Pilot Study: Effort on Clean Labelled Plant-Based (AWE) diet on Human Gut Microbiome

Executive Summary

OsomeFood is a Singapore food and beverage (F&B) company that started in 2021. It produces Clean-Labelled Plant-Based ready-to-eat meals (AWE Meals). Their meals are 100% plant-based and without any additives or preservatives. They sourced their raw ingredients from naturally occurring fungi, including various mushrooms, which then undergo fermentation to produce proteins that have the same essential amino acid profiles as meat.

The gut microbiome is an ecosystem of millions of bacteria species that colonize the gastrointestinal tract. The gut microbiome plays an instrumental role in cardiovascular, metabolic and gastrointestinal health (Valdes et al. 2018) and there is growing recognition of the role of diet in modulating the composition and metabolic activity of the human gut microbiota, which in turn can impact health (Conlon & Bird 2014).

Ten healthy participants with omnivorous dietary habits were recruited for a 21-day study to **examine the effect of AWE meals on human gut microbiome composition.** All participants completed the study dietary plan which consisted of 5 days of plant-based diet and 2 days regular diet (participants are free to choose and there were no restrictions applied) every week for 3 weeks. Stools samples were collected at 3 different timepoints to observe the changes in the gut microbiome resulting from adopting AWE meals into their diets.

The participants daily meal intakes and general wellbeing were monitored via WhatsApp. Stool samples were obtained from each subject prior to the intervention as baseline, on day 7, and finally on day 21. DNA were extracted from their stool samples and sequenced. The bacterial species and functional pathway composition were annotated using BioBakery3 workflows.

All participants reported higher wellbeing scores (feeling satisfied with their meals, feeling more energetic, and feeling healthier) at the end of the study What they reported were aligned with functional pathway analysis that showed increased potential for Short Chain Fatty Acids and γ-Aminobutyric acid (GABA) production as well as better insulin signalling throughout the study period.

Two groups were observed based on their response to the diet intervention. One group (Moderately Responsive) had a reduced abundance of 16 disease-related bacteria at the end of the intervention period, with no gain in diversity by D21. Another group (Strongly responsive) showed the suppression of disease related bacteria starting from D7 which was followed by the emergence of 30 health related species not seen before the diet intervention.

There were 7 overweight (BMI 23-27.4kg/m2) participants (both gender and across all ages), who responded better (strongly responsive) to the AWE diet compared to other weight groups. These responders did not possess certain microbiota signals (*Bacteroides phage B124-14* and *Enterobacter bugandensis*) that might have contributed to the stronger response from this group of participants.

The findings of this study indicate that short term clean-labelled plant-based consumption have the potential to improve wellbeing through higher signalling of SCFAs and GABA production as well as insulin signalling, which may be beneficial for the cardiometabolic health of overweight adults. In addition, whilst diet preferences can be notoriously hard to change, cue cravings for hometown food when abroad for long, all participants reported remarkable ease in adopting AWE food into their regular diets.

This pilot study observed a link between AWE diet and gut microbiome modulation in the short term but to integrate into a person regular dietary habit for a longer term, a more in-depth study is needed to investigate the optimal frequency of AWE meals consumption to sustain the desired positive gut microbiome effects.

1. Background/Objectives

An adult human harbour ten times as much bacterial and other microbial cells in the body compared to human cells, most of which are in the gastrointestinal tract. Considering the large bacterial population in the human gut, it is thus not surprising that the modification of the gut microbiome has significant impacts on human health.

Many studies have reported that diet is a major driver for gut microbiota composition. A rich and balanced diet is essential to promote the maintenance of diversity and proper functioning of a healthy gut microbiota

Many Singaporean dietary intakes are rich in fats, cholesterol, animal proteins, sugars, salt and a wide range of processed foods that can influence the gut microbiota composition with possible affect health. Plant-based (PB) diets, defined in terms of replacing dietary requirements based on plants, plant extract and modified plants with no animal protein consumption, have been increasingly recommended for health benefits. Numerous studies have found plant-based diets, especially when rich in high quality plant foods such as whole grains, fruits, vegetables, and nuts, to be the primary driver to alter microbiota composition and at same time, increase bacterial richness and diversity.

OsomeFood offers Clean labelled started offering PB ready-to-eat meals (AWE Meals) in 2021. The foods are 100% PB, without additives and preservatives. AWE meals are designed using various types of plant-based proteins, including mycoprotein - a nutritious protein with a meat-like texture that is high in dietary fibre and low in saturated fat.

In this research, the team has recruited 10 participants and observe the gut microbiome induced changes resulting from switching the animal-based diet to 5 days AWE meals (Refer to Annex A for full menu) and 2 days non-AWE meals. The study aims **to report the changes and differences in the gut microbiome when healthy adult adopts AWE meals into their diets.**

2. Methods

A total of 10 heathy participants (50% Male & 50% Female), aged between 21 to 46 years old, with BMI between 18 and 30 kg/m2, were recruited from social media. None of the participants recruited for the study had chronic diseases, neither had been treated with antibiotics and probiotic within the last 2 months before the study started. Baseline height and weight were measured, and BMI was calculated. Written informed consent was obtained from all participants, and the study protocol was approved by AMILI Institutional Research Ethics Committee.

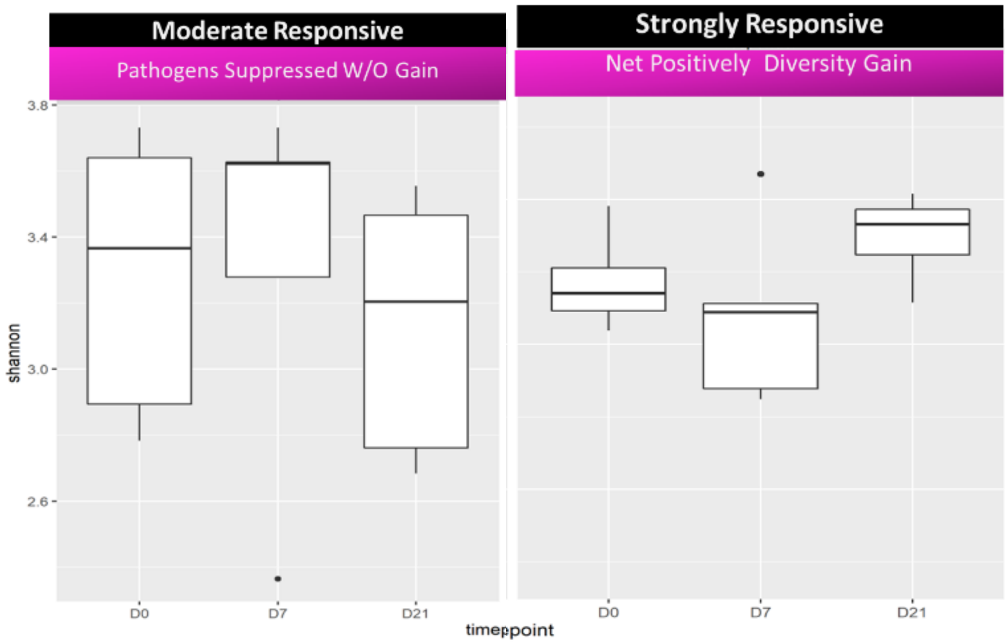
Basic demographic information was collected upon recruitment. The participants captured their daily foods intakes by sharing the photo of the before and after meals via WhatsApp. General wellbeing of the participant was monitored daily using 3-points Likert scale.

Baseline stool samples were collected from the participants before intervention. Subsequent samples were collected on 7, and 21 days. Total of 21 stool samples were collected for this study. The samples were stored at -80°C freezer in AMILI laboratory prior to DNA extraction and shotgun sequencing.

Microbial composition and functional pathways annotation were conducted using the BioBakery3 workflows pipeline. All statistical analyses were conducted in R version 4.1.2, while all figures were created using the ggplot2 package (Wickham, 2011). Gut bacterial diversity (i.e., Shannon diversity index) was computed using the microbiome R-package (Lahti & Shetty, 2018). To have an overview of the gut bacterial composition, taxonomic bar plot at the phylum level was created using the phyloseq package (McMurdie & Holmes, 2013).

3. Results

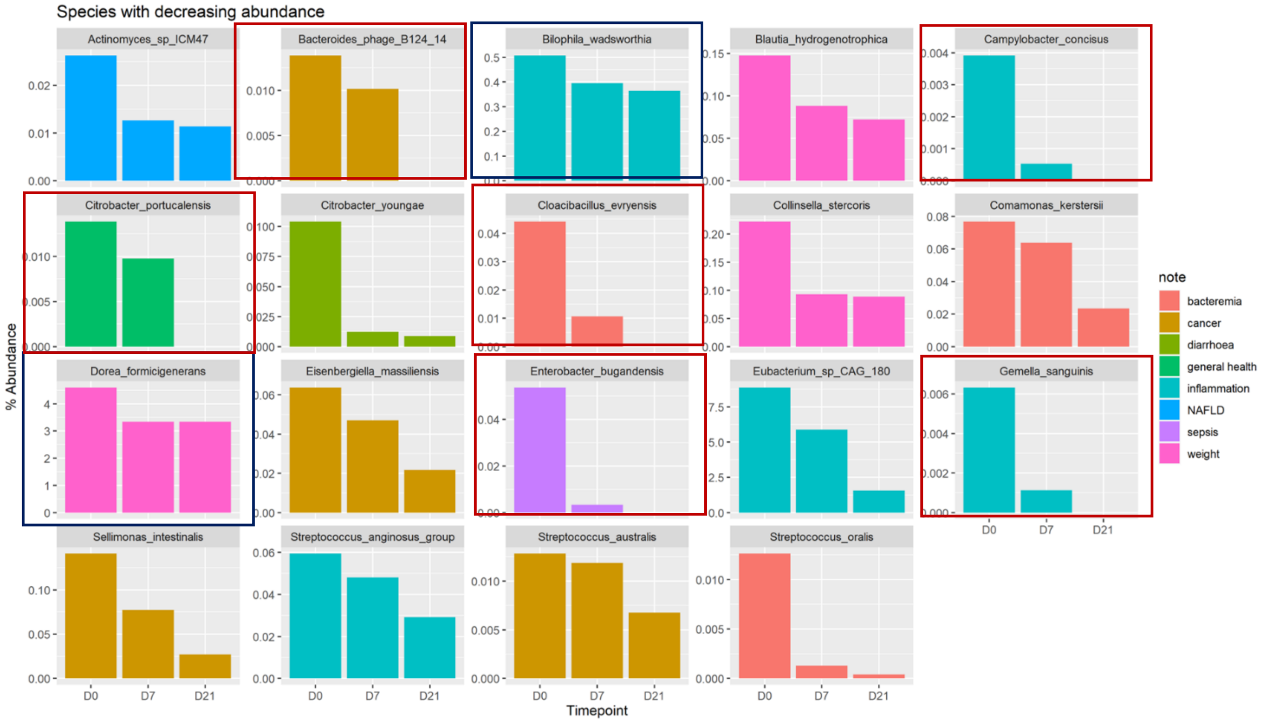
Graph 1: All the participants experience positive gut microbiome changes



The participants were classified based on the net diversity gain/loss at the end of the intervention period. Compared to baseline, participants n the moderately responsive group had lower diversity while strongly responsive subjects exhibited higher diversity at D21.

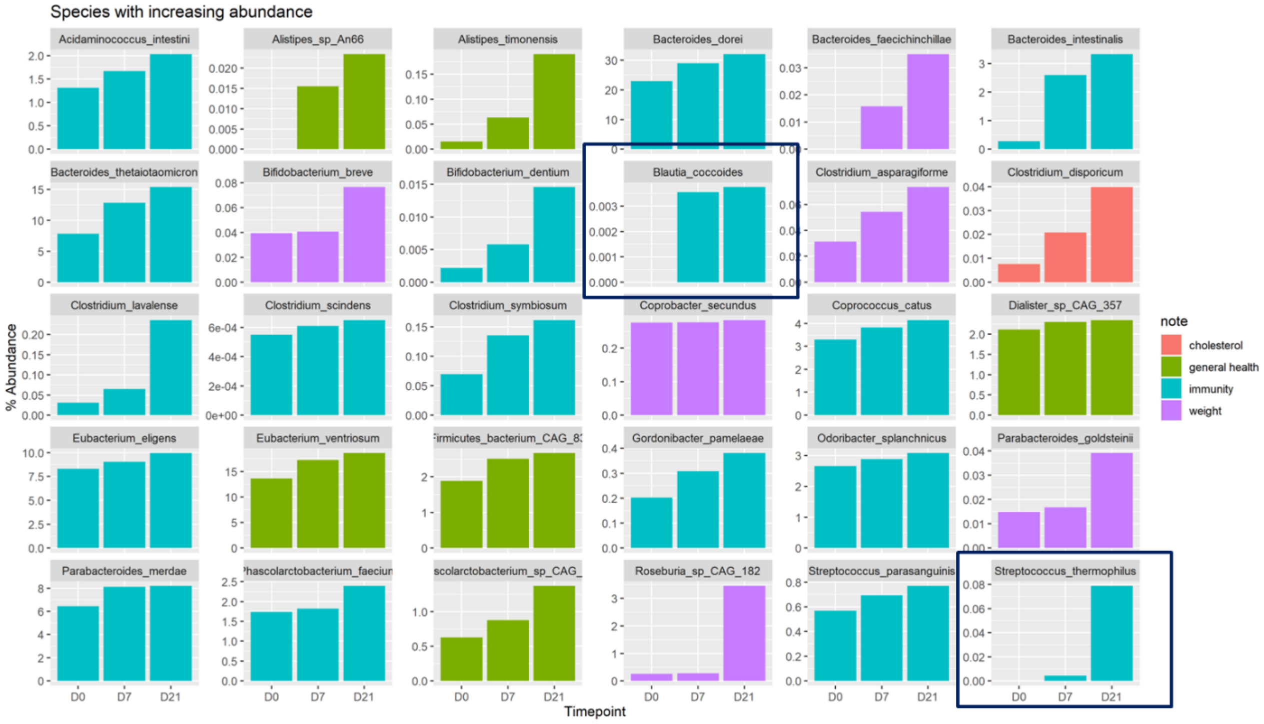
We further analysed the species that were differentially abundant throughout the study period. At the end of the intervention, moderately responsive group exhibited a reduced abundance in 19 disease related species throughout the study period (Graph2). Similar signals were also observed for those in the strongly responsive group by D7, in addition to the increased abundance and emergence of health-related species not observed at D0. Based on this result, we hypothesised that AWE diet is negatively associated with disease related species, however only strong responders exhibited higher abundance of health-related species at D21 (Graph3), explaining the net positive diversity gain of strong responders but not moderate responders at end of study period (graph1).

Graph 2: Species suppressed after intervention



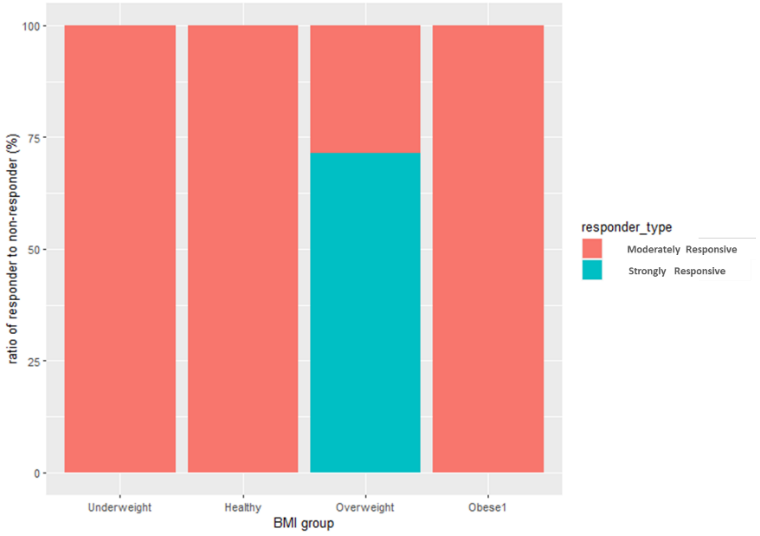
Microbiota Composition According to Responsiveness: 19 disease-related species suppressed throughout the interventions reported under the moderately responsive participants (60%). Of those suppressed were *Bilophila wadsworthia* and *Dorea formicigenerans*, which have been associated with higher inflammation and obesity, respectively (Graph 2- Blue Box). Same trend was also observed for 6 disease-related species (Cancer, Inflammation, Sepsis, weight management) that were totally eradicated by the end of the interventions (Graph 2- Red Box).

Graph 3: New Species after intervention



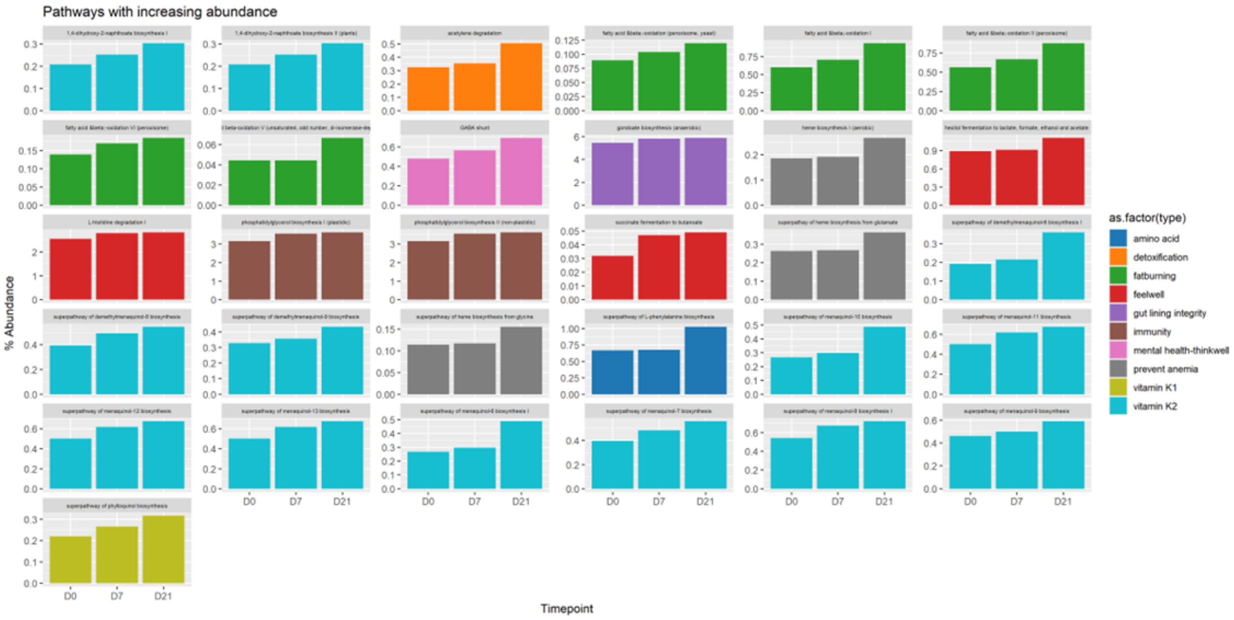
Microbiota Composition According to Responsiveness: The strongly responsive participants (40%) continue to report the trend of suppressing, eradicating disease-related bacteria and continue to promote growth of beneficial species throughout the interventions. A total of 30 species which are known for health benefits such as Cholesterol, Immunity & weight management were reported (Graph 3). Meanwhile, 2 novel Probiotic species such as *Blautia Coccoides* and *Streptococcus thermophilus* (Graph 3- Blue box) which have been associated with immunity were found by the end of the intervention. Probiotic species such as Bifidobacterium breve, Coprococcus catus and Eubacterium eligens were higher in abundance on day 21.

Graph 4: Overweight Participants have the most benefit



Microbiota Composition According to BMI: Significantly higher level of highly responsive group are from the overweight group. Further analysis revealed two distinct baseline microbiota profiles of the subjects, which dictated the response towards the intervention. The absence of *Bacteroides phage B124-14* and *Enterobacter bugandensis* in the baseline profile was associated with a higher diversity score for this group of participants.

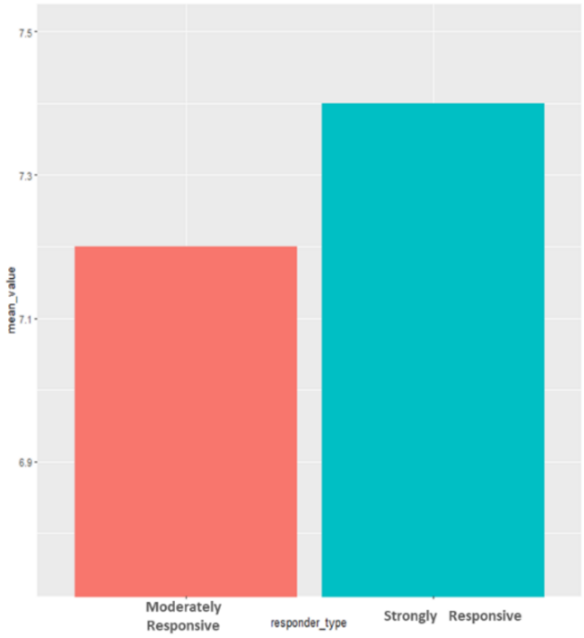
Group 5: Correspond to the Function Pathway elevation by end of the intervention



Functional Pathway: The intestinal microbiota plays an important role in the maintenance of host health. Several studies have linked changes in the gut microbial community to metabolic disorders, such as obesity and insulin resistance. Throughout the intervention, these pathways showed continuous increase and were associated with a better health profile of the subjects. Most of these pathways relate to vitamin K production (aqua bluer Bar), with some pathways relating to immunity (brown Bar), gut lining integrity (purple Bar) and detoxification (orange Bar).

Three pathways associated with the AMILI Gut health Test scores were also improved, relating to higher insulin signaling and SCFA production (red Bar) and GABA production (pink Bar). The observation is aligned with reports on high fiber diet was linked to a higher abundance of bacteria, which produce short chain fatty acids: these acids help control inflammation and protect the integrity of the cells lining the gut.

Group 6: Further supported by General Wellness (Likert Scale) reported positive by the end of the intervention.



Survey General Welling Score: Weekly score of all participants general welling (Feel Satisfy with the meal, Feel more energetic and Feel Healthier) reported that all participants scored more satisfied with the meal, more energetic and feeling healthier as the study progress. The strongly responsive group (0verweight participants) consistently scored higher than the moderately responsive group.

4. Conclusion

The study reports the effect of AWE meals altering the participants gut microbiota. Moreover, the role of AWE in modulating gut microbiota towards the overweight participants by increasing *Blautia coccoides* and *Streptococcus thermophilus* which have been associated with immunity has indicated a favourable health outcome for wider population if the AWE meals can be tailored to meet different dietary restrictions and needs.