

# **Feed the Future Innovation Lab for Collaborative Research on Grain Legumes (Legume Innovation Lab)**

## **FY 2017 WORKPLAN**

**Project Code and Title:** Legumes and growth

**Lead U.S. Principal Investigator (PI) and affiliated Lead U.S. University:**

Mark Manary MD, Helene Roberson Professor of Pediatrics  
Washington University School of Medicine in St. Louis

**Host Country and U.S. Co-PIs and Institutions:**

- Ken Maleta MBBS PhD, Professor in Community Health, University of Malawi College of Medicine
- Chrissie Thakwalakwa PhD Lecturer in Community Health, University of Malawi College of Medicine
- Indi Trehan MD, Assistant Professor of Pediatrics, Washington University School of Medicine in St. Louis

### **I. Project Problem Statement and Justification:**

Each year millions of children in Africa die from malnutrition and even more are stunted due to nutritional and absorption deficiencies, interventions to help children affected and at risk are urgently needed to improve the lives of these children. Environmental enteric dysfunction (EED), a pervasive chronic subclinical gut inflammatory condition is prevalent amongst these children and places them at high risk for stunting, malabsorption, and poor oral vaccine efficacy. EED is characterized by T-cell infiltration of the intestinal mucosa leading to a chronic inflammatory state with increased intestinal permeability, translocation of gut microbes, micro- and macronutrient malabsorption, poor weight gain, stunted physical and cognitive development, frequent enteric infections, and decreased response to enteric vaccines. EED often develops within the first three years of life, a high-risk period marked also by the transitions from exclusive breastfeeding to mixed feeding with complementary foods to the complete reliance on adult foods for sustenance. In traditional sub-Saharan African societies, complementary foods are dominated by protein-poor and micronutrient-poor starches such as maize, cassava, and sorghum. Alternative, yet culturally acceptable, complementary foods that could provide a better and more palatable balance of nutrients would potentially decrease in EED and improve growth amongst these at risk children. Legumes provide just such an opportunity, as their protein content is significantly higher than cereals, and they are rich in dietary fiber, starch, minerals, vitamins, and antioxidants.

### **II. Project Activities for the FY 2017 Workplan Period (October 1, 2016 – September 30, 2017)**

We will complete all components of two randomized, controlled clinical trials investigating the effect of cowpea or common bean consumption on infant and young child growth and gut health.

**Objective 1:**

Completion of intervention delivery and specimen collection in infants with a dietary legume.

**Collaborators:**

University of Malawi, College of Medicine

**Approaches and Methods:**

Evaluate changes in childhood anthropometry (height-for-age and weight-for-height z scores), biomarkers of EED (lactulose:mannitol and a panel of human mRNA messages predictive of EED) and the characteristics of the microbiome (population taxonomy from phyla to genus, and the collective metabolic capacity expressed as Kyoto Encyclopedia of Genes and Genomes (KEGG) categories) after inclusion of either cowpeas or common beans as an integral component of complementary feeding for 6-11 month-old rural Malawian children.

*Study population.* Approximately 300 healthy children aged 6-11 months in villages surrounding Mitondo in the Chikwawa District of southern Malawi and Llmela in Machinga District were randomized to receive a legume-based complementary food made from cowpeas, common beans or an isoenergetic amount of corn flour, a traditional Malawian complementary food. These villages are very similar in that the residents are subsistence farmers growing maize on small plots, live in mud huts with thatch roofs, and use boreholes or nearby streams as their water source.

These infants were recruited between the ages of 5.5 and 6.5 months, and their participation will last for 6 months. Enrollment was ongoing, and extended over a 9 month period and involved health surveillance assistants, midwives, and other local health staff and village leaders to maximize outreach into the community. Given our extensive prior experience working in this community and our excellent working relationship with the Ministry of Health and District Health Officers in this area, we have been successful with subject retention.

In addition to consuming legumes daily, these children were tested for EED using a dual sugar test at the ages of 6, 9 and 12 months of age. Stool samples were collected at 6, 6.5, 7.5, 9, 10.5 and 12 mo of age suitable for microbiota and microbiome analyses.

At the onset of Oct 2017 there will only be 15 children remaining in the study, and their participation is scheduled to be completed by 15-Oct-16. Thus the bulk of the work associated with this study is sample analyses and data analyses. Urine tests for lactulose and mannitol are done monthly in batches, so the September 2016 batch and October 2016 batch will be sent to the lab at Children's Nutrition Research Center in Houston. Data are tabulated and processed upon receipt of the results. Analyses of stool samples for 16s and metagenomic sequencing will be ongoing through March 2017, and data analyses will follow thereafter.

**Objective 2:**

Analyses of complementary foods

**Collaborators:**

Elizabeth Ryan PhD, Assistant Professor,  
Department of Environmental and Radiological Health Sciences,  
Colorado State University, Fort Collins, CO

**Approaches and Methods:**

Dr. Ryan's lab will deliver a comparative analysis of food composition as well as a comparative analysis of small molecules and metabolite pathways. This includes all known toxins and contaminants as well as bioactive small molecules for the cowpea and common bean supplements fed to the children in Malawi. This will identify the 40 major nutrients (macro and micronutrients) as well as hundreds of other small molecules. This information is helpful and necessary to understand the feasibility of operational implementation of this complementary feeding.

**Objective 3:**

Completion of intervention delivery and specimen collection in young child project

**Collaborators:**

University of Malawi

**Approaches and Methods:**

Evaluate changes in childhood anthropometry (height-for-age and weight-for-height z scores), biomarkers of EE (lactulose:mannitol and a panel of human mRNA messages predictive of EE) and the characteristics of the microbiome (population taxonomy from phyla to genus, and the collective metabolic capacity expressed as Kyoto Encyclopedia of Genes and Genomes (KEGG) categories) after inclusion of either cowpeas or common beans as an integral component of complementary feeding for 12-24 month-old rural Malawian children.

*Study population.* Approximately 300 healthy children aged 12-24 months in villages surrounding Mitondo in the Chikwawa District of southern Malawi and LImela in Machinga District were randomized to receive a legume-based complementary food made from cowpeas, common beans or an isoenergetic amount of corn flour, a traditional Malawian complementary food. These villages are very similar in that the residents are subsistence farmers growing maize on small plots, live in mud huts with thatch roofs, and use boreholes or nearby streams as their water source.

These infants were recruited between the ages of 12-24 months, and their participation will last for 12 months. Enrollment occurred in 3 month period and involved health surveillance assistants, midwives, and other local health staff and village leaders to maximize outreach into the community. Given our extensive prior experience working in this community and our excellent working relationship with the Ministry of Health and District Health Officers in this area, subject retention was successful.

Eligible children were screened by the research supervisors and physicians from our team. Specific exclusion criteria were severe or moderate acute malnutrition, severe developmental delay or congenital malformations (including congenital heart disease) or any other known chronic disorder. After a thorough, tiered informed consent process presented to the community and parents, written as well as oral consent will be sought from the primary caretaker, who is almost always the mother or another matriarchal figure. Attempts will be made to engage any paternal figures in the household in the consenting process as well in order to maximize compliance with the study interventions and decrease attrition. Any caretakers reluctant to participate were not be encouraged to do so, and any participant desiring to leave the study after enrollment was allowed to do so without coercion. This method of informed consent has been used successfully by the research team in the past, and been endorsed by the University of Malawi College of Medicine Research and Ethics Committee and the Washington University Human Research Protection Office.

In addition to consuming legumes daily, these children were tested for EED using a dual sugar test at enrollment and after 3, 6, 9 and 12 months of feedings. Stool samples were collected at 10 time points during the 12 months of participation and these fecal samples are suitable for microbiota and microbiome analyses.

In October 2017 78 children will be remaining in this study, and their participation will be complete by 15-Dec-16. Most of the work in 2017 associated with this objective is sample analyses and data analyses. Urine tests for lactulose and mannitol are done monthly in batches, so the September-December 2016 batches will be sent to the lab at Children's Nutrition Research Center in Houston. Data are tabulated and processed upon receipt of the results. Analyses of stool samples for 16s and metagenomic sequencing will be ongoing through May 2017, and data analyses will follow thereafter.

**Objective 4 :** Increase the capacity, effectiveness and sustainability of agriculture research institutions which serve the bean and cowpea sectors in Malawi.

**Collaborators:**

University of Malawi  
LUANAR

While completing Study Aims, the PI and his research team will promote sustainable research through relationships with the Malawi College of Medicine and with colleagues at LUANAR. The research team recognizes how integral it is that local Malawi institutions be

equipped to initiate and conduct operational health, nutrition and agriculture studies to improve the health and wellness of its population, and extensive training and support will be offered. Chrissie Thakwalakwa of the College of Medicine, who received her PhD with support from the LIL, completed her charge to develop the study procedures, guidelines and materials and has acted as an administrative supervisor for the clinical trial, she has been under the guidance of the PI and his research team. The Agriculture Department at LUANAR, led by Vernon Kambambe and Agnes Mwangwela, were engaged developing formulations and recipes using cowpeas and common beans, the PI and his team trained the **only** two student LUANAR food scientists on the development processes used in the Washington University food science labs.

#### *Trainees*

Chrissie Thakwalakwa – PhD, Malawi College of Medicine -**completed**

Tereza Ngoma from LUANAR to develop recipes lead a food quality workshop - **completed**

Ulemu Chimimba from LUANAR to develop recipes -**completed**

Yankho Kaimila PhD candidate Malawi College of Medicine

Oscar Divala - PhD candidate Malawi College of Medicine

### **III. Contribution of Project to USAID Feed the Future Performance Indicators:**

This project supports the US Government's Feed the Future commitment to a multifaceted approach to nutrition and sustainably reducing global poverty and hunger. EE is estimated to cause about one third of the child stunting seen worldwide and the causes of EE are multifactorial. Our project aligns with these goals: developing a dietary intervention for children at risk for malnutrition and enteropathy using legumes, a local and common Malawian crop, is an opportunity to harness a local crop to resolve widespread condition afflicting children across the developing world. In the first year of the project we will set forth the methodology and training to develop a food that can treat this condition, and also train local universities and students on the methods to conduct this kind of research.

### **IV. Outputs:**

- Report on nutrient properties and food safety of the cowpea and common bean complementary foods
- Clinical trial results

### **V. Engagement of USAID Field Mission(s)**

Continued communication, engagement and collaboration are planned with Crispin Magombo from the FTF team at the USAID mission in Lilongwe, Malawi.

### **VI. Partnering and Networking Activities:**

The PI and his team will work with the Ministry of Health and the College of Medicine to understand the results from the clinical trial and how they might be implemented in an operational setting.

### **VII. Leveraged Resources:**

Ken Maleta is a lead member of the Investigation of Lipid Nutrient Supplements (iLiNS) project, a large Bill and Melinda Gates Foundation-supported effort in Malawi. He provides a direct link between this legumes project and any other international nutrition programs in Malawi.

Equipment will be shared with the iLiNS project, reducing the costs. All results will be presented at international nutrition and food research meetings focused on FTF themes, which will allow an opportunity to synergize with other projects.

Samples collected through the clinical trials will be assessed for serum metabolomics at Johns Hopkins University to better understand EED.

**VIII. Timeline for Achievement of Milestones of Technical Progress:**

*See attached Milestones*

## Appendix 1: Workplan for Training and Capacity Strengthening (FY 2017).

### Degree Training:

1. William Cheng, USA, Male  
Washington University in St. Louis, MA in Biological Sciences  
Supervisor: Mark Manary  
Participant Trainee: Yes  
Host Country Institution to Benefit from Training: College of Medicine  
Research Area: Assisting with the completion of the clinical trial  
May 2016 – March 2017  
Training status: Ongoing  
Type of LIL Support: Indirect
2. Theresa Ngoma, Malawi, Female  
LUANAR, MSc in Food Science and Technology  
Supervisors: Mark Manary, Ken Maleta, Indi Trehan  
Participant Trainee: Yes  
Host Country Institutions to Benefit from Training: LUANAR  
Research Areas: Development of common bean and cowpea flour recipes ;  
Production and quality control of common bean and cowpea flour recipes  
January 2015 – December 2015  
Training status: Active  
Type of LIL Support: Direct
3. Ulemu Chimimba, Malawi, Female  
LUANAR, MSc in Food Science and Technology  
Supervisors: Mark Manary, Ken Maleta, Indi Trehan  
Participant Trainee: Yes  
Host Country Institutions to Benefit from Training: LUANAR  
Research Areas: Development of common bean and cowpea flour recipes ;  
Production and quality control of common bean and cowpea flour recipes  
January 2015 – December 2015  
Training status: Active  
Type of LIL Support: Direct
4. Yankho Kaimila, Malawi, Female  
University of Malawi College of Medicine, PhD in Epidemiology  
Supervisors: Mark Manary, Ken Maleta, Indi Trehan  
Participant Trainee: Yes  
Host Country Institutions to Benefit from Training: LUANAR  
Research Areas: Clinical trial of flours to improve EED and stunting; Laboratory  
techniques to measure biomarkers of EED  
August 2015 – July 2017 (estimated)  
Training status: Active  
Type of LIL Support: Direct
5. Oscar Divala, Malawi, Male  
University of Malawi College of Medicine, PhD in Epidemiology  
Supervisors: Mark Manary, Ken Maleta, Indi Trehan  
Participant Trainee: Yes

Host Country Institutions to Benefit from Training: LUANAR  
Research Areas: Clinical trial of flours to improve EED and stunting; Laboratory techniques to measure biomarkers of EED  
August 2015 – July 2017 (estimated)  
Training status: Active  
Type of LIL Support: Direct

Short-term Training: Staff Field Training

Type of training: Field training for research activities  
Description of training activity: Training study research nurses, drivers, research assistants and staff on the field study guidelines. Trainees will receive training in "Good Clinical Practice" guidelines, anthropometric data collection skills, biological sample collection methods and community engagement.  
Location: Malawi College of Medicine  
Duration: 1 week  
When will it occur? ongoing  
Participants/Beneficiaries of Training Activity: Research team Anticipated numbers of Beneficiaries (male and female): 10  
PI/Collaborator responsible for this training activity: Indi Trehan and Ken Maleta List other funding sources that will be sought (if any): None  
Training justification: this training is necessary to conduct the research projects, having a knowledgeable and capable staff is imperative to conducting this research.

Short-term Training Food safety course

Type of training: In country course on food safety for agronomists and food processors (open enrollment)  
Description of training activity: A sets of seminars and field experience set up to run over 1 week are arranged through the school of public health at the College of Medicine, University of Malawi  
Location: Malawi College of Medicine  
Duration: 1 week  
When will it occur? ongoing  
Participants/Beneficiaries of Training Public and private sector individuals in Malawi. Anticipated numbers of Beneficiaries (male and female): 75  
PI/Collaborator responsible for this training activity: Indi Trehan and Ken Maleta List other funding sources that will be sought (if



any): None

Training justification: this training will heighten awareness of food safety issues and how they can be minimized.