 g of high-quality protein with approximately 10 g of IAA to maximise MPS and maintain muscle mass in the elderly, with higher intakes being energetically inefficient as the excess is oxidised (232).

An effective eating strategy to achieve protein requirements for the ageing population is to consume foods that provide higher protein density in relation to energy (222). This is undeniably easier to achieve from ASFs (e.g., dairy) than PSFs. As it is, dietary protein intake in the USA decreases with advancing age and likely falls below the current protein EAR (229). Intake greater than 30 g/meal may be required to compensate for the lower protein density in vegan diets, although more evidence is required (70, 216). However, the poorer digestibility and greater protein-satiating effect of high-fibre PSFs, coupled with the reduced appetite commonly observed in older adults, could increase the difficulty of achieving utilisable protein intake within a vegan diet (18, 233) (**Figures 2.2 and 2.3**). Caloric restriction, weight loss and anorexia in ageing lead to reduced food consumption (18). Energy requirements also decrease with age (225, 234). Lower energy and protein intake below the requirement was observed in the vegan arm of a short-term randomised controlled crossover study compared to the omnivore diet (216). The study attributed this observation to significantly higher dietary fibre intake, which reduced portion sizes in the study cohort that already exhibited age-associated early-onset satiety.

A way to circumvent these problems is to ensure that the composition of meals and snacks throughout the day is appropriately balanced. This helps vegan older adults achieve IAA intake closer to the requirements and maintain elevated levels before the next protein-dense meal, without consuming excessive amounts of food **(Figure 2.3)**. The intake of complementary plant proteins (e.g., legumes with cereals), fortified PBAs, and supplementation with beverages and snacks that are enriched with limiting IAAs in appropriate concentrations are practical options for the elderly (71, 163).

Evidently, recent assessments of 16 older adults over 2 test days found higher postprandial MPS rates with an isocaloric, isonitrogenous whole-food omnivorous meal as compared to whole-food vegan meals (125). In contrast, Domić et al. (2024) demonstrated that anabolic stimulation of muscle can be achieved at a similar rate on a well-balanced vegan diet with lower protein quality compared with an omnivorous diet (163). The diet was protein-dense, with diverse sources from cereals, legumes, nuts, soy-based PBBs, and PBMAs consisting of protein isolates, which likely promoted sufficiently high protein intake and favourable AA profiles, comparable to an omnivorous diet. The authors have acknowledged that these are highly controlled diets, so habitual vegan diets that are less balanced across FGs may not exhibit similar protein digestibility kinetics.

Taken together, strategic plant protein complementation in a vegan diet should be emphasised as a key solution to achieve higher protein quality **(Figure 2.3).** Appropriate cooking methods to increase the palatability and digestibility of plant proteins are also effective measures (235). Food formulations by industry partners, dietetic advice, and guidance on physical activity would optimise nutritional intake and support the maintenance of body composition and overall health of older adults who choose to adopt vegan diets.

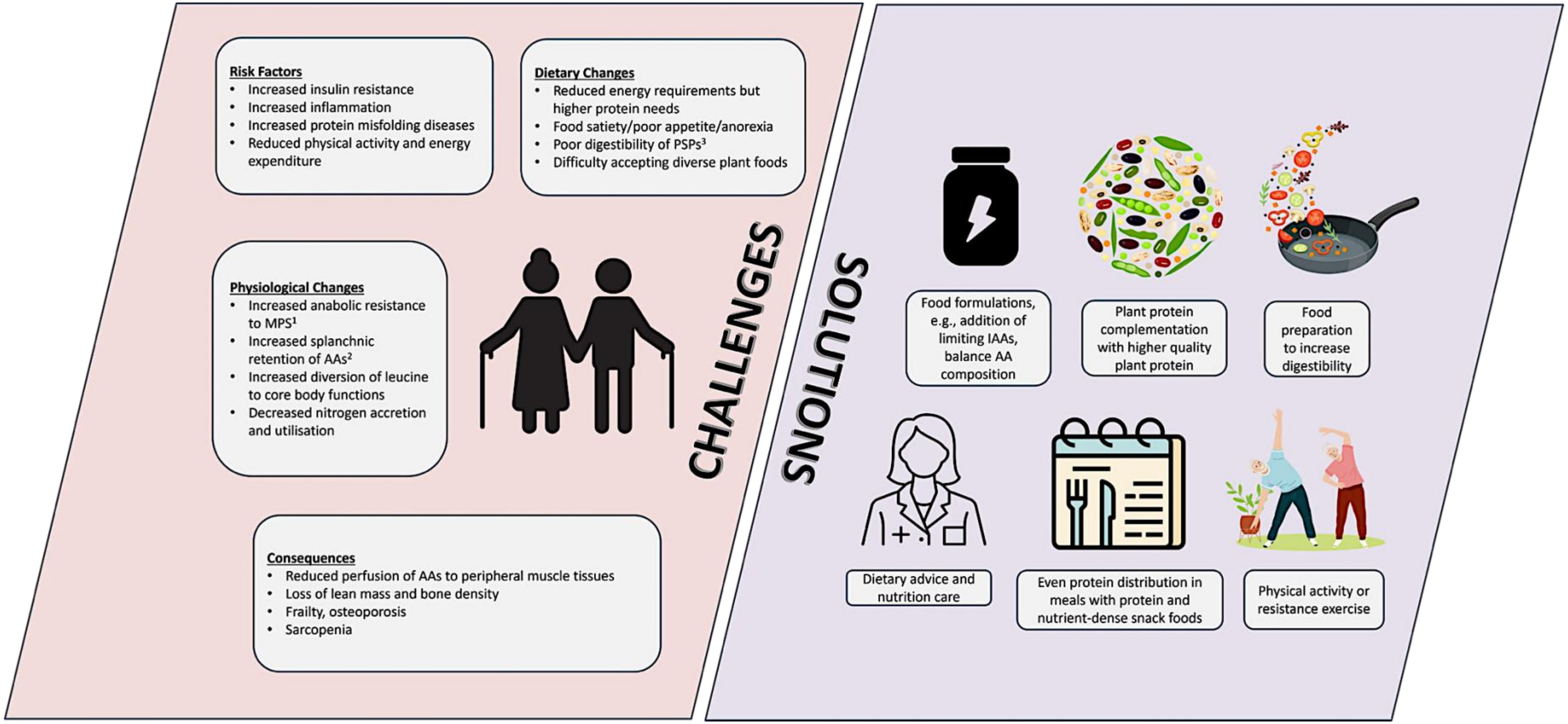

Figure 2.3 Challenges and practical solutions for protein intake by vegan older adults
[1]MPS, Muscle Protein Synthesis; [2]AAs, Amino Acids; [3]PSP; Plant-Sourced Proteins
The design was created with images from Canva Pro and PowerPoint, and supported by the literature discussed in section I.

2.4 Section II: Protein adequacy of PB dietary scenarios

This section explores the impact on protein intake and protein quality in hypothetical diet models where ASFs are replaced with PSFs. A total of 31 studies were included at the time of this review. Baseline diets were derived from dietary recalls and food composition datasets of populations, predominantly from high-income Western countries. Protein content in PSFs was commonly examined among all the studies. Studies that examined only crude protein intake are represented in **Table 2.2.** Five studies included diet quality indices to evaluate the nutrient quality of modelled diets **(Table 2.3)**. Six studies included the protein intake from novel PBAs (**Table 2.4)**. Ten studies analysed nutritional outcomes in scenarios involving special populations, and four are presented in **Table 2.5**; the remaining studies overlap with those summarised in other tables (19, 236-240). Seven studies examined protein quality by accounting for utilisable protein and IAA intake **(Table 2.6)**.

Methods utilised were heterogeneous across studies, but most substitutions of protein sources were based on gram-for-gram replacement of ASFs with PB protein sources (19, 236, 240-252). This does not account for higher rates of carbohydrates, fat, dietary fibre, and thus, higher caloric density per unit of protein mass (55) in different foods. A more detailed substitution was utilised by a few studies, which replaced an equivalent mass of protein or AAs from ASFs (124, 253, 254). One study examined isocaloric substitutions in an energy-adjusted model (253) and another constrained the PB diets to within ± 5 % of energy difference (238). In an isocaloric comparison, PB protein sources generally provide poorer protein quality than ASFs. Such a method is therefore limited in practicality, as increased caloric intake of PSFs is needed to achieve the same quantity of AAs as provided by ASFs (140).

2.4.1 Protein intake in simulated PB dietary scenarios

In most dietary scenarios where animal proteins were substituted with plant proteins, total protein intake was lower but remained above the country-specific nutrient reference values (NRV) **(Table 2.2)**.

Table 2.2. Dietary models which only assessed crude protein intake when replacing ASFs with PSFs

Reference	Study Aim and Target Population	Simulation Design	Protein Assessment
1.Westhoek et al. (2014) (244)	Examine the large-scale consequences in the EU when replacing ASFs with PB foods	25 % or 50 % decrease in meat, poultry, eggs, and dairy, compensated by a higher intake of cereals and pulses	The alternative diet resulted in 10 % lower protein intake than the baseline diet, but the mean protein intake was still at least 50 % higher than dietary requirements
2. Seves et al. (2017) (245)	Examine the effect on nutrient intake when shifting towards more PB diets for the Dutch adult population, as compared to the current diet	1. Replacement of 30 % meat and dairy products with PBAs 2. Replacement of 100 % of meat and dairy	For 100 % replacement, protein intake decreased by 21 g/day for males and 15 g/day for females, but the percentage below EAR was 3 % for females and 1 % for males. For 30 % replacement, lower protein intake was found, but was not significantly different to the baseline diet
3. Röös et al. (2020) (246)	Explore the role of grain legumes in Swedish adults	50 % reduction in meat, and replaced by 55 g of cooked grain legumes	Protein intake was 6 % lower than in the current Swedish diet but still within the Nordic Nutrient Recommendation for adults
4. Ferrari et al. (2020) (28)	Develop an optimised diet for adult Italian population to meet national DRIs with minimum GHGE	Linear programming model to minimise GHGE while meeting protein intake, acceptability, and health constraints	Total protein intake of the optimised diet is lower than the observed diet (67 g/day compared to 76 g/day)
5. Hess (2022) (249)	Determine the nutritional adequacy of dairy-free and vegan diets in varied caloric intakes for American adults	Model 1(dairyALT): Replace dairy with soy milk and fortified yogurt Model 2: Replace cup equivalent of dairy and eggs with PSPs	Decrease in protein by 10 % to 20 % in both models for 1800- and 2000- kcal/day plans, within AMDR, protein meets the DRI in all models for all calorie levels and both sexes
6. Benvenuti et al. (2022) (239)	Assess the carbon footprint of optimised vegan, vegetarian and omnivorous menus for Italian school lunches	Two-week schedule, adhering to Italian RDA with constraints set for protein intake	Weekly protein for all models above lower bound (100 g). Protein intake increases from vegan to vegetarian to omnivorous menus
7. Kaartinen et al. (2023) (8)	Examine the impact on average intakes of selected nutrients and changes to population shares below DRI when replacing meat	Model 1: < 70 g/day red and processed meat	Decrease in absolute protein intake (as a percentage of energy) was only evident in males for 50 g and 30 g scenarios, and in females for the 30 g scenario. No

	with legumes for Finnish adults	Model 2: < 50 g/day red and processed meat Model 3: < 30 g/day red and processed meat	significant difference in absolute protein intake between all models and the reference diet
8. Auclair (2024) (255)	Assess the impact of partial substitutions of red and processed meat or dairy with plant protein foods on nutrient inadequacy, health outcomes and GHGE in Canadian adult diets	Doubled intake of plant protein foods when half of red and processed meat was replaced, which increased to 4 x when half of dairy was replaced	The replacement of red and processed meat, but not dairy, increased protein inadequacy from 0.8 % to 1.9 %
9. Vellinga et al. (2025) (256)	Harmonised assessment of environmental impact and protein intake in 11 European countries	8 scenarios, including reducing meat by 50 % and 100 %, replacing meat with legumes and replacing meat with PB meat substitutes	Reducing meat consumption by 50 % reduced protein intake by 12 to 21 %. Reduction by 100 % reduced protein intake by 25 to 41 %. Legumes as a replacement had a greater reduction in protein intake compared to replacing with PB meat substitutes

EU, European Union; ASFs, Animal-Sourced Foods; PB, Plant-Based; PBAs, Plant-Based Alternatives; EAR, Estimated Average Requirement; DRI, Dietary Reference Index; GHGE, Greenhouse Gas Emissions; ALT, Alternative; PSPs, Plant-Sourced Proteins; AMDR, Acceptable Macronutrient Distribution Range; RDA, Recommended Dietary Allowance

In a few studies, protein deficiencies were observed when ASFs were completely replaced with plant foods. Three separate studies on US populations that utilised similar methodologies saw a decrease in protein intake for all age groups, with an increase in the proportion of the population below the EAR (241-243). In PB dietary scenarios from Australia, the complete replacement of meat and milk led to more undesirable changes in protein intake of > 5 % from baseline diet for young children (2 to 3 years), adults (19 to 30 years), and older adults (71 years) (19). It was unclear if these changes were below NRVs, but in scenarios with smaller amounts of replacements, non-significant decreases in protein intake were observed. Protein inadequacy was reported to increase when red and processed meat, but not dairy, was substituted with plant protein foods, although such replacements improved life expectancy (255). This observation thus highlights the need to consider the emergence of trade-offs among nutrient adequacy, health outcomes and environmental impacts when different ASFs are replaced by PB foods.

Overall, a complete transition to PB dietary patterns reduced protein intake in the studies and may increase the risk of deficiency compared with country-specific NRVs. Protein sufficiency from diets where only smaller proportions of animal protein were substituted is further supported by evidence from an Indonesian study (237) (presented in **Table 2.6**). Three increasingly PB diet scenarios (no-dairy diet, fish-and-poultry diet, low-food-chain diet) were designed to be culturally aligned and tailored to the health concerns of the Indonesian population. Improved nutritional compositions were evident in these modelled diets compared with the current Indonesian diet, which provides excessive energy from rice and has poorer diet diversity and nutrient density (237). While lower protein intake was observed in these PB diets, it was not markedly different from the current diet. Most of the protein provided by the PB diet scenarios was from pulses, nuts and seeds.

It is important to note that protein intake adequacy was likely achievable in the Indonesian dietary scenarios because staples and protein-rich foods were adjusted to meet protein criteria, and the absolute quantity of protein was set to 69 g/day for an average individual, in alignment with dietary guidance of 10 to 15 % of energy from protein consumption per day (237, 257). However, many PSFs have poorer protein quality due to at least one limiting AA - lysine from grains and SAAs from legumes (258). A single average score for IAAs was provided for the Indonesian scenarios, and a score of > 100 showed that the requirement for all IAAs was achieved for an average individual (237). The accuracy of this summation, however, remains unknown because AA profiles and bioavailability differ among foods (259).

Nutritional outcomes from dietary models depend on the reference diets used as the comparison. Another study found a meaningful decrease in protein intake when there was a 30 g and 50 g substitution of meat for legume intake only in males, but not in females (8). Such a differentiated outcome between the sexes was also observed in a simulated diet for Finnish adults (252) (Presented in **Table 2.6**). As baseline meat consumption is typically higher in males than in females, comparable reductions in meat intake may result in smaller relative declines in total protein intake among females than males (8, 252). In a dairy-free vegetarian and vegan dietary model for US males and females (≥ 19 years), soy milk, soy products and fortified soy milk were food items chosen, but these were not preferred milk alternatives among US individuals (249). These scenarios show that modelled nutrient outcomes may not represent the population’s diet (249) and deviate from the habitual intake. In determining the proportion of plant protein required within daily food quantity ranges for older adults, Fouillet et al. (2025) (238) (**Table 2.6**) identified legumes, nuts, fruit and vegetables as key dietary levers for achieving nutrient adequacy when PB diets exceed 75 %

of plant protein. However, meeting these targets likely requires consumption levels that surpass the assumed food consumption limits of the population.

2.4.2 Diet quality of simulated PB diets

Diet quality indices are often used to assess the healthiness of PB dietary patterns (264), and were used in some models **(Table 2.3**). One example is the Healthy Eating Index Score-2015 (HEI-2015), which was utilised to determine the diet quality of ovo-vegetarian and vegan diets, relative to the Healthy Vegetarian Dietary Pattern (HVDP) among healthy US adults (253). The HEI is a 100-point metric system that measures adherence of these diets to the Dietary Guidelines of Americans (DGA) (20, 265). A HEI-2015 score of 100 % was attained for protein intake, and protein was within the AMDR (10 to 35 %) in both ovo-vegetarian (16 %) and vegan diets (15 %) (253).

Another study on US adults analysing PB diets using the HEI-2015 similarly showed higher HEI scores with decreased intake of animal protein, with the highest HEI score from vegetarian diets (20). However, HEI as a diet quality metric favours seafood, plant protein, dairy and foods which are elevated in unsaturated fatty acids, and penalises saturated fatty acids (SFAs), added sugars, and sodium (20). It should not be seen as a tool solely for assessing the adequacy of protein in diets. Furthermore, plant proteins and seafood were combined into a single group for analysis when a vegan or vegetarian diet should not contain any seafood (253).

Table 2.3 Dietary models using diet quality measurements

Reference	Study Aim and Target Population	Simulation Design	Protein Assessment
1. Vatanparast et al. (2020) (247)	Effects of increasing plant proteins with decreasing processed meat in Canadians aged 1 year and above	Increase currently consumed plant proteins from legumes, nuts, and seeds by 100 % while halving red and processed meat consumption	At baseline, plant foods contributed lower protein than red and processed meat. Doubling consumption of PBMAs and halving intake of red and processed meat led to a significant decrease in protein: 78 ± 0.6 g/day to 73 ± 0.6 g/day ($P < 0.05$). Higher NRF score in modelled diet (from 517 to 526, $P < 0.05$)
2. Hess and Comeau (2022) (250)	Test nutritional adequacy and diet quality of ovo-vegetarian and vegan	Five-day menus for ovo-vegetarian and vegan diet of 2000 kcal	Protein was 15 % (AMDR 10 to 35 %) for vegans and 16 % for ovo-vegetarians. The average protein intake was 74 g per day for ovo-veg and

	diet models in American adults		72 g for vegan. Perfect HEI-2015 scores (100 %) for protein intake
3. Salomé et al. (2022) (262)	Impact of optimised meat substitute, made of pulses and minimally processed ingredients, on French adults	One optimised meat substitute and 43 existing meat substitutes with constraints set for nutritional, technological, and inter-individual variability	6-point increase in PANDiet score in replacing meat with optimised meat substitute. A quarter of the meat substitutes increased the PANDiet score above the observed diet value. Protein intake remains unchanged between all modelled and reference diets
4. Vasiloglou et al. (2023) (20)	Simulate meal plans with different levels of animal protein reduction in US adults (31 to 50 years old), and assess diet quality	MILP to create 100 7-day meal plans (breakfast, lunch, dinner, snack) for US reference diet, vegetarian, pescatarian, flexitarian diets of 25 %, 50 % and 75 %	Highest animal protein foods found in US reference diet, higher meat, and poultry intake for males than females in all models. More contribution of egg protein in pescatarian and vegetarian models for both sexes. The highest contribution of protein was from seafood in the pescatarian model. The highest contribution of protein was from dairy in vegetarian and pescatarian models
5. Aidoo et al. (2023) (263)	Build composite diet scenarios between current and optimal scenarios by partially replacing meats with legumes to identify environmental and nutritional trade-offs in the PB diet transition in the US	Food4HealthyLife Calculator used to derive composite diets, with the use of trade-off analysis to identify the optimal composite diet scenario	Nutritional quality as measured by Food Compass and HENI score increased from current to optimal diet Contribution of protein to Food Compass score from current to optimal diet scenario varied minimally. Diet scenario with 76 % of total meat replaced with legumes (10 % legumes, < 1 % red meat, < 3 % white meat) provided high nutritional quality with half the environmental impact of the current diet

PBMAs, Plant-Based Meat Alternatives; NRF, Nutrient Rich Food; AMDR, Acceptable Macronutrient Distribution Range; HEI, Healthy Eating Index; PANDiet, Probability of Adequate Nutrient Intake; MILP, Mixed Integer Linear Programming; HENI, Health Nutritional Index; PB, Plant-Based

While protein intake decreased in PB diet scenarios for a Canadian population, scores for the NRF index increased (247) **(Table 2.3)**. NRF scores are calculated with similar beneficial and detrimental nutrient patterns to HEI (247). These indices may provide a gauge on the overall quality of diets based on the summation of composite nutrients, but provide less information about the contributing weightings and bioavailability of each nutrient towards the overall nutritional value of food (264).

Possibly due to the varied scope and focus of these studies, few have accounted for AA composition in their analysis. Hence, the bioavailability of protein in the above PB scenarios has not been examined in detail. The consideration of protein quality in PB diets is therefore of greater importance than accounting for protein intake alone in populations that are generally not limited in gross protein intake. Given that meat and dairy alternatives are often marketed as sustainable and healthy, the nutritional value of PBAs must be established. This is especially crucial if current formulations of PBAs are designed to simply emulate the appearance, texture, taste and comparable protein content of meat, without considerations of protein quality and micronutrient content (262, 265).

2.4.3 Novel PBAs in simulated PB diets

Table 2.4 summarises findings from several studies examining the protein intake and overall diet quality following the substitution of meat and dairy with novel PB foods. Overall, these substitutions were generally associated with reduced protein intake, except when soy-based alternatives were used. Diet quality tended to improve alongside increased consumption of plant foods, but the extent of improvement depended on the type of PBA. Cereal-based and sweetened products tended to contribute less consistently to diet quality improvements.

Table 2.4 Dietary models evaluating protein contribution by PBAs

Reference	Study Aim and Target Population	Simulation Design	Protein Assessment
1. Farsi et al. (2022) (248)	Evaluate nutritional implications of complete and partial meat replacements, and with different alternative products, as compared to the current intake in the UK adult population	Model 1: Replacement with 25 %, 50 %, 75 % and 100 % in quantity Model 2: 100 % replacement of meat with alternatives of varied ingredients (vegetable, mycoprotein, legume, tofu, nut, soya) Model 3: 100 % replacement with fortified and unfortified alternatives	Model 1: Graded decrease in protein intake with increasing substitution Model 2: Significant reduction of protein intake across all alternatives, but particularly from nut, vegetable, and legume Model 3: Fortification results in smaller projected reduction in total protein intake from current intakes

2. Salomé et al. (2021) (240)	Assess how nutritional impact varies with different plant substitutes in a French adult population (18 to 79 years)	Model 1: Meat substitution with 56 PBAs Model 2: Milk substitution model with 16 PBAs Model 3: Dairy dessert substitution with 24 PBAs	Model 1: Significant increase in PANDiet score for soy and pulse-based substitutes but significant decrease with cereal-based substitutes Model 2: Diet quality score for protein decreased with sweet PB milk, cereal, and nut-based milk Model 3: Diet quality score for protein improved with fortified and soy-based products but significantly decreased in non-soy-based substitutes
3. Temme et al. (2015) (236)	Model the impact of diets with less or no meat and dairy on nutrient intakes in Dutch children (2 to 6 years old) compared with reference diet	Model 1: 30 % replacement Model 2: No meat and dairy	Model 1: 3 % decrease in total protein intake but 0 % of population consuming below EAR levels Model 2: 8 % decrease in total protein intake but 0 % of population consuming below EAR levels
4. Tso and Forde (2021) (266)	Determine if replacement of animal products with novel PBMA and dairy alternatives will lead to equivalent nutrient intakes when compared to standard omnivorous diet in a US adult population	Flexitarian, vegetarian and vegan diets where animal foods were substituted with whole plant foods (Traditional) or with PBMAs, coconut- or soy-based dairy alternatives (Novel)	Similar protein intake in flexitarian, vegetarian and vegan diets as compared to reference diet. Protein intake met dietary guidelines for all diets. PBMAs and dairy alternatives were matched for protein intake with the other diets
5. Lawrence et al. (2023) (19)	Estimate nutritional implications of substituting meat and dairy milk with alternatives in Australian population of young children (2 to 3 years), adults (19 to 30 years) and older adults (71 years and older)	1. Total replacement: increase of PB 'meat' by 42 g/person/day and PB 'milk' by 1 L/person/day 2. Partial replacement: increase of PB 'meat' by 6.5 g/person/day for and PB 'milk' by 6 g/person/day	Substantial decrease in protein intake for total replacement scenarios, of 8 % for young children but not for other groups. Insignificant decrease for partial replacement scenarios PB meat products have lower protein than beef Predicted 2 to 10 % decrease in protein intake for total replacement scenarios for all populations
6. Marchese et al. (2024) (251)	Model the impact of partial and complete substitution of meat and dairy with equivalent weight of	Model 1: Replace 50 % of ASFs with traditional PB foods	Protein intake decreased across all sex and age groups but were still above the EAR, largely because baseline

	traditional or novel PBAs on diet quality (Dietary Guideline Index) and nutrient adequacy in Australian adults	Model 2: Replace 100 % of ASFs with traditional PB foods Model 3: Replace 50 % of ASFs with same weight of traditional and novel PB mixture Model 4: Replace 100 % of ASFs with same weight of traditional and novel PB mixture	intakes were at 127 to 225 % of the EAR The diet quality increased across all models for all sex and age groups.

PB, Plant-Based; PBAs, Plant-Based Alternatives; PANDiet, Probability of Adequate Nutrient Intake; EAR, Estimated Average Requirement; PBMAs, Plant-Based Meat Alternatives; ASFs, Animal-Sourced Foods

Substituting meat and dairy from a reference US omnivorous diet with PBAs led to reduced protein contribution and inadvertent consequences related to increased energy and sodium intake due to the inclusion of these UPFs (266). In the case of vegan cheese, coconut-based yoghurts, and ice cream, a higher intake of fat and a lower intake of protein were observed (266). In comparison, the inclusion of eggs and dairy in a traditional flexitarian model did not face the same nutritional outcomes (266). The study did not make any associations between the compositions of each alternative and its protein contribution. In contrast, in the dietary scenarios of French adults, the type of ingredient used in each substitute was evaluated in the calculation of the diet quality score using the Probability of Adequate Nutrient Intake (PANdiet) (240).

The PANDiet study showed that diet quality depended on the type of plant substitutes used. For example, scores increased significantly ($P < 0.0001$) when substitutions were soy-based products (+ 0.57), tofu, tempeh or soy protein (+ 1.58), but decreased with PB sausages (- 0.60) and cereal-based foods (- 0.72) (240). Similar improvements in diet quality were observed for milk and dairy substitutes that were derived from soy products (240). For example, for dairy dessert substitutes, PANDiet scores significantly improved with soy-based substitutes (+ 0.06, $P < 0.0001$) and calcium-fortified plain PB desserts (+ 1.35, $P < 0.0001$) but decreased significantly for PB mousses (- 2.85, $P < 0.0001$), and non-soy-based substitutes (+ 1.31, $P < 0.0001$) (240). These findings indicate that the implications of substitution will vary depending on the ingredients used to formulate substitutes.

In a further evaluation, meat was substituted with an equivalent quantity of pulse-based replacement (262). This meat replacement was then optimised to meet the nutritional requirements of French adults, utilised in one diet model, and compared with other market-derived meat substitutes in 43 other diet models. The exclusion of soy products (tofu or textured soy protein) resulted in a lower protein content (8.5 g/100g) than that of meat and other meat substitutes on the market, highlighting the benefit of soy products as a protein-dense substitute. Soy-based PBMAs are often formulated with processed soy products such as isolates, textured soy and concentrates, which can provide for higher protein content and quality if utilised in a meat substitute (267). Furthermore, soy was found to have the highest DIAAS score among plant proteins (12), and is a good source of lysine (268). However, the digestibility and long-term health effects of these newly formulated soy products warrant further research, as do their acceptability in cultures where soy is not traditionally consumed.

Further evidence was provided when examining the replacement of meat with different varieties and quantities of PBAs (248). As compared to meat, the greatest projected decrease in protein intake was from the nut source (- 19.79 g/day; $P \leq 0.001$), followed by vegetable (- 19.33 g/day; $P \leq 0.001$), then legume (- 15.43 g/day, $P \leq 0.001$) (248). Although tofu, which is soy-based, had a significant reduction of total daily protein intake (- 4.87 g/day; $P \leq 0.001$) (248), this reduction was smaller than the other alternatives, further supporting soy products as a better PB option for protein contribution. However, the models still included eggs and dairy, so the loss of 20 g/day of protein was exclusively due to the removal of meat from the diets. This large reduction could result in negative consequences, especially for more vulnerable age groups (very young and very aged) (248), in the absence of careful diet planning (269).

A few simulation studies have compared the implications of gradual increments or intake frequency of ultra-processed PBAs in diets represented in this review (19, 236, 248, 251). A study in Australia showed that a partial replacement of meat and milk with PBAs resulted in a small reduction in protein intake, with less than a 5 % difference in nutritional intake from baseline diets, compared to complete replacement (19). These findings were corroborated in another study, in which the complete replacement of meat and dairy with PBAs in children aged 2 to 6 led to lower protein intakes compared to baseline diets or partial replacement (236). However, these differences were only significant among boys when complete replacement was compared to baseline. When compared to the EAR, dietary protein still met the requirements for all age-sex categories (236).

Interestingly, the presence of novel PBMAs in replacement scenarios resulted in higher protein adequacy as compared to when only traditional PB foods - including the protein-dense legumes - were used (251, 256). This further highlight the potential benefits of incorporating PBMAs to ensure high protein intake. However, when compared to the baseline diet, all models had reductions in the percentage meeting protein adequacy (251). Based on the replacement models of graded increase from 25 % to 100 % in the UK population, significant reductions in protein intake ($P < 0.05$) were already observed at the 25 % replacement (248). A Canadian study that modelled the nutritional impacts of increasing PBMAs and decreasing red and processed meat intake also found a significant reduction in protein (77.8 ± 0.6 g to 73.4 ± 0.6 g) when PBAs were doubled while red and processed meat were halved. However, the NRF score, which reflects diet quality (247) significantly increased (517.4 to 525.8, $P < 0.05$) in the simulated diet. This could suggest an improvement in overall nutritional value when reducing red and processed meats and increasing PBA, even if protein decreases.

There are contrasting views on the nutritional and health outcomes of PBAs. While moderate replacements of red and processed meat with traditional plant proteins may be beneficial, the same may not be true of PB substitutes, due to a higher quantity of sodium and sugars, and poorer provision of protein from some types of substitutes (248, 266, 270, 271). This point was raised by Marchese et al. (2025), who found greatest improvements in diet quality with traditional PB food substitutions, but only small increments when novel PBAs were used (251). These nuanced outcomes are further influenced by consumer choice, including the selection of, and the presence of, fortification, and the frequency and quantity of both traditional and processed PBAs. These factors lead to complexities in achieving a balanced PB diet. While nutritional risks may exist if diets contain a high proportion of these processed products (266), smaller or occasional consumption may not produce the same effects and therefore require closer investigation, not only for protein intake but for other nutrients. Higher replacement quantities may incur more negative implications, not only for protein intake but also for other nutrients, depending on the ingredient composition, fortification level, and the prevalence of consumption of these foods.

The lack of clinical evidence on the impacts and long-term health consequences of PBAs (180, 272) means that analysis of their nutritional composition is required before they can be recommended as a staple in meals or a complete replacement of ASFs. Compared with a vegan diet high in whole fruits and vegetables, one that includes consistent intake of processed PB products high in sodium and SFA may lead to adverse outcomes. This is particularly true for older adults who are more susceptible to risk factors for hypertension (273). More research is also needed to consider both the IAA composition and digestibility of PBAs, thereby establishing their sufficiency as a dietary source of protein. These were areas not analysed by most studies in this review, apart from one (274) and require experimental validation.

2.4.4 Simulated diets for special populations

The dietary transition to PB diets must involve the consideration of individual variability and examine the impact on groups who have different nutritional needs (19, 57). The current protein intake recommendation of 0.8 g/kg/day (49) is based on NB studies in healthy young males to avoid loss of lean body mass (243) and prevent deficiency (275). Protein intake based on this requirement needs to be high quality, with DIAAS of 100 or more (276). 0.8 g/kg/day is likely inadequate for populations with higher protein demands, such as pregnant and lactating females (16, 276), infants and young children (16), and older adults (16, 246). Higher protein intake is also more difficult to achieve on a PB diet, and certain micronutrients are at risk of deficiency. For example, Fouillet et al. (2025) demonstrated that vitamin B12, riboflavin, EPA and DHA, rather than total protein, became limiting in a 100 % PB diet for older adults, even with high intake limits for legumes and nuts (238). It therefore becomes imperative that fortified foods and supplementation be included in vegan diets to avoid such consequences.

The limiting IAA of protein sources can vary depending on the age of the consumer, due to differing IAA requirements (57). While most limiting IAAs for children, adolescents and adults are lysine, methionine and cysteine (SAAs), it is phenylalanine and tyrosine that are more commonly limiting among infants (57). In contrast, leucine, the BCAA that is important for MPS, may be more limiting for older adults (18, 243) who experience increased loss of skeletal muscle mass and higher resistance to postprandial muscle protein metabolism (130, 227, 258, 277). The risks of protein and AA deficiency in older adults become more pronounced in PB diets because plant proteins may not induce the same intensity of MPS as ASPs (277).

Among the special populations, the replacement of ASFs with PSFs is more commonly examined in children and older adults, with limited investigation in pregnancy, lactation, and disease states. Examples of protein inadequacy were simulated by replacing animal foods with habitually consumed plant foods among US children, adolescents, and older adults **(Table 2.5)**. In particular, the proportion of children not meeting the EAR for protein tripled as compared to baseline (1.9 % ± 0.3 to 5.6 % ± 0.6) in scenarios of complete replacement (241). A reduction of 10 g/day of protein intake was observed among adolescent girls (63.7 g ± 0.9 to 54.0 g ± 1.0) in scenarios of complete replacement (242). These observations were attributed to inadequate consumption of high-protein plant foods, such as LNS and soy, and to higher consumption of grain-based foods (243), which have higher energy-to-protein ratios than LNS (253). These findings illustrate that shifts toward a lower-protein-quality PB diet, based large on grain-based foods, rather than LNS could increase the risk of protein deficiency for these populations.

Table 2.5 Dietary models evaluating protein requirements in special populations

Reference	Study Aim and Target Population	Simulation Design	Protein Assessment
1. Cifelli et al. (2016) (241)	Determine nutrient intake and adequacy in American children (2 to 18 years old) and adults (19 + years old), with increasing plant foods or dairy, as compared to usual intake (baseline)	Model 1: 100 % increase in currently consumed PB foods with a corresponding decrease in animal foods Model 2: 100 % increase of protein-rich plant-based foods (e.g., legumes) with a corresponding decrease in animal foods Model 3: Doubling currently consumed dairy products	Model 1 showed a decrease in protein intake across all groups, tripling the number of 2 to 18-year-olds below EAR and a 321 % increase in 19 + year-olds below EAR. Model 2 showed no difference in macronutrient intake or EAR between the two groups compared to baseline. Model 3 showed increased intake of energy, protein and SFA with decreased % of individuals in both groups below protein EAR.
2. Demmer et al. (2017) (242)	Determine energy and nutrient adequacy in American adolescent girls (9 to 18 years old) with increasing plant foods or dairy, as compared to baseline	Identical models as Cifelli et al. (2016)	Model 1 showed a decrease in protein intake with a 3 x increase in % not meeting the EAR for protein. Model 2 showed no differences in meeting macronutrient and EAR targets between both groups and the baseline. Model 3 showed an increase in intake of energy, protein and SFA with decrease in the % of individuals in both groups below protein EAR.
3. Houchins et al. (2017) (243)	Determine protein intake in older Americans of 51 to 70 years and above 70 years when increasing PB foods or dairy products, as compared to baseline	Identical models as Cifelli et al. (2016)	Protein-rich plant foods were not a major component of the baseline diet; dairy intake was half the recommended intake. Model 1 showed a decrease in total protein intake and an increase in % below EAR for both age groups and sexes. Model 2 showed no meaningful change in protein intake as compared to baseline for both age groups and sexes.
4. Borkent et al. (2024) (124)	Assess how transitioning towards a more PB diet impacts	Two flexitarian models with 40 % and 80 % of meat replaced, one pescetarian, one	The difference in mean daily protein intake between baseline and simulations was

	protein availability in Dutch older adults	vegetarian and one vegan.	small but decreased by 35 % in the vegan diet scenario.
		PBMAs were included as food options in the PB scenarios	Utilisable protein intake was 15 % lower than intake based on digestible protein in the vegan scenario.
			Utilisable protein intake decreased by 28 g in the vegan scenario compared to the original diet, and 80 % of older adults had an intake below the adjusted EAR.

EAR, Estimated average Requirement; SFA, Saturated fatty Acid; PB, Plant-based

As addressed in the Indonesian study (237), the nutritional needs of some individuals may not be met in diets lower in animal protein, which provide valuable nutrients beyond protein, needed in elevated quantities during growth stages of life. To meet specific protein and micronutrient (iron) requirements for adolescent and lactating females, the complete elimination of meat was unachievable in optimised diets, as more diverse protein sources (including ASFs such as red meat and poultry, totalling up to 150 g of protein per day) were required (237). A similar outcome was observed in another diet optimised for nutrition, where the relative contribution of animal protein had to be higher for females than males, to meet higher iron requirements (278).

The US studies modelled dietary scenarios of increasing PB foods and dairy products on nutrient intake in several demographic groups: older adults (51 to 70 years, and 71 years and older) (243), adults and children (241), and adolescent girls of 9 to 18 years (242) and compared these results with the baseline diets. Doubling dairy consumption to meet the required servings for each age group per day showed marked improvements in protein intake and reductions in the proportion of the population that was consuming below the EAR (241-243). For example, among older adults, the dairy model increased protein intake by 12 %, and the proportion falling below EAR decreased to 1 % (243). However, the concomitant decrease in energy intake from nutrient-poor foods to displace higher caloric intake from dairy was not modelled, resulting in a lack of information about energy balance (243).

Milk is an important provider of not only protein and IAA, but also other essential nutrients (279, 280). ASPs such as lean meat and milk are protein-dense whole foods that supply a higher proportion of absorbable AAs as compared to PSPs, to efficiently fulfil the physiological requirements of humans (55, 148, 149). Indeed, the authors of this US study highlighted the importance of dairy products to supply critical nutrients. For example, 18 % of protein could be provided by two daily servings of dairy products (milk, cheese and yoghurt), alongside small proportions of caloric intake (10 %), total fat (14 %), SFA (26 %) and sodium (11 %) (241). Dairy is thus valuable as a FG to efficiently contribute vital nutrients, particularly for certain populations (older adults and young children) who may experience difficulties with the lower digestibility of plant proteins (281). These benefits are not available in a vegan diet.

The decrease in protein intake from milk was more pronounced among Australian young children (2 to 3 years) under scenarios of replacement, with an estimated 7.4 % decrease in dietary protein if all dairy milk was replaced with PB 'milk' comprising 47.6 % soy, 44.2 % almond and 8.2 % 'other', with no changes to the original intake of meat (19). Alongside this decrease was the reduction of other vital nutrients (e.g. vitamin B12, calcium and iodine), highlighting challenges in meeting the requirements of these micronutrients when ASPs are excluded from the diet (282). These results may carry more adverse nutritional implications for populations with elevated nutrient needs.

In two other PB scenarios on young children in the Dutch population, it was observed that while partial and complete replacement of meat with plant alternatives led to no differences in meeting age- and sex-specific EAR, the authors had assumed sufficient intake of protein would lead to adequate provision and balance of EAAs (236). However, this conclusion may be erroneous without protein quality analysis. In corroboration, several studies have found evidence of poor protein and AA content in PBBs, which varied greatly depending on the ingredient (283) and consumer behaviour (shaken or unshaken containers) (198). Almond and oat milk were found to have less than half the protein of soy, goat and cow's milk (284). In an analysis of 148 PBBs, only 26 % of PBBs had at least 5 g of protein per serving, and these were beverages formulated with soy and pea protein (283).

2.4.5 Protein quality in simulated PB dietary scenarios

Protein quality measurements consider the quantity and composition of IAAs in the protein source, and the digestibility and physiological utilisation of each AA (44, 140, 258, 276). To improve dietary protein quality in vegetarian and vegan diets, different protein sources with complementary IAA profiles can be combined in meals (12, 285) or be consumed in higher quantities to increase AA uptake in the plasma (152).

Few studies have analysed the quantity of IAAs in PB dietary scenarios **(Table 2.6)**. Three studies optimising diets for French adults (238, 253, 286) found that protein was never the limiting factor for either sex in all three models, even though total protein decreased in optimised diets with lower ASFs (286). Instead, micronutrient content was the deciding factor in final diet composition.

Table 2.6 Dietary models evaluating protein quality

Reference	Study Aim and Target Population	Simulation Design	Protein Assessment
1. De Gavelle et al. (2017) (253)	Assess sufficiency for protein and lysine in association with varying plant: animal protein intake, achieved with different methods of replacing animal with plant foods, for French adults	Model P: Replacement of ASFs with the same quantity of PSPs Model A: Replacement of ASFs with the same energy from habitually consumed PSPs Model B: 100 % isocaloric replacement with LNS, with intermediate models adjusting the proportion of high-protein PSPs and habitual PSPs: 0 to 100 %	Protein and AA intake met population requirements at baseline. Model P: Increment in energy intake of ~ 300 kcal per 10 % increase in % of PSP Model A: Increased probability of protein inadequacy (5 % and 50 % for PSP of 50 % and 85 %, respectively). Lysine inadequacy increased with increasing PSP % Model B: The greater the proportion of LNS, the lower the prevalence of inadequacy for protein and lysine
2. Barre et al. (2018) (286)	Assess the impact of nutrient bioavailability in 3 modelled sustainable diets in French adults	Diet optimisation departing least from the observed diet	Food composition was not altered when protein bioavailability was accounted for. Protein was never limiting in either males or females. Overall protein digestibility: 95 % for both males and females; total protein was 117 % of the RDA for females and 122 % for males; PDCAAS was 112 % for females and 116 % for males.
3. de Pee et al. (2021) (237)	Determine how alternative dietary scenarios compare to current food patterns in Indonesia for nutrient content, contribution to climate change, and cost	1) No-dairy diet 2) Fish and poultry diet 3) Low food-chain diet 4) EAT-Lancet diet 5) Optimised to individual nutrient needs within a household 6) Current diet	Optimised diets: a) met requirements for all nutrients and IAA b) higher proportion of ASFs for growing children, and adult males c) higher cost and higher GHGE diets for adolescent girls and lactating females compared to males due to higher ASFs.

4. Marinangeli et al. (2022) (254)	1. Assess protein sources, intake, quality and cost across quartiles of protein intake for US adults (≥ 19 years old) 2. Model effects of replacing AA from grain protein sources with non-grain plant proteins across quartiles	1. Increasing intakes of PSPs across four quartiles 2. Replacing 25 % and 50 % of AAs from grains with an equivalent amount from legumes, lentils and other non-grain plant proteins	1. IAA score and PDCAAS were lower across increasing quartiles ($P_{quartile\ trend}$ < 0.05). Total protein intake and PDCAAS decreased with increasing PSP intake 2. Effect of replacing AAs for legumes and lentils: Quartiles 3 and 4 had the greatest improvements in IAA and PDCAAS scores. Absence of protein complementation led to 25 % decrease in PDCAAS of diets from the lowest to the highest defined level
5. Simojoki et al. (2023) (252)	Assess protein (g/kg/day) and IAA intake across genders and age groups in Finnish adults after partial replacement of red and processed meat with legumes or cereals, compared to the reference diet	Six replacement scenarios to replace meat that exceeded 70 g/d and 30 g/d with an equivalent amount of legumes, cereals or their combination	Decreases in protein and IAA intakes with greatest impact on adult males 70 g scenario: Decrease in protein, histidine and lysine (cereal and combination); decrease in histidine, lysine and SAAs (legume) 30 g scenario: Decrease in protein and all IAAs (but not AAAs)
6. Fouillet et al. (2025) (238)	Assess the extent to which % plant protein can be increased in older French adults relative to two different protein requirement levels, and identify dietary levers for increasing % of plant protein	Diet optimisation to model diets with increasing % of plant protein while constraining for energy and nutrient intakes	Within the nutrient-adequate and healthy % plant protein range (up to 70 %), protein intake meets the 0.83 g/kg/day requirement, but below the 1 g/kg/day, particularly for males Intakes of all IAA were always above the 98 % safe intake threshold in all cases. Legumes and nuts were important dietary levers but arrived at the maximum acceptable intake at 25 % of plant protein.
7. Wanders et al. (2025) (274)	Assess the impact on protein adequacy for the Dutch adult population when animal meat is replaced with PBMA	Model 1: No protein replacement scenario, where all meat is removed Model 2: Replacing all meat with PBMA Model 3: Increase the total protein content of	Model 1 had the lowest protein adequacy of 85 % and only 50 % of utilisable protein adequacy. 25 % mycoprotein, soy and wheat scenarios had the highest adequacy of 100 % and high utilisable protein > 92 %

	PBMA to be similar to meat	10 and 25 % wheat scenarios had lower utilisable protein
	Model 4: Lowered the digestibility of the current PBMA to 75 %	Lysine was the main limiting IAA in all meals except dinner, for all scenarios

ASF, Animal Sourced Food; PSP, Plant Sourced Protein; AA, Amino Acid; LNS, Legumes, nuts and seeds; RDA, Recommended Dietary Allowance; PDCAAS, Protein Digestibility Corrected Amino Acid Score; IAA, Indispensable Amino Acid; GHGE, Greenhouse Gas Emission; SAA; sulphur amino acids; AAA, Aromatic Amino Acid; PBMA; Plant-Based Meat Alternative

Lysine adequacy is the key challenge with higher intake of plant foods, as shown in some studies listed in **Table 2.6**. Lysine inadequacy exceeded protein inadequacy when plant protein constituted > 70 % of protein intake (253). Lysine contribution from habitually consumed cereal foods within the population is relatively poor (243, 253, 279). Different outcomes were attained when animal foods were replaced with high-protein plant foods in the form of LNS (253). Due to their higher protein density compared to cereals, greater inclusion of these plant protein sources in energy-adjusted diets would allow higher overall plant protein contributions without increasing the probability of protein inadequacy (253). Such outcomes were not emulated in the US studies (241-243) when LNS were used to replace animal foods, as the model setup was constrained by low LNS consumption in the baseline diet. Further increments would have been unrealistic projections of dietary and energy intake and would have deviated greatly from the population's habitual diet (243, 253).

In another US study utilising the same population database (254), protein quality as measured by IAA score and the PDCAAS, calculated from intakes of each IAA per gram of protein (138), generally decreased across increasing quartiles of plant protein intake. However, in models where 25 % and 50 % of AAs from grains were replaced with those from legumes, lentils and non-grain plant protein, protein quality improved. These results thus corroborate conclusions that protein quality in PB diets improves when animal proteins are substituted with LNS rather than with grains (253).

PDCAAS was used in some studies (254, 274, 286) as a protein quality metric, while a small generic penalty was applied in another study to emulate reduced digestibility of IAAs (238). These approaches may overestimate protein quality. The limitations of PDCAAS have been widely highlighted in the literature, including the truncation of protein quality scores and determination of digestibility at the protein rather than IAA level (44, 105, 259). Current methods in the simulation studies reviewed here (8, 241-243, 246, 248) rarely account for protein quality in complementary sources of protein (258) and instead focus on comparing protein adequacy in isolated foods, limiting representation of true protein quality from mixed diets. Biases are introduced in the selection of plant protein in each simulation model when plant foods have different AA profiles (e.g., grains compared to soy or legumes). Combining plant proteins in a meal (e.g. grains and lentils) can overcome the differing limiting AAs of each plant source and improve protein quality, making it comparable to the AA profile of animal proteins (258, 285). Future simulation studies should address this limitation by simulating mixed-diet scenarios and accounting for overall protein quality measurements in mixed-diet settings.

2.5 Conclusions and future directions

Section I of the literature review provided insight into the complexities of adopting vegan diets. The key challenge lies in the body's poorer utilisation of plant protein sources compared to an ASF of equivalent food weight or energy content. Diets containing PB foods with lower protein quality necessitate higher food quantities, which can increase the proportion of protein calories in the diet with a consequence of imbalances in contribution of energy from fats and carbohydrates (287). This is more prevalent in the case of populations with higher protein requirements but lower energy requirements (e.g., older adults). The current understanding of the long-term feasibility of vegan diets, especially in vulnerable populations, is hampered by limited studies determining the effects of prolonged vegan diets on metabolic functions in these populations (10, 18). Several studies conflate vegan results with less restrictive PB diets, which are unlikely to incur similar metabolic repercussions as vegan diets. It is most informative to specifically examine vegan diets for protein adequacy and quality, because all protein sources are plant-derived, and assessments will therefore reveal whether exclusive reliance on PB foods can still meet IAA requirements. The development of targeted solutions to close gaps in protein quality needs to be a central goal of vegan diet research, including this present work, to inform evidence-based dietary guidelines.

Section II of the literature review outlined the main nutritional outcomes of protein as a consequence of animal-to-plant protein substitution. Protein intake from PB diets was examined in all studies, but protein quality was not the focus for many. Novel PBAs are promising foods that can help close nutrient gaps. As the risks of deficiencies for utilisable protein and many micronutrients are elevated in certain populations, the inclusion of PBAs may be a pivotal strategy to support these needs. However, dietary scenarios incorporating novel PBAs demonstrate inconsistencies in protein intake, protein quality and overall diet quality due to variability in their formulated ingredients.

Apart from protein, the addition of PBAs into the diet needs to consider balanced micronutrient profiles, including limitations on saturated fat, added sugar and sodium, if healthy PB diets are to be achieved. While soy appears to be the best plant protein for inclusion, a combination of plant foods is optimal to accommodate the limiting AA of different plant sources. This is an underexplored area in the dietary simulation literature. Furthermore, the choice of a balanced PB diet is highly dependent on the consumer. For example, a PB diet higher in LNS will provide more suitable AA profile than one that is higher in refined grains. There is therefore a clear gap in characterising FG patterns associated with higher protein quality in completely PB diets – this evidence provides important dietary guidance for vegan consumers.

Distributing protein-dense foods across meals and snacks throughout the day is another key strategy to meet the daily protein requirements without necessitating excessive energy or dietary fibre intake. The metabolic use of IAAs depends on their synchronised availability within a narrow time window, and since IAAs are not stored in the body, the combined provision of IAAs from complementary plant foods within individual meals is highly relevant for vegans. Examination of protein quality at the meal level remains relatively unexplored in vegan diet research. Emerging evidence suggests that meals with more balanced protein distribution or greater protein intake earlier in the day are associated with a higher likelihood of achieving daily protein adequacy (165, 233). This highlights opportunities for meal design and identifying optimal plant FGs that collectively supply a complete AA profile.

2.6 Aims of the thesis

Given the available evidence, it remains unclear whether vegan diets can adequately meet protein quality requirements at the population level. If protein quality represents a potential nutritional challenge, then strategies must be developed to support consumers who choose a vegan diet, so that the associated health and environmental benefits can be realised without compromising protein and IAA adequacy. While the existing evidence presented in this literature review outlines the challenges of protein quality in plant proteins, it remains unclear to what extent combinations of different PB foods consumed in mixed meals in real-world eating patterns can compensate for these limitations. Addressing this knowledge gap is central to understanding whether protein quality constraints in vegan diets represent a practical nutritional concern or one that can be effectively mitigated through appropriate food selection and dietary design.

In the research portion of this thesis, the first aim is to evaluate the protein adequacy and protein quality in the diets of a NZ vegan cohort. Establishing the extent of any inadequacies, if any, is a necessary step before the second aim of identifying strategies to improve protein quality in the diet of the target population. The research chapters are presented in two major parts. Part I describes the protein adequacy and protein quality of vegan diets in NZ. Part II focuses on solution-driven approaches to optimise meals and daily diets of New Zealand vegans and determine whether protein adequacy and protein quality can be achieved in well-planned vegan diets.

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Part I
Investigating the intake of total protein and protein quality in current New Zealand vegan diets

By identifying the extent and nature of any protein and IAA inadequacies in vegan diets against the requirements, this part of the thesis serves as a foundational step to establish the evidence base required to inform subsequent analyses. These findings are used to identify key protein quality gaps within vegan diets and to guide the development of targeted strategies in the subsequent Chapters.

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STATEMENT OF CONTRIBUTION DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certifythat all co-authors have consented to their work being included in the thesis and they have accepted the student'scontribution as indicated below in the Statement of Originality.			
Student name:	Bi Xue Patricia Soh		
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In which chapter is the manuscript/published work?		Chapter 3	
Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1] Bi Xue Patricia Soh: Conceptualisation, data curation, formal analysis, investigation, methodology, writing of original draft, review and editing. Dr Matthieu Vignes and Dr Nick W Smith: Formal analysis, methodology, supervision, review and editing. Professor Pamela R von Hurst and Professor Warren C McNabb: Conceptualisation, funding acquisition, investigation, project administration, supervision, review and editing			
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Chapter 3 Evaluation of protein intake and protein quality in New Zealand vegans

This Chapter provides the first assessment of protein intake and protein quality of the diets of NZ vegans, evaluated against established requirements for total protein and IAAs. It examines whether observed dietary patterns align with the literature which suggest that vegan diets may be limited in protein quality.

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Abstract

Dietary protein provides IAAs that the body cannot synthesise. Past assessments of total protein intake from vegan populations in Western, developed countries were found to be low but not necessarily below daily requirements. However, PSPs generally have lower quantities of digestible IAAs than ASPs. Simply accounting for protein intake without considering AA profile and digestibility could overestimate protein adequacy among vegans. This study quantified protein intake and quality, as compared to reference intake values, among 193 NZ vegans using a four-day food diary. Protein and IAA composition of all foods was derived from the NZ Food Composition Database and the United States Department of Agriculture (USDA) and adjusted for TID. Mean protein intakes for males and females were 0.98 and 0.80 g/kg/day, respectively, with 78.8 % of males and 74.5 % of females meeting the EAR for protein. PSPs provided on average 52.9 mg of leucine and 35.7 mg of lysine per gram of protein and were below the reference scoring patterns (leucine: 59 mg/g, lysine: 45 mg/g). When adjusted to individual body weight (BW), average intakes for all IAAs were above daily requirements, but lysine just met requirements at 31.2 mg/kg of BW/day (reference: 30 mg/kg/day). Upon TID adjustment, the percentage of vegans meeting protein and IAA adequacy decreased, and only approximately 50 % of the cohort could meet lysine and leucine requirements. Hence, lysine and leucine were the most limiting IAAs in the vegan cohort's diet. Legumes and pulses contributed most to overall protein and lysine intake. An increased proportion of legumes and pulses could potentially increase these intakes, but must be considered in the context of the whole diet. AA composition and digestibility are important aspects of protein quality when assessing protein adequacy, particularly in restrictive diets.

3.1 Introduction

A vegan diet is defined as one which excludes all ASFs and products, such as meat, dairy, eggs, and honey, and includes only foods of plant origin (1, 2). Recent analysis from the NZ Health Survey found that 0.74 % of the surveyed population (n = 23,292) were self-declared vegans (3). Nevertheless, some studies have reported a growing trend in the adoption of vegan diets among younger adults (1, 4) and the increased popularity of PB diets among females (5). Despite discussions of the potential of PB diet transitions to promote environmental sustainability and health, assessing their nutritional quality is essential before making these recommendations.

Lower total protein intake has been reported among vegans in some studies compared to other diets, with one study highlighting crude protein intake at 9.2 % of total energy intake, below the threshold of 10 % (6). However, others found protein intake to be between 13 to

15 % of energy intake (1, 7). Few studies have evaluated AA intake among vegans nor accounted for the digestibility of AAs in the analysis (8).

As explained in Chapter 2, dietary sources of protein provide AAs, nine of which cannot be supplied endogenously or have excess stored within the body over long periods (9). These are the IAAs which are often available in lower concentrations in PB foods compared to ASFs (10). Regular high-quality protein intake throughout the day is essential to ensure IAA quantities are at the threshold level to stimulate protein synthesis, grow and maintain muscle mass, as well as regulate other body functions (2, 11, 12). PSPs have varied AA profiles and contain anti-nutritional elements, which often reduce the quantities of digestible AAs (13, 14). As compared to diets inclusive of ASFs, a diet consisting only of PSPs usually supplies lower concentrations of digestible AAs to meet individual requirements efficiently and is thus considered lower in protein quality (7, 15, 16).

The presence of at least one limiting IAA (the AA in the lowest quantity) could hamper protein synthesis and deposition within the body, with excess IAAs oxidised and contributing to other metabolic pathways (12, 17, 18). As PB proteins share several common limiting IAAs, complementary protein sources in the diet add value by overcoming these limitations and improving overall protein quality. FG combinations, however, require consideration of the trade-off among protein quality, energy, and fibre increment. Energy and fibre content are higher in PB foods than in ASFs, with the latter contributing to satiety, thereby limiting how much food a vegan can consume to meet nutritional requirements.

Examining the current dietary status of long-term vegans, as well as the distribution of different FGs in the diet, is necessary to uncover potential limitations of PB foods in their supply of IAAs. Only then can there be subsequent development of appropriate dietary interventions to help vegans achieve nutritionally balanced diets with optimal protein quality. The main aim of this Chapter was to determine protein adequacy among a cohort of vegans in NZ and assess how protein and IAA consumption among these vegans compares to individual daily protein and IAA requirements. A secondary aim was to explore how the dietary vegan pattern (in terms of FG contribution) impacts protein intake and protein quality.

3.2 Methods

3.2.1 Study design

Dietary intake data were obtained from the Vegan Health Research Programme conducted at Massey University, Auckland, NZ, from 2022 to 2023. The study was cross-sectional and aimed to explore vegan dietary patterns and determine the associations between nutrient intake and multiple health outcomes. Ethical approval was granted by the Health and Disability Ethics Committee (HDEC 2022 EXP 12312). All participants provided informed written consent prior to data collection.

3.2.2 Study population

Using convenience sampling, healthy adults 18 years and above who live in NZ were recruited through university advertisements, local vegan societies, and word-of-mouth. The key inclusion criterion was that participants must have followed a vegan diet for at least two years. Pregnant and lactating women were excluded from the study. Recruitment began on 01/06/2022 and ended on 1/08/2023.

3.2.3 Data collection

All participants visited the Human Nutrition Research Unit at Massey University once. Height and weight measurements were completed by trained researchers using the standardised operating procedures. Body height (cm) to the nearest 0.1 cm was measured without shoes with a stadiometer. BW (kg) to the nearest 0.1 kg was measured without shoes using floor scales. The Body Mass Index (BMI) kg/m^2 was calculated by taking the individual's BW in kilograms divided by the square of his or her height in meters. Body fat percentage was assessed alongside bone tissue examination with dual-energy X-ray absorptiometry.

Participants were instructed to provide a detailed record of all food, beverage and supplement consumption using a food diary spanning four consecutive days. Descriptions of records included the date and time of consumption, portion size, brand, variety, and cooking method. Completed records were returned to the research team for processing.

3.2.4 Data processing

Food intake data was processed by supervised and trained Master of Dietetics students at Massey University using the FoodWorks Professional nutrient analysis software (Xyris, Australia, 2022) that is based on FOODfiles, the reference food composition table for NZ that provides values for various nutrients in NZ food items (19).

Protein was calculated as total nitrogen multiplied by a specific nitrogen-to-protein conversion factor of 6.25 (19). As IAA composition data is not currently available in the food composition table for NZ, each food item was also matched to the food composition data from the USDA (20). When bulk recipes were recorded, the composite ingredients with their respective portion sizes were used to calculate total protein (g) and IAA (g) for the weighed recipe, and these values were then adjusted to the weight of the consumed portion. Within a home-made or commercial recipe where the composite ingredients were recorded, each ingredient was matched to the most similar food item in the databases and adjusted for TID. If composite ingredients were absent, the ingredient that provided the highest source of protein (e.g., pea protein isolate in a burger patty) - the food component of interest in this study - was selected and matched to the most similar food item in the USDA data.

To improve the quality of the estimations of nutrient intake and obtain the most appropriate match, food matching guidelines from FAO/INFOODS were followed (21). In cases where an exact match was unattainable, the food item was matched to the closest available items to tabulate an average value for protein and IAA. To accommodate the influence of cooking methods on digestibility values for protein and AAs, a food item prepared in the most similar method from the USDA data was chosen as a match for the reported food item. To account for the disparity in food compositions between the two countries, AA values from the USDA food composition data were normalised to the protein content of reported foods in NZ where the USDA IAA quantity was multiplied by the ratio of New Zealand protein to USDA protein.

Values for protein and IAA were adjusted for digestibility using currently available TID values from the literature (22-24). This involved categorising each food item into an appropriate FG that has an estimated digestibility coefficient (between 0 and 1), which is multiplied by the total protein and AA content of each food item (22-24). To condense the number of food items for analysis, food items that contributed < 0.5 g of protein in each of the four-day food diaries were excluded. The remaining data captured more than 98 % of the total cohort's protein intake and at least 92 % of each participant's protein intake.

3.2.5 Data analysis

The percentage of energy from protein was calculated and expressed against the AMDR, which, when using the reference BW of 57 kg for women and 70 kg for men using the mean estimated energy requirement (EER) of 36.5 kcal/day is between 10 to 35 % of caloric intake or protein intake of 1.05 to 3.67 g/kg/day (25). The protein intake for each participant in g/kg of BW was calculated by dividing their mean protein intake over four days by their BW (assumed to be maintained across the days of dietary recall). This was compared to the Australian and NZ EAR for protein (0.68 g/kg/day for males and 0.60 g/kg/day for females) and to the RDI (0.84 g/kg BW/d for males and 0.75 g/kg BW/d for females) for adults aged 19 to 70 years (26). For older adults above 70 years, the EAR followed for males and females were 0.86 g/kg/day and 0.75 g/kg/day, respectively, and RDI for males and females were 1.07 g/kg/day and 0.94 g/kg/day, respectively (26).

To determine IAA contribution (mg) in one gram of digestible protein, the quantity of each IAA (mg) consumed in a day was divided by total protein consumed in the day and the mean of four days was derived for each individual. This allowed comparison of the IAA content of each food to its protein content (IAA mg/g protein). This IAA scoring pattern (mg/g protein) is a derivation of each IAA requirement (mg/kg/day) divided by protein or nitrogen requirement (g/kg/day) following the age group of 18 and above (27, 28). When incorporated with digestibility coefficients, the protein quality of a specific diet could be examined (27).

To compare IAA intake to daily requirements (mg/kg/day), the quantity of each IAA (mg) consumed in a day was divided by each individual's body weight (kg) and the mean of four days was compared to the IAA requirement (27). Percentage adequacy was calculated to determine the proportion of participants who met the daily EAR, RDI and IAA requirements.

Measurements of body composition, BMI and percentage body fat were tabulated and explored alongside protein and IAA adequacy. A normal BMI range of 18.5 to 25 kg/m^2, established for a NZ European population, was applied as it was representative of the surveyed cohort (29, 30). While there is no clear definition of normal body fat percentage for males and females of different age groups for the NZ population, the cut-off points of body fat for overweight were taken to be 20.1 to 24.9 % for males and 30.1 to 34.9 % for females, and for obesity were equal to or greater than 25 % for men and 35 % for females (31).

To determine how absolute IAA intake changes with total protein intake, linear regression of each TID-adjusted IAA (g) as a function of TID-adjusted total protein intake (g) was used. The value of the slope coefficient of the line was used to assess how IAA composition provided by the vegan diet changes with overall protein intake. To evaluate how effectively individuals achieve daily IAA requirements relative to protein requirements, the ratios of the actual intake values of each nutrient to its respective requirement were calculated. Ratios < 1 indicate that requirements are not met, and ratios ≥ 1 indicate meeting and/or exceeding requirements. A heat map of four quadrants was used to visualise the distinct categories of intake adequacy between protein and each IAA.

To assess the contribution of individual FGs to the percentage energy (MJ), dietary fibre (g), protein (g) and IAA (mg), each food item from the four-day food diary was matched to its respective FG, as defined in the 2008/2009 NZ Adult Nutrition Survey (32). For example, wheat and cereal products were categorised as "Grains and pasta" while beans, peas and soy products were classified as "Legumes and pulses". Protein sources used in novel meat and dairy alternatives were matched to the FG of their principal protein-contributing ingredient (33).

To assess the percentage contribution of foods to energy and dietary fibre, ingredients contributing most to energy and dietary fibre were identified and categorised into the relevant FGs.

Evaluation of energy misreporting was undertaken using the Goldberg cut-offs, and the energy-adjusted protein intake was calculated using the residual method, with energy intake as the independent variable and TID-adjusted protein intake as the dependent variable (34, 35). The Basal Metabolic Rate (BMR) for each participant was estimated using the Schofield equations with sex, age, body weight and body height, and expressed in MJ/day (26). The Physical Activity Level (PAL) was determined with The International Physical Activity Questionnaire (IPAQ), which recorded the number of metabolic equivalent task (MET) minutes for each individual (36). Age- and PAL-specific Goldberg cut-offs were assigned for low, moderate and high physical activity (34, 37). Participants were classified as under-reporters when the ratio of mean energy intake to BMR fell below the lower cut-off, and over-reporters when the ratio exceeded the upper cut-off. Plausible reporters fell between the cut-offs. Energy adjustment was undertaken statistically using the residual method and did not involve rescaling reported food quantities to the energy requirements.

All statistical analyses were conducted using R Studio (version 4.3.1) (38). Continuous variables were described as mean and standard deviation (SD), and differences between sexes for energy and protein intake were calculated using the Student's t-test. Differences were regarded as statistically significant when $P < 0.05$. Discrete variables were represented as counts with percentages.

3.3 Results

3.3.1 Characteristics of the study population

In total, 212 vegans were recruited in the Vegan Health Research Programme. Subjects who did not submit completed food records were excluded from the dietary analysis. A total of 193 participants, consisting of 52 males and 141 females, completed the food diary for at least 3 days and were included in the analysis. **Table 3.1** describes the demographic and anthropometric characteristics of the study population. Females and New Zealanders of European descent form the majority. A large percentage of participants were highly educated, with more than 80 % completing tertiary education. Females had lower BW and height than males but had a higher percentage body fat.

Table 3.1 Characteristics of study subjects

Characteristics	Male	Female
n (% of total cohort)	52 (26.9)	141 (73.1)
Ethnicity, *n* (%)		
European	45 (86.5)	117 (83)
Others	7 (13.5)	24 (17)
Highest education level, *n* (%)		
Tertiary education	45 (86.5)	129 (91.5)
High school/ lower	7 (13.5)	12 (8.5)
Duration of vegan diet in years, *n* (%) [1]		
2 to 4 years	84 (39.6)	
5 to 10 years	93 (43.9)	
More than 10 years	35 (16.5)	
Age in years (SD)	41.3 (11.9)	39.5 (12.7)
Body weight in kg (SD)	79.3 (12.5)	66 (10.5)
Height in cm (SD)	179.4 (7.9)	166.6 (6.7)
BMI in kg/m^2 (SD)	24.6 (3.0)	23.8 (3.2)
Within BMI range, *n* (%)	33 (63.5)	92 (65.2)
Body fat in % (SD)	22.9 (5.0)	33.4 (6.0)
Overweight or obese for body fat, *n* (%)	35 (67.3)	100 (70.9)

SD, standard deviation. [1] Based on consolidated data of 212 subjects. Continuous values are expressed as mean (SD), and categorical values are expressed as counts, *n* (percentage). The BMI normal range is established at 18.5 to 25 kg/m^2. The cut-off points of body fat for overweight were taken to be 20.1 to 24.9 % for males and 30.1 to 34.9 % for females, and for obesity were equal to or greater than 25 % for men and 35 % for females (31).

3.3.2 Energy and protein consumption

Table 3.2 presents the mean energy and TID-adjusted protein intake for males and females. Females had lower energy ($P < 0.01$) and protein (g/kg/day) ($P < 0.05$) consumption compared to males. **Table B1 of Appendix B** provides a summary of energy intake differentiated by sex and age group, compared with daily energy requirements. The percentage of caloric intake as protein is calculated and compared to the AMDR. The results did not differ significantly between males and females (P = 0.44). When compared to the reference values for daily dietary protein intake, a larger proportion of males than females met both the EAR and the RDI.

Table 3.2 Energy and TID-adjusted protein consumption among vegan participants

Intake	Male	Female
Energy intake in MJ/d	10.4 (2.6)	7.5 (1.9)
Protein intake in g/d	76.3 (27.5)	52.1 (16.3)
Protein intake in g/kg BW/d	0.98 (0.36)	0.80 (0.27)
Protein as % of energy intake	12.4 (3.5)	12.0 (3.7)
Percentage meeting (%)		
EAR	78.8	73.0
RDI	59.6	53.9
AMDR for protein	82.7	72.3

Intake values are expressed as mean (standard deviation). EAR and RDI for males and females were obtained from the NRV of Australia and NZ (26). The reference value of the AMDR for protein was obtained from the literature (25).

3.3.3 Impact of TID-adjustments on protein and IAA supply

Adjustments for TID showed clear differences for overall protein intake compared to requirements. Digestible protein intake (g/kg/day) was 0.98 for males (compared to gross protein intake of 1.25) and 0.80 for females (compared to 1.05), demonstrating that when TID is accounted for, mean protein is lower relative to reference values (**Figure 3.1**). After TID adjustment, the percentage of the population meeting the EAR for protein adequacy decreased from 92.3 % to 78.8 % for males and from 92.2 % to 73.0 % for females.

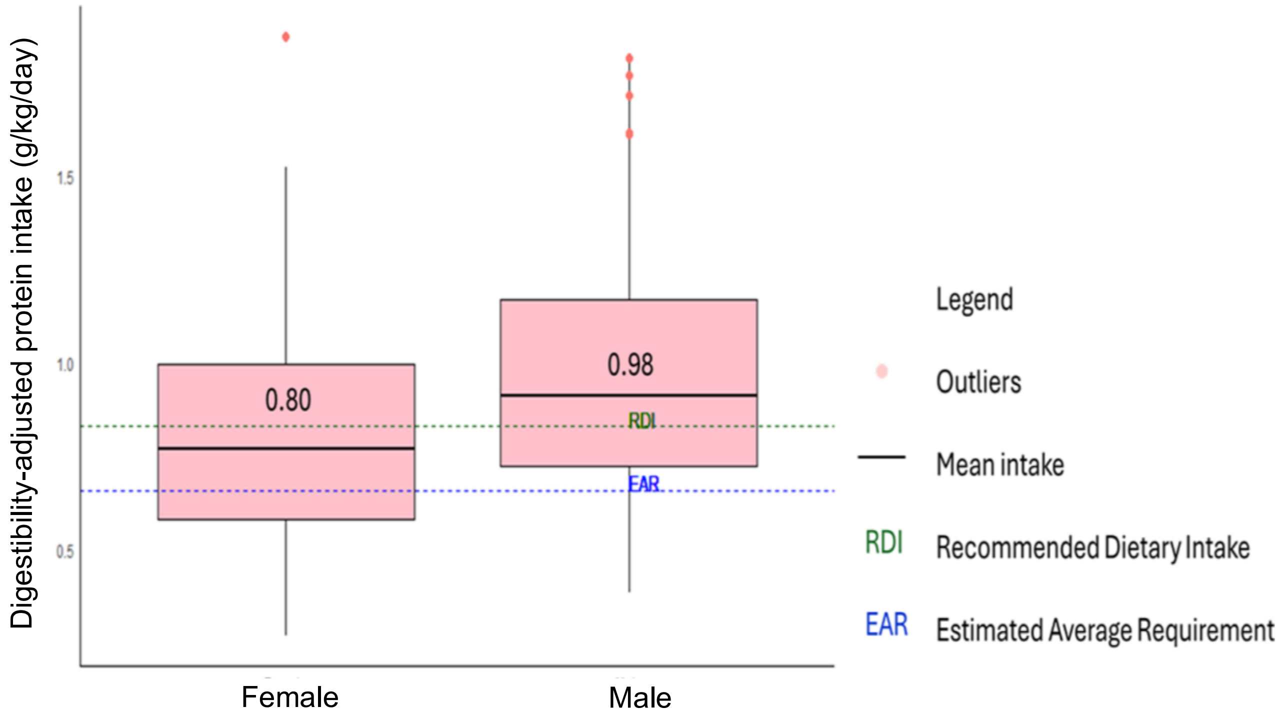

Figure 3.1 TID-adjusted protein intake for males and females.
The mean is represented by the horizontal black line and the numeric value in each box. The box represents the interquartile range (IQR), and the whiskers show the 10th to 90th percentile range. Outliers in the study consumed protein above 1.70 g/kg/d.

Table 3.3 summarises IAA consumption relative to AA scoring patterns (mg/g of protein) and daily AA requirements (mg/kg/day). When compared to the AA scoring pattern (mg/g), the analysed diets supplied lower lysine, leucine, isoleucine, and valine per gram of protein. However, when adjusted to individual BW, all IAAs met AA requirements in mg/kg/day.

Table 3.3 AA consumption patterns as compared to AA scoring pattern and reference AA requirements, before and after TID adjustments

	His	Ile	Leu	Lys	SAA[4]	AAA[5]	Thr	Trp	Valine
AA scoring pattern (mg/g protein requirement)	15	30	59	45	22	38	23	6	39
Reference AA requirements (mg/kg/d)	10	20	39	30	15	25	15	4	26
Unadjusted values									
AA intake (mg/g protein)	18.2 (2.8)	28.4 (4.3)	50.8 (7.3)	35.2 (5.9)	22.5 (3.5)	55.6 (7.9)	25.7 (3.8)	8.4 (1.7)	34.5 (5.3)
AA intake (mg/kg/d)	20.1 (7.7)	31.2 (11.6)	55.9 (20.8)	39.3 (16.0)	24.8 (9.3)	61.1 (22.9)	28.3 (10.5)	9.1 (3.5)	37.8 (13.8)
Percentage adequacy (%)[1]	96.4	84.0	75.6	66.3	88.6	99.5	95.3	97.9	78.2
Digestibility-adjusted values[2]									
AA intake (mg/g protein[3])	19.1 (2.8)	-	52.9 (7.6)	35.7 (6.2)	24.0 (3.8)	-	26.1 (3.9)	8.5 (1.8)	-
AA intake (mg/kg/d)	16.4 (6.5)	-	45.0 (17.5)	31.0 (13.6)	20.3 (7.6)	-	22.1 (8.5)	7.0 (2.7)	-
Percentage adequacy (%)	86.5	-	56.0	43.5	71.5	-	78.2	90.0	-

His, histidine; Ile, isoleucine; Leu, leucine; SAA, sulphur amino acids; AAA, aromatic amino acids; Thr, threonine; Trp, tryptophan; Val, valine. AA scoring pattern mg/g protein requirement and reference AA requirements (mg/kg/day) are obtained from FAO (28). Values of AA intake (mg/g protein) and AA requirement (mg/kg/day) are expressed in mean (SD). Dashes are indicated for Ile, AAA, and valine in TID-adjusted values, because digestibility values of food for these AAs are not currently available. [1] Percentage adequacy describes the percentage of the study population consuming each AA at or above the AA requirements (mg/kg/day). [2] Digestibility coefficients were available for histidine, cystine, methionine, leucine, lysine, threonine and tryptophan (22-24). [3] Total protein (g) was multiplied by the digestibility coefficient (22-24). [4] SAAs are cystine and methionine. Intake values for cystine and methionine were added for each participant on each day. Values for IAA in mg/g after TID-adjustments increase because of different denominators (TID-adjusted protein intake) used in the calculation, to reflect the portion of digestible IAA per gram of digestible protein. [5] AAA, aromatic amino acids are phenylalanine and tyrosine.

TID adjustment decreased all IAA intakes (mg/kg/day) (**Table 3.3**). Before adjustment, the percentage of individuals meeting adequacy for histidine, threonine, and tryptophan was close to 100 %, while the percentage adequacy for leucine and lysine was lower. Digestibility adjustment decreased the percentage adequacy for all IAAs, especially for leucine, lysine, SAAs and threonine. Notably, the decreases in lysine and leucine were at least 15 %. Consequently, only approximately half of the cohort met IAA requirements for lysine and leucine after adjustment. The impact on utilisable AA quantity following TID adjustment is also observed in **Figure 3.2,** as the mean value of each AA (mg) reduced after digestibility adjustment.

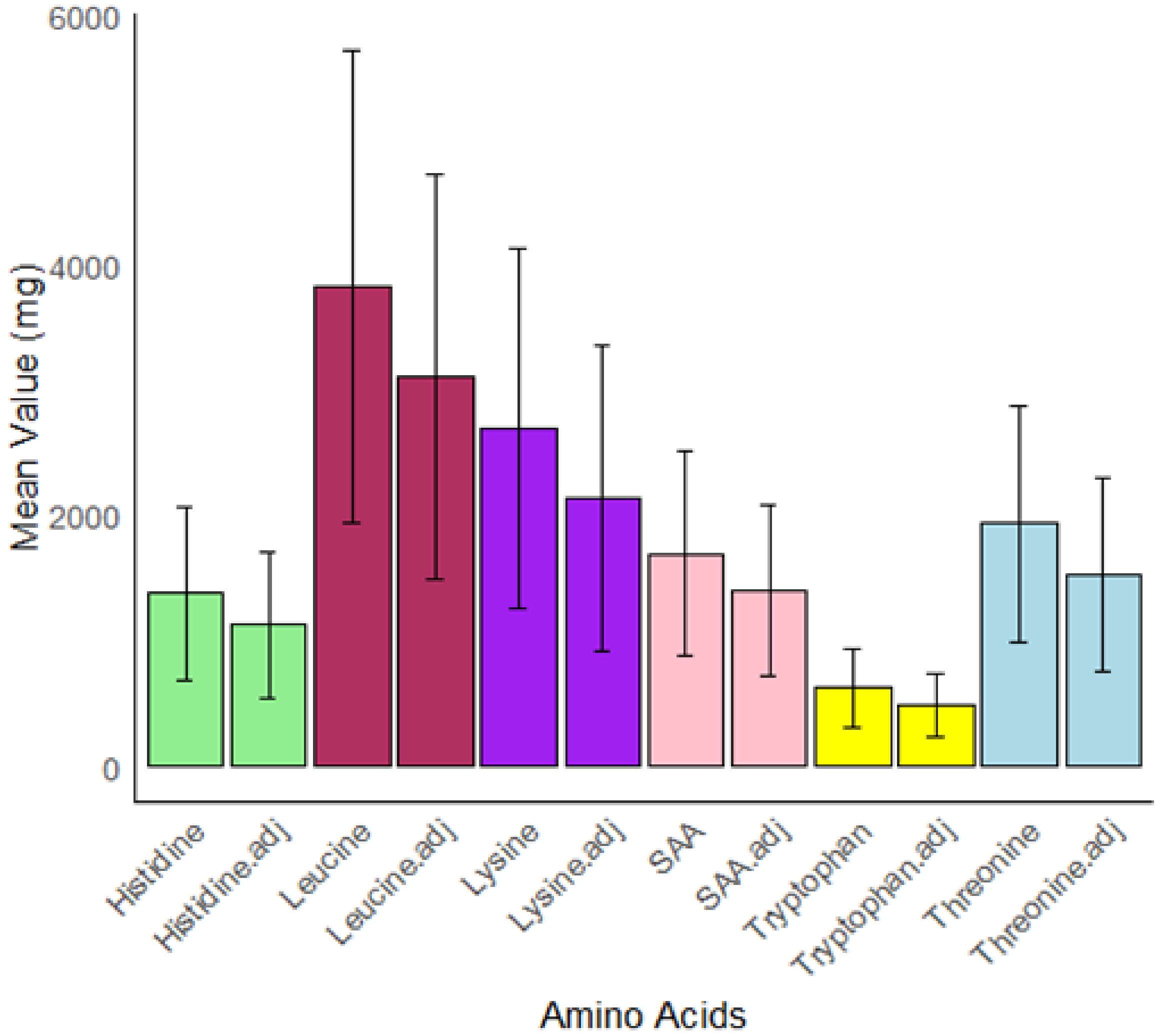

Figure 3.2 Comparison of mean daily IAA intake quantity before and after digestibility adjustments.
Error bars represent standard deviations. adj, AA TID-adjusted values; SAA, sulphur-containing amino acid.

3.3.4 Comparison of TID-adjusted IAA intake to reference patterns and daily requirements

Table 3.3 and **Figure 3.3** show that while the other TID-adjusted IAAs were meeting reference scoring patterns, TID-adjusted lysine and leucine were below their reference scoring patterns of 45 mg/g of protein and 59 mg/g of protein, respectively. This indicates that lysine and leucine are the two most limiting AAs provided by PB protein sources in the vegan cohort.

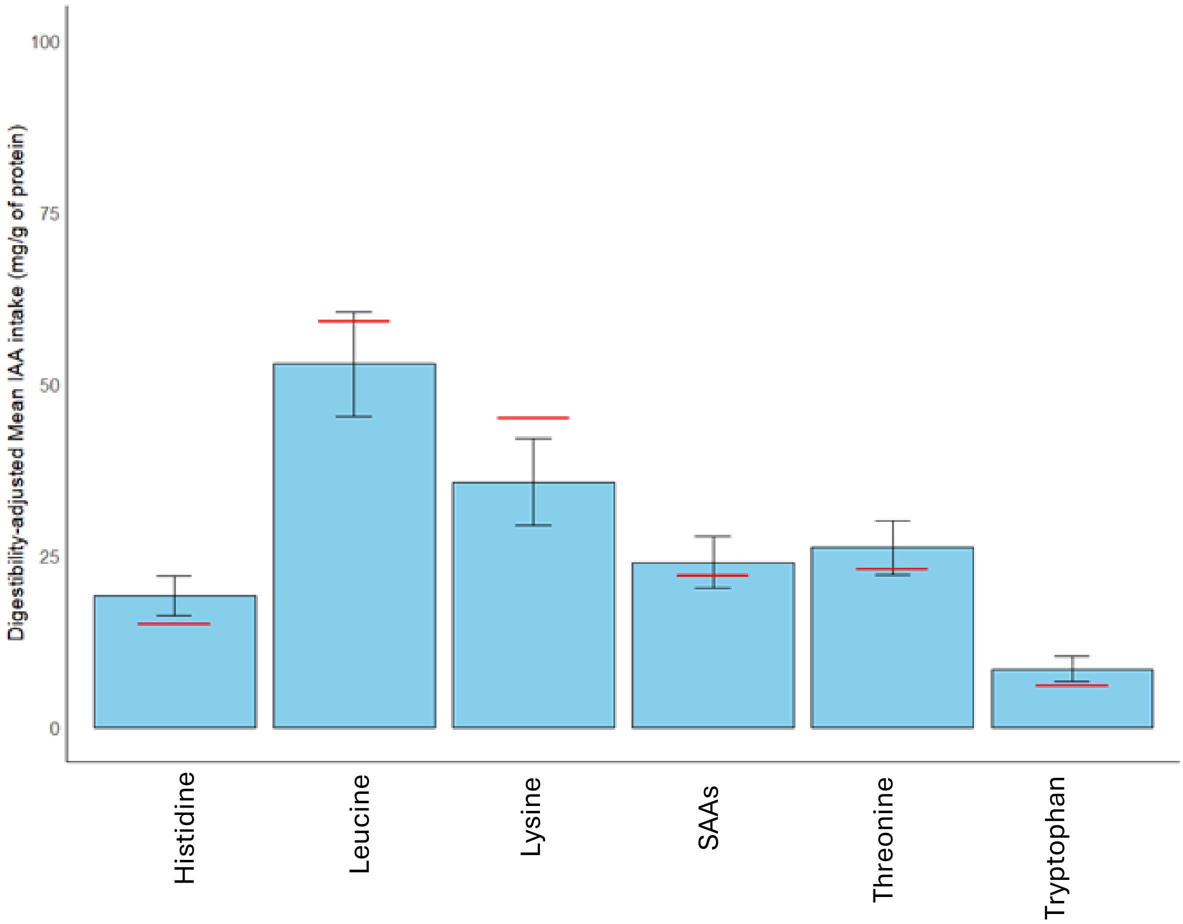

Figure 3.3 Comparison of each IAA intake with its respective AA scoring pattern.
The scoring pattern is represented by the red horizontal lines. The error bar represents the SD. Blue bars that meet or are above the red line indicate that the mean IAA intake pattern in mg/g of protein met the AA scoring pattern.

The mean IAA intake adjusted to BW met daily IAA requirements, but for lysine, the intake (31 mg/kg/day) only just met the requirement (30 mg/kg/day) (**Figure 3.4 and Table 3.3**). Approximately half the cohort did not meet BW-adjusted requirements for digestible lysine (56 %) and leucine (43.5 %) (**Figure 3.4**). In investigating protein intake among individuals who did not meet both lysine and leucine requirements, 56.6 % did not meet the protein EAR (g/kg/day).

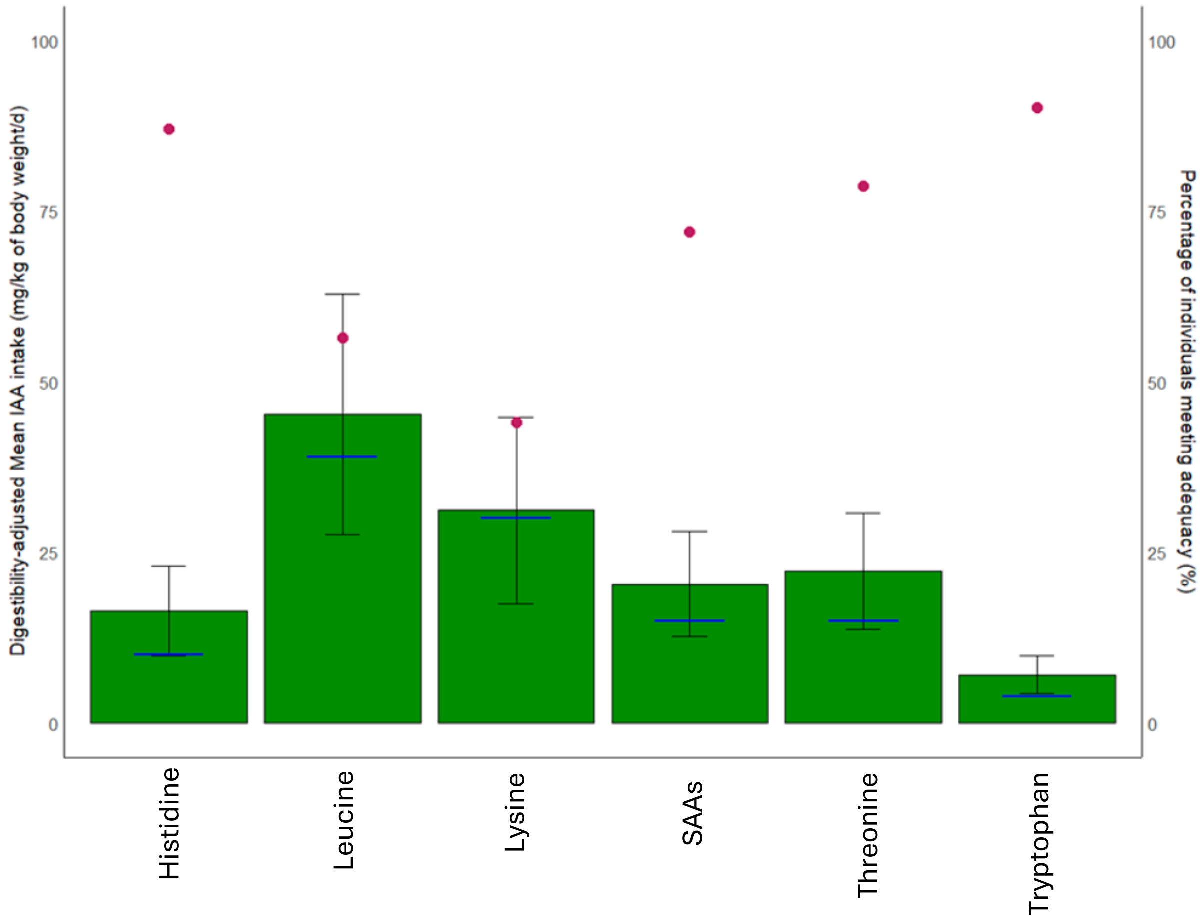

Figure 3.4 Comparison of TID-adjusted IAA intake compared to its respective requirement. The requirement is represented by the blue horizontal lines. Each green bar represents the mean IAA intake in mg/kg BW/d (left-hand vertical axis), and the error bar represents the SD. Red dots represent the percentage of individuals in the cohort meeting daily IAA adequacy (right-hand vertical axis).

BMI and percentage body fat were explored to assess the relationship between body composition and protein intake. The results are presented in **Table B2 of Appendix B**. Approximately 50 % of the group who were inadequate in TID-adjusted leucine (g/kg/day) had a BMI above the normal BMI range. Approximately 80 % were in the overweight or obese range for body fat. Among the individuals inadequate for TID-adjusted lysine, 42 % had a BMI above the normal range, and 85 % were in the overweight and obese category for body fat. The energy intake and AMDR for protein were also examined in individuals who were below the requirements for leucine and lysine **(**see **Table B3 of Appendix B)**. Mean energy intake and mean AMDR for protein were lower in this group than in the cohort.

A small percentage (7.2 %, n = 14) of participants were below the requirement for all the digestibility-adjusted IAAs. Within this group, BMI was above the normal range for all but two females, and the percentage body fat was in the overweight and obese category for all individuals.

Regression models were applied to determine how increasing the quantity of TID-adjusted protein intake was associated with the change in the quantity of each TID-adjusted AA (**Figure 3.5**). Generally, an increase in each IAA intake was associated with an increase in total protein intake. Wider variation in IAA intake was observed with total protein intakes above 50 g. The coefficient of determination, as denoted by the R^2 value, was similar for all IAAs and total protein, except for tryptophan, which has the smallest R^2 value of 0.64. This shows that protein and tryptophan intakes have a poorer association than that of protein and the other IAAs. Greater variability in tryptophan levels was observed at elevated protein levels as compared to other IAAs.

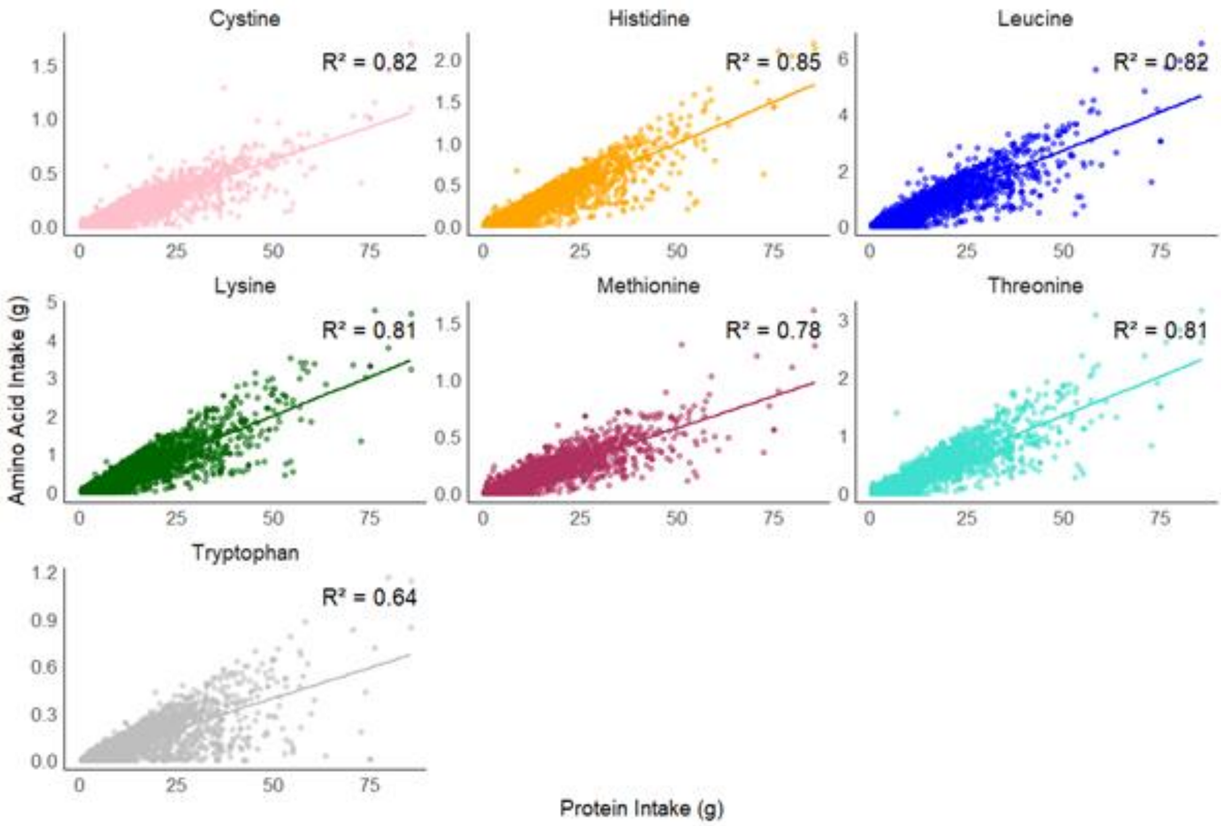

Figure 3.5 Regression analysis describing the relationship between TID-adjusted total protein (g) and each TID-adjusted IAA consumption (g).
Data points represent all individuals in the cohort for each day of the food diary. The R^2 value quantifies the proportion of variance in IAA intake with increasing protein intake.

Figure 3.6 demonstrates how participants were distributed across distinct categories of intake adequacy for protein and each respective IAA. More participants achieved the requirements for IAAs like histidine, tryptophan, threonine, and SAAs, but fewer achieved the requirements for lysine and leucine. For example, when protein intake was above adequacy, more individuals were above histidine adequacy (n = 142) than above lysine (n = 84) and leucine (n = 108). The largest numbers below adequacy, even at higher protein consumption (above adequacy), were found in the lower right quadrants for lysine (n = 61) and leucine (n = 37).

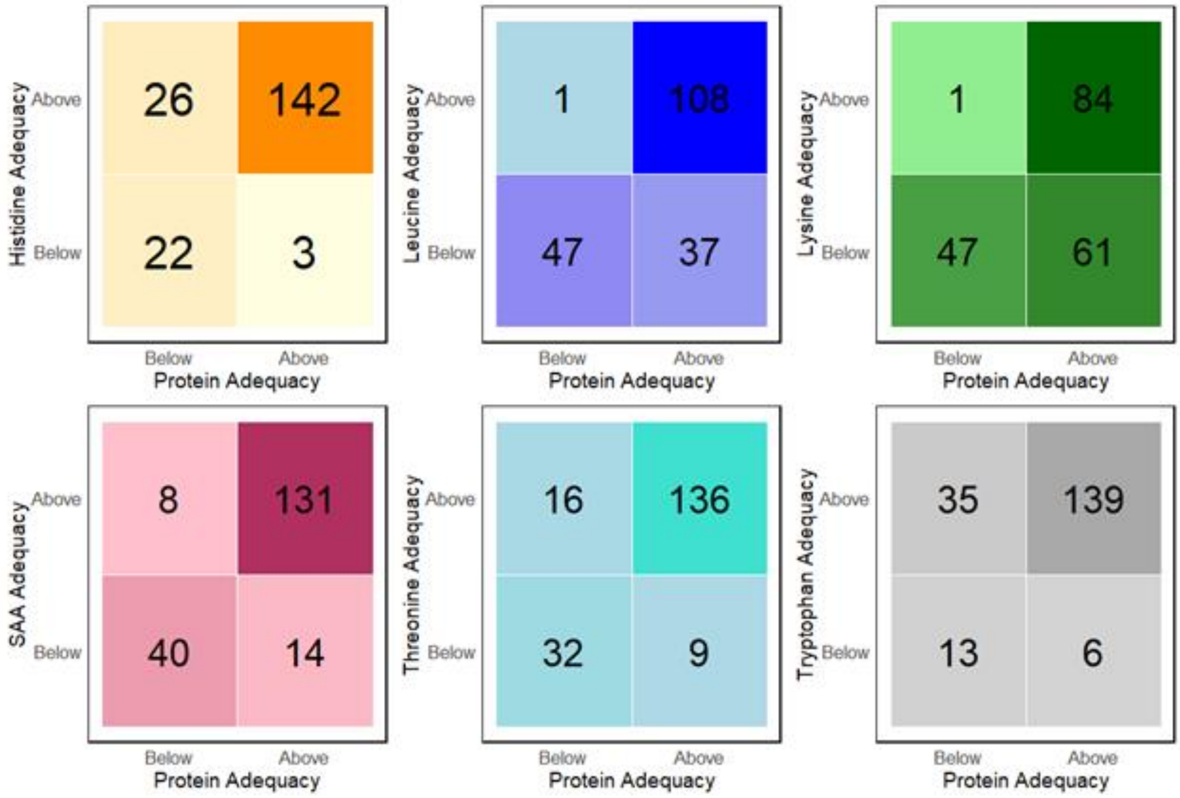

Figure 3.6 Visualisation of quadrants for the comparison of each IAA adequacy relative to protein adequacy.
Each colour gradient represents an IAA (histidine, leucine, lysine, SAA, threonine, and tryptophan), all of which had been adjusted for TID. The numbers on the quadrants represent the number of vegans distributed for each distinct combination of IAA and protein adequacy. For example, in histidine quadrants, 142 individuals achieved adequacy for both total protein and histidine when compared with daily requirements adjusted for BW. Adequacy is expressed as a ratio of intake / requirement. Ratio < 1 indicates below requirement, and ratio ≥ 1 indicates meeting and above requirement. The total number of vegans represented in each quadrant collection is 193.

3.3.5 Energy misreporting

Several approaches were explored to identify implausible energy reporting. For example, fixed energy intake thresholds (< 500 kcal/day, > 4000 kcal/day for females, and > 5000 kcal/day for males) adapted from a previous dietary study on the protein intake distribution of older adults were used to identify potential outliers (39). These thresholds are recognised as being derived from a different study population. One male and one female were above the upper boundary of caloric intake with protein intake being equally high (**Figure 3.1).** Three females were below the lower boundary for caloric intake. Potential under-reporting was defined as participants reporting mean energy intake below the EER (accounting for individual PAL), in combination with the BMI or body fat being above the respective thresholds. 18.4 % of females and 21.2 % of males were classified as under-reporters.

These approaches were viewed to be screening measures and do not explicitly account for inter-individual variation in BMR and PAL. Consequently, the Goldberg cut-offs were used as the primary method for classifying reporting plausibility. Using the Goldberg cut-offs, however, only one male was identified as an over-reporter and 16 participants (2 males and 14 females) were classified as under-reporters, while the remaining 176 participants were classified as plausible reporters. Under-reporters had significantly lower protein intakes than plausible reporters (0.56 versus 0.87 g/kg/day, $P < 0.05$).

Mean protein intake after energy adjustment remained similar in males and females, at 0.94 g/kg/day and 0.82 g/kg/day, respectively. Energy-adjusted mean protein intake among under-reporters was 0.82 g/kg/day, compared with the unadjusted intake of 0.56 g/kg/day. This was closer to the mean protein intake of plausible reporters (0.85 g/kg/day). The difference in protein intake between under-reporters and plausible reporters was attenuated following adjustment for reported energy intake, suggesting that the lower observed protein intake among under-reporters was partly associated with their lower reported energy intake. Participants identified as potential outliers or implausible reporters were not excluded from subsequent analyses. While Goldberg cut-offs provide a validated approach for identifying implausible energy reporting, they do not definitively establish that an individual has under-or over-reported dietary intake. Given the relatively modest cohort size (n = 193), removal of approximately 10 % of the participants may induce reduced statistical power or compromise the representativeness of the cohort.

3.3.6 Contribution of FGs to protein and IAA profile

Figure 3.7 shows the percentage contribution of each FG to cohort mean intake of energy, fibre, TID-adjusted protein, and TID-adjusted IAA. The highest contributing FG to total energy intake was “grains and pasta” (38.4 %), followed by “legumes and pulses” (19.3 %) and “nuts and seeds” (15.6 %). The highest contribution to total protein intake and total lysine intake was from “legumes and pulses” (45.9 % and 57.5 %, respectively). In contrast, “grains and pasta” contributed less to total protein intake (32.0 %) and total lysine (21.4 %).

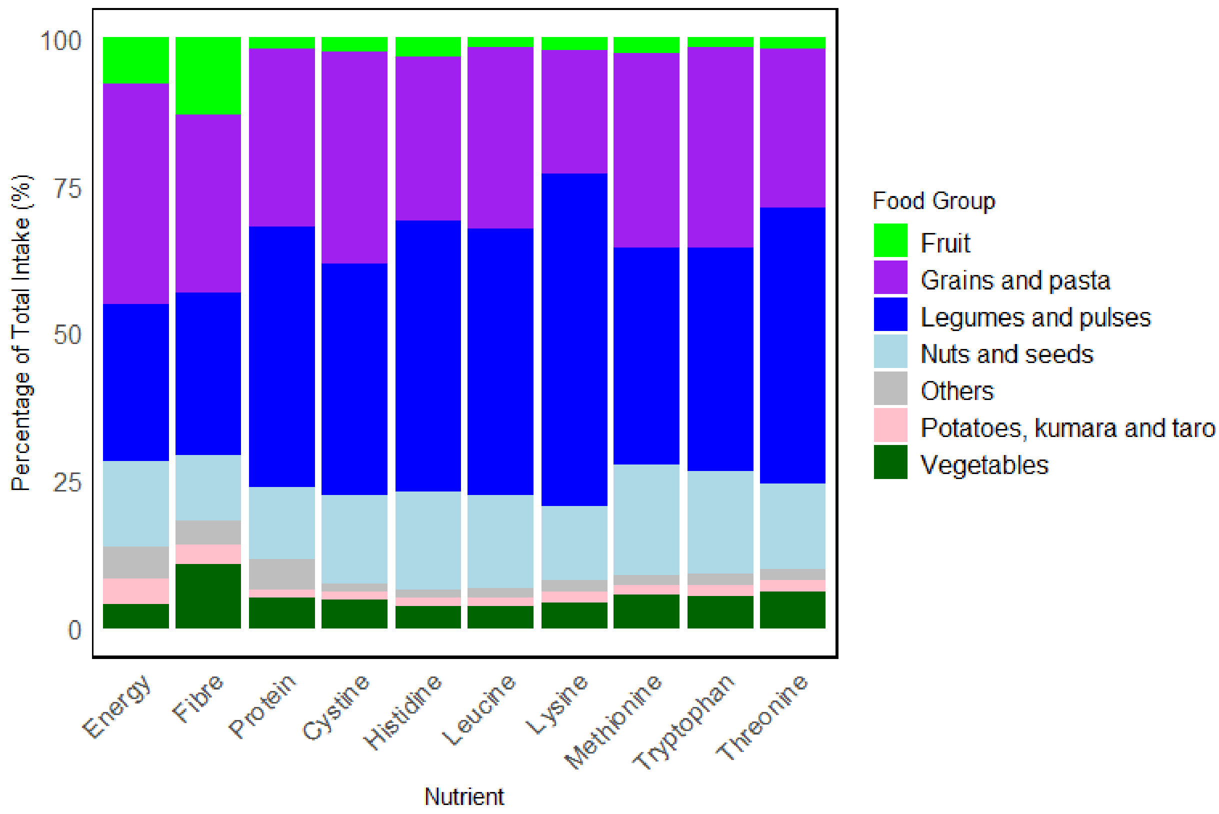

Figure 3.7 Contribution of FGs to energy, dietary fibre, TID-adjusted protein, and TID-adjusted IAA intake.
“Others” indicate FGs such as “Fats and oil”, “Sugar and sweets”, “Savoury sauces and condiments”, that contribute materially to energy and fibre intake but provide less than 1 % of total intake for protein and IAAs.

Analysis of consumption patterns of novel PB foods as compared to traditional sources was out of the scope of this Chapter, but protein and IAA contribution from novel foods was estimated. Ingredients used in novel PBAs varied, but extruded protein sources from soy and pea (e.g., isolates) were the main protein-contributing ingredient from PBMAs and powdered supplements. The main contributing FG to the novel alternatives was “legumes and pulses”, which provided more than 90 % of the total TID-adjusted protein and lysine from this group.

3.4 Discussion

The present study assessed the intake of digestible protein and IAA in a cohort of vegan individuals in NZ and is the first comprehensive dietary analysis for protein adequacy in long-term vegans residing in NZ. This study adds to past findings of protein and IAA intake assessments among vegans from other regions of the world (40-44) and contributes the perspective of protein quality assessment relative to daily requirements.

The key finding of this Chapter corroborates findings from past research on PB diets: utilisable protein intake is at risk of deficiencies, especially when accounting for protein quality. Lysine, followed by leucine, were the two most limiting IAAs in the vegan diet of this NZ cohort, with a large proportion of the cohort consuming insufficient amounts, despite most individuals achieving adequate daily protein intake (when compared to the EAR). As such, assessments of protein intake must consider IAA intake and protein digestibility. As evident from this study, digestibility adjustments reduced protein and IAA values compared with a composition-only approach.

3.4.1 Comparison of protein and IAA intakes relative to requirements

As observed in **Figure 3.1**, total protein intake is of small concern within the vegan cohort, as even after digestibility adjustments, mean protein intake for both males and females met the EAR. Compared with the RDI, the mean protein intake for males also met the requirement, while the mean protein intake for females was close to it. However, only 55.6 % of individuals who met this protein requirement achieved adequacy for all digestibility-adjusted IAAs, indicating that protein quality is not always assured, even when protein adequacy is met.

As described in Chapter 2, the EAR and RDI values were derived from NB studies based on the attainment of the lowest possible AA oxidation or a minimum value for nitrogen balance, with excess AA intake unnecessary for metabolic advantage (12, 45). More recent work with IAAO methods, although conducted only on young males, noted an increase in the RDA from 0.8 g/kg/day of protein to approximately 1.2 g/kg/day to better meet the functional properties of the AAs (2, 12, 25, 46). These conclusions were still based on high-quality protein that can provide adequate IAA to match requirements (2, 47).

Based on the above theories, the vegans in this present study, especially the females (with a mean protein intake of 0.81 g/kg/day), will be below the RDA. A different protein requirement specified for vegan populations is currently absent. Due to the diet's restrictive nature and where protein quality considerations are not included, an elevation of the recommended daily protein requirement may be required to meet metabolic or utilisation needs (48). Considering protein quality may be superfluous in diets that provide protein sources of high biological value. However, in diets that exclude all ASFs, simply comparing crude protein intake to the EAR or RDA values and assuming adequacy will neglect the overall digestible AA profile and its impact on adequacy (49).

Protein quality assessments among vegan populations, even without adjustments for digestibility, have also found reduced intakes of total lysine and BCAAs such as leucine, when compared to omnivorous diets (44, 50-54). A similar cross-sectional study on 40 Danish vegans also found that lysine was the most limiting IAA, only 50 % of participants met daily requirements, followed by SAAs and leucine as the next limiting IAAs (55). While the SAAs appeared adequate (but were the third limiting IAA) in this Chapter's results, the percentage of individuals meeting lysine and leucine requirements (mg/kg/day) was the lowest (**Table 3.3** and **Figure 3.4**). As these AAs have important metabolic functions, such as DNA methylation, bone health and MPS (particularly for leucine) (56-58), a deficiency will have negative implications for long-term health.

When adjusted for individual BW, intake for all IAAs adequately met daily requirements, even for lysine and leucine, albeit at lower levels than the other IAAs. Currently, BW is used as the predictor for, and is assumed to have a linear relationship with human protein requirement (59, 60). The group with adequate protein intake, compared with the EAR (g/kg/day), had higher energy intake than the group with inadequate protein intake **(Table B2 of Appendix B)**. This suggests that higher daily energy consumption may contribute to higher protein consumption, as similarly depicted by higher protein as a percentage of energy **(Table B2 of Appendix B)**. Interestingly, the group that was inadequate for protein had higher BW, BMI, and body fat despite lower energy intake than the group adequate for protein.

3.4.2 Exploration of body composition on protein and IAA intake

All individuals in the NZ cohort who met IAA requirements had a normal BMI. This could indicate a healthy BW. As protein requirements are expressed on a BW basis (g/kg/day), individuals with lower BW may more readily achieve daily protein and IAA requirements when comparisons are made on a BW basis alone. Similarly, higher dietary protein intake in these individuals could have a role in promoting satiety, maintaining a healthy weight and lean muscle mass (16, 61). Conversely, individuals with lower lean muscle mass may have a lower protein requirement (62). In individuals who were below leucine and lysine requirements but had higher BMI and percentage body fat, underreporting of energy intake and thus lower reported protein intake could be the cause, as reflected in the misreporting analysis. Yet, these results could also reflect true intake deficiency and thus, lower dietary protein intake (57.1 % of these individuals under the EAR), the latter being key for lean MPS (63, 64). Since adipose tissue contributes less to the BMR than lean body tissue, these individuals may physiologically require lower daily energy (65).

In developed countries, protein requirements can generally be achieved with adequate energy intake and diverse diets (66). This is not always the case if diets lack protein-dense foods, as is the case with many PB foods (grains, fruits and vegetables). Lower % energy from protein (~ 10 %) among individuals below lysine and leucine requirements (see **Table B3 of Appendix B**) in this cohort may suggest unbalanced macronutrient distribution, which could contribute to poorer body composition outcomes (higher BMI and percentage of body fat), but further analysis of FG contribution to carbohydrates and fats is required. A study with NHANES data on American adults similarly found an inverse correlation with BMI and protein intake and attributed this finding to mechanisms in lipid and glycaemic regulations (67).

Clinically significant differences in protein requirement tested with different approaches exist, such as BW and fat-free mass, but further assessments are required to determine how these measurements differ for different genders and population groups (62). Geisler et al. also described that body composition, which has large variability, leads to large variances in protein intake recommendations as compared to using only BW (60). There are currently no cut-off values for protein intake recommendations based on body composition metrics, such as fat-free mass. Clearly, more large-scale, well-controlled studies comparing individuals on different long-term diets could provide more precise guidance on protein and IAA intake based on body requirements and composition, rather than relying solely on BW. Ascertaining the effect of varied protein sources and macronutrient distribution on body composition would be valuable, but is not the emphasis of this study.

3.4.3 FG contribution to protein and IAA

Figure 3.7 demonstrated that “legumes and pulses” was the FG providing the largest proportion of overall protein and lysine intake. This was similarly reported in a systematic review of four PB diets, which found that legumes (including soy) contributed most to total protein intake in vegans as compared to other dietary patterns (68). Plant sources higher in leucine include dried seaweed, roasted pumpkin seeds, dry-roasted soybeans, cooked lentils and dry-roasted peanuts (69). These foods may be consumed in insufficient quantities in the cohort or in forms that limit digestibility. To improve total protein and lysine intake to match requirements, the intake of “legumes and pulses” could be increased, thereby increasing the protein-to-energy ratio of the diet. However, this approach must consider the implications on dietary fibre and its contribution to satiety, as well as the overall balance of other nutrients.

Cereals, such as wheat and maize, contribute less to digestible lysine. Moreover, as indicated by **Figure 3.7**, SAAs are more limiting in legumes like soy, peas, beans and lentils than in non-legume proteins (70, 71). Hence, the strategy of complementing legumes and cereals within each vegan meal to supply more balanced profiles for SAAs and lysine could be an effective approach for this vegan cohort. However, this is not always straightforward, as the content of these AAs varies substantially across PB foods as compared to ASFs. For example, the methionine:cysteine ratio varies notably across maize and wheat, even though they are categorised in the same FG in this study (72).

In support, one analysis suggested that pairings of PB foods should be conducted on an individual food level rather than broader food categories, which may offer low specificity in predicting IAA patterns (71). This implies that studies modelling nutritionally balanced vegan diets must critically examine the different permutations of PB foods at the food-item level, in their appropriate proportions towards nutrient contribution. The current dietary pattern of this vegan cohort compared poorly to the IAA scoring patterns for lysine and leucine, which indicates a poorer match between IAA intakes from the current vegan protein sources and the body's needs (27, 73, 74).

3.4.4 Optimising total protein and IAA intake

Generally, increased total protein consumption was associated with increased absolute IAA intake. Absolute tryptophan intake showed the weakest association with protein intake (R^2 = 0.64). This could be related to a lower tryptophan content in protein structures, such as in cereals and maize (75, 76). In corroboration, studies have found a 50 % decrease in tryptophan intake in both male and female individuals on a vegan diet compared with an omnivorous diet (44, 50). Another reason could be related to the methods used in tryptophan analysis, and these are addressed in the limitations.

Higher numbers in the top right quadrant for all IAA and protein adequacy comparisons in **Figure 3.6** clearly indicated common achievement of the digestible IAA requirement when the protein requirement was met or exceeded. These findings suggested that meeting protein quality in a vegan diet can likely be achieved when the total protein requirement is met. However, it was more difficult to achieve adequacy for lysine and leucine as compared to the other IAAs, even when protein adequacy was achieved. This was not unexpected as these IAAs, particularly lysine, have low concentration in cereals as compared to other PB foods, such as legumes (77). To follow up on this study, examining the most appropriate PB protein combinations and proportions across the cohort is crucial to optimise both protein and IAA intake to achieve daily requirements. Investigating dietary patterns among individuals at risks of deficiencies (below requirements) and overcoming IAA shortfalls in the diet are critical steps forward.

Overall, this Chapter shows that while the majority of vegans in the cohort were meeting total protein intake compared to the EAR, about a quarter had insufficient intake (**Table 3.2**), as similarly reported in past surveys on vegan populations (7, 44). If requirements were increased to the level of the RDI, 50 % of the cohort would be inadequate. Beyond examining total protein, the critical examination of individual AAs is more critical because of their direct roles in protein metabolism and other chemical pathways within the body. Uncovering potential deficiencies in lysine and leucine within this vegan cohort is a key finding of this study.

Generally, the vegans in this study followed diets that included a variety of plant foods, which could imply moderate acceptability and knowledge of diverse food combinations. This could be related to a high level of education among participants, but requires further confirmation with nutritional knowledge scores. Yet, the IAA intake profiles (especially for lysine and leucine) could reflect inappropriate proportions of cereals to legumes. Exploring the optimisation of the cereal-to-legume ratio could improve leucine and lysine profiles while simultaneously increasing overall protein intake. Food combinations occur at the meal level so these strategies must be implemented within such contexts. These methods must also account for overall macronutrient and micronutrient balance, and consider the impacts of dietary fibre intake, which contributes to fullness and gastrointestinal discomfort in excess (78).

Novel PB meat and dairy alternatives could offer opportunities to narrow the dietary gaps in protein and certain micronutrients, as evidenced by the lysine contribution from these foods in this study. However, novel alternatives have highly variable ingredient and nutrient composition, as well as processing methods, which have differing impacts on digestibility (79). A review of product formulations is necessary to uncover how limiting IAAs and overall nutritional content may be improved in novel alternatives.

3.4.5 Strengths and limitations

High protein quality diets cannot be assumed in vegan populations, even if total protein intake meets daily requirements. Varied digestibility among PSPs must be accounted for, and this is the first strength of this study. However, digestibility scores provided from the literature may be imprecise due to simplified classifications of many food items into one FG. While normalising IAA quantities from matched foods in the USDA database to those consumed in this cohort provides a representation of actual intake, the degree of accuracy in this normalisation also remains uncertain. Neither have all food preparation techniques been differentiated for digestibility variations.

Methodological inconsistencies in obtaining tryptophan digestibility values could further explain the greater variance (R^2 = 0.64) observed at higher protein intake (**Figure 3.5).** Firstly, tryptophan cannot be analysed by standard amino acid hydrolysis, which degrades its labile indole ring and even with alkali hydrolysis, only small quantities (16 to 20 %) can be recovered (80-83). Secondly, varying protein sources could explain the variation in the digestibility values of tryptophan (84). Finally, tryptophan digestibility is impacted by competition from large neutral amino acids and terminal ileum absorption kinetics, which also vary across protein sources; higher tryptophan absorption is strongly correlated with higher tryptophan concentrations in foods (85).

The pig is the most reliable animal model for obtaining TID values across a wide range of protein sources, but digestibility data for many fruits and vegetables remain lacking (84). However, this may be of lower consequence in this study, as these foods do not contribute large amounts of protein and IAAs. An effort has also been made in this thesis to distinguish several common PB foods. For example, to reflect the higher digestibility of extruded proteins, more recently available TID values for pea and soy concentrates and isolates (23) were used for the novel PBMAs instead of using poorer TID values from peas and soy beans. While this step does not account for all aspects of bioavailability, such as the overall food matrix and intra-individual variations in absorption and utilisation, it indicates the proportion of nutrients that are digestible (28, 86). This also accounts for differences between traditional and more novel plant foods made with protein isolates and concentrates, with the expectation that more specific measurements will be used when updated databases become available.

Misreporting is always an inherent limitation in dietary records, with studies finding underreporting of carbohydrates, protein, and snack foods among adults (87), which could account for low energy intake in this cohort. Little information is known about which foods are misreported within each macronutrient group (87). A similar problem was highlighted by Waldman et al. (2003), who found a large proportion of surveyed German vegans falling below the recommended daily energy requirement, even when lower values were used as a reference (88). Without a controlled feeding study or doubly labelled water techniques to assess the accuracy of self-reported recalls, it is unclear which foods may be misreported in this study. The Goldberg cutoffs that were utilised are a reasonable method to determine misreporting in the cohort (37).

For outliers in this study, the food diaries were checked for accuracy and anthropometric measurements were validated. Excluding outliers did not affect the significance of the results. To check whether these outliers followed a high-protein-quality vegan diet alongside a high-protein diet, they were not excluded from the analysis. Similarly, participants with a likelihood of misreporting were still included in the study to avoid selection bias (89). Another limitation was at the calculation step of protein content in food, where the nitrogen-to-protein conversion factor of 6.25 was used, based on the assumption that all proteins contain 16 % nitrogen (90). This is imprecise, but it is the widespread current practice when quantifying protein.

The four-day food diary of long-term vegans was used to calculate the mean protein and energy intake over four days. As nutrient intake can vary from day to day, even within the same individual, a four-day food diary may be insufficient to accurately reflect true protein intake. However, it provides a reasonable estimate to obtain dietary intake while limiting participant burden. Within- and between-person variability in daily protein intake was quantified using a random-intercept linear mixed model, which estimated 61 % of the total variance in daily protein intake between individuals and 39 % within individuals. The estimated reliability coefficient attained for the four-day mean was reasonably high, indicating that 86 % of the variance in the four-day mean protein intake reflects true between-person differences in usual protein intake, with 14 % attributable to residual day-to-day variation.

There was also an over-representation of females in the surveyed cohort, who have lower energy and total protein intake than males in the results found here, potentially biasing the overall energy and protein intake of the cohort. However, the sampled population reflects the typical demographics of the NZ vegan population, with a high female-to-male ratio (3). Similarly, a few studies that evaluated the diets of vegans with more than 100 participants also found a domination by females (1). Nevertheless, longer-term studies with more balanced male-to-female ratios and larger cohort sizes in long-term vegan populations would validate these results. Lastly, plasma AA levels were not evaluated or compared with those from other studies that have collected both intake and metabolite measurements (44, 53, 91, 92). This was outside the scope of this thesis, but is a potential area for exploration to ascertain if insufficient intake of IAAs would manifest in nutritional status.

3.5 Conclusions

Generally, higher consumption of total protein to achieve daily protein requirement is associated with higher protein quality and achievement of daily IAA requirements in NZ vegans. However, these conclusions vary across IAAs and could be impacted by the combination of different plant protein sources. Meeting adequate total daily protein intake in a vegan diet does not always guarantee a high-protein-quality diet, and simply considering total protein intake without delving into protein quality will overestimate protein adequacy among vegans. Digestibility-adjusted leucine and lysine were the two most limiting AAs when compared with AA scoring patterns and daily requirements in this cohort of NZ vegans. Given their critical metabolic functions, dietary recommendations to improve the overall IAA composition and increase leucine and lysine intake should be considered. Increasing intake from “legumes and pulses” could improve the overall IAA profile, but determination of ratios between cereals and legumes needs to be carefully examined for energy, dietary fibre and overall nutritional profile.

This Chapter showed the importance of accounting for digestibility in dietary studies, as simply utilising the IAA composition of food items can overestimate protein adequacy. As the body is incapable of producing or storing excess IAAs, there are limited time windows throughout the day for effective protein utilisation. A few studies have discussed the potential of an even distribution of protein and AAs in meals to maximise muscle protein accretion (12, 64, 93). To this end, assessing protein intake and protein quality of different plant food combinations would be more suitable at the meal level. However, there is still no consensus on the appropriate protein and IAA requirements for each meal, or whether these may differ among sexes, age groups and body composition patterns. To improve protein quality in vegan diets, future Chapters will focus on identifying optimal combinations of traditional and novel PB foods to achieve balanced intakes of all digestible IAAs, particularly for lysine and leucine, and this should ideally be examined within the context of meals.

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In which chapter is the manuscript/published work?		Chapter 4	
Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1] Bi Xue Patricia Soh: Conceptualisation, data curation, formal analysis, investigation, methodology, writing of original draft, review and editing. Dr Matthieu Vignes and Dr Nick W Smith: Formal analysis, methodology, supervision, review and editing. Professor Pamela R von Hurst and Professor Warren C McNabb: Conceptualisation, funding acquisition, investigation, project administration, supervision, review and editing			
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Chapter 4 Protein intake and protein quality patterns in New Zealand vegan diets: An observational analysis using Dynamic Time Warping

This Chapter provides an assessment of dietary protein intake and protein quality across eating occasions in the day and identifies three dietary pattern clusters. While Chapter 3 has only assessed daily protein intake and protein quality, this Chapter provides additional evaluation of how protein and IAA intake change over 24 hours and outlines the impact of meal protein distribution on protein metabolism.

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Abstract

Inadequate intake of IAAs is a significant challenge in vegan diets. Since IAAs are not produced or stored for long periods in the human body, regular, balanced dietary protein consumption throughout the day is essential for metabolic function. The objective of this Chapter was to investigate variation in protein and IAA intake over 24 h among NZ vegans with time-series clustering, using Dynamic Time Warping (DTW). This data-driven approach objectively categorised vegan dietary data into distinct clusters to analyse protein intake and protein quality. Mean total protein consumed per eating occasion (EO) was 11.1 g, with 93.5 % of the cohort falling below the minimum threshold of 20 g of protein per EO. The mean protein intake for each EO in cluster 1 was 6.5 g, cluster 2 was 11.4 g, and only cluster 3 was near the threshold at 19.0 g. IAA intake was highest in cluster 3, with lysine and leucine intakes 3 times higher than in cluster 1. All EOs in cluster 1 were below the reference protein intake relative to BW, closely followed by cluster 2 (91.5 %), while cluster 3 comparatively had the number of lowest EOs under this reference (31.9 %). DTW produced three distinct dietary patterns in the vegan cohort. Further exploration of plant protein combinations from clusters with more adequate protein intake could inform future recommendations aimed at optimising protein quality in vegan diets.

4.1 Introduction

Dietary protein ingestion and its subsequent digestion within the GIT is crucial to the provision of IAAs, which cannot be endogenously supplied by the human body (1-3). IAAs are mostly absorbed through the ileal membrane into the bloodstream and then utilised for protein synthesis, the maintenance of net protein balance, and other metabolic functions (3, 4). One unique trigger for MPS is the postprandial elevation of IAAs, particularly leucine, in the plasma and intracellular spaces, and this needs to be achieved through protein consumption (1, 5, 6). Plasma AAs are absorbed by body tissues such as the skeletal muscle until metabolic capacity (i.e., “muscle-full effect”) is achieved (6, 7). The synchronous supply of all IAAs within a single EO is essential, as the effectiveness of protein synthesis may be constrained by the least available AA (8). As the body is incapable of storing excess AAs for later metabolic function, the need for the simultaneous presence of all IAAs within a limited timeframe underscores the importance of carefully balancing dietary protein consumption within each EO (9, 10).

Foods in the human diet are rarely consumed in isolation (11), so including an ASF of higher protein quality is an efficient method to raise the overall IAA composition and digestibility of a mixed meal. This is, however, not an option in a vegan diet, which excludes all ASFs. Combining complementary PB foods with different limiting IAAs within each EO is therefore an efficient method for the simultaneous delivery of all IAAs to achieve threshold levels necessary for protein utilisation in a vegan diet. Understanding the current temporal distributions of dietary protein and IAAs, and how food combinations vary within such contexts, is the first step in identifying practical meal guidelines to improve overall protein intake and protein quality in vegan diets.

The protein adequacy of diets is currently considered by comparing total protein and IAA intake with their respective daily requirement values (12). However, when protein adequacy is examined on a per-day instead of per-EO perspective, misleading conclusions about meeting protein requirements may occur (13). The distribution and protein quality across meals of the day are masked with such an analysis, and thus cannot account for how the evenness of protein distribution over time can impact physiological functions (14). A high daily protein intake is the most significant factor in impacting lean body mass, but the combination of total protein quantity, protein quality, and meal (and snack) distribution may become pertinent in restrictive diets or more vulnerable populations (1).

Research on meal-pattern analysis for protein distribution is often hampered by the lack of well-defined per-meal requirements of total protein and IAA relative to body requirements. The current recommendation by a few experts for absolute protein intake per meal in a three-meal-per-day scenario is between 20 and 30 g to achieve metabolic benefits (15-17), but the target population in these studies varied, so it is unclear if this recommendation applies to the general adult population.

Past studies assessing the nutritional quality of meals have used time-of-day approaches, with meals generally designated as breakfast, lunch, and dinner (14, 18, 19). Such methods are problematic, as pre-defined time windows for meals are inconsistent across studies and subject to researcher and classification bias - often associated with personal and social constructs - as compared to more novel data-driven methods (20-22). Conventional techniques are also unlikely to capture meals outside of these time windows (such as for shift workers) and are open to debate on whether an EO should be classified as a meal or snack, depending on energy, nutritional content, or the situational context (11, 23). A more objective methodology is necessary to examine high-dimensional dietary and nutrition data (11, 24). Data-driven techniques allow for the detection of patterns in the diet independent of time of day (25) that may otherwise be missed with traditional approaches. It is then possible to assess whether groups of individuals with similar temporal dietary patterns have the same nutritional and health outcomes.

The aim of this Chapter was to evaluate protein and IAA intake patterns among a sample of NZ vegans and identify the temporal variation in total protein and IAA distribution across 24 hours. DTW and hierarchical clustering (HC), two unsupervised methods in data analytics, were used to assess and classify distinct protein and IAA consumption patterns. A secondary aim was to investigate PB food combinations that emerged from the categorised dietary patterns. This Chapter thus offers quantitative insights into how varied dietary patterns can affect protein metabolism and, consequently, inform future interventions to improve the protein content and protein quality of vegan meals.

4.2 Methods

4.2.1 Study design and data processing

Steps undertaken in the collection and processing of dietary intake data have been detailed in Chapter 3. Date and time stamps from the processed dietary data were examined for accuracy for all EOs of each participant. When possible, missing date or time records were imputed by cross-checking with actual food records provided by participants. When manual imputation was not possible or accurate, the food records for that entire day were excluded. Six days with unclear or missing records were excluded. The time of food intake was absent in one food diary record for the first day. For the remaining five, records for the 4th day were absent. Total protein and IAA quantities, adjusted for digestibility, were summed at each EO for each day and each participant. EO is defined as the time point at which all foods were consumed together.

4.2.2 Data analysis

DTW and subsequent clustering of time-series data were used to uncover patterns in the data and group them based on similarity (24). The temporal distribution of intake patterns within the vegan cohort and how protein and IAA quantity vary as a function of time can thus be objectively categorised and subsequently examined (26).

Unlike the well-established daily requirement patterns for protein and IAA (12), there are no well-defined reference or requirement values for protein and IAA intake per EO. As described previously, reported reference values varied between 20 and 30 g of protein per meal, or 0.24 to 0.4 g/kg/day (19, 27, 28), but are dependent on the protein source, population tested, and the parameters measured (e.g., lean mass or muscle strength). However, the intake levels in this study cohort need to be compared with a suitable reference measurement to evaluate the protein adequacy of a vegan diet throughout the day, and must be especially adjusted for BW (29). The lowest possible threshold values obtained from the aforementioned studies were thus selected: 20 g per meal and 0.24 g/kg/day. Mean protein intake per EO for each individual was compared to this threshold, which was established as the minimum threshold to stimulate MPS (22, 30, 31). This was justified by a significant increase in leucine oxidation above 20 g, suggesting a "muscle-full effect" and no further protein anabolism above this threshold (30). The percentage of individuals in the cohort with mean EOs below these thresholds was calculated as an indication of adequacy at the EO level.

Total protein intake at each EO across the day represented the data points within one time series for each individual. This is denoted as *individual.day,* to represent the combinations of EOs per day for each participant. To group similar time series, a similarity measure was calculated by DTW (26) with the dtw package in R (32, 33). Briefly, this approach obtains a warping path of minimum distance between two time series to align patterns of the same size (34-36). To account for the varying lengths of time-series sequences, the normalised distance was used (32). Using total protein consumed per EO as the comparison parameter, a distance matrix was tabulated that captured the similarity between participants' daily protein patterns (*individual.day*) across all four days. Lower DTW values indicated greater similarity between time series, showing more similar protein consumption patterns. Self-comparison was avoided by ensuring that DTW calculated distance between distinct *individual.days*. To validate the similarity measure calculated by DTW, several time series were selected from each cluster, and the temporal protein distribution patterns were mapped and compared against the computed pairwise distances.

Figure 4.1 and **Table 4.1** below provide a visual representation of a subset of participants and an example of how protein intake patterns were validated against the DTW-computed distance matrix. For example, 2.1 and 2.2 represent the same individual's (individual 2) dietary pattern over two days (day 1 and day 2, respectively). These samples were more similar to each other than to 4.2 (individual 4 on day 2); their pairwise distance was smaller (2.34) than that between 2.1 and 4.2 (25.0). This was similarly observed when comparing individual 2 to individual 3. Sample 5.3 (individual 5 on day 3) was more similar to both 2.1 and 2.2 than it was to 4.2. By warping their time axes, the DTW method computed a minimal distance between two time series. This computed distance did not consider the duration between time points where the peaks occurred (for e.g., differences in time of first meal), or the duration of each EO (how long foods were consumed), but aligned the overall "shape" of dietary intake between each *individual.day*.

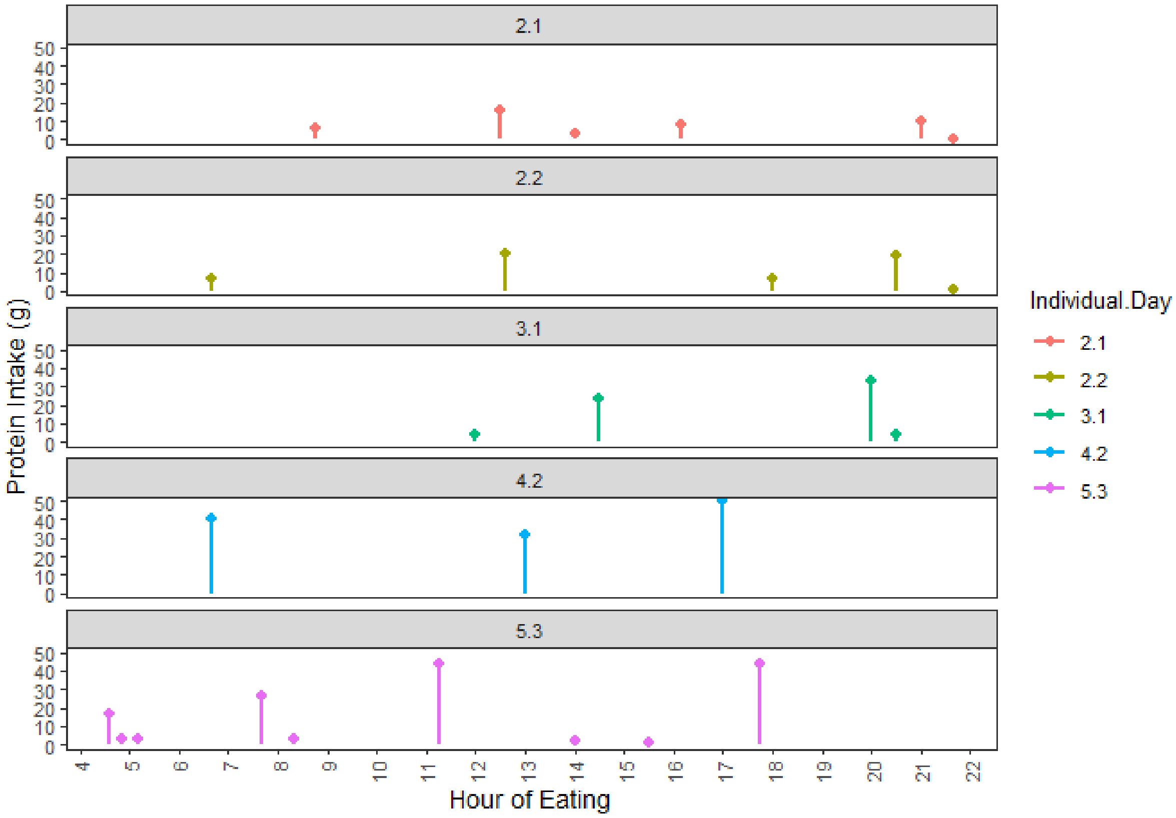


Figure 4.1 Protein intake as a function of time of the day for several sample *individual.days*. 2.1 represents individual 2's protein intake pattern for day 1, and 2.2 represents the same individual on day 2. 3.1 represents individual 3's protein intake pattern for day 3. 4.3 represents individual 4's protein intake pattern on day 3, and 5.3 represents individual 5's protein intake pattern for day 3.

Table 4.1 Sample representation of a two-dimensional distance matrix of pairwise normalised distance as computed using DTW.

	2.1	2.2	3.1	4.2
2.2	2.34			
3.1	5.45	5.70		
4.2	25.0	21.9	14.0	
5.3	8.07	7.99	8.44	19.6

Each participant's unique protein intake distribution across the day was depicted as one time series (*individual.day*). The application of DTW compared and matched the patterns of two time series. For example, 2.34 is the resulting distance between the *individual.days*, 2.1 and 2.2. This is smaller than the distance between *individual.days* 2.1 and 3.1, which is 5.45, indicating greater similarity between 2.1 and 2.2 than either is to 3.1. Based on the calculated distance, *individual.day* 4.2 differs most from the other time-series patterns.

Each *individual.day* was placed as a data object in a dendrogram with the agglomerative method of HC (37), which grouped these objects based on their similarity as calculated using DTW. Ward's minimum variance method was applied to merge clusters that yielded the smallest increase in the sum-of-squares variance (36). The output of the HC is visualised in the dendrogram in **Figure 4.2**, which was enhanced by colouring branches to distinguish clusters, with k = 3 clusters. The appropriate number of clusters (k) was determined using domain knowledge and guided by an average silhouette width (see **Figure C1 of Appendix C**). This is the optimal ratio that maximises inter-cluster distance and minimises intra-cluster distance, thus providing an evaluation of clustering validity (38).

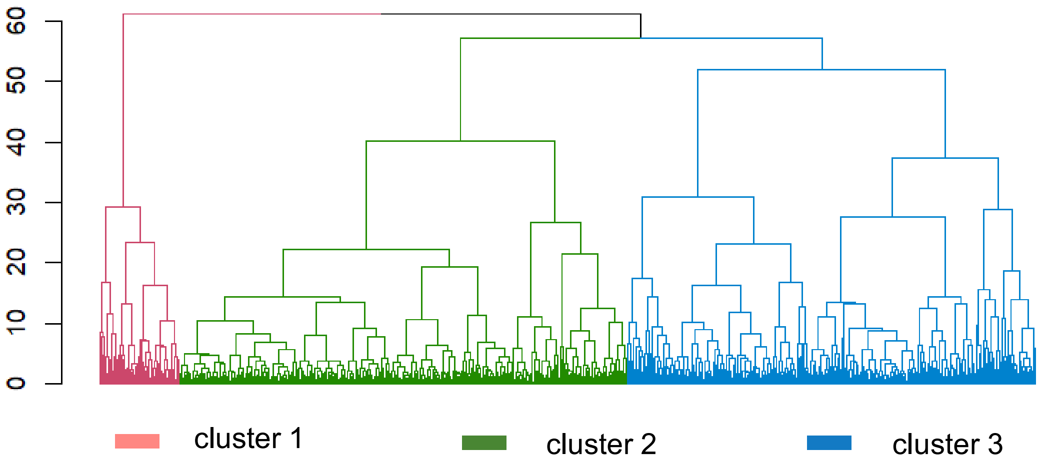


Figure 4.2 Dendrogram clusters for each *individual.day.*
A total of 766 time series/observations using HC with Ward.d2 method (36). Using the agglomerative method, each data point was merged to form a cluster, and merging continued until a single data point was formed, at height 60. Vertical lines represent the branches, and horizontal lines represent fusions of clusters or data points. The longer the vertical line, the larger the height, and the more dissimilar the data points or clusters.

To further visualise the clusters, the DTW distance matrix was projected into a two-dimensional space using multidimensional scaling (MDS) (39). This step transforms the DTW distance matrix into coordinates in a 2D plane to interpret cluster separation and represent the relative distances between participants based on patterns of EOs.

While Chapter 3 evaluated protein and IAA adequacy based on total daily intake, this approach did not capture the distribution of protein across EOs. Considering the vegan cohort as a single group also obscure heterogeneity in dietary behaviour which can affect protein intake. This Chapter thus extends the assessment from daily adequacy to temporal distribution of protein intake. The use of DTW therefore enables quantification of dissimilarity in the timing of EOs to reflect differences in overall temporal patterns. HC was then appropriate as it operates directly on dissimilarity matrix and does not require the observations to first be represented within a Euclidean space. This was relevant as DTW uses pointwise differences when aligning two time series and do not satisfy the Euclidean geometry. Together, DTW and HC provided added value beyond assessing daily protein intake by identifying if subgroups existed within the cohort based on when and how much protein was consumed across the day.

4.2.3 Cluster analysis

Each cluster represented collections of *individual.days* with similar patterns for protein intake. To investigate how the DTW algorithm identified similarities between time series, clusters derived from the computed distance matrix were analysed for variations in protein quantity, IAA composition, frequency of EOs per day and FG contribution.

Variation across clusters

The contribution of individual FGs to protein and IAA intake was evaluated for each cluster. This step allowed determination of how the composition and proportion of PB protein sources differed between clusters and their influence on protein and IAA intake. Each food item from the four-day food diary was matched to its respective FG, as defined in the 2008/2009 NZ Adult Nutrition Survey (40). This step has been elaborated in Chapter 3 (Section 3.2.4).

Statistical analysis

To assess differences in protein and individual IAA intake, as well as body composition across clusters, and to identify which FGs exhibited significant differences in nutrient contributions among clusters, the Kruskal–Wallis (KW) test was used. A significant difference was identified when the P-value was < 0.05. Subsequent pairwise comparisons were conducted using the post hoc Dunn's test with a Bonferroni adjustment to determine the specific groups contributing to observed significant differences.

Temporal variation in each cluster

To capture the fluctuation of dietary behaviour over time and across different clusters, the time data were transformed into 24-hourly intervals. Total protein intake per EO was computed for each time segment and dietary cluster. To establish how protein quality differed across clusters, heat maps were created to visualise the distribution of mean IAA intake across hourly time segments for each cluster.

4.3 Results

4.3.1 Total protein distribution over time

A total of 193 individuals with complete dietary intake data for at least three days were included in the temporal analysis of protein intake across the day. Distinct protein intake patterns were observed across the cohort, and protein intake was also uneven throughout the day.

Figure 4.3A presents the overall distribution pattern for protein intake (y-axis) for all EOs (whose time is depicted on the x-axis) for all 193 participants across the four days of dietary recall. A high density of EOs contains protein content lower than 10 g. Fewer EOs contained 10 to 20 g, and very few contained > 30 g of protein. These findings were corroborated by the histogram illustrated in **Figure 4.3B**, which showed a gradual decrease in density as protein intake increased. The highest density was observed in the lowest protein-intake bin of < 5 g per EO. Less than 2.5 % of EOs contained protein above 40 g and were omitted in **Figure 4.3,** but can be observed from **Figure C2 in Appendix C.** As a cohort, the mean protein intake was 11.1 g (SD = 5.3 g) per EO, and 93.5 % of EOs contained < 20 g of protein. **Figure 4.3A** and the histogram illustrated in **Figure 4.3C** showed three main peaks for total protein intake, which occurred at approximate time intervals of 07:00 to 08:00, 12:00 to 14:00, and 18:00 to 20:00 h. Total protein intake per EO was slightly higher in the later hours of the day as compared to the morning.

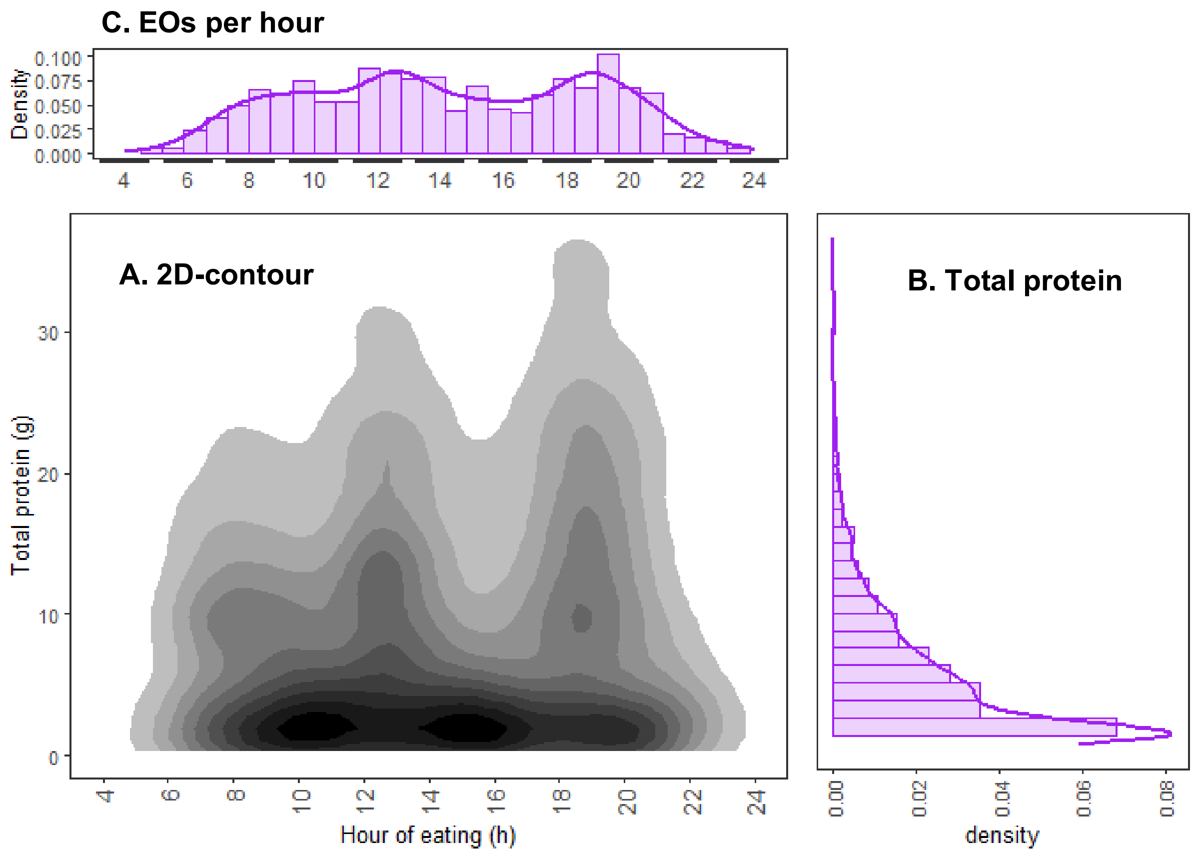


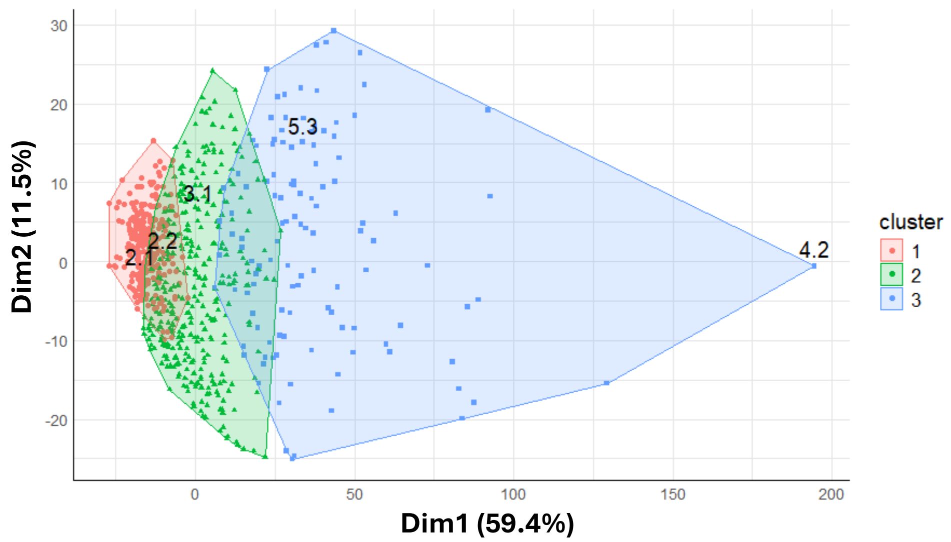
Figure 4.3 Two-dimensional contour estimate of temporal distribution of total protein (g) per EO for all 193 participants over four days.
The varying density of total protein intake is represented by a grey colour ramp; darker areas have more EOs (**A**). Histograms (purple) represent density of total protein consumed (g) (**B**) and density of EOs per hour of eating (**C**). The temporal distribution of protein intake per EO showed that the vast majority of EOs occurred between 04:00 and 24:00. In total, < 10 EOs were observed outside of these hours (presented in **Figure C2, Appendix C**).

4.3.2 Cluster analysis: variation in intake patterns

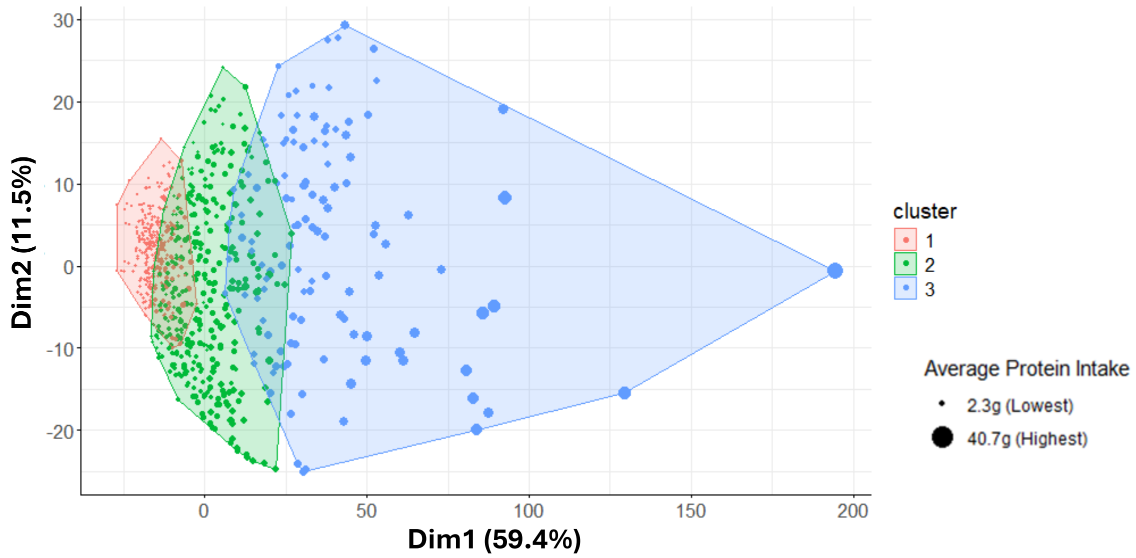
A total of 766 time series (193 individuals × 4 days, with 6 *individual.days* excluded due to incomplete time records) were tabulated. Each *individual.day* was placed in its respective cluster based on the calculated DTW distance matrix (**Figure 4.2**). The agglomerative clustering stopped at a height of 60, when all observation clusters were merged. The results from the silhouette analysis (**Figure C1 of Appendix C**) indicated that the optimal number of clusters is $k = 2$, as determined by the largest average silhouette width. However, as two large clusters were observed sharing the same merging node in **Figure 4.2**, $k = 3$, three clusters were selected for greater differentiation of the dataset (**Figure 4.2 and Figure C3 of Appendix C**).

Across the cohort, individuals had an average likelihood of 72.8 % that their diets would be assigned to the same cluster on different days. There was a 65.4 % chance that an individual experienced at least one shortfall in protein or an IAA on any given day within the four-day diet record. Together with findings from Chapter 3 on within-individual variation, participants tended to follow broadly similar dietary patterns across the four-day food records, but there was some fluctuation in protein intake. The three clusters were visualised on an MDS plot (**Figure 4.4**). Each point represents an individual time series, which corresponds to a unique *individual.day*. Dim 1 and Dim 2 accounted for 59.4 % and 11.5 % of the variance in these time series, respectively. The emergence of clusters was based on normalised DTW distances between time-series data. Clusters were analysed to determine how mean protein intake and number of EOs differed across dim 1 and 2. The specific exemplar *individual.days* as described in **Figure 4.1** and **Table 4.1** are noted in the MDS plot (**Figure 4.4A**). Their separation in the MDS space is consistent with the DTW dissimilarities, illustrating that exemplar i*ndividual.days* assigned to different clusters are positioned further apart than those within the same cluster.

A. Cluster visualisation of protein intake (g) on MDS plot



B. Mean protein intake (g) per EO, across dim 1 and dim 2



C. Number of EOs across dim 1 and dim 2

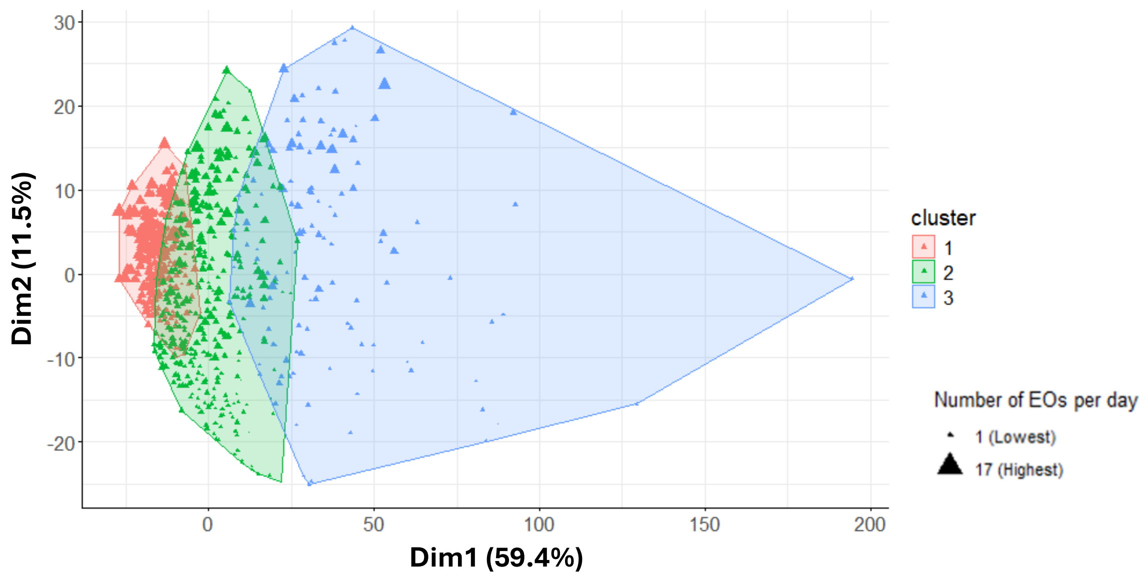


Figure 4.4 Cluster visualisation of the mean protein intake pattern per EO for each *individual.day* on the MDS plot.
Convex envelopes as shown by coloured lines around each cluster represent the boundaries of each cluster. (**A**) Examples of *individual.days* described in **Figure 4.1** and **Table 4.1** are noted. (**B**) A cluster visualisation of how mean protein intake changes across clusters. The larger the point (shown as a dot), the higher the quantity of protein per EO. The size of dots is the largest in cluster 3, and smallest in cluster 1. Mean protein intake increases towards the right of dim 1. (**C**) Cluster visualisation in which each point on the MDS plot represents the number of EO for each *individual.day*. The larger the point (shown as a triangle), the higher the number of EOs. The size of points is smallest in cluster 3, and larger in cluster 1, as well as larger at the top of dim 2. This indicates that the frequency of EOs decreases across dim 1 and down dim 2.

Mean daily protein intake per cluster was tabulated to be above the EAR for both males and females, taken to be 0.68 g/kg/day for males and 0.60 g/kg/day for females (41), but was lowest in cluster 1 (0.64 g/kg/day) compared to clusters 2 (0.88 g/kg/day) and 3 (1.3 g/kg/day). The mean absolute protein intake per EO for cluster 1 was also the lowest (6.5 g), followed by cluster 2 (11.4 g), and highest in cluster 3 (19.0 g).

When adjusted to individual BW, the lowest protein intake (g/kg/day) per EO was in cluster 1, at 0.10 g/kg/day, followed by cluster 2, at 0.17 g/kg/day. The mean protein intake per EO was highest in cluster 3, at 0.27 g/kg/day, and above the selected reference value of 0.24 g/kg/day. Consequently, no EO in cluster 1 met the target of 0.24 g/kg/day, while clusters 2 and 3 had 91.5 % and 31.9 % of EOs below the target, respectively. In tabulating the mean total of all IAAs per EO, cluster 1 also exhibited the lowest quantity of total IAA, with a mean of 1.3 g (0.4) per EO, followed by cluster 2, at 2.2 g (0.7) and cluster 3, at 3.8 g (1.4).

The number of EOs per *individual.day* was similar across clusters but highest in cluster 1 and lowest in cluster 3 (cluster 1 mean = 6.5 EOs/day; cluster 2 = 5.2 EOs/day; cluster 3 = 5.0 EOs/day). A larger number of EOs were found towards the top of dim 2, although the distribution down dim 2 was inconsistent (**Figure 4C**), which could again be a consequence of the smaller variance exhibited by dim 2. Despite the high EOs per day in cluster 1, the mean protein per EO remained low, whereas in cluster 3, there were fewer EOs across the day but with higher protein intake per EO (**Figure 4C**).

Differences in body compositions were explored across the clusters. The mean BMI of individuals across clusters was similar. Cluster 1 was 23.8 (3.3), cluster 2 was 24.0 (3.1), and cluster 3 was 24.2 (2.7) and were not significantly different ($P = 0.19$). However, the mean percentage body fat was highest in cluster 1, at 32.0 % (6.5), followed by cluster 2 at 30.0 % (7.6) and cluster 3 at 25.4 % (6.8), with a significant difference across all clusters, as demonstrated by the KW and pair-wise comparisons with a post hoc Dunn's test ($P < 0.05$).

4.3.3 Cluster analysis: contribution of FGs to total protein and IAA intake

Figure 4.5 shows the percentage contribution of each FG to the total intake of protein and each IAA across the entire dataset. “Legumes and pulses” provided the highest contribution to total protein and most IAAs, particularly for lysine, across all clusters but was higher in clusters 2 and 3 than cluster 1. “Grains and pasta” was the next largest contributing FG to total protein and IAA **(Table C1 of Appendix C,)** and, contributed most to the SAAs (cystine and methionine) in cluster 1, more so than for clusters 2 and 3.

The KW test showed that for “legumes and pulses” and “grains and pasta”, significant differences in absolute intake were observed across most clusters for protein and all IAAs, but were not significantly different for most of the other FGs. **Table C2 of Appendix C** presents the P values from the post hoc Dunn’s test for pairwise comparisons between clusters for all nutrients and FGs. Clusters 1 and 3 differed significantly in many nutrients across most food groups compared with 1–2 and 2–3 (**Table C2 of Appendix C**). Protein and IAA contributions from novel PBAs such as PBMAs and PBBs were explored for each cluster **(Figure C4 of Appendix C**). As compared to clusters 1 and 2, where protein and IAA contributions from novel PB foods were mostly from grains, the contributions from cluster 3 were provided almost entirely by “legumes and pulses”.

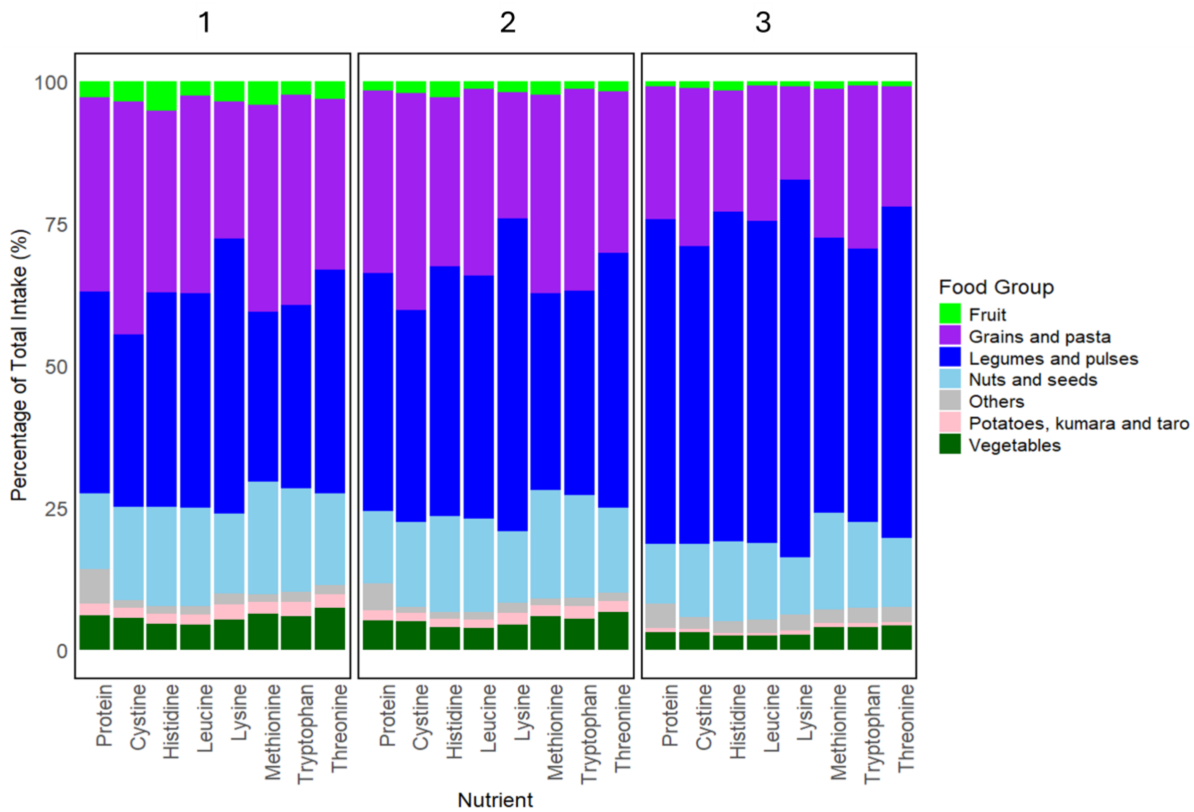


Figure 4.5 The contribution of food groups to TID-adjusted protein and IAA intake per cluster (numbered 1 to 3) as a percentage of total intake.
"Others" included "beverages", "savoury sauces and condiments", and "sugar and sweets" that each contributed < 5 % of total protein intake.

4.3.4 Cluster analysis: temporal variation in total protein and IAA intake

Based on the spline curves in **Figure 4.6**, cluster 3 showed greater variability. As shown by the colour gradient of individual plot points, total protein intake per EO was slightly higher in clusters 2 and 3. A slightly higher intake of protein in the later hours of the day than in the morning was observed, more so for clusters 2 and 3. Overall, cluster 1 portrays EOs with the lowest total protein, which were more evenly distributed throughout the day as compared to clusters 2 and 3.

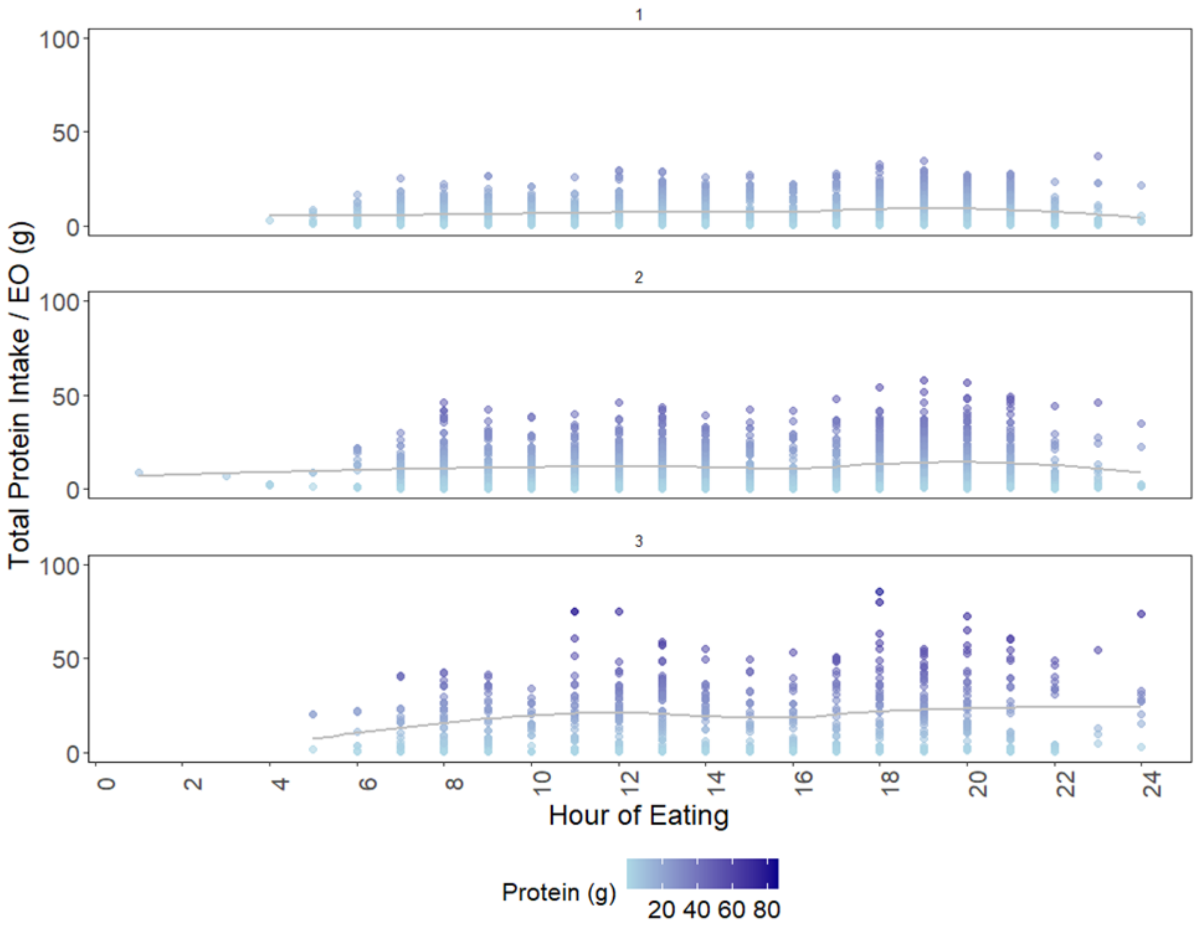


Figure 4.6 Temporal patterns showing the distribution of protein intake over 24 h of the day for all *individual.days* in each cluster (numbered 1 to 3).
Points represent total protein intake (g) from each EO. Grey lines indicate a spline curve to outline the relative trend over time.

Figure 4.7 shows that the lowest mean intake of all IAAs across the day was observed in cluster 1, followed by clusters 2 and 3. This was particularly true for threonine, lysine, and leucine. Mean IAA intake was slightly higher in the later part of the day, as indicated by the colour scale. This was most apparent for clusters 2 and 3, where the highest intake for these IAAs was between 18:00 and 21:00 h.

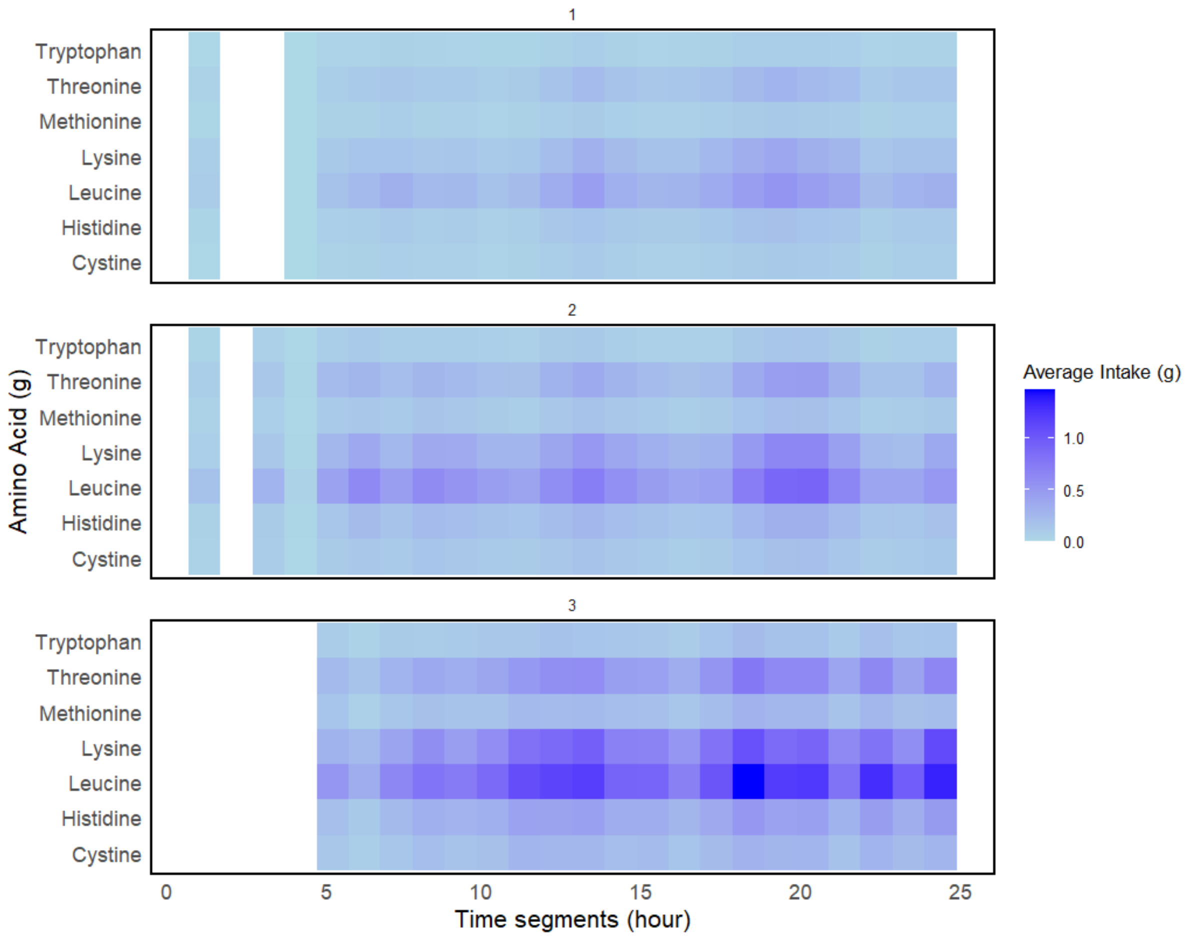


Figure 4.7 Comparing the mean IAA intake (g) per EO across 24-hour time segments among clusters 1 to 3 (numbered).
A gradient colour scale was used, with darker shades indicating higher intake. IAAs with higher requirements (threonine, lysine and leucine) appear denser in each cluster.

4.4 Discussion

This Chapter evaluated the temporal distribution of protein and IAA intake among a sample population of NZ vegans. The findings showed that total protein intake is unevenly distributed throughout the day, with higher intake in the later hours. When examining absolute protein intake as a cohort (**Figure 4.3**), most vegans consumed less than 20 g of total protein per EO. Using DTW and HC, participants were objectively classified into three distinct clusters that differed in total protein intake, IAA intake, and EO frequency throughout the day. This application of DTW aligns with prior research that also used this method to identify unique temporal patterns in energy intake across clusters (20).

4.4.1 Cluster analysis

Clusters 1 and 2 contained 38.4 % and 46 % of the total *individual.days* respectively, while cluster 3 contained 15.7 % *individual.days*. Only cluster 3 was close to the stipulated reference intake of 20 g and 0.24 g/kg/day for mean protein intake per EO and had the highest intake of most IAAs. Leucine and lysine intakes were at least 3 times higher in cluster 3 than in cluster 1 (**Figure 4.7**). When using *k* = 2 for HC, as indicated by the silhouette analysis, clusters 2 and 3 were combined **(Figure C3 of Appendix C)**, while differences between clusters 2 and 3 were captured when k = 3 (**Figure 4.2**). Overlaps were observed between clusters 1 and 2 and between clusters 2 and 3 (**Figure 4.4**), indicating some similarity in dietary patterns, but the DTW algorithm distinguished protein intake and protein quality patterns, with the greatest differences between clusters 1 and 3.

Chapter 3 showed that in this vegan cohort, increasing protein intake is associated with increasing IAA intake, although with varying degrees of association strength as protein intake increased above 50 g. Assessing total protein intake on a per-day basis tends to overestimate protein adequacy, as IAAs provided by dietary protein sources can only be utilised within a short time window following ingestion (13, 42). Unlike carbohydrates, fats, and some micronutrients, IAAs cannot be stored in the body apart from in skeletal muscle, which serves as a short-term reservoir (1, 42). Despite achieving the mean daily protein intake per kg of BW for each cluster, this vegan cohort may be challenged in achieving the minimum threshold of 20 g or 0.24 g/kg/day of protein per EO, especially for individuals in cluster 1. This underscores the importance of evaluating protein adequacy at the EO level, as a per-day analysis tends to overestimate it. These findings align with Cardon-Thomas et al. (2017), who found a sub-optimal supply of protein in each meal despite the total protein intake per day being above RDI for older adults (14).

4.4.2 Temporal distribution of dietary protein

Even though protein intake in cluster 1 appeared to be more evenly distributed as compared to the other clusters (**Figure 4.6**), none were above the minimal threshold of 0.24 g/kg BW. This may resemble a “grazing” or “snacking” pattern, as similarly observed among Australian females (25). Cluster 1 is thus observed to have frequent but small EOs containing low protein levels spread throughout the day, with few individuals meeting the protein threshold. Cluster 3, on the other hand, had higher absolute protein intake and protein adjusted to BW, at lower EO frequency. This raises the question of whether it is more effective to have protein intake throughout the day at a higher frequency in a grazing pattern but below the required threshold, or to have less frequent meals that meet the protein threshold.

An even distribution of protein intake throughout the day, compared with a skewed pattern, has been associated with potential benefits in physiological health, such as in MPS (14, 43). In opposition to this, a study by Arnal et al. (1999) found a positive impact on MPS in older adults using a pulse feeding strategy as compared with an even distribution of protein throughout the day (44). Bollwein et al. (2013), however, stressed that each meal must still contain a minimum threshold of absolute protein content of at least 20 g to potentially achieve such a metabolic benefit (22). A grazing pattern consisting of many EOs below the threshold (as seen in cluster 1) could more negatively impact MPS or other health outcomes than when more EOs are meeting this threshold (clusters 2 and 3). A well-controlled study is required to test this hypothesis and validate previously asserted meal protein thresholds.

An unbalanced intake pattern is metabolically inefficient, with only EOs at one point of the day meeting or exceeding the threshold. The presence of any excess IAAs is futile in the presence of at least one limiting IAA, as excess IAAs are lost to oxidation instead of being maximally utilised within body tissues (29). There was no clear difference in temporal variation across the clusters in this study, which may be due to the small sample size in each cluster. However, the trends in **Figure 4.3** show that protein intake was higher at the later parts of the day as compared to the start of the day and consistent with several studies examining protein distribution across the day in various populations (14, 18, 19, 45).

In Johnson et al. (2022), among healthy adult women (18 to 79 years old) who consumed at least 0.24 g/kg or 25 g of protein at least once in three meals, a positive association was observed with lean mass, upper-body strength, and lower-body endurance (19). Two other studies that examined dietary protein distribution to MPS, lean mass, and muscle performance also identified that more frequent and even consumption of approximately 30 g of protein per meal had the greatest association with leg lean mass, strength (10), and MPS stimulation (43). Other studies have suggested that the optimal protein intake per meal is 0.24 g/kg/day for younger adult men and 0.4 g/kg/day for older adult men, based on a breakpoint analysis of MPS data (28, 29). However, these conclusions were based on the ingestion of high-quality protein and the absence of middle-aged individuals between 38 and 64 years (19).

Hence, sufficient protein intake is linked to the maintenance of lean mass, not just on a daily basis, but also at a per-meal level (10). The BMI between cluster 1 and the other clusters was not significantly different, but body fat percentage was significantly different across all clusters, with cluster 1 having the highest body fat percentage. This also appeared to align with findings from Chapter 3, in which low protein intake and protein quality were observed in individuals with high body fat percentage. Cluster 3, with individuals having the highest mean protein intake (g/kg/day) and the lowest percentage of EO below the threshold, had the lowest mean body fat percentage.

Without considering other confounders such as PAL and energy expenditure, it is challenging to identify an association between protein intake and temporal distribution on body composition. This finding was similar to results from a cross-sectional study which found higher fat-free mass in adults whose protein intake was at least 0.24 g/kg BW in each of three meals across the day, although these meals were unevenly distributed (27). While this vegan cohort study was not intended to assess the relationship between protein intake and body composition, this may be an interesting avenue for future work.

Stable isotope techniques have concluded that IAA availability is the most important factor for MPS stimulation, with a dose-dependent mechanism (46, 47). Individuals who consistently fall below the EO threshold for dietary protein may receive insufficient concentrations of IAAs to meet requirements and face risks in depleting intramuscular stores of IAAs. Taken together, it would be ideal for all EOs to meet the protein threshold. If this objective is not met, then at least meeting the quantity for one EO per day is important for MPS stimulation. Based on results from the ingestion of whey protein (a rapidly digested protein), the deprivation of IAA intake induces the catabolic process of skeletal MPB around three hours postprandially, and MPB is likely more significant between an overnight fast and the first meal of the day when the body is in a catabolic state (1). Hence, it is perhaps more important for the protein threshold to be met at the first meal.

However, slower-digesting proteins may induce the prolonged elevation of IAA concentration in plasma (30) and negate the risk of MPB during post-absorptive periods. Studies that found elevated leucine levels at four to five hours postprandially were based on isolated leucine supplementation (7, 48). Hence, the type of protein source may matter in its delivery and maintenance of IAAs in the body. The plasma response of leucine and other IAAs may differ in mixed diets of varying protein quality, as compared to isolated protein sources which may induce greater aminoacidemia (10), but these differences are not sufficiently examined in the literature.

4.4.3 FG contribution to dietary protein

To assess how different FGs contribute to protein and IAA intake across the clusters, the percentage of total intake contributed by each FG to protein and each IAA was tabulated. **Figure 4.5** and **Table C1 (Appendix C)** demonstrated that across all clusters, “legumes and pulses” was the major group contributing to total protein and most of the IAAs, such as lysine, leucine, threonine, and histidine. As supported by **Table C2 (Appendix C)**, the contribution of “legumes and pulses” to absolute intakes of protein and IAA was significantly higher in clusters 2 and 3 than in cluster 1. Other evaluations highlighted the importance of legumes for total protein and lysine intake (49-52). “Grains and pasta” was the next-largest contributing FG, most notably to methionine and cystine in cluster 1, and significantly different between clusters 1 and 3. These results suggest the potential that individuals in cluster 1 may consume a larger proportion of “grains and pasta” than “legumes and pulses”, while the opposite is true for clusters 2 and 3.

In analysing the contribution of the main protein-containing ingredient from novel PB foods, cluster 3 showed “legumes and pulses” as the dominant FG, while a smaller proportion of “grains and pasta” was observed in cluster 1. Most of the PBMA consumed by the NZ vegan cohort was derived from soy and pea protein concentrates and isolates. Furthermore, these isolated proteins were processed with techniques that could increase the availability of AAs, as shown by the digestibility coefficients utilised in this study, as well as other protein quality measures (53, 54). Soy and pea-based PBBs have higher IAA quantities than those made from oat and rice, particularly for lysine, and are closer to meeting the FAO IAA requirements (55, 56). Hence, it could be valuable to prioritise “legumes and pulses” as the primary protein-contributing ingredient if novel alternatives are included within a vegan diet, especially if lysine and leucine quantities must be increased. However, this must account for overall consumer acceptability, the balance of other micronutrients, and non-nutritional factors such as cost.

4.4.4 Strengths and limitations

The Chapter presents several limitations, the first of which has a relevant impact on measuring protein adequacy at the meal level. The minimum threshold of protein per EO was based on literature values, but these values ranged from 15 to 45 g of protein per EO across numerous studies and depended on the type of protein tested and the study population. The minimum threshold of 20 g of protein per EO taken in this current study was based on findings from only six young men after resistance exercise, so the minimum threshold may be elevated in other populations, especially in sedentary older adults, who face the highest risks of protein deficiency (12). Furthermore, this minimum quantity was based on high-quality protein containing 10 g of all IAAs (57, 58). Higher levels may be warranted in diets providing lower concentrations of utilisable IAAs, such as in a vegan diet. Setting the minimum threshold at a conservative 20 g provided a benchmark to assess protein adequacy and avoid overstating inadequacy that could occur with a higher threshold. Well-defined thresholds for utilisable protein and IAA intake would be valuable, especially for populations on restrictive diets or at more advanced ages.

This cross-sectional study relied on a four-day food diary, which provided a brief view of dietary behaviour that may not represent true temporal variations in nutrient intake for individuals (23).

Weekday and weekend differences in meal patterns were not considered but could be important given that various social and lifestyle behaviours may affect the type of food consumed at each EO and the number of EOs and thus impact the results. However, the tabulation of the mean nutrient intake per EO over four days was conducted to account for intra-individual variations during the food-recording period.

One strength of this study was the use of novel quantitative techniques to objectively identify meal patterns, rather than pre-defining time points for meals and snacks. This is especially useful because definitions of meals and snacks are not clear in the literature, and segregating consumption points based on conventional "breakfast, lunch and dinner" time patterns is subject to bias and inter-individual variation. The optimal number of clusters was initially assessed using the silhouette width but the final optimal number of clusters, k, was determined by balancing the quantitative criterion with the nutritional interpretability of the clusters, wherein each cluster represented a distinct composition with sufficient observations for meaningful analysis, and avoiding fragmented clusters with very small groups. the selection of three clusters was also validated by comparing to results from $k = 2$.

The mechanism behind DTW is the computation of the mean distance based on the alignment of time series of different lengths (33). However, DTW does not distinguish time series by daily consumption duration or the specific timing of meal initiation in the day. Consequently, the analysis does not capture differences in daily meal duration or starting points across individuals.

As was the case for Chapter 3, adjustments for TID for both protein and individual IAAs are crucial to assess the adequacy of utilisable protein and are a strength of this thesis. Despite limitations with data paucity for TID values and other factors (apart from digestibility) that can impact protein and IAA utilisation in the human body, digestibility adjustments taken at the terminal ileum serve as a valid proxy for the quantity that can be utilised by the body (59). This is an important step in evaluating how adequately a vegan diet can provide high-quality protein, since plant foods are known to have lower protein digestibility due to anti-nutritional compounds and inherent structural configurations within their protein structures.

4.4.5 Implications for future research

The emergence of dietary patterns that vary in protein intake and protein quality in this Chapter suggests that certain consumption patterns of a vegan diet may be more effective in increasing the daily protein intake and protein quality to requirement levels. This was observed especially in cluster 3, where individuals had higher total protein and IAA intake in EOs across the day while maintaining lower EO frequency. Balanced intake of diverse high-quality plant protein sources in meals or snacks within narrow time windows could be the key to achieving protein adequacy in vegan diets without excess energy consumption. Meals and snacks found in the dietary data of cluster 3 could provide information on healthy vegan diets that meet protein requirements and can be adapted to dietary patterns in clusters 1 and 2. The compositions and proportions of how each FG influences protein quality are explored in the subsequent research chapters.

4.5 Conclusions

This Chapter highlights the impact of dietary patterns on protein and IAA adequacy within a NZ vegan population through novel quantitative techniques to objectively categorise distinct variations. DTW distinguished three dietary patterns in this vegan cohort, with cluster 3 being most different from cluster 1. Cluster 1 displayed markedly lower total protein and IAA intake and compared poorly to the stipulated absolute and relative protein reference values. Individuals who follow dietary patterns in cluster 1 may be most at risk for protein deficiency, especially if these daily diets are followed over longer periods. The greater prevalence of these inadequacies suggests this group may require more substantial dietary modifications than clusters 2 and 3 to achieve adequacy. For example, larger proportion of "legumes and pulses" added to the diets for cluster 1 may improve total protein and IAA intake. Dietary modifications required to achieve adequacy for protein and protein quality could be expected to differ across clusters, providing a rationale for the optimisation analyses in later Chapter 5. Importantly, Chapter 4 further corroborates earlier research indicating that protein consumption tends to peak in the later hours of the day, regardless of cluster assignment, raising concerns about protein distribution and metabolic inefficiencies over the day with potential negative impacts on lean muscle mass and MPS stimulation.

PSFs often have lower concentrations of digestible IAAs that can meet individual requirements as compared to ASFs. Identifying appropriate PB protein sources in the right proportions is a practical way to achieve combinations of foods that can complement the limiting IAAs and improve the protein adequacy of a vegan diet. This complementary effect may occur only within the same EO or within a narrow time window. Every EO thus presents an opportune moment in the day to mix PB foods in the most optimal combinations to achieve adequate protein and overall nutrient intake. Given the potential risks of protein and IAA deficiencies identified at the daily level and across EOs in Chapters 3 and 4, there is a need to develop strategies to address these limitations within vegan dietary patterns. The following Chapters will focus on identifying individual nutritional shortfalls and implementing dietary modelling interventions to close these gaps.

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Other supporting data

Other supporting information for Chapter 4 that is absent in the Appendix of this thesis can be downloaded at: **https://www.mdpi.com/article/10.3390/nu17111806/s1**.

Part II
Deriving solutions to improve protein intake and protein quality of vegan diets

Clearly, the current dietary patterns observed in the NZ vegan cohort are suboptimal in terms of protein quality. The selection of protein-dense foods, together with appropriate strategies to combine them, is pivotal in overcoming limitations in inadequate utilisable protein. The following Chapters explore targeted dietary optimisation approaches to improve protein quality while remaining consistent with realistic dietary practices.

> "Nutritional adequacy in plant-based diets is perhaps not a question of possibility, but of design."
>
> - The author of this thesis

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DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

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In which chapter is the manuscript/published work?	Chapter 5

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1 Doctoral Research Committee May 2023

Chapter 5 Improving the protein quality of New Zealand vegan diets: An optimisation modelling approach incorporating energy constraints and diet acceptability

This Chapter explores an initial strategy using linear programming to modify daily vegan diets that fall below the protein and IAA requirements. The approach evaluates the trade-offs and constraints encountered when attempting to improve suboptimal diets. In doing so, the Chapter provides insight into both successful and failed optimisation scenarios, highlighting the practical limits of dietary modifications to inform future dietary strategies.

The following Chapter is currently under revision in Frontiers of Nutrition:

Soh, B. X. P., Vignes, M., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2026). Improving the protein quality of New Zealand vegan diets: An optimisation modelling approach incorporating energy constraints and diet acceptability.

Abstract

Targeted dietary modifications are necessary to improve nutrient adequacy in vegan diets for optimal health. Optimisation modelling was applied to identify combinations of PB foods within an individual's existing diet to address protein and IAA shortfalls in the NZ vegan cohort. Shortfalls for protein and IAAs in the existing diets were calculated by comparing daily intakes to individual requirements. An energy-tailored optimisation was applied. Diets with lower energy intake had foods added, while those with excess energy had energy-dense and low-protein foods replaced with protein-rich alternatives. Linear programming was used to add suitable foods to the baseline diets. The modified diets had to 1) meet protein and IAA requirements, 2) respect serving-size constraints for added foods, and 3) remain within individual energy boundaries while minimising the weight of food added. Post-optimisation analysis assessed changes in protein, IAAs, dietary fibre, and selected micronutrient intakes, with results compared across clusters. Protein and IAA shortfalls were more prevalent in cluster 1 (85 % of daily diets) than in clusters 2 (61.1 %) and 3 (30.8 %). "Legumes and pulses" contributed most to total protein and lysine with lower energy costs, while "nuts and seeds" contributed most to methionine and leucine, but with higher energy costs. Optimisation resolved shortfalls in 90 % of daily diets. The remaining 52 diets - mainly from clusters 1 and 2 - could not reach adequacy due to large protein and IAA deficits and limited unallocated energy capacity, highlighting the need for more substantial dietary restructuring beyond food substitutions. Post-optimisation micronutrient analysis showed continued risks of shortfalls for calcium, vitamin B12 and iodine, although quantities of most micronutrients improved following optimisation. Mathematical optimisation can enhance the protein adequacy of vegan diets while preserving individual preferences. However, full nutritional adequacy remains challenging in energy-constrained diets with large nutrient deficits.

5.1 Introduction

Foods are not equal in their nutrient provision. For example, the quantities of IAAs are generally lower in PSPs as compared to animal-sourced proteins (ASPs) (1). This disparity is particularly consequential in vegan diets, where the exclusion of all ASFs means that all protein must come from PB sources. Appropriate quantities and a diverse range of PB foods must be consumed to provide sufficient quantities of these essential nutrients to match human requirements. PB protein sources can be strategically combined to complement one another's AA profiles, hence mitigating shortages of these IAAs in individual foods (2). Food choices are governed by various factors, including cultural acceptability, individual preferences, and food costs, leading to the emergence of varied dietary patterns with different diet quality and nutritional profiles.

Devising vegan diets that are both acceptable and high in protein quality can be challenging in situations where FG diversity is limited, or where dietary choices are dominated by energy-dense but nutrient-poor foods. Such combinations restrict the extent to which a diet can be modified to meet nutrient requirements without substantial food substitutions (3). The health benefits of nutrient adequate diets can only be realised if they are accepted and adhered to by the population. However, defining acceptability constraints is challenging due to the subjectivity associated with individual food preferences and cultural practices (4).

Mathematical optimisation using linear programming (LP) offers an efficient approach to identify the most suitable combinations of available foods that can satisfy nutritional constraints while minimising deviation from the original diet (3, 5, 6). Past modelling techniques have incorporated various strategies to ensure dietary realism, such as setting upper and lower bounds for food intake (3, 4), imposing penalties for deviations from current energy and serving-size intakes (4, 7), using each individual's current dietary intake as the baseline (3, 8), and benchmarking against healthier diets within the same population group (4, 9). LP can be applied in the design of high protein quality vegan diets by identifying the extent to which total protein and utilisable IAA intake can be obtained within the boundaries of individual energy requirements, while implicitly accounting for dietary preferences by incorporating some of the above acceptability constraints.

In Chapter 3, lysine and leucine deficiencies were observed in the NZ vegan cohort, where approximately half of the participants were below the daily requirements for these IAAs (10). Several studies have also identified lower intake and plasma concentrations of several IAAs in vegans compared to non-vegans (11) with analysis showing lysine intake in a vegan diet falling below the reference values (12). Subsequently, cluster analysis was used in Chapter 4 to explore the distribution of protein and IAA across the day. Only one cluster was close to achieving the total protein requirement at the meal level (13). The current or baseline diets of these clusters can be modified through LP to bridge nutritional gaps in protein and IAAs, but the extent of modification would vary across individuals and clusters, depending on the protein intake and protein quality of the baseline diets.

Prolonged deficiencies in AAs can impair metabolic functions in the body, including protein synthesis and muscle mass maintenance, with certain populations (such as the elderly) being at higher risk (14). It is often more challenging to deliver comparable protein quality within the same energy allowance as many PB foods have lower protein-to-energy ratios than ASFs, and this challenge is compounded by higher fibre intake and satiety of PB diets, as described in section I of Chapter 2 (15-17). As described in section II of Chapter 2, while past studies have examined substitutions of ASPs with PSPs in varying proportions (18-20), no studies have applied optimisation methods to systematically improve deficient vegan diets to the point of nutrient adequacy.

The overall aim of this Chapter was to generate dietary modifications based on each individual's existing food intake, with the goal of rectifying shortfalls in total protein and IAA, commonly observed in vegan diets, within individual energy limits. Through the incorporation of familiar foods into the diet, this individualised framework allows diet modifications to remain culturally and behaviourally relevant to each individual (3). A secondary aim was to quantify the deviation in food intake and nutrient contribution required to achieve adequacy by comparing the modified and baseline diets of the population and analysing variation across clusters. Modified diets should be feasible, so Food-Based Dietary Guidelines (FBDG) data for serving sizes (6) and energy constraints served as boundaries that promote the increased likelihood of real-world acceptability.

5.2 Methods

5.2.1 Calculation of individual nutrient requirements and shortfalls

The daily protein and IAA intakes for each recorded day for all participants, following TID adjustments, were tabulated as described in Chapters 3 and 4. After excluding days with missing consumption or time data, 766 daily diets were included to quantify nutrient shortfalls relative to individual requirements.

Individual daily protein requirements were calculated based on sex, age and BW, provided by the NRV for Australia and NZ (21). The EAR values were used for this computation. This was 0.68 g/kg/day for males and 0.60 g/kg/day for females between 19 and 70 years of age. The EAR was 0.86 g/kg/day for males and 0.75 g/kg/day for females above 70 years of age. IAAs with currently available TID values (histidine, leucine, lysine, cystine, methionine, SAAs, threonine and tryptophan) were included in this study. Individual daily IAA requirements were calculated based on individual BW **(See Table 3.3)**, using the reference values from the FAO (22).

The minimum daily energy requirement in MJ for each individual was calculated by accounting for the sex, age, BW and height, as well as the PAL (21). The IPAQ was used to compute the daily PAL (23). Briefly, IPAQ records the number of MET minutes, which represents a multiple of the estimated resting energy expenditure. For example, 1 MET indicates energy expended at rest. 3.3 METS, 4 METS and 8 METS represent energy expended during walking, moderate physical activity (PA) and vigorous PA, respectively (23). The PAL was then calculated by adding 1 MET to the sum of the average MET values for each PA, weighted by the average number of minutes per week spent on that activity (24, 25), as shown:

[Equation 1] $$\mathrm{PAL} = 1 + \frac{[\text{Walk (mins)} \times 3.3 + \text{Moderate Activity (mins)} \times 4 + \text{Vigorous Activity (mins)} \times 8]}{10080 \text{ mins (per week)}}$$

The Estimated Energy Requirement (EER) value was then assigned to each individual, based on the sex, BW, height, age, and the calculated PAL (21). While an upper limit to dietary energy intake has not been established (21), a 20 % increase above the EER was applied as a pragmatic upper boundary (see sensitivity analysis). This allowance provided the model with greater flexibility to choose a wider range of foods for individuals who met or exceeded their EER but had significant shortfalls for protein and/or IAAs. Shortfalls in protein, IAA, and energy (for individuals with daily intake < the minimum requirement for EER (EER_{min})) were tabulated for each day by deducting the total daily intake of each nutrient from the daily requirement. Only daily diets with at least one shortfall either in protein or any IAA were optimised and analysed. **Figure 5.1** presents the overall methodology, highlighting the processes in the data preparation and subsequent diet optimisation.

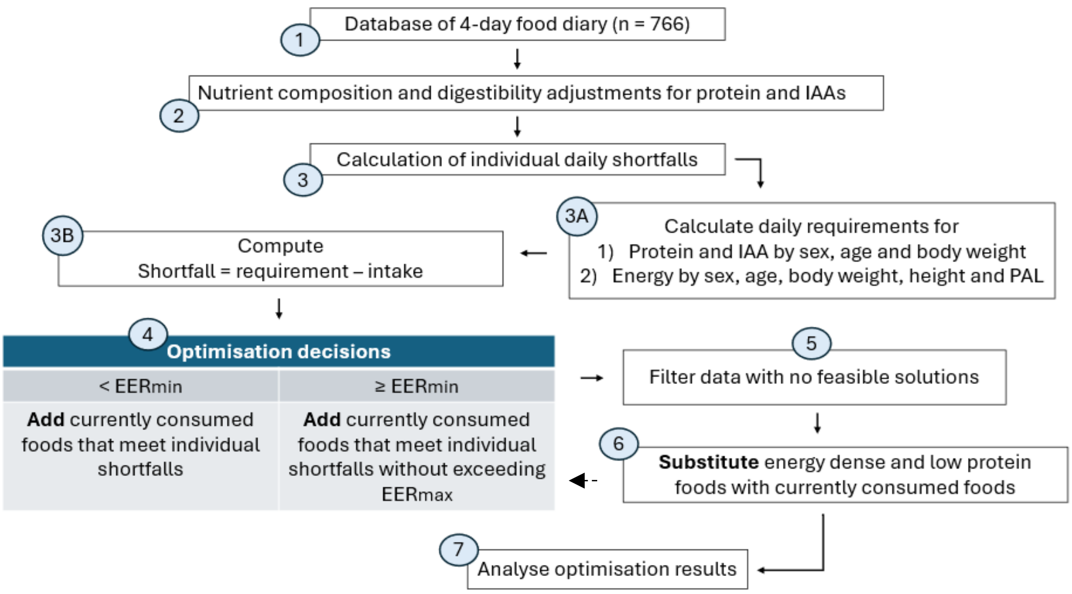


Figure 5.1 Methodology of LP optimisation.
The numbers indicate the steps taken in the optimisation process. (1) Dietary data was in the form of 766 food diaries, self-reported by a cohort of 193 NZ vegans. Days with missing records were excluded from analysis. (2) Each food item was matched to its nutrient composition for protein, IAA, energy, dietary fibre and micronutrients in NZ and USDA food composition tables. Total protein and IAAs were adjusted for digestibility. (3) Shortfalls were calculated for each of the 766 daily diets by first, (A) calculating the daily requirements for energy, protein and IAA, with demographic and anthropometric data of each participant, and (B) computing the daily shortfalls. (4) LP optimisation methods depended on the baseline

energy intake of the daily diets. Should energy intake be below (<) EER_{min}, energy formed part of the shortfall to be filled, whereas when intake met or exceeded (≥) EER_{min}, foods could only be added within the residual energy allowance up to the EER_{max}. (5) After optimisation was completed for all clusters, cases which had no feasible solutions were filtered and combined with cases where added foods had resulted in daily energy intake above $EER_{max.}$ (6) For these cases, a substitution strategy was used to remove foods with the highest energy-to-protein ratios to provide energy headroom for further food additions in step 4. (7) All cases with feasible solutions from steps 4 and 6 were analysed for nutrient intake after optimisation. IAA, indispensable amino acids; PAL, physical activity level, EER_{min}, minimum estimated energy requirement per day, EER_{max}, maximum estimated energy requirement per day

5.2.2 Diet optimisation using LP

One of the constraints of the LP model was that foods added should fulfil any protein and IAA shortfalls. Hence, for each nutrient, *i* (where *i* may be TID-adjusted protein or one of the TID-IAAs), the quantity, x_j (in grams) of all foods *j* in the optimised diet, must together supply at least the daily requirement, R_i. Formally:

[Equation 2]

$$\sum_{j \in J} a_{ij} x_j \geq R_i$$

for each nutrient *i*, where:

a_{ij} is the quantity of nutrient *i* per gram of food *j*,

R_i is the daily nutrient requirement for nutrient *i*.

J represents the set of all possible food items in the optimised diets.

Foods in the optimised diet must contribute energy that is within the lower (EER_{min}) and upper (EER_{max}) energy boundaries for that individual. This is expressed as:

[3]

$$EER_{min} \leq \sum_{j \in J} e_j \, x_j \leq EER_{max}$$

where e_j represents the energy (MJ) per gram of food selected, x_j.

As shown in **Figure 5.1**, the optimisation decision depends on the daily energy intake (step 4). In all cases, the model is constrained not to exceed the $EER_{max.}$ If the daily diet does not meet the EER_{min}, then energy is falling short of what is required to support metabolic function and the model chooses foods that will simultaneously cover the shortfalls for protein, IAA and energy. The model may not return feasible solutions in every case (step 5). For example, if the current energy intake is close to EER_{max} but significant deficits exist for protein and IAA, it may not be possible to add foods to fill the protein and IAA deficits while respecting the energy constraint. A substitution strategy was then utilised to remove the most energy-dense but protein-poor foods from the diet, to accommodate the addition of foods that can fill nutrient gaps (step 6).

To do so, an energy-to-protein ratio was calculated for each food in these cases. Foods were ranked within each individual day according to their energy-to-protein ratio, a proxy for protein density. The first substitution strategy involved removing the 25th percentile of this ratio to exclude the least protein-efficient foods from the baseline diet. New shortfalls were recalculated, and the LP model was re-run to add the substitutions. Cases which still had no feasible solutions were then identified. From here, food items with energy-to-protein ratios above the 50th percentile were removed from the baseline diet, and the food addition process was performed again. This approach allowed direct identification of foods that were suboptimal contributors to protein quality while remaining within the energy constraints of a vegan diet.

Any remaining cases without feasible solutions were not further addressed, since removal of > 50 % of food from the baseline diet was considered too great a deviation to be acceptable to consumers. Additionally, further removal may not yield significant improvements in energy alignment, nutrient contributions, or food diversity (e.g., the model may end up choosing very high servings of nuts and seeds because there were no alternatives). Substantial dietary restructuring, ideally supported by practical dietary guidance may be necessary to improve protein quality while maintaining acceptability.

An acceptability constraint was implicitly introduced through the proxy of adding foods already in an individual's diet. However, certain foods were intentionally omitted at this point. Brazil nuts were removed due to their high potential to exceed selenium upper limits (one seed from a high-selenium area is sufficient to meet the daily allowance) and the narrow window between selenium deficiency, adequacy and toxicity (26); seaweed, which has a wide range of iodine content depending on species (0.02 to 428 µg per gram across species in the vegan cohort's food inventory), could exceed the upper limit of iodine intake (27), especially when adding on to the baseline intake. Herbs and spices were mostly used as flavouring in the dietary data, and were not considered feasible foods to address protein shortfalls in the diet.

The objective function of the LP model was to minimise the total weight of food added to the diet, thereby limiting the addition of large volumes of food that cannot be administered in realistic diets. Weight-based constraints corresponding to serving sizes were set for each FG and introduced as continuous variables to prevent excessive addition of each food item, as guided by the NZ Eating and Activity Guidelines (28). For example, "grains and pasta" included foods such as wheat products, cereal grains, bread, rice and processed corn products such as corn chips. “Legumes and pulses” included the PBMA products that were legume-based or consisted of protein isolates, as well as traditional legumes like lentils, tofu and beans. Protein isolates which are typically used as supplemented powder are also part of this group. The maximum allowable serving sizes for each FG are listed in **Table 5.1**.

Table 5.1 Weight of food per serving size for each FG

FG	Maximum weight (g) per serving
Fruit	150
Grains and pasta	120
Legumes and pulses	150
Nuts and seeds	30
Potatoes, kumara and taro	75
Sugar and sweets	30
Vegetables	75
Yeast and condiments	30

The serving constraint for spirulina was capped at 3 g, according to recommended guidelines from the literature (29, 30). To encourage dietary diversity, the LP optimisation was constrained to adding up to one serving for each unique food item (but not FG). For example, up to 30 g of sesame seeds and 30 g of cashew nuts could be added to address shortfalls. These are different food items, but belong to the same FG, “nuts and seeds”. The characteristics of the optimisation model are described in **Table 5.2**. Optimisation was conducted separately for all individual diets in each cluster within the vegan cohort, and the solutions were compared across clusters. The LP optimisation was conducted using R Studio (version 4.3.1) with the *lp solver* package (31).

Table 5.2 Characteristics of the optimisation model

Constraints	Objective function	Decision variable	Solution
1. Meet protein and IAA requirements 2. Serving size per food item, with maximum of one serving per food item 3. Optimised diet does not exceed EER_{max}	Minimise the weight of food added	Food items reported in any of the four days in each individual's food diary	Weight, energy, and nutrient contribution of food items added

Sensitivity analyses

Sensitivity analyses were applied to test how changing certain parameters in the diet optimisation will impact feasibility. For example, energy constraints for the optimised diets were tested at 5 %, 10 %, 20 %, 50 %, and 100 % above EER_{min}. This step provides a suitable indication of the upper boundary of energy intake that can fulfil protein and IAA shortfalls. The safe upper thresholds for each individual IAA were explored in the model and set at 24 % higher than the individual IAA requirements (in mg/kg of BW) (22). Given the limited empirical evidence defining true upper limits for IAAs, these thresholds were not treated as constraints but rather used to evaluate optimisation outcomes under more conservative intake assumptions. As plant protein isolates, such as soy and pea, were important dietary levers for protein quality (32), the impact of their removal from the inventory was also evaluated.

5.2.3 Data Analysis: Post-optimisation assessment of total protein and IAA

Results were analysed (**Figure 5.1**, step 7) and compared across clusters 1 to 3. The mean weight in grams of added foods for each FG was tabulated. The mean contributions to energy, protein and IAA from each added FG were computed as a percentage of the minimum daily requirement. To obtain the final nutrient content after optimisation, the quantity of nutrients from foods introduced by optimisation was added to the baseline nutrient content. For cases where foods had to be substituted (**Figure 5.1**, step 6), the diets after removing energy-dense foods were used as the new baseline diets for this computation.

The baseline diets (dietary data in step 1 of **Figure 5.1**) and the final optimised diets for all individuals were then compared to determine how the contributions of energy, protein, and IAA per FG changed after the diet modification. Total nutrient content for the baseline and final diets was also computed as a percentage of the minimum daily requirement. The final protein and IAA content for all optimised diets were computed with respect to individual BW (kg) and compared with the reference values. For total protein, the final quantity was compared to the EAR and the AMDR, which is between 10 to 35 % of caloric intake (21, 33).

For IAAs, the final intake was compared to requirement values in **Table 3.3**. Risks of exceeding the upper limit intakes for IAAs were evaluated by comparing quantities against the currently available values of Tolerable Upper Intake Level, No-observed-adverse-effect-level (NOAEL) or Lowest-observed-adverse-effect level (LOAEL) (34) (**Table D1 of Appendix D**).

5.2.4 Data Analysis: Post-optimisation assessment of changes in other nutrient levels

Finally, dietary fibre, ALA (the most prominent long-chain fatty acid from PSFs) and selected micronutrient quantities in the optimised diets were computed to assess how these nutrients changed from baseline. Vitamin B12, calcium, iodine, iron and zinc were the micronutrients focused on in this study because of their increased risk of deficiencies associated with a vegan diet (35-37). The total sodium content in the optimised diets was also analysed against reference values to assess whether the modelled diets had inadvertently added excess sodium. The EAR (or adequate intake (AI) when EAR was not available) was used as the minimum daily requirement and the upper limit per day was also provided when applicable (21) (**Table D2 of Appendix D).** As one of the main aims of the thesis was to provide solutions to improve protein quality in vegan diets, micronutrient intake was not constrained during optimisation. The evaluation of micronutrient intake was included as further contextual information about the quality of the optimised diets and post-optimisation evaluations were conducted to assess the potential for modified diets to exceed the safe upper limits of micronutrients.

5.3 Results

5.3.1 Optimisation results with targeted food additions and substitutions

The number of daily diets with at least one shortfall in cluster 1 was 250 (total of 294 daily diets), cluster 2 was 215 (total of 352), and cluster 3 was 37 (total of 120). More severe shortfalls, with simultaneous shortfalls in protein and all IAAs, were observed in 10 % of daily diets in cluster 1 and 4 % in cluster 2. No daily diets in cluster 3 showed concurrent shortfalls for protein and all IAAs. Lysine, leucine and methionine had the largest average deficits relative to the daily requirement. The first round of LP optimisation yielded feasible solutions for 303 of 502 daily diets. For the remaining 199 daily diets, 99 had insufficient residual energy capacity (mean of 1.07 MJ) to accommodate inclusion of additional foods to meet nutrient shortfalls, while the rest (n = 100) had exceeded the $EER_{max.}$ To address these shortfalls, it was necessary to first remove some foods from the baseline diets.

Hence, food substitution was conducted for these 199 cases. When the top 25 % of foods by energy-to-protein ratios were removed prior to optimisation, feasible solutions were obtained for 118 of the 199 cases. The foods removed were consequently fruits, savoury sauces, grains and sugary foods and beverages. Before optimisation, these diets had a mean energy of 3.09 MJ below the EER_{max} and relatively smaller shortfalls, mainly in the IAAs. Contrastingly, for the remaining 81 unsolved cases, the diets had a mean energy of 2.14 MJ below the EER_{max}, but with larger shortfalls, especially for protein. For these 81 cases, further food substitutions were required. The top 50 % of foods with the highest energy-to-protein ratios were removed to provide a higher residual energy capacity (mean 4.20 MJ) for new food additions. After this substitution, the LP model found feasible solutions using currently consumed foods in 29 cases, but in 52 cases, it found no feasible solutions.

Overall, the optimisation process obtained solutions for 90 % of daily vegan diets which had at least one shortfall at baseline. This was 219 cases in cluster 1, 195 in cluster 2 and 36 in cluster 3. Of the cases where no feasible solutions could be obtained from the current diet (n = 52), cluster 1 diets accounted for the largest proportion at 60 %, followed by cluster 2 (38 %). These diets may benefit from other modification strategies (explored in the sensitivity analyses).

The mean mass of total food added per person per day was highest for clusters 1 and 2, at 44.9 g (SD = 26.4) and 43.7 g (SD = 27.3), respectively, while the mean mass added for cluster 3 was 39.3 g (SD = 25.8). The mean number of unique foods added per individual was again higher in clusters 1 and 2, at 3.5 and 3.2 items, respectively, while for cluster 3, 2.8 items were added. The most frequently added food items were from the FGs “legumes and pulses” and “nuts and seeds”, with peanut, pumpkin seed, almond, PBMAs, tofu and pea protein isolate (such as in protein powdered beverages) the most commonly added foods in all clusters.

Table 5.3 presents the mean weight and nutrient contribution expressed as a percentage of daily requirements for all added food items in cases where LP optimisation could find solutions. Food items were categorised into their respective FGs. Large standard deviations indicated large variation in the quantities of food items added across daily diets.

Table 5.3 Mean added weight in grams (SD) and mean percentage contribution to energy, protein and IAA from each FG across clusters. Examples of food items were similar across clusters and listed.

Food group	Examples of food items	Mean weight in grams (SD)	Energy and nutrient contribution to minimum daily requirement [1] (%)									
			Energy	Protein	Cys	His	Leu	Lys	Met	SAA	Thr	Tryp
Cluster 1												
Fruit	Currants, dates	90.5 (73.2)	10.9	2.40	3.51	0.59	0.38	0.34	0.53	1.29	0.58	0.27
Grains and pasta	Wheat products, cereals, popcorn	75.7 (42.5)	18.8	21.3	55.1	28.9	22.8	13.9	20.8	28.6	23.7	36.1
Legumes and pulses	PBMA, pea protein isolate, tofu, beans, lentils, chickpeas	78.6 (48.3)	11.1	35.3	70.5	48.1	31.3	37.9	21.5	33.2	40.5	30.0
Nuts and seeds	Pumpkin seed, sunflower seed, walnut, peanut, sesame	26.2 (7.92)	16.2	24.0	58.8	40.1	27.4	18.2	29.6	35.4	33.4	49.3
Potatoes, kumara and taro	Potato chips	32.0 (29.0)	9.13	2.75	6.07	4.09	2.82	3.86	3.00	3.62	4.25	6.66
Sugar and sweets	Chocolate, marshmallows	26.8 (7.98)	7.41	5.85	1.36	0.75	0.61	0.51	0.58	0.75	0.66	0.81
Vegetables	Spirulina, green leafy veg	26.5 (31.7)	2.61	5.96	11.4	4.29	3.22	3.04	5.06	6.42	7.40	7.94
Yeast and condiments	Yeast extract, soy sauce	22.1 (9.62)	3.75	13.0	24.3	17.7	14.6	19.5	11.8	14.3	24.1	26.3

						Cluster 2						
Fruit	Currants, banana	86.9 (74.8)	7.38	3.70	8.23	2.30	1.42	1.16	2.22	3.67	3.29	0.62
Grains and pasta	Wheat products, pasta, rice, oats, corn chips	88.0 (41.9)	19.0	19.9	56.8	29.6	24.0	12.8	21.5	29.5	24.5	35.6
Legumes and pulses	PBMA, pea protein isolate, tofu, beans, lentils	73.7 (54.3)	9.38	29.7	58.9	39.2	25.3	30.9	17.9	27.6	33.1	23.9
Nuts and seeds	Pumpkin, sunflower, peanut, sesame	26.0 (8.23)	14.0	21.1	51.1	35.7	24.9	16.2	26.3	31.2	29.3	46.5
Potatoes, kumara and taro	Kumara, chips and cripss	51.3 (26.3)	10.8	3.53	8.08	5.40	3.77	5.00	3.92	4.77	5.76	8.83
Sugar and sweets	Chocolate	19.3 (10.9)	4.95	2.93	1.31	0.61	0.48	0.32	0.46	0.65	0.48	0.80
Vegetables	Spirulina, green leafy veg, corn	54.0 (26.9)	2.66	5.02	8.37	5.52	5.24	3.97	5.55	5.93	7.26	7.30
Yeast and other condiments	Yeast extract and flakes	23.8 (10.4)	1.97	7.26	11.3	8.36	6.96	9.33	5.60	6.75	11.4	12.8

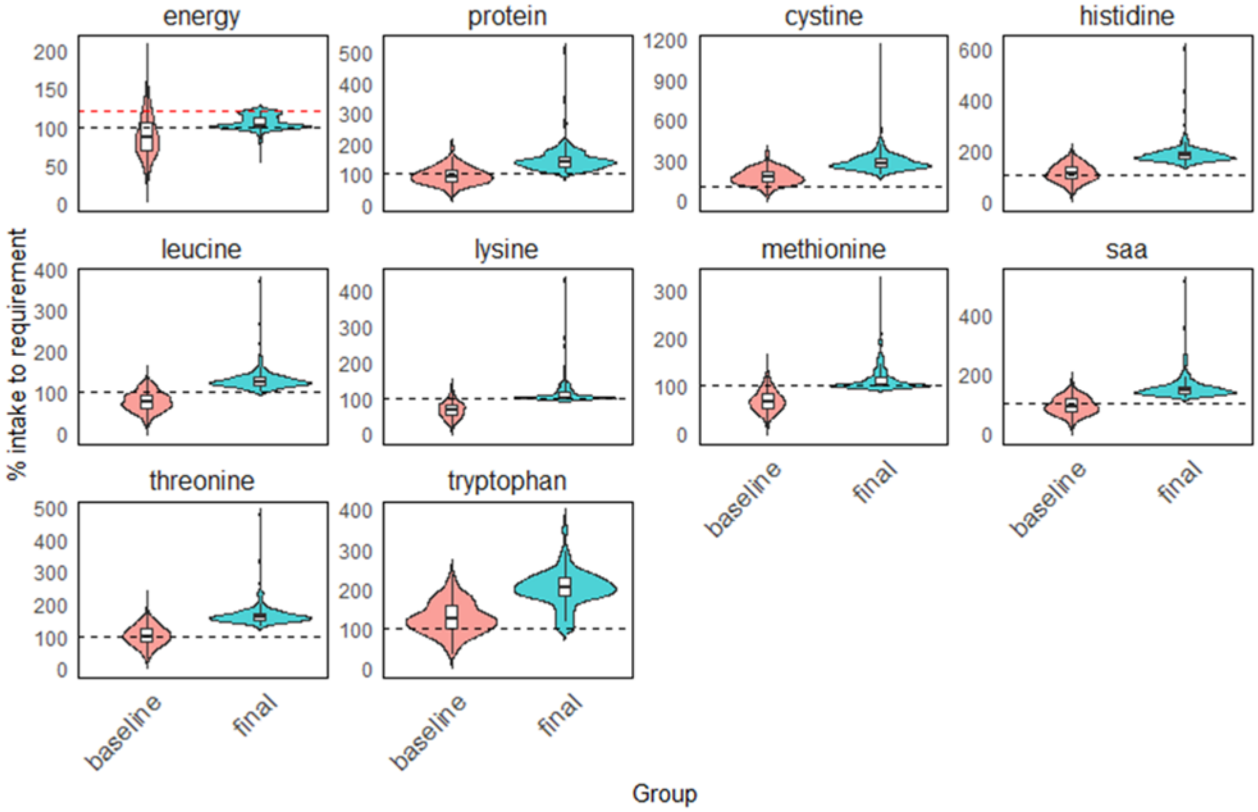
				Cluster 3								
Fruit	-	0	0	0	0	0	0	0	0	0	0	0
Grains and pasta	Wheat products, corn chips, rice	70.5 (40.2)	16.2	12.8	31.7	17.7	14.7	8.76	13.9	17.7	15.7	21.7
Legumes and pulses	PBMA, pea protein isolate, tofu, beans, lentils	55.4 (40.9)	8.05	25.1	50.2	32.2	20.0	25.2	14.0	22.7	26.1	16.1
Nuts and seeds	Pumpkin, sunflower, peanut, sesame	26.2 (7.89)	13.9	17.7	39.1	29.7	20.6	13.0	18.3	22.6	22.4	33.1
Potatoes, kumara and taro	Potato chips	75.0 (0)	15.0	5.36	13.2	8.9	6.13	8.38	6.53	7.87	9.23	14.5
Sugar and sweets	Chocolate	25.0 (12.2)	5.91	3.68	2.23	1.04	0.82	0.54	0.77	1.11	0.81	1.37
Vegetables	Spirulina	3 (0)	0.71	3.77	6.70	2.95	3.08	2.25	4.77	4.97	6.73	7.44
Yeast and other condiments	-	0	0	0	0	0	0	0	0	0	0	0

SD, standard deviation; Cys, cystine; His, histidine; Leu, leucine; Lys, lysine; Met, methionine; SAA, sulphur-amino acids; Tryp, tryptophan; Thr, threonine. [1]Minimum daily requirement: protein was based on EAR (differentiated by sex and age), IAA was based on requirement values described in **Table 3.3**, and energy was based on minimum energy requirement with the consideration of PAL.

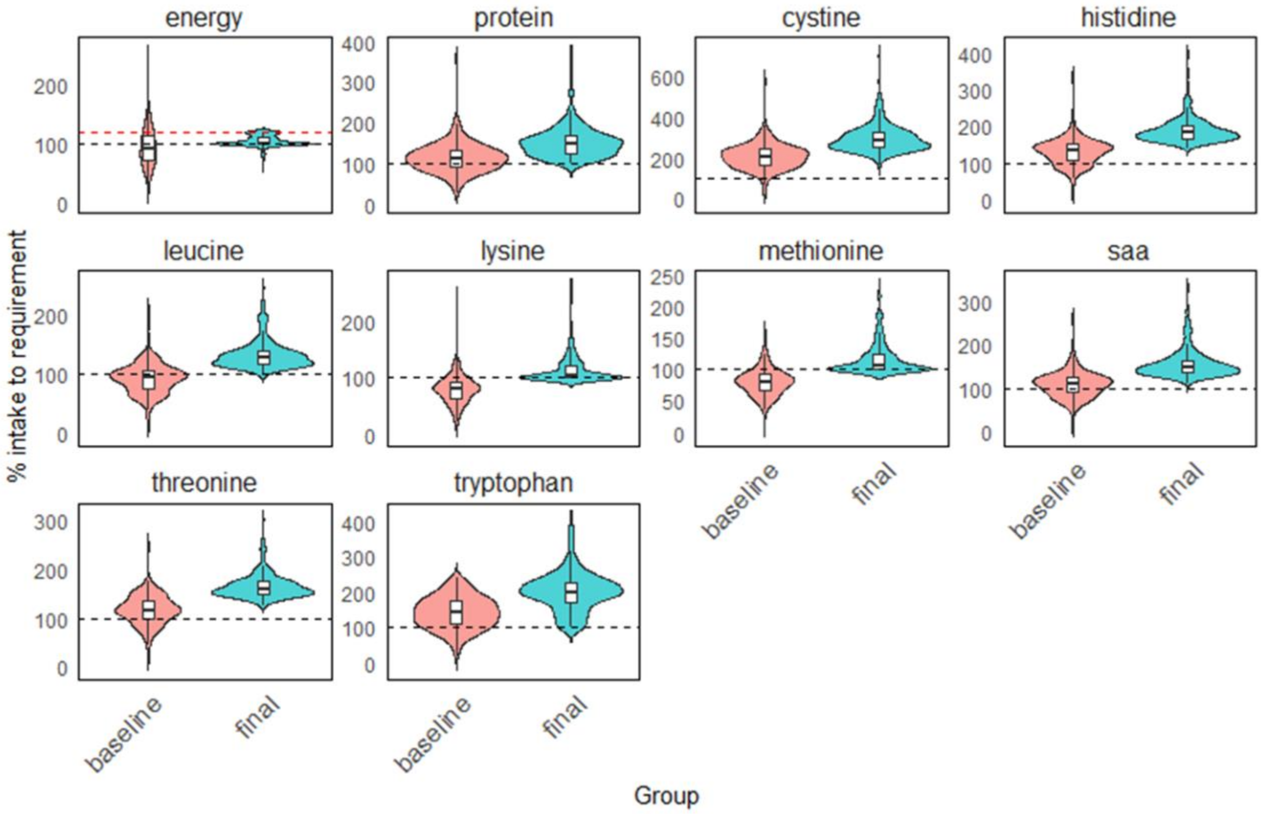
Across all clusters, “legumes and pulses” contributed the most to total protein and IAAs such as threonine, lysine, and histidine, with the smallest energy contribution. While “nuts and seeds” contributed more energy per gram of added food, their contribution to total protein, tryptophan, methionine, leucine, and SAA was higher than that of “legumes and pulses”. Yeast-containing foods contribute notably to lysine, suggesting that foods could be added to the diet with small increases in energy intake. The contribution of novel PBAs was approximately 10 to 15 % for total protein and IAAs and < 10 % for energy across all clusters. Almost all PBMAs included pea and soy isolates in the ingredients. These isolates belong to the “legumes and pulses” FG. As “fruit” was only added in a few cases (two in cluster 1 and four in cluster 2), the average quantity added was inflated and driven by large portions (up to 150 g per fruit) in a few individuals. “Fruit” and “sugar and sweets” were only added to individuals with an energy shortfall. Given their relatively high energy content, these foods may have been added by the LP model to close the energy gap when other FGs – “nuts and seeds”, “legumes and pulses” - have reached the maximum serving-size limits.

5.3.2 Assessment of protein and IAA intake after optimisation

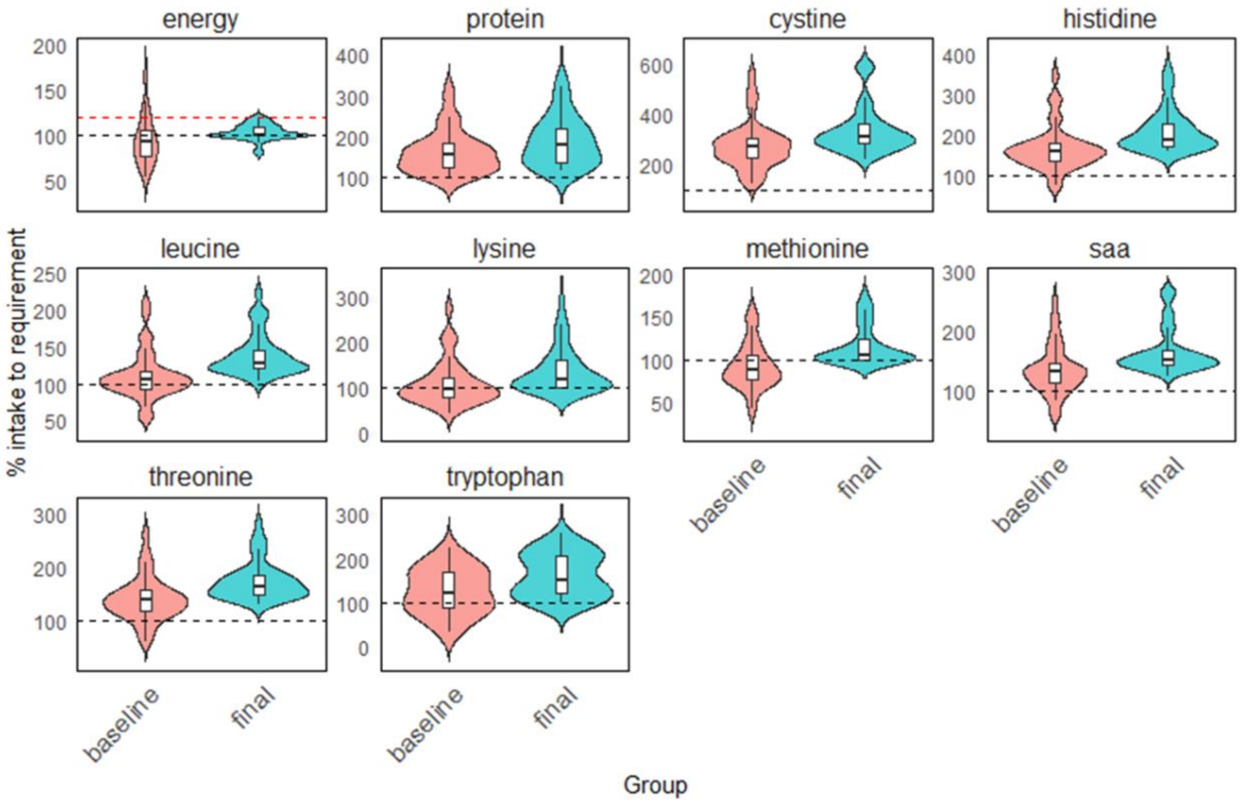
Comparisons of the baseline and final diets are presented in **Figures 5.2 A to C.** The violin plots illustrate the cohort distribution of nutrient intake relative to daily requirements. Requirements are demarcated at 100 % of the y-axis, and plot points above the 100 % line indicate fulfilment of the daily nutrient requirement.



(A) Cluster 1



(B) Cluster 2



(C) Cluster 3

Figure 5.2 Comparison of adequacy in energy, protein and IAA, at baseline intake (unmodified diets) and final modified diets in the cluster 1 (**A**), cluster 2 (**B**) and cluster 3 (**C**).
The dotted black line at 100 % indicates the average minimum daily requirement for energy and each nutrient. The dotted red line at 100 % and 120 % for the energy panel depicts the EER_{min} and EER_{max}. Each violin plot, together with its box plot, represents the overall distribution of energy or nutrient intake in each cluster, with wider portions of the violin plot indicating a larger proportion of daily intake. The top and bottom of the violin plots correspond to the maximum and minimum observed values of the cohort, smoothed by a density estimate. The horizontal line in the box plot represents the median (50^{th} percentile) of the data with the IQR at 25^{th} and 75^{th} percentiles (lower and upper edges of the box plot, respectively). The lines extending from the percentiles represent the range (smallest to largest non-outlier values), within 1.5 times the IQR. As the violin plots represent kernel density estimates, their proportions may appear to fall below the 100 % requirement. However, all successfully modified diets meet at least 100 % of the protein and all IAAs.

As evident from **Figure 5.2**, all final diets, except for the 52 unsolved cases, met the constraints set by the LP model and achieved at least 100 % of the daily required intake for protein and all IAAs. Cluster 1 had the largest proportion of baseline daily diets below the requirement as compared to the other clusters. This is most apparent for leucine (83.6 %), lysine (92.7 %) and methionine (90.9 %). For the baseline diets in cluster 2, similarly large proportions of daily diets fell below the nutrient requirements, especially for lysine and methionine, at 84.1 % and 87.1 %, respectively, but less so for leucine, where 62.6 % were below requirements. In contrast, baseline diets in cluster 3 were closer to daily requirements for all nutrients, with fewer daily diets falling below the requirements, even for leucine (41.7 %), lysine (52.8 %) and methionine (69.4 %).

Table 5.4 presents the mean protein and IAA intakes of all clusters relative to BW at baseline and in final diets. In the final diets, the EAR was achieved for all individuals and satisfied the model's protein requirement constraints. Most individuals were within the AMDR for protein (10 to 35 %), but 14.1 % and 9.2 % in clusters 1 and 2, respectively, were below this range, indicating a smaller contribution of protein intake to daily energy. The mean daily protein consumed in grams per individual increased to 60.9 g (SD = 16.0) from 39.8 g (SD = 11.8) at baseline in cluster 1. The value was 68.6 g (SD = 16.7) in Cluster 2 compared to 51.4 g (SD = 15.3) at baseline, and 88.9 g (SD = 23.4) in Cluster 3 compared to 76.7 g (SD = 19.5) at baseline.

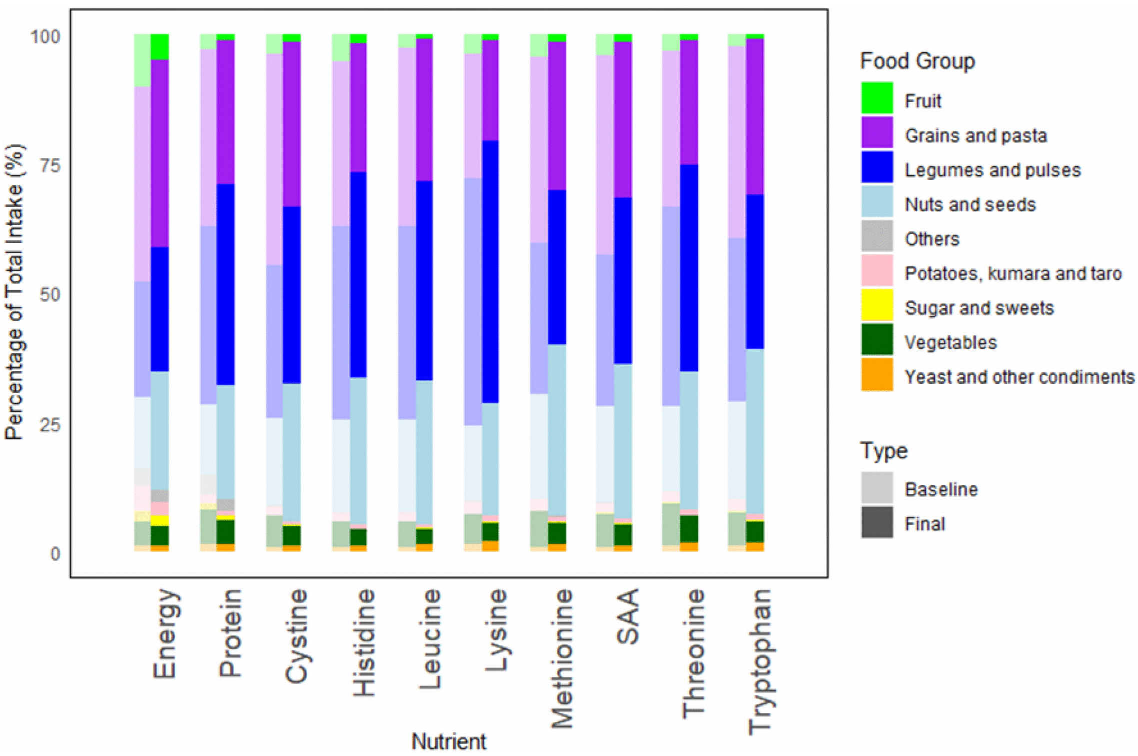
Table 5.4 Mean daily nutrient intake (SD) in the baseline and optimised diets, relative to individual BW across clusters 1 to 3

	Protein		IAAs (mg/kg/day)							
	Protein (g/kg/day)	AMDR in % of energy from protein	Cys	Hist	Leu	Lys	Met	SAA	Trp	Thr
Cluster 1										
Baseline	0.59 (0.17)	10.2 (2.43)	6.99 (2.29)	10.9 (3.41)	30.2 (9.30)	20.0 (6.99)	6.92 (2.28)	13.9 (4.39)	5.03 (1.63)	15.2 (4.75)
Final	0.90 (0.23)	12.6 (2.98)	11.6 (3.38)	18.4 (4.23)	50.6 (9.75)	33.8 (8.99)	11.5 (2.71)	23.1(5.73)	8.04 (1.84)	24.7 (4.8)
Cluster 2										
Baseline	0.72 (0.23)	11.7 (3.41)	8.32 (2.53)	13.0 (3.67)	36.1 (10.2)	23.8 (7.80)	8.04 (2.21)	16.4 (4.55)	5.68 (1.83)	17.5 (4.57)
Final	0.96 (0.23)	13.5 (3.45)	11.9 (2.79)	18.9 (3.5)	52.2 (9.54)	34.3 (7.03)	11.7 (2.52)	23.6 (4.94)	8.07 (2.17)	24.9 (3.8)
Cluster 3										
Baseline	1.02 (0.31)	17.6 (7.21)	11.1 (3.83)	16.8 (5.58)	43.3 (12.9)	33.4 (15.0)	9.30 (2.69)	20.3 (6.20)	5.06 (2.07)	21.1 (6.23)
Final	1.18 (0.34)	17.7 (4.93)	13.5 (3.76)	21.1 (5.17)	54.3 (11.2)	41.0 (14.5)	11.6 (2.28)	25.1(5.72)	6.51 (1.97)	26.2 (5.28)

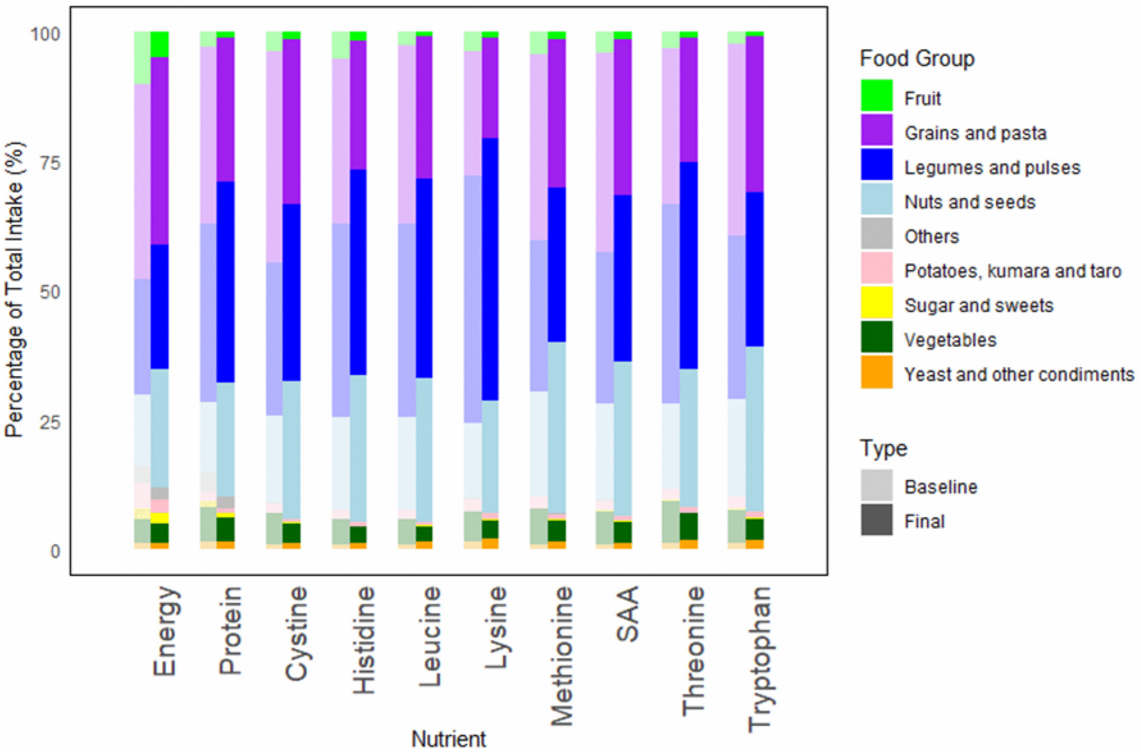
AMDR, Acceptable Macronutrient Distribution Range, SD, standard deviation; BW, body weight; Cys, cystine, His, histidine; Leu, leucine; Lys, lysine; Met, methionine; SAA, sulphur amino acids; Trp, tryptophan; Thr, threonine

The hypothesised IAA intake in the final diets was compared to the upper limits. Only two daily diets had lysine intakes above the NOAEL but below the LOAEL. These cases were from cluster 1 (female) and cluster 3 (male). The former case also had an elevated protein intake relative to BW, at 3.03 g/kg/day. This result could be attributed to a large serving (150 g) of nutrient-dense pea protein isolate selected by the model, which contributed a high amount of lysine. For the latter case, protein intake was 1.67 g/kg/day. The high lysine intake could again be attributed to the addition of one more serving of pea protein during optimisation, on top of 3 servings at baseline.

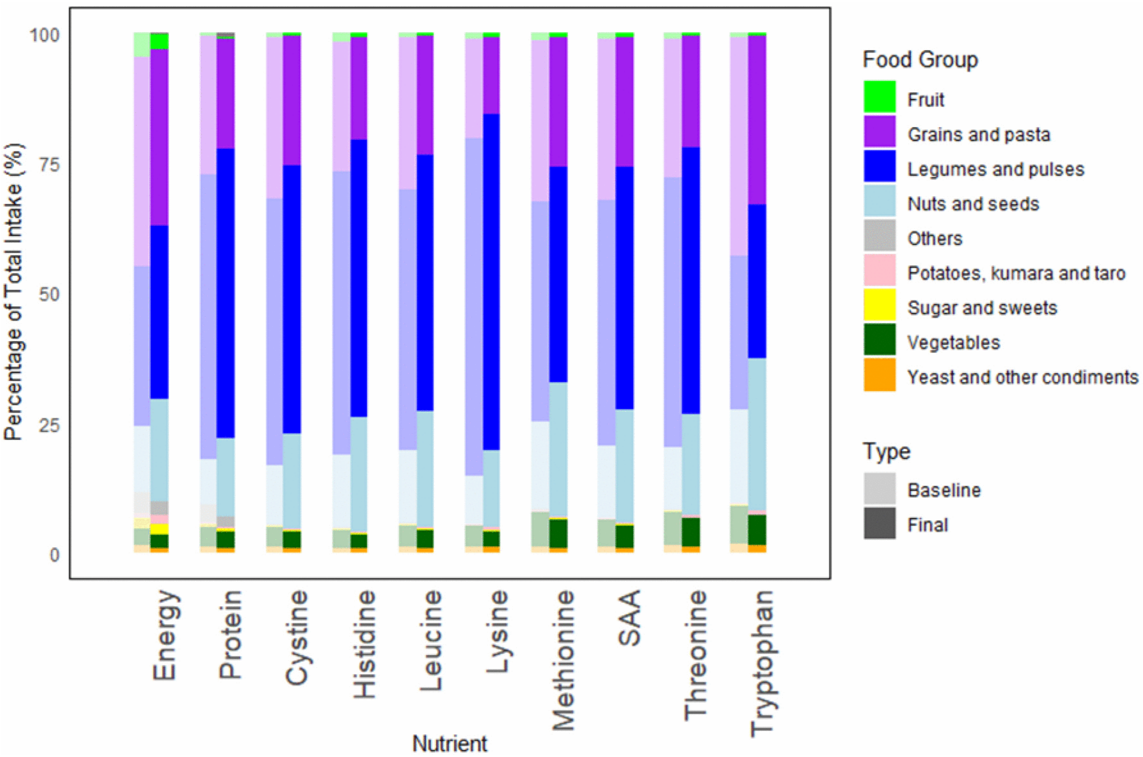
The final optimised diet composition was compared to the baseline diet in **Figure 5.3A to C**. A decrease in energy, protein and IAA contribution from "fruit", "vegetables" and "grains and pasta" was observed when shifting from the baseline to the final diet, largely due to substantial increases in other FGs. "Nuts and seeds" and "yeast and condiments" provided greater contributions to energy, total protein and all IAAs from baseline to final diets. In contrast, apart from increases in the contribution to protein and lysine, the nutrient contributions by "legumes and pulses" remain relatively unchanged in baseline and final diets.



(A) Cluster 1



(B) Cluster 2



(C) Cluster 3

Figure 5.3 Comparison of the final modified diet with the original diet across clusters 1(**A**), 2(**B**) and 3(**C**) Stacked bar graphs show the contribution of each FG to energy, total protein, and IAAs as a percentage of total intake. “Others” consist of alcoholic and non-alcoholic beverages.

The breakdown of the proportion based on the total weight of food consumed, for each FG, is shown in **Table 5.5**. “Grains and pasta” and “legumes and pulses” contributed the largest proportion of total food mass in roughly a 1:1 ratio. When comparing how the total weight of each FG changed from the baseline to the final diet, FGs that had at least a 10 % increase in all clusters were “legumes and pulses” and “nuts and seeds”. FGs that had a reduction in total weight (noting that this only occurred in cases where foods were required to be removed from the diet to provide residual energy capacity) were mainly energy-dense, from “potatoes, kumara and taro”, and “others”, which consisted of alcoholic and non-alcoholic beverages such as fruit juices and sugar-sweetened beverages (SSBs). In cluster 3, the decrease in total weight of “yeast and condiments” was due to the removal of energy-dense sauces. The increase in total weight of “potatoes, kumara and taro” occurred in two cases in cluster 3 to meet energy shortfalls.

Table 5.5 Daily mean food weight (%) contributed per FG, for each cluster

FG	Cluster 1	Cluster 2	Cluster 3
Fruit	7.93	6.38	5.42
Grains and pasta	27.0	29.1	29.0
Legumes and pulses	28.7	31.2	33.1
Nuts and seeds	10.5	9.81	11.5
Potatoes, kumara, taro	2.94	3.26	0.50
Vegetables	10.5	11.3	7.99
Yeast and other condiments	1.31	0.78	0.94
Sugar and sweets	0.63	0.38	0.51
Others[1]	10.4	7.62	10.9

[1] Others denote beverages, not including PBA, that have been classified into the FGs, “grains and pasta”, “legumes and pulses” and “nuts and seeds” (e.g. oat, almond and soy-based beverages) group

5.3.4 Sensitivity analyses

When the upper boundary for energy was reduced from + 20 % to + 10 % and + 5 % of the EER_{min}, the number of unsolved optimisation cases increased (n = 60 and n = 77, respectively) as cases which exceeded the + 20 % threshold inevitably also exceeded the tighter energy boundaries (+ 10 % and + 5 %). However, the mean weight of additional foods required to achieve adequacy among the solvable cases remained similar, indicating feasible solutions were largely unaffected. Importantly, 91.1 % of solvable days required substantially less than the + 20 % energy margin. This boundary could be interpreted as a flexible safety margin to improve model feasibility, rather than a prescriptive increase of energy intake.

For the 52 daily diets that had no solutions, assessments were first made to determine whether increasing the energy allowance (EER_{max}) and removing serving-size restrictions would improve the percentage of each nutrient's shortfall that can be met. Even with increments in energy up to twice the EER_{min} – a level that is likely unrealistic - feasible solutions could not be achieved for most cases. Only five individual diets met at least 70 % of the nutrient intake in short supply. This was only possible because these diets had fewer nutrient gaps and smaller shortfall magnitudes, making it more attainable to meet requirements within the relaxed energy constraint and limited food options available in the baseline diet.

Without serving-size limits, the LP solver selects foods from existing diets to meet nutrient targets, by adding any quantity of available dense protein sources (seeds, pulses and isolates). Low-protein foods such as green-leafy vegetables were also added in larger amounts. These are unrealistic in practice to cover nutrient shortfalls. When energy and serving-size constraints were eliminated, most of the foods in the 52 unsolved diets were suboptimal in reconciling protein and IAA shortfalls. Together, the exploration of these scenarios confirms that the model formulation is robust and provides predictable changes in the outcomes when constraints are altered.

An alternative approach was explored for the 199 problematic cases where the model could select foods from any diet in the cohort and add them to the original diets. Extruded plant proteins – notably pea, faba bean and hemp isolates – as well as spirulina and various nuts and seeds (hemp, pine nut, walnut, sesame) were frequently selected in this approach. These foods were also commonly found in the daily diets of those who had no protein or IAA shortfalls (n = 264). Nutrient shortfalls were resolved with small increases in mean energy intake of 0.71 (SD = 0.81) MJ with this approach.

A scenario in which all isolates were removed from the model's food selection was explored for all daily diets in this study, with the aim of understanding whether that changes the number of feasible solutions and the impact on the added weight of FGs. Removal of isolates resulted in 11 additional daily diets with no feasible solutions, with 8 cases from daily diets meeting or exceeding the EER_{min}. Among the daily diets that remained solvable, removing isolates caused small deviations in the average weight of each FG. Notable shifts in particular FGs were likely reflections of the model's need to substitute or reduce certain items once isolates were excluded. Taken together, there is a potential that baseline diets can be largely maintained, provided that these protein-dense foods are included into the diets as supportive additions to improve protein quality, and modest changes in overall energy intake are acceptable.

When upper constraints for IAAs were introduced, no feasible solutions could be obtained for nearly all cases. The only exception was a single instance in which wheat was added to contribute protein and energy, but not IAAs, to a baseline diet that had only shortfalls in protein and energy. No solutions can be found for other cases; cystine and histidine intakes were often already above the upper IAA constraint even at baseline. Hence, the model could not identify suitable combinations of baseline foods that simultaneously resolve nutrient shortfalls while remaining within all imposed constraints.

5.3.5 Assessment of micronutrient intake after optimisation

The influence of food addition on the quantity of vitamin B12, calcium, iron, iodine, zinc, ALA, sodium and dietary fibre was examined post-optimisation to determine how adequacy was affected and assess whether the optimisation process introduced risks of exceeding the upper limit. **Table D4 of Appendix D** also presents an evaluation of changes in other micronutrient levels from the baseline to the optimised diets.

More than 90 % of daily diets met or exceeded the daily requirements for dietary fibre, iron and zinc in all clusters. This should however be interpreted with caution, as adequacy was assessed against the EAR only, without broader adjustments for bioavailability and accounting for skewed distributions among menstruating women for iron intake. Hence, adequacy may be over-estimated. Many optimised diets were still below the minimum intakes for calcium, vitamin B12, and iodine across all clusters, but particularly for cluster 1. Hence, food additions based solely on constraints in protein, IAA and energy content cannot correct for shortfalls in these micronutrients.

The mean and percentage intake of daily diets meeting the requirement for each micronutrient, however, increased from baseline to the final diets, indicating that dietary optimisation not only reduced shortfalls in protein quality but to some extent, provided a small co-benefit for some micronutrients of concern. The exception was an increase in dietary sodium. Interestingly, the dietary clusters stratified by dietary protein intake at the EO level also reflected similar trends in micronutrient intake, with cluster 1 exhibiting the lowest nutrient intake, relative to the requirements. **Table 5.6** illustrates the mean intake of these nutrients at baseline and the percentage of the daily diets that meet the requirements in the optimised diets.

Table 5.6 Comparison of the mean intake of micronutrients (SD) and % adequacy[1] between baseline and final diets

	ALA (g)	Dietary fibre (g)	Calcium (mg)	Iron (mg)	Iodine (µg)	Sodium (g)[2]	Vitamin B12 (µg)	Zinc (mg)
Cluster 1								
Baseline								
Mean intake	1.48 (1.3)	35.7 (12.7)	673.4 (367.5)	14.1 (5.7)	101.5 (95.5)	2.08 (1.0)	1.44 (2.6)	7.71 (4.7)
Adequacy[1] (%)	55.3	77.6	20.5	92.7	35.6	46.6	20.5	49.8
Final								
Mean intake	3.62 (3.78)	43.8 (13.2)	799.4 (376.3)	19.1 (6.35)	117.7 (103.9)	2.45 (1.16)	1.87 (3.34)	11.5 (6.92)
Adequacy[1] (%)	75.3	94.1	33.8	98.6	46.6	61.6	26.0	94.1
Cluster 2								
Baseline								
Mean intake	1.92 (1.8)	39.6 (12.5)	769.0 (374.4)	16.2 (5.91)	144.7 (479.8)	2.28 (1.5)	1.41 (1.8)	8.89 (2.5)
Adequacy[1] (%)	63.1	87.2	31.8	95.4	35.9	47.7	22.6	60
Final								
Mean intake	3.34 (3.59)	45.1 (13.7)	869.1 (349.1)	20.2 (6.96)	154.2 (479.1)	2.55 (1.47)	1.62 (1.78)	11.9 (3.16)
Adequacy[1] (%)	75.9	94.4	45.1	99.5	43.1	59.0	26.7	91.8

Cluster 3								
Baseline								
Mean intake	1.59 (1.8)	35.8 (12.8)	838.4 (445.9)	18.9 (7.23	134.9 (105.1)	2.50(1.4)	4.72 (11.1)	10.5 (3.3)
Adequacy[1] (%)	47.2	77.8	33.3	94.4	55.6	52.8	44.4	72.2
Final								
Mean intake	2.43 (2.18)	40.2 (13.8)	903.4 (417.6)	21.7 (8.07)	133.5 (103.3)	2.46 (1.16)	5.32 (13.4)	12.2 (3.57)
Adequacy[1] (%)	61.1	88.9	41.7	100	58.3	55.5	47.2	77.8

ALA, alpha-linolenic acid; SD, standard deviation

[1]% adequacy describes the percentage of daily diets that meet or are above the daily nutrient requirement, at the EAR level (**Table D2 of Appendix D**).

[2]For sodium, the percentage indicates the proportion that exceeds the recommended daily intake of 2 g.

The mean contribution of nutrients as a percentage of the daily requirement by each added FG is presented in **Table D3 of Appendix D.** This analysis was used to identify the key food sources responsible for the high contribution of specific micronutrients. For example, relatively large quantities of spirulina (15 g) in the baseline diet, fortified yeast products, and fortified PBMA were the primary contributors to vitamin B12 intake. ALA contribution was mostly from "nuts and seeds" and "grains and pasta". Sodium contributions were mainly from "sauces and condiments", protein powders and PBMAs. At least 50 % of cases in the final diets, across all clusters, were above the recommended intake of 2000 mg.

Despite the mean calcium content in modified diets approaching the EAR of 840 mg across all clusters, substantial variation remained. One case from cluster 1 was above the upper limit for calcium, but was already above it at baseline. Similarly, large variations were observed for iodine, with two cases from cluster 2 exceeding the upper limit, but this was also observed in the baseline diets of these individuals due to seaweed consumption. For zinc, one case in cluster 1 exceeded the upper limit and was already the case at baseline due to protein powder consumption. Three cases from cluster 2 exceeded the upper limit for iron in the final diets and were close to, but not above, the upper limit at baseline. The addition of iron-rich nuts, seeds and legumes during optimisation increased dietary iron and ALA content.

Although micronutrient adequacy was not prioritised as a primary optimisation constraint in this study, additional analyses were conducted to characterise which micronutrients were most difficult to achieve from habitual vegan diets. Specifically, for each daily diet in which these micronutrients were present in foods already consumed, and where additional energy intake was theoretically feasible, the maximum achievable adequacy was quantified under energy and serving-size constraints. This analysis was intended to estimate the highest level of micronutrient adequacy achievable from foods alone. The mean achievable adequacy was lowest for vitamin B12, calcium, and iodine (35.0 %, 38.9 % and 41.3 % of the nutrient requirements, respectively).

When micronutrients were incorporated as optimisation constraints along with protein and IAAs, the number of feasible solutions declined substantially. Only 131 of 502 daily diets were successfully resolved, compared with 303 diets achieved in the previous optimisation. Moreover, the optimised solutions required approximately twice as many added food items and total added food weight, indicating substantial dietary change. This degree of modification could also compromise the acceptability of the optimised diets and reduce their real-world feasibility. As mentioned, specific foods such as spirulina, yeast products and novel PBAs emerged as key contributors to these limiting micronutrients, the latter two could be related to fortification strategies.

5.4 Discussion

The main objective of this Chapter was to address inadequate protein and IAA found in the current diets of a vegan cohort in NZ. Mathematical optimisation resolved nutritional shortfalls in 90 % of daily diets while minimising the weight of added food, using food items found in each individual's existing diet. Consequently, the model generated outputs for the weight (g) and nutrient contribution for total protein, energy, and IAAs. At the same time, some beneficial impact to certain micronutrients was observed after diet optimisation.

5.4.1 Diet optimisation and key findings

The weight of added food must be guided by both the energy and serving-size constraints, as these parameters ensure that optimised diets are feasible in reality. Designing optimal vegan diets can be challenged by the need to correct nutrient shortfalls while simultaneously maintaining energy intake within limits – two constraints that may be incompatible within the baseline diet (3, 38). This was the case in this study for individuals who were already meeting or exceeding the maximum boundary of EER but had shortfalls in protein and/or IAAs.

Substitution of foods in step 6 of **Figure 5.1** enabled 147 daily diets, out of an unsolved 199, to meet protein and IAA shortfalls, while keeping below the EER_{max}. The majority of removed foods were "fruit", "beverages" (alcoholic, SSBs and fruit juices), "grains and pasta" and "potatoes, kumara and taro". Eliminating these foods provided residual energy capacity to accommodate the addition of more protein-dense foods, from "legumes and pulses" and "nuts and seeds" - FGs which had a larger increase in total weight in the optimised diets as compared to other food groups. This observation was aligned with a Swedish diet optimisation study, where pulses, meat and dairy substitutes (pea, soy and mycroprotein-based products), but not nuts, had a ten-fold increase compared to the baseline diet (18).

As reviewed in section II of Chapter 2, LNS are considered good sources of lysine in a vegan diet. Since lysine was a commonly deficient IAA in the baseline diet of this vegan cohort, the increased intake of these foods resolved this nutrient gap. Replacement of ASFs with LNS in other PB diet scenarios produced similar observations (19, 39). Nuts as valuable protein sources were also highlighted in food replacement scenarios among pregnant women in Australia and identified to contribute at least twice the protein of tofu, legumes and pulses (40).

Compared to the current Danish diet, the *NutriHealthGHGE* diet - an optimised diet modelled to meet nutritional adequacy, health and GHGE goals - showed substantial shifts in protein source composition. To compensate for the displacement of ruminant meat, the intake of "nuts and seeds" showed a marked increment from 6 to 20 g per day (+ 230 %), alongside increases in other ASFs (fish, eggs and poultry) (41). "Nuts and seeds" were observed as a key source of total protein, leucine and methionine in the optimised diets in this study, contributing higher quantities of these nutrients per gram of food than "legumes and pulses".

Given that fruits and vegetables are an excellent source of vitamin C (42) and other essential micronutrients, these foods are central components of FBDGs around the world (43, 44). Hence, the reduction of 20 to 30 % weight of fruit in the final diets of the problematic cases in this study is a concern. Vegetables were less affected, with a decrease of less than 7 % in weight across the final diets of the clusters. As an indication, vitamin C in the final diets remained within an acceptable range despite reductions or substitutions in fruits and did not vary from the baseline diets **(Table D4 of Appendix D)**. Vitamin A, however, showed high levels of inadequacy in the final diets, although inadequacy was already high in the baseline diet.

Identifying energy-dense, low-protein foods for removal was a pragmatic strategy to explore the trade-off between protein quality and energy constraints. This approach highlighted a critical challenge in PB diet optimisation. When the baseline diets contain foods that are relatively energy-dense but protein-poor (as exemplified by the problematic cases in this study), substantial dietary change may be required to accommodate food substitutions with higher protein density. The broader limitation of the substitution approach was the complete removal of certain foods, like fruits which contribute to micronutrients of importance. This was albeit applied only in the problematic cases. While the approach was consistent with the study's aim of examining how vegan diets may be optimised within energy boundaries, a more sophisticated method could incorporate partial displacement of foods and/or incorporate additional constraints to better reflect realistic dietary modification. This is further explained in the strengths and limitations section.

On average, in the optimised diets, the mean intake per individual across the whole vegan cohort for" legumes and pulses" was 129 g, for "nuts and seeds" was 38 g and for "grains and pulses" was 108 g. This was roughly equivalent to the quantity of protein-rich foods (consisting of beans, peas, lentils, soy products, nuts and seeds) in a modelled 2000 kcal-per-day vegan diet in America, but the quantity of grain was higher at 184 g per day (45). This difference could be attributed to the narrower individual energy boundaries in the NZ study cohort, which would favour less energy-dense but protein-rich foods. The modelled American vegan diet met the reference value for protein, but AA intake was not analysed, so the proportions of these foods may require adjustments if protein quality is included. For example, another diet simulation on American adults found that the IAA scores increased when 50 % of AAs from grains were replaced by AAs from lentils and legumes (46).

Given the high protein density and protein quality of LNS, and their complementary effect with grains to achieve protein adequacy, the proportions and balance of these foods within the contexts of vegan diets warrant further investigation. Subsequent updating of serving-size guidelines in FBDGs, such as the Eating and Activity Guidelines for NZ adults (28), could provide clearer guidance for vegan consumers on meeting all IAA requirements. The optimal ratio of grain to legume in PB protein complementation remains unclear and varies across foods. For example, substituting 200 g of cooked chickpeas with 120 g of cooked lentils improved the protein quality score because lentils have a higher protein content (47). This shows variation in protein quality even within a food group. An approximate 1:1 ratio is evident from this study, but a 2:1 ratio may be more effective for optimal protein quality (17). More experimental verification or modelling at the meal level is necessary to establish more precise ratios of these foods.

Extrusion processes in the manufacture of protein isolates improve protein digestibility by reducing the ANFs in these foods (48, 49). Hence, their addition to a vegan diet could be beneficial for promoting protein adequacy. However, as compared to nuts and seeds, which are versatile additions to meals and snacks, protein isolates and spirulina are more challenging to incorporate into vegan diets without adding sweeteners - such as fruit or PBBs - to enhance palatability. This is an opportunity for innovation in the formulation of PB meat and dairy alternatives, where the integration of high-quality protein isolates as ingredients can improve nutrient shortfalls in an acceptable form. The value of these isolates was evident in the sensitivity analyses of the 52 unsolved cases, where their addition to the baseline diet was more effective in fulfilling shortfalls than restricting the model to add or substitute foods that were present in the individual's diet.

The post-optimisation analysis of dietary fibre and micronutrients was examined. The most concerning nutrients at risk of deficiency were vitamin B12, iodine, calcium, and selenium, for which the percentage of daily diets achieving adequacy was approximately 50 % or below. Results were poorer in the baseline diets of clusters 1 and 2. PB foods are not always reliable sources of bioavailable versions of these micronutrients. Limited amounts of vitamin B12 for example is obtained from seaweed, protein powder and yeast products (50) - foods found in the baseline diets of this vegan cohort. Spirulina is a rich source of vitamin B12 (29) and contains the largest quantity of the micronutrient at 3 mg per gram in the vegan dietary records of this study. It is also a high contributor of iodine (22 mg per gram). Concomitant improvements in micronutrient intake were observed with the addition of protein-dense foods. For example, the addition of tofu, soy-based foods and seeds simultaneously increased calcium intake in the optimised diets.

When the optimisation was constrained to meet the EAR for selected micronutrients, fortified foods in the forms of PBMAs, PBBs and protein powders were the largest contributors to several micronutrients, particularly vitamin B12, iodine, zinc and iron. Similar observations were reported in other studies. For example, the inclusion of a fortified soy drink in a vegan diet scenario in the Netherlands increased calcium intake to meet requirements (43). Similarly, optimised vegan diets in Sweden achieved calcium and vitamin B12 adequacy through high quantities of fortified PBAs (18). However, these benefits must also be considered alongside potential trade-offs. Depending on formulation, these foods may have high caloric density and contribute to excess sodium, as compared to traditional PB sources (51-53). Again, the role of food formulation and product innovation is important in developing fortified PB foods that can support the provision of bioavailable micronutrients and utilisable protein.

A substantial reduction in the number of feasible solutions after including micronutrient constraints was an expected observation. Few foods within the existing baseline diet could meet protein quality and all micronutrient requirements within the required energy boundaries. Given the challenges of attaining nutrient adequacy in this vegan cohort for vitamin B12, iodine and calcium from whole foods alone, supplementation is likely needed to fill remaining gaps. Without appropriate documentation of supplement use in the baseline diets and its inclusion in diet optimisation, total micronutrient intake in the cohort may be underestimated. In support, Storz et al (2023) observed adequate vitamin B12 status among vegans due largely to > 90 % of the cohort consuming a daily median intake of 250 mg of a vitamin B12 supplement (54). Supplementation expenditure is sizeable in vegan populations, and about 2 to 4 times higher than in omnivores and lacto-ovo-vegetarians, as indicated by one study on German vegans (55). Despite its importance, vegan dietary studies (including this present thesis) often exclude supplement use in the food recall data (56, 57). Reliable records of supplement usage in vegan dietary records could improve modelling outcomes for micronutrient adequacy in vegan diets.

Across all clusters, a high intake of dietary fibre was also introduced in the optimised diets, with more than 90 % of individuals in all clusters consuming quantities above the requirements.

While dietary fibre intake is associated with several health benefits, including enhanced satiety, which has a beneficial role in appetite regulation, it can impair nutrient digestion and utilisation by the body (58). The introduction of large quantities of dietary fibre in the modelled diets may present a practical challenge in reality, as overall food intake may become limited due to satiety (40, 59). Hence, this may restrict the consumption of protein, IAAs and essential micronutrients.

5.4.3 Strengths and limitations

A strength of this study is correcting protein quality shortfalls at the individual-level by incorporating calculations of energy, total protein and IAA requirements based on each participant's BW, sex, age and PAL. This enabled the identification of specific nutrient shortfalls for targeted optimisation. Compared to population-level modelling, this individualised approach could potentially improve the acceptability and adherence to dietary interventions aimed at improving the protein quality of vegan diets (60).

The limitation of the model is its dependence on foods within the individual's existing dietary records. For example, spirulina provides a supply of several micronutrients and protein, at a low energy density. However, it was not commonly consumed within this vegan cohort and additional food preparation may be necessary to improve its palatability. More broadly, the usefulness of this optimisation approach is tied to the diversity and nutritional quality of the baseline diet. For diets characterised by energy-dense and nutrient-poor options (in the case of the 199 problematic cases), modelling using benchmarking of peer diets may be more optimal to devise diets with a favourable balance of "more-is-better" versus "less-is-better" nutrients (4). By leveraging the dietary habits of peers within the same population, such approaches enable modifications of less optimal diets using culturally appropriate alternatives to improve both nutritional quality and feasibility (4, 9). However, within the context of vegan diets, this may be challenging in practice due to the limited availability of diverse, nutritionally adequate vegan dietary records in the population to serve as peer benchmarks.

Diet variety was considered by introducing maximum serving limits based on each FG, as guided by NZ dietary guidelines (28) and disallowing duplicated selections for each food item. A similar approach that applied serving-size constraints, as guided by the Australian Dietary Guidelines (21), was adopted in an LP optimisation exercise for various diet scenarios in Australia (6). By placing limits on the daily intake of each food item, the model ensured that some diversity was introduced to the diet while keeping to a practical volume of food item. The model in this Chapter chooses up to 150 g for "legumes and pulses", but this serving-size constraint may not be applicable for legume-based protein powders, which are typically consumed in quantities of 20 to 35 g per serve (61). This suggests that more specific serving size guidelines for these foods are required for future optimisation modelling.

One limitation of the current model is that, during substitutions, entire baseline food items are removed rather than their portion sizes reduced or displaced by alternative foods. This means that all nutrients contributed by that food are eliminated as well. Although the model may introduce the same food at a smaller serving size, perhaps a more effective approach might involve modifying the food's recipe or formulation to improve the protein quality, rather than removing it completely. Among the 199 problematic cases, 41 cases with feasible solutions ended up with diets that were below the EER_{min} (**Figure 5.2**, energy panel). This suggests that removing the top 25 % and 50 % of energy-dense foods may be too restrictive in these scenarios, so enabling the partial removal rather than full elimination of some foods could be a more intuitive and realistic approach. While the 25 % and 50 % thresholds used were arbitrary, further empirical testing is needed to determine optimal cut-off points for dietary feasibility.

A displacement model could simultaneously reduce portions of energy-dense foods while adding alternative foods, thus being closer to real-world food substitution. Such a framework would be more complex and require assumptions for how foods can be partially displaced (e.g., 17 g of white rice calculated by the solver may not be realistic in practice) and how much dietary change is acceptable. Aligned with the overall aim of investigating tensions between energy intake and protein density in this Chapter, the present approach, although simpler, is more transparent in identifying foods that are less efficient providers of utilisable protein. Implementing a solver that iteratively adjusts food quantities rather than eliminating foods altogether could increase computational complexity and runtime, but highlights a methodological limitation and directly informs the development of a more flexible optimisation framework in the later part of the thesis.

Notably, the EAR for protein was applied instead of the RDI for protein, which may underestimate the total protein intake needed to meet adequacy in our optimised solutions. However, post-optimisation evaluation indicated that among individuals whose protein intake remained below the RDI, the majority still met the AMDR for protein (10 to 35 % of total energy intake), indicating that protein intake is proportional to total energy intake. In several cases, individuals had approached the upper limit of energy intake, indicating limited scope to further increase food intake without exceeding realistic energy constraints. Only a small proportion of cases (< 5 %) had sufficient energy capacity to increase food intake further to meet both the RDI and AMDR simultaneously. This suggests that applying the RDI as a rigid lower-bound constraint across the entire cohort would have led to the prescription of unnecessarily large food quantities, despite IAA requirements already being satisfied.

The feasibility of this optimisation model follows the assumption that the added foods will be acceptable because they are obtained from the current diets of each individual (62). Additionally, the solutions are only reliable when dietary intake data is reported accurately, but misreporting is a common limitation of dietary records, as covered in Chapter 3. There is also no measurement to determine satiety from the consumption of protein and fibre-dense foods (59, 63, 64), or validate the extent of acceptability by the cohort. Furthermore, the calculation of the optimal nutrient intakes by LP is dependent on the quality of the food and nutrient composition databases used in the optimisation (6, 38) which may not have exact matches to AA composition in NZ foods.

Defining "currently consumed foods" from the four-day records may underestimate the variety of foods individuals consume over longer periods. Yet, foods outside of the records also do not guarantee acceptability. The vegan diet is inherently restrictive in high-quality protein sources, and, using the dataset available, captured all major FGs that feature in these diets. An alternative approach was explored where the model could draw from all available food sources. However, the optimisation consistently defaulted to a narrow set of food items for inclusion in everyone's existing diets, such as protein isolates, to maximise protein quality with only small increases in energy (such as in the scenario for the problematic cases), but this offers an oversimplistic solution and a lack of diversity.

5.5 Conclusion

This Chapter has optimised protein intake and protein quality with respect to daily requirements, but did not explicitly account for how foods are distributed across EOs. Distributing protein-dense foods in appropriate quantities across multiple EOs is better tolerated than consuming large quantities of these foods in one meal. The limitations experienced in this Chapter provide several important learning points that inform subsequent solutions in vegan diet planning. In particular, the application of uniform nutrient targets across individuals highlights the constraints of fixed-threshold approaches to nutrient adequacy. Dietary acceptability inferred solely from short-term dietary records can be a flawed assumption.

Hence, diet optimisations to improve the nutritional adequacy of vegan diets could move beyond static optimisation approaches to more flexible frameworks that account for individual preferences, meal structure, and changes to serving sizes. This may better allow requirements to be met in ways that align with real-world dietary behaviour.

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In which chapter is the manuscript/published work?	Chapter 6

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Bi Xue Patricia Soh: Conceptualisation, data curation, formal analysis, investigation, methodology, writing of original draft, review and editing. Dr Matthieu Vignes and Dr Nick W Smith: Formal analysis, methodology, supervision, review and editing. Professor Pamela R von Hurst and Professor Warren C McNabb: Conceptualisation, funding acquisition, investigation, project administration, supervision, review and editing

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Chapter 6 Beyond daily totals: Meal-level DIAAS reveals how food groups shape protein quality in vegan diets

This Chapter characterises the protein quality of vegan meals using the DIAAS calculation and analyses the impact of FG composition on protein quality. This serves to identify suitable PB dietary levers to overcome limiting IAAs in the vegan diet.

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Abstract

Vegan diets rely solely on PB proteins, which often supply lower quantities of IAAs than omnivorous diets. Low protein quality can leave IAAs undersupplied even if the protein intake meets the recommendation. Mixtures of PB foods in meals can complement limiting IAAs and improve the synchronised delivery of all IAAs for optimal metabolic function. Assessing how FG compositions differ among meals of varying protein quality is essential for determining the ideal proportions of PB foods to improve the DIAAS of vegan meals. The DIAAS was calculated for each meal found in the 4-day food diaries of 193 New Zealand vegans. Principal component analysis (PCA) and k-means clustering were applied to identify FGs associated with meals of varying DIAAS, and meal-level clusters were compared to participants' daily protein intake. Eight distinct meal clusters emerged. Lower-DIAAS meals were characterised by higher quantities of fruit, grain foods, potatoes and sugary condiments, with significantly lower ($P < 0.001$) weight share of legumes than higher-DIAAS meals, which had a ~ 2:1 legume:grain profile. IAA contribution differed across FGs as grains, nuts and seeds supplied more SAAs, while legumes and extruded plant protein isolates (such as protein powders) supplied more lysine. When these analyses were related back to Chapters 4 and 5, lower-protein-quality meals appeared most often in daily patterns with lower total protein and IAA intake, but also occurred in some instances when daily protein was high. This confirms that assessing protein adequacy only at the daily level can mask meal-level deficits. Deliberate meal-level complementation of PB foods is necessary to ensure protein quality in vegan dietary patterns.

6.1 Introduction

As established in Chapter 2, interest in vegan diets is rising within nutrition and public health due to their environmental sustainability potential, ethical considerations regarding animal welfare, and potential health benefits (1, 2). However, a parallel body of literature has emerged to highlight the risks of nutrient deficiencies of vegan diets, when nutrient-dense and highly bioavailable ASFs are entirely replaced with PBAs – particularly if the diet is poorly planned or relies on a limited variety of foods (3). Hence, while the motivations to adopt such diets may be compelling for some individuals, ensuring that fully PB diets meet nutrient requirements demands close examination.

As clarified in the earlier Chapters of this thesis, IAAs may be at risk of deficiencies in a poorly planned vegan diet and are thus the nutrients of interest in this study. The IAAs must be sufficiently provided through dietary protein, owing to the body's inability to synthesise them endogenously (4). Plant sources generally have inferior protein quality compared to ASFs - a concept encompassing the content of digestible and utilisable/bioavailable IAAs to match the body's requirements (5, 6).

It takes knowledge and intention, but gaps in the IAA profile of vegan meals can be fulfilled through the combinations of diverse plant foods (7). This may be more practical at the meal-level, where different foods are mixed and consumed in the same EO. Even so, achieving high protein quality in vegan meals is further challenged by the lower digestibility of plant proteins, as compared to animal proteins, due to the presence of ANFs such as phytates, dietary fibre, and enzyme inhibitors in traditional and minimally processed plant foods (8-10). These ANFs limit the postprandial metabolic availability of IAAs in the body (11). As described in Chapter 2, while certain processing mechanisms, such as heat treatment, may reduce the presence of ANFs, this effect varies across different foods and types of ANF (12). Therefore, when evaluating the protein quality of vegan meals, the extent of IAA digestibility in the human body must be properly accounted for to avoid overestimating protein adequacy in vegan diets (13).

The DIAAS is currently the recommended metric for protein quality (14). While DIAAS is designed to indicate the protein quality of single foods, it can be applied in the context of meals due to the additive nature of the underlying TID-adjusted IAA values for various foods in a meal (15). A DIAAS of at least 100 % indicates that 1 g of the protein from the meal contains at least the quantity of IAA present in 1 g of the reference IAA pattern (15, 16). This provides a straightforward way to identify high-protein-quality vegan meals and subsequently evaluate the proportions of PB foods needed to achieve highly utilisable protein. The relative quantities of component foods in high-protein-quality meals can then be adjusted to meet an individual's specific protein and IAA requirements.

The overall aim of this Chapter was to analyse the protein quality of vegan diets at the meal level, using real-world meal consumption data from the NZ vegan four-day food diaries. By comparing FG compositions across meals of varying protein quality, the ideal proportions of PB food sources needed to derive meals of higher protein quality can be evaluated. When protein adequacy is assessed solely by comparing total daily protein intake with daily requirements, protein distribution across meals and the protein quality of individual meals are not considered. Considering the potential implications of dietary protein distribution over time on body composition and metabolism (17, 18), determining protein quality at both daily and meal levels is necessary. Thus, the secondary aim of this Chapter was to quantify the association between meal-level protein quality and daily protein intake and distribution (Chapters 4 and 5). This was achieved by estimating the probability that meals of high protein quality will be observed on days that met protein targets.

6.2 Methods

6.2.1 Data processing

A four-day food diary that detailed each individual's food and beverage consumption was used in the analysis (See Chapters 3 and 4 for details of data processing). Foods with the same date and time stamps were assumed to be consumed together and were categorised into one EO. Daily records containing food items with missing time stamps were excluded. A total of 766 daily dietary records were included in the analysis. Single foods that were not consumed as part of an EO, with low digestible protein (< 1 g / 100g of food), were excluded from the analysis. This mostly included beverages (coffees, teas, alcoholic beverages, juices, PBBs) and fruits. 603 single foods (not part of any meals) with mostly low protein content (< 1 g / 100g of food) were removed from a list of 4345 possible meal combinations.

6.2.2 Protein quality of meals

In this Chapter, mixed meals refer to the combination of all foods and beverages consumed in the same EO. The TID-adjusted IAA content was calculated and expressed as mg of AA per gram of dietary crude protein per meal (19, 20). This value was then divided by the same dietary IAA in 1 g of the reference protein, also referred to as the AA scoring pattern (see **Table 3.3**) - the ideal IAA composition required to meet the body's requirements (21). Considering that the baseline vegan diets were obtained from adult participants, aged 18 years and above, the AA requirement pattern for adults (> 18 years of age) was used (19).

The lowest value of the digestible IAA reference ratio thus represents the most limiting IAA in the mixed meal and denotes the DIAAS. The above-described calculation using an example of a vegan mixed meal consumed by one participant is shown in **Table 6.1**. Each meal component was categorised into its respective FG. For example, the "gravy", which is primarily made of cashew nuts, was classified as "nuts and seeds". Meal weight (g), crude protein (g), TID-adjusted protein (g), and IAA (mg) contents were listed. The digestible IAA reference ratio was expressed as digestible IAA in 1 g protein from the mixed diet per mg of the same dietary IAA in 1 g of the reference protein **(Table 6.1)** (19). In this example, the food items "gravy" and "meatballs" were the result of a home-cooked recipe, and the TID-adjusted values reflected in **Table 6.1** were a summation of the nutrient content of each recipe. "White rice" was a single food item (not part of a recipe; consumed on its own), with a TID value of 0.9 that was multiplied by the crude protein value to provide a TID-adjusted protein value of 0.81 g.

Table 6.1 Sample calculation of one baseline vegan mixed meal with leucine as the most limiting IAA

Food	FG	Weight in meal (g)	Crude protein in meal (g)	TID-adjusted protein (g)	TID-adjusted AA content (mg) for each food in the mixed meal								
					Cys	His	Leu	Lys	Met	SAA*	Thr	Tryp	
Gravy	Nuts and seeds	17.3	2.08	1.58	36.5	41.2	129.4	82.2	34.7	71.1	61.3	24.0	
“Meatballs”	Legumes and pulses	92.6	12.6	8.08	113.9	209.9	573.3	488.3	100.5	214.5	312.1	120.2	
White rice	Grains and pasta	29.0	0.90	0.81	18.0	20.9	60.3	29.9	18.2	36.1	26.3	9.41	
Totals													
		138.9	15.6	10.5	168.4	272.1	762.9	600.5	153.4	321.7	399.7	153.6	
					Digestible IAA reference ratio								DIAAS
					*	1.16	0.83	0.86	*	0.94	1.11	1.64	83% (Leu)
			Weight proportion of each FG to nutrient contribution										
Gravy	Nuts and seeds		0.13	0.15	0.23	0.15	0.17	0.14	0.23	0.22	0.15	0.15	
“Meatballs”	Legumes and pulses		0.80	0.77	0.68	0.77	0.75	0.81	0.66	0.67	0.78	0.78	
White rice	Grains and pasta		0.06	0.08	0.11	0.08	0.08	0.05	0.12	0.11	0.07	0.06	

Abbreviations: FG, Food group; DIAAS, Digestible Indispensable Amino Acid Score; TID, True-ileal digestibility; Cys, Cystine; His, Histidine; Leu, Leucine; Lys, Lysine; Met, Methionine;; SAA, Sulphur-amino acids; Tryp, Tryptophan; Thr, Threonine. *SAA = met + cys. TID-adjusted values for the AAAs (phenylalanine and tyrosine), isoleucine and valine were not calculated due to insufficient TID data for these IAAs. Example of weight proportion of TID-adjusted tryptophan from "gravy" was computed by taking 24.0 mg divided by 153.6 mg. Proportions may not sum exactly to 1 due to rounding.

6.2.3 Meal component analysis

Baseline mixed meals were classified into two strata based on their DIAAS scores: higher protein quality (DIAAS ≥ 75 %) and lower protein quality (DIAAS < 75 %) (19, 20). The precise rationale for this cut-off is not fully established and could vary across different food matrices (22, 23). This cut-off, however, provides a pragmatic approach in this study to categorise meals into different levels of protein quality for systematic comparison and subsequent modification of meals to increase DIAAS.

Meal compositions within each DIAAS stratum were evaluated with the following steps. The proportional contribution of each contributing FG to protein and IAA within a mixed meal was computed as the ratio of the nutrient quantity of each FG to the total nutrient quantity of the mixed meal. Specifically, protein contribution was expressed as grams of protein provided by each FG divided by the total protein (g) of the meal. The same approach was applied to each IAA, where the IAA quantity (mg) provided by each FG was divided by the total IAA of the meal. Contributions thus summed to 1 across FGs within each mixed meal for each nutrient. **Table 6.1** provides a sample of the calculated weight proportion. To summarise across all meals, the mean contribution to each nutrient for each DIAAS stratum was computed and displayed as a continuous scale using a heat map.

The variation in FG weight (g) contributions when present in meals was explored with ridgeline density plots, which illustrated the frequency of a given FG as a proportion of each meal's weight for each DIAAS stratum. The Wilcoxon rank-sum test was used as the nonparametric test to compare the relative contributions of each FG between higher- and lower- DIAAS meals. FG shares were expressed as ratios (e.g., legumes:grains, legumes:nuts/seeds; grains:nuts/seeds) to provide a quantitative estimation of the balance required among FGs to achieve higher protein quality meals.

6.2.4 Meal pattern analysis with Principal Component Analysis (PCA)

Each mixed meal was expressed as the proportional contribution of FGs to total meal weight. To reduce sparsity and improve interpretability, minor groups such as "beverages", "yeast and other condiments", and "sugars and sweets", that often had no weight share, were collapsed into a single category, "others". As the FG data was compositional (all shares of the FGs sum to one), PCA was performed on data that had undergone centred log-ratio transformation (24).

The formula is provided as:

$$CLR(x_i) = \ln\left(\frac{X_i}{g(\mathbf{x})}\right),$$

where x_i is the proportion of food group i and $g(\mathbf{x})$ is the geometric mean of all FG proportions within the meal. The CLR transformation converts each FG to a log-ratio relative to the meal's geometric mean composition, permitting the removal of unit-sum constraint inherent to the compositional data so that subsequent PCA analysis can be performed on appropriately transformed variables. The two principal components (PC1 and PC2), which explained the largest proportion of variance in the meal compositions, were retained for visualisation. Each meal was plotted in a two-dimensional map for further interpretation (25, 26).

FGs with large positive PC1 loadings drive points to the right of the plot, while negative loadings position corresponding points to the left. Likewise, large positive PC2 loadings push points upward and strong negative loadings pull points downward. Meals with very low DIAAS < 10 were excluded from the final analysis. Inspection of these meals indicated that extremely low DIAAS values were driven by the absence or near-absence of one or more IAAs. For example, tryptophan was not recorded for some foods, resulting in a DIAAS of 0. There were also instances where meals provided negligible protein or IAA content. Nevertheless, to assess the impact of this exclusion, PCA was rerun to include all meals, with no notable shifts in PC loadings for all FGs, apart from slight shifts in "legumes and pulses" and "others" FGs as a result of soy milk and other beverages, respectively.

To explore the clustering of meals by their FG profiles, k-means clustering was applied to the PCA scores of PC1 and PC2, and the average silhouette width was computed for candidate values of k = 2 to 15. The value of k with the highest average silhouette width provides guidance for determining the number of clusters to which each meal fits, relative to neighbouring clusters. Domain knowledge was also utilised to determine the optimal k value. Cluster assignments were then visualised in the PCA space and differentiated by point shapes and colour gradient, which represented DIAAS. Cluster centroids were calculated to approximate the mean protein quality of meals, as represented by the DIAAS of each cluster.

6.2.5 Linking meal pattern analysis to daily diets

In Chapter 4, cluster analysis of daily protein intake patterns was performed, and individuals were categorised according to the distribution of protein and IAA intake across EOs of the day. In Chapter 5, daily diets were identified in which participants had at least one shortfall in either protein or any IAA as compared to their daily requirements. However, these analyses did not consider the composition of each meal or EO. With PCA and k-means clustering in this study, distinct FG patterns were identified at the meal level and related back to the findings from the past Chapters. This aimed to determine the probability of consuming a high-protein-quality meal among individuals in clusters with higher average per-EO protein intake and with no daily protein or IAA shortfalls.

Each meal was assigned back to the daily temporal cluster from which it originated – the cluster assigned to that participant's meals for that day (27). Cluster 1 contained participants whose mean intake of protein and IAA per EO was the lowest, and cluster 3 contained participants whose mean intake of these nutrients per EO was the highest, and cluster 2 was an intermediate cluster. Meal compositions were not examined for protein quality in Chapters 4 and 5, so this Chapter aims to address this. The probability that a meal from a PCA-derived meal cluster fell into each pre-assigned daily cluster was calculated. A similar probabilistic approach was also applied to test whether specific meal compositions were more common among individuals who exhibited no nutrient shortfalls (28). A chi-square test of independence was applied to determine the association between PCA-derived meal clusters and specific daily dietary patterns.

6.3 Results

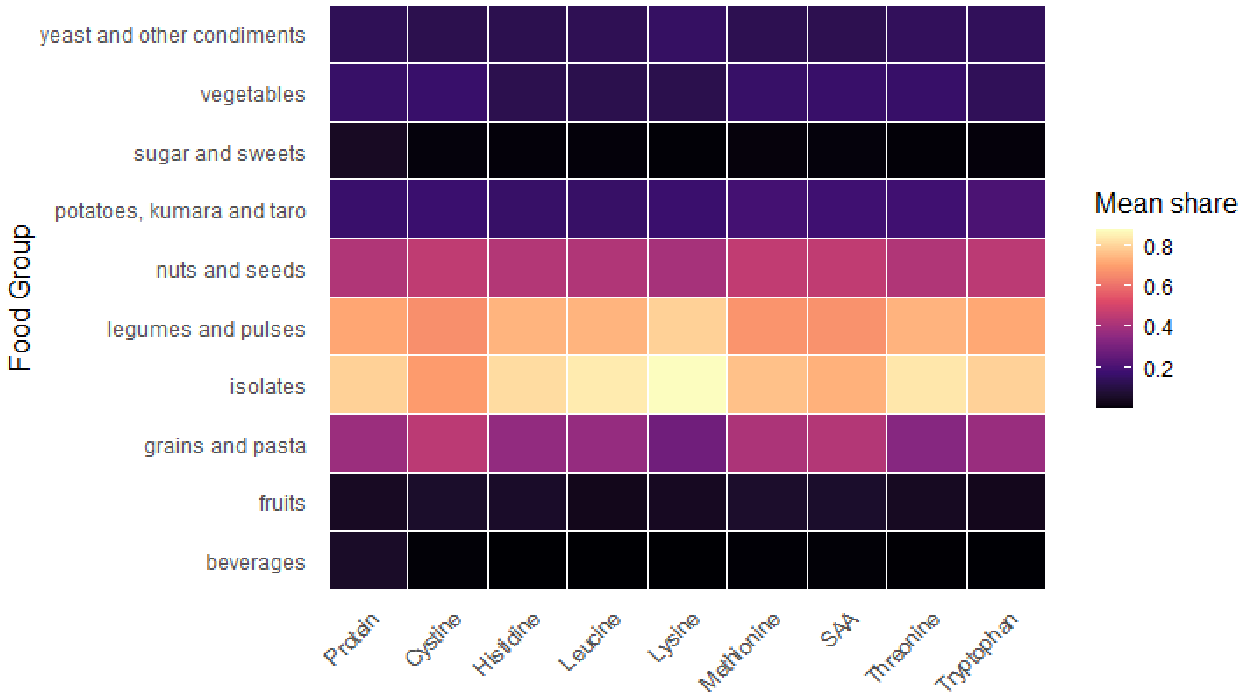
6.3.1 Meal component analysis

The meals in this vegan cohort were predominantly lower in protein quality: 822 meals had a DIAAS ≥ 75 %, and 2920 had a DIAAS < 75 %. The mean contribution of FGs to total nutrient content when present in meals with higher (DIAAS ≥ 75 %) and lower protein quality (DIAAS < 75 %) are shown in separate heat maps **(Figure 6.1).** Lysine was the most frequently limiting IAA in meals (56.6 % of meals), followed by SAAs (21 %), leucine (13.4 %) and tryptophan (8.7 %). Histidine and threonine were rarely limiting.

In meals with higher DIAAS, the largest contributions to total protein and overall protein quality were from "isolates" and "legumes and pulses", with ≥ 0.6 mean share of protein and each IAA. Lower mean shares were observed for "nuts and seeds" and "grains and pasta" (approximately 0.2 – 0.5) **(Figure 6.1A)**. In particular, lysine was mostly contributed by "isolates", and "legumes and pulses". Conversely, "grains and pasta" contributed less to lysine than to other IAAs but provided a larger share of the SAAs. "Grains and pasta" were more dominant in meals of lower than higher protein quality.

As expected, "sugar and sweets", "fruit", and "beverages" did not contribute meaningfully to protein content or quality. In particular, "sugar and sweets" and "beverages" formed < 0.1 mean share of protein in higher DIAAS meal scores, in contrast to 0.7 and 0.4, respectively, in lower DIAAS meals **(Figure 6.1B).** The contribution of “isolates” to the nutrient totals was fairly similar in both strata. As indicated by the mean share (colour gradient) of both heat maps in **Figure 6.1A**, “legumes and pulses” and “isolates” were dominant FGs providing protein and IAAs in higher DIAAS meals. Conversely, the provision of these nutrients was more equally distributed across FGs in lower DIAAS meals.

A. Meals with a DIAAS ≥ 75%.



B. Meals with a DIAAS < 75%

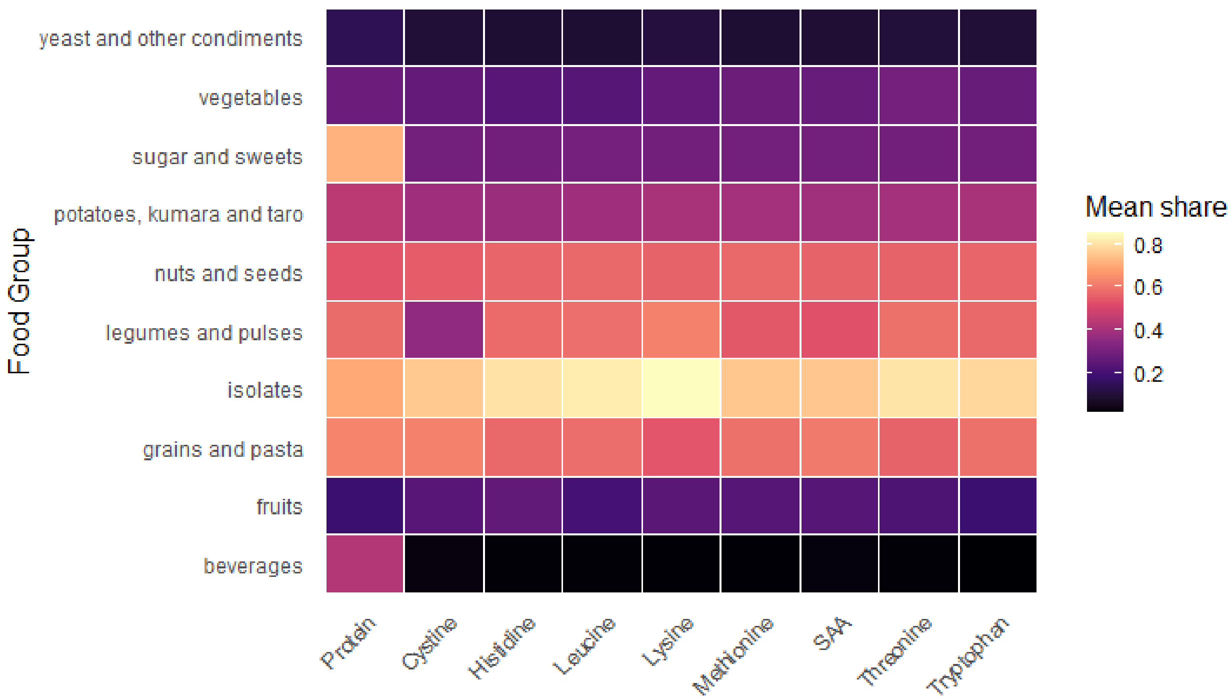


Figure 6.1 Heat map showing the mean share of protein and IAA content contributed by each FG when present in meals.
Lighter colours show larger share. Meals with higher protein quality (DIAAS ≥ 75 %) are characterised by higher contributions to protein and IAA from "isolates", "legumes and pulses" than from other FGs **(A),** as compared to meals with lower protein quality (DIAAS < 75 %) where several FGs contribute more equally to total protein and IAAs **(B)**.

FG contributions by weight were analysed with two complementary approaches. Within each DIAAS stratum, the distribution of each FG to the weight proportion of a meal was visualised with ridgeline plots in **Figure 6.2**, which showed higher weight proportions for a few FGs in the lower DIAAS than in the higher DIAAS meals. In this case, the distributions of within-meal FG weight proportions were examined conditional on the presence of the FG. For example, for "sugar and sweets", the curve for higher DIAAS meals peaked near zero, indicating that these foods were minimal in a large share of higher-protein-quality meals, in direct contrast to the curve for lower-protein-quality meals. For "potatoes, kumara and taro", and "grains and pasta", peaks were observed above 0.9 in the lower DIAAS meals but not in the higher DIAAS meals. In the higher DIAAS meals, the curves for these FGs peaked between 0.0 and 0.6. Interestingly, the distribution of the more protein-dense FGs – "legumes and pulses", and "nuts and seeds" – showed less distinction between higher and lower DIAAS meals, indicating that the quantity, when present in meals, did not vary systematically with DIAAS.

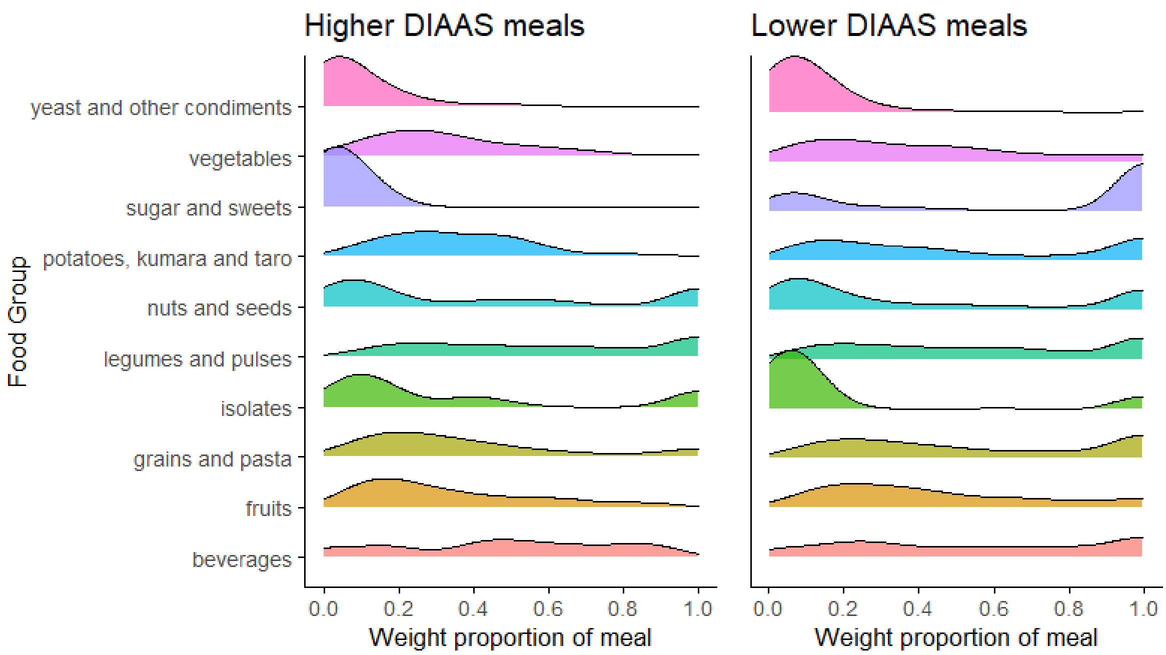


Figure 6.2 Ridgeline plots showing the distribution of FG weight shares (grams in meals) when present in meals, in higher and lower protein quality strata.
The x-axis represents the proportion of the meal's weight contributed by each FG. The height of the ridge (y-axis) reflects the relative frequency of meals. Taller ridges show a higher concentration of meals at a particular weight proportion, and flatter ridges show a wider spread of meals across different weight proportions.

Bar plots (**Figure 6.3**) compared FG contributions between higher- and lower DIAAS meals after meals in which a FG was absent were retained as zero values. While results in **Figure 6.2** did not consider prevalence, the step here computed the mean FG share by averaging weight proportions across all meals, with absent FGs in meals assigned a value of zero. This approach thus incorporated FG-prevalence, defined here as the frequency at which a FG appeared in meals. The estimates in **Figure 6.3** therefore reflect the combined influence of both the frequency with which a FG occurred and its proportional contribution when present. Wilcoxon rank-sum test indicated significant differences in the mean proportion of FG contribution between higher and lower DIAAS strata for all FGs, except “nuts and seeds” (P = 0.622) and “potatoes, kumara and taro” (P = 0.699).

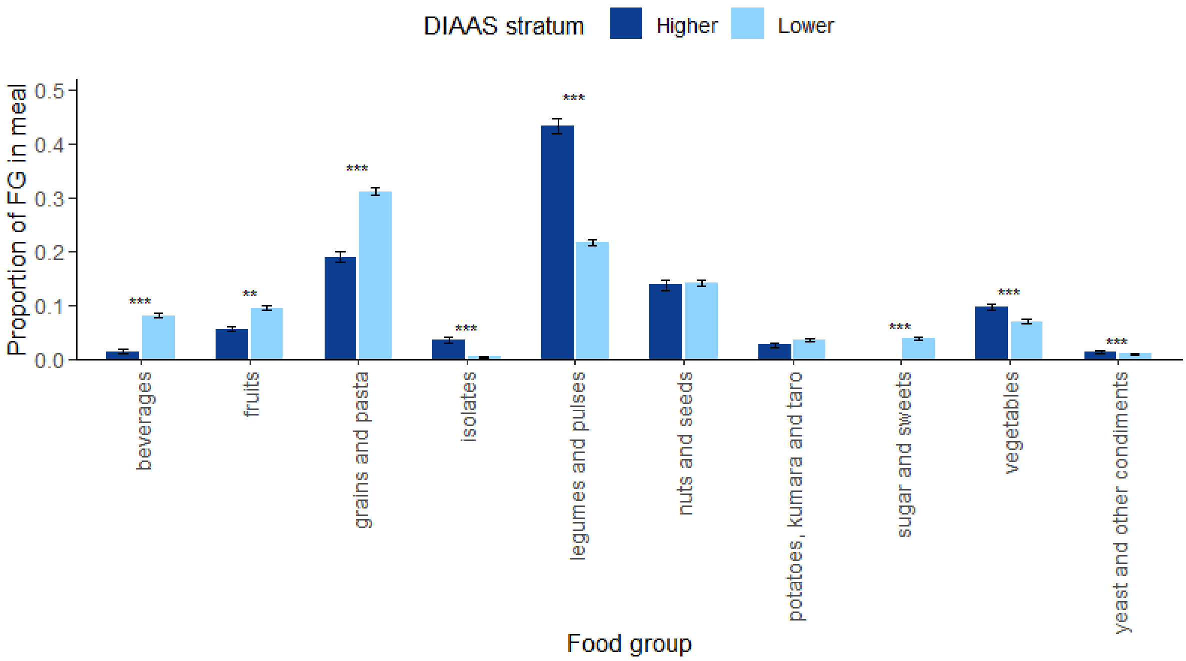


Figure 6.3 Bar plots comparing the mean meal proportion in % contributed by each FG between the higher and lower DIAAS strata.
Error bars represent standard error, with ** representing P < 0.01 and *** representing P < 0.001. Proportions of FGs, accounting for their weight contribution and prevalence, are computed for each DIAAS stratum and sum to 1.

When prevalence was not considered (**Figure 6.2**), “legumes and pulses” had a similar mean weight proportion of approximately 0.6 in higher DIAAS meals and lower DIAAS meals, indicating comparable portion sizes in meals from different DIAAS strata. However, the prevalence of “legumes and pulses” appearing as a component in higher DIAAS meals was 0.7 as compared to 0.4 in lower DIAAS meals, hence explaining the significant difference observed in **Figure 6.3**. This reflects more frequent inclusion of this FG in higher DIAAS meals. In contrast, while “nuts and seeds” also contributed similar weights to meals (**Figure 6.2**), the difference in their weight proportions was not statistically significant between higher and lower DIAAS meals, when prevalence was accounted for (**Figure 6.3**). These observations reflect comparable prevalence and within-meal weight proportions across DIAAS strata for “nuts and seeds”.

Relative FG proportions that characterise higher and lower-protein quality meals are presented for the top representative FGs in **Table E1 of Appendix E**. Proportions were expressed as ratios relative to a reference FG, which was set to 1.0, and other FGs were expressed relative to that baseline. For example, when "legumes and pulses" was set as the reference, "grains and pasta" had a mean value of 0.45 in the higher DIAAS meals, as compared to 1.39 in the lower DIAAS meals. This indicated a higher ratio of "legumes and pulses" to "grains and pasta" in higher protein quality meals. When nuts and seeds were set as the reference, "legumes and pulses" still dominated in higher DIAAS meals, while "grains and pasta" rose relative to "nuts and seeds" in poorer DIAAS meals. "Fruits" and "vegetables" contributed smaller weight proportions than "grains and pasta", "legumes and pulses" and "nuts and seeds" in both DIAAS strata. However, their relative contributions were less extreme in higher-DIAAS meals as compared to other FGs.

6.3.2 PCA and k-means clustering

PCA and k-means clustering were data-driven techniques used to identify clusters of meal composition and how these clusters related to protein quality. Based on the silhouette analysis in **Figure E1 of Appendix E**, the optimal number of clusters was k = 6, where the mean silhouette width was the highest. However, the PCA plot showed more than 6 distinct clusters. To have a clearer distinction of meals with varied protein quality, k = 8 clusters, which also had a high mean silhouette width, was chosen and visualised in **Figure 6.4.** Cluster differentiation with k = 6 found convergence of cluster A with B and a small portion of cluster E, while C and D were combined. Overall, with approximately 50 % of the cumulative variance (as shown in PC1 and PC2 of **Figure 6.4**), the PCA resolved eight visually distinct meal types.

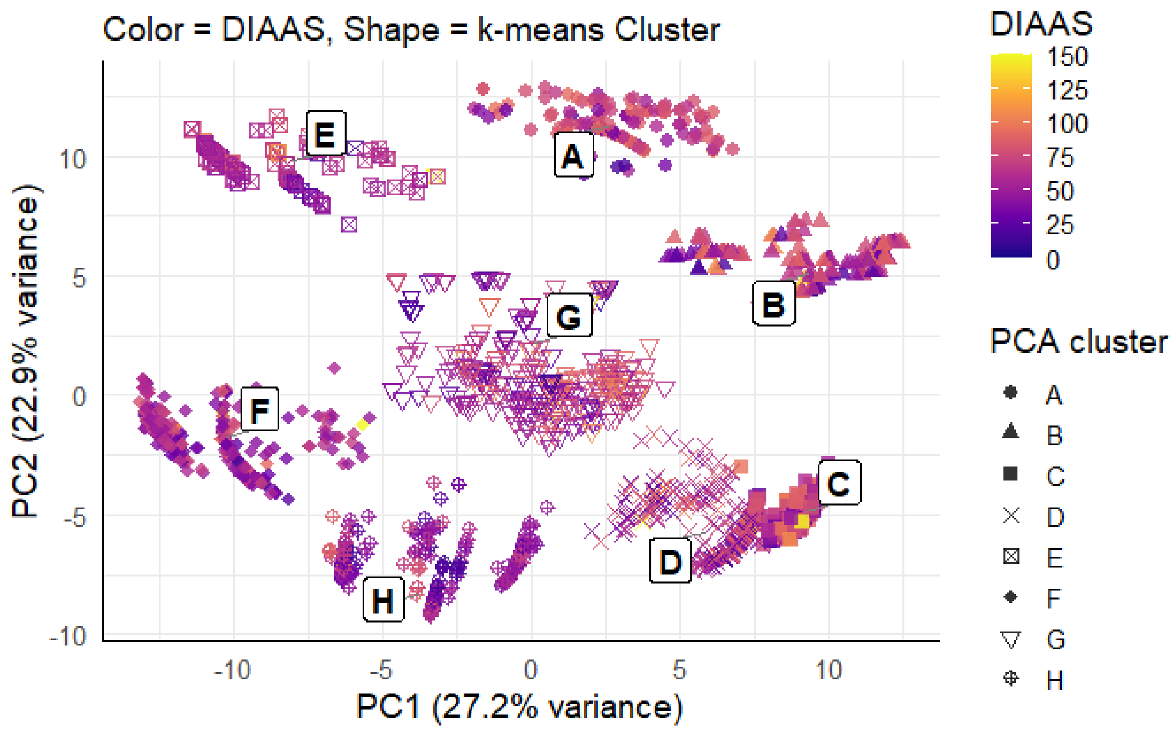


Figure 6.4 Cluster visualisation of meal protein quality in a 2-dimensional plot.
Shapes of plot points representing meals of different protein quality as distinguished by k-means clustering. The colour of plot points represents the meal DIAAS in the range of 10 - 150. Each cluster is labelled “A-H”. Cluster centroids were derived by calculating the centroid of each k-means cluster by taking the mean PC1 and PC2 values of all meals belonging to that cluster (**Table E3 of Appendix E**).

A sensitivity test was undertaken to evaluate the robustness of clustering based on two PCs. K-means clustering was repeated using the first five PCs which accounted for 88.8 % of total compositional variance. The resulting clusters showed substantial agreement with the original PC1-PC2 solution, with similar dominant FG patterns and mean DIAAS for most clusters. The principal difference was the subdivision of one heterogenous cluster into more compositionally homogenous meal patterns which revealed greater variation in secondary FGs, particularly for fruits and vegetables, while the broad protein-source meals remain relatively unchanged. These findings suggest that clustering based on PC1 and PC2 captured the principal dietary patterns within the dataset. However, incorporating additional PCs would provide a more detailed representation of the finer compositional differences among vegan meal patterns.

The loadings indicated the relative weight and direction of each FG along the axis (**Table 6.2**). PC1 thus distinguished meals that were legume- and vegetable-rich from those dominated by grain and nut-based foods. PC2 was driven by positive loadings for “nuts and seeds”, “fruit”, “legumes and pulses”, and “isolates”, and negative loadings for “grains and pasta”. Together, the two axes capture contrasts in meal structure within the dataset. The strongest differentiating factor in the data appears to be between meals dominated by “legumes and pulses” and those dominated by “grains and pasta”. **Figures E 2A to H in Appendix E** provide a two-dimensional binned density heatmap to visualise the contribution of each FG as meal proportions across the PCA score space.

Table 6.2 PC loadings of all FGs, indicating the relative weight and direction of each FG along the axis.

Food group	PC1 loading	PC2 loading
Fruits	-0.244	0.083
Grains and pasta	-0.199	-0.852
Isolates	-0.054	0.066
Legumes and pulses	0.76	0.143
Nuts and seeds	-0.544	0.48
Potatoes, kumara and taro	0.055	0.059
Vegetables	0.20	0.06
Others (sugar and sweets, yeast and condiments, beverages)	-0.007	-0.041

Table 6.3 shows the mean DIAAS calculated for each of the eight clusters, which exhibited distinct FG compositions, some of which were more aligned with higher protein quality. Clusters A to D, which had higher DIAAS, were positioned along the positive end of PC1, where “legumes and pulses” had the strongest loadings. Clusters E, F, and H, with the lower DIAAS, were positioned in the negative end of PC1, where “fruit”, “nuts and seeds”, and “grains and pasta” load more strongly. Meals on the positive end of PC2, namely clusters A, B and E, contain a larger share of “nuts and seeds”. In contrast, meals on the negative end of PC2 had strong loadings for “grains and pasta”.

Table 6.3 Mean DIAAS (%) and number of meals for each cluster

Cluster	Mean DIAAS (SD)	Number of meals
A	67.7 (21.7)	133
B	67.6 (32.1)	558
C	67.4 (21.9)	175
D	63.1 (24.2)	423
E	62.8 (18.8)	430
F	56.3 (18.8)	362
G	54.6 (24.0)	506
H	47.7 (19.1)	694

Total number of meals = 3281 as meals with DIAAS < 10 were not represented in the PCA plot. SD = standard deviation.

Considering PC1 and PC2 jointly, variation in meal protein quality appeared to be driven predominantly by “legumes and pulses” (strong positive on PC1), “nuts and seeds” (positive on PC2, negative on PC1), and “grains and pasta” (negative on both PC1 and PC2). The remaining FGs show comparatively small absolute loadings, so they contribute less to the discrimination among meals.

6.3.3 Association between meal types and daily clusters

Cross-tabulation of meal composition clusters A to H (derived from k-means on PCA scores) against daily dietary pattern clusters 1 to 3, as derived from DTW and HC (27) was conducted in Chapter 4. This provided a probability distribution of meal protein quality clusters conditional on daily cluster membership: how likely is it that a given meal composition is consumed by someone in a specific daily cluster? A similar probability analysis was repeated to test whether specific meal compositions were more common among individuals who exhibited no daily shortfalls in either protein or any IAA, presented in Chapter 5 (28). Results are presented in **Table 6.4**. The conditional probabilities provide a summary of the distribution of meal-pattern clusters across daily clusters and shortfall status. However, probabilities derived from smaller strata may be more susceptible to sampling variability than those based on larger numbers of meal observations. Hence, these probabilities should be interpreted as descriptive summaries of meal-pattern distributions rather than precise estimates of effect magnitude.

Table 6.4 Conditional probability table showing relative frequency of each meal composition type (PCA clusters A to H) within (i) three daily dietary clusters and (ii) with or without shortfalls in protein or IAA.

Daily cluster	Presence of at least one shortfall	P_A	P_B	P_C	P_D	P_E	P_F	P_G	P_H
	N	133	558	175	423	430	362	506	694
1	Yes (n = 250)	0.027	0.169	0.032	0.123	0.167	0.096	0.126	0.259
	No (n = 44)	0.031	0.177	0.046	0.116	0.174	0.085	0.158	0.212
2	Yes (n = 215)	0.043	0.134	0.068	0.134	0.082	0.136	0.170	0.233
	No (n = 137)	0.056	0.161	0.073	0.140	0.140	0.120	0.149	0.161
3	Yes (n = 37)	0.043	0.190	0.069	0.147	0.052	0.086	0.207	0.207
	No (n = 83)	0.053	0.252	0.050	0.119	0.109	0.106	0.194	0.117

"Yes" denotes the presence of a shortfall, and "No" indicates no shortfall with the number of *individual.days* with and without shortfalls represented by n in parentheses. Probabilities (columns labelled "P_") represent the likelihood that a meal type belonged to each daily cluster and whether there was a shortfall. Key probabilities for discussion are highlighted in red. The number of contributing meal observations is represented by N.

Daily clusters ranked by total protein intake and protein quality were 3 > 2 > 1, with daily diets in cluster 3 exhibiting 3 times higher protein and IAA intake per EO than cluster 1. As shown in **Table 6.3**, protein quality of meal types was in the order of DIAAS: A > B > C > D > E > F > G > H. Meal types A and B, characterised by the legume- and nut-dense foods, showed the highest probability in daily clusters 2 and 3 and among individuals with no shortfalls (probabilities in red in columns P_A and P_B). In contrast, meal type H, characterised by grain-rich, legume- and nut-poor foods, was most strongly associated with daily cluster 1 and with individuals who had at least one shortfall (P_H, values in red). Interestingly, high-quality meal types were not exclusive to daily cluster 3, nor were low-quality meals confined to daily cluster 1. The presence of meal pattern H was observed in clusters 2 and 3 as well, but more so for individuals with at least one shortfall (P_H = 0.233 for cluster 2 and 0.207 for cluster 3).

Similarly, meals of higher protein quality (A, B, C) were also found in daily cluster 1, but at a higher probability for individuals with no shortfalls.

The chi-square test indicated a significant association between daily clusters and PCA-derived meal types (χ^2 (14, N = 3281) = 111.63, P < 0.001), suggesting that certain meal types were more common in specific daily dietary protein intake patterns. Similarly, a significant association was also obtained between meal types and the presence of at least one shortfall (χ^2 (7, N = 3281) = 43.3, P < 0.001). **Table E2 of Appendix E** shows the contingency table of PCA-derived meal types A to H vs daily dietary clusters and presence of at least one shortfall.

The adjusted Pearson residuals were scaled to be comparable across cells. Pearson residuals with values > 2 indicate significant over-representation (observed > expected) and values < - 2 showed significant under-representation (observed < expected) (38). As expected, meal types with lower DIAAS, such as E and H, were over-represented in daily cluster 1 (E: 3.81; H: 3.10), indicating that low protein quality meals occurred more frequently in this cluster than would be expected under independence (**Table E2 of Appendix E**). In contrast, these meals were underrepresented in cluster 3 (Pearson residual of - 3.55 for meal H). Meals of higher protein quality, such as A, B and C, were underrepresented in cluster 1 (A: - 2.38; C: - 2.93) but overrepresented in cluster 3 (B: 3.62). These similar patterns were also observed when comparing the distribution of meal types between individuals with and without daily shortfalls. To highlight, meal type H was overrepresented in individuals with at least one shortfall (Pearson residual of 3.35 in H) but underrepresented in individuals with no shortfalls (- 4.15).

6.4 Discussion

6.4.1 Meal compositions in meals of varying protein quality

Comparisons between vegan meals with higher (DIAAS ≥ 75 %) and lower protein quality (DIAAS < 75 %) found differences in FG compositions. While “legumes and pulses”, “nuts and seeds”, “grains and pasta”, and “isolates” were singled out as the primary FGs in vegan meals that contributed most to protein intake, their relative nutrient contribution differed between higher and lower protein quality meals (**Figures 6.1 and 6.2**). “Legumes and pulses” were present in higher proportions than “grains and pasta”, “potatoes, kumara and taro”, “fruits” and “sugar and sweets” in higher protein quality meals and were thus key PB food sources to improve IAA content of meals, but more so for lysine, leucine, tryptophan and threonine than for SAAs. “Grains and pasta” and “nuts and seeds” provided more SAAs and could be suggested as suitable food sources to complement “legumes and pulses” and to elevate IAAs in meals closer to reference values.

While the PCA did not use meal DIAAS in its construction, the mean DIAAS by cluster indicated a correspondence between legume and nut-dominant patterns and higher protein quality, while grain-dominated patterns showed lower protein quality (**Figure 6.4**). This was further supported by calculated weight proportions of the key protein-providing FG in each meal cluster. The largest proportions for “legumes and pulses” and “nuts and seeds” were observed in clusters A and B, with no contribution from grains. The proportion of “legumes and pulses” and “nuts and seeds” was 1.0 in cluster B and E, respectively, while the proportion of “grains and pasta” was 1.0 for cluster H. These results indicate the potential importance of considering appropriate FG ratios that combine to provide vegan meals of high protein quality.

Particular attention is often paid to the complementation of “legume and pulses” and “grains and pasta” as they are common staples in PB meals. As shown in **Table E1** of **Appendix E,** higher-protein-quality meals appear to have approximately a 2:1 ratio of legumes to grains, which is associated with improved IAA profiles. Similar to findings from the vProtein project, where different plant foods were combined in a simulation exercise, the optimal proportion for high protein quality was found in an exemplar of 60 % soy to 25 % rice (29). These patterns are also consistent with previous studies examining two- and three-PB food combinations, where legumes often appear in greater quantities than grains to achieve higher utilisable protein and DIAAS (20, 30).

This current study found that, in vegan meals of higher protein quality, “nuts and seeds” also appeared to be at higher quantities than “grains and pasta”, but not higher than “legumes and pulses” (**Table E1 of Appendix E**). However, the PCA results showed that meal cluster E, which was dominated by “nuts and seeds”, had a lower DIAAS compared to other meal clusters. This was because “nuts and seeds” consumed in this cluster were snacks and mostly consumed in smaller quantities in isolation rather than part of a mixed meal with other complementary foods that together provided higher DIAAS. Other snack foods, like fruits and beverages made of isolates, were also prevalent in this cluster. This pattern may thus reduce the overall protein quality of the EOs in this cluster.

Protein quality can be adequate even in minimally diverse diets (31, 32), but as diets become increasingly PB, or in vegans, the proportions of “legumes and pulses”, “grains and pasta” and “nuts and seeds” need to be critically examined (33). Even within the “legume and pulses” group, food items vary in their contribution to protein quality. For example, when a study on vegan menus replaced chickpeas with lentils, a higher protein quality was attained with a lower replacement quantity (34). PBMAs consumed by the NZ vegan cohort study frequently used pea or soy protein isolate as an ingredient and had high DIAAS. Processing mechanisms in the manufacture of PBMAs often reduce the quantities of ANFs and transform these foods to highly digestible sources of protein, with AA profiles close to the reference values, albeit more limited in SAA (35-37). Yet, traditional "legumes and pulses", such as soy products, lentils, black beans and chickpeas, were also part of high DIAAS meals that contained no PBMAs. This demonstrated the value of traditional plant protein sources in improving protein quality. Verification with specific digestibility coefficients for different types of legumes and their proportions in mixed meals is a further area of research to uncover the impact on protein quality and micronutrient provision.

6.4.2 Protein quality at the meal and daily level

The cross-tabulation of meal composition clusters against daily dietary pattern clusters highlighted clear links between meal composition and daily dietary patterns. Meals of higher protein quality, characterised by higher "legume and pulses" to "grain and pasta" ratios (A, B and C), were significantly overrepresented in cluster 3 - the cluster with daily diets that had the highest mean protein and IAA intake per EO. Contrastingly, meals of lower protein quality, such as meal H, were significantly overrepresented in cluster 1 – the cluster with daily diets that had the lowest mean protein intake and protein quality per EO. Cluster E, which was dominated by "nuts and seeds", was also overrepresented in daily cluster 1. This could coincide with a 'grazing' or snack-like pattern of < 10 g per EO that was observed in Chapter 4.

While there was an association between meal protein quality, daily cluster patterns, and the presence of shortfalls, meals with higher protein quality were not confined to specific daily patterns, and the converse was true for meals with lower protein quality. These findings underscore that daily dietary patterns do not always translate to consistent meal-level practices, necessitating the evaluation of protein quality at the meal level. The main reason driving research interest in meal-level protein distribution and protein quality lies in the postprandial rise in circulating IAAs, and more acutely for leucine following dietary protein ingestion, which directly impacts MPS (38). Assessing only the daily total will obscure the evenness of protein and AA intakes and absorption across meals. This may have greater implications for metabolic function within certain demographics (e.g., the elderly) and those on long-term diets high in PB foods, which generally exert lower anabolic responses in skeletal muscle mass (39, 40).

The extent to which IAAs must be complemented at the meal or day level is still debated. Young and Pellet argued that complementation at each EO is unnecessary and that IAA profiles can be complemented across the day to maintain net NB, even for PB food sources

(41). However, the time window of this complementation remains unknown, with some evidence that plasma IAA availability remains elevated for a shorter period in PB proteins than ASPs (42). This may be more relevant in vegan diets, where many foods have lower protein quality when consumed alone but achieve substantially higher protein quality when combined in the context of meals. Pending definitive evidence on the appropriate temporal window and the impact of PB mixtures on anabolic responses, recommending complementarity at the meal level is likely beneficial to improve daily adequacy.

However, one controlled trial comparing isonitrogenous meals of complete, complementary or incomplete protein profiles in adult women found no significant differences in postprandial and 24-hour MPS rates across the meal types (42). Nonetheless, the study found that greater response was elicited from complete (animal-based) and complementary (plant) sources than from the incomplete plant protein meal. The incomplete meal also resulted in higher glucose and insulin levels, showing that differences in protein quality at the meal level influence metabolic outcomes beyond MPS. Collectively, this suggests that fully PB meals can elicit metabolic responses comparable to those of animal-based meals, with the caveat that AA complementarity must be ensured so that utilisable protein intake aligns closely with human requirements.

Relying solely on daily protein and IAA intake as an estimation for protein adequacy (as explored in Chapters 3 and 5) overlooks meal-level composition. The approach in this Chapter to contextualise meal-level protein quality within overall dietary intake does not assume dependence between individual meals, as people realistically consume different types of foods throughout the day. Given that more than 50 % of daily diets in the cohort had a shortfall in either protein or one of the IAAs, an important aim of this Chapter was to understand which meal compositions tended to occur within broader daily dietary contexts. In the context of restrictive diets such as the vegan diet, this analysis provided insight into whether individuals who consistently met protein or IAA requirements tended to consume higher-protein-quality meals throughout the day.

Overall, meals composed mostly of “legumes and pulses” were more associated with daily diets with no shortfalls, reiterating a finding highlighted throughout this thesis that the inclusion of this FG contributes substantially to protein adequacy. However, the majority of plant protein sources in the human diet appears to come from grain-based foods, especially in dietary shifts towards predominantly PB diets (43, 44). PB diets characterised by poor diversity of PB foods therefore incur the risks of deficiencies in bioavailable IAAs, as well as other micronutrients. Complementarity among various PB protein sources, such as those from grain and legumes, and consumed within narrow time windows, such as at the meal level, is a means of overcoming such challenges (2).

The long-term physiological consequences of repeatedly consuming vegan meals with incomplete IAA profiles are unknown. If protein quality is not consistently ensured, there is a risk that the EAR/RDI – defined on the basis of high-quality reference proteins - may not be achieved in practice (6). Chapter 3 uncovered that overall protein - even after adjustments for bioavailability – did not indicate a high risk of deficiency for the majority (> 75 %) of participants when compared to the EAR (13). However, lysine and leucine emerged as critical nutritional concerns, with approximately half of the cohort failing to meet the daily requirements (Chapter 3). These findings emphasise the importance of analysing meal composition for protein quality, a central aim of this Chapter. By analysing the composition and proportions of FGs in vegan meals, this work sought to identify patterns that provide higher protein quality and thus inform strategies to optimise vegan meal planning to better meet individual nutrient requirements.

6.4.3 Strengths and limitations

All diets can incur nutrient shortfalls if FGs are poorly balanced. For restrictive dietary patterns, such as vegan diets, where nutrients are obtained solely from fewer FGs, evidence-based research is needed to provide practical solutions to address nutritional limitations. A key contribution of this current Chapter was the use of meal-level analysis, which uncovered critical FGs such as “legumes and pulses” and “isolates” to achieve high protein quality in vegan meals.

The analysis was limited by the foods that vegan participants reported consuming. While this provided a realistic snapshot of what NZ vegans typically eat, it does not capture the full range of PB products, including more novel foods such as algae and mycoprotein, which may have nutritional potential as meat substitutes and contribute to higher protein quality (45). The PB combinations reported by the cohort and the food pairings within each EO may not generalise to other vegan populations. However, the food diaries obtained are representative of a Western-style vegan diet, due to its similarity in patterns with other vegan populations in the West, where legumes, textured soy and other meat analogues, grains, nuts and seeds dominate protein intake (46-48).

Time interval analysis is an effective means of assessing IAA complementation within EOs (43). As per Chapter 4, a meal was strictly defined as only foods co-reported at the same timestamp, rather than using a proximity window. Given the lack of consensus on optimal inter-meal intervals for metabolic outcomes, using a time-stamp definition had greater certainty.

However, this approach may miss foods eaten shortly before or after an EO that could have been part of the same meal and influenced its protein quality.

Another limitation of this study was the reliance on incomplete or imprecise IAA composition data and on broadly grouped TID values for many PB foods. Owing to current data paucity, individual food items were assigned the digestibility coefficients of the broader FG, which can overlook variation within FGs and lack granularity in calculating DIAAS. Composition data for certain IAAs (such as tryptophan) were absent for some foods, making it impossible to calculate an accurate DIAAS value.

As observed in previous Chapters, the lack of TID adjustments for isoleucine, valine and the aromatic AAs meant that DIAAS calculations did not incorporate the protein quality measurements of these IAAs. However, these IAAs are not commonly limiting in PB foods. For example, valine is most limiting in pork and beef, while lysine is identified as most limiting for several PB foods (2, 37). The mean intakes as assessed without digestibility adjustments were also higher than their requirement levels, as observed in **Table 3.3**. Consequently, in the context of this study, the exclusion of TID values for these IAAs is unlikely to substantially affect protein quality assessments, as protein quality is primarily constrained by lysine and SAAs. Nevertheless, an explorative analysis was conducted and discussed in Chapter 8.

Insufficient or inaccessible TID and AA data continue to hamper research in protein quality (49). More detailed composition and digestibility data for PB foods are becoming available (50), so replacing current estimates with these values will enable more precise conclusions in future work. As each IAA was expressed per gram of protein, foods with inherently low protein density, such as vegetables, will appear artificially inflated in IAA contribution (16). However, these foods contributed minimally to protein quality in the vegan meals in this study and were often present as part of a larger meal or recipe.

This study is limited by a lack of standardisation on total protein intake. Interpretation of meal protein quality based on the habitual eating patterns of vegans may be confounded by energy consumption or energy under-reporting. This was identified in Chapter 3, where under-reporters had significantly lower protein intakes than plausible reporters (0.56 versus 0.87 g/kg/day, $P < 0.05$). These findings will therefore benefit from verification from well-controlled clinical trials (e.g., isonitrogenous diets), although conclusions may still be based on short-term diets (51).

One strength of the study was the use of unsupervised data-driven techniques such as PCA and k-means clustering, applied to real-world meal records and linked to a robust protein quality metric (DIAAS). This approach identified the FGs most strongly associated with protein quality in vegan meals and outlined possible ranges of food proportions that underpinned meal patterns that had higher protein quality. This lays the foundation for the strategic complementation of various plant foods to improve the protein quality of vegan meals – the next phase of this research.

The quantity needed to meet individual requirements should be considered in addition to the DIAAS, which only accounts for protein quality in 1 g of a protein source. This provides limited insight as, without sufficient intake, even proteins that are completely utilisable will fall short of meeting IAA requirements (52). This will be accounted for in Chapter 7. Lastly, protein quality is only one area of concern in vegan diets. Several micronutrients are often lacking in these diets, especially if fortified foods and supplementation are absent (3, 53). While this is not the area of focus in this thesis, meal-level analysis of vegan diets should take into consideration the daily requirements of certain vitamins and minerals, as well as potential meal-level requirements if applicable.

6.5 Conclusions

The analyses here indicate that vegan meals with lower protein quality are characterised by higher contributions from fruit, grain foods, potatoes and sugary condiments. Meals with greater proportions of legumes and pulses – often exceeding grains – and to a lesser extent nuts and seeds, are associated with higher DIAAS. However, conclusions from these composition analyses should be interpreted with caution. Protein quality was evaluated using a single metric (DIAAS), without further analysis on the utilsation of different IAAs in the body which needs to consider other measurements such as oxidation rates (30). The inferences are also specific to the foods and proportions of these foods in this NZ vegan cohort, rather than a universal reflection of the diets of other populations. Further work should test a wider range of food combinations and ratios and relate meal compositions not just to IAA reference values, but also evaluate the implications for metabolic outcomes.

Comparing meal-level and daily patterns suggests that assessing protein adequacy only at the daily scale can overestimate true adequacy because within-day protein distribution and complementation are unknown. Individuals may achieve similar total daily protein intake yet consume meals of varied numbers and quality. This may have potential physiological implications. Evaluation of protein adequacy should thus account for both daily totals and meal quality. A practical implication is the need to provide approximate quantities of these foods in mixed meals to construct realistic protein-adequate vegan meals. The thesis research concludes in Chapter 7 with a solution that presents meal complementation and diet optimisation in an interactive digital tool that tailors mixed meals to individual requirements and preferences.

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Chapter 7 Designing smarter vegan meals with high protein quality: introducing the Vegan Protein Quality (VPQ) tool

This final research Chapter applies a comprehensive pairwise iteration approach to all possible meal combinations in the vegan dietary dataset to identify meal combinations that achieve high protein quality. These combinations are then incorporated into a novel, interactive digital tool that dynamically adjusts food quantities based on individual preferences and protein requirements.

The following Chapter is currently under revision in Scientific Reports:

Soh, B. X. P., Vignes, M., Smith, N. W., von Hurst, P. R., & McNabb, W. C. (2026). Development of the Vegan Protein Quality (VPQ) tool to derive smarter vegan meals with high protein quality.

Abstract

Targeted complementation of diverse plant foods in optimal proportions can overcome different limiting IAAs in vegan meals. This should be coupled with methods to determine the optimal food quantities needed to meet individual targets for total protein and protein quality. Meals with a DIAAS < 100 % (n = 3623) were targeted for improvements in protein quality using a systematic pairwise matching procedure. Conditional weight shares contributed by each FG per meal were computed to determine the most important FGs in improving the protein quality of baseline meals. Meal pairs attaining DIAAS of ≥ 100 % were incorporated into the Vegan Protein Quality (VPQ) tool, an interactive web application that optimises food quantities to match user-set energy, total protein and IAA requirements. The pairwise matching approach improved the AA profile of > 99 % of baseline meals to a DIAAS of at least 75 %, with 41 % achieving a DIAAS of ≥ 100 %. Across these successful meal pairs, "legumes and pulses" accounted for 33.8 % of the total weighted participation, while "grains and pasta" and "nuts and seeds" accounted for approximately 25 %, and "isolates" accounted for a smaller proportion (14.9 %). Composite meals achieving high protein quality were characterised by specific FG pairings. For example, when "grains and pasta" was the focal FG, "legumes and pulses" was the most dominant complementary partner to (55 %), followed by "isolates" (23 %). In legume-containing meals, the most frequent complementary partners were "nuts and seeds" (42.8 %) and "grains and pasta" (40.4 %). Test scenarios in the VPQ tool demonstrated the application's ability to optimise meals that fulfil the requirements for energy, total protein, and all IAAs, within serving-size constraints, even for individuals with higher energy and protein requirements (> 0.8 g/kg of body weight per day). Total protein and energy were the most limiting constraints in the test scenarios, rather than IAA. Expanding the tool to include a wider range of PB foods and optimising it for micronutrient adequacy will provide better guidance for designing vegan meals to meet individual nutrient requirements.

7.1 Introduction

As described in the literature review (Chapter 2), dietary protein is needed chiefly for its supply of IAAs that the body cannot synthesise. IAAs are precursors in various metabolic pathways in the human body, including protein synthesis, but the excess cannot be stored within body tissues. Instead, regular intake of dietary protein throughout the day is essential to maintain protein balance, where continuous protein breakdown is offset by synthesis (1).

A well-recognised challenge in vegan diets is that plant protein sources often have at least one IAA that is supplied in a lower utilisable proportion than required. When digestible protein and IAA intake of the NZ vegan cohort was examined in Chapter 3, approximately 50 % of individuals had deficiencies in lysine and leucine, even when total protein intake met the EAR for protein in three-quarters of the cohort (2). This is concerning as consequences of protein and IAA deficiency can manifest in, but are not limited to: increased muscle breakdown, decreased MPS, impaired homeostatic functions, and disrupted metabolic functions (e.g. post-translational modifications) (3, 4). These effects may be more pronounced in older adults, who experience greater anabolic resistance to AA intake, potentially requiring higher protein intake to stimulate the same metabolic responses as younger individuals (5).

For vegans, consuming larger portions of PB foods is one way to mitigate challenges with protein and IAA deficiency, but can inadvertently be accompanied by excess energy, with negative health connotations (6). Protein quality varies among PB foods. In general, legumes are comparatively rich in lysine, often the most limiting IAA in vegan diets, relative to grain foods and potatoes (7). Chapters 5 and 6 showed that methionine is readily supplied by grains, nuts, and seeds. These foods can thus compensate for the lower methionine content of legume-based foods. Meal-level complementation of PB foods is therefore a practical approach for vegans to improve the synchronised supply of all IAAs (8, 9). When a meal is energy-adequate, as is the case for most individuals in high-income countries, all IAAs can be provided to fill gaps that single PB foods cannot fulfil alone (10). Previous studies have shown improvements in IAA intake compared to reference patterns when there are optimal plant protein combinations (9, 11-13), albeit with limited diversity in food combinations.

The DIAAS is a protein quality metric that distinguishes foods based on their ability to supply digestible IAAs that match the human AA requirements in 1 g of the protein source (14). The DIAAS for a meal containing complementary protein sources can be calculated from the underlying IAA values for the constituent foods (15). However, in a diet or meal-planning context, applying the DIAAS without accounting for food quantity offers little insight, as it overlooks whether the amount consumed meets individual nutrient requirements (16). Consuming foods with high protein quality in small quantities can still result in inadequate IAA intake.

By leveraging real-world consumption data collected from 193 NZ vegans, this Chapter aims to formulate meal pairings of varied PB foods that, when combined, provide high protein quality, as indicated by a DIAAS of at least 100 %. These meal combinations were integrated into an interactive digital tool, the Vegan Protein Quality (VPQ) tool. This is a novel web application that selects potential meal pairings and calculates the optimal quantity (g) of each food within the meal pair to meet user-specified protein, energy, and IAA requirements.

7.2 Methods

7.2.1 Meal complementation with pairwise iteration

Following the data processing steps in the previous Chapters, baseline meals with DIAAS < 100 % were selected for improvement using a pairwise matching approach. Each meal was systematically paired with every other meal in the dataset, resulting in all possible combinations. The objective was to investigate how different food items in the two meal combinations could complement each other's limiting IAAs and raise the overall DIAAS to ≥ 100 %. For clarity, the resulting combinations from the pairwise iteration are termed "meal pairs".

DIAAS was recomputed for each meal pair by summing digestible IAAs and protein across both meals and then comparing each AA to its reference pattern. The DIAAS calculation follows the same steps as exemplified in **Table 6.1**. Pairs were retained as successful combinations if DIAAS ≥ 100 %. FGs of each composite meal in these meal pairs were examined to determine which specific FG-pairings drive high protein quality. A weighted pairing approach was applied. For each meal within the meal pair, the contribution of each FG to total protein and IAA content was estimated. IAA content was calculated as the sum of each IAA divided by its corresponding AA requirement value. This step provided a relative indicator of IAA contribution so that IAAs with a lower relative contribution to the requirement exerted less influence on the score. However, one limitation with this calculation is that the resulting index is an additive one, so the excess of one IAA can increase the total score without compensating for the deficiencies in another IAA. Within each meal, the proportional contribution of each FG to total protein and IAA was calculated. These two components were combined using the geometric mean to generate a composite score that reflected the joint contribution of each FG to both total protein intake and protein quality. Scores were normalised within each meal so that FG contributions summed to one. (See **Appendix F** for equations).

All cross-pairings of FGs within each meal pair were generated and assigned a weight equal to the product of the normalised contribution scores from the two meals. Pairings were represented directionally so that each FG is treated as a focal group (A) with a paired partner group (B). Partner distributions are interpreted conditionally for each focal FG. The largest aggregated FG-FG weights indicate the combinations that most often underpin these high-DIAAS meals.

Conceptually, when these meal pairs are consumed together in one EO, they can complement one another's IAA shortfalls to achieve sufficient protein quality (DIAAS ≥ 100) on a per-gram basis. However, this screening step does not consider portion sizes or quantities of each food item, energy content, or whether all food items in the meal pair were necessary. Taken at face value, this could result in impractically large amounts or the inclusion of every food item. To address these limitations, a digital tool was designed that incorporates meal-level optimisation to determine feasible quantities of each food item in each meal pair to meet per-meal targets for energy, protein, and each IAA, subject to serving-size constraints and user preferences.

7.2.2 Digital tool: The Vegan Protein Quality (VPQ) tool

Software and data sources

The interactive meal-planning tool was implemented in R version 4.3.1 using Shiny (17) and the interactive app is available at: vegan-diet-tracking.shinyapps.io/Vegans/. A supplementary file containing a video demonstration is submitted alongside this thesis.

Two datasets underpin the tool:

1. Meal-pair dataset: Foods placed together in meal pairs from the pairwise analysis, each identified by a meal-pair ID. For every unique food item, the nutrient composition is expressed per gram of food: energy (kJ/g), protein (g/g), and each IAA (mg/g).
2. Recipe dataset: Ingredients and quantities for homemade or store-bought recipes as reported by participants in the vegan cohort. Nutrients are recorded per ingredient weight (g) for energy (kJ), protein (g) and each IAA (mg).

User input and requirements

Users of the tool input age (years), biological sex, BW (kg) and height (cm), which are used in the calculations of the BMR using the Mifflin-St Jeor equation (18), and the total protein requirements. EER is then obtained by multiplying the BMR by the user-specified PAL, selected on a 1.2 to 2.4 scale (19). The user's daily protein requirement is calculated by multiplying BW by the user-set protein requirement, expressed as g/kg/day. The default is 0.66 g/kg/day, as guided by FAO/ WHO guidelines for adults ≥ 18 years (20), but the user may change this target depending on specific needs. For example, an athlete or older adult may require a protein intake of 1.0 g/kg/day or more. Daily IAA requirements are determined by multiplying BW by the IAA requirement patterns (mg/kg) for adults ≥ 18 years.

There is currently no consensus on the per-meal protein and IAA requirements, although some studies have proposed protein targets per EO of 0.2 to 0.3 g/kg/day, as discussed in Chapter 4 (16, 21). Instead of prescribing fixed meal-level targets in this study, the tool partitions daily requirements across the selected number of meals and evaluates each meal's content against these proportional targets, with the objective that the sum of meals meets or exceeds the daily requirement. Selecting more meals, therefore, lowers the protein and IAA targets, while fewer meals raise the per-meal target. However, as the per-meal energy limit also decreases, the range of meals to simultaneously meet all targets also reduces. In snack-based patterns with high meal frequency, for example, protein and IAA targets per meal are low, but as energy allowance per EO also shrinks, all nutrient targets may be harder to satisfy, especially if foods are energy-dense.

Three modes of use: fully optimised, semi-manual, manual

The digital tool offers three modes to give users progressive control over food quantities in each meal pair. In all three modes, serving sizes for each FG were enforced as constraints within each meal pair, as guided by the NZ Eating and Activity guidelines (22) (i.e., a maximum of 30 g of "nuts and seeds", 75 g of "potatoes, kumara, taro" and "vegetables", 120 g of "grains and pasta", 150 g of "legumes and pulses" and "fruits"; see Chapter 5). "Isolates" used in beverages, such as pea protein powders, were capped at 30 g, following standard serving-size guidelines on the back-of-pack labels for these foods. These serving constraints are applied to each candidate food based on its mapped FG.

In the fully optimised and semi-manual modes, the solver calculates the quantities of foods needed in grams for each meal pair to meet or exceed per-meal targets for energy, protein, and IAA, while respecting serving-size constraints via LP. The objective function minimised the total mass of foods selected while applying a small additive penalty proportional to the number of foods included. This is formulated as $\min \sum_j g_j + \lambda \sum_j y_j$, where g_j represents the grams of food j and y_j represents whether the food j is used. λ represents a penalty weight of 0.05 for each additional food used with the aim of reducing excess number of food types selected to achieve individual requirements. The user-selected food is guaranteed to appear by setting a minimum constraint of 5 g.

The semi-manual mode allows the user to adjust the included quantity of one or more foods as preferred. These changes are then locked; the model does not readjust these foods but re-optimises the quantities of the remaining foods to meet per-meal requirements. The food quantities set in the user adjustment become additional constraints on the model solution. If the re-optimisation is feasible, a new solution with updated food quantities is shown. If infeasible, the tool retains the user-selected quantities, preserves the previous amounts for foods that were solved in the fully optimised mode, but highlights the largest remaining nutrient shortfalls to guide further edits. The manual mode in the tool allows the user to have full control over the desired quantities of all foods, and the graphs reflect the percentage of requirement met for each nutrient, based on the selected food quantities. The user can reset both the semi-manual and manual modes to restore the solutions obtained from the fully optimised mode.

The focus of this Chapter is on illustrating the functionality of the optimiser rather than conducting large-scale user evaluation. Nevertheless, to systematically evaluate the behaviour of the optimisation framework, the fully optimised model embedded in the VPQ tool was applied to all available meal pairs under predefined benchmark scenarios. Four representative individual profiles with different energy, protein and IAA requirements were specified to generate per-meal targets (**Table F2 of Appendix F**). The model was then executed sequentially for every candidate meal pair in the VPQ database. Outputs from each optimisation run were aggregated to evaluate solution feasibility, total food mass required for each FG and nutrient adequacy ratios.

7.3 Results

7.3.1 Meal complementation

Using the pairwise matching procedure, each base meal was paired with a complementary partner, and the meal-pair combination was stored for further analysis whenever the two-meal combination achieved an IAA ratio of at least 100 %. The aggregated FG-FG weights of all high-DIAAS meals are visualised in **Figure 7.1**.

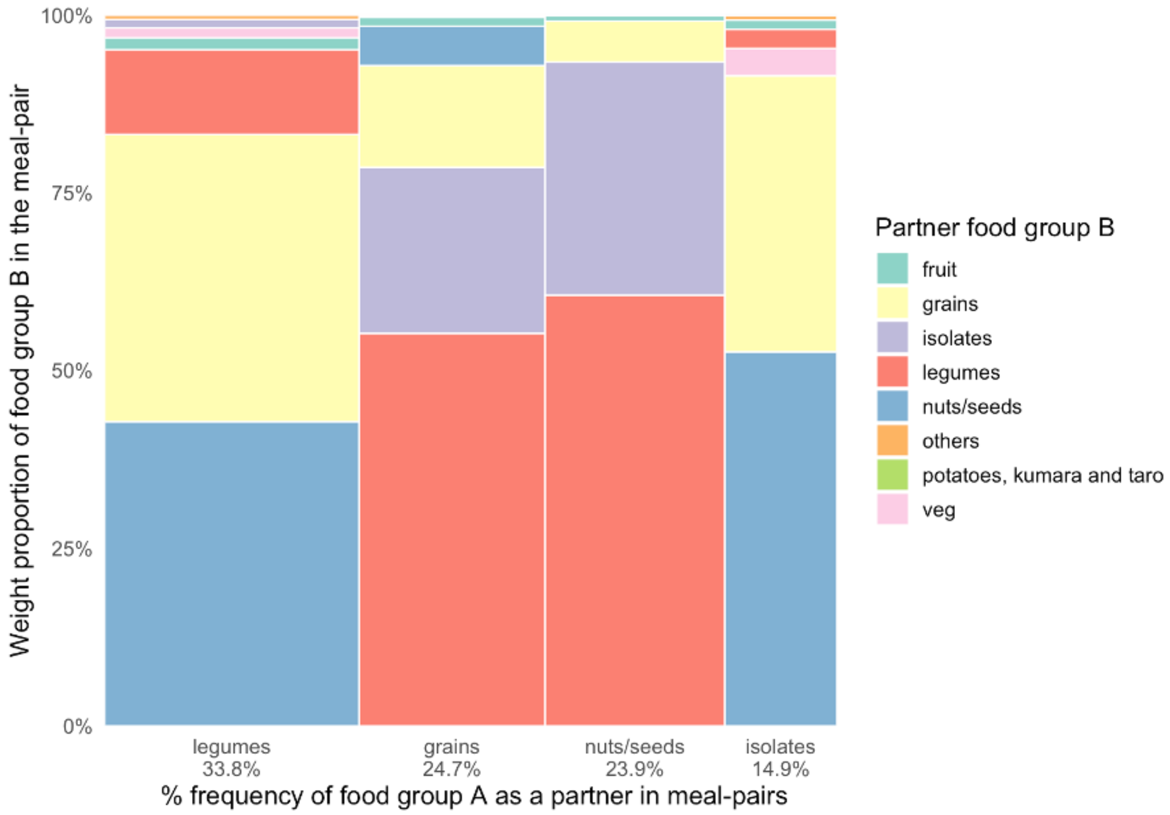


Figure 7.1 FG complementation (represented bi-directionally) after pairwise iteration
The x-axis shows the top four **FG-A** by overall prevalence in successful meal pairings (combined DIAAS ≥ 100 %) with each partner **FG-B**. The column width of the x-axis shows how often (%) FG-A appears across successful meal pairs (Only FGs contributing at least 2 % of total weighted participation are visualised). The y-axis shows the percentage contribution of the partner **FG-B** to the total aggregated FG-FG weight for a given **FG-A**. Each FG is represented by a different colour. "Others" comprise *beverages*, *sugar and sweets,* and *yeast and other condiments* that do not contribute substantially to meal complementations. "Veg" represents "vegetables"

Only baseline meals with DIAAS < 100 % were considered for meal-pairing. **Figure 7.1** showed that "legumes and pulses" accounted for the largest weighted share of FG participation across meal pairs, at 33.8 %, indicating their importance as an FG in meal pairs of high protein quality. When "legumes and pulses" were the focal FG, the dominant partners were "nuts and seeds" and "grains and pasta", at 43 % and 40 %, respectively. When "grains and pasta" were the focal FG, the dominant partners were also "legumes and pulses" at 55 % and were also well complemented by "isolates" at 23 %. The percentage distribution in **Figure 7.1** highlights the complementary roles of protein-dense FGs in vegan diets. Individually, these foods may be constrained by one or more limiting IAA, but combining them within meal pairs complement their IAA profiles and improve the overall DIAAS.

Meals with a base of DIAAS very close to 100 % were most frequently able to achieve DIAAS > 100 % when combined with other meals and were typically "legumes and pulses" and “isolates” (**Table F1 of Appendix F**). Owing to their contribution to a high DIAAS, meals containing PBMAs also stood out as commonly chosen complements. However, successful PB mixtures without the reliance on PBMAs or isolates were obtained in 66.2 % of meals that featured traditional PB foods, often legumes (beans, peas and soy products) and a broad variety of wheat-based foods. After complementation, lysine, SAA and leucine remained the top three limiting IAAs despite their ratios improving to values above 1. Systematic pairwise complementation thus enabled consideration of all possible pairings and demonstrated the potential to achieve sufficient protein quality with a wide range of foods.

Of 3623 baseline meals with DIAAS < 100 %, 41% had at least one pairing that achieved a DIAAS of at least 100 %. A further 54.1 % of baseline meals had at least one pairing that achieved DIAAS ≥ 90 %. A further 4.5 % could be complemented to achieve a DIAAS of at least 75%. Only 0.4 % of meals did not achieve 75 % even with complementation. This was attributed to baseline DIAAS values of 0 due to the absence of tryptophan values. Meals became more complex after complementation, with the mean number of distinct FGs in meals increasing from 2.06 before complementation to 3.20 after complementation. At the food-item level, complemented meals contained a median of 6 items (IQR: 4-8, range 1-24), as compared to 3 items (IQR: 1-4, range 1-16) in baseline meals. This indicated that many different plant foods were brought together to produce meals of high protein quality.

7.3.2 Scenarios from the VPQ tool

This section demonstrates the functionality of the tool. For each candidate meal pair, the Explorer interface displays bar charts showing the percentage of the per-meal requirement achieved for energy, protein, and each IAA. A dashed line at 100 % denotes the per-meal target (computed by taking the daily requirement divided by the user-selected number of meals). Bar charts above the line indicate the target is exceeded, while values below indicate a shortfall.

Similarly, the Recipe interface displays ingredient-level stacked bar charts showing each ingredient’s percentage contribution to daily requirements for energy, protein, and each IAA, again with a 100 % dashed line as a reference. These percentages are computed as if the entire recipe were consumed. In practice, the Explorer optimiser selects a portion of the recipe based on its per-gram composition and serving-size constraints. The nutrient contribution from the recipe will thus be in proportion to the optimised quantity.

Users can adjust ingredient quantities to reflect preferences or goals, such as increasing ingredients that supply more protein or IAAs, or reducing ingredients that supply more energy. When this happens, the tool rescales the recipe’s per-gram nutrient composition and automatically updates all meal pairs that include the recipe, so that optimisations and visualisations reflect the new compositions. Hence, recipes that deliver more limiting nutrients per gram may be favoured by the model, but it is also possible to see reduced quantities of the selected food, as protein and IAA targets can be met with lowered amounts of the recipe.

User outputs from the fully optimised and semi-manual mode, as well as user interactions with the recipes, are demonstrated in this section. A daily diet can be constructed from valid meal pairings, highlighting that success in meeting daily nutrient targets is sensitive to user decisions, including meal frequency (e.g., 2 versus 3 meals/day) and specific combinations of foods selected. Worked examples are presented, showing how meal components can be scaled to protein and IAA requirements, even beyond the RDI of 0.8 g/kg/day and within serving limits of each FG, thereby supporting its application to populations with higher protein needs. The demonstration of user-interaction of the tool is illustrated under two settings:

1) Older adult female: 65 years, 75 kg, 165 cm, PAL of 1.5, with protein requirement of 1.0 g/kg/day; 3-meal scenario per day
2) Young adult male: 30 years, 95 kg, 180 cm, PAL of 2.0, with protein requirement of 1.2 g/kg/day; 3-meal scenario per day

The VPQ tool is currently presented as a prototype designed to demonstrate the feasibility of integrating optimisation modelling with an interactive dietary planning interface. Part of the tool’s user interface is shown in **Figure 7.2.**

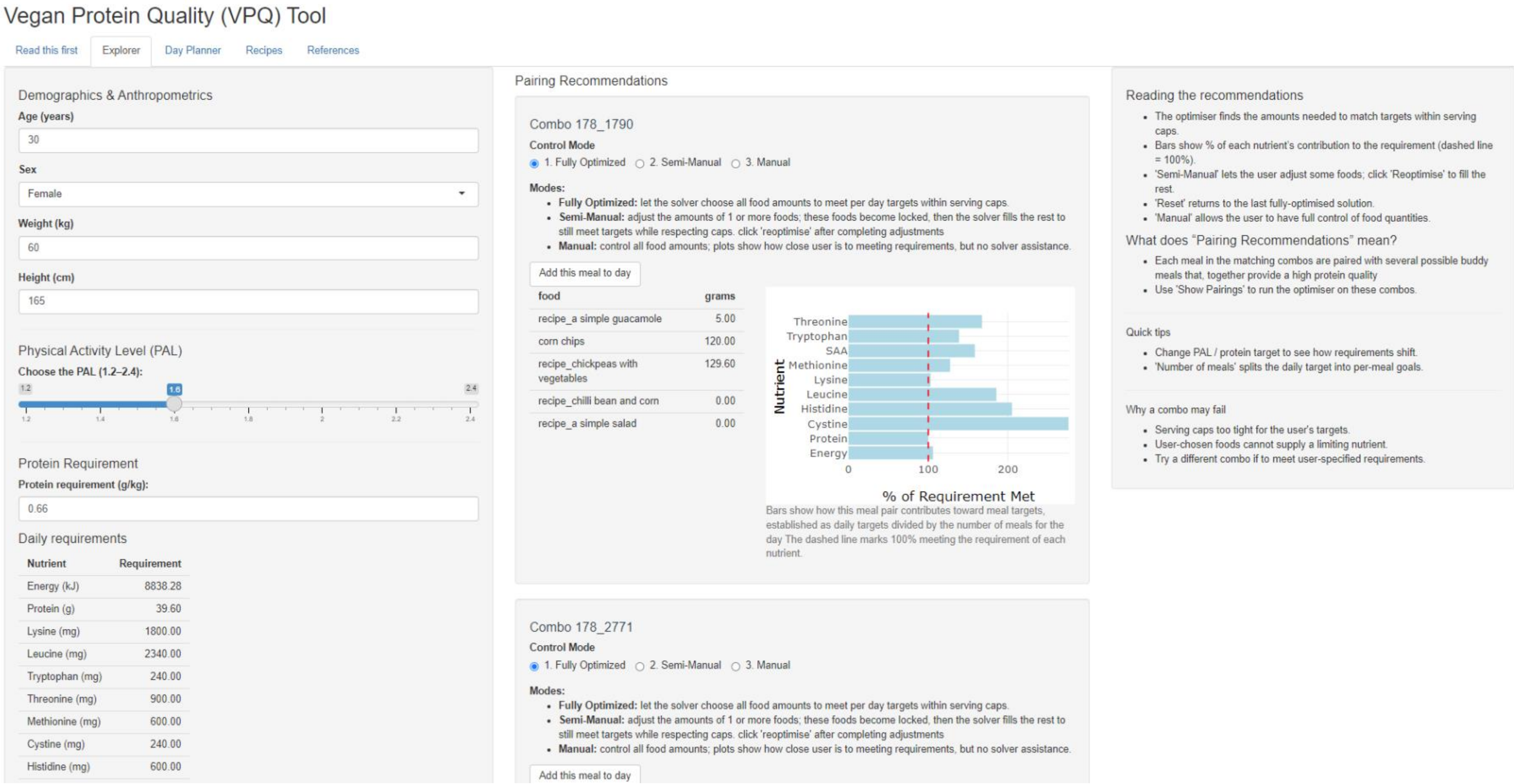


Figure 7.2 User interface of the VPQ tool

Scenario 1

Table 7.1 shows the target energy, protein, and IAA intake for a sample meal-pair, and **Figure 7.3** shows the % of requirement met for each nutrient in the fully optimised mode

Table 7.1 Simulated daily requirements for Scenario 1

Nutrient	Target quantity
Energy (kJ)	8128.99
Protein (g)	75
Lysine (mg)	2250
Leucine (mg)	2925
Tryptophan (mg)	300
Threonine (mg)	1125
Methionine (mg)	750
Cystine (mg)	300
Histidine (mg)	750
SAA (mg)	1125

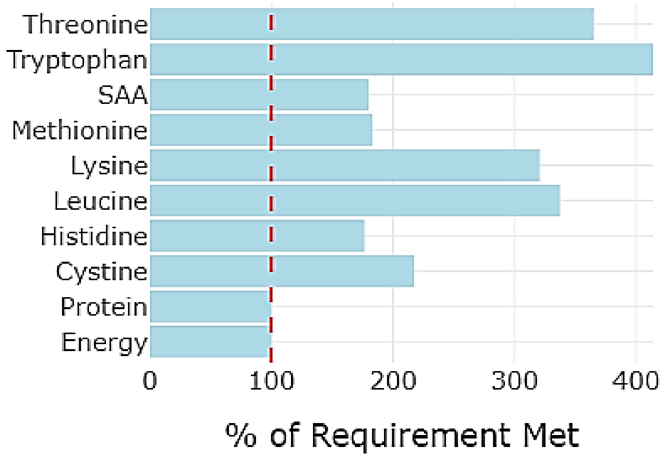


Figure 7.3 Percentage of requirement met for each nutrient.
The minimum requirement is denoted by the dotted line at 100 %, for meal pair code 7_2771. Bars passing the dotted line indicate that the target is exceeded.

In the fully optimised mode, per-meal targets for energy, protein, and all IAAs are set to meet the required fraction of the daily intake. Protein and energy were the binding constraints, while all IAAs exceeded the minimum requirement. **Table 7.2** lists the food weights within meal pair 7_2771 needed to meet one-third of the daily protein and IAA target.

Table 7.2 List of foods for meal pair 7_2771, with the optimised quantity in grams for each food

Meal ID	Food	Quantity (g)
7	recipe_ pesto pasta with pumpkin and tofu	59.7
	white rice	0
	recipe_cashew nut gravy	30
	recipe_swedish-style meatballs	0
2771	wholemeal bread	5.7
	recipe_chicky salad	150

The fully optimised mode selects quantities of food (g) to meet per meal-targets, subject to serving-size restrictions. The user is notified if any meal pairs are infeasible under the current nutrient target or serving-size constraints. Since the objective penalises total grams and number of foods, solutions in the fully optimised mode may choose fewer items and sometimes max out a single food which has high protein or IAA density per gram, thus yielding limited diversity within a meal pair (e.g., in **Table 7.2** where *recipe_chicky salad* reaches the maximum serving size and *white rice* and *meatballs* were 0 g).

A user may opt to reduce or increase food quantities based on preference, concerns about satiety or energy intake. **Table 7.3** shows the user's reduction in foods.

Table 7.3 User adjusted and re-optimised food quantities under the semi-manual mode

Meal ID	Food	Quantity (g)	Action
7	recipe_ pesto pasta with pumpkin and tofu	36.1	User changed
	white rice	0	No change
	recipe_cashew nut gravy	15	User changed
	recipe_swedish-style meatballs	53.2	Reoptimised
2771	wholemeal bread	109.6	Reoptimised
	recipe_chicky salad	59.9	User changed

Figure 7.4 shows the resulting impacts on the nutrient intake relative to % requirements after user adjustment. Energy, protein, SAA, methionine, and histidine are now below the nutrient targets.

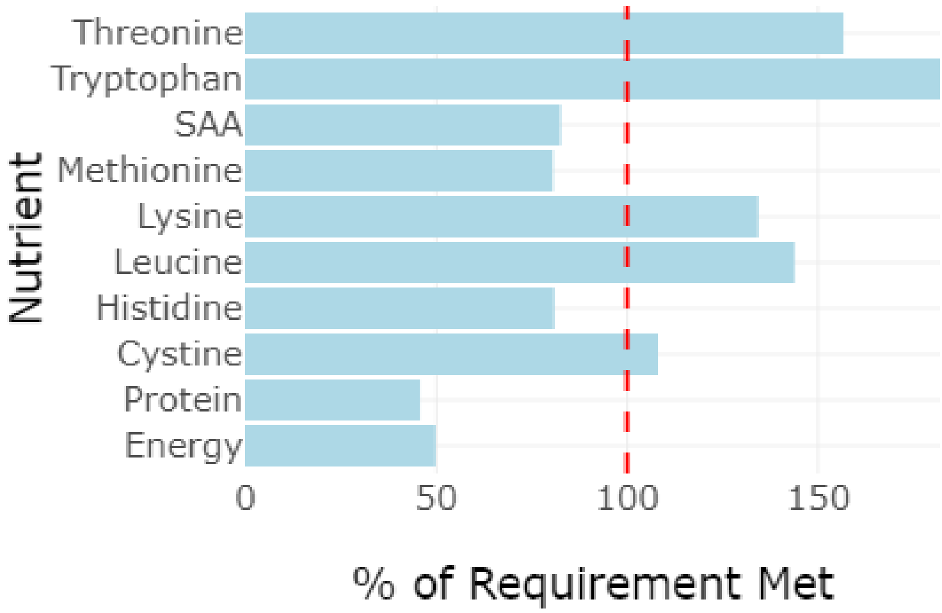
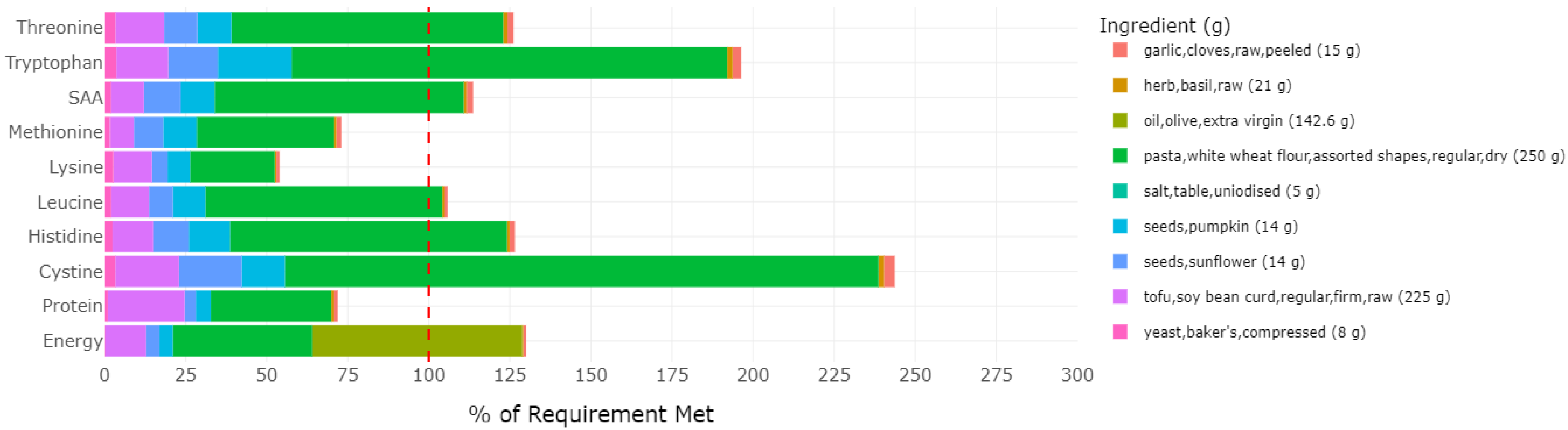


Figure 7.4 Simulated outcome of nutrient intake relative to requirement, after user reduced quantities (g) of selected foods in the meal pair.

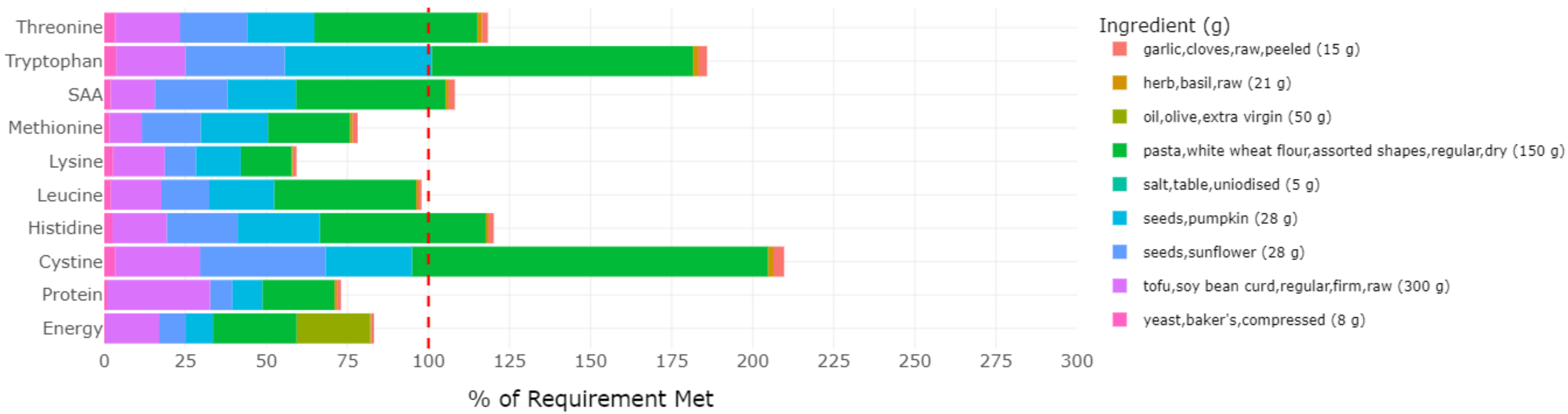
Re-optimisation in the semi-manual mode then readjusted the quantities of other foods that the user had not modified to increase nutrient intake to the requirement level shown in **Table 7.1**. The reoptimised quantities are shown in **Table 7.3**. Energy is a common limiting constraint in simulations. In this case, the reduced quantity of the energy-dense foods, *pasta* and *gravy,* resulted in an energy deficit which had to be covered by an increase in the quantity of *wholemeal bread*.

The manual mode disables optimisation and gives the user full control over the food quantities in each meal pair. Slider adjustments update nutrient intake immediately, allowing users to see how selected quantities perform against per-meal requirements. Finally, the user can add any meal pair to the daily plan, where the nutrients across the added meals are aggregated as the day total and compared to the % of daily requirement (dashed line at 100 %) in the Day Planner tab of the tool.

The interactive recipe tab of the VPQ tool allows changes to ingredient weights, and a new per-gram nutrient profile is automatically calculated. For example, we demonstrate how to increase the protein quality of *recipe_pesto pasta with pumpkin and tofu* by increasing the protein-dense ingredients (*tofu* and *seeds*). **Figures 7.5A** and **7.5B** show the changes in ingredient quantities and their resulting contributions to daily nutrient requirements. Changing ingredient quantities reordered the optimiser's allocations within the example meal pair. The portion of *recipe_pesto pasta with pumpkin and tofu* increased to 120 g while *chicky salad* and *wholemeal bread* decreased to 93.9 g and 7.8 g, respectively (updated relative to **Table 7.2**). Recipe edits can re-rank foods by nutrient density (nutrients per gram), prompting the model to increase the quantities of food items that now contribute more nutrients per gram.



A. Nutrient contribution to requirement from original recipe quantities for olive oil, pasta, pumpkin seeds, sunflower seeds and tofu



B. Nutrient contribution to requirement after change in ingredient quantities for olive oil, pasta, pumpkin seeds, sunflower seeds and tofu

Figure 7.5 Ingredient list of *recipe_pesto pasta* with *pumpkin and tofu* with the original (**A**) and changed (**B**) quantities.
Ingredients are differentiated by colour, as listed in the legend. The respective weight of each ingredient is indicated beside the name of each ingredient in the legend. Stacked bars show each ingredient's contribution to daily requirements and update when the ingredient quantities or the individual nutrient requirements change. Percentages are computed and compared to the daily requirement, as if the entire recipe were consumed. In practice, the optimiser often selects smaller portions based on nutrient composition and serving-size constraints. Charts should not be interpreted as per-meal contribution unless the full recipe is consumed.

The % contribution of each ingredient to the daily nutrient requirements shifted from the scenario illustrated in **Figure 7.5A** to that of Figure **7.5B**. Overall, the highest contributions for all nutrients were most apparent from *pumpkin* and *sunflower seed*, and from *tofu* for protein and lysine (protein: from 23.7 to 36.8 %, lysine: from 11.9 to 18.5 %). Energy decreased notably from ~ 125 % to ~ 75 % of the daily requirement due to reduced quantities of *olive oil* and *pasta*.

Changing ingredient quantities reordered the optimiser's allocations within the example meal pair. The portion of *recipe_pesto pasta with pumpkin and tofu* increased to 120 g while *chicky salad* and *wholemeal bread* decreased to 93.9 g and 7.8 g, respectively (updated relative to Table 1). Recipe edits can re-rank foods by nutrient efficiency (nutrients per gram), prompting the model to increase the quantities of items that now contribute more nutrients per gram.

Multiple recipe adjustments were further explored in increments of protein-dense ingredients in *chicky salad*. The quantities of *almonds* and *PBMA* were increased while the quantity of *bread* was reduced (**Figure 7.6**). With an increase in the protein quality of this recipe, a smaller serving portion is now sufficient to meet IAA requirements (decrease in the *chicky salad* from 93.9 to 63.3 g). *Pasta* remained unchanged, whereas *wholemeal bread* increased from 7.8 g to 30.3 g, likely to satisfy the energy constraint, which remained the most limiting.

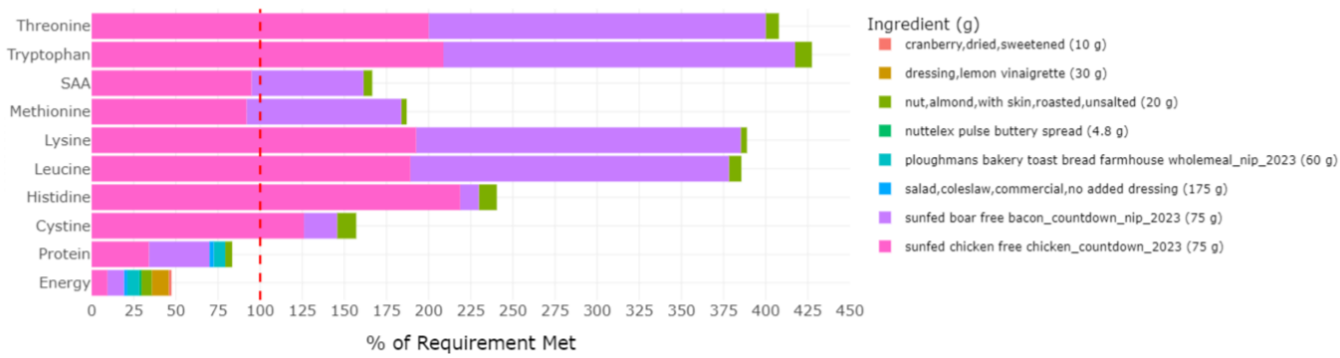


Figure 7.6 Contribution of ingredients in *recipe_chicky salad* to daily requirements after adjustment to the quantity of some ingredients.
Changes made to the quantity of *sunfed chicken free chicken* (PBMA) (25 to 75 g), *almond* (10 to 20 g) and *toast* (85 to 60 g). The primary ingredient in the PBMAs, *sunfed boar free*

bacon and *sunfed chicken free chicken* is a legume-based protein isolate. This recipe with the simulated ingredient quantities thus contributes highly to protein quality, as shown by bars exceeding 100 % requirement for all TID-adjusted IAAs.

Scenario 2

For the same meal pair as in Scenario 1 (7_2771) in the fully optimised mode, the optimiser increased the quantity of several foods for the 30-year-old male. This was largely driven by higher energy and protein requirements, as a result of elevated PAL and protein target (**Table 7.4A**). The solution still met all per-meal nutrient targets, demonstrating that a high-protein-quality and energy-adequate meal is achievable under higher requirements, with energy and protein emerging as the binding constraints. While this is a feasible meal plan, multiple food items have hit the maximum serving size limit, which may pose practical challenges for satiety. **Table 7.4B** shows the optimised quantities for each food item, with several reaching the maximum serving size.

Table 7.4 Daily requirements of the second simulated user for energy, protein and all IAAs (**A**), food quantities in the fully optimised mode for meal pair 7_2771 (**B**)

(A)

Nutrient	Quantity
Energy (kJ)	16150.24
Protein (g)	114
Lysine (mg)	2850
Leucine (mg)	3705
Tryptophan (mg)	380
Threonine (mg)	1425
Methionine (mg)	950
Cystine (mg)	380
Histidine (mg)	950
SAA (mg)	1425

(B)

Meal ID	Food	Quantity (g)
7	recipe_ pesto pasta with pumpkin and tofu	120
	white rice	12.4
	recipe_cashew nut gravy	30
	recipe_swedish-style meatballs	150
2771	wholemeal bread	120
	recipe_chicky salad	150

Relative to Scenario 1, more meal pairs were infeasible under the current serving-size boundaries, because energy was the most limiting constraint. As a test, when PAL increased to a maximum of 2.4 and the protein requirement to 2.0 g/kg/day, the same meal pair was infeasible under the 3-meal /day plan but feasible under a 4-meal/day plan. While it was still possible for vegan meals to satisfy the nutrient constraints for individuals with very high protein and energy requirements, a user needs to navigate decisions around distributing food intake over more meals (or inclusion of protein-dense snacks) in a day, exceeding serving constraints of each FG in one EO, or choosing more protein-dense foods such as traditional legumes, extruded protein isolates or nuts and seeds. **Figure 7.7** provides a visualisation of how the total intake from all meals in the day contributes to the daily nutrient requirements in the Day Planner of the VPQ tool.

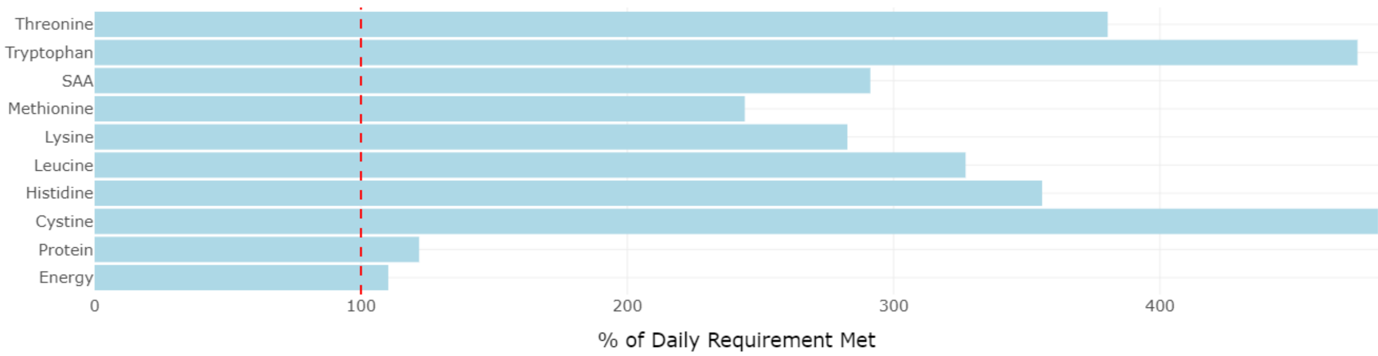


Figure 7.7 Total intake across all meals as a percentage of daily requirements for energy, protein, and each IAA.

Table 7.5 shows a breakdown of food quantities required to meet the energy, protein and IAA requirements of the individual in Scenario 2. Several foods reached the maximum allowable serving size across the three meals. Importantly, the modelling framework allows users to explore alternative meal configurations by adjusting food combinations and meal quantities, thereby enabling identification of nutritionally adequate solutions that are acceptable at the individual level.

Table 7.5 Examples of meals and quantities of each food item, as calculated in the fully optimised mode for scenario 2.

Meal	Food items	Quantity (g)
Breakfast	Recipe_protein shake with pea protein isolate strawberry	30
	Hemp seeds	30
	Chia seeds	30
	Linseed	30
	Oat milk	86.2
	Recipe_buckwheat, coconut and seed muesli	120
	Banana	150
Lunch	Recipe_frooze balls	30
	Recipe_coconut chocolate cake	120
	Corn chips	120
	Black bean chilli soup	147.6
	Sausage rolls	150
Dinner	Recipe_cashew nut gravy	30
	Recipe_swedish-style meatballs	90.4
	Wholemeal bread	120
	Recipe_pesto pasta with pumpkin and tofu	120
	Recipe_chicky salad	150

An exploratory validation of the fully optimised mode was conducted using the same meal pair database and optimisation model across four scenarios with varying per-meal nutrient requirements. This step assessed how outcomes may change depending on the user-set constraints. Each of the 3623 baseline meals could be paired with multiple partner meals, resulting in 23,539 candidate meal pairs available for optimisation. Scenario testing showed that the proportion of feasible meal pairs decreased as protein and / or energy requirements increased, and when these requirements were distributed across a smaller number of meals (**Table F2 of Appendix F**).

Across feasible solutions, energy and total protein were most frequently identified as the limiting constraints. IAAs were less commonly limiting although lysine and SAAs appeared more often. This could be a result of effective meal complementation to generate high-DIAAS combinations before incorporation into the VPQ tool. **Table F3 of Appendix F** showed the mean meal mass (g) and the mean proportional share (%) of each FG in the meal pairs. The absolute food quantities remained broadly similar across scenarios, although “nuts and seeds” and “isolates” frequently approached their maximum serving-size constraints. FG contributions were also examined using the mean proportional share, representing the composition of each meal pair. “Grains and pasta” and “legumes and pulses” accounted for the largest mean share of meal mass, followed by “nuts and seeds” and “isolates”. “Fruit” and “vegetable” contributions were comparatively small, which may reflect the model’s design in prioritising protein quality over overall diet diversity. Standard deviations of FG shares appeared large relative to the means, reflecting skewed distributions in which certain FGs appeared infrequently but contributed substantial quantities when selected.

7.4 Discussion

Using a systematic pairwise-matching approach with IAA-deficient meals, vegan meals were generated in which dietary protein is fully utilisable; such that the first limiting IAA meets or exceeds the body’s physiological requirements (20, 23, 24). The combination of diverse PB foods, with the most frequent complementary pairings between “grains and pasta” and “legumes and pulses”, provided meal pairs with a DIAAS of at least 100 %. “Legumes and pulses”, “nuts and seeds”, and protein “isolates” consistently emerged as key complements. These generated meals did not prescribe quantities, so the VPQ tool was developed to convert these high-quality pairings into personalised meal plans by optimising the required food quantities per meal to meet user-defined targets for energy, protein, and IAAs, while accommodating user preferences.

As observed in the test scenarios outlined in this Chapter, meeting nutritional targets often does not require every food in a meal pair, apart from cases where protein and energy requirements are high. The model generally prioritises nutrient-dense options to close shortfalls. IAAs, especially lysine, leucine and SAAs, are often the most limiting nutrients in food combination simulations (8). However, as observed across the VPQ tool scenarios, protein quality is not the binding constraint; energy and total protein often determine the feasibility of the optimisation. This is largely a result of the pairwise matching approach, which ensured that even the most limiting IAA can meet the reference value. This affirms that meal-level complementation to improve protein quality is synergistic with the tool's optimisation of quantity to achieve specific requirements. In these cases, total protein may become more limiting rather than protein quality – contrary to the commonly held view that vegan diets are primarily constrained by inferior protein quality.

Even at elevated protein requirements (e.g., 2.0 g/kg/day), the fully optimised mode of the VPQ tool could still derive feasible solutions that satisfied all nutrient constraints. However, this generally required larger portions, often approaching the upper limits of serving-size constraints. In practical terms, if vegan diets are not sufficiently diverse, one alternative is simply consuming more of the same PB foods to reach protein and IAA targets (23, 25). This approach is seldom practical as higher-fibre PB foods promote early satiety. The high carbohydrate and lipid content of grains, nuts, and seeds also means that increasing portions raises energy intake. This may be problematic for individuals with higher protein requirements but relatively lower energy requirements (e.g., older females) (20). Pragmatically, distributing nutrient intake across meals and diversifying PB food choices with complementary limiting IAAs helps avoid overconsumption of any single PB food beyond recommended portion sizes (26).

Meal patterns throughout the day may have consequences on protein metabolism (27, 28). Past research emphasised the benefit of evenly distributing sufficient protein intake (20 to 30 g of protein per meal) across 2 to 3 meals, compared to skewed intakes (29-32). Such a pattern was associated with greater stimulation of MPS, leg lean mass and muscle strength (31, 33, 34). Yet, one study found that older women who consumed a higher-protein breakfast, rather than dinner, experienced greater improvements in muscle mass (35). This may be explained by the fact that a higher protein dose in the first meal of the day helps to attenuate the body's catabolic state following an overnight fast, thus facilitating an improved anabolic response (36). The gap between meals and the protein sources of these meals may also influence the required quantity. For example, while 20 g of digestible protein should be consumed if meals are spaced 3 to 5 hours apart, approximately double that amount may be required if EOs are further apart and if protein sources are PB (37). Discrepancies across studies may stem from methodological differences, variation in participant demographics such as sex and age, the associated anabolic sensitivity of skeletal muscle, PAL, and the type of protein source (animal or PB).

While meal requirements for protein remain uncertain, the VPQ tool is adaptable to curate diverse eating patterns that can at least achieve the more clearly defined daily targets for protein and IAAs. Since there are no established per-meal requirements for protein and amino acids, the VPQ tool estimates the optimal nutrient distribution based on the user-defined number of meals per day, where compositions of all meals collectively meet the daily requirements. When new evidence emerges to more precisely define per-meal requirements, these parameters can be integrated into the tool. The interactive interface allows users to visualise the impact of their dietary choices on protein, energy, and IAA intake in real time. This is supported by a distinctive feature of the tool that facilitates the interaction between the optimisation engine and user autonomy. While the optimiser can generate solutions, the users still retain the flexibility to adjust these decisions.

Mixing PB foods to achieve higher protein quality is not a novel concept in vegan diet research; novel tools such as LP and substitution techniques have been implemented to determine optimal pairings to improve nutrient quality (8, 38-40). A diverse list of PB foods has been identified as promising complementary protein sources. Foods such as soy isolate, Brazil nuts, potato protein, and mixtures of beans and peas combined with various other foods can attain balanced IAA profiles that match the reference patterns, and which are at least 85 % comparable to the amino acid profiles of ASFs (8, 39). Novel legume-based products (e.g., PBMAs) were frequently chosen by the tool to meet individual requirements. In snack-like meals, pea protein isolates, nuts and seeds appeared to be critical food choices to fulfil all nutrient constraints in the optimisation but could reach the maximum serving size allocations in scenarios with higher protein requirements (e.g. 2.0 g/kg/day).

PBMAs often contain either soy or pea protein isolate, which provides higher protein quality per serving than whole foods. Processing mechanisms disable ANFs and transform these leguminous foods to highly digestible sources of protein with AA profiles close to the reference values, albeit more limited in SAA (41-43). Individual protein requirements for vegans may thus be more easily achieved with the inclusion of these foods. Indeed, one study found that a nutrient adequate diet can only reach up to 70 % plant protein if novel foods were excluded (13). However, recommendations on the consumption of these products must be made with caution. Their formulations vary widely, and plant proteins are often combined with flavourings, emulsifiers, binding agents, sweeteners and salt, with many products containing higher levels of saturated fat as compared to meat and traditional PB foods (44). Consequently, uncertainty remains regarding their long-term health impacts relative to a whole-food PB diet. Hence, it may be more prudent to position them as being complementary rather than a replacement option in diets (45).

Traditional foods such as "legumes and pulses" and "nuts and seeds" were also included as potential complements in the pairwise exercise and chosen by the tool to meet individual requirements. This study found that traditional plant food sources, when combined in varying proportions, can also achieve similarly high protein quality (mean DIAAS 1.09, SD 0.10) to that achieved when PBMAs were used as the main complementary food (mean DIAAS of 1.16, SD 0.2). However, uncertainty remains regarding their metabolic impact. For example, Pinckaers et al. (2024) reported greater MPS after an isonitrogenous omnivorous meal compared to a legume-based vegan meal, which may be associated with differences in postprandial plasma AA concentrations - such as leucine that directly impacts MPS - even if the IAA profiles in these meals were similar (46). Contrastingly, the researchers observed no differences in MPS when a protein-dense blend of wheat, pea and corn was provided in comparison to milk protein in younger males (47). Further verification may thus be necessary, particularly in relation to different whole-food combinations and across demographics.

The pairwise iteration technique for meal complementation is complete, enabling the generation of fully complementary meals using real-world consumption data and, consequently, identifying the FGs that were useful in obtaining improved IAA profiles. The VPQ tool shares similarity with the Alpha-Tool (48) in the ability to formulate vegan meals of high protein quality. The Alpha-Tool computes a single Meal Protein Quality Score (MPQS), based on the first limiting IAA, with a score ≥ 100 % to indicate all IAAs meeting requirements (16). The same objective was obtained with a meal-level DIAAS in this Chapter, which is calculated with similar underlying TID-adjusted amino acid data.

7.4.1 Strengths and Limitations

A key strength of the VPQ tool is its two optimisation modes (levels of automated guidance), which compute the minimum portion sizes for pre-identified high-protein-quality meals. Compared to the Alpha-Tool, the VPQ tool offers less freedom for users to assemble different foods for meals, since meal pairs and recipes were already curated. However, these meals reflect what is consumed by a vegan population, thus providing familiar recommendations for combining PB foods. Amidst global attention on the shift towards PB diets, these tools offer practical data-driven approaches to ensure that nutrient-adequate diets remain the priority for all individuals, even in more restrictive diets. Overall, vegan mixed meals of high-protein-quality are achievable through AA complementation from diverse PB foods.

Several limitations exist with this approach. Some high-DIAAS meal pairings identified by the tool may not reflect practical and acceptable dietary habits (e.g., combining two large recipes is unlikely to be feasible in practice). Although the VPQ tool constrains solutions to avoid oversizing portions and including unnecessary food items, this may undercut the intent of meal pairing, with imbalanced and edge-case solutions (e.g., reaching maximum serving limit for one food, and a small serving for another food, or choosing only the most protein-dense foods and ignoring other essential FGs like vegetables). The model also assumes equal distribution of daily requirements across meals throughout the day, when in reality, an individual may consume three main meals and two snacks, which would not be expected to contribute equally to daily requirements.

The current approach with plant protein pairings and optimising the quantity for each food focused on ensuring that the lowest IAA ratio reached at least 100 %, and that protein and energy constraints were also met. However, this did not consider how high ratios for other IAAs become and the potential that protein quality is overestimated with the pairings of several different food items within each meal pair. The surplus of some IAAs may be oxidised rather than stored or utilised (39, 49). However, it should be noted that in most omnivorous diets in higher-income regions, the protein and IAA intake also surpasses requirements, and that this is rarely deleterious (23, 50). Furthermore, excess IAAs, in the context where all other IAAs are above requirements, may provide precursors for DAA synthesis (12). While the pairwise meal matching is a thorough technique, identical meal combinations may recur when participants consume the same meals across different days. These repetitions reflect the underlying dietary patterns such as the more commonly consumed foods in the NZ vegan cohort, but future refinements could incorporate additional steps to consolidate repeated meal complementations.

Future work could investigate food ratios or portion patterns that align with real-world behaviour, incorporate evidence from focus groups, and potentially leverage artificial intelligence to incorporate the complexities in eating patterns. The pairwise iteration in this study achieved a relatively low proportion (~ 40 %) of meals that could be matched to yield a DIAAS of ≥ 100 %. This could be related to limitations in the variety of protein sources as represented by a single vegan population, so further permutations of PB consumers of various cultures and nationalities could be explored.

Moreover, future versions of the VPQ tool could incorporate meal pairings beyond the strict DIAAS ≥ 100 % to include combinations achieving DIAAS ≥ 75 %, which are generally considered to represent good protein quality (11, 24). Examining such scenarios would allow investigation of how modest improvements in protein quality could influence the required food quantities, energy and overall diet feasibility.

Importantly, the current tool does not optimise for the adequacy of other micronutrients, nor compare how portion sizes will change if ASFs are considered. The tool has also not incorporated supplement use or highlighted the presence of fortified foods in the baseline dietary data. Consequently, it cannot assess micronutrient adequacy, which is crucial given the known deficiency risks in unbalanced vegan diets (51). Hence, a meal plan could score well on protein quality but be inadequate in certain micronutrients. Future extensions of the tool should aim to cover priority micronutrients in vegan diets and allow supplement and fortification inputs.

7.5 Conclusions

The VPQ tool is a proof-of-concept optimiser with the goal of designing meals for protein adequacy and protein quality with minimal surplus, tailored to user requirements and food preferences. It is not intended for general consumer use; rather, it should serve as an initial decision aid for nutrition or dietetic professionals who can interpret outputs along with clinical judgment. As the VPQ tool outlines the ingredients used in PB recipes and their nutrient contribution, it serves as a useful guideline for food industries that may consider the use of specific ingredients in novel PB food product formulation to improve protein quality.

Chapter 5 demonstrated that protein adequacy can be achieved across the whole day in vegan diets. The approaches taken in this Chapter show novel approaches to pair incomplete protein sources so that the resulting meals closely approximate the reference IAA profiles, followed by the application of an interactive tool to fulfil specific user requirements. With nearly half the vegans in this cohort found to be inadequate in lysine and leucine (2), there is a clear need for strategies that address protein quality so that total protein requirements are not limited by specific AA shortfalls. Simply increasing food intake to compensate is not a sustainable solution, as it would inevitably lead to infeasible food and excess energy consumption. Instead, improving the quality of meals directly – through the strategic use of complementary plant foods – offers a more efficient solution to protein adequacy.

Once again, “legumes and pulses”, “grains and pasta”, and “nuts and seeds” were identified as dietary staples that, when combined in the right proportions, drive protein quality. Such insights are highly relevant for vegan diets, where the focus must shift from quantity alone to the balance and quality of protein intake, specific to individual requirements.

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Chapter 8 General discussions

This Chapter provides an overview of the findings of this thesis, discusses their implications, and summarises the strengths and limitations. The thesis concludes with an outline of directions for future research.

8.1 Key findings and implications

This thesis contributed to several key areas of understanding protein intake and protein quality in vegan diet research. The assessments undertaken in Chapters 3 and 4 highlighted concerns about the adequacy of utilisable protein intake in the NZ vegan cohort. While protein intake appeared sufficient in three quarters of the cohort when compared to the ERA, half the cohort was consuming inadequate utilisable protein when compared to the RDI. A similar proportion was reported by Bartholomae et al. (2026), who examined protein adequacy in healthy, lightly active women who habitually consumed a vegan diet (1). In Chapter 4, the assessment of protein adequacy, stratified by dietary clusters, identified a concerning number of daily diets below the minimum threshold of 0.24 g/kg at the EO level. This pattern was observed mostly in clusters 1 and 2, which comprised daily diets with the poorest total protein and IAA intake. Taken together, utilisable protein intake is a concern for a proportion of vegans in the studied population.

Assessment of protein adequacy must also consider protein quality. Among individuals who met the RDI, only half achieved adequacy for all IAAs (Chapter 3). Lysine and leucine were the two most limiting IAAs, with half the cohort falling below the daily requirement. SAA was noted as the next limiting IAA, with a quarter of the individuals below the requirement. Clearly, modifications to the diets of these vegans are necessary to improve the provision of protein and limiting IAAs. Evidence from Chapters 3 to 6 identified that protein-dense PB foods are present in the daily diets and meals of some vegans in the study. For example, in Chapter 4, individuals in cluster 3 obtained significantly more protein and IAA from “legumes and pulses”, “nuts and seeds” (**Table C2 of Appendix C**).

Several reviews on PB diet research suggested that high protein quality can be achieved in diets that limit or exclude ASFs when diverse plant foods are consumed in complementary combinations (2, 3). To test this hypothesis, Chapters 5 to 7 investigated the quantities and proportions of different PB foods required to improve baseline vegan diets and resolve shortfalls in protein and IAA for each individual. PB foods vary substantially in their contribution to protein and IAAs for a given energy intake. This influences the combinations and quantities required in daily diets and meals to achieve high protein quality while staying within energy boundaries. **Figure 8.1** summarises the overall flow and principal findings of the thesis.

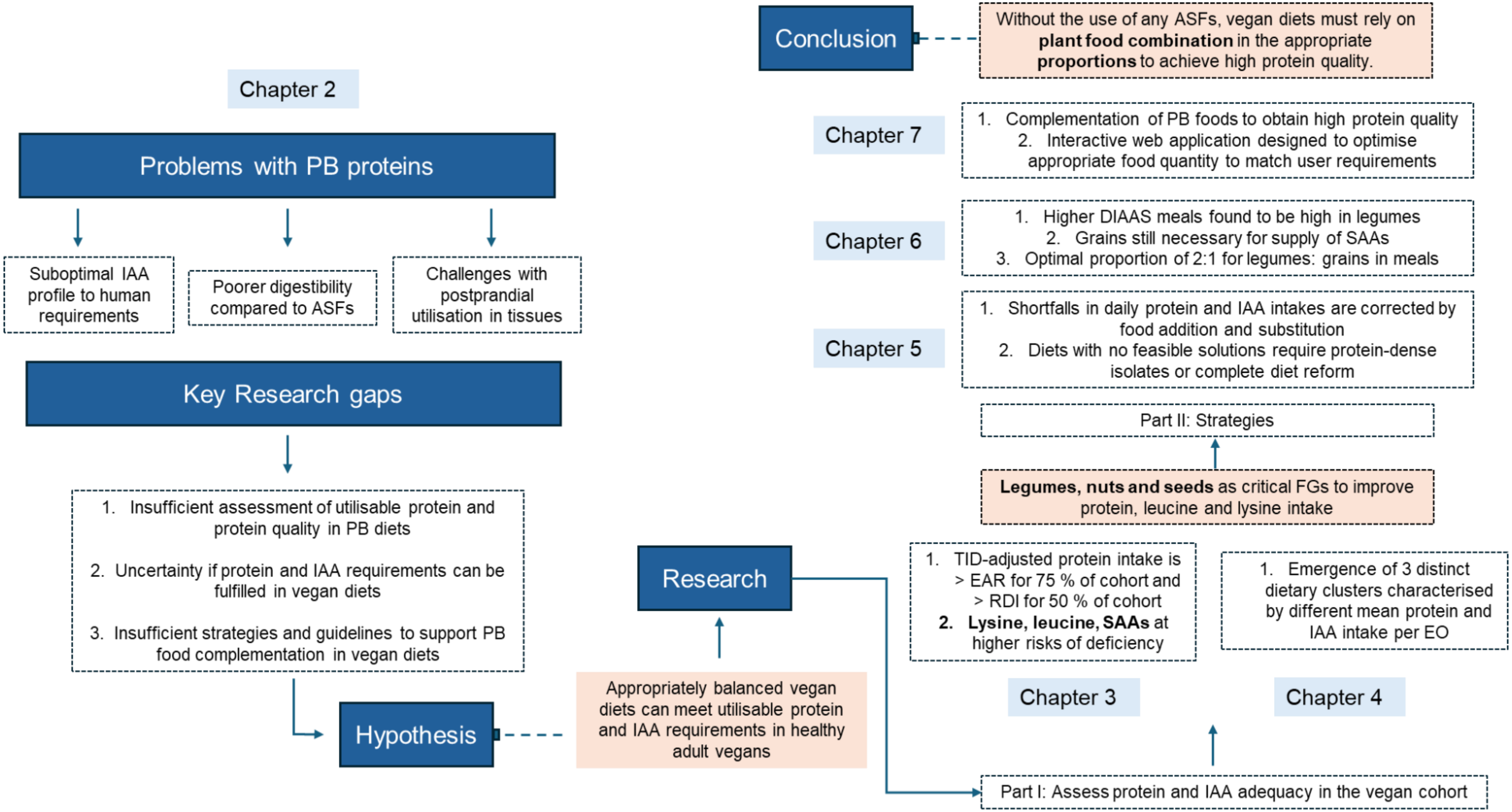

Figure 8.1 Summary of the main results of the thesis.
ASF, Animal-sourced food; EAR, Estimated Average Requirement; DIAAS, Digestible Indispensable Amino Acid Score; IAA, Indispensable Amino Acid; PB, plant-based; RDI, Recommended Dietary Intake; SAA, Sulphur Amino Acid

Veganism can be understood as a deliberate lifestyle choice shaped by ethical, environmental, and/or health-related concerns. This orientation is expressed in part through dietary practice, where vegans avoid the consumption of ASFs and their by-products. Beyond food choice, veganism may be embedded within broader systems of identity, moral values, social, cultural, and gender norms (4, 5). Consistent with this framing, motivations among NZ vegans whose diets were analysed in this thesis varied along these aspects, with ethical concerns for animals and environmental considerations most commonly prioritised relative to health-related motivations (6). Participants who identified health as the most important motivator tended to be older and more highly educated. Additional influences that motivated the adoption of a vegan diet included family and social networks, as well as concerns about food intolerance and allergies.

While ethical and environmental motivations often underpin the adoption of vegan diets, nutrient adequacy and health outcomes should remain the critical determinants of long-term sustainability and diet adherence. Vegan diets are inherently restrictive in that all nutrients can only be attained from plant and fungi sources, within which several nutrients are absent or limited. Critical nutrients of concern include, but are not limited to iron, zinc, iodine, vitamin B12, calcium and omega-3 fatty acids. Some evidence indicates that dietary deficiencies of these nutrients are linked to adverse health outcomes such as anaemia, neurological dysfunctions, osteoporosis, and increased risks of bone fractures (7, 8). Evidently, some risks can emerge when the elimination of nutrient-dense ASFs is not offset by nutritionally equivalent supplements or fortified alternatives.

Dietary protein intake is commonly measured in cross-sectional studies assessing the nutritional adequacies of vegan diets (9). Despite insufficient TID data on many AAs, protein quality intake within vegan diets has also been evaluated in several observational studies (Section I of Chapter 2). Many studies comparing IAA intake in vegan diets with omnivorous or control diets have reported lower intakes of several IAAs, with lysine most frequently identified as the limiting IAA. However, it remains unclear whether these lower intakes were also below the individual physiological requirements. While diet quality indices in PB diet models revealed potential increases in several nutrients that are commonly associated with positive health outcomes, analyses of protein quality in simulated PB diets remain limited (Section II of Chapter 2). These uncertainties represent notable gaps. Protein quality in diets is a critical determinant for human health, but can be compromised in overly restrictive or poorly balanced diets. Complexities associated with digestibility and utilisation of nutrients from plant proteins, even for the more superior soy foods, indicate clear challenges when diets exclude all ASFs. This uncertainty highlights an important research gap in determining whether fully PB diets can meet protein quality requirements and be comparable to omnivorous diets with respect to protein adequacy and protein quality.

As discussed in Section I of Chapter 2, reliance on PB foods as the sole source of dietary protein poses specific challenges in providing sufficient quantities of digestible IAAs to meet individual requirements. Food complementation has therefore been investigated as a strategy to overcome limiting IAAs in different plant foods, with different studies demonstrating that combining specific plant sources can enhance protein quality (10-13). These findings align with the dietary modelling studies reviewed in Section II of Chapter 2, which identified legumes, pulses, nuts and seeds as valuable PB food groups to improve protein quality. Much of the existing evidence is derived from isolated food ingredients or controlled combinations rather than habitual dietary patterns. In reality, everyday eating patterns are complex and heterogeneous, characterised by substantial variation in food combinations.

This thesis, therefore, offered the opportunity to address parts of this research gap by leveraging real-world vegan meal data, which comprised multiple foods combined within EOs. In this context, there was the opportunity to utilise computational modelling approaches as an efficient, cost-effective, and ethically appropriate means of examining protein quality without subjecting individuals to restrictive diets. Through Chapters 5 to 7, this thesis employed modelling and data analytics to assess how overall protein adequacy and protein quality can be achieved through realistic food combinations and substitutions, relying only on PB foods.

Findings from Chapter 3, together with cluster analyses in Chapters 4 and 6, demonstrated that both protein adequacy and protein quality are influenced by food combinations consumed over the day. A proportion of individuals were able to combine complementary PB foods in ways that ensured higher protein quantity and quality, but the majority of the cohort required diet modifications (Chapters 5 and 6). These results reinforce the well-established principle that dietary choice is a key determinant of nutrient adequacy.

Although adequate protein intake and protein quality can be achieved in vegan diets, this depends on the deliberate selection and combination of PB foods. In the absence of thoughtful food pairing, protein quality may be compromised even if total protein intake appears sufficient (Chapter 3). Targeted dietary planning, often supported by nutrition professionals, is important to ensure sufficient nutrition provision, especially for populations at greater risks of deficiencies due to dietary restrictions and/or relatively higher requirements. At present, however, guidance to support vegans in achieving a well-planned, nutritionally adequate diet remains limited within the NZ dietary guidelines (14). Following the recent position paper from the Academy of Nutrition and Dietetics, which aimed to inform healthcare practitioners about the evidence-based benefits and considerations of PB diets, the development of succinct educational resources for vegan and PB consumers, as well as the practitioners who support them, represents an important first step towards improving nutritional knowledge and supporting healthy dietary practices (15). Such guidance should extend beyond protein quality to include key micronutrients and reflect culturally appropriate dietary recommendations relevant to NZ populations.

Chapters 3 and 4 were foundational to this thesis in characterising the baseline patterns of protein and IAA intake and in identifying the most limiting IAAs in the habitual vegan diet. Chapter 5 further identified protein and nutritional shortfalls that were specific to each individual. Empirical evidence generated from Chapters 3 to 4 identified the constraints that should be applied within the diet optimisation framework in Chapters 5 and 7. Lower bounds were set to ensure that individual-specific protein and IAA requirements could be achieved after diet improvements. Diet optimisation approaches in Chapters 5 and 7 also enabled dietary solutions to preserve some extent of individual acceptability through the proxy of using only foods currently recorded in the food diaries (Chapter 5), or through user-interaction with the VPQ tool (Chapter 7).

Energy intake frequently emerged as the limiting constraint in the optimised diets. For some individuals in the NZ vegan cohort, maintaining energy intake within the upper energy boundaries while achieving adequate protein quality proved challenging unless energy-dense and low-protein foods were first removed from the baseline diet (Chapter 5). Given the lower IAA density of many plant foods, higher overall energy intake may be required to meet protein quality targets in vegan diets, compared to diets containing ASFs (16). This challenge may be more pronounced among older and sedentary populations who have lower energy requirements but higher protein needs (17).

Among the various PB FGs examined in this thesis, “legumes and pulses” and “nuts and seeds” were shown to play complementary roles to “grains and pasta” in providing all the IAAs. However, the contributions to protein quality differ in energy cost, with “grains and pasta” and “nuts and seeds” generally contributing protein and IAAs at a higher energy cost than “legumes and pulses” (Chapters 3 to 5). By systematically examining these trade-offs, the analyses presented throughout this thesis highlight the inherent complexity of balancing protein quality and energy intake within vegan diets. Chapter 7 however demonstrated that when PB foods are deliberately matched to achieve a DIAAS of at least 100 %, PB meals can be optimised to attain high protein quality without exceeding energy consumption.

Central to discussions of attaining protein quality in modern vegan diets is the contribution of novel meat and dairy alternatives, which are UPFs that often mimic the sensory properties of their animal-sourced counterparts. These foods were commonly found in the daily diets of the NZ vegan cohort and contributed important sources of many IAAs, particularly if the main ingredient was derived from “legumes and pulses” (Chapters 3 and 4). Yet, the suitability of novel PBAs to ASFs and traditional plant foods remains an ongoing debate. These products offer potential advantages, including high digestibility and dense IAA profiles to support protein intake and protein quality (17). Evidence on their ability to elicit comparable MPS responses to ASFs remains inconsistent (18-20) as findings were influenced by differences in study populations (age and sex), product formulations, and the diet that was used as the control or comparison.

An association between higher consumption of UPFs and increased BW and BMI has been reported previously (21). These public health implications could be driven, in part, by the high sodium, sugar, and saturated fat content of many UPFs, as well as comparatively low protein density to fat and sugar content in some dairy alternatives (22, 23). Surprisingly, higher BMI values and body fat percentage were observed in a subset of participants despite relatively low reported energy intakes (Chapter 3). While this may be related to underreporting, it may also reflect unmeasured metabolic or other behavioural factors. Associations between UPF consumption patterns and anthropometric outcomes were beyond the scope of the thesis but would benefit from investigation. This likely requires further adjustments for PAL, energy misreporting, and other confounders in future studies.

As discussed in Chapter 2, many PBAs are classified as UPFs under the NOVA classification which raise concerns regarding their potential health implications. However, UPFs represent an extensive group of foods. For example, health impacts from frequent consumption of certain subgroups such as processed meat, alcoholic beverages, SSB, ready meals and snack foods may differ from PBAs that provide nutritionally useful quantities of protein, IAAs or fortified micronutrients. Hence, treating all UPFs as a single exposure to health impact can obscure important differences among these subgroups. Health associations observed for total UPF consumption may be inappropriately generalised to individual or smaller subgroup of UPFs. Further empirical evidence examining PBAs as distinct UPFs to the other foods under the same NOVA category is necessary to establish their long-term health impact.

Nevertheless, novel food products appeared to be more popular among consumers who are younger and among those who have more recently transitioned to PB diets (21, 23). Their use may therefore reflect the reliance on convenient foods or limited familiarity with planning nutritionally balanced vegan meals. Consequently, food manufacturers face both opportunities and challenges in developing PBAs that deliver complete AA profiles, high protein digestibility, and adequate fortification with micronutrients commonly limiting in PB foods, all while also maintaining acceptable quantities of sodium, sugars, and fats, and ensuring palatability and affordability.

Findings from Chapter 7 suggest that varying proportions of traditional PB foods can achieve comparable improvements in protein adequacy and protein quality. This often requires intention, nutritional knowledge, and careful food combination. Dietary diversity, through the combination of multiple plant products, appears to be integral to achieving protein quality. Building on this principle, the VPQ tool introduces meal-level food combinations and user-specific optimisation modes to automate the calculation of food quantities to achieve high protein quality. Further development of the tool to incorporate a wider range of both traditional and novel PB foods, alongside additional nutrients of concern, would enhance its applicability and usefulness for vegans seeking practical guidance on daily meal and snack choices. Beyond vegan dietary applications, the optimisation framework may be extended to broader contexts of nutritional planning, including culturally diverse dietary patterns and population-specific nutrition guidance. Such expansion presents opportunities for interdisciplinary collaboration between nutrition scientists, health practitioners and other food systems researchers.

Consistent with observations from vegan and PB diet research (21, 24), females constituted the majority of the NZ vegan cohort examined in this thesis (Chapter 3). This pattern is discussed in relation to gendered food norms, including psychological and sociocultural associations between meat consumption and constructs of masculinity (24, 25) However, emerging evidence suggests that these demographic patterns may be shifting. One Swedish study reported increased engagement of younger males with PB diets, driven primarily by concerns about climate change and environmental sustainability (5). This could point to a shift in the motivations for adopting a vegan diet across generations.

The predominance of females within the vegan population has additional nutritional relevance, given that women may continue a vegan diet through pregnancy – a life stage characterised by increased protein requirement and other nutrient demands. Lower birthweight and small-for-gestational age have been reported in neonates born to vegan mothers as compared to omnivores, partly related to the leaner body composition of vegan mothers (26-28). While these outcomes remained within normal ranges and may not indicate adverse health risks, they underscore the importance of dietary guidance for vegan diets during pregnancy to support optimal maternal and infant health.

Meal-level and time-related protein intake was a central focus of this thesis (Chapters 4, 6 and 7), motivated by ongoing debate on whether PB protein sources must be complemented within narrow time windows or whether complementation across the day is sufficient. Habitual protein consumption in many studied populations is observed to be unevenly distributed across the day, with the largest proportion consumed in the later part of the day (29, 30). Such patterns were similarly observed in Chapter 4, indicating that assessing only daily protein adequacy masks variability of protein intake at the meal level.

Arguments supporting meal-level optimisation centre on the finite effect of postprandial MPS response, which lasts for 2 to 3 hours after a meal (31). Protein distribution across meals may also have broader metabolic roles, such as mitochondrial dynamics and tissue fuel partitioning, but these are still not fully understood (32). Contrastingly, a more recent cross-over analysis on short-term diet comparisons reported no differences in acute or 24-hour MPS between meals containing 1) ASFs, 2) complementary plant proteins, or 3) incomplete plant proteins, suggesting the redundancy of complementing PB foods within a meal as long as total daily IAA requirements are met (33). However, the study demonstrated that only meals with ASFs and complementary plant-protein combinations elicited a significantly greater MPS response than low-protein meals (< 5 g). In contrast, meals composed of incomplete protein sources did not produce a comparable anabolic response. The longer-term physiological responses of repeatedly consuming incomplete protein profiles of PB meals remain unclear, but these findings highlight potential metabolic limitations of such dietary patterns.

When protein intake is well above the requirement, protein quality and meal distribution may have reduced relevance (31, 34). Yet, this perspective is less applicable to the NZ vegan cohort. Most individuals clustered within groups characterised by mean protein and IAA intake per EO fell below the proposed optimal thresholds (Chapter 4). Analysing meal compositions becomes important in such contexts, as meals are the primary opportunity for food combination and present a timely point of intervention to increase postprandial IAA availability and subsequent net protein balance in the body (16, 35). Such strategies may be more relevant for older individuals who have higher nutrient demands but greater sensitivity to satiety. Nevertheless, ensuring that all meals achieve the optimal IAA thresholds is not always realistic. As observed in Chapter 6, while strong associations were observed between meal composition and per-day protein adequacy, instances of meals with high protein quality were still identified among individuals whose overall daily intake fell below adequacy. Diets are thus inherently varied; suboptimal meals at one time point do not preclude adequate intakes at subsequent EOs or adequacy against daily targets.

This thesis provides practical strategies to optimise protein intake in vegan diets to meet both daily and meal-level requirements in adults (> 18 years). From a dietary perspective, the findings highlight the critical role of “legumes and pulses”, “nuts and seeds” and “isolates” as key nutritional levers for achieving adequate protein quality in diets that exclude all ASFs. Collectively, the results provide evidence in support of the study’s central hypothesis: a well-planned vegan diet with appropriate food combinations from protein-dense PB foods can meet total protein requirements and achieve high protein quality.

8.2 Methodological considerations and future directions

This thesis, building on and extending prior evidence in the literature (20, 36) demonstrated that the appropriate use of both traditional and novel plant foods can support high protein quality within vegan diets. While protein adequacy can be achieved through strategic food selection and combination, this thesis did not establish whether such dietary approaches translate to concurrent adequacy across all micronutrients. Micronutrient sufficiency, therefore, remains an ongoing challenge and an unresolved aspect of vegan dietary planning that was beyond the scope of this work.

Proteinaceous foods, whether obtained from plant or animal sources, do not just provide protein and AAs, but also many micronutrients, some of which are either absent or limited in plant foods. ASFs are dense sources of vitamin B12, omega-3 fatty acids, iodine, bioavailable iron, calcium and zinc. Diet optimisation studies have proposed that fortification and supplementation be mandated to achieve full nutrient adequacy if protein contribution from ASFs to total dietary protein falls below the range of between 30 to 60 % of the total diet (37, 38).

In Chapter 5, deficiencies for some micronutrients were observed following dietary optimisations. This can be attributed to the absence of lower-bound constraints within the optimisation framework. These limitations also partly reflect the trade-offs involved in dietary optimisation under fixed energy constraints. In several cases, the model prioritised higher-protein foods over micronutrient-fortified PB dairy alternatives to be added within energy limits inadvertently lowering micronutrient provision in the modified diets. Another conundrum is highlighted here for novel PBAs, especially for meat alternatives: while these foods could offer advantages in protein density and micronutrient fortification, their inclusion can also increase excessive intakes of other dietary components such as sodium, sugars, and fats, which are typically discouraged in a healthy dietary pattern. For PB-dairy alternatives, benefits in micronutrient provision may be outweighed by its lower protein density and higher caloric content. Determining the appropriate frequency and quantities at which such fortified foods should be incorporated, alongside analysis of how food formulation and processing influence their nutritional profiles, represents an important area for future research (39).

One could argue that a straightforward resolution to micronutrient challenges is to allow limited quantities of ASFs back into the diet. If this were an acceptable solution, there would be little need for scientific inquiry into vegan nutrition, including the present thesis. The substantial and growing body of research on vegan diets, encompassing nutritional, health, environmental, and social dimensions, reflects ongoing efforts to address challenges in vegan dietary practice through solutions that align with the values of individuals who adopt veganism. In line with the position of some nutrition organisations, such as the US Academy of Nutrition and Dietetics, well-planned vegetarian and vegan diets can be healthy and appropriate across the life course, with the caveat that supplementation of key micronutrients remains a central recommendation (40-42). This will be particularly important during specific life stages with higher nutrient demands, such as pregnancy, early life, and older adulthood, where age-related declines in nutrient absorption (e.g., iron and B12) increase the risks of inadequacy (43).

Supplement use and adherence were not analysed in this thesis, so it remains unclear whether micronutrient requirements within the vegan cohort could be met if supplementation were included in the diet optimisation. This represents an additional gap in the current evidence base, highlighting the need for further research to evaluate whether fortification and supplementation can reliably deliver adequate nutrition. Moreover, future research should consider the economic implications and accessibility of fortified foods and supplements, particularly the long-term feasibility and equity of such strategies in vegan dietary patterns.

In NZ, the cost of a vegan diet is 35 % higher than the current average diet, with the purchase of milk alternatives contributing largely to this increased cost (44). Supplementation expenditure is sizeable in vegan populations, and about 2 to 4 times higher than for omnivores and lacto-ovo-vegetarians, as indicated by one study on German vegans (45). Despite its importance, studies looking at the association between food costs and the vegan diet often exclude supplement use in the food recall data (46, 47). Cost is a key component of diet sustainability. Future extensions of the VPQ optimisation framework could incorporate both (fortified and unfortified) food and supplement use, alongside price data, to identify the least-cost combinations to meet all nutrient requirements. This would enable evaluation of the economic trade-offs associated with achieving nutritionally adequate vegan diets relative to other dietary patterns.

One of the key methodological advances of the DIAAS framework is its use of digestibility coefficients for individual IAAs, measured at the end of the small intestine, rather than relying on faecal protein digestibility, as in the PDCAAS. This approach more accurately quantifies AA absorption and circulation but remains limited in its ability to measure utilisation by the body systems (16). Furthermore, digestibility data for individual IAAs are not available for many foods. Accordingly, in this thesis, digestibility adjustments for IAAs were applied at the level of broader FGs or representative food items where IAA-specific digestibility data were available.

Other assumptions were also present in the use of the underlying digestibility coefficients. Firstly, it is assumed that the IAA compositions and TID coefficients from the reference foods are an adequate representation of the consumed foods in the cohort, when these can be impacted by differences in food preparation and cultivars. Secondly, it was assumed that the digestibility is the same across all individuals in the cohort when the food matrix and the physiological aspects of digestion may differ across individuals. Thirdly, it was assumed that the TID-adjusted IAA values of individual foods remain the same even when foods are combined within a meal, when meals were paired (Chapters 5 and 7), or when portion sizes were disaggregated from the recipes. In reality, the meal matrix and interactions between foods can potentially influence digestion.

In the absence of complete data for estimating protein quality, the use of crude protein digestibility as a proxy for individual IAA digestibility could be an alternative (11). However, applying uniform protein digestibility coefficients across all IAAs may obscure variability in AA-specific digestibility between foods and limit the precision of protein quality estimates. While IAA-specific digestibility would be preferable, the gains in accuracy may be modest in practice. Evidence from human studies evaluating the true oro-ileal nitrogen digestibility found that IAA digestibility from both plant- and animal-derived proteins only varied by a few percentage points from the protein digestibility (36). Thus, protein digestibility, as a proxy for protein quality, may be sufficient until TID data become more widely available across foods and nutrients. Nevertheless, this thesis errs on the side of caution by incorporating IAA-specific digestibility information wherever possible. Given the central hypothesis that vegan diets may be more limiting in certain IAAs (lysine, leucine and the SAAs), greater methodological precision was prioritised to avoid limiting granularity of measures of protein quality. TID data for individual IAAs across varied PB foods were therefore applied when available or at least approximated at the broader FG level.

While the assessment of isoleucine, valine and AAAs in the baseline diets was not included in the main Chapters of the thesis due to limited digestibility values for many plant foods, a brief exploratory analysis was conducted. Food items were matched to coarse digestibility estimates derived from available literature (48, 49). Where direct matches were unavailable, approximate values were applied with the most similar food item. Consequently, these analyses should be considered as indicative rather than directly comparable to the more detailed assessments performed for the other IAAs in this thesis.

The results suggested that AAA intake was unlikely to be limiting in meeting requirements. Contrastingly, intakes of isoleucine and valine decreased relative to the requirements after digestibility adjustments, although the mean intakes remained above the recommended levels. Despite this, a substantial proportion of individuals fell below requirements (approximately 50 % for valine and 40 % for isoleucine). Consistent with these findings, both IAAs occasionally emerged as limiting in the meal DIAAS calculations, although lysine remained the predominant limiting AA across meals. These observations highlight the importance of considering the BCAAs in evaluations of vegan diets, which may also be at risk of deficiencies (**Table 2.1**).

As digestibility and AA composition data continue to emerge, methodological refinements were incorporated across Chapters where feasible. For example, the availability of AA composition data specific to NZ-sourced plant protein isolates (50) allowed these data to be incorporated into the DIAAS calculations in Chapters 6 and 7, improving the protein quality estimates for novel PB foods. In earlier Chapters, the AA profiles for these foods were approximated from broader protein sources (e.g., the composition of pea protein isolate was based on whole peas, as available in the USDA database).

Progressive improvements in digestibility and AA composition data can meaningfully refine model outputs. In this thesis, more accurate estimates of protein quality allowed for more precise quantification of the food quantity needed to meet protein and IAA requirements. Where protein quality was underestimated (or overestimated) due to reliance on less specific compositional data, diet optimisation models risked prescribing incorrect quantities of food. Improved protein quality data therefore supports provision of evidence-based dietary guidelines. More consolidated and accessible databases of AA composition and digestibility for foods are emerging as a result of global collaboration among food scientists (48, 51, 52).

This progress advances future protein quality research to reliably inform guidelines and nutrition policy.

As discussed in Chapter 2, there remains ongoing debate regarding the most appropriate reference values for daily protein requirements. The EAR may underestimate the needs of 50 % of individuals, and even the RDA or RDI may not adequately reflect the optimal protein requirements for all populations. This is illustrated by AMDR calculations, in which the lower bound of recommended protein intake already surpasses the RDA (53). Additional complexity arises from potential variation in protein and IAA requirements across sex and age groups, with some evidence pointing to age-related changes in protein metabolism, and sex-specific differences in the utilisation of some IAAs (54). Consequently, uncertainty surrounding the selection of EAR or RDA as reference values can meaningfully influence dietary modelling outcomes.

In Chapter 5, the EAR was used as the reference value. This choice resulted in some optimised diets falling below the lower bound of the AMDR values. This reflects potential risks as total protein intake falls below the “safe” intake level necessary to support NB. However, the use of the RDA or RDI in this context would have required substantially larger food quantities to meet protein targets within a fixed energy and serving-size constraint, thereby limiting the number of feasible solutions, despite already meeting protein quality targets. A more flexible approach was later implemented in Chapter 7 using the VPQ tool to circumvent these limitations and allow protein requirements to be adjusted to individual user needs. This approach still introduces practical challenges, as individuals may not be aware of their personal protein requirements.

Regardless of whether the EAR or RDA is used, evaluation of protein adequacy should account for digestibility, as dietary protein sources differ in metabolic activity within the body. The interpretation of dietary shortfalls relative to reference requirements warrants caution. Individual protein requirements are unknown and potentially requires consideration of energy intake, physical activity and body composition. Hence an intake below the EAR for example may indicate an increased probability of inadequacy rather than confirming inadequacy in a particular individual. The actual requirement may be higher than the requirement guideline if the variation of protein intake within the population is large. Accordingly, the prevalence of shortfalls reported in this thesis is most appropriately interpreted as an indicator of potential nutritional vulnerability and for comparison between different dietary patterns in this vegan cohort, rather than as evidence of clinical deficiency.

Computational modelling could offer a complementary approach. Structured scenario analyses could be conducted with varied protein and IAA targets, with and without digestibility adjustments, to evaluate whether requirements can be met across different life stages under varying constraints (e.g., energy and serving-size limits). For example, if higher protein targets are unattainable for certain populations (e.g., older women) within these constraints, modelling could identify when dietary strategies such as fortification, supplementation, or inclusion of isolates and novel PB foods become necessary and then quantify the resulting trade-offs in cost, energy intake, and diet acceptability. Extended versions of the VPQ tool or a similar predictive modelling framework may be a means of integrating current evidence and exploring the feasibility of varied diet scenarios to meet protein and other nutrient adequacy.

While the diet optimisation in this thesis was conducted with observed diets at the individual level, the acceptability of the dietary solutions cannot be assumed. Acceptability is influenced by factors beyond nutrient adequacy. Taste preferences, satiety responses, food preparation burden, cultural and social norms often play important roles in food and diet acceptability. This may be particularly relevant for older adults who are vegans, where emphasis on protein-dense foods may conflict with early satiety. The improved diets in Chapters 5 and 7 should therefore be assessed by vegan participants to ascertain true acceptability. User-centred methods such as focus groups or interviews can help researchers identify preferred combinations of foods in mixed meals that meet nutrient adequacy while remaining acceptable to the target population.

Dietary assessment is subject to methodological limitations and potential errors in dietary recall. Under-reporting of energy intake and consequent misreporting of macronutrients, such as protein, as well as certain micronutrients, is a recognised limitation, as evident in this vegan cohort (addressed in Chapter 3) and well documented in dietary research using self-reported dietary intake data (55, 56). Energy standardisation, such as scaling all diets to a fixed daily energy intake, can facilitate comparison of dietary composition across individuals. One objective of this thesis was to determine absolute protein and IAA intakes and compare the intakes to individual requirements. Energy standardisation was thus not applied to avoid obscuring meaningful variation in protein intake relative to energy consumption. Instead, individual energy requirements, based on BMR, age, height, BW and PAL, were incorporated into the optimisation framework in Chapters 5 and 7. This approach was considered more appropriate for the study aims, which focused on whether protein and IAA requirements can be met within energy constraints. Nevertheless, future research could benefit from triangulating dietary intake data with food frequency questionnaires and biomarkers of energy intake and nutritional status to characterise the extent of misreporting and improve confidence in modelling outcomes (57).

Determining associations between socioeconomic factors, nutritional knowledge, and nutrient adequacy was beyond the scope of this thesis. Nevertheless, understanding these relationships would be valuable for translating the present findings into effective dietary and public-health strategies. Nutritional adequacy in vegan diets, is unlikely to depend only on individual knowledge and motivation, but extend to accessibility, affordability and cultural acceptability of nutritionally appropriate foods and the wider food environment where dietary choices are made. Support in these areas are important so that people choosing vegan diets have access to an enabling food environment and evidence-based guidelines to meet individual nutritional needs, support favourable health outcomes, and strengthen public confidence in the viability of PB diets (58). This thesis may contribute to such guidance by identifying dietary patterns and food combinations associated with greater protein and IAA adequacy. Rather than providing universal recommendations for vegan diets, these findings support more targeted approaches that account for differences in dietary patterns, energy requirements, and existing protein and IAA deficiencies.

The representativeness of the study population must however be considered when interpreting the findings of this thesis. While the cohort provided detailed dietary input of individuals following vegan diets in NZ, it cannot be assumed to be representative of the wider NZ vegan population, considering the small sample size (n = 193), over-representation of females, narrow age-group distribution and limited ethnic diversity. These constrain population-level generalisability. Additionally, the convenience sampling within a particular geographical and social context may impose selection bias; for example, individuals within a university or health-conscious community may differ from the wider vegan population on motivation, nutritional knowledge and food access to various PB foods.

Finally, generalisability is further limited by cultural differences. The dietary patterns represented in this cohort largely reflect foods available and commonly consumed within a NZ or Western food environment but vegan diets in other cultural settings may be structured around substantially different staple foods and traditional sources of plant protein. The acceptability or utilisation of novel PB alternatives as part of the habitual diet may differ across populations. Cultural familiarity, food preparation practices and familiar combinations of different PB proteins may influence the protein and IAA intake. Hence, the feasibility or mathematically optimal food combinations identified in this thesis should not be assumed to be equally applicable to Asian, Latin American, African or other dietary contexts without further validation with culturally representative food composition databases and dietary patterns.

8.3 Final conclusions

Overall, total protein intake, even when adjusted for digestibility, was generally adequate in the majority of the vegan cohort. However, leucine, lysine, and the SAAs (particularly methionine) were identified as the more limiting IAAs. These findings highlight the importance of moving beyond assessments of total protein intake alone and toward evaluating individual AAs that have direct metabolic significance in the human body. Assessing individual IAA intake provides a more meaningful indicator of utilisable protein intake as these AAs ultimately determine the capacity of dietary protein in supporting key physiological processes such as MPS and metabolic regulation.

Building on the concept that IAAs are not stored in the body and therefore require regular dietary provision, this thesis then examined protein intake across EOs with a novel DTW approach to characterise temporal variation in protein intake patterns. Consistent with previous research, protein intake was unevenly distributed throughout the day, with the largest proportion consumed in the evening meals. Although the metabolic implications of this pattern are not fully understood, this finding highlights a dietary characteristic that may be relevant for protein utilisation in PB diets.

Clustering analyses further demonstrated that vegan diets are heterogeneous, even within a small population. Distinct dietary patterns emerged among individuals, with some more likely to achieve adequate protein intake and protein quality at the meal level. These findings emphasised the importance of examining food combinations and identifying patterns that are more effective in supporting protein adequacy. Legumes, pulses, nuts and seeds, both in traditional and novel food products, were beneficial in achieving protein adequacy in a vegan diet. Meals of higher protein quality were characterised by more frequent inclusion of these food groups.

This thesis demonstrated practical strategies for evaluating and improving protein quality in vegan diets at both the daily and meal levels using dietary optimisation approaches. Protein quality shortfalls were addressed while staying within realistic energy and serving-size constraints, thereby promoting dietary plausibility. The development of the VPQ tool further enabled user-led exploration of how targeted dietary adjustments can improve adequacy at both the meal and daily level, while retaining some flexibility in food choice. Through strategic FG complementation of different PB foods, this thesis provided potential solutions that can overcome the challenges associated with achieving protein quality in vegan diets.

In principle, improving diets to meet certain nutritional constraints is possible, but may entail inevitable trade-offs with other parameters (e.g., energy intake, food volume, micronutrient intake, environmental impact, and diet cost). These trade-offs were not examined in depth in this thesis but represent critical considerations for evaluating the overall sustainability and practicality of vegan diets. It remains uncertain whether the optimised diets would be acceptable in real-world settings, particularly for populations with lower energy requirements or reduced appetites, such as older adults. Although the VPQ tool accommodates higher-protein targets (with more limited meal pairings), the extent to which individuals can adhere to these meal patterns and their increased reliance on protein-dense isolates remains to be validated.

The findings of this thesis should be interpreted within the specific context of protein adequacy and protein quality of NZ vegan diets, rather than as an assessment of overall nutrient adequacy, or of PB diets across different cultural or dietary contexts. In addition, the applicability of these findings is limited to the population studied, as the dietary intake data and physiological requirements for children, adolescents, pregnant or lactating individuals, and older adults were not included in the analysis. The protein and IAA reference requirements applied in this thesis were population reference values rather than requirements derived specifically for the demographic and ethnic composition of the study cohort. Potential variation associated with factors such as age, body composition and physical activity could not be fully captured. Hence, estimates of inadequacy and food quantities generated through optimisation are conditional on the reference requirements applied. Consequently, the results cannot be used to determine whether vegan diets optimised for protein quality are appropriate across all life stages and different populations. Translation of the optimised dietary solutions into practice requires intervention studies to assess acceptability, adherence and metabolic outcomes in diverse populations. Further extensions of this work must also integrate micronutrient constraints to provide a more comprehensive assessment of nutrient adequacy in vegan diets.

The transition towards more sustainable, PB dietary patterns is a promising but complex pathway to improve both planetary and human health. While PB diets are often positioned at the forefront of food system transformation, this thesis demonstrates that nutrient adequacy, in particular protein quality, cannot be assumed, especially when the diet is restrictive and inadequately planned. Current FBDG could provide more explicit guidance, not only on consuming plant-protein foods, but also on selecting and combining complementary protein sources to improve protein and IAA adequacy.

The contribution of legumes and pulses to protein quality, and the approximate 2:1 ratio of legumes to grains provides practical dietary guidelines to supplement FBDGs like the NZ Eating and Activity Guidelines, following validations in population studies. Collaborations with dietitians, chefs, food-service providers, and public facing content creators can facilitate the development of affordable and palatable meals in everyday cooking. Digital tools such as the VPQ, other dietary apps and AI may allow recommendations to be adapted to individual energy, protein and nutrient requirements, and tailored for individual preferences. At the food-system level, these findings may also inform engagement with food manufacturers regarding the protein density, IAA composition and fortification of PBAs.

Together, these approaches could shift guidance from broad recommendations of increasing PB foods for sustainability and health towards more practical information to consumers on the quantities, combinations and dietary contexts in which these foods can support nutritional adequacy and positive impacts on human health. By advancing methods to evaluate and improve protein quality in vegan diets, this thesis contributes to a growing body of evidence needed to support informed dietary transitions that balance sustainability goals with nutrient adequacy.

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Appendices

Appendix A

Table A 1 Summary of challenges in the digestion and utilisation of IAAs from PSFs

Challenge		Key impact	Reference
Digestibility	Structural hindrance	• Hydrophobic β-conformations within plant proteins increase protein aggregation	(1-8)
		• Steric hindrance due to intercalation of plant proteins with fibre	(9)
	ANFs	• Inhibitors of trypsin, chymotrypsin, carboxypeptidases, elastase and α-amylase	(10, 11)
		• Fractionation of trypsin inhibitors within plant products during processing	(11, 12)
		• Chelation of phytates with carboxypeptidases and aminopeptidases	(11)
	Processing techniques	• Heating reduces protein digestibility • Phytates may be heat-resistant, but heating damages phytase	(11, 13)
		• Inaccuracy in measured TID values for some industrially or home-based processed foods	(14, 15)
	Current assessments	• Under-representation of novel PBAs in DIAAS databases • Reference values of IAA quantity used in PDCAAS still used in DIAAS • Current RDA is based on assumption of high-quality protein intake, and higher values may be required for poorer-quality protein in PSFs • Under-representation of digestibility measurements for mixed vegan diets, with the current focus mainly on isolated proteins • Continual use of the same nitrogen-to-protein conversion factor of 6.25 in DIAAS, but larger discrepancies can emerge for PSFs	(14-21)

Utilisation	Reduced utilisation	• Redirection of AA (e.g., sulphur-containing IAAs) from protein synthesis to enzyme production	(10, 11)
		• Increased splanchnic retention of AAs, leading to reduced bioavailability to peripheral tissues	(20, 22)
		• Increased urea conversion for some PSPs (e.g., soy and wheat) as compared to ASPs (e.g., whey), leading to reduced bioavailability for metabolism	(20, 23-26)
		• Presence of limiting IAAs in commonly consumed plant foods halts protein synthesis even in a surplus of other IAAs, which becomes oxidised	(27-29)
		• Post-translational modification of some IAAs leading to reduced protein synthesis	(30-32)
		• Evidence of lower plasma AA concentrations and myofibrillar protein synthesis or muscle mass retention with PSPs as compared to ASPs	(33-37)
	Protein distribution across the day	• Uneven distribution of protein intake throughout the day may influence the sensitivity of signalling pathways to postprandial AA concentration and MPS • Skewed protein intake, with higher intake in evening meals, could mean protein threshold is only met once a day or surplus IAAs are oxidised	(38, 39)

TID, True Ileal Digestibility; DIAAS, Digestible Indispensable Amino Acid Score; PBAs, Plant-Based Alternatives; IAA, Indispensable Amino Acid; PDCAAS, Protein Digestibility Corrected Amino Acid Score; RDA, Recommended Dietary Allowance, PSF, Plant-Sourced Food; AA, Amino Acid; PSP, Plant-Sourced Protein; ASP, Animal-Sourced Protein; MPS, Muscle Protein Synthesis

Table A 2 Summary of challenges in protein quality for novel PBAs

Type of PBA	Challenge	Key impact	Reference
Protein isolates	• Attachment of phytates to proteins when extracting isolates • Certain processing techniques and heat treatments, the presence of plant-cell walls and ANFs	Reduces AA bioavailability	(40, 41)
	• Other nutritional components, e.g., fat	Inhibit protein denaturation Adverse nutritional/health outcomes	(42)
	• Food manufacturing processes in industrial or home settings can chemically alter AA function (e.g., Maillard reaction)	• Reduce nutritional viability of certain AAs such as lysine, cysteine, tryptophan and threonine • TID measurements may be inaccurate	(15, 43-46)
PBMA	• Variable protein content and IAA composition across different brands and regions • Limited understanding of digestibility	Without further nutritional analysis, supported by sufficient databases, the nutritional suitability of replacing traditional PSFs and ASFs with PBMAs is inconclusive	(42, 47, 48)
PBB	• Poorer contribution of IAAs from PBBs as compared to milk	Inappropriate food sources for more vulnerable populations with higher nutrient needs, from a protein quality perspective	(49-51)
	• Raw forms of PSF, rather than as part of the product's formulation, were used in the analysis	Misrepresentation of the true impact of protein intake and protein quality from these foods	(50-53)

AA, Amino Acid; ANF, Anti-Nutritional Factor; TID, True Ileal Digestibility; IAA, Indispensable Amino Acid; PSF, Plant-Sourced Foods; ASF, Animal-Sourced Food; PBMAs, Plant-Based Meat Alternatives; PBBs, Plant-Based Beverages

Appendix B

Table B 1 Energy intake as distinguished by sex and age

Sex	Energy requirement (MJ)		Mean energy intake (MJ)	Percentage adequate (%)
	Age (years)	Range from PAL of low to moderate activity (MJ)		
Male	19 – 30 (n = 9)	10.8 – 13.8	11.2(1.9)	55.6
	31 – 50 (n = 29)	11 – 16.1	10.7(2.4)	37.9
	51 – 70 (n=14)	9.5 – 12.1	9.4(3.5)	28.6
	> 70 (n=0)	7.4 – 13.6	-	-
Female	19 – 30 (n=43)	8.1 – 10.5	7.5 (1.6)	30.2
	31 – 50 (n=65)	7.9 – 10.1	7.7 (2.0)	41.5
	51 – 70 (n=31)	7.6 – 9.6	7.0 (1.6)	35.5
	> 70 (n=2)	7.1 – 9.1	10.1 (3.2)	100

PAL, Physical Activity Level. Energy requirement values are obtained from the NRV of Australia and New Zealand, which provided a low to moderate activity range (1) and based on 76 kg male and 61 kg female. Percentage adequacy is defined as the proportion of individuals at or above the low end of the energy requirements range. For males, this would be 10.8 MJ (19 to 30 years), 11 MJ (31 to 50 years), 9.5 MJ (51 to 70 years), 7.4 MJ (above 70 years), and for females, this would be 8.1 MJ (19 to 30 years), 7.9 MJ (31 to 50 years), 7.6 MJ (51 to 70 years) and 7.1 MJ (above 70 years). Dash lines indicate that the calculations are not applicable, as there are no males aged 70 or older in the study.

Table B 2 Protein intake and body composition

Sex	*n*	Mean Energy intake in MJ	Mean AMDR for protein in percentage	Mean weight in kg	Mean BMI in kg/m^2	Mean body fat in percentage
Adequate for protein						
Female	103	7.9 (1.8)	12.8 (3.9)	63.7 (8.6)	22.9 (2.7)	31.8 (5.8)
Male	41	11.1 (2.5)	12.9 (3.6)	77.0 (11.1)	24.1 (2.8)	22.0 (4.8)
Inadequate for protein						
Female	38	6.3 (1.4)	9.9 (1.9)	72.1 (12.6)	26.0 (3.4)	37.5 (4.8)
Male	11	8.1 (1.7)	10.3 (1.8)	88.4 (13.5)	26.5 (3.2)	26.1 (4.4)

n represents the number of individuals. Values represented as mean (standard deviation) unless stated. Adequacy for protein is derived by comparing individual protein intake in g/kg/day with the EAR provided by the NRV of Australia and New Zealand (54) which is 0.60 g/kg/day for females and 0.68 g/kg/day for males, between 19 and 70 years of age, and 0.75 g/kg/day for older females.

Table B 3 Energy and AMDR of individuals who were below the daily requirements for lysine and leucine

	Male			Female		
Age (years)	*n*	Mean Energy intake (Percentage adequacy in %)	Mean AMDR (Percentage adequacy in %)	*n*	Mean Energy intake (Percentage adequacy in %)	Mean AMDR (Percentage adequacy in %)
Inadequate for lysine						
19 - 30	3	9.6 (0)	10.2 (33.3)	22	6.7(13.6)	10.5 (59.1)
31 – 50	15	9.4 (20.0)	10.7 (73.3)	40	7.5 (37.5)	10.6 (57.5)
51 – 70	7	7.8 (14.3)	10.4 (71.4)	22	6.6 (27.2)	10.7 (68.2)
> 70	-	-	-	-	-	-
Inadequate for leucine						
19 - 30	1	9.8 (0)	9.8 (0)	18	6.5 (5.5)	10.2 (50.0)
31 – 50	10	9.2 (10)	11.6(70.0)	30	7.2 (26.6)	10.3 (50.0)
51 – 70	6	7.3 (0)	10.5 (83.3)	20	6.4 (20.0)	10.6 (70.0)
> 70	-	-	-	-	-	-

n represents the number of individuals. Energy requirement values are obtained from the NRV of Australia and New Zealand (54) and based on a 76 kg male and a 61 kg female. Percentage adequacy is defined as the proportion of individuals who meet or exceed the lower range of energy requirements. For males, this would be 10.8 MJ (19 to 30 years), 11 MJ (31 to 50 years) and 9.5 MJ (51 to 70 years). For females, this would be 8.1 MJ (19 to 30 years), 7.9 MJ (31 to 50 years) and 7.6 MJ (51 to 70 years). Dash lines represent not-applicable calculations, as there are no males or females aged 70 or older who are inadequate for TID-adjusted lysine and leucine in the study. AMDR (Acceptable Macronutrient Distribution Range) for protein is between 10 % and 35 % of caloric intake. (55) Percentage adequacy is represented by individuals who meet or exceed the lower end of the AMDR for protein (10 %).

Appendix C

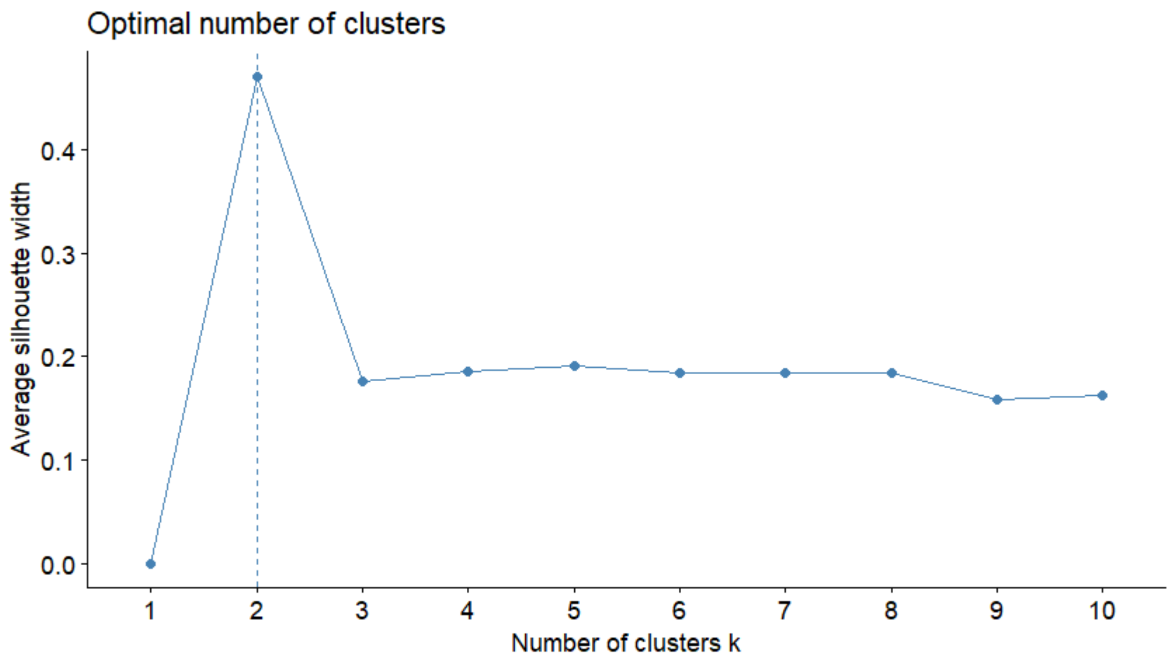


Figure C 1 Mean silhouette coefficient
The mean silhouette coefficient is calculated to objectively guide the optimal number of clusters (k). The dashed vertical line represents the optimal number of clusters.

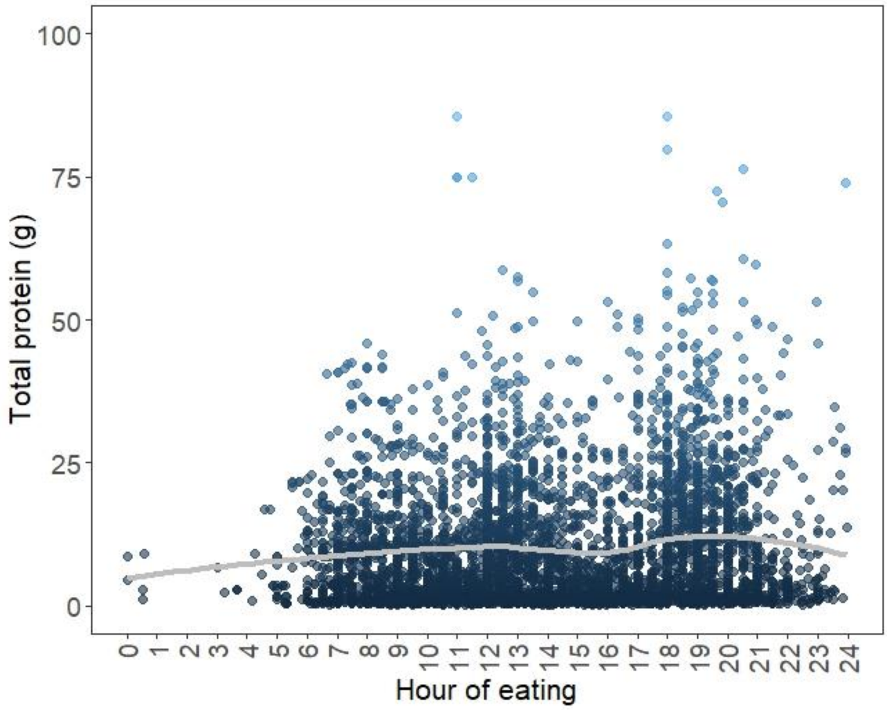


Figure C 2 A scatter plot and spline curve representing total protein distribution across 24 hours for all the EOs of 193 participants
A higher number of EOs contain a low protein intake of < 10g per EO. The fewest EOs contain > 40 g per EO and were not represented in **Figure 4.3**. The spline curve shows a gradual increase in EOs over 24 hours, with higher intake in the later hours of the day.

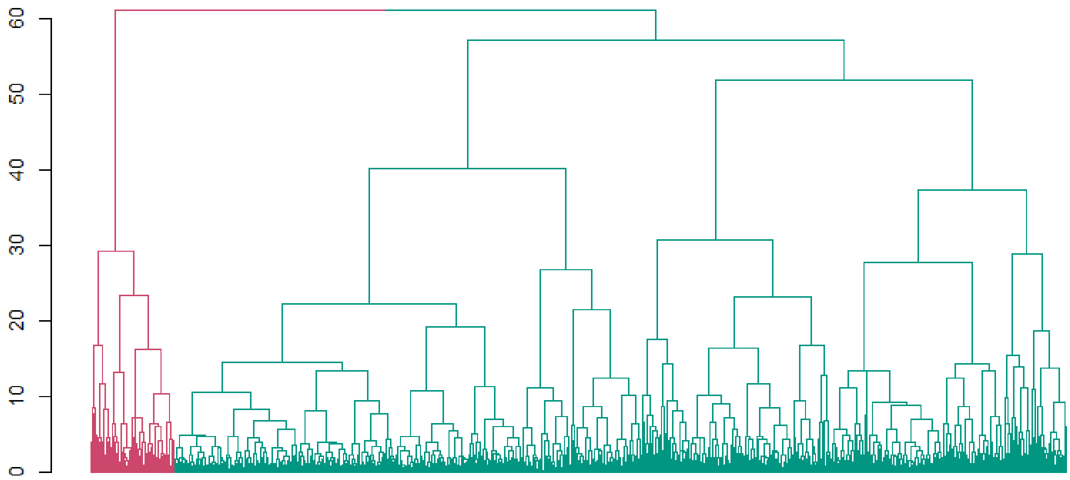


Figure C 3 Dendrogram clusters using k = 2
Colours denote two different clusters.

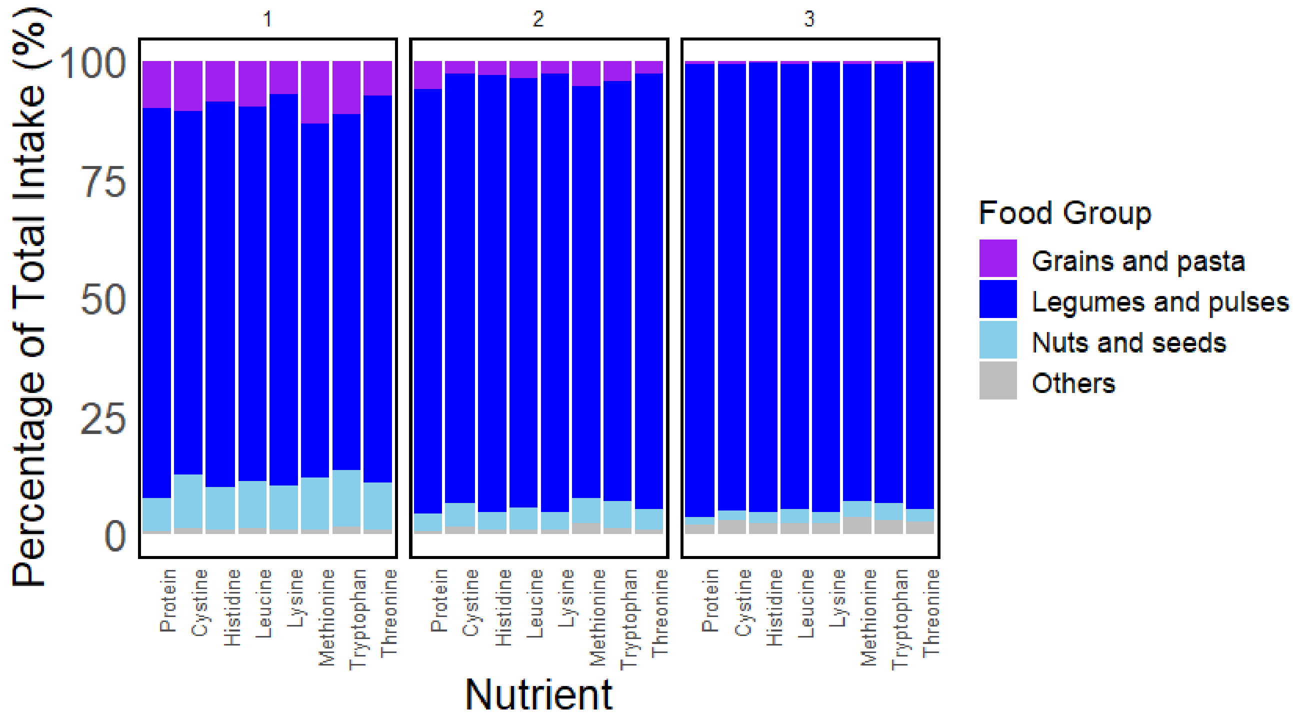


Figure C 4 The contribution of food groups that are the primary protein contributors in novel PBAs to total protein and IAA intake

Analysis was conducted within clusters, using the scaled percentage of total intake. Novel PBAs include PBMA and PBB. “Others” represent “vegetables” and “savoury sauces and condiments” that were the main ingredients in these novel alternatives. Numbers 1 to 3 represent clusters 1, 2 and 3.

Table C 1 FG contribution to the percentage of total intake for protein and each IAA across clusters 1 to 3

Food Group	Nutrient	% of Total intake across clusters		
		1	**2**	**3**
Fruits	Protein	2.8	1.6	0.9
	Cystine	3.7	2.1	1.2
	Histidine	5.2	2.9	1.7
	Leucine	2.6	1.4	0.8
	Lysine	3.6	1.9	0.9
	Methionine	4.2	2.4	1.3
	Tryptophan	2.4	1.3	0.8
	Threonine	3.2	1.8	1.0
Grains and pasta	Protein	34.1	32.2	23.5
	Cystine	41.0	38.1	27.9
	Histidine	31.9	29.7	21.2
	Leucine	34.7	32.8	23.8
	Lysine	24.1	22.2	16.4
	Methionine	36.3	34.9	26.3
	Tryptophan	37.0	35.5	28.7
	Threonine	30.0	28.4	21.1
Legumes and pulses	Protein	35.5	41.7	57.0
	Cystine	30.3	37.3	52.4
	Histidine	37.8	44.0	58.1
	Leucine	37.7	42.8	56.7
	Lysine	48.4	55.0	66.5
	Methionine	29.8	34.6	48.3
	Tryptophan	32.1	36.0	48.0
	Threonine	39.4	44.8	58.3

Nuts and seeds	Protein	13.3	12.7	10.4
	Cystine	16.4	14.9	12.8
	Histidine	17.5	16.9	14.1
	Leucine	17.3	16.4	13.5
	Lysine	14.0	12.7	10.1
	Methionine	19.8	19.1	17.1
	Tryptophan	18.3	18.1	15.1
	Threonine	16.0	14.9	12.2
Potatoes, kumara, taro	Protein	2.0	1.8	0.6
	Cystine	1.8	1.5	0.6
	Histidine	1.9	1.5	0.5
	Leucine	1.9	1.5	0.5
	Lysine	2.7	2.1	0.7
	Methionine	2.2	1.9	0.7
	Tryptophan	2.6	2.1	0.8
	Threonine	2.3	2.0	0.7
Vegetables	Protein	6.1	5.2	3.2
	Cystine	5.6	5.0	3.1
	Histidine	4.5	4.0	2.4
	Leucine	4.4	3.8	2.5
	Lysine	5.3	4.4	2.6
	Methionine	6.3	5.9	4.0
	Tryptophan	5.9	5.5	4.0
	Threonine	7.4	6.6	4.2
Others	Protein	6.1	4.7	4.4
	Cystine	1.3	1.1	2.1
	Histidine	1.3	1.1	2.0

Leucine	1.5	1.3	2.3
Lysine	1.9	1.7	2.8
Methionine	1.4	1.3	2.3
Tryptophan	1.7	1.5	2.7
Threonine	1.7	1.5	2.6

"Others" included "beverages", "savoury sauces and condiments", and "sugars and sweet".

Table C 2 P values from Dunn's test comparing the absolute intake of protein and IAA contributed by each FG across clusters.
P values (after Bonferroni adjustment) < 0.05 show a statistically significant difference and are in bold. The table shows P values for FGs across the clusters. NS indicates no significant difference as determined by the KW test.

Food Group	Nutrient	P values in clusters		
		1–2	**2–3**	**1–3**
Grains and pasta	Protein	**< 0.001**	1.00	**< 0.001**
	Cystine	**< 0.001**	1.00	**0.001**
	Histidine	**< 0.001**	1.00	**0.001**
	Leucine	**< 0.001**	1.00	**< 0.05**
	Lysine	**< 0.001**	1.00	**< 0.001**
	Methionine	**< 0.001**	1.00	**< 0.05**
	Tryptophan	**< 0.001**	1.00	**< 0.05**
	Threonine	**< 0.001**	1.00	**0.02**
Legumes and pulses	Protein	**< 0.001**	**< 0.001**	**< 0.001**
	Cystine	**< 0.001**	**< 0.001**	**< 0.001**
	Histidine	**< 0.001**	**< 0.001**	**< 0.001**
	Leucine	**< 0.001**	**< 0.001**	**< 0.001**
	Lysine	**< 0.001**	**< 0.001**	**< 0.001**
	Methionine	**< 0.001**	**< 0.001**	**< 0.001**
	Tryptophan	**< 0.001**	**< 0.001**	**< 0.001**
	Threonine	**< 0.001**	**< 0.001**	**< 0.001**
Nuts and seeds	Protein	0.06	0.30	**< 0.01**
	Cystine	0.12	**0.03**	0.82
	Histidine	0.05	**<0.01**	0.28
	Leucine	0.07	0.28	**< 0.01**
	Lysine	0.22	0.46	**0.02**
	Methionine	**0.04**	1.00	**0.02**

	Tryptophan	0.08	1.00	**0.03**
	Threonine	0.20	0.63	**0.03**
Vegetables	Protein	0.14	0.39	**0.01**
	Cystine	0.08	0.28	**< 0.01**
	Histidine	0.17	0.23	**< 0.01**
	Leucine	0.40	0.25	**0.02**
	Lysine	0.23	0.18	**< 0.01**
	Methionine	0.25	0.17	**< 0.01**
	Tryptophan	0.06	0.31	**< 0.01**
	Threonine	0.14	0.23	**< 0.01**
Fruit	Protein	NS	NS	NS
	Cystine	0.49	0.16	**0.01**
	Histidine	NS	NS	NS
	Leucine	NS	NS	NS
	Lysine	0.55	0.06	**< 0.01**
	Methionine	0.18	0.06	**< 0.001**
	Tryptophan	0.46	**0.04**	**< 0.01**
	Threonine	0.71	0.15	**0.02**
Others	Protein	0.09	**0.03**	**< 0.001**
	Cystine	NS	NS	NS
	Histidine	0.06	0.88	0.23
	Leucine	NS	NS	NS
	Lysine	NS	NS	NS
	Methionine	0.06	0.81	0.26
	Tryptophan	NS	NS	NS
	Threonine	NS	NS	NS

“Others” included “beverages”, “savoury sauces and condiments”, and “sugar and sweets”. “Potatoes, kumara and taro” are not represented in the table as the KW test showed no significant differences across all nutrients among the clusters.

Appendix D

Table D 1 Upper limits of IAAs

Amino acid (g/day)	UL	NOAEL[1]	LOAEL[2]
Leucine (young adults)	35		
Tryptophan	4.5		
Methionine		3.2	6.4
Lysine		6	7.5
Histidine		8	12

UL, tolerable upper intake level; NOAEL, no-observed-adverse-effect-level; LOAEL, lowest-observed-adverse-effect-level, as adapted from Elango (2023) (56)

[1] indicates the highest intake or experimental oral dose of a nutrient where no adverse effects have been observed

[2] indicates the lowest intake or experimental oral dose of a nutrient where an adverse effect has been observed

Table D 2 Minimum daily requirements and upper limits of nutrients

Nutrient	Sex	Age	AI / EAR / day	Upper limit / day
ALA	Male	19 and above	1.3 g	-
	Female	19 and above	0.8	-
Dietary fibre	Male	19 and above	30 g	-
	Female	19 and above	25g	-
Vitamin B12	Male and female	19 and above	2.0 µg	-
Calcium	Male	19 to 70	840 mg	2500 mg
		Above 70	1100 mg	
	Female	19 to 50	840 mg	
		Above 51	1100 mg	
Iodine	Male and female	19 and above	100 µg	1100 µg
Iron	Male	19 and above	6 mg	45 mg
	Female	19 to 50	8 mg	
		Above 51	5 mg	
Sodium	Male and female	18 and above	2000 mg	-
Zinc	Male	19 and above	12 mg	40 mg
	Female		6.5 mg	

AI, Adequate intake; EAR, Estimated Average Requirement; ALA, Alpha-Linolenic Acid

Table D 3 Mean contribution of dietary fibre, ALA and micronutrients (%) for each added FG during optimisation

Cluster 1								
	Nutrient contribution to daily requirement [1] (%)							
Food group	ALA	Dietary fibre	Calcium	Iron	Iodine	Sodium	Vitamin B12	Zinc
Fruit	0	19.3	8.46	33.6	0.29	1.85	0	4.76
Grains and pasta	30.5	18.7	6.73	29.3	18.3	13.6	2.84	19.2
Legumes and pulses	41.2	14.3	10.8	38.2	7.96	16.2	14.9	23.7
Nuts and seeds	144.0	12.3	4.14	21.2	0.98	1.28	0.91	15.1
Potatoes, kumara and taro	3.69	4.16	0.75	5.43	1.68	13.0	0	3.72
Sugar and sweets	1.99	4.52	1.60	19.4	1.24	0.35	1.54	7.44
Vegetables	5.27	9.31	2.59	18.6	28.1	1.41	196.3	27.6
Yeast and condiments	0.17	11.0	1.63	17.7	2.42	14.1	0	26.1
Cluster 2								
Fruit	7.33	18.2	7.69	25.2	0.04	3.85	0	2.32
Grains and pasta	15.7	15.8	5.99	28.0	12.5	13.1	1.10	14.3
Legumes and pulses	29.3	12.9	11.6	41.3	9.53	14.4	15.6	18.9
Nuts and seeds	102.1	10.3	3.73	21.6	1.22	1.40	0.76	14.8
Potatoes, kumara and taro	4.55	6.08	1.19	8.66	1.95	10.9	0	4.83
Sugar and sweets	1.56	4.08	1.29	21.6	1.03	0.19	1.43	5.50
Vegetables	13.5	5.61	3.03	9.00	4.68	3.16	26.2	4.17

Yeast and condiments	0	7.05	1.04	46.6	1.25	22.3	33.6	13.6
Cluster 3								
Grains and pasta	24.2	15.2	4.21	17.3	6.89	8.90	2.80	12.6
Legumes and pulses	22.8	9.11	10.2	34.3	7.97	12.4	9.20	12.0
Nuts and seeds	65.4	9.73	3.64	15.3	0.98	1.40	0	12.6
Potatoes, kumara and taro	5.77	8.50	1.92	13.8	3.90	26.4	0	5.31
Sugar and sweets	3.75	7.73	1.84	34.0	1.84	0.42	2.25	7.60
Vegetables	0	1.50	0.55	17.8	66.0	1.58	471.0	0.92

ALA, Alpha-Linolenic Acid.

Nutrient contribution (%) is calculated as the total intake of each nutrient per FG as a percentage of the daily nutrient requirement at the EAR level (54).

Table D 4 Mean baseline and final intake, and percentage adequacy of other micronutrients across clusters 1 to 3

	Vitamin A (mcg)	Vitamin B6 (mg)	Niacin (mg)	Thiamine (mg)	Folate (mcg)	Vitamin C (mg)	Vitamin E (mg)	Potassium (mg)	Magnesium (mg)	Phosphorus (mg)	Selenium (mcg)
Cluster 1											
Baseline											
Mean intake	864.32	2.09	13.4	1.47	363.4	120.2	15.6	3213.4	416.4	1083.9	39.9
	(932.8)	(1.79)	(7.08)	(1.01)	(363.4)	(129.0)	(10.4)	(1209.3)	(154.2)	(377.9)	(31.1)
% adequacy	53.0	72.6	54.8	70.8	53.9	81.7	85.8	53.9	82.2	96.3	13.7
Final											
Mean intake	834.7	2.20	18.1	1.90	454.5	107.6	24.5	3551.02	592.0	1525.6	54.2
	(899.8)	(1.69)	(7.70)	(1.10)	(237.2)	(105.1)	(19.3)	(1051.4)	(177.8)	(427.5)	(32.8)
%adequacy	51.1	83.6	84.5	89.5	72.1	78.5	94.5	71.2	98.2	100	37.9
Cluster 2											
Baseline											
Mean intake	781.6	1.92	15.4	1.74	384.7	118.6	16.6	3252.8	479.7	1304.3	42.7
	(707.3)	(1.22)	(7.9)	(1.19)	(384.7)	(112.7)	(11.9)	(1102.8)	(156.4)	(380.7)	(27.4)
% adequacy	51.2	76.9	67.2	85.1	58.5	84.6	83.1	51.8	90.8	97.4	18.5
Final											
Mean intake	725.3	2.05	19.4	2.07	456.7	111.7	25.1	3450.4	627.4	1677.2	54.6
	(671.9)	(1.32)	(9.36)	(1.28)	(232.7)	(119.0)	(23.3)	(1089.5)	(190.1)	(441.9)	(30.0)
% adequacy	48.7	84.1	86.7	95.4	69.7	82.1	92.8	57.9	98.5	99.5	40.5

Cluster 3											
Baseline											
Mean intake	723.1 (817.0)	1.86 (1.10)	17.4 (7.56)	1.60 (0.76)	430.4 (430.4)	77.6 (70.6)	19.4 (22.4)	2966.2 (923.0)	447.6 (164.0)	1394.4 (402.13)	53.2 (39.5)
% adequacy	27.8	75.0	83.3	77.8	66.7	63.9	91.7	41.7	83.3	100	22.2
Final											
Mean intake	727.5	1.95	21.0	1.82	459.2	76.5	26.8	3172.9	545.5	1631.9	59.2
	(820.8)	(1.07)	(8.80)	(0.70)	(209.2)	(71.6)	(34.1)	(898.3)	(164.9)	(452.7)	(38.6)
% adequacy	38.9	75	88.9	88.9	69.4	66.7	88.9	50.0	91.7	100	47.2

Appendix E

Table E 1 Relative FG ratios characterising higher and lower- protein quality vegan meals

Each row uses one FG as the reference ratio, set to 1.00, and the values in the row indicate the proportional contribution of other FGs relative to that reference. For example, in higher DIAAS meals, when grains were set to 1.00, legumes appeared, on average, 2.205 times more often. Ratios for isolates are not shown in this table because other FGs have very elevated ratios (> 100) relative to isolates, possibly because isolates contribute comparatively less weight than other foods.

	Meal protein quality based on DIAAS									
	Higher	Lower	Higher	Lower	Higher	Lower	Higher	Lower	Higher	Lower
FGs	Ratio to grains		Ratio to legumes		Ratio to nuts/seeds		Ratio to fruits		Ratio to vegetables	
Grains	1.00	1.00	0.45	1.39	1.37	1.88	3.31	7.02	2.03	13.1
Legumes	2.20	0.72	1.00	1.00	3.03	1.36	7.29	5.06	4.47	9.41
Nuts/seeds	0.73	0.53	0.33	0.74	1.00	1.00	2.41	3.73	1.48	6.92
Fruits	0.30	0.14	0.14	0.20	0.42	0.27	1.00	1.00	0.61	1.86
Vegetables	0.49	0.08	0.22	0.11	0.68	0.14	1.63	0.54	1.00	1.00

Table E 2 Contingency table

The table shows daily clusters (1 to 3) x PCA meal composition clusters (A to H) and presence/absence of shortfall (Yes/No) x PCA meal composition clusters. Key Pearson residuals selected for discussion are highlighted in red.

Daily cluster		A	B	C	D	E	F	G	H	Total
Cluster 1	Observed	37	230	47	164	227	126	178	337	1346
	Expected	54.6	228.9	71.8	173.5	176.4	148.5	207.6	284.7	1346
	Pearson residual	-2.38	0.07	-2.93	-0.72	3.81	-1.85	-2.05	3.10	
	Adjusted Pearson residual	-3.16	0.10	-3.92	-1.00	5.32	-2.55	-2.91	4.54	
Cluster 2	Observed	71	211	101	197	156	186	231	289	1442
	Expected	58.4	245.3	76.9	185.9	189.0	159.1	222.4	305.0	1442
	Pearson residual	1.64	-2.19	2.75	0.81	-2.40	2.13	0.58	-0.92	
	Adjusted Pearson residual	2.24	-3.21	3.77	1.12	-3.44	3.02	0.84	-1.38	
Cluster 3	Observed	25	117	27	62	47	50	97	68	493

	Expected	20.0	83.8	26.3	63.6	64.6	54.4	76.0	104.3	493
	Pearson residual	1.12	3.62	0.14	-0.20	-2.19	-0.60	2.40	-3.55	
	Adjusted Pearson residual	1.24	4.31	0.15	-0.23	-2.55	-0.69	2.84	-4.34	
Presence of a shortfall										
Yes	Observed	68	311	96	256	252	221	294	489	1987
	Expected	80.5	338	106	256.1	260.4	219.2	306.4	420.3	1987
	Pearson residual	-1.40	-1.46	-0.97	-0.01	-0.52	0.12	-0.71	3.35	
	Adjusted Pearson residual	-2.27	-2.56	-1.59	-0.02	-0.89	0.20	-1.23	6	
No	Observed	65	247	79	167	178	141	212	205	1294
	Expected	52.5	220	69	166.8	170	142.8	199.6	273.7	1294

	Pearson residual	1.73	1.82	1.21	0.01	0.65	-0.15	0.88	-4.15
	Adjusted Pearson residual	2.27	2.56	1.59	0.02	0.89	-0.20	1.23	-6

*Pearson value > 2 or 3 (highlighted in red) has a significant deviation from expectancy (57)

Table E 3 Cluster centroids dimensions on PC1 and PC2

Cluster	PC1	PC2
A	2.93	11.3
B	9.68	5.18
C	8.85	-4.96
D	6.21	-5.60
E	-8.40	9.68
F	-10.6	-1.82
G	-0.26	2.02
H	-3.46	-8.20

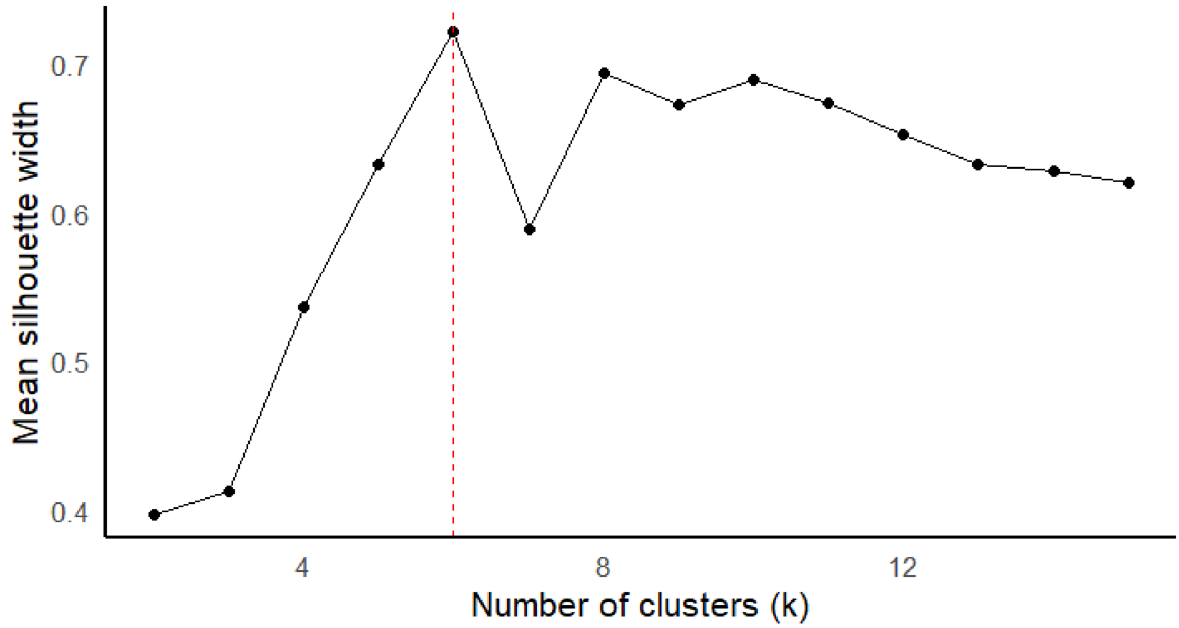
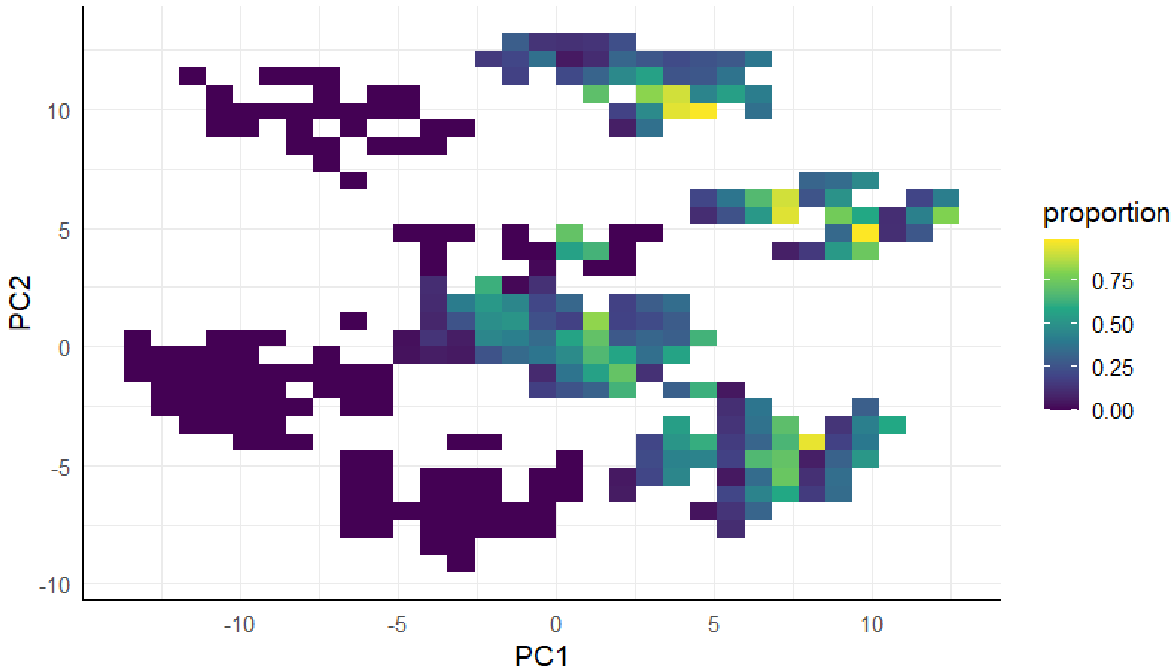
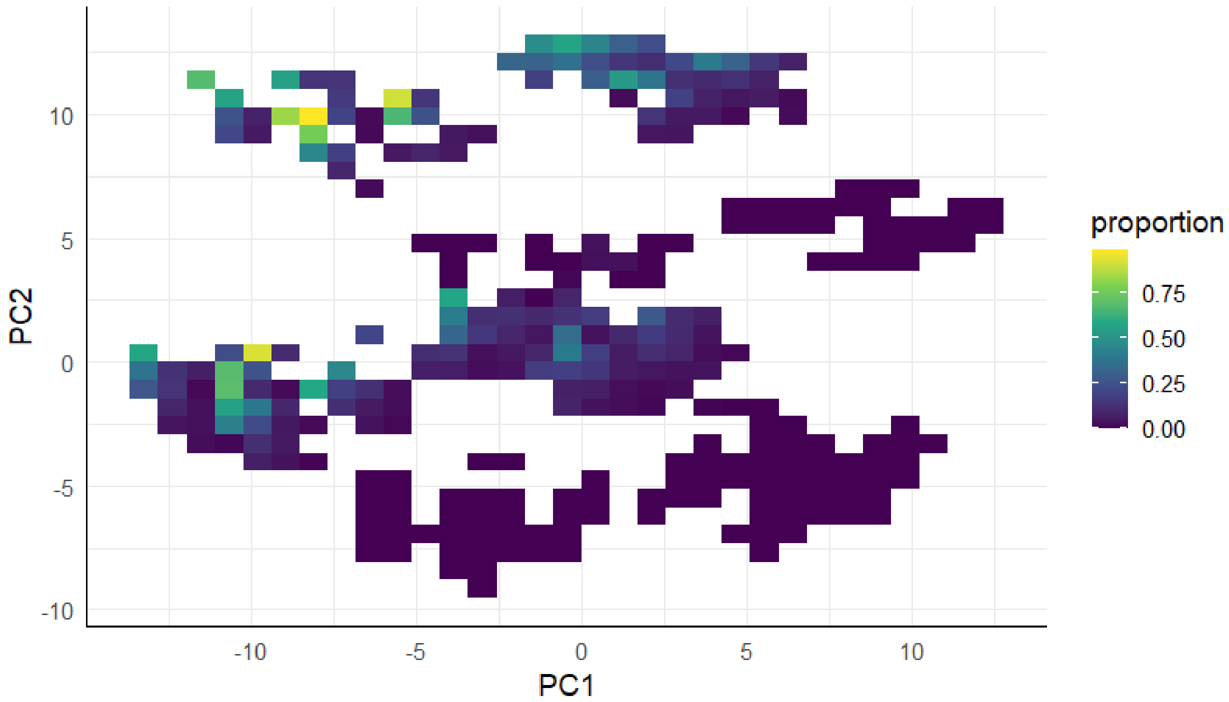


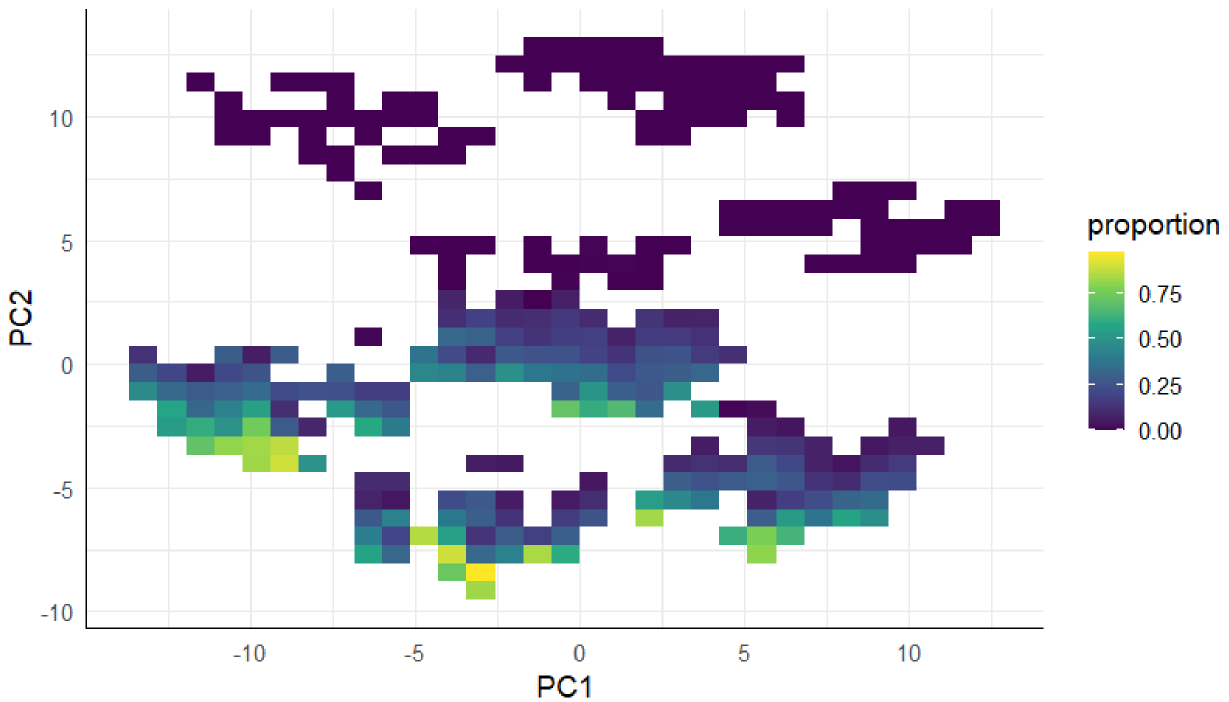
Figure E 1 Silhouette analysis to guide the optimal number of clusters for distinction with PCA The dashed red line indicates that optimal k = 6, which has the highest mean score of 0.72.



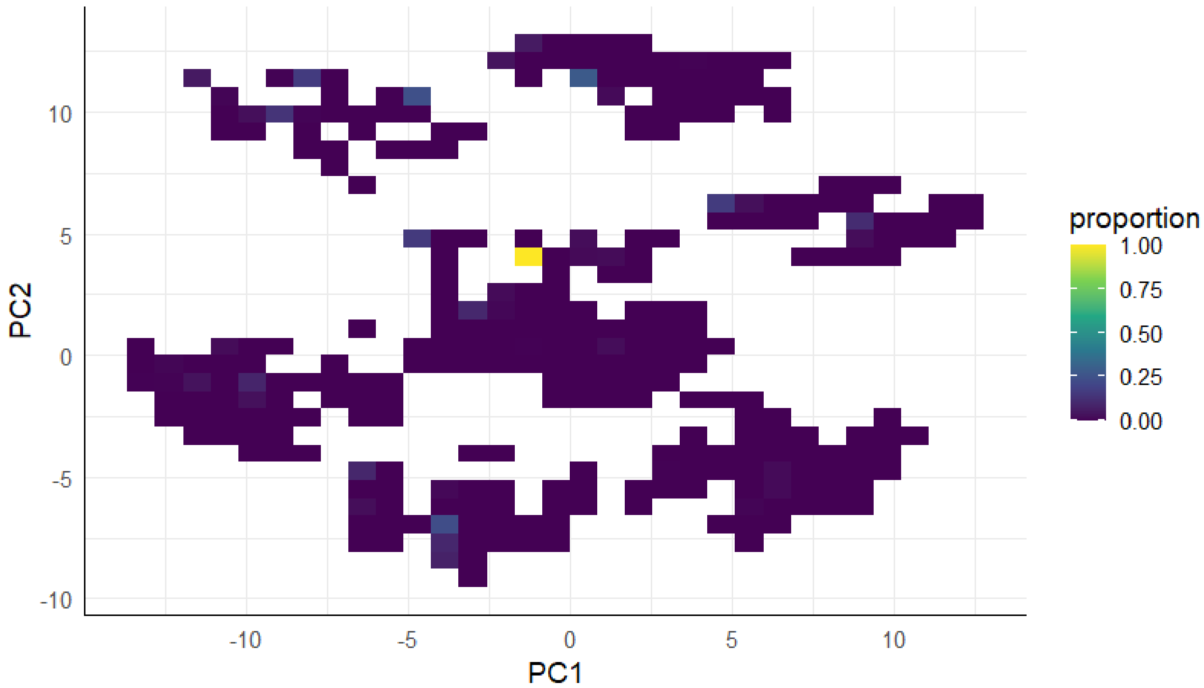
2a. Proportion of legumes across the PCA axes. A higher proportion of legumes within meal compositions was observed in the positive sides of PC1 and PC2.



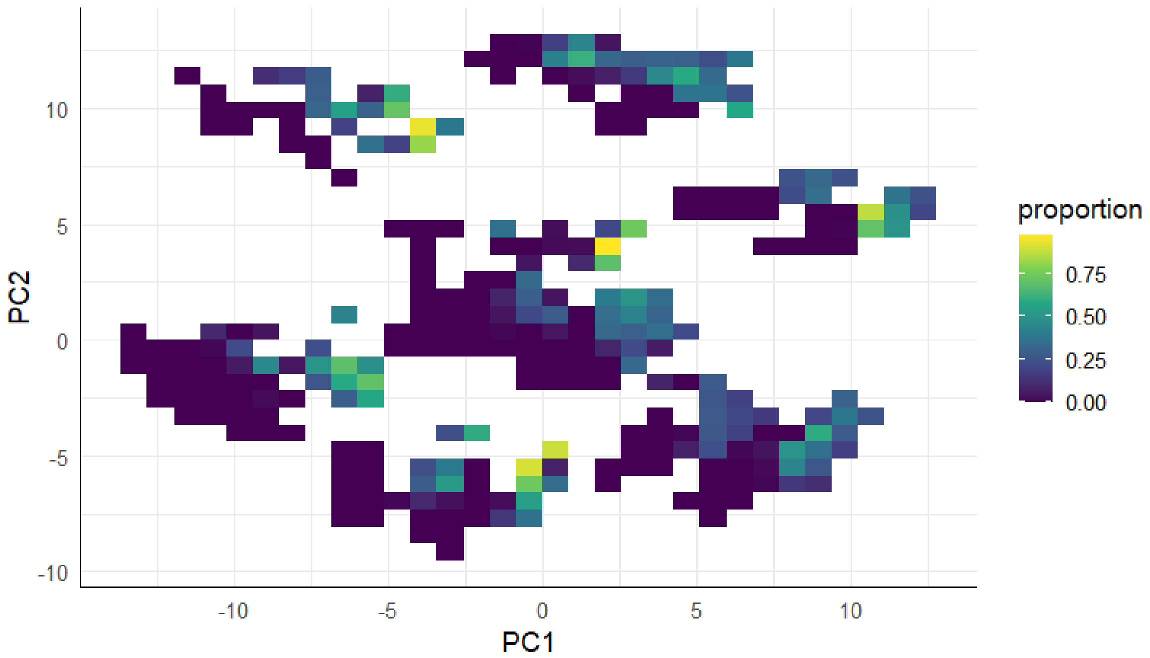
2b. Proportion of nuts and seeds across the PCA axes. A higher proportion of nuts and seeds within meal compositions was observed in the negative side of PC1, but in the positive side of PC2.



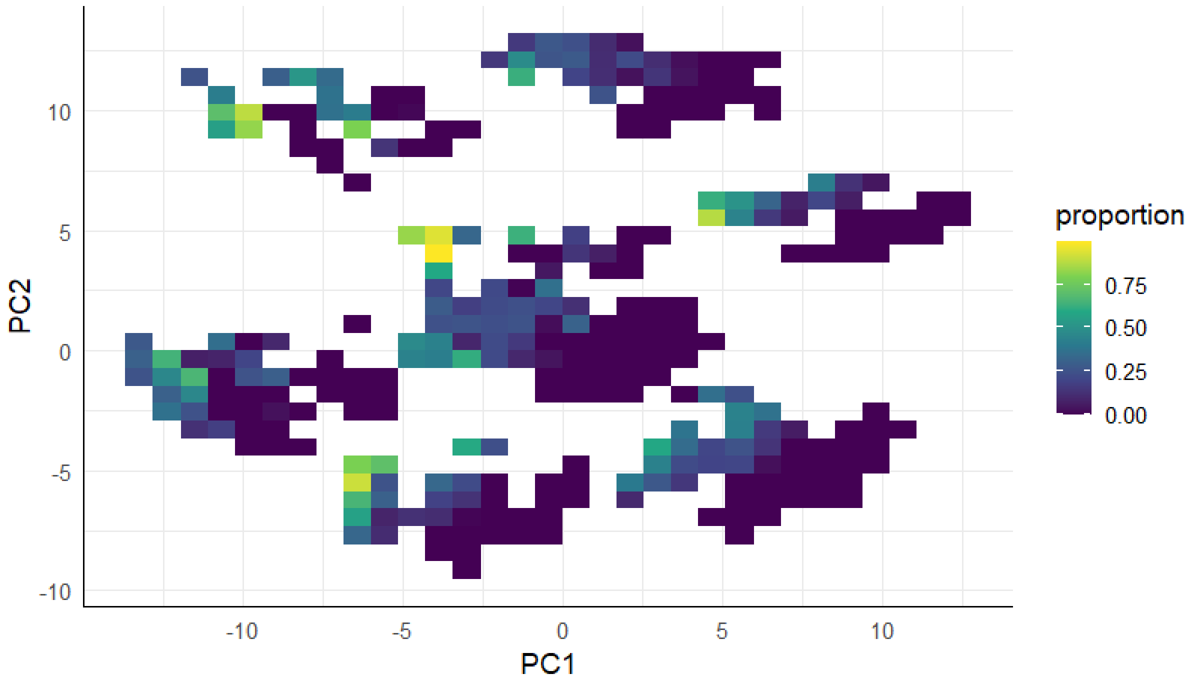
2c. Proportion of grains across the PCA axes. A higher proportion of grains within meal compositions was observed in the negative sides of PC1 and PC2.



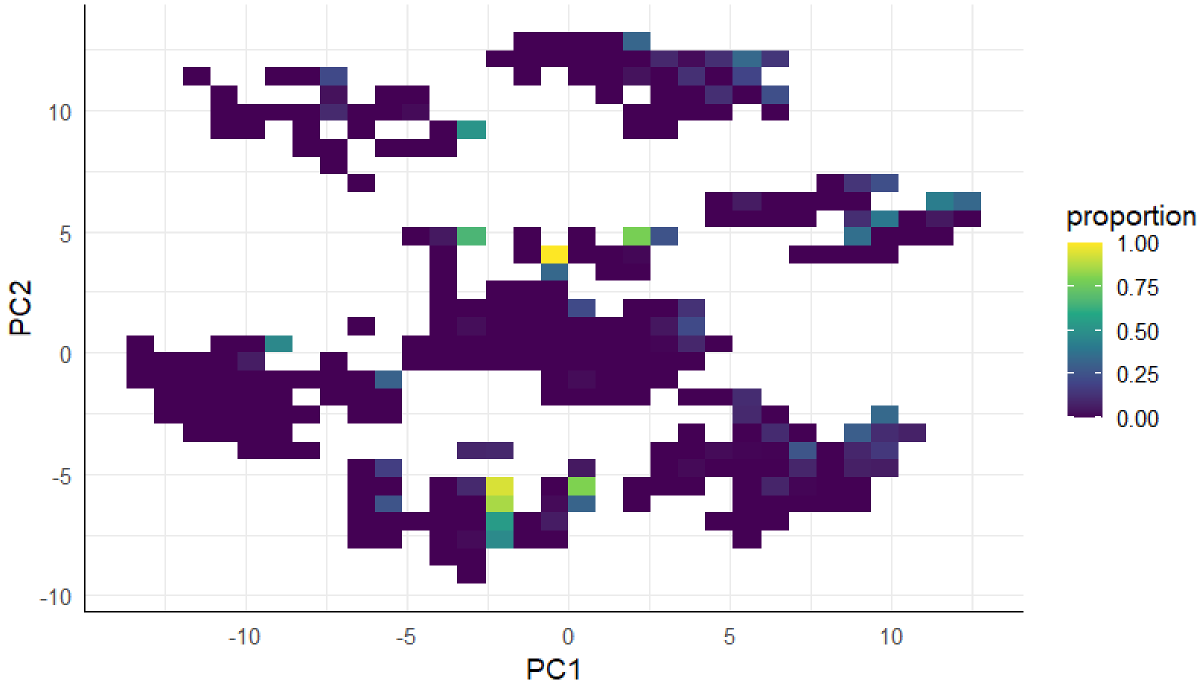
2d. Proportion of isolates across the PCA axes. Very low proportions were observed across all meal compositions and were evenly distributed across the PCA axes, indicating isolates were not a dominant FG in any clusters.



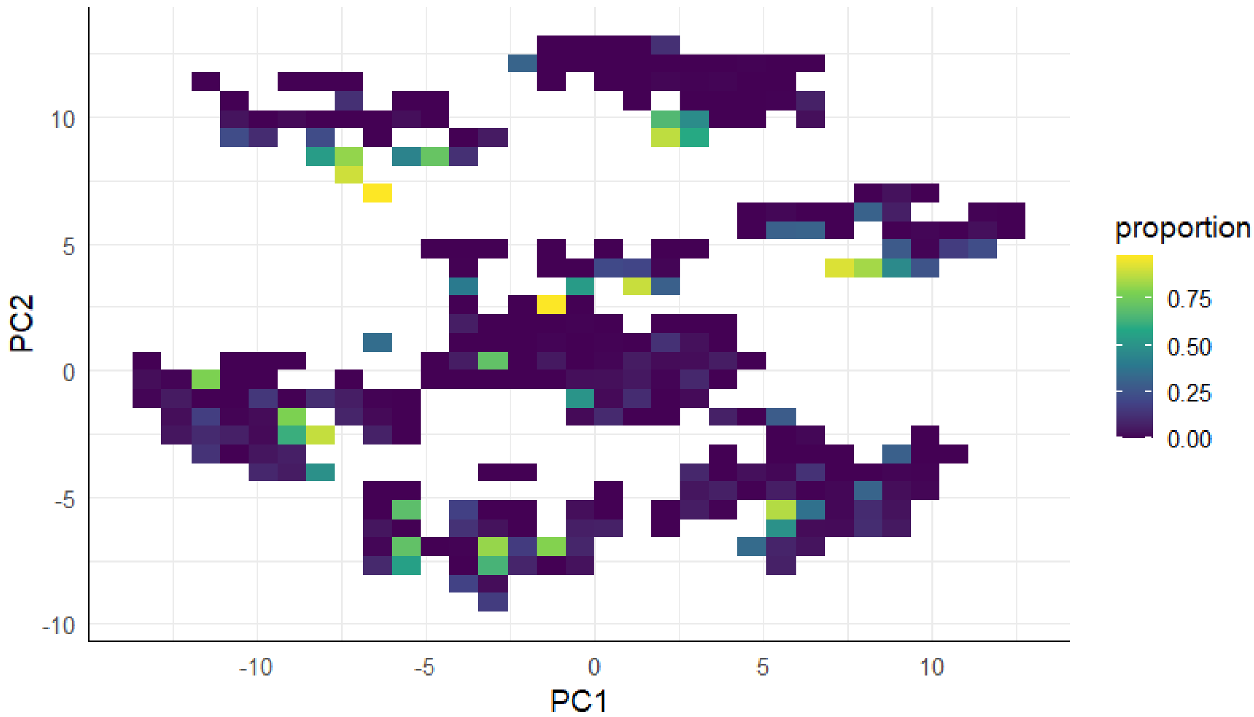
2e. Proportion of vegetables across the PCA axes. Relatively equal contribution of vegetables spread across all clusters, indicating substantial proportions in each meal.



2f. Proportion of fruits across the PCA axes. All clusters were characterised by some proportions of fruits, but higher proportions were observed in meal compositions found in the negative side of PC1.



2g. Proportion of potatoes, kumara and taro across the PCA axes. Slightly higher proportion in meal compositions in the negative side of PC1 and PC2, but generally do not contribute highly to meals.



2h. Proportion of "others" comprising minor FGs, "beverages", "sugar and sweets" and "sauces and condiments". The distribution of these foods appears to be equal across all clusters, but meal compositions aligned with higher protein quality in the positive PC1 and PC2 spaces have lower proportions.

Figure E 2 Contribution of all FGs across meal composition patterns
Meal composition patterns are presented in a two-dimensional binned density heat map in a PCA score space that was divided into 30-by-30 bins. Lighter tiles show a higher proportion of the FG in the meal composition. Distribution of FGs across the principal components (PCs) was visualised for legumes and pulses **(A)**, nuts and seeds **(B)**, grains and pasta **(C)**, isolates **(D)**, vegetables **(E)**, fruits **(F)**, potatoes, kumara and taro **(G)** and other minor FGs **(H)**.

Appendix F

Meal complementation equations

1) Weighted IAA for each food item, relative to each IAA requirement

$$Q_i = \sum_{a=1}^{A} \frac{IAA_{ia}}{R_a}$$

Where:

IAA_{ia} = amount of IAA a in food item i

R_a = requirement of each IAA based on FAO adult requirements (58).

2) Aggregate to the FG level for each meal side, in each meal pair

Protein share:

$$P_{g,m} = \sum_{i \epsilon g,m} Protein_i$$

$P_{g,m}$ = Summing protein from all food item *i* that were categorised to FG *g*, in meal side, *m.*

IAA share:

$$Q_{g,m} = \sum_{i \epsilon g,m} Q_i$$

$Q_{g,m}$ = Total requirement-weighted IAA contribution of FG *g*, in meal side, *m.*

3) Within-meal shares

Protein share:

$$p_{g,m} = \frac{P_{g,m}}{\sum_g Pg,m}$$

$p_{g,m}$ = protein contribution from each FG *g*, in meal *m,* as a proportion of total protein in meal *m*.

IAA share:

$$q_{g,m} = \frac{Q_{g,m}}{\sum_g Qg,m}$$

$q_{g,m}$ = requirement-weighted IAA contribution from each FG *g*, in meal *m,* as a proportion of total protein in meal *m*.

4) Geometric mean to give equal weighting to protein intake and protein quality

$$s_{gm}^{raw} = \sqrt{p_{gm}\ x\ q_{gm}}$$

5) Normalisation of FG contribution, *g* within each meal side and all FG scores, *s,* summed to 1 in each meal side, *m*

$$s_{gm} = \frac{s_{gm}^{raw}}{\sum_g s_{gm}^{raw}}$$

6) Cross-pairing weight of both meal sides

$$w_{gh} = s_{g,1}\ x\ s_{h,2}$$

Code: https://github.com/Pattybx/VPQtool_FGcontribution.git

Table F 1 Identity of food items in the most frequently selected meal combos

ID of combo2	Food items in meal combo	Total frequency	DIAAS
2161	Chia, flaxseed, pea isolate, fruit	3608	97
3797	PBMA, peanut butter	3400	98.6
3173	PBMA sushi, noodle soup	4716	85.7
2771	PBMA in salad, toast	4180	98.6
3007	Fruit, cereal, PB yogurt, pea isolate	3576	95.3
3817	PBMA patty	1695	97.8
4173	Fruit, oats, oat beverage, pea isolate	1468	85.6
3425	Black bean, lentil, PBMA, vegetables	3390	95.5
4021	Fruit, pea isolate, flax	1077	81.1
4328	PBMA, corn, rice, fruit	964	96.2
3889	PBMA, nuts and seeds, vegetable	550	85.5
2908	Bread, chickpeas, PBMA	507	97.9
1803	Chickpea, corn, bread, sesame seed	300	69.4
4195	Potato, bread, PBMA sushi	48	43.8
3370	Hemp, fruit, flaxseed, pumpkin seed	476	69.1
3613	Hemp, oat, flaxseed, pumpkin seed	32	63.9
3482	Vegetables, peanut, seaweed, sesame seed	32	47.0
3223	Oats, grains, fruit, walnut, flaxseed	32	57.6
3188	Tofu, seaweed, vegetables, rice	32	87.4
3150	Fruit, popcorn	38	69.9

PB: Plant-Based; PBMA: Plant-Based Meat Alternative. Main contributing ingredient to PBMA was pea protein or soy isolate. PB yogurt consisted of a mixture of rice, coconut and oat.

Table F 2 Descriptions of scenarios

Scenario	Age (years)	Sex	Height (cm)	Weight (kg)	Physical activity level	Protein requirement (g/kg/day)	Number of meals per day
1. Moderate protein intake; adult	30	Female	160	50	1.8	0.66	3
2. High protein intake; adult athlete	30	Male	180	90	2.0	1.6	3
3. High protein intake distributed over more meals; adult athlete	30	Male	180	90	2.0	1.6	5
4. Moderate-to-high protein intake, older adult	65	Female	165	75	1.5	1.0	3
Scenario	**Feasible meal pairs[1], n (%)**	**Mean FGs per meal (SD)**	**Most limiting nutrients[2] (%)**				
			Energy	Protein	Lysine	SAA	Other IAAs
1	14248 (60.5)	3.34 (0.94)	94.3	1.09	4.23	0.21	0.20
2	2976 (12.6)	5.47 (1.33)	72.1	18.7	0.1	-	-
3	11454 (48.7)	3.76 (1.02)	77.8	22.1	-	0.07	0.08
4	14404 (61.2)	3.31 (0.95)	76.1	23.5	0.41	0.06	-

SD, Standard Deviation; IAAs, Indispensable Amino Acids

[1]Total possible meal pairs are 23,539; [2]For the feasible meal pairs, all nutrients can be met in the fully optimised mode. Limiting here refers to the nutrient that is at the lowest ratio with respect to the requirement of the modelled scenario;

Table F 3 Mean mass and mean share of each FG for each scenario

Scenario	FG	Mean mass in grams (SD)	Mean share in % (SD)
1. Moderate protein intake; adult	Legumes and pulses	98.6 (50.8)	25.6 (28.6)
	Grains and pasta	99.6 (33.6)	45.3 (28.6)
	Fruits	84.4 (51.2)	5.23 (13.8)
	Vegetables	61.4 (21.7)	1.79 (0.07)
	Potatoes, kumara and taro	54.0 (23.3)	0.16 (1.99)
	Nuts and seeds	29.5 (3.2)	17.0 (21.0)
	Isolates	28.1 (6.5)	4.27 (6.70)
	Yeast and other condiments	27.6 (6.8)	0.69 (3.78)
	Sugar and sweets	22.0 (11.3)	< 0.1
	Beverages	9.38 (1.37)	0
2. High protein intake; adult athlete	Legumes and pulses	126.5 (38.6)	37.1 (22.1)
	Grains and pasta	110.3 (25.1)	37.8 (18.7)
	Fruits	109.2 (45.6)	6.26 (12.1)
	Vegetables	63.4 (21.5)	2.97 (6.84)
	Potatoes, kumara and taro	51.3 (28.7)	0.17 (1.46)
	Nuts and seeds	29.7 (2.20)	12.4 (13.3)
	Isolates	29.5 (2.78)	2.21 (3.55)
	Yeast and other condiments	29.3 (3.12)	1.11 (3.09)
	Sugar and sweets	27.8 (5.90)	< 0.1
	Beverages	9.86 (0.39)	< 0.1
3. High protein intake distributed over more meals; adult athlete	Legumes and pulses	110.5 (45.2)	30.1 (27.8)
	Grains and pasta	98.8 (32.7)	40.6 (24.8)
	Fruits	89.1 (48.1)	5.27 (13.2)
	Vegetables	64.6 (18.4)	2.19 (7.9)
	Potatoes, kumara and taro	54.5 (23.8)	< 0.1

	Nuts and seeds	29.3 (3.63)	16.0 (19.1)
	Isolates	27.6 (5.42)	4.97 (6.44)
	Yeast and other condiments	26.6 (7.50)	0.67 (3.21)
	Sugar and sweets	26.0 (5.51)	< 0.1
	Beverages	8.72 (2.56)	0
	Legumes and pulses	101.8 (47.0)	29.9 (30.0)
4. Moderate-to-high protein intake, older adult	Grains and pasta	93.6 (33.9)	40.0 (27.9)
	Fruits	82.2 (48.4)	3.34 (11.4)
	Vegetables	60.7 (20.8)	1.92 (8.11)
	Potatoes, kumara and taro	46.4 (27.3)	< 0.1
	Nuts and seeds	29.3 (3.59)	18.2(22.1)
	Isolates	25.6 (7.15)	5.85 (7.69)
	Yeast and other condiments	26.2 (8.05)	< 0.1
	Sugar and sweets	27.0 (7.06)	< 0.1
	Beverages	7.51 (3.84)	< 0.1

SD, Standard deviation

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