AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2018 – 2020

TITLE: Efficacy of sea vegetable dulse as a prebiotic crop for metabolic health

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EXECUTIVE SUMMARY:

Pacific dulse (*Palmaria mollis*) is a type of red algae that is increasingly cultured in the U.S. coastal states including Oregon. With its recently gained publicity with its culinary application based on the work of Oregon State University (OSU) researchers, dulse is developing as a new promising crop for local sea vegetable producers. As consumer interest towards sea vegetables is growing, this presents an opportunity to further our understanding of its potential as a valuable food crop for Oregon coast.

Amongst its various nutritional properties, dulse is a rich source of dietary fibers including polysaccharides. Dietary fibers are the indigestible part of plant food by human enzymes and a main type of prebiotic that can modulate gastrointestinal microorganisms by promoting bacterial fermentation in the gut. Evidences suggest that dysbiosis, a state of imbalance and alteration of the intestinal flora composition, links to the development of various diseases including obesity, diabetes, inflammatory diseases as well as impaired immunity and infection. In this regard, fiber-rich food such as dulse deserves a particular attention as soluble fibers serve as a prebiotic that can positively alter the composition of gut microorganisms by acting as a substrate for bacterial fermentation and alleviate dysbiosis. Further, foods rich in dietary fibers are shown to improve metabolic health in obesity and reduce chronic inflammation, which may be associated with modulation of gut microbiome. Although sea vegetables such as dulse are a valuable source of dietary fiber occurring at higher level than most plants food and with a potential as a powerful prebiotic, their impact on the gut microbiome and its possible connection to metabolic inflammation has not been investigated.

Given the exceptionally high content of dietary fiber in dulse, we hypothesized that it may positively regulate the gut microbiome and that this regulation substantially contributes to the overall beneficial effect of dulse consumption on health and metabolism. Therefore, the study was designed to verify the effect of dulse consumption on metabolic health and inflammation in obesity and its link to the change in gut microbiota population and function.

OBJECTIVES:

The project aimed to investigate the effect of Pacific dulse (*Palmaria mollis*) consumption on metabolic health and inflammation in obesity in an animal model and address how the effect is linked to the gut microbiome. This was in order to assess the value of dulse to be promoted as healthy food crop and support creating new opportunity of market/revenue for Oregon fisheries. The specific objectives of the project included:

1. Determine the effect of dulse consumption on metabolic health and inflammation in a mice model.
2. Characterize the effect of dulse consumption on the gut microbiome and elucidate its link to the elicited physiological effect.

PROCEDURES:
1. Determine the effect of Pacific dulse (*Palmaria mollis*) consumption on metabolic health and inflammation in a mice model.
   a. Dulse was provided by Dr. Christopher Langdon at Hatfield Marine Science Center and study diet was formulated to contain 5% (w/w) of dulse in the diet.
   b. To demonstrate the physiological effect of dulse consumption, the status of metabolic health and systemic inflammation was monitored by measuring metabolic parameters and circulating level of cytokines in high fat diet-induced obese (DIO) mice fed with or without dulse.
2. Characterize the effect of dulse consumption on the gut microbiome and elucidate its link to the elicited physiological effect.
   a. To determine whether the consumption of dulse alters the gut microbiome, we characterized the gut microbiome in DIO mice fed with or without dulse via 16S rRNA gene sequencing.

SIGNIFICANT ACCOMPLISHMENTS:
In our study, we aimed to test the hypothesis that consumption of dulse (*Palmaria mollis*) would offer positive metabolic benefit against obesity and also influence intestinal microflora balance. For this study, we conducted mice feeding study with 8 weeks old male C57BL6J mice separated into designated test diet groups. Test diets containing 5% freeze-dried and milled dulse in high fat diet (HFD) were provided ad libitum for 8 weeks. Wakame (*Undaria pinnatifida*), another species of sea vegetable, which is well-studied for its metabolic health benefits, has been included in the study in comparison of the overall effect with dulse. Weekly body weight and daily food intake were monitored, and blood and fresh fecal samples were collected at the 8th week for further analysis.

1. Dulse and wakame suppress obesity induced by HFD and increase fecal fat secretion.
Over the course of 8 weeks of designated diet feeding, modest but statistically significant decrease of body weight was observed in both dulse and wakame-fed groups compared to HFD-fed group (Figure 1A). Intriguingly, daily dietary intake values indicated that dulse diet group consumed significantly more food than the HFD group, which is indicative of better palatability of the diet (Figure 1B). The different level of daily dietary intake prompted us to compare the food efficiency of each diet in order to determine how much actual weight is gained from same amount of diet. Food efficiency is determined by calculating the amount of weight gained per fixed amount of diet, and lower food efficiency indicates less weight gain from same amount of diet. Of interest, the results showed that dulse-fed group had significantly lower food efficiency compared to HFD group (Figure 1C). Lower food efficiency in the animal may indicate lower energy absorption or higher energy expenditure. To confirm this postulation, we examined the level of fat excretion in the feces. The fecal triglyceride level

significantly increased in both dulse and wakame-fed group at 2.4-fold and 6.2-fold, respectively, compared to HFD group (Figure 1D). The increase in dulse group was particularly dramatic, which strongly indicates reduced level of fat absorption. These data suggest dulse may elicit anti-obesity effect through reduction of intestinal fat uptake. To better address the physiological effect of sea vegetable-supplemented diet and achieve accurate comparison between groups, controlling the diet intake to same level in each group will be necessary, and this will be part of the design of our future study. In addition, increased number of animals in each group is required to improve the data dependability and reduce interference of biological variance.

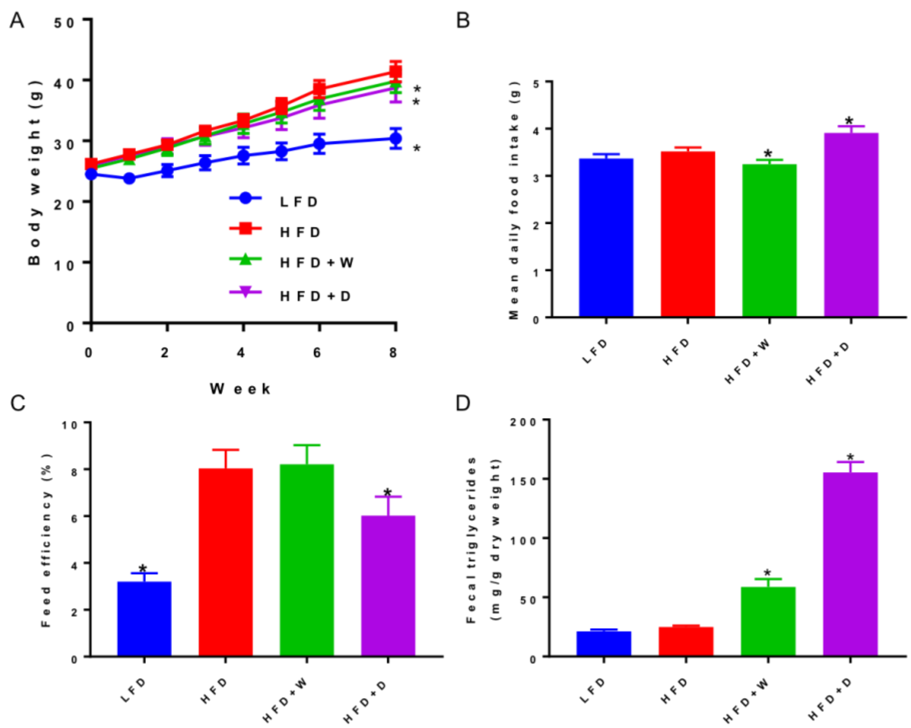

Figure 1. Effect of wakame and dulse supplementation on body weight and energy intake in C57BL6J mice under HFD. (A) Change of body weight, (B) average daily food intake, (C) % food efficiency, (D) triglyceride content in feces. LFD, control low fat diet; HFD, high fat diet; HFD+W, 5% wakame-supplemented diet; HFD+D, 5% dulse-supplemented diet. Significant difference (*, P < 0.05) was determined compared to HFD group based on one-way ANOVA followed by Tukey's multiple comparison.

2. Dulse and wakame consumption displays anti-inflammatory activity in DIO mice

To examine the effect of wakame and dulse on systemic inflammation in obesity, we analyzed the level of circulating monocyte chemoattractant protein-1 (MCP-1) in DIO mice. MCP-1 is a chemokine implicated in proinflammatory signaling, in particular, macrophage-linked inflammation in adipose tissue. Both wakame and dulse diet dramatically suppressed the HFD-induced elevation of MCP-1 level (Figure 2), suggesting strong anti-inflammatory effect of these sea vegetables in obesity. This aspect will be further investigated in our proposed project to demonstrate the effect of wakame and dulse in systemic and local inflammation, its underlying mechanism, and its potential link to gut microbiota.

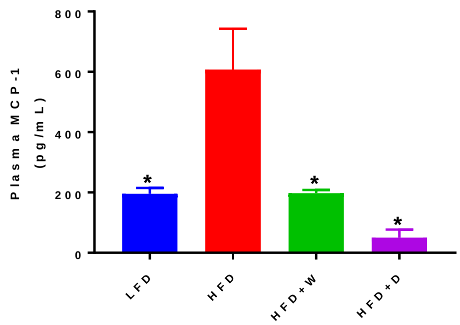

Figure 2. Effect of wakame and dulse supplementation on plasma MCP-1 level in C57BL6J mice under HFD. LFD, control low fat diet; HFD, high fat diet; HFD+W, 5% wakame-supplemented diet; HFD+D, 5% dulse-supplemented diet. Significant difference (*, P < 0.5) was determined compared to HFD group based on one-way ANOVA followed by Tukey's multiple comparison.

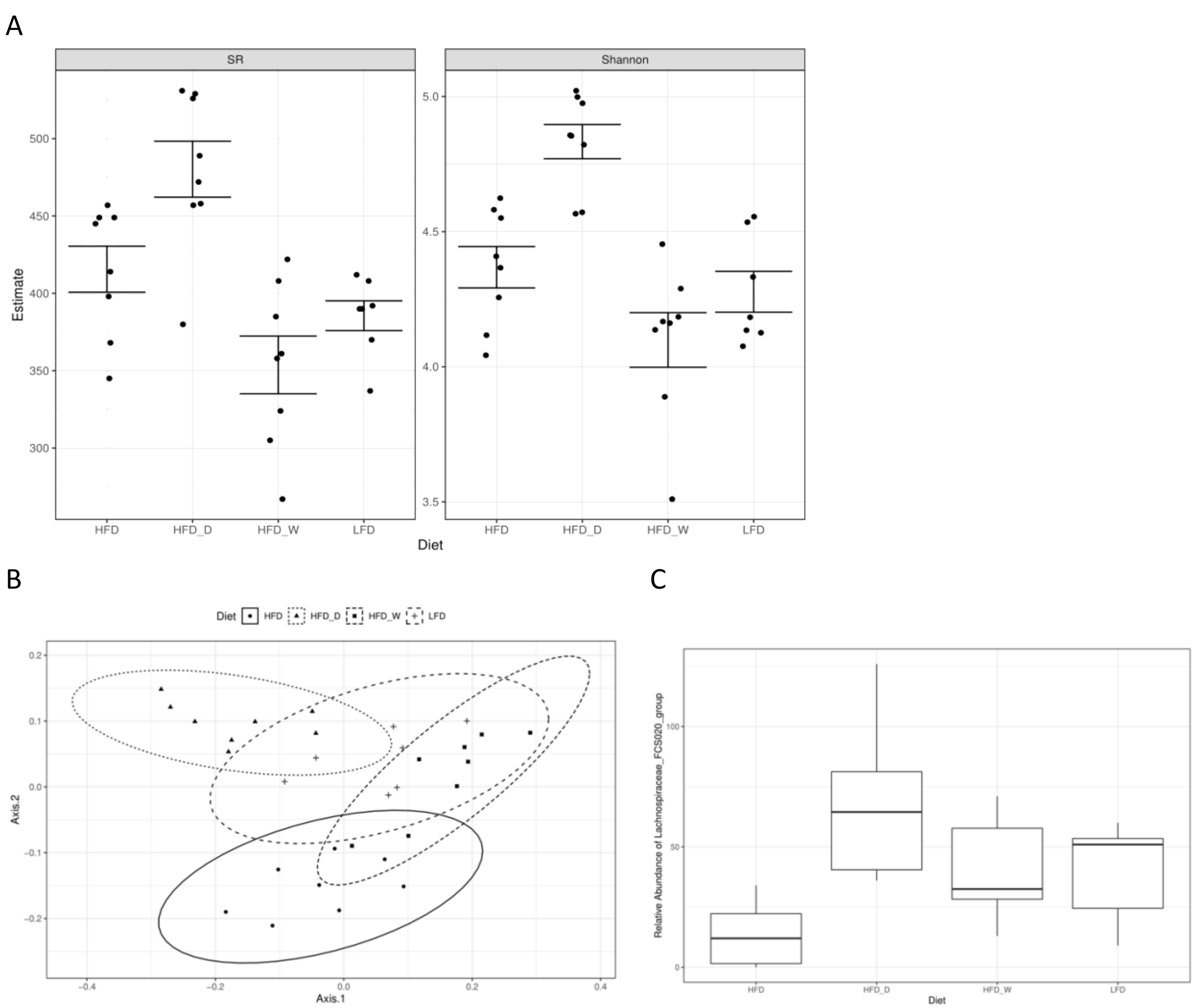

Figure 3. Effect of wakame and dulse supplementation on gut microbiome in C57BL6J mice under HFD. (A) Microbiome alpha-diversity analyzed by species richness (SR; left) and Shannon entropy (Shannon; right), (B) microbiome ordinations analyzed by Bray-Curtis dissimilarity beta-diversity metric, where ellipses represent 95% confidence intervals, (C) the rarefied abundance

of Lachnospiraceae_FCS020_group. LFD, control low fat diet; HFD, high fat diet; HFD+W, 5% wakame-supplemented diet; HFD+D, 5% dulse-supplemented diet.

3. Dulse and wakame consumption modify gut microbiome in DIO mice

To examine the correlation between the effect of sea vegetable consumption on physiology and lipid uptake and intestinal microflora composition, we characterized gut microbiome from the fresh fecal samples collected from each animal at the end of 8-week feeding period. The microbiome analysis was based on the sequencing of the V3V4 hypervariable region of the 16S rRNA gene. Wakame or dulse-fed mice manifested significant differences in the diversity and structure of the gut microbiome relative to controls based on alpha- and beta-diversity analyses (Figure 3). For example, both community species richness and Shannon entropy, which is a summary statistic of community evenness, vary as a function of diet (Figure 3A; ANOVA $p < 0.001$). Notably, mice fed sea vegetables not only differ in these measures of alpha-diversity from high-fat diet controls, but each sea vegetable manifests a significantly different effect on microbiome diversity, indicating that each vegetable elicits a unique impact on the microbiome. Species richness, but not Shannon entropy, also significantly correlated with fecal fat content (ANOVA; $p = 0.006$). Diet also significantly impacts the composition of the gut microbiome as measured by the Bray-Curtis dissimilarity beta-diversity metric (Figure 3B; PERMANOVA $p < 0.001$; consistent results observed for Canberra and Weighted Unifrac distances). In fact, mice fed HFD supplemented with sea vegetables manifested microbiome compositions that were more similar to mice fed LFD than those fed HFD. Moreover, microbiome Bray Curtis dissimilarity significantly associates with an individual’s weight gain, supporting the hypothesis that diet induced alterations to the gut microbiome impact host obesity status (Capscale $p = 0.037$). Sea vegetable supplementation impacted the relative abundance of 34 genera (FDR corrected Kruskal-Wallis $p < 0.05$), including the Lachnospiraceae_FCS020_group, whose relative abundance increased to levels more consistent with LFD in sea vegetable supplemented mice (Figure 3C). This result shows that despite each vegetable’s unique effect on the microbiome, there exist common trends in how sea vegetable potentially rescues a HFD’s impact on the microbiome. We plan to expand this analysis to additional taxonomic levels and resolve taxa that associate with physiological covariates.

* An abstract was submitted based on this study to *Nutrition 2019* conference organized by American Society for Nutrition which led to a poster presentation.
* A manuscript was produced from this study and submitted to the journal of *Current Development in Nutrition* and is currently under review.

BENEFITS & IMPACT:

The market for sea vegetables in the U.S. is rapidly growing due to consumer recognition of their health benefits. Local sea vegetable production is at the beginning stage with a great potential of growth. Dulse is an excellent target for promotion with increasingly ongoing production in the U.S. coastal states including Oregon. Our study provided initial evidences of health value of dulse which will facilitate validation of dulse as an emerging healthy food source. A continued scientific research verifying its biological function will increase in market demands and new revenue for fisheries. Especially, by building up on the current research at

OSU for its cultivation and culinary application, establishing the research of nutritional aspect of dulse will set Oregon forward as a global leader in dulse research and production.
Although dulse is an excellent source of dietary fiber, its impact on gut microbiome and its potential connection to metabolic inflammation remains unknown. Our study revealed the potential of dulse as an effective prebiotic, which will support improving human gastrointestinal and metabolic health through sustainable natural product.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: None

FUTURE FUNDING POSSIBILITIES: ODA, USDA