

Research Proposal for the Oklahoma Wheat Research Foundation (OWRF) and Oklahoma Wheat Commission (OWC)

Title: Has Genetic Improvement of Wheat Resulted in Negative Effects on Gut Health?

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Funding History with OWRF/OWC: None

Abstract: In the U.S., wheat consumption per capita has declined over the last two decades. This has been driven by the increasing incidence of celiac disease in North America, concerns regarding gluten sensitivity, and claims that high carbohydrate intake is responsible for the obesity epidemic. Growing consumer awareness about the importance of the gut mucosal immune system and microbiome in overall health has also resulted in many consumers experimenting with gluten-free or reduced-gluten diets. Despite the fact that genetic manipulation has improved the nutritional properties and yield of wheat, the effects of these genetic changes on gut inflammation have not been adequately explored. Therefore, the *purpose* of this project is to determine if genetic improvements in wheat have led to gut inflammation, which is contributing to the increase in gluten-related disorders. Our *objective* is to investigate the effects of the heirloom variety (Turkey) and the modern variety (Gallagher) on gut inflammation when incorporated into a normal vs. a typical Western diet. Studying these varieties will enable us to determine if genetic alterations are having unanticipated, negative health consequences. Importantly, investigating the gut immune response in a well-controlled animal experiment will allow us to determine the response to the wheat varieties in the context of a healthy diet as well as the more typical Western diet (high in fat and refined sugars). Our findings will provide evidence to counter misinformation about wheat's effects on the gut or clearly show that modern varieties present a problem. If the Gallagher variety is shown to increase gut inflammation, we will aggressively work to determine when in the development of these wheat varieties the changes occurred and the responsible wheat component(s). The findings of this study will be published in appropriate nutrition-biomedical journals and the *Oklahoma Wheat Brief*.

Objective(s): The study will provide an important opportunity to determine if modern wheat breeding contributes to gut inflammation and gluten-related disorders. Specifically, the *objective* is to compare the effects of the heirloom (Turkey) with the modern (Gallagher) varieties of wheat on: 1) the gut barrier integrity and function; and 2) the immune response using an animal model.

The gut mucosal immune system houses more than 70% of the body's immune cells at any given time. These tissues are arranged to allow complex interactions between epithelial cells that line the mucosa and the underlying immune cells. Gut barrier integrity and function plays an

important role in that components of the diet essential for normal metabolic processes must be distinguished from potentially pathogenic chemicals or microorganisms. Therefore, dietary components that trigger an immune response and compromise the gut mucosal barrier can induce both a local and systemic inflammation that results in negative health consequences.

Our research team, made up of nutrition scientists who study gut health and immunity, and plant geneticists, are uniquely positioned to conduct this research. Utilizing an animal model will allow us to examine the direct impact of the wheat varieties on the gut under well-controlled dietary conditions. Importantly, it is necessary to investigate these gut changes due to wheat varieties under normal dietary conditions as well as in a diet that more closely relates to the typical Western diet. This study is an essential first step in determining the effects of a popular modern wheat variety on gut health that will provide important insights to the OWRF and OWC, and establish the foundation for future federally-funded grant applications.

Procedures:

To accomplish our objective, we will conduct an animal study using young growing, male mice (6 wks of age) that will be housed at Oklahoma State University’s environmentally controlled Laboratory Animal Research facility. After acclimation, mice will be randomly assigned to six different treatment groups (n=12 mice /group; **Table 1**). The diets will be formulated to be the same, with wheat cultivar being the distinguishing factor. The 10% by weight dose of wheat is based on estimated routine human consumption.

Wheat samples will be obtained from Dr. Brett Carver, a member of our research team and the *Wheat Genetics Chair in Agriculture* at OSU. The control diet will provide 10% kcal from fat compared to the western diet which provides 45% kcal of fat and is high in refined sugars. The mice will be fed their respective diets for 45 days. During this period, food intake and body weights will be monitored. At the end of the study, blood and gastrointestinal sections will be collected to investigate the effects of the different wheat varieties on the gut barrier function and inflammation.

Table 1. Experimental Groups

Groups	Treatments
1	Control
2	Control + Turkey
3	Control + Gallagher
4	Western Diet
5	Western Diet + Turkey
6	Western Diet + Gallagher

Gut Barrier Integrity and Function. To investigate the effects of wheat on gut integrity and barrier function (small and large intestine), changes in gene expression and protein will be assessed by evaluating: a) markers of cellular injury, b) regulators of mucus synthesis which protects the intestine; c) tight junction proteins that maintain integrity; and d) antimicrobial peptides that limit the invasion and adherence of pathogenic bacteria. Additionally, histological evaluation of gut mucosal structures will be performed.

Local (gut) and Systemic Immune Response. Sections of the small and large intestine will also be processed for the evaluation of protein levels of inflammatory mediators as an indication of the local immune response. Blood samples will be used to determine the alterations in systemic biomarkers of inflammation.

Statistical Analyses. Data will be analyzed to compare the effects of the different wheat varieties under normal and Western dietary conditions using factorial analyses (SAS version 9.4).

Timeline: One year is sufficient to complete animal feeding and proposed laboratory analyses.

Months

Activity

- 1-2 Animal use approval, preparation and characterization of animal diets
- 3-5 Animal feeding study
- 6-12 Laboratory analyses

*Additional 6 months will be requested (with no additional costs) for data and statistical analyses as well as preparation of manuscripts and reports.

Justification: The findings of this study will provide organizations like the Oklahoma Wheat Commission and Wheat Foods Council the necessary science-based information to reverse negative perceptions of wheat and wheat digestibility. This will be critical for our nation’s wheat industry to reverse the two-decade decline in wheat consumption and restore confidence in the health benefits of wheat products. This is a big deal to marketing organizations like our wheat commission, and to OSU’s wheat improvement team if findings from this study call for prompt corrective measures in wheat variety development.

Report of Accomplishments: NA

Budget:

CATEGORY	
<u>PERSONNEL SALARIES & BENEFITS</u>	
Graduate Research Assistant, Ph.D.	
25% plus benefits	11,342
Total Personnel Salaries and Benefits	11,342
<u>Materials and Supplies</u>	
Mice (6 wk C57BL/6 mice plus shipping @ \$35 ea n=72)	2,520
Animal Diets (Ingredient Analyses and 20 kg diet @ \$35 ea plus shipping)	700
Inflammatory Mediators (ELISA kits)	4,000
Real Time PCR (Primers, Reagents & Supplies)	3,200
Western Blot Reagents and Antibodies	3,500
General Laboratory Supplies	1,510
Subtotal for Materials and Supplies	15,430
<u>OTHER EXPENSES</u>	
Vivarium & Veterinarian Services	1,350
Laboratory Equipment Maintenance	1,587
Subtotal for Other Expenses	2,937
TOTAL PROJECT COSTS	29,709

Relation to Other Research: This proposed project is a continuation of our work on the role of foods and their bioactive components in preventing inflammation and promoting gastrointestinal health. We have collaborated for more than 20 years and are well prepared to undertake the proposed research because we have done similar studies and have assembled a team with Dr. Carver that has extensive experience in all the different aspects of the proposed research.

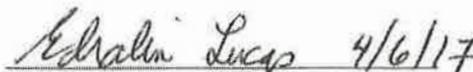
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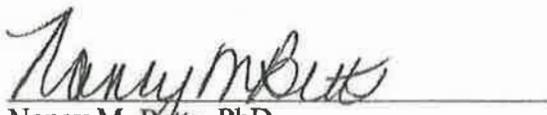
Requested Funding Level: \$29,709



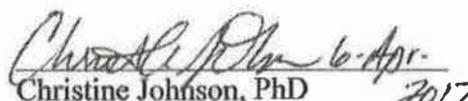
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