

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

**FY 2017 Annual Project Technical Progress Report  
(October 1, 2016 – September 29, 2017)**

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

**Lead U.S. Principal Investigator and University:**

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

**Collaborating Host Country and U.S. 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, Associate Professor of Pediatrics, Washington University School of Medicine in St. Louis

**I. Abstract of Research and Capacity Strengthening Achievements**

*(A succinct narrative on the technical progress of the project, including key research and capacity strengthening achievements and outcomes, during the FY 2017 performance and report period. 1200 character limit.)*

In FY17, complete sample collection for Study 2 was accomplished. Samples were sent to the University of California, San Diego, for sequencing and analysis. The local team implementing the clinical trial continued ongoing training in the principles of “Good Clinical Practice” until the trial was completed. Two Malawian PhD students enrolled at the University of Malawi-College of Medicine furthered their knowledge and education by learning extraction and sequencing techniques in the laboratory at Washington University and by attending classes and other training/seminars during this fiscal year. Three articles with results were published in American Society for Nutrition journals.

**II. Project Problem Statement and Justification**

*(Present a concise problem statement and justification for the research and capacity strengthening activities carried out in this project. Please **do not provide a cut-and-pasted version of the project problem statement** from the Technical Application. 2000 character limit.)*

Successful interventions to help prevent children from becoming malnourished and achieve their full growth potential remain lacking. Environmental enteric dysfunction (EED), a pervasive chronic subclinical gut inflammatory condition, places rural children at high risk for malabsorption, stunting, and acute malnutrition. Minimizing EED is an essential step in improving the survival and growth of at-risk children. EED is

characterized by T-cell infiltration of the intestinal mucosa leading to a chronic inflammatory state with increased intestinal permeability, translocation of microbes, nutrient malabsorption, poor weight gain, stunted physical and cognitive development, frequent enteric infections, and decreased response to enteric vaccines. EED often begins to develop shortly after the transition away from exclusive breastfeeding and increases progressively during the first several years of life, a high-risk period marked by 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. In this study, we tested two different legume foods as complementary food products, given that their protein content is significantly higher than cereals, and they are rich in dietary fiber, starch, minerals, vitamins, and antioxidants. The active engagement of several Malawian graduate students as part of the capacity-building activities is essential to this work, as their local insights and knowledge of food systems and cultural feeding practices will help guide the optimal development and implementation of these

### **III. Technical Research Progress**

*(Describe the research activities (research methods, studies conducted, analyses completed, and significant findings) completed under each objective during the FY 2017 reporting period. Present sufficient detail so that the reviewers will understand and have confidence that the research was carried out in a manner that meets high scientific standards. Briefly discuss primary results, findings, and/or technological achievements that give evidence of technical progress toward objectives. Please be reminded to highlight significant outputs that have potential for impact.)*

**Objective 1:** Enrollment, intervention delivery and specimen collection in infants with a dietary legume.

This objective began in July 2015 after several months preparation and completed in December 2016. Two legume interventions were tested, common bean and cowpea. Children were enrolled as they turned 6 mo of age and participated until they were 12mo of age. Four hundred children were assessed for eligibility, 355 randomized to one of 3 interventions; cowpea, common bean or control. Children were followed every 6 weeks, assessed for anthropometric status and compliance with consumption of the legume supplement. Of the 291 children that completed the entire 6 mo of participation, daily compliance was high, mothers reported giving the legume on 98% of the study days. Among those children that did not complete the participation, the most common reason was development of acute malnutrition in 42/64 children, which required therapy with supplementary or therapeutic feeding. Over 2000 fecal specimens were collected, snap frozen in liquid nitrogen and delivered to the analyzing labs. Three dual sugar permeability tests were done; at 6mo, 9mo and 12mo of age. Almost all fecal samples were sequenced for 16s rDNA and metagenomics, as well as having fecal metabolites measured. The findings are discussed under the achievement section.

**Objective 2: Recipe Development**

The LUANAR graduate students developed food recipes using cowpeas and common beans in accordance with WHO specification earlier in the research project. The candidate recipes underwent acceptability testing in Malawian infants with the support of the Malawi College of Medicine. Simple roasted and milled cowpea and common bean were chosen as the intervention on the basis of infant acceptability, safety and practicality. The dosages of legumes given daily were 80 kcal/d when 6-9 mo old, 120 kcal/d when 10-12 mo, 155kcal/d when 12-23 mo and 200kcal/d when 24-35 mo. Children avidly took these legume supplements as powder poured on top of their cereal porridge. Mothers offered no complaints regarding the supplements.

**Objective 3: Enrollment, intervention delivery and specimen collection in young children with a dietary legume.**

This objective began in July 2015 after several months preparation and completed in December 2016. Two legume interventions were tested, common bean and cowpea. Children were enrolled during the ages of 12-24 mo and participated for 12 subsequent months. Four hundred thirty-five children were assessed for eligibility, 383 randomized to one of 3 interventions; cowpea, common bean or control. Children were followed every 6 weeks, assessed for anthropometric status and compliance with consumption of the legume supplement. Of the 329 children that completed the entire 12 mo of participation, daily compliance was high, mothers reported giving the legume on 98% of the study days. Among those children that did not complete the participation, the most common reason was development of acute malnutrition in 35/54 children, which required therapy with supplementary or therapeutic feeding. Almost 4000 fecal specimens were collected, snap frozen in liquid nitrogen and delivered to the analyzing labs. Five dual sugar permeability tests were done: upon enrolment, after 3mo, 6mo, 9mo and 12mo of participation. A representative group of fecal samples were sequenced for 16s rDNA and metagenomics, as well as having fecal metabolites measured. The findings are discussed under the achievement section.

**Objective 4: Increase the capacity, effectiveness and sustainability of agriculture research institutions in Malawi.**

The PI and the research team continued to promote sustainable research through relationships with the University of Malawi College of Medicine and with colleagues at LUANAR. In addition to the training of four graduate students, a junior faculty member, Chrissie Thakwalakwa at the College of Medicine, continued to be supported by this project and provided overall supervision of the field studies. The Agriculture Department at LUANAR was engaged in developing the formulations and recipes using cowpeas and common beans, and the Washington University team trained two student LUANAR food scientists on the development processes used in the Washington University food science lab. The two PhD students recruited at the Malawi College of Medicine attended classes, learned lab techniques and attended other seminars/lectures/conferences to enhance their knowledge base at Washington University in St. Louis, they then returned to Malawi to utilize these new skills to complete their educational training.

#### **IV. Major Achievements**

*(Present a list of significant research achievements and/or technical advances resulting from project activities during the FY 2017 performance period. The description of each achievement need not be more than three sentences long. Quantitative information on or a technical description of the research achievement would be appreciated because it adds credibility to the importance of the achievement.)*

1. We proved that cowpea supplementation improves linear growth in 6-12 mo Malawian children
2. We proved that common bean supplementation improves gut health in 12-36 mo Malawian children
3. 100% sample collection in Study 2, requiring a large amount of effort and resources, including collaboration in the districts where the studies were conducted.
4. Two Malawian PhD students successfully completed classes at Washington University and were taught ddPCR and other lab techniques.
5. Analyzed all data from the field including anthropometric data and its relationship to L:M showing the change in Environmental Enteropathy.
6. 4048 fecal samples were sequenced and characterized to determine the bacterial and metabolic signature for growth and gut health.
7. Three articles have been published and 4 more are in preparation describing this work.

#### **V. Research Capacity Strengthening**

*(Describe how collaborative research activities supported by the project during FY 2017 have contributed to the strengthening of institutional capacity to carry out multidisciplinary research on grain legumes and to solve the problems facing the legume sectors in host countries and regions. Appropriate capacity strengthening items to present in this section include research equipment procured (>\$5,000), laboratory and analytical facilities developed, participation in professional meetings or other networking activities, etc. Please also identify in this section the activities completed and equipment procured during the past fiscal year with supplemental Institutional Capacity Strengthening funds received by host country institutions in the respective project.)*

The PI and the research team continue to promote sustainable research through relationships with the Malawi College of Medicine and with colleagues at LUANAR. The training provided to the four Malawian graduate students continued and helped them to develop into investigators able to continue research on childhood malnutrition, especially in the use of grain legumes. Chrissie Thakwalakwa of the College of Medicine, with support from Drs. Manary, Trehan, and Maleta, continued to supervise the field team, honing and improving her skills in conducting large collaborative clinical trials aimed at improving the nutritional status of impoverished rural children. The Agriculture Department at LUANAR was engaged in developing the formulations and recipes using cowpeas and common beans, and the Washington University team trained two student LUANAR food scientists on the development processes used in the Washington University food science lab. One of these students continues to be engaged in by supervising production and quality control of the flours using the food science and safety knowledge she obtained as part of her training. The two Malawian PhD students attended

classes at Washington University that are not offered at the University of Malawi increasing their research knowledge base. These students have also been trained in ddPCR lab techniques utilized in Dr. Manary's lab at Washington University and attended a Scientific Nutrition Conference in Chicago, Illinois.

## **VI. Human Resource and Institution Capacity Strengthening**

*(This section is a compilation of short-term and long-term degree training activities completed by the project during the performance period. This section is intended to be independent of research capacity strengthening activities described in the previous section.)*

### **1. Short-Term Training**

*(Provide the following information for each short-term training activity completed. If a training was repeated in several places, each training must have a separate entry. Short term training is defined as a minimum of two consecutive class days or more in duration, or 16 contact hours or more scheduled intermittently.)*

#### **Short-Term Training: Staff Field Training**

- a. *Purpose of Training:* Study research nurses, drivers, research assistants and staff received training in study guidelines, anthropometric data collection skills, biological sample collection methods and community engagement. Having a knowledgeable and capable staff is vital to conducting research.
- b. *Type of Training:* Field Training for research activities
- c. *Country Benefiting:* Malawi
- d. *Location and dates of training:* Malawi, 2016
- e. *Number receiving training (by gender):* 6 female nurses, 4 male drivers, 15 village health workers (11 male, 4 female)
- f. *Home institution(s) (if applicable):* Nurses and drivers are from the University of Malawi College of Medicine; village health workers are employed by the Ministry of Health
- g. *Institution providing training or mechanism:* University of Malawi College of Medicine

#### **Short-Term Training: Malawi PhD Visiting Researchers**

- a. *Purpose of Training:* Visiting Malawian researchers attending conference focused on areas of anatomy, biochemistry and molecular biology, investigative pathology, nutrition, pharmacology and physiology allowing access to the latest research impacting life sciences.
- b. *Type of Training:* Experimental Biology Meeting
- c. *Country Benefiting:* Malawi
- d. *Location and dates of training:* Chicago, IL USA, April 22-26, 2017
- e. *Number receiving training (by gender):* 2 PhD candidates (1 male and 1 female)
- f. *Home institution(s) (if applicable):* University of Malawi College of Medicine

- g. *Institution providing training or mechanism:* American Association of Anatomists, American Physiological Society, American Society for Biochemistry and Molecular Biology, American Society for Investigative Pathology, ASPECT

#### **Short-Term Training: Malawi PhD Visiting Researchers**

- a. *Purpose of Training:* Visiting Malawian researchers training in Good Clinical Practices and basic Human Subject Training
- b. *Type of Training:* Good Clinical Practices, HRPO-Basic Human Subject Education for BioMed IRB members, CITI training, HIPPA Privacy and Information Security
- c. *Country Benefiting:* Malawi
- d. *Location and dates of training:* St. Louis, MO August 28<sup>th</sup> - September 1<sup>st</sup>
- e. *Number receiving training (by gender):* 2 PhD candidates (1 male and 1 female)
- f. *Home institution(s) (if applicable):* University of Malawi College of Medicine
- g. *Institution providing training or mechanism:* Washington University in St. Louis

#### **1. Degree Training**

- i. *Name of trainee:* Lucy Bollinger
- ii. *Country of Citizenship:* USA
- iii. *Gender:* Female
- iv. *Host Country Institution Benefitting from Training:* University of Malawi College of Medicine
- v. *Institution providing training:* Washington University
- vi. *Supervising CRSP PI:* Mark Manary and Indi Trehan
- vii. *Degree Program:* Masters
- viii. *Field or Discipline:* Biological Sciences
- ix. *Research Project Title (if applicable)*
- x. *Start Date:* May 2015
- xi. *Projected Completion Date:* May 2016
- xii. *Is trainee a USAID Participant Trainee and registered on TraiNet?* No
- xiii. *Training status (Active, Completed, Pending, Discontinued, or Delayed):* Completed

#### **2. Degree Training**

- i. *Name of trainee:* William Cheng
- ii. *Country of Citizenship:* USA
- iii. *Gender:* Male
- iv. *Host Country Institution Benefitting from Training:* Washington University
- v. *Institution providing training:* Washington University
- vi. *Supervising CRSP PI:* Mark Manary and Indi Trehan
- vii. *Degree Program:* Masters
- viii. *Field or Discipline:* Biological Sciences
- ix. *Research Project Title (if applicable)*

- x. *Start Date:* May 2016
- xi. *Projected Completion Date:* May 2017
- xii. *Is trainee a USAID Participant Trainee and registered on TraiNet?* No
- xiii. *Training status (Active, Completed, Pending, Discontinued, or Delayed):*  
Active

### **3. Degree Training**

- i. *Name of trainee:* Oscar Divala
- ii. *Country of Citizenship:* Malawi
- iii. *Gender:* Male
- iv. *Host Country Institution Benefitting from Training:* University of Malawi College of Medicine
- v. *Institution providing training:* University of Malawi College of Medicine
- vi. *Supervising CRSP PI:* Mark Manary, Ken Maleta, Indi Trehan
- vii. *Degree Program:* PhD
- viii. *Field or Discipline:* Epidemiology
- ix. *Research Project Title:* N/A
- x. *Start Date:* August 2015
- xi. *Projected Completion Date:* June 2018
- xii. *Is trainee a USAID Participant Trainee and registered on TraiNet?:* No
- xiii. *Training status (Active, completed, pending, discontinued or delayed):*  
Active

### **4. Degree Training**

- i. *Name of trainee:* Yankho Kaimila
- ii. *Country of Citizenship:* Malawi
- iii. *Gender:* Female
- iv. *Host Country Institution Benefitting from Training:* University of Malawi College of Medicine
- v. *Institution providing training:* University of Malawi College of Medicine
- vi. *Supervising CRSP PI:* Ken Maleta
- vii. *Degree Program:* PhD
- viii. *Field or Discipline:* Epidemiology
- ix. *Research Project Title:* N/A
- x. *Start Date:* August 2015
- xi. *Projected Completion Date:* June 2018
- xii. *Is trainee a USAID Participant Trainee and registered on TraiNet?:* No
- xiii. *Training status (Active, completed, pending, discontinued or delayed):*  
Active

### **5. Degree Training**

- i. *Name of trainee:* Chrissie Thakwalakwa
- ii. *Country of Citizenship:* Malawi
- iii. *Gender:* Female

- iv. *Host Country Institution Benefitting from Training:* University of Malawi  
College of Medicine
- v. *Institution providing training:* Tampere University in Finland
- vi. *Supervising CRSP PI:* Ken Maleta
- vii. *Degree Program:* PhD
- viii. *Field or Discipline:* Community Health
- ix. *Research Project Title:* N/A
- x. *Start Date:* August 2015
- xi. *Projected Completion Date:* July 2017
- xii. *Is trainee a USAID Participant Trainee and registered on TraiNet?:* No
- xiii. *Training status (Active, completed, pending, discontinued or delayed):*  
Active

## **6. Degree Training**

- i. *Name of trainee:* Theresa Ngoma
- ii. *Country of Citizenship:* Malawi
- iii. *Gender:* Female
- iv. *Host Country Institution Benefitting from Training:* The Lilongwe  
University of Agriculture and Natural Resources (LUNAR)
- v. *Institution providing training:* LUNAR
- vi. *Supervising CRSP PI:* Mark Manary, Indi Trehan, Ken Maleta
- vii. *Degree Program:* Masters
- viii. *Field or Discipline:* Food Science and Technology
- ix. *Research Project Title:* N/A
- x. *Start Date:* January 2015
- xi. *Projected Completion Date:* December 2015
- xii. *Is trainee a USAID Participant Trainee and registered on TraiNet?* No
- xiii. *Training status (Active, completed, pending, discontinued or delayed):*  
Active

## **7. Degree Training**

- i. *Name of trainee:* Ulemu Chimimba
- ii. *Country of Citizenship:* Malawi
- iii. *Gender:* Female
- iv. *Host Country Institution Benefitting from Training:* LUNAR
- v. *Institution providing training:* LUNAR
- vi. *Supervising CRSP PI:* Mark Manary, Indi Trehan, Ken Maleta
- vii. *Degree Program:* Masters
- viii. *Field or Discipline:* Food Science and Technology
- ix. *Research Project Title:* N/A
- x. *Start Date:* January 2015
- xi. *Projected Completion Date:* December 2015



- xii. Is trainee a USAID Participant Trainee and registered on TraiNet?: No
- xiii. Training status (Active, completed, pending, discontinued or delayed):  
Complete

## **VII. Achievement of Gender Equity Goals**

*(Describe progress in achieving gender equity goals set for the project during the performance period. This includes training, involvement in project activities, and research approaches. )*

Beneficial findings and knowledge gained from these studies will benefit both women and men in these societies, including parents and children. Farming work is generally carried out by both men and women in this agrarian culture, so this will benefit both genders. Improvements in child health are most likely to benefit women in Malawi, as they have the primary role in childrearing. Health improvements that lead to improved survival and intellectual development of girls will also likely translate into improved school performance and capacity for careers. Demonstrating achievement of such goals is beyond the scope of the current project.

In terms of training future scientists, all but one of our Malawian graduate students is female. One of our American graduate students is female. One of our non-degree American students is female.

Particular care was extended to women for inclusion in the Food Safety training course, with approximately 46% of attendees being female.

## **VIII. Implementation of Data Management Plan**

*(Describe efforts to implement of project's Data Management Plan, including a summary of data sets that have been submitted to Open Data Access sites, including USAID DDL. Indicate your Open Data Access sites for future submissions as well.)*

At this time data sets have not been submitted to the USAID DDL, however, they will be submitted prior to November 30<sup>th</sup>.

## **IX. Scholarly Accomplishments**

*(Identify all **publications**, theses and/or dissertations, presentations, professional recognitions, awards, patents, and Plant Variety Protection Certificates that evidence scholarly accomplishments by U.S. and Host Country scientists as well as degree trainees during the performance period. Please send electronic copies of publications to the MO for sharing with USAID and include URL or DOI information where available.)*

1. Borresen, et al. "The nutrient and metabolite profile of three complementary legume foods with potential to improve gut health in rural Malawian children". Curr Dev Nutr 2017;1:e001610; DOI: 10.3945/cdn.117.001610
2. Stephenson, et al. "Complementary feeding with cowpea reduces growth faltering in rural Malawian infants: a randomized, blinded, controlled clinical trial". Am J Clin Nutr (accepted).
3. Agapova, et al. "Additional common bean in the diet of Malawian children does not affect linear growth, but reduces intestinal permeability". J Nutr (accepted)

4. Ngoma, et al. “Effect of cowpea flour processing on the chemical properties and acceptability of a novel cowpea blended maize porridge”. PLoS ONE (under review).

## **X. Achievement of Impact Pathway Action Plan**

*(At the project planning and workplan development stage, each project team prepared an Impact Pathway identifying major research outputs, users of these outputs, a vision of success, and necessary steps to achieve the vision of success. In the Impact Pathway worksheet, your project also identified strategies and an action plan to be undertaken by the project team over the 4.5 year life of the project to translate the outputs into outcomes. Please provide an update on your team’s efforts in implementing the action plan and progress towards achieving the ‘vision of success’ as laid out in the Impact Pathway strategy. Discuss any constraints encountered and steps taken to overcome them.)*

We remain on track with the Impact Pathway developed during the project planning and workplan stage. Both Goal 1 (capacity building) and Goal 2 (clinical trial decreasing stunting and EED) are being carried out as planned. The measurements of success (Steps 3 and 4) are still several years away from completion, as originally planned.

## **ANNEXES**

### **Annex 1. Tables, Figures, and Photos Cited in the Report**

### **Annex 2. Literature Cited**

*(List all literature cited in the body of the technical progress report in full bibliographic form.)*