16/02/2026

1. **Project title:** Danish Pulses: Tasty, Healthy, and Safe Danish Pulses with Added Value for The Users
1. **Danish title:** Dansk Bælg: Velsmagende, sunde og sikre danske bælgfrugter med merværdi for brugerne

GUDP grant number: 34009-23-2191

2. Rationale and purpose

Fermentation of legumes has the potential to enhance the health benefits associated with increasing daily legume consumption to 100 grams, as recommended by the Danish Veterinary and Food Administration (1). Despite this, the current average daily intake among Danes remains as low as 2–3 grams (1). Legumes are widely recognized for their contribution to satiety, thereby supporting weight management and their role in the prevention of non-communicable diseases (2–6). These health-promoting effects are largely attributed to their content of bioactive compounds, including phenolics, peptides, and dietary fiber, which positively modulate the gut microbiota (7,8). However, legumes also contain antinutritional factors such as lectins, phytic acid, and protease inhibitors, which can hinder the bioavailability of essential nutrients, including proteins, vitamins, and minerals, ultimately diminishing the nutritional value of legume-based foods (7–10).

Food processing techniques—particularly fermentation—offer promising strategies to mitigate these antinutrients, as fermentation has been shown to reduce antinutrient content by 70–90%, depending on the method and substrate used (10–13). This process involves microbial metabolism, in which carbohydrates are oxidized to generate energy while simultaneously enhancing the bioavailability of minerals in plant-based foods (10–13). Fermentation has also been demonstrated to increase the levels of free amino acids, including essential amino acids such as lysine, methionine, and tryptophan, in cereals (14), and similar effects may be observed in legumes—though this remains to be fully elucidated (15).

The nutritional enhancement of fermented foods is largely due to the enzymatic breakdown of complex macronutrients—carbohydrates, proteins, and lipids—into more digestible forms such as simple sugars, fatty acids, and amino acids. However, fermentation can also produce resistant proteins, which are poorly soluble in water and may influence food matrix viscosity, similar to dietary fibers (16). Additionally, the fermentation of amino acids by these microbes can lead to the production of short-chain fatty acids (SCFAs), which are associated with improved glucose tolerance through reduced hepatic glucose output and lower circulating free fatty acid levels (6,9,17–19). Furthermore, intervention studies involving lactic acid bacteria (LAB) fermented foods such as kimchi have demonstrated reductions in health parameters such as body weight, body fat, waist circumference, fasting blood glucose levels, C-reactive protein levels (CRP), and blood pressure compared to their unfermented counterparts (16,20,21). These findings suggest that fermentation may play a pivotal role in enhancing the nutritional and functional properties of plant-based protein sources.

In this context, the fermentation of legumes may support the dietary transition from animal-based to plant-based proteins by improving the nutritional quality of legume-derived proteins. Fermented foods are deeply rooted in traditional diets worldwide, with legumes such as soybeans, black gram, chickpeas, and mung beans commonly used in fermentation practices (22–24).

This study focuses on locally grown Danish legumes - including fava beans, peas, and lentils ***to explore the potential of LAB fermentation in enhancing their nutritional and functional properties.*** While the health benefits of legumes and LAB-fermented fruits and vegetables are well-documented, the specific effects of LAB-fermented legumes remain a relatively novel area within food science.

It is hypothesized that a daily intake of 100 grams of LAB-fermented or unfermented legumes will lower the levels of blood glucose, inflammation, and cholesterol, change gut microbiome composition, and increase SCFAs in adults, compared to a control period consuming a habitual diet. Furthermore, it is hypothesized that fermented legumes have additional health effects in terms of changes in body composition compared to unfermented legumes.

3. Methods

Study design

A parallel two-armed randomized trial.

The participants are randomly assigned to two experimental groups based on a randomized trial design: two weeks of habitual dietary intake (control period), two weeks of LAB fermented foods, or two weeks of unfermented foods.

The control period is from zero to 14 days, and after these two weeks, baseline data will be collected from all subjects. The second intervention period lasts from day 14 to day 28 (Fig. 1), during which participants will be instructed to consume 100 g of either fermented or unfermented legumes every day, seven days a week, for two weeks.

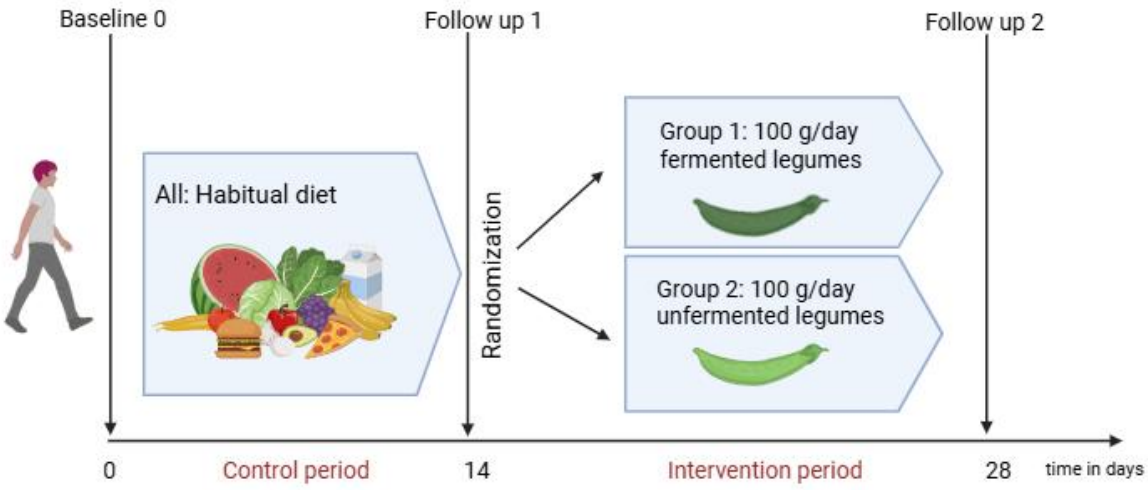


Fig. 1: Trial design

Study population

Healthy adults are recruited from the University College Absalon or the University of Copenhagen canteens from two locations: University College Absalon in Slagelse, Denmark, and University of Copenhagen in Frederiksberg, Denmark.

Inclusion criteria:

Adults, 18 – 60 years.
No diabetes.
Biological sex includes both males and females.

Exclusion criteria:

Treatment with antibiotics within the last three months.
Ongoing treatment with non-steroidal anti-inflammatory drugs.
Metabolic diseases, including diabetes.
Inflammatory bowel disease (IBD), such as Crohn's disease or ulcerative colitis.
Allergies or intolerance to legumes.
People that report eating > 25 g per day of legumes as part of their habitual diet.

Sample size calculation:

Initially, the power calculation is performed by a parallel two-armed comparison of the change in continuous blood glucose levels from baseline (control period). A total of **50 participants (25 participants in each group)** should be recruited to be able to detect a clinically relevant difference of approximately 9 mg/dL in fasting blood glucose (American Dietetic Association (ADA) 2024) between the groups adjusted (fermented vs. unfermented legumes) (18,23–25), assuming a standard deviation of approximately 10-11 mg/dl, a power of 0.8, and a significance level of 0.05, and includes a 10% dropout adjustment. There will be a running inclusion from September 2026.

Control period

All participants are instructed to adhere to their habitual diet during the 14-day control period.

Intervention period

Participants in the treatment groups are given two weeks of LAB-fermented or unfermented foods, containing 100 g of legumes, including fava beans, peas, and lentils. Fermented and unfermented legumes will be provided and packed as standardized food items to their habitual meals e.g. as hummus, or pasta with legume flour, adding up to 100 g of legumes per day, seven days a week, for two weeks. These test foods can be eaten throughout the day; however, intake of other legumes, except those included in the foods, should be avoided. If participants eat other legumes, this should be noted and accounted for in data analysis. If participants do not consume the full 100 g of legumes, they must weigh the leftovers and take a photo of the remaining food and report to the researchers.

Primary outcome:

Continuous Glucose Monitoring

Continuous Glucose Monitoring (CGM) will be measured in real-time and intermittently scanned CGM (isCGM) bi-weekly with an isCGM sensor (model Abbott Freestyle Libre PRO). This method is a minimally invasive method, as the sensor is placed on the participant's arm (26–30), by participants themselves after careful instructions, incl. videos. The Abbott Freestyle Libre PRO is blinded to the participants to avoid habitual changes, and it measures CGM in real-time throughout the entire 14-day period. The study follows the Standardized CGM metrics for Clinical Care, and the 14-day duration is aligned with the international recommendations (26,30). To ensure accurate and meaningful interpretation of CGM, adequate glucose data is collected according to the recommendation (70% of data from 14 days) (26,30). Data from participants with a CGM activity < 70% will be analyzed with the intention to treat principles.

Secondary Outcomes

The secondary outcome includes changes in inflammation, cholesterol, long-term blood glucose levels, systolic and diastolic blood pressure, body composition, the fecal microbiomes and metabolome, and dietary intake. Digestive abnormalities will also be assessed.

Inflammation monitoring

Fasting C-reactive proteins (CRP) monitoring will be measured at baseline, 14 days, and at day 28 by a finger prick test using a QuickRead go PLUS Instrument (#154580-4).

Cholesterol monitoring

Fasting cholesterol monitoring will be conducted at baseline, 14 days, and at day 28 by a finger prick test using a QuickRead go PLUS Instrument (#154580-4).

Long-term blood glucose monitoring

Fasting hemoglobin A1c (HbA1c) monitoring will be conducted at baseline, 14 days, and at day 28 by a finger prick test using a QuickRead go PLUS Instrument (#154580-4).

Systolic and diastolic blood pressures

Systolic and diastolic blood pressures will be measured at baseline, day 14, and at day 28. Blood pressures will be measured in millimeters of mercury using an automatic blood pressure monitor (Digital Automatic Blood Pressure Monitor, Omron). Participants will be asked to lie down and relax for approximately 8 to 10 minutes, and 3 blood pressure measurements will be recorded at 5-minute intervals.

Anthropometric measurements

Anthropometric measurements, such as body weight, height, waist, and hip circumference, will be measured by trained clinical dietitian students at baseline, day 14, and at day 28.

Weight, total fat mass (FM) (kg), fat-free mass (FFM) (kg), and percent body fat (%) will be measured using a calibrated Body Impedance Analytics (BIA) (TANITA MC 780 MA S, Japan).

Height (m) will be measured using a standard dynamometer without shoes and is recorded to the nearest 0.1 cm.

Body mass index (BMI) is calculated by dividing weight (kg) by height (m) squared (kg/m^2). BMI is categorized following WHO and ESPEN guidelines: underweight: BMI < 18.5 kg/m^2; normal weight: BMI 18.5 kg/m^2 < 24.9 kg/m^2; overweight: BMI 25 kg/m^2 < 29.9 kg/m^2; and obesity: BMI > 30 kg/m^2 (31,32).

Waist and hip circumferences will be measured using a standardized tape measure that is calibrated before use. Waist circumference (cm) will be measured at the midpoint between the lowest rib and the upper part of the iliac bone in a standing position. Hip circumference (cm) will be measured at the site of the largest circumference between the waist and thighs.

Fecal samples

Fecal samples (approximately 10 g) will be collected from all participants at baseline, day 14, and at day 28. Participants are collecting the samples by themselves using the EasySampler tool kit,

including a thorough written introduction(33,34). Fecal pH will be determined by mixing fecal matter 1:2 with sterile MilliQ-water after which pH is measured. Total fecal DNA will be extracted using standard methods. The gut microbial metagenome will be determined by shotgun high-throughput sequencing using long-read Oxford Nanopore-based sequencing(33,34). Before any further bioinformatics processing the raw reads will be purged of any human DNA-related sequences.. Fecal short chain fatty acids (SCFA) and other fecal metabolite concentrations, incl. free amino acids, are determined using high-field nuclear magnetic resonance NMR (34) on fecal samples..

Dietary intake

Data on dietary intake will be collected by three-day dietary records with photos to evaluate adherence to the intervention. The dietary records will be collected once during the control period and once during the intervention period. Photos will be used for main meals and leftovers of legumes.

Physical activity will also be documented as part of the dietary records and used to estimate energy expenditure. Furthermore, data on dietary intake will be used to assess compliance with the intervention.

Digestive abnormalities

Digestive abnormalities are identified through IBS symptoms and will be assessed using self-reported questionnaires at baseline, follow-up 1, and 2, using the IBS Severity Scoring System (IBS-SSS) for assessment of the following symptoms: abdominal distension, abdominal pain, satisfaction with bowel habits, and the extent to which symptoms affect the quality of life (QoL) (35).

Overall data collection

Table 1: Data collection

Variables	Methods	Baseline Day 0	Follow-up 1 Day 14	Follow-up 2 Day 28
Age		x		
Sex		x		
Bio markers				
Blood glucose	Continuous blood glucose levels by glucometer: Abbott Freestyle Libre PRO	x	x	x
Cholesterol levels	Prick-test	x	x	x
C-reactive protein	Prick test	x	x	x
Long-term blood glucose	Prick test	x	x	x
Blood pressure	Systolic and diastolic blood pressures	x	x	x
Body composition				
Body composition incl. body weight, kg	Body Impedance Analytics (BIA)	x	x	x
Fat mass (FM), kg		x	x	x
Fat free mass (FFM), kg		x	x	x
Height, meters	Dynamometer	x		
Body Mass Index (BMI)	BMI is calculated by dividing weight (kg) by height (m) squared (kg/m^2).	x	x	x
Waist and hip circumference (Waist/hip ratio), cm	Waist circumference was measured at the midpoint between the lowest rib and the upper part of the iliac bone in a standing position. Hip circumference was measured at the site of the largest circumference between the waist and thighs.	x	x	x
Gut microbiome and metabolites				
Gut microbiome composition	Near-full length 16S rRNA gene amplicon sequencing	x	x	x
Short chain fatty acids (SCFA) and other metabolites	^1H NMR spectroscopy	x	x	x
Other variables				
Dietary intake	Three days' dietary records with photos	x		x
Digestive abnormalities	IBS Severity Scoring System questionnaire	x	x	x

4. Statistical analysis

Stages of data processing will begin with descriptive analyses of variables secondary to outcomes for all participants at baseline. These characteristics will be tested using analysis of covariance (ANCOVA) when the data are normally distributed, and the Mann–Whitney U test when the data are not normally distributed. Comparisons of the incremental area under the curve (AUC) in the control (baseline) period will be performed against the two groups (group 1 and group 2), and differences between groups will also be assessed.

Continuous variables will be presented as the mean and 95% confidence interval (CI) in the format [lower limit, upper limit] when the data are normally distributed. Differences between continuous variables will be tested using ANCOVA. Categorical variables will be presented as percentages (e.g., sex), and differences will be tested using Fisher's exact test to compare groups (group 1 and group 2). A P-value < 0.05 will be considered statistically significant. Statistical analyses will be conducted using a statistical software program for scientific research, such as R (RStudio; © 2009–2023 Posit Software, PBC).

For 16S rRNA gene amplicon sequencing data, raw FASTQ data will be subjected to second-level demultiplexing, quality filtering, and amplicon sequence variant (ASV) definition, as well as taxonomy assignment, using the in-house established LACA pipeline (https://github.com/yanhui09/laca). Subsequently, the effects of the intervention on alpha- and beta-diversity metrics, compositional changes, and correlations with anthropometrics, metabolites, and intervention arm will be evaluated as previously described (34,36).
For ^{1}H NMR spectral data, fecal metabolites are assigned, and their concentrations are quantified using the Chenomx software. Subsequently, statistical analysis will be conducted on the individual metabolites as continuous variables are described above.

5. Risks, side effects, and disadvantages in the short and long term

In the study, no serious adverse events or risks are expected. There are no known serious adverse effects associated with the consumption of either non-fermented or fermented legumes in healthy adults, and the official dietary guidelines recommend an intake of 100 g of cooked legumes per day. All participants participate voluntarily and can withdraw from the study at any time. All participants are informed about the product content, and potential participants with food allergies or intolerances are excluded before starting the study.

The finger-prick test is a simple and nearly painless tool to measure inflammation, cholesterol, and blood glucose. No blood is collected, and the test is performed by trained researchers. A disinfectant is used to reduce the already small risk of bleeding, bruising, or infection.

Stool samples is safe to collect when EasySampler is used. Only a small sample is needed (approximately 10 g). There is no health risk when following the instructions.
This project meets the guidelines of the Declaration of Helsinki II, as well as the guidelines of the Regional Ethics Committee.

The study has been submitted to the Science Ethics Committees for Region Zealand
j.nr .: SJ-1132-120770.
The study is covered by the Danish Patient Compensation scheme (In Danish: Patienterstatningen)

6. Biological material is collected.

Throughout the study, each participant is donating a total volume of maximum 40 grams of feces. The samples will be stored in the research-biobank and batch analyzed at the end of the trial for primary and secondary efficacy variables. Some fecal samples will also be stored for delivery for analysis at collaborators' laboratories. Collaborators are described in section 9.0. The objective of these analyses is to establish the gut microbial composition and metabolites of the enrolled subjects. The metabolic activity of the gut microbiome of the enrolled subjects will be examined through fecal metabolomics analyses. These studies are performed at University of Copenhagen, Department of Food Science (UCPH-FOOD) and Aarhus University, Department of Food Science (AU-FOOD).

UCPH-FOOD, Rolighedsvej 26, 1958 Frederiksberg C
AU-FOOD, Agro Food Park 48, DK-8200 Aarhus N.

Fecal samples will be self-collected by participants at home. Faeces samples (approximately 10 g) are collected at home by the subjects. Equipment and containers are provided to the subjects beforehand, and thorough information will be given both verbally at the information meeting and on an information sheet (Appendix EasySampler® Stool Collection Kit. Subjects are instructed to keep their samples in the refrigerator before delivering at Absalon or the UCPH, at baseline, day 14, and the final visit at day 28. Faeces samples will be stored in the research-biobank at minus 80 degrees at the study site (Absalon or UCHP) for metabolomics analysis, DNA extraction, and fecal pH at UCPH-FOOD and AU-FOOD. Feces samples will be stored at minus 60- 80 degrees until analysis and stored in a locked freezer for 5 years after study completion. Samples will be destroyed as clinical waste.

7. Information from patient records

7. a No patient records will be used. No data will be collected before the consent procedure has been completed.

8. Processing of personal data in the project:

Data Protection Regulation and the Data Protection Act are complied with.
Data collection is performed by the principal investigator and trained students. The students will practice data collection before the study. It is the principal investigator who is the responsible data manager. Only data on the above-mentioned information will be transferred by the principal investigator into the Absalon secured drive (R-drive) when written consent is received. All data will be reviewed by the principal investigator. Data managers in the project are University College Absalon, Sdr. Stationsvej 30, 4200 Slagelse. absalon@pha.dk. Data Protection Advisor (DPO): dpo@pha.dk.

The information is collected and processed in accordance with legal authority in Article 6 (1) of the Data Protection Regulation, 1, letter A, and Article 9, para. 2, letter al:

The information is processed in accordance with the University College Absalon's privacy policy, including requirements for technical and organizational security - read more here: https://www2.phabsalon.dk/studienet/studiehjaelp/informationssikkerhed/politikker-og-vejledninger/privatlivspolitik/

A data management plan has been prepared in accordance with the guidelines from Good Clinical Practice (GCP) standards, the general data protection regulation with guidance from the DPO.
A data management plan is prepared following the guidelines of Good Clinical Practice standards (37), the Data Protection Regulation, and the Data Protection Law (38). All data retrieved during the study will be encrypted after collection and will be handled according to Good Clinical Practice standards.
The principal investigator and the students will continuously document all data on each participant. All participants receive a personal ID number, which can only be read through a code file that contains their full name. When data collection is completed, the code file with identifiable personal data is destroyed, and the data sets will be pseudo-anonymized or anonymized. All data is treated as anonymized using the anonymous ID number, which appears when data is processed. When the project is completed, data is stored on a specially secured research drive with the data controller in Absalon University College for 5 years after the publication of scientific articles. Relevant anonymized data can only be shared on a reasonable request following the Data Protection Regulation and the Data Protection Law (38).
No personal information is sent abroad the country.

9. Economy

The study is part of a project supported by the public fund Green Transition and Demonstration Program (GUDP) under the Ministry of Food, Agriculture and Fisheries (Grant No. 34009-23-2191), and it is not sponsored by private companies. University College Absalon initiated the project. Project partners include UCPH-FOOD, AU-FOOD, KMC Amba, Organic Plant Protein, and AM Nutrition.

Total budget: DKK 14,929,281

GUDP grant: DKK 12,278,068

Co-funding from universities and University College Absalon: DKK 1,247,974

Co-funding from industry partners: DKK 1,403,239

All project-related expenses are covered by the GUDP grant. Dava Food contributes by supplying legumes for the trial but has no influence on the study design, conduct, data analysis, or dissemination/publication of results, and will be acknowledged in publications only. None of the investigators has any financial or other competing interests related to the project.

10. Fee and /or other benefits to the participants

Participants will receive DKK 1,000 and a health consultation as compensation for the time, inconvenience, and any discomfort associated with sample collections. The compensation is taxable.

Compensation will only be provided if participants complete the entire study. No compensation will be given for partial participation.

11. Recruitment of subjects and informed consent

Recruitment of project participants takes place from the Absalon or the UCPH canteens. The first step in recruitment involves the principal investigator writing to inform all campus employees and students about the project's purpose, content, and course. Posters are displayed to inform people about the project. In the next step, oral information in the canteens is provided by the principal investigator and students. Participant information (written information) is provided as well as the leaflets "Subjects' rights in a health science research project", and "Before you decide - to be in health science experiments". The written information contains a description of the project, its purpose, method, and possible disadvantages associated with the participants' participation. Participants will receive both verbal and written information by email and will have 24 hours to decide before signing the consent form. There will be an information meeting where participants can ask questions, and they may bring a support person if needed. The meeting will be held individually according to participants' needs—by phone, online, or as an in-person meeting in a private, undisturbed room. Participation in the project is voluntary, and the participants have the opportunity, without further justification, to interrupt the collaboration and their participation in the project. In the third step, the residents then have 24 hours to decide whether they want to participate in the project and sign a declaration of consent. The content is obtained at day 1 (baseline). There is a running inclusion. During the project period, the participants can obtain further information about the project from the principal investigator. Participants have the opportunity to withdraw consent at any time during the project.

12. Publication of results

Based on the project's results, it is expected that two scientific publications will be submitted.

Table 2: Expected publications

Title proposal	Scientific journals proposal
Implementing a high-legume diet in everyday meals: a feasibility study of 100 g/day legume consumption in Denmark	Nutrients
Health effects of introducing locally sourced legumes into regular Danish diet: A parallel trial comparing fermented and unfermented legumes	Frontiers in Nutrition

The results obtained will be published in anonymous form in international scientific journals and may be presented at conferences. Positive, negative, and inconclusive results will be published. If, contrary to expectations, the results are not published in a scientific journal, they will be published on www.clinicaltrials.gov and www.clinicaltrialsregister.eu

13. Ethics

In the study, no serious adverse events or risks are expected. There are no known serious adverse effects associated with the consumption of either non-fermented or fermented legumes in healthy adults. All participants are informed about the product content, and potential participants with food allergies or intolerances are excluded before enrollment. . This project meets the guidelines of the Declaration of Helsinki II, as well as the guidelines of the Regional Ethics

Committee. The research project has been submitted to the Science Ethics Committees for Region Zealand j.nr.:SJ-1132-120770.

a. Why the therapeutic benefit for the subjects or future patients justifies the studies

Danish pulses is a project aimed at getting more Danish-grown legumes—such as beans, peas, and lentils—into the food we eat daily. Legumes have a distinctive composition of proteins, – which can replace some of the meat in a meal, dietary fiber, which supports digestion and provides longer-lasting satiety, and carbohydrates. This combination makes legumes particularly interesting from a health perspective. The fiber and protein content may help slow the rise in blood sugar after a meal and contribute to satiety for a longer time.

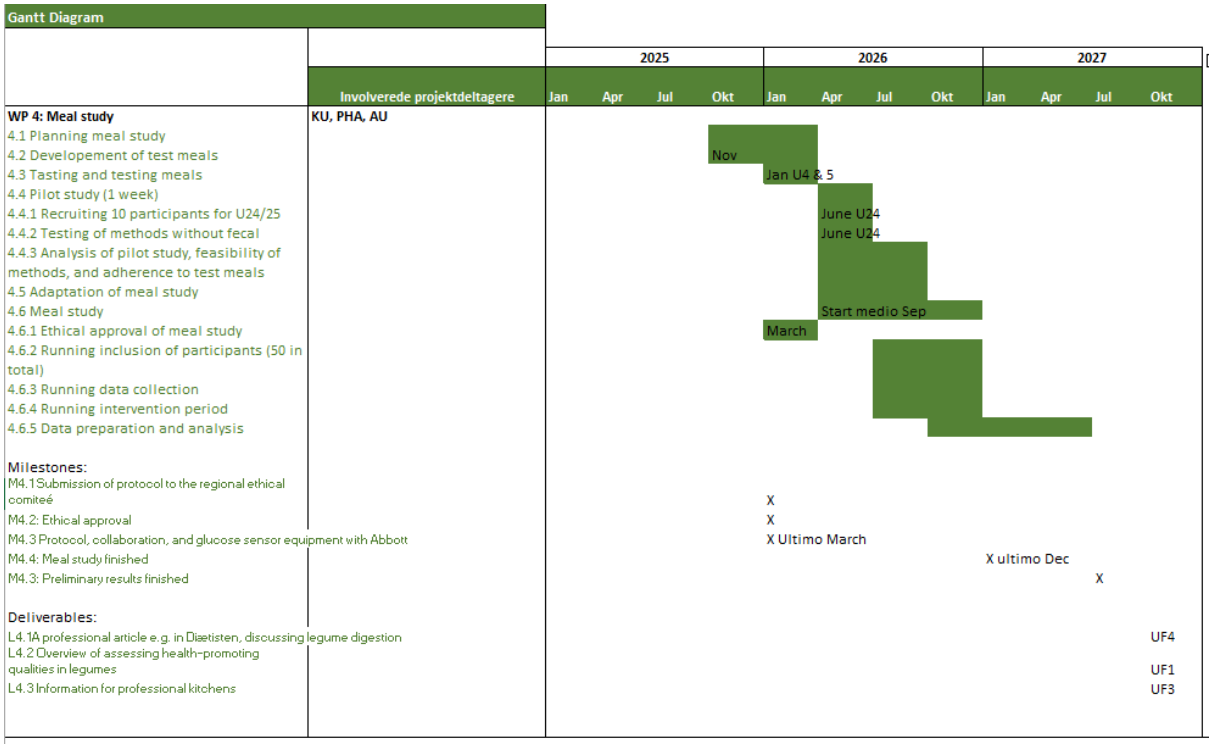
The project covers the entire "journey" of legumes—from field to plate—and works with several different species and varieties. The knowledge generated from the study may therefore be relevant to many Danes and support a greener future.

13. Information regarding compensation

See. Pkt. 10.

Timeline perspective

The Gantt diagram presents a structured project timeline from 2025 to 2027, progressing from preparatory work to data collection and final analysis. Early 2025 was dedicated to planning and the early development of test meals. The main intervention and data collection occur in 2026, marked by several defined milestones and deadlines. From late 2026 through 2027, the focus shifts to data processing, analysis, and the production of final deliverables. Overall, the timeline reflects a clear and sequential progression that ensures methodological readiness, efficient implementation, and thorough dissemination of results.



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