Diminishing Inflammatory Bowel Disease and Metabolic Syndrome Severity through Naturally Occurring Fibers

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Diminishing Inflammatory Bowel Disease and Metabolic Syndrome Severity through Naturally Occurring Fibers

by

Rachael Ott

Under the Direction of Andrew Gewirtz, Ph.D.

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

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ABSTRACT

Diets rich in fiber are known to have significant health benefits, but patients with inflammatory bowel disease (IBD) have reported an increase in disease symptoms while on such diets. Thus, IBD patients are recommended to eat low fiber foods and are unable to gain health benefits from fiber. While it has been shown that genetic factors can play a role in the occurrence of IBD, environmental factors are thought to further induce IBD, specifically diet and its effect on the gut microbiota. The self-reporting from IBD patients has been supported with studies in which the fiber inulin added to a low fiber diet has caused increased severity of colitis; conversely, a diet of a standard mouse chow containing naturally occurring dietary fiber has shown resistance to the same colitis murine models. Similarly, metabolic syndrome is a cluster of health conditions related to inflammation and diet, with studies finding dietary fiber capable of reducing metabolic syndrome symptoms. From these findings, wheat and oat fibers have been analyzed to determine if they provide protection against colitis and diet-induced obesity in murine models. After performing the in vivo models, wheat fiber provided protective benefits against IBD and metabolic syndrome. With these results, patients with IBD or metabolic syndrome will be able to consume a dietary fiber that will significantly reduce their disease severity or potentially eliminate manifestations of IBD and reverse metabolic syndrome conditions.
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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract that is increasing in prevalence and incidence around the world, especially in Western countries (Ananthakrishnan, 2015; Baumgart & Carding, 2007). In America, more than 1 million people have been diagnosed with IBD (Ananthakrishnan, 2015). IBD is differentiated into two main diseases, Crohn’s disease (CD) and ulcerative colitis (UC). These diseases are associated with diarrhea, abdominal pain, rectal bleeding, and bowel urgency, which lead to a decrease in quality of life (Fakhoury, Negrulj, Mooranian, & Al-Salami, 2014). Additionally, the development of IBD is multifactorial and has been linked to genetics and environmental factors, such as childbirth method, antibiotics, NSAIDs, smoking, diet, and the gut microbiota (Ananthakrishnan et al., 2018; Baumgart & Carding, 2007). There is no available cure for IBD, which is costly for patients and healthcare systems, as patients require continual access to care and treatments (Ananthakrishnan, 2015). The current treatments include medications, surgery, and lifestyle changes, such as change in diet, exercise, and smoking status (Fakhoury et al., 2014; Seyedian, Nokhostin, & Malamir, 2019). Due to the multifactorial and chronic nature of IBD, diet has become a potential therapeutic candidate, with an emphasis on dietary fiber.

Similarly, metabolic syndrome is associated with diet interventions that may reduce inflammation and other conditions associated with metabolic syndrome, including hyperglycemia, hypertension, excess fat around the midsection, and insulin resistance (Aboonabi, Meyer, & Singh, 2019). Metabolic syndrome can cause heightened risk of heart disease, stroke, and diabetes and is extremely common in the U.S., comprising about 33% of adults diagnosed with the syndrome (Aboonabi et al., 2019; Aguilar, Bhuket, Torres, Liu, &
Wong, 2015). Unlike IBD, metabolic syndrome can be reversed through diet changes, exercise, smoking status, and weight loss, making dietary fiber an ideal modifier of disease conditions (Aboonabi et al., 2019; Stone & Saxon, 2005).

Gut health, and consequently the gut microbiota, are key factors in the pathogenesis of IBD and metabolic syndrome and are heavily impacted by diet (Chassaing, Vijay-Kumar, & Gewirtz, 2017). The gut microbiota is a diverse collection of microorganisms that encompass bacteria, viruses, and fungi (Fan & Pedersen, 2021). The microbiota is incredibly robust and can impact the host through several mechanisms (Fan & Pedersen, 2021). The mechanism of focus in this paper will be on secondary metabolites from bacterial fermentation, which include secondary bile acids (BAs) and short chain fatty acids (SCFAs).

Secondary BAs, including deoxycholic acid and lithocholic acid, are produced when primary BAs from the liver are transported to the small intestine and deconjugated and dehydroxylated by bacterial metabolism (Chiang, 2009; Zeng, Umar, Rust, Lazarova, & Bordonaro, 2019). The SCFAs in the gut, butyrate, acetate, and propionate, are created from fermentation of dietary fiber (Zeng et al., 2019). BAs and SCFAs differ in their functions and impact as primary BAs assist in lipid digestion and cholesterol uptake with their secondary successors being related to inflammation and negative alterations in the gut microbiota, whereas SCFAs are linked to anti-inflammatory effects and positive modifications to the microbiota (Zeng et al., 2019). Specifically, SCFAs foster the differentiation of anti-inflammatory T cells (Arpaia et al., 2013; Singh et al., 2018; Zeng et al., 2019). In addition, SCFAs function as an energy source for colonocytes, thereby affecting host metabolism (Donohoe et al., 2011; Singh et al., 2018). Inversely, high-fat diets can cause a proliferation of secondary BAs that can act on signaling pathways and immune system receptors, including farnesoid X receptor (FXR) and
pregnane X receptor (PXR) (Zeng et al., 2019). When FXR and PXR are inactivated, a pro-inflammatory environment is initiated (Zeng et al., 2019). Ideally, dietary fiber would counteract the negative effects of high-fat diets and secondary BAs and cause a rise in SCFAs.

Dietary fiber is specified as a nondigestible, complex carbohydrate that can come from plants (Holscher, 2017; Slavin, 2013). Fiber can be grouped into several categories based on the key characteristics of solubility, fermentability, and viscosity (Holscher, 2017; Slavin, 2013). Solubility is defined as the capability of a fiber to change stool weight, transit time of the digestive system, and serum lipid levels, whereas fermentability is determined by a fiber’s ability to be fermented by the gut microbiota to produce SCFAs (Holscher, 2017; Slavin, 2013). Additionally, due to their ability to increase transit time, insoluble fibers can be partly metabolized in the descending colon and sigmoid colon compared to soluble fibers fermented in the proximal colon (Holscher, 2017). Viscosity is the ability to form gels (Slavin, 2013).

Typically, soluble fibers will be fermentable and viscous, while insoluble fibers will be non-fermentable and non-viscous (Slavin, 2013). Nevertheless, there are exceptions to these grouped fiber properties, as seen with psyllium and inulin (Holscher, 2017; Slavin, 2013).

The relationship between diet, dietary fiber, and IBD is complex as it has been noted that diet is a trigger and treatment related to IBD. In epidemiological studies investigating diet and IBD, people diagnosed with a form of IBD consumed fewer foods rich in dietary fiber (Ananthakrishnan, 2015; Hou, Abraham, & El-Serag, 2011). On the contrary, it has been reported that patients with IBD have detailed an increase in disease symptoms after consumption of high fiber diets. This self-reporting was backed by studies with the fiber inulin, in which colitis symptoms were worsened after ingestion of the fiber (Miles et al., 2017). In the same study, a typical mouse chow containing fiber provided protection against colitis (Miles et al.,
2017). Similarly, in a separate study, various fibers had differing effects on colitis development in SPF mice, with psyllium, cellulose, and pectin reducing colitis symptoms and methylcellulose worsening colitis symptoms (Llewellyn et al., 2018). These findings have led to the investigation of fibers that lessen gut inflammation and symptoms associated with IBD and deliver fiber benefits. In addition, due to the connection between diet, inflammation, and gut health, it has been postulated that fiber could reduce metabolic syndrome symptoms. In several studies, fiber, specifically inulin, added to high-fat diets decreased metabolic syndrome severity in mice (Chassaing et al., 2015; Zou et al., 2018). Although inulin has differing effects on colitis and metabolic syndrome, by worsening colitis severity and protecting against metabolic syndrome, it is curious if there were fibers that could alleviate both inflammation-related diseases.

Oat and wheat fibers have been selected as the initial fibers to examine based on their availability as naturally occurring fibers and their high total of dietary fiber, as observed in previous fiber reviews and confirmed by preliminary fiber testing (Dhingra, Michael, Rajput, & Patil, 2012). In addition, and shown in the preliminary testing, the solubility percentages in oat and wheat fibers range from 86-90% insoluble fiber and 1-6% soluble fiber and align with previously determined cellulose percentages. Since oat and wheat fibers resemble cellulose as being largely insoluble and contain a substantial amount of dietary fiber, it is hypothesized that these fibers would mimic cellulose’s effects in an *in vivo* model as promoters of stool movement through the gut and of partial fermentation. Due to their beneficial effects based on these properties, it is postulated that oat and wheat fibers will provide protection against IBD and metabolic syndrome in a dextran sulfate sodium (DSS)-induced colitis model and a diet-induced obesity model in mice.

**METHODS**
Fiber Testing

Minimally refined oat fiber, highly refined oat fiber, and wheat fiber were purchased from J. Rettenmaier USA LP (JRS), Schoolcraft, MI. Samples of the oat and wheat fibers were sent to Medallion Labs, Minneapolis, MN for gravimetric fiber testing to identify the percentages of total dietary fiber, insoluble fiber, and soluble fiber. The fiber information is in Appendix A.

Mice and Diets

C57BL/6J male mice were purchased from Jackson Laboratories. All mice were housed at Georgia State University, Atlanta, GA under institutionally approved protocols (IACUC #A17047 and A20043). All experimental groups were housed with 5 mice per cage.

Mice were ordered at 6 weeks of age and were acclimated for approximately a week. At 7 weeks of age, mice were given a grain-based rodent chow (GBC) from PMI Nutrition International, LLC, Brentwood, MO or a compositionally defined diet (CDD) produced from purified ingredients from Research Diets, New Brunswick, NJ. The CDDs and their individual ingredients are listed in Appendix B, C, and D. The high-fat diets for the diet-induced obesity studies were composed of roughly 20 gm% fiber while the low-fat diets for the DSS-induced colitis studies were composed of roughly 15 gm% fiber, which is comparable to GBC at 15-25 gm% fiber. An exception to these percentages were the low-fat and high-fat low cellulose-enriched diets, which consisted of roughly 4-7 gm% fiber. Additionally, the high-fat diets were comprised of 60 kcal% fat, while the low-fat diets were made up of 10 kcal% fat. The fibers added to the purified diets consisted of highly refined oat fiber, minimally refined oat fiber, and wheat fiber that were previously tested. Cellulose was added to the two control diets.

Mice were fed GBC prior to the study start date and during acclimation. For the diet-induced obesity studies, the mice were given GBC or a specific high-fat CDD for 29 days. For
the DSS-induced colitis studies, the mice were given GBC or a specific low-fat CDD for 17 days.

**Food Consumption Measurement**

Mice were given appropriate diet based on treatment group and study type. For the diet-induced obesity studies, the diets were measured alternative days. For the DSS-induced colitis studies, the diets were measured ranging from every day to alternative days prior to DSS administration and daily after DSS administration.

**Administration of DSS**

DSS was given to mice in the DSS-induced colitis studies to stimulate the development of colitis. For the initial exploratory study, each treatment group was administered 2.5% DSS in drinking water at day 8 until day 17. For the subsequent study, each treatment group was administered 2.5% DSS in drinking water at day 7 until day 17.

**Water Consumption Measurement**

After mice were administered DSS in drinking water, the water was weighed daily until the study end date.

**Disease Activity Index Measurement**

After administration of DSS, mice were monitored daily for the development of colitis. The disease activity index (DAI) parameters were weight loss, stool consistency, and the presence of blood in feces (Carvalho et al., 2009). The DAI system is tabulated in Appendix E.

**Body Weight Measurement**

For the DSS-induced colitis studies, all mice were weighed alternative days until DSS administration. Post DSS administration, body weights were taken daily until the study end date.
For the diet-induced obesity studies, all mice were weighed alternative days until the study end date.

**Feces and Sera Collection**

To collect feces, mice were placed in individual containers for approximately 1-2 hours. To collect sera, mice were bled from the retro-orbital sinus.

For the initial colitis study, feces and sera were collected at the study end date at day 17. For the subsequent colitis study, feces were collected prior to and post DSS administration at day 7 and day 15.

For the initial obesity study, feces were collected at the study midpoint and near the study end date at day 16 and day 28. Sera was collected at the study end date at day 29. For the subsequent obesity study, feces were collected at the study start date, study midpoint, and near the study end date at day 0, day 16, and day 28.

**Glucose Measurement**

For the initial obesity study, glucose tolerance was measured at the midpoint of the study at 10 and 11 days and near the study end date at 25 and 26 days. Due to the number of treatment groups, 3 treatment groups were performed one day, while the remaining 3 groups were performed on the consecutive day for the 2 timepoints. For the subsequent obesity study, glucose tolerance was tested at the midpoint of the study and near the study end date at 11 and 25 days.

Prior to fasting, mice were placed in clean cages with water, and food was removed for 5 hours. After 5 hours of fasting, blood glucose at baseline was measured using a novaMax blood glucose monitor. Mice were administered glucose via intraperitoneal injection (2 mg of glucose/gm body weight). Following administration, blood glucose levels were measured at 30-, 60-, and 90-minute post administration timepoints.
Insulin Measurement

For the initial obesity study, inulin tolerance was measured at the midpoint of the study at 21 and 22 days. Due to the number of treatment groups, 3 treatment groups were performed one day, while the remaining 3 groups were performed on the consecutive day. For the subsequent obesity study, insulin tolerance was tested at 21 days after the study start date.

Prior to fasting, mice were placed in clean cages with water, and food was removed for 5 hours. After 5 hours of fasting, blood glucose at baseline was measured using a novaMax blood glucose monitor. Mice were administered insulin from Sigma via intraperitoneal injection (0.5 U of insulin/kg body weight). Following administration, blood glucose levels were measured at 30-, 60-, and 90-minute post administration timepoints.

Postmortem Measurement and Collection

For the initial colitis study, the mice were humanely euthanized at 17 days post study start date and 9 days post administration of DSS. For the subsequent colitis study, the mice were euthanized at 17 days post study start date and 10 days post administration of DSS. A section of liver and a section of colon were collected from each mouse. Spleen weight, colon weight, cecum weight, and colon length were measured.

For the obesity studies, mice were euthanized at 29 days post study start date. A section of liver, a section of colon, a single epididymal fat pad, and cecal contents were collected. Spleen weight, fat pad weight, colon weight, cecum weight, and colon length were measured.

RNA Extraction and qPCR

RNA was extracted from mid-colonic tissue from the colitis studies using TRIzol Reagent, ambion, Carlsbad, CA. Quantitative reverse-transcriptase PCR was performed using the iTAQ Universal SYBR Green One Step Kit from Bio-Rad, Hercules, CA in a Bio-Rad CFX96
real-time system thermocycler with the primers listed in Appendix F. The transcript levels were calculated against the housekeeping gene 36B4.

**Lipocalin-2 ELISA**

Feces from the second colitis study were used for analysis of lipocalin-2 levels. The fecal samples were prepared and tested using DuoSet Mouse Lipocalin-2/NGAL from R&D Systems, Minneapolis, MN. A linear curve was determined from the standards and used for calculating the concentration of lipocalin-2 in each fecal sample.

**Statistical Analysis**

Results were expressed as mean ± SEM. Statistical significance was analyzed by Kruskal-Wallis test for the two initial studies and Mann-Whitney test for the two subsequent studies using GraphPad Prism 8. Differences between experimental groups were considered significant at *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

**RESULTS**

To determine if oat and wheat fibers could provide protection against IBD and metabolic syndrome, a DSS-induced colitis study and a diet-induced obesity study in mice were performed simultaneously. The diets of the two studies were supplemented with highly refined oat fiber, minimally refined oat fiber, and wheat fiber.

**Initial DSS-Induced Colitis Study**

Weight loss is a common indicator of the inducement of colitis from DSS in mice (Chassaing, Aitken, Malleshappa, & Vijay-Kumar, 2014). For the initial DSS study, wheat fiber in a highly purified diet minimized body weight loss in comparison to the other fibers after DSS administration (Figure 1A). Moreover, this included reduced weight loss compared to the GBC group that characteristically exhibits less weight loss compared to purified diets.
Rectal bleeding is a frequent symptom of colitis in mice and humans (Chassaing et al., 2014). This manifestation of colitis, as well as other symptoms of colitis, were monitored daily through DAI measurements that accounted for the appearance of blood in stool, stool consistency, and weight loss (Carvalho et al., 2009). Wheat fiber had a lower DAI than the other added fibers in purified diets, displaying lower levels of diarrhea, rectal bleeding, and weight loss (Figure 1B). Additionally, GBC had the lowest DAI of all groups, while the low-cellulose diet had the highest score.

To assess the development of colitis, gross organ measurements were taken postmortem. Typically, markers of inflammation in the gut include a shorter colon, a shrunken cecum, and an enlarged spleen (Chassaing et al., 2014; Miles et al., 2017). Of the treatment groups, GBC displayed less gut inflammation in the spleen, colon, and cecum after ingestion of DSS, with the wheat fiber-enriched diet showing modest, but reduced inflammation in relation to the other purified diets (Figures 1C-F). Conversely, the low cellulose-enriched diet had exacerbated inflammation compared to all diets.

After DSS administration, mice will typically have increased levels of pro-inflammatory cytokines and chemokines in the colon that include IL-6, KC, and TNF-α (Chassaing et al., 2014). The relative expression of IL-6, KC, and TNF-α were lower with the CDD with wheat fiber compared to the other diets, including GBC (Figure 1G). Wheat fiber reduced pro-inflammatory cytokines and chemokines in the colon and had an impact on the inflammatory response caused by DSS. These results suggest a mechanistic effect of wheat fiber on inflammation to investigate in future studies.
**Initial Diet-Induced Obesity Study**

Contrary to colitis studies, weight gain is a widely used method of determining the development of obesity in mice (Kleinert et al., 2018). In the initial obesity study, GBC resulted in the least amount of weight gain in comparison to the other diets (Figure 2A). This was anticipated, as GBC has significantly less fat than the purified diets. Alternatively, the low cellulose-enriched high-fat diet caused the greatest weight gain in mice. The high fiber-enriched diets resulted in appreciably less weight gain than the low fiber diet and increased weight gain.

**Figure 1. Wheat fiber provided protection against colitis compared to oat fiber.**
Mice were fed grain-based chow (GBC) or a specific low-fat diet (LFD) for 8 days and then administered 2.5% DSS for 9 days prior to euthanasia.

- (A) Body weight loss post DSS administration (n=5).
- (B) Disease activity index (DAI) measurement by cage post DSS administration.
- (C-F) Spleen weight, cecum weight, colon weight, and colon length measured postmortem after DSS administration (n=5).
- (G) Colon RNA tested by qPCR (n=5). Data are expressed as mean +/- SEM. Statistical significance was assessed by Kruskal-Wallis test. *p < 0.05, **p < 0.01, ***p < 0.001.
compared to GBC. These results indicate that higher amounts of fiber can partially protect against weight gain due to a high-fat diet.

Insulin resistance is associated with the consumption of high-fat diets that lead to the progression of obesity (Kleinert et al., 2018). Glucose tolerance tests were performed throughout the study. After consumption of high-fat diets for 10 and 11 days, GBC, the wheat fiber-enriched diet, and the high cellulose-enriched diet rescued the distorted glucose response caused by fat intake (Figure 2B). This was further supported by calculations of area under the curve (AUC) (Figure 2C). Likewise, wheat fiber displayed a lower level of fasting glucose in comparison to the remaining groups (Figure 2D). These trends were observed after 25 and 26 days of being fed high-fat diets, where the low cellulose and oat fiber groups had an impaired glucose response and GBC, wheat fiber, and high cellulose had a normal response to glucose administration (Figures 2E and 2F). Due to these results, wheat fiber and a higher amount of cellulose can ameliorate the altered glucose response caused by the ingestion of increased fat.

Similar to the colitis model, postmortem tissue measurements were performed to determine levels of inflammation, with the addition of the epididymal fat pad to ascertain adiposity (Chassaing et al., 2014; Kleinert et al., 2018; Miles et al., 2017). The fat pad weight was significantly increased in the low cellulose group, while the other diets had sizable reductions in fat accumulation (Figure 2G). Of the purified diets supplemented with fiber, wheat fiber drastically decreased fat pad mass that typically enlarge due to high-fat diets. Additionally,
the diet supplemented with a lesser amount of cellulose resulted in shrunken ceca compared to GBC and the high-fat fiber diets (Figure 2H).

After performance of initial exploratory studies, wheat fiber was revealed to be a potential therapeutic candidate for IBD and metabolic syndrome in comparison to oat fiber. A DSS-induced colitis study and diet-induced obesity study were repeated with a wheat fiber-enriched diet to examine reproducibility of the initial studies.

**Subsequent DSS-Induced Colitis Study**

As observed in the exploratory colitis study, wheat fiber prevented body weight loss in mice after DSS administration compared to GBC and cellulose, and therefore, provided...
protection against colitis (Figure 3A). Furthermore, this protective trend was observed in DAI measurements, cecum weight, and colon length (Figures 3B-3D). In addition, wheat fiber had significantly lower relative expression levels of IL-6 and KC, as well as a considerably lower level of TNF-α (Figure 3E). Of note, an outlier of the 5 mice in the wheat fiber-treated group was noticed to have markedly increased inflammation, which was confirmed through the expression of pro-inflammatory cytokines by qPCR. Due to this variation, this outlier was thereby removed from the dataset.

Figure 3. Wheat fiber prevented colitis in a reproducibility study.
Mice were fed grain-based chow (GBC) or a specific low-fat diet (LFD) for 7 days and then administered 2.5% DSS for 10 days until euthanasia.
(A) Body weight loss post DSS administration (n=5).
(B) Disease activity index (DAI) measurement by cage after DSS administration.
(C and D) Cecum weight and colon length measured postmortem after DSS administration (n=5).
(E) Colon RNA tested by qPCR (n=5). Data are expressed as mean +/- SEM. Statistical significance was assessed by Mann-Whitney test. *p < 0.05, **p < 0.01.
Subsequent Diet-Induced Obesity Study

For body weight gain, the results of GBC and the high-fat fiber diets were similar to the initial study (Figure 4A). GBC caused little weight gain, low cellulose in a high-fat diet resulted in the largest weight gain, and a higher amount of wheat fiber and cellulose in a high-fat diet caused moderate weight gain.

For the glucose measurements at 11 days and 25 days post diet administration, wheat fiber did not have as noticeable of an effect of recovering the glucose response as the fiber did in the initial obesity study (Figures 4B and 4C). Similarly, wheat fiber did not recover the adiposity observed in the fat pad or the shrunken ceca at the level of the initial studies, but the fiber was able to provide partial protection of these tissues (Figures 4D and 4E).

Figure 4. Wheat fiber prevented metabolic syndrome in a reproducibility study. Mice were fed grain-based chow (GBC) or a specific high-fat diet (HFD) for 29 days. (A) Body weight gain post diet administration (n=5). (B) At 11 days post diet administration, mice (n=5) were fasted for 5 hours and administered 2mg/g of glucose via intraperitoneal injection. Blood glucose levels were measured at 0, 30, 60, and 90 minutes. (C) At 25 days post diet administration, mice (n=5) were fasted for 5 hours and administered 2mg/g of glucose via intraperitoneal injection. Blood glucose levels were measured at indicated timepoints. (D and E) Epididymal fat pad and cecum weights measured postmortem (n=5). Data are expressed as mean +/- SEM. Statistical significance was assed by Mann-Whitney test.

The appreciable results of wheat fiber in the initial colitis and obesity studies were reproduced in the subsequent studies, thus identifying a potential fiber for diminishing IBD and
metabolic syndrome symptom severity. Although the oat fibers did not protect against IBD and metabolic syndrome as wheat fiber exhibited, the highly refined oat fiber was able to modestly protect against the two inflammation-related diseases, whereas the minimally refined oat fiber was similar to the low cellulose group. Additionally, for the prevention of weight gain due to a high-fat diet, these studies showed that a reasonable amount of fiber in a diet can partially rescue inflammation-related events in comparison to a low amount of fiber, as seen with the high cellulose-enriched diet compared to the low cellulose-enriched diet. Furthermore, GBC, which is known to contain naturally occurring fibers and a low quantity of fat, will result in a reduction of IBD and metabolic syndrome symptoms, suggesting that the intake of other potential fibers and a low-fat diet is a healthy option for individuals with these diseases.

**DISCUSSION**

To further evaluate the inducement of colitis via DSS and to observe protective effects by wheat fiber intake, performing the measurement of lipocalin-2 in feces is recommended for future studies, as it is a robust inflammation biomarker capable of offering quantitative levels of inflammation and colitis development (Chassaing et al., 2014). Also, histologic scoring and staining techniques could be used for further assessment methods to observe prevention of neutrophil infiltration, loss of crypt structure, and absence of goblet cells in mice fed wheat fiber compared to other control groups (Chassaing et al., 2014). Additionally, other pro-inflammatory cytokines from colon RNA could be examined, such as IL-18 and IL-1α/β, which are typically upregulated in DSS models (Chassaing et al., 2014).

Although the DSS-induced colitis model is widely used, conducive for easy manipulation of colitis development, and similar to human UC, the model does not encompass all mechanistic effects of human IBD nor may it comprise the mechanisms of wheat fiber (Chassaing et al.,
2014). Because of these potential deficiencies, it is proposed that other colitis models in mice are used in future studies to fully evaluate the beneficial effects of wheat fiber consumption against IBD and its ensuing mechanisms. As stated in the Gewirtz grant proposal for the Crohn’s and Colitis Foundation of America, two colitis models can be used, which consist of the spontaneous colitis model in mice that lack IL-10 and the T cell transfer colitis model. For the IL-10-/− mice model, mice deficient in the anti-inflammatory cytokine IL-10 can spontaneously develop chronic colitis after a period of time, which is comparable to human CD (Zhang, Fu, Sun, Li, & Guo, 2014). For the T cell transfer colitis model, mice lacking T and B cells, Rag 1-/− mice, are inoculated with CD4+CD45RBhigh T cells, resulting in colitis development akin to human CD (Ostanin et al., 2009). In comparing these colitis models, DSS can cause damage to gut epithelial cells causing infiltration of the gut microbiota and immune cells, while the absence of IL-10 leads to inflammasome and IL-1β stimulation and IL-17 secretion (Zhang et al., 2014). The transfer of specific T cells results in helper T cells that can trigger colitis, specifically Th1 and Th17 cells (Ostanin et al., 2009). While it is possible that the differing mechanisms of colitis development in the DSS-induced, IL-10 deficiency-mediated, and T cell transfer models can have an impact on whether wheat fiber is protective, it is anticipated that this fiber can shield against multiple forms of IBD.

As for the diet-induced obesity model methods, it is suggested that colonic histology is performed to observe crypt structure and the impact of wheat fiber on rescuing altered gut tissues caused by increased fat intake (Zou et al., 2018). Also, other areas of concentrated fat tissue can be harvested post euthanasia, including the mesenteric fat pad and subcutaneous fat deposits, for additional assessments of wheat fiber’s effects on obesity (Zou et al., 2018). Furthermore, insulin tolerance tests are advised for use and were performed on mice in both metabolic syndrome
studies. As the data was inconclusive, it is proposed that the current insulin used for insulin tests is insufficient, and therefore, it is recommended that another source or lot of insulin is obtained for future obesity studies.

Male mice were used in the metabolic syndrome studies, which are prone to becoming more obese than female mice in diet-induced obesity models (Kleinert et al., 2018). This is ideal for observing diet-induced obesity when comparing diets and fibers but can be limiting when translating data to the human population, as women tend to be more obese than men (Kleinert et al., 2018). To provide relevant information for both sexes and the entire human population, it is proposed that male and female mice be used in future obesity study designs.

To account for variable body weights, food weights, and glucose measurements in the high-fat diet experiments, there are several limitations to consider. Firstly, the composition of high-fat diets can lead to varied results. For example, high-fat diets can lack food diversity, which may result in an absence of overeating (Kleinert et al., 2018). In studies, mice have shown the tendency to binge initially when administered a high-fat diet but will gradually consume a similar amount like mice given GBC (Kleinert et al., 2018). Fat source and texture of the diets are also factors to be considered (Kleinert et al., 2018). In the high-fat diets used in these studies, lard and soybean oil were used, with the animal-based fat added at a larger amount than the plant-based fat. It has been observed that animal-based fat can have a marked impact on accumulation of fat and insulin resistance (Kleinert et al., 2018). Additionally, texture is an aspect that could alter the development of obesity, as one study showed that a powdered low-fat diet led to an increase in fat in mice (Kleinert et al., 2018). Secondly, when C57BL6/J mice are administered high-fat diets or treated with other obesity methods, roughly 60% gain weight, which may be due to epigenetics (Kleinert et al., 2018). Lastly, the number of procedures
performed during a study can affect data, such as body weight, by the handling and fasting of mice during glucose and insulin tolerance tests and feces and sera collections (Kleinert et al., 2018).

The mechanisms of wheat fiber’s protection against IBD and metabolic syndrome are recommended for investigation in future studies and include the effect of wheat fiber on the gut microbiota and its metabolites. In previous studies, the fiber inulin provided protection via the microbiota (Chassaing et al., 2015; Zou et al., 2018). Also noted in the introduction, different fibers, such as psyllium, cellulose, and pectin, decreased colitis severity in mice, with psyllium’s effective protection attributed to the microbiota through use of germ-free mice (Llewellyn et al., 2018). It was determined that psyllium decreased bacterial density, while diversifying the microbiota composition (Llewellyn et al., 2018). Due to these results, it is recommended that the gut microbiota composition and density is assessed through 16S and metagenomic sequencing and qPCR, respectively, as described in the Gewirtz grant proposal. Furthermore, germ-free mice or the use of antibiotics can be utilized for further understanding of the microbiota in fiber’s protective benefits (Chassaing et al., 2015; Llewellyn et al., 2018; Zou et al., 2018).

Alternatively, and as proposed by the Gewirtz lab, mice with a restricted microbiota, termed altered Schaedler flora (ASF) mice, could be utilized (Henderson et al., 2015).

The metabolites of the microbiota from ingestion of dietary fiber and high-fat diets could be possible mechanisms of wheat fiber’s ability to protect. As noted in the introduction, dietary fiber is fermented by the microbiota and produces SCFAs, which create anti-inflammatory conditions by stimulating regulatory T cell differentiation (Arpaia et al., 2013; Singh et al., 2018; Zeng et al., 2019). It is feasible that wheat fiber increases the production of SCFAs, so it is recommended that the levels of SCFAs are measured. As observed in previous studies, gas
chromatography could be used on cecal and fecal samples (Zheng et al., 2013; Zou et al., 2018). Additionally, and as described in the grant proposal from the Gewirtz lab, the function of SCFAs compared to the function of the bacteria in the gut that ferment SCFAs can be examined. This action could be performed using several different techniques. Firstly, β-acids from the hops plant, *Humulus lupulus*, can be given to mice, which have been observed to prevent or reduce SCFA formation through restriction of fermenting bacteria (Flythe & Aiken, 2010; Harlow, Lawrence, Kagan, & Flythe, 2014; Singh et al., 2018; Zou et al., 2018). Alternatively, the antibiotic metronidazole can be administered to mice to eradicate bacteria that produce butyrate (Kaiko et al., 2016; Louis & Flint, 2007; Singh et al., 2018; Zou et al., 2018). One final approach could be to utilize mice that do not possess SCFA receptors, specifically Toll-like receptor 5 (Singh et al., 2018). In using the techniques mentioned, the ability of wheat fiber to reduce colitis symptoms would be ablated, therefore confirming that the primary mechanism of wheat fibers lies in the production of SCFAs.

As described previously, high-fat diets increase the creation of secondary BAs, which can cause pro-inflammatory conditions (Zeng et al., 2019). It has been observed that some fibers, specifically psyllium, is capable of binding bile acids possibly due to psyllium’s chemical structure or psyllium’s distinctive viscosity (Niu, Xie, Zhang, Sheng, & Yu, 2013). This binding ability could decrease reabsorption in the small intestine and interrupt the cycling process of bile acids (Buhman, Furumoto, Donkin, & Story, 1998). Although wheat fiber lacks the viscosity unique to psyllium and has a different chemical structure, it is possible that wheat fiber can affect bile acids in the gut. A protocol proposed by the Gewirtz lab and shown in a previous study can be performed to measure fecal bile acid levels, which consists of a chemical extraction of the feces followed by quantification (Bhat et al., 2003). Additionally, specific bile acids can be
determined through gas-liquid chromatography (Bhat et al., 2003). Furthermore, cholestyramine can be administered to mice, which precludes reabsorption of bile acids, and could mimic wheat fiber’s possible mechanism of protection (Singh et al., 2018).

As for cytokine-related mechanisms of wheat fiber in reducing inflammation, testing the cytokine IL-22, known for cell proliferation and host protection, is proposed, as inulin was found to protect against metabolic syndrome upon IL-22 upregulation (Dudakov, Hanash, & van den Brink, 2015; Zou et al., 2018). This cytokine is recommended for testing in both colitis and metabolic syndrome models to assess wheat fiber’s potential anti-inflammatory mechanisms via specific cytokine expression.

CONCLUSION

Wheat fiber is a possible dietary fiber for decreasing inflammation and IBD severity, as seen with protecting against weight loss, rectal bleeding, and diarrhea. Additionally, when added to a high-fat diet, wheat fiber can rescue the symptoms associated with metabolic syndrome, including reducing weight gain and preventing dysglycemia. From these conclusions, wheat fiber has the potential to be an added ingredient in processed foods to offer the key benefits associated with fiber, such as stool regularity and metabolism, to the greater public and to people with IBD and metabolic syndrome.
REFERENCES


Kleinert, M., Clemmensen, C., Hofmann, S. M., Moore, M. C., Renner, S., Woods, S. C., . . .


doi:10.1053/j.gastro.2017.11.030


doi:10.1128/AEM.02561-06

Miles, J. P., Zou, J., Kumar, M. V., Pellizzon, M., Ulman, E., Ricci, M., . . . Chassaing, B.


doi:10.1097/MIB.0000000000001155


doi:10.1152/ajpgi.90462.2008


Zhang, J., Fu, S., Sun, S., Li, Z., & Guo, B. (2014). Inflammasome activation has an important role in the development of spontaneous colitis. Mucosal Immunol, 7(5), 1139-1150. doi:10.1038/mi.2014.1


APPENDICES

Appendix A. Fiber information, including percentages of total dietary fiber, insoluble fiber, and soluble fiber.

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Source</th>
<th>Product #</th>
<th>Total Dietary Fiber</th>
<th>Insoluble Fiber</th>
<th>Soluble Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat, Minimally</td>
<td>JRS</td>
<td>Vitacel HF 401</td>
<td>86.70%</td>
<td>85.60%</td>
<td>1.10%</td>
</tr>
<tr>
<td>Refined</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oat, Highly</td>
<td>JRS</td>
<td>Vitacel HF 600-30</td>
<td>93.50%</td>
<td>87.60%</td>
<td>5.90%</td>
</tr>
<tr>
<td>Refined</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>JRS</td>
<td>Vitacel WF 600</td>
<td>92.70%</td>
<td>90.10%</td>
<td>2.60%</td>
</tr>
</tbody>
</table>
### Appendix B. Diets used throughout studies.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Source</th>
<th>Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodent GBC (LabDiet)</td>
<td>PMI Nutrition International, LLC</td>
<td>Product # 5001</td>
</tr>
<tr>
<td>Low-fat, low cellulose-enriched diet</td>
<td>Research Diets</td>
<td>Product # D12450J</td>
</tr>
<tr>
<td>Low-fat, high cellulose-enriched diet</td>
<td>Research Diets</td>
<td>Product # D13081109R</td>
</tr>
<tr>
<td>Low-fat, minimally refined oat fiber-enriched diet</td>
<td>Research Diets</td>
<td>Product # D20030501</td>
</tr>
<tr>
<td>Low-fat, highly refined oat fiber-enriched diet</td>
<td>Research Diets</td>
<td>Product # D20030502</td>
</tr>
<tr>
<td>Low-fat, wheat fiber-enriched diet</td>
<td>Research Diets</td>
<td>Product # D20030503</td>
</tr>
<tr>
<td>High-fat, low cellulose-enriched diet</td>
<td>Research Diets</td>
<td>Product # D12492</td>
</tr>
<tr>
<td>High-fat, high cellulose-enriched diet</td>
<td>Research Diets</td>
<td>Product # D13081107G</td>
</tr>
<tr>
<td>High-fat, minimally refined oat fiber-enriched diet</td>
<td>Research Diets</td>
<td>Product # D20030504</td>
</tr>
<tr>
<td>High-fat, highly refined oat fiber-enriched diet</td>
<td>Research Diets</td>
<td>Product # D20030505</td>
</tr>
<tr>
<td>High-fat, wheat fiber-enriched diet</td>
<td>Research Diets</td>
<td>Product # D20030506</td>
</tr>
</tbody>
</table>
Appendix C. Low-fat diet formulations.

<table>
<thead>
<tr>
<th>Product #</th>
<th>D12450J</th>
<th>D13081109R</th>
<th>D20030501</th>
<th>D20030502</th>
<th>D20030503</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet Name</td>
<td>LFD-50 Cell</td>
<td>LFD-200 Cell</td>
<td>LFD-MR Oat</td>
<td>LFD-HR Oat</td>
<td>LFD-Wheat</td>
</tr>
<tr>
<td></td>
<td>gm% kcal%</td>
<td>gm% kcal%</td>
<td>gm% kcal%</td>
<td>gm% kcal%</td>
<td>gm% kcal%</td>
</tr>
<tr>
<td>Protein</td>
<td>19 20</td>
<td>17 20</td>
<td>17 20</td>
<td>17 20</td>
<td>17 20</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>67 70</td>
<td>59 70</td>
<td>71 70</td>
<td>59 70</td>
<td>59 70</td>
</tr>
<tr>
<td>Fat</td>
<td>4 10</td>
<td>4 10</td>
<td>4 10</td>
<td>4 10</td>
<td>4 10</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50 0</td>
<td>200 0</td>
<td>50 0</td>
<td>50 0</td>
<td>50 0</td>
</tr>
<tr>
<td>Oat Fiber, Minimally Refined</td>
<td>0 0</td>
<td>0 0</td>
<td>150 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Oat Fiber, Highly Refined</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>150 0</td>
</tr>
<tr>
<td>Wheat Fiber</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>150 0</td>
</tr>
<tr>
<td>Lard</td>
<td>20 180</td>
<td>20 180</td>
<td>20 180</td>
<td>20 180</td>
<td>20 180</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>25 225</td>
<td>25 225</td>
<td>25 225</td>
<td>25 225</td>
<td>25 225</td>
</tr>
<tr>
<td>Total Fiber</td>
<td>50 4.7</td>
<td>200 16.6</td>
<td>180.1 14.9</td>
<td>190.3 15.8</td>
<td>189.1 15.7</td>
</tr>
<tr>
<td>Insoluble Fiber</td>
<td>50 4.7</td>
<td>200 16.6</td>
<td>178.4 14.8</td>
<td>181.4 15.1</td>
<td>185.2 15.4</td>
</tr>
<tr>
<td>Soluble Fiber</td>
<td>0 0</td>
<td>0 0</td>
<td>1.7 0.1</td>
<td>8.9 0.7</td>
<td>3.9 0.3</td>
</tr>
</tbody>
</table>
### Appendix D. High-fat diet formulations.

<table>
<thead>
<tr>
<th>Product #</th>
<th>D12492</th>
<th>D13081107G</th>
<th>D20030504</th>
<th>D20030505</th>
<th>D20030506</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet Name</td>
<td>HFD-50 Cell</td>
<td>HFD-200 Cell</td>
<td>HFD-MR Oat</td>
<td>HFD-HR Oat</td>
<td>HFD-Wheat</td>
</tr>
<tr>
<td></td>
<td>gm% kcal%</td>
<td>gm% kcal%</td>
<td>gm% kcal%</td>
<td>gm% kcal%</td>
<td>gm% kcal%</td>
</tr>
<tr>
<td>Protein</td>
<td>26 20</td>
<td>22 20</td>
<td>22 20</td>
<td>22 20</td>
<td>22 20</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>26 20</td>
<td>22 20</td>
<td>38 20</td>
<td>22 20</td>
<td>22 20</td>
</tr>
<tr>
<td>Fat</td>
<td>35 60</td>
<td>29 60</td>
<td>29 60</td>
<td>29 60</td>
<td>29 60</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50 0</td>
<td>200 0</td>
<td>50 0</td>
<td>50 0</td>
<td>50 0</td>
</tr>
<tr>
<td>Oat Fiber, Minimally Refined</td>
<td>0 0</td>
<td>0 0</td>
<td>150 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Oat Fiber, Highly Refined</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>150 0</td>
</tr>
<tr>
<td>Wheat Fiber</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>150 0</td>
</tr>
<tr>
<td>Lard</td>
<td>245 2205</td>
<td>245 2205</td>
<td>245 2205</td>
<td>245 2205</td>
<td>245 2205</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>25 225</td>
<td>25 225</td>
<td>25 225</td>
<td>25 225</td>
<td>25 225</td>
</tr>
<tr>
<td>Total Fiber</td>
<td>50 6.5</td>
<td>200 21.6</td>
<td>180.1 19.5</td>
<td>190.3 20.6</td>
<td>189.1 20.5</td>
</tr>
<tr>
<td>Insoluble Fiber</td>
<td>50 6.5</td>
<td>200 21.6</td>
<td>178.4 19.3</td>
<td>181.4 19.6</td>
<td>185.2 20</td>
</tr>
<tr>
<td>Soluble Fiber</td>
<td>0 0</td>
<td>0 0</td>
<td>1.7 0.2</td>
<td>8.9 1</td>
<td>3.9 0.4</td>
</tr>
</tbody>
</table>
## Appendix E. Disease activity index system.

<table>
<thead>
<tr>
<th>Score</th>
<th>Weight Loss</th>
<th>Stool Consistency</th>
<th>Blood in Stool</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>1-5%</td>
<td>Loose stool</td>
<td>Presence with Hemoccult test</td>
</tr>
<tr>
<td>2</td>
<td>5-10%</td>
<td>Watery diarrhea</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10-20%</td>
<td>Slimy diarrhea, slight appearance of blood</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&gt;20%</td>
<td>Severe watery diarrhea with blood</td>
<td>Visible blood</td>
</tr>
</tbody>
</table>
### Appendix F. Primer sequences for qPCR.

<table>
<thead>
<tr>
<th>Primer Sequence</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>36B4:</strong> 5’TCCAGGCTTTGGGCATCA-3’ and 5’TCTTTATCAGCTGCACATCAGTCTCA-3’</td>
<td>Invitrogen</td>
</tr>
<tr>
<td><strong>IL-6:</strong> 5’GTGGCTAAGGACCAAGACCA’ and 5’GGTTTGCCGAGTAGATCTCA-3’</td>
<td>Invitrogen</td>
</tr>
<tr>
<td><strong>KC:</strong> 5’TGTGTGCAGAAAGAAGTGCAG-3’ and 5’TACAAACACAGCCTCCCACA-3’</td>
<td>Invitrogen</td>
</tr>
<tr>
<td><strong>TNF-α:</strong> 5’CGAGTGACAAGCTGTAGCC-3’ and 5’CATGCGTGGGCCAGGA-3’</td>
<td>Invitrogen</td>
</tr>
</tbody>
</table>
Appendix G, related to figure 1. Additional measurements of wheat fiber’s protection against colitis. (A) Body weight loss post DSS administration (n=5). Data are expressed as mean +/- SEM.
Appendix H, related to figure 2. Additional assessments of the protective effect of wheat fiber on metabolic syndrome in comparison to oat fibers.

(A) At 21- and 22-days post diet administration, mice (n=5) were fasted for 5 hours and administered 0.5 U/kg of insulin via intraperitoneal injection. Blood glucose levels were measured at 0, 30, 60, and 90 minutes.

(B) At 25- and 26-days post diet administration, mice (n=5) were fasted for 5 hours, and blood glucose levels were measured at baseline.

(C-E) Spleen weight, colon weight, and colon length measured postmortem (n=5).

(F) Body weight gain post diet administration (n=5). Data are expressed as mean +/- SEM. Statistical significance was assessed by Kruskal-Wallis test. *p < 0.05.
Appendix I, related to figure 3. Further examinations of the protective impact of wheat fiber on colitis.
(A and B) Spleen weight and colon weight measured postmortem (n=5).
(C) Body weight loss post DSS administration (n=5). Data are expressed as mean +/- SEM. Statistical significance was assessed by Mann-Whitney test.
Appendix J, related to figure 4. Additional analysis of wheat fiber effects on metabolic syndrome.

(A and B) At 11 days post diet administration, mice (n=5) were fasted for 5 hours and administered 2mg/g of glucose via intraperitoneal injection. Blood glucose levels were measured at 0, 30, 60, and 90 minutes. Area under the curve (AUC) was calculated (A).

(C) At 21 days post diet administration, mice (n=5) were fasted for 5 hours and administered 0.5 U/kg of insulin via intraperitoneal injection. Blood glucose levels were measured at 0, 30, 60, and 90 minutes.

(D and E) At 25 days post diet administration, mice (n=5) were fasted for 5 hours and administered 2mg/g of glucose via intraperitoneal injection. Blood glucose levels were measured at 0, 30, 60, and 90 minutes. AUC was calculated (D).

(F-H) Spleen weight, colon weight, and colon length measured postmortem (n=5).

(I) Body weight gain post diet administration (n=5). Data are expressed as mean +/- SEM. Statistical significance was assessed by Mann-Whitney test. **p < 0.01.
VITAE

EDUCATION

MASTER OF INTERDISCIPLINARY STUDIES IN BIOMEDICAL SCIENCE & ENTERPRISE
GEORGIA STATE UNIVERSITY, ATLANTA, GA
GPA: 4.08/4.00
- Research Thesis: Diminishing inflammatory bowel disease (IBD) and metabolic syndrome severity through naturally occurring fibers
- Relevant Course Work: Crosscutting Concepts in the Sciences, Effective Science Communication, Leadership and Organizational Behavior, Translational Immunology
- Honors:
  - Science ATL Communication Fellowship (Fall 2020)
  - Learned new communication skills and improved upon former skills for professional environments by working with a team of fellows
  - Interviewed local winemaker and wrote a scientific article centered on winemaking for public education and engagement purposes
  - Created a short scientific presentation based on research thesis for a general audience to teach, empower, and inspire
  - Women’s Leadership Experience (Fall 2020)
  - Practiced communication techniques and leadership skills with fellow students through development workshops
  - Received guidance from women in professional leadership roles

MAY 2013
BACHELOR OF SCIENCE IN BIOCHEMISTRY
KANSAS STATE UNIVERSITY, MANHATTAN, KS
GPA: 3.4/4.0
- Relevant Course Work: Biochemistry I, Biochemistry II, Physical Studies of Biomacromolecules
- Honors: Johnson Center for Cancer Research Award (2011 and 2012)
  - Tested the delivery of heme to trigger apoptosis in cultured cells
  - Researched an alternative selection protocol for peptide vesicle assisted gene transfer

EXPERIENCE
AUGUST 2021 – CURRENT
ADJUNCT ASSISTANT PROFESSOR, BIOLOGY
JOHNSON COUNTY COMMUNITY COLLEGE, OVERLAND PARK, KS
Instructor for Principles of Cell and Molecular Biology at the undergraduate level, which includes teaching the following objectives and requirements through a hybrid course:
- Explain levels of biological organization, connection between cell structure and function, cell metabolism and reproduction, Mendelian and molecular genetics, and evolution
- Students perform labs that include core concepts of biology
- Students are required to complete lab handouts, lab exams, lecture exams, video presentation, online quizzes, and discussion posts
• Communicate with students and colleagues to assist with scheduling and educational needs
• Utilize educational technology, such as Canvas, Zoom, and Yuja

Instructor for Microbiology Lab at the undergraduate level, which includes teaching the following project and techniques:
• Students conduct laboratory projects that involve searching for antibiotic candidates from soil and offers relevant research experience
• Students perform aseptic technique to culture and isolate environmental bacterial candidates
• Students complete PCR, staining techniques, and metabolic tests to identify bacterial candidates
• Students are required to complete lab notebook, exams, online quizzes, group research paper, and oral and written research presentation

JUNE 2021 – CURRENT
ACCOUNTING CLERK/FARM WORKER
OTT LAND AND GRAIN, MULVANE, KS
• Input, review, and manage business records and transactions
• Reconcile bank statements and create financial reports
• Utilize accounting software
• Responsible for employee payroll
• Operate heavy machinery, including tractors, forklifts, and trucks

AUGUST 2019 – MAY 2021
GRADUATE RESEARCH ASSISTANT
INSTITUTE FOR BIOMEDICAL SCIENCES, GEORGIA STATE UNIVERSITY, ATLANTA, GA
Performed thesis research on fiber and the microbiota, which included the following responsibilities:
• Trained rotating graduate students on mouse handling, laboratory protocols, and ongoing research
• Conducted murine studies that involved alterations in mice diets, dextran sulfate sodium administration for the development of colitis, and high-fat diet administration for the development of diet-induced obesity
• Performed necropsies for tissue collection, blood and feces collections, body and food weight data compilation, and glucose and insulin tolerance tests
• Extracted RNA from tissues including the colon
• Performed assays including lipocalin-2 ELISA and qPCR
• Managed breeding of mice strains for research purposes

SEPTEMBER 2014 – AUGUST 2019
RESEARCH TECHNICIAN II
CEVA BIOMUNE, LENEXA, KS
Completed the research and development of inactivated and live attenuated bacterial vaccines for poultry, which included the subsequent responsibilities:
• Trained new coworkers on laboratory protocols, animal handling, and government regulations
• Led groups tasked with time and financially sensitive projects relating to regulatory compliance
• Designed and implemented in vivo studies with chickens and mice
• Performed necropsies for tissue collection, vaccinations, bacterial challenges, blood collections, and body weight data compilation
• Wrote protocols and reports for biologics licensure in compliance with federal regulations for the USDA
• Conducted bacterial isolation and identification of *Salmonella* and *Escherichia coli* in Biosafety Level 2 and Animal Biosafety Level 2 areas
• Performed assays including ELISA, LAL, and classic PCR
• Formulated and batched experimental vaccines

**AUGUST 2013 – SEPTEMBER 2014**
**EXTRACTION TECHNOLOGIST**
**QUEST DIAGNOSTICS, LENEXA, KS**
• Performed toxicology screening by extracting drug analyte from urine and oral fluid samples using solid phase and liquid/liquid extractions

**FEBRUARY 2011 – AUGUST 2013**
**LAB ASSISTANT**
**BIOTECHNOLOGY CORE LAB, KANSAS STATE UNIVERSITY, MANHATTAN, KS**
• Trained incoming undergraduate student on cell culture protocols
• Assisted graduate student projects that involved *in vitro* experiments
• Performed cell culture methods on breast and cervical cancer cell lines including MCF-7 and HeLa
• Executed bacterial transformation and transfection of mammalian cells
• Purified, cloned, and analyzed DNA using gel electrophoresis

**JUNE 2011 – JULY 2011**
**LAB ASSISTANT**
**BIOMATERIALS AND TISSUE ENGINEERING LAB, UNIVERSITY OF KANSAS, LAWRENCE, KS**
• Assisted graduate student projects that involved *in vitro* experiments
• Prepared hydrogels and performed stress compression tests

**PUBLICATIONS**
1. **Ott, R.** (2020). Divine Wine Starts on Earth. *ALEX Explores Science ATL.*

**CONFERENCE PRESENTATIONS**


**TRAININGS AND SYMPOSIA**
• One Health Innovations Symposium “Preventing the Next Pandemic,” Kansas City, MO (August 2017)
• Veterinary Biologics Training Program by Institute for International Cooperation in Animal Biologics, Ames, IA (May 2016)
• Symposium on Gut Health in Production of Food Animals, Kansas City, MO (November 2015)

**VOLUNTEER ACTIVITIES**
• Big Brothers Big Sisters: mentor and foster as a Big Sister (November 2022 – current)
• Georgia Science and Engineering Fair: judged microbiology projects in the senior division (March 2021)

**SKILLS**
• Adept at creative and critical thinking
• Practiced at professional communication
• Teamwork focused
• Adaptable with projects, timelines, and resources
• Highly organized