



## Introduction

- The human gut microbiome is understood to be greatly affected by changes to the content of an individual's diet.
- Protein, while consumed in great excess in the typical adult Western diet, is poorly understood regarding its exact effects on the gut microbiome.
- Bacterial fermentation of dietary protein results in several products, which are either excreted, utilized by colonic microbes, or further detoxified by the colonic epithelium.
- We designed a randomized controlled trial that investigates the effect of excess protein on gut microbiota and its metabolites.

## Goal

To determine if our participants' dietary protein intake is increased by a significant amount so we can further assess for the effect of protein excess on an individuals' gut microbiome

## Methods

- Recruitment efforts were exclusively remote
- Interested individuals were screened
- Eligible individuals randomized to either the whey supplement group or pea supplement group
- At the start of the study, individuals completed the National Cancer Institute's Automated Self-Administered 24-Hour Dietary Assessment Tool (ASA24), recorded demographic information, and completed international physical activity questionnaire (IPAQ)
- Participants collected a fecal sample, consumed 50 g of protein supplementation daily for seven days, then collected a second fecal sample on day 8
- After collecting the second fecal sample, the ASA24 and IPAQ were completed again
- Reported dietary intakes were summarized using median and interquartile range (IQR) and compared using the Wilcoxon signed rank and ranked sum tests.

## Results

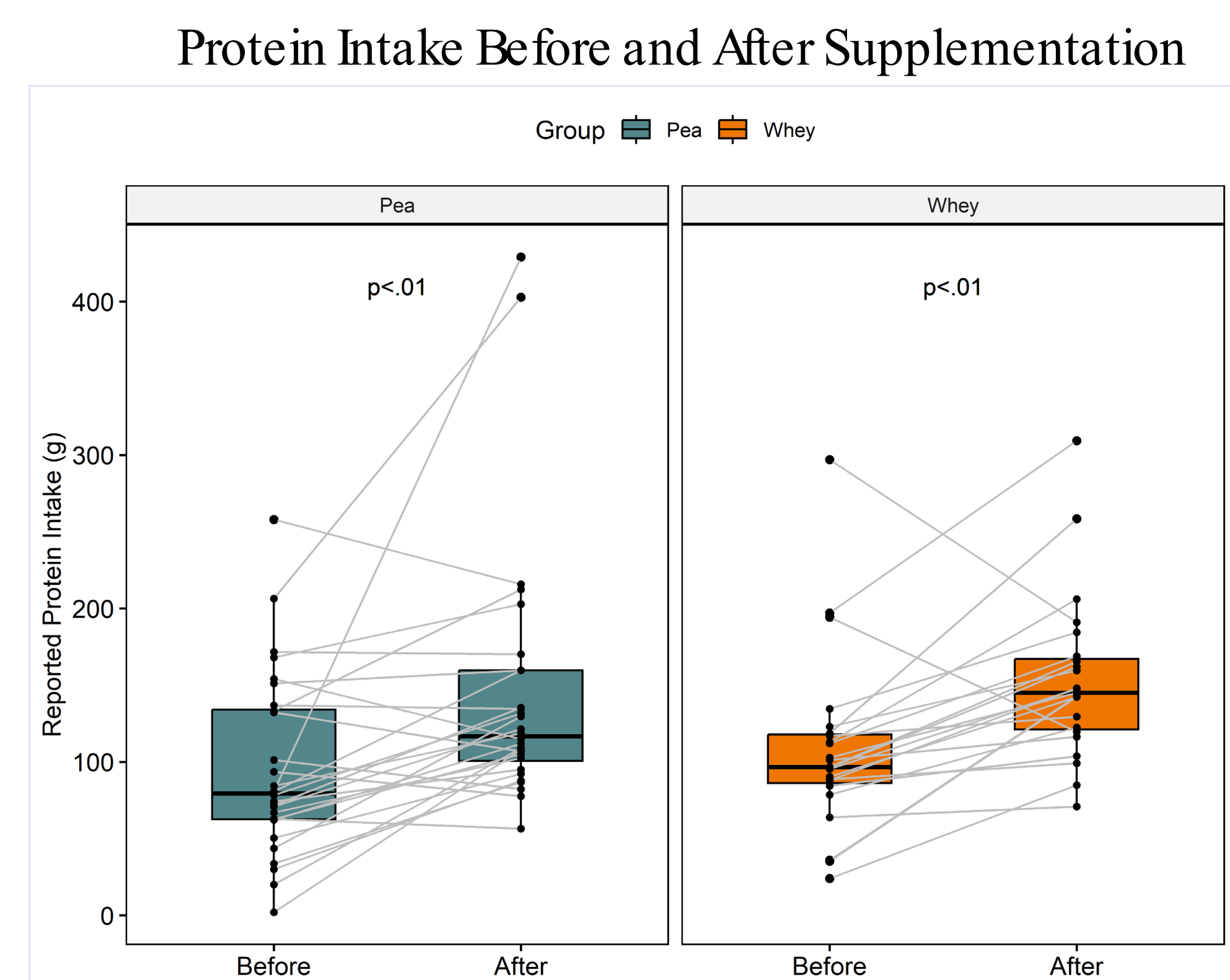


Figure 2. Individual protein intake based on ASA24 before and after whey or pea protein supplementation. Grey lines connect reported dietary intake for each participant.

- 51 total participants completed the study.
  - 23 consumed the whey supplement.
  - 28 consumed the pea supplement.
- There was no significant difference in physical activity levels between the two groups.
- Dietary protein intake before adding in the supplement was not significantly different between the whey and pea protein groups.
- After seven days of supplement consumption
  - Participant's dietary protein intake without the supplement was not significantly different from baseline.
  - Both groups showed a significant increase in dietary protein.
  - No significant difference in the increase in dietary protein between the whey and protein supplement groups

## Results

### Demographics

	All (n=51)	Pea (n=28)	Whey (n=23)	p-value
Age, years				0.644
mean, std	36.9 (7.9)	36.4 (8.2)	37.4 (7.7)	
Sex, n (%)				0.781
Female	26 (51.0)	15 (53.6)	11 (47.8)	
Male	25 (49.0)	13 (46.4)	12 (52.2)	
Race, n (%)				0.868
American Indian or Alaska Native	2 (3.9)	1 (3.6)	1 (4.4)	
Asian	1 (2.0)	1 (3.6)	0 (0)	
Black or African American	6 (11.8)	4 (14.3)	2 (8.7)	
Native Hawaiian or Pacific Islander	1 (2.0)	1 (3.6)	0 (0)	
White	41 (80.4)	21 (75.0)	20 (87.0)	
Ethnicity, n (%)				0.035
Hispanic or Latino	4 (7.8)	0 (0)	4 (17.4)	
Not Hispanic or Latino	47 (92.2)	28 (100.0)	19 (82.6)	

Figure 3. Participant demographics

### Dietary Intake Before and After Supplementation

		Pea (n=28)	p-value <sup>a</sup>	Whey (n=23)	p-value <sup>a</sup>	p-value <sup>b</sup>
Calories, median (IQR)	Baseline	2008 (1371,2718)		1899 (1503,2845)		0.502
	Change (without supplement)	-287 (-646,419)	0.348	-72 (-565,512)	0.746	0.551
	Change (with supplement)	42 (-312,807)	0.293	435 (-325,752)	0.176	0.947
Protein, g, median (IQR)	Baseline	80 (62,135)		97 (84,119)		0.222
	Change (without supplement)	-10 (-52,9)	0.075	-2 (-38,43)	0.860	0.276
	Change (with supplement)	42 (-2,71)	<.001	52 (15,69)	<.001	0.410
Fat, g, median (IQR)	Baseline	68 (48,113)		87 (44,131)		0.359
	Change (without supplement)	-5 (-45,17)	0.314	0 (-54,14)	0.459	0.932
	Change (with supplement)	3 (-38,24)	0.790	4 (-53,21)	0.637	0.828
Carbohydrates, g, median (IQR)	Baseline	219 (133,281)		203 (153,284)		0.872
	Change (without supplement)	-6 (-120,60)	0.397	-7 (-112,33)	0.220	0.842
	Change (with supplement)	25 (-98,92)	0.773	-3 (-83,52)	0.535	0.502
Fiber, g, median (IQR)	Baseline	19 (14,25)		23 (18,26)		0.268
	Change (without supplement)	0 (-8,6)	0.790	-5 (-10,1)	0.033	0.208
	Change (with supplement)	4 (-5,12)	0.162	-2 (-7,5)	0.516	0.182

<sup>a</sup>Wilcoxon signed rank test comparing dietary intake before and after the intervention within treatment groups.

<sup>b</sup>Wilcoxon rank sum test comparing treatment groups.

Figure 4. Calories and macronutrients based on daily dietary intake reported on ASA24 before and after consuming either the whey or pea protein supplement for 7 days

### Methods Timeline

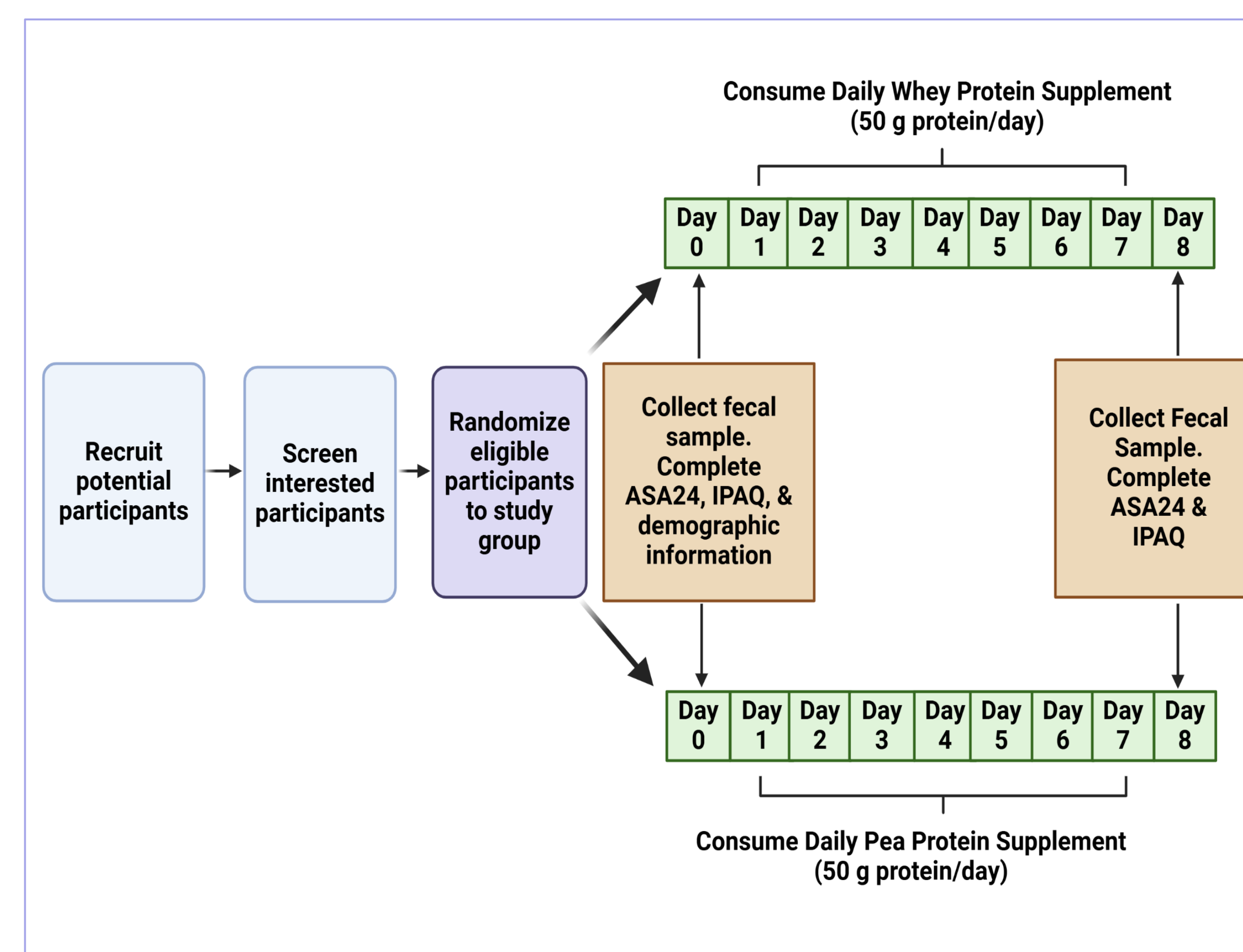


Figure 1. Methods timeline

## Conclusion

- Participants successfully significantly increased their protein intake, demonstrating that the study intervention was effective.
- We are now able to use the data from these participants' samples and survey responses to further assess the impact of protein intake on the composition of the gut microbiome and its resulting metabolites.