Fiber Force: A Fiber Diet Intervention in an Advanced Course-Based Undergraduate Research Experience (CURE) Course†

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Course-based undergraduate research experiences (CUREs) are an effective way to introduce students to contemporary scientific research. Research experiences have been shown to promote critical thinking, improve understanding and proper use of the scientific method, and help students learn practical skills including writing and oral communication. We aimed to improve scientific training by engaging students enrolled in an upper division elective course in a human microbiome CURE. The “Fiber Force” course is aimed at studying the effect of a wholesome high-fiber diet (40 to 50 g/day for two weeks) on the students’ gut microbiomes. Enrolled students participated in a noninvasive diet intervention, designed health surveys, tested hypotheses on the effect of a diet intervention on the gut microbiome, and analyzed their own samples (as anonymized aggregates). The course involved learning laboratory techniques (e.g., DNA extraction, PCR, and 16S sequencing) and the incorporation of computational techniques to analyze microbiome data with QIIME2 and within the R software environment. In addition, the learning objectives focused on effective student performance in writing, data analysis, and oral communication. Enrolled students showed high performance grades on writing, data analysis and oral communication assignments. Pre- and post-course surveys indicate that the students found the experience favorable, increased their interest in science, and heightened awareness of their diet habits. Fiber Force constitutes a validated case of a research experience on microbiology with the capacity to improve research training and promote healthy dietary habits.

INTRODUCTION

Incorporating research experiences into the undergraduate curriculum is a major emphasis of national educational reform (1, 2) and the American Society for Microbiology (ASM) (3). The educational field proposes integration of research experiences into traditional lab courses in the form of course-based undergraduate research experiences (CUREs) (4). CUREs enable students to experience research first-hand, providing a more accurate understanding of how scientific research is conducted. Authentic research experiences lead to student-reported gains in general skills (e.g., oral, visual, and written communication) and more specific research-associated skills (e.g., research design, hypothesis formation, data analysis) (5–8). In addition, students in CURE courses develop scientific reasoning skills, begin to identify themselves as scientists, are more inclined to pursue graduate education or careers in science (9–11), and have increased graduation rates (12).

To maximize student engagement in research and in the classroom, CURE curricula should focus on relevant research topics and questions. One of the fastest growing research areas in the last 10 to 15 years has been the relationship between human health and the human microbiome, or the consortia of commensal microorganisms living in and on our bodies. The gut microbiome alone encompasses more than 1,000 resident microorganisms, including bacteria, viruses, fungi, and protozoa (13). The majority of these microorganisms inhabit the colon, where they contribute to human health through the biosynthesis of vitamins and essential amino acids and the generation of metabolic

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byproducts through fermentation of low- or non-digestible dietary fiber (previously termed fiber) (14–19). The type and proportion of fiber that reaches the colon is an important factor that can drive alterations of gut microbial composition (20–22). Diets low in fiber reduce total bacterial diversity and abundance in the gut microbiome (23), while diets high in fiber increase microbiota richness (24, 25). Interest in the role of dietary fiber in regulating the gut microbiome has led to diet interventions, which suggest that a high-fiber diet can alter metabolic parameters (21, 26) and ameliorate clinically relevant colon cancer biomarkers (27).

Given the importance of fiber for a healthy gut, further dietary studies are still needed. Especially in healthy individuals, there is a need to understand not only the stability and resilience of the “normal” microbiota, but also the amount and type of fiber that can result in positive microbial changes. Therefore, we developed a research-based learning laboratory course at the University of California (UCI) to study the effect of a high-fiber diet on the gut microbiome of college students. The Microbiome laboratory course at UCI, or Fiber Force, aimed to engage students in research activities by actively participating in a safe diet intervention. Students manipulated their own gut microbiomes through a safe high-fiber diet intervention and actively participated in the sample processing and data analysis. Students analyzed their own samples, gained molecular experience in the laboratory and became computational tools to analyze the data. Similar to other microbiology modules described recently (27–30), a goal of Fiber Force was for students to develop critical reasoning and problem-solving skills related to microbiology and microbiome research.

Prerequisite student knowledge

Students were expected to have completed all core biology courses (including Chemistry, Biology, Molecular Biology, and Biochemistry), a common practice for upper-division laboratories at UCI. Students had a basic knowledge of microbial cell biology, the central dogma of biology, as well as metabolic and phylogenetic diversity. Many students also had experience in pipetting, PCR, southern blotting and DNA gel electrophoresis. Since this experience was not assumed, and we covered basic laboratory principles and practices in the course. Advanced analyses, such as phylogenetics, were covered with active lectures and discussions (Appendix 5).

Learning objectives

Upon completion of this activity, students will be able to:

• Design and execute a dietary intervention plan to study the gut microbiome, with proper sample collection and storage.
• Become proficient in microbiome laboratory and computational techniques and skills.
• Apply basic microbiome principles and concepts to solve experimental problems.
• Apply the scientific method in different ways, including writing hypotheses, analyzing results, reporting data, and proposing further experiments.
• Report hypothesis, proposed research, results, and conclusions both orally and in writing to an audience of scientists and peers.
• Interpret and evaluate results from lab experiments, including writing hypotheses, analyzing results, filtering data, 4) denoising data, and 5) calculating diversity and other statistical metrics.

The first day of the workshop consisted of an introduction for food science students. Then a Mac OS or Linux operating system (Windows users were instructed to enable Windows Subsystem for Linux, a standard feature on Windows 10). Students followed installation instructions for QIIME (https://docs.qiime2.org/2019.4/install/) through a standard Miniconda installation. During installation, students discussed the QIIME2 pipeline and the vocabulary that comes with microbial sequence data (see ideas for discussion topics in Appendix 3). Students learned Linux-based commands, such as navigating, creating, and removing directories. After installation, students completed an abridged version of the “Moving Pictures” tutorial (https://docs.qiime2.org/2019.4.tutorials/moving-pictures/), which skips time-intensive steps such as denoising. The workshop emphasized the interpretation of data, such as alpha and beta diversity plots.

The R and QIIME2 workshops described above can be replaced by computational and data analysis training. For resources and training on microbiome analysis we recommend checking the Human Microbiome project webpage (https://www.hmpdacc.org/ourworkshops/phase2.php) (42). Bioinformatics Inquiry through Sequencing (35, 43). Galaxy resource (https://usegalaxy.org/) (44), and the Program for Unifying Microbiome Analysis (45).

Student instructions

Instructions and protocols were provided to students via electronic files on our learning management system. The protocol for DNA extraction was used directly from the manufacturer’s manual (Zymo, https://www.zymoresearch.com/collections/zymobiomics-dna-kits/products/zymobiomics-dna-miniprep-kit). Each student carried out the protocols individually or external samples were used according to the class schedule. The R and QIIME2 workshops described above can be replaced by computational and data analysis training. For resources and training on microbiome analysis we recommend checking the Human Microbiome project webpage (https://www.hmpdacc.org/ourworkshops/phase2.php) (42). Bioinformatics Inquiry through Sequencing (35, 43). Galaxy resource (https://usegalaxy.org/) (44), and the Program for Unifying Microbiome Analysis (45).

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tants are in charge of facilitating discussions, teaching active lessons, supervising group work, answering questions, and demonstrating techniques (when needed). The weekly discussion (active lecture) handouts are provided in Appendix 6. To ensure students properly practice research and oral communication skills, we recommend adding “laboratory or group meetings” to the schedule to discuss research questions, hypotheses, research plans, preliminary results, and troubleshooting. At the end of the course, students share their findings as a poster presentation, to which instructors, teaching assistants, students, and research laboratories are invited. The protocols (Appendix 8) and the respective required laboratory preparation, including materials and kits (Appendix 9) are described in the appendices.

A significant prerequisite to implementing the Fiber Force course is getting Institutional Review Board (IRB) approval in advance, a process that can take up to six months. UCI IRB reviewed the educational (educational research) and diet intervention (clinical study) components separately. To ensure timely approval of IRB requests, we recommend 1) providing a detailed description of how the data will be anonymized for privacy protection, 2) writing a detailed consent form for students that includes a protocol for opting out of the study without affecting course performance (see the consent form in Appendix 16), and 3) using a third-party (other than the instructor) as holder of the key that connects student’s names with sample codes. The IRB background should cite previous studies performed in the context of classrooms (see, for example, 46–48) or the present manuscript (UCI IRB # 2018-4297) as precedent.

**Suggestions for determining student learning**

An essential component of research involves the ability to write a research proposal, conduct experiments in a rigorous and reproducible manner, and communicate scientific findings in oral and written forms. Accordingly, the assessment task for Fiber Force involved 1) a step-by-step proposalwriting activity (Appendix 3) to evaluate ability to design and propose new research; 2) weekly discussion handouts and lab notebook evaluations to assess laboratory performance; 3) data meeting reports and oral poster presentations to evaluate science communication. The assessments are described in full in the course syllabus (Appendix 5). The data meeting reports and poster format followed structural conventions of a scientific publication. The activities required students to outline the background of the field, provide the aims and hypotheses of the study, present results in both text and graphical forms with descriptive legends, and discuss the validity and significance of their findings. The integration of this assessment task with learning activities in this project directly align with our learning objectives (Table 1). The marking rubric for the proposal writing spanned numerous criteria including quality of aims, literature support, effective introduction of project aims, validity of research questions and aims, and validity of proposed research. These rubrics are part of a manuscript that is currently under preparation (contact the corresponding author). The rubrics used to evaluate lab notebooks, lab meeting presentations and poster presentations are included in Appendix 13.

**Sample data**

Students were required to read the literature on the effects of diet on the gut microbiome. This led to predictions of and/or hypotheses on the impact of fiber before and after the diet intervention. After the samples were sequenced, students were expected to interpret OTU tables, use QIIME2/R to visualize data, and compare samples to validate (or not) their predictions. Students then presented their results during a poster session (samples in Appendix 19). One example predicted that the intervention would lead to an increase in fiber-degrading bacteria (for student-led literature review see Appendix 19) as these bacteria should possess a specialized ability to degrade complex carbohydrates. After analysis, the students found that the > 40 g fiber intervention caused no significant change in diversity or abundance of microbes at the genus or phylum levels, but registered increases in some relevant bacterial genera. A second example hypothesized that a 40- to 50-g fiber intervention would exhibit increases in short-chain-fatty-acid-producing bacteria known to be responsible for gastrointestinal fermentation. However, the students concluded that there was no significant difference in the relative abundance of short-chain-fatty-acid-producing bacteria before versus after the diet intervention. This shows that the same dataset can be used by different groups of students to study different predictions or hypotheses.

**Safety issues**

The Fiber Force course involves collection of fecal samples from healthy individuals (according to answers from the health survey Appendix 16), but potentially pathogenic bacteria can be present in fecal samples. As part of the course, instructors and teaching assistants receive BSL2 safety training to instruct students on the necessary personal protective equipment (PPE), the safe handling of samples, and the proper disposal of biological waste in accordance with BSL2 regulations. Aliquoting of fecal samples, as well as bead-beating (the first step in DNA extraction) should be conducted inside a biosafety cabinet, and DNA extraction should be conducted in a BSL2 laboratory. The subsequent steps (PCR, electrophoresis) can be performed in a BSL1 laboratory. Students with health conditions such as pregnancy, allergies, or immune-compromised status should not directly handle or come into contact with fecal samples. Laboratory bench surfaces and notebooks were decontaminated with 70% ethanol before and after each session and all students washed their hands with antibacterial detergent. Electronic devices were used (if needed) inside sealed plastic bags that were wiped before and after each use. All waste was disposed of in accordance with BSL2 regulations and decontaminated by autoclaving. The biosafety rules and policies for the laboratory are included in the syllabus (Appendix 5).

**FIGURE 1.** Course performance between 2017 and 2018 courses were compared using the following grade scores: A) grade on a step-by-step research task for Fiber Force involved; B) weekly discussion handouts to assess data analysis performance; C) data meeting reports and oral poster presentation to evaluate science communication.

**FIGURE 2.** Experimental design for the high-fiber study intervention and collection of fecal samples.
Field testing

Fiber Force was tested in an Advanced Molecular Biology course at the University of California, Irvine, with an enrollment of 18 students. The project protocols and interventions were cleared in accordance with the UCI IRB, and participants provided consent with regard to their de-identified microbiome data and responses to health and course surveys. Teaching and lab assistants increased participants to 22 (Fig. 2). To ensure participants consumed a diet rich in wholesome fiber for two weeks, each participant was provided with 10 meals per week from a food delivery company called Thistle. These lunch and dinner meals had pre/menu/). For breakfast and snacks, as well as on weekends, students supplied their own meals. In our experience, supplying ready-made meals ensured that participants ate a varied and wholesome diet. However, this was not a necessary part of the course, as instructors can provide resources for students to design their own diets (see Appendix 14). For instance, legumes such as split peas, lentils, lima beans, black beans, and chickpeas are particularly rich in fiber (12 to 15 g of fiber per cup) and are economical for students' budgets. Students frequently discussed their diet choices during class, sharing ideas and recipes with each other, and the diet intervention also raised awareness about healthy food habits. Participation in the study as a subject was not required to take the course, nor was it needed to receive meal compensation. In addition, course grades were not attached to participation as a study subject (i.e., samples were de-identified).

The first week of class consisted of discussing the intervention and designing health surveys with the students to evaluate their diet intervention. Students were asked to find examples of surveys in the literature and design (as a class) a 10- to 25-question survey about health status, usual (pre-intervention) food habits, and other relevant questions for the study (e.g., body mass index [BMI]). Students answered their designed survey (Appendix 16), indicating that all students were generally healthy. After the intervention, we asked students to complete an open-ended survey that included questions about their experience in the Fiber Force course (see questions in Appendix 17 and answers in Appendix 18).

Participants in the intervention increased their fiber ingestion from an average of approximately 15 g (lowest pre-intervention individual averaged 1.8 g) to an average of approximately 40 g of fiber per day (highest intervention individual averaged 54 g) with a concomitant increase in carbohydrate consumption (Fig. 3B). The intervention minimally increased fat and protein consumption with similar caloric intake (Fig. 3B). As a consequence of this dramatic increase in dietary fiber, 42% of students reported an increase in flatulence and bloating, and 31% reported appetite loss (Appendix 18). These are expected secondary effects due to the increase of bulk and fermentation. Students did not report increased time in meal preparation or cooking during the intervention, suggesting that the intervention does not drastically increase time commitment outside the classroom.

Evidence of student learning

To determine whether students in the Fiber Force course mastered important research-based skills, we measured course grade on important skill-based assignments. We also compared performance in the Fiber Force course (2018) with a previous version of the course (2017) that used a research module (37) using synthetic biology to study bacterial promoters (Fig. 4). In the 2017 course, students designed primers to study their own promoters and made predictions, but they did not participate in the experimental design nor were they active participants in the study. The types of course assignments, rubrics and grade weight in 2017 and 2018 (Table 1) were the same, except that the topic of proposal writing and discussions in 2017 was synthetic biology. The 2018 cohort exhibited a lower GPA in prior lower-division biology core courses than the 2017 cohort (Fig. 4A). The 2018 cohort also had less laboratory experience, with 62% of the students indicating that they had taken zero or one laboratory before Fiber Force, compared with 42% in 2017. This means that the 2018 cohort had less preparation for this upper-division research course. However, both groups showed similar average final course grades at the end of the quarter (Fig. 4B). The 2018 cohort also performed better in the Fiber Force course than

FIGURE 3. Fiber type and quantity and dietary changes by participants in the fiber intervention study. A) Quantities (in grams) of fiber, protein, carbohydrates, fat, and calories pre- and post-intervention (N=24). B) Participants in the diet intervention were asked to answer the following: "What were your ‘go to’ or staple high-fiber foods? Name your top 3 and provide a description." Answers were tallied and plotted by frequency (n=18).

FIGURE 4. Performance of students in the Advanced Molecular Biology course M130L in Fiber Force (2018) compared with control (inquire module, studying promoters using pClone by 37) 2017 course. In 2017, each group picked a guided inquiry project module and collected data. Students studied their own promoters but did not participate in experimental design. In the 2018 course, the class as a whole volunteered as participants in interventions, designed surveys, and discussed data. They also worked in small groups for presentation and discussion. Panel A compares the students' GPA in biology-related core courses (Biochemistry, Molecular Biology) followed before the 2017 and 2018 courses, as a way to compare incoming level. Panel B compares the average final course grade in the 2017 and 2018 courses. Panel C compares the grade average in the 2017/2018 courses with grades in other courses that students followed simultaneously that quarter. Grade anomaly is the average GPA on other courses that quarter minus M130L course grade.
TABLE I. Alignment between learning goals and course assessments

<table>
<thead>
<tr>
<th>Learning Outcomes</th>
<th>Assessment</th>
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<tbody>
<tr>
<td>Design and execute a dietary intervention plan to study the gut microbiome, with proper sample collection and storage</td>
<td>Assessed through data meeting reports, lab notebook points, and poster presentation</td>
</tr>
<tr>
<td>Become proficient in microbiome laboratory and computational techniques and skills</td>
<td>Assessed through data meeting reports, lab notebook points, and poster presentation</td>
</tr>
<tr>
<td>Apply basic microbiome principles and concepts to solve experimental problems</td>
<td>Weekly quizzes</td>
</tr>
<tr>
<td>Apply the scientific method in different ways, including writing hypotheses, analyzing data, reporting, and proposing further experiments</td>
<td>Assessed by poster presentation, lab meeting reports, and grant proposal writing</td>
</tr>
<tr>
<td>Report hypotheses, proposed results, and conclusions both orally and in writing to an audience of scientists and peers</td>
<td>Discussions of research articles, lab reports, and grant proposal writing</td>
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<tr>
<td>Interpret and evaluate results from lab experiments and primary research articles</td>
<td>Lab reports, lab meetings, poster presentations</td>
</tr>
<tr>
<td>Critically analyze scientific publications, research proposals and results</td>
<td>Lab discussions and problems and analysis of research articles, grant proposal writing, data meetings, poster presentations</td>
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Appendix 1: CURE workshop | Panel discussions and team projects |
Appendix 2: Qimera workshop | Lab activities and assignments |
Appendix 3: Grant proposal writing timeline | Weekly quizzes |
Appendix 4: 10-week course schedule | Lab reports and posters |
Appendix 5: Fiber Force course syllabus | Lab experiments and discussions |
Appendix 6: Discussion handouts | Lab exercises and projects |
Appendix 7: Stool collection instructions | Lab reports and presentations |
Appendix 8: Fiber Force protocols | Lab reports and preparation instructions |
Appendix 9: Laboratory preparation instructions | Lab reports and presentations |
Appendix 10: Guide to laboratory notebooks | Lab reports and presentations |
Appendix 11: Lab meeting guidelines | Lab reports and presentations |
Appendix 12: Poster presentation guidelines | Lab reports and presentations |
Appendix 13: Grading rubrics | Lab reports and presentations |
Appendix 14: Diet definitions and resources | Lab reports and presentations |
Appendix 15: UCI IRB consent form | Lab reports and presentations |

Appendix 16: Health questionnaire
Appendix 17: Post-microbiome survey study
Appendix 18: Post-microbiome survey study responses
Appendix 19: Sample student posters

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