Legumes and Growth (SO3.1)

Lead U.S. Principal Investigator (PI) and affiliated U.S. University
Mark Manary MD, Helene Roberson Professor of Pediatrics, Washington University School of Medicine in St. Louis, St. Louis, Missouri

U.S. and HC Co-PIs/Collaborators and Institutions
Ken Maleta, University of Malawi College of Medicine, Malawi
Chrissie Thakwalakwa, University of Malawi College of Medicine, Malawi
Indi Trehan, Washington University School of Medicine in St. Louis, Missouri

Abstract
Because millions of children die annually due to undernutrition and hundreds of millions more are stunted, interventions that decrease the burden of childhood malnutrition are urgently needed. Environmental enteropathy (EE), a pervasive chronic subclinical inflammatory condition among children that arises when complementary foods are introduced, places them at high risk for stunting, malabsorption, and poor oral vaccine efficacy.

Two randomized, controlled clinical trials to determine if common beans or cowpeas improve growth, ameliorate EE, and alter the intestinal microbiome during this high-risk period are proposed. The first study involves 6–11-month-old children who will receive common beans, cowpeas, or standard local complementary foods for six months. Anthropometry will be compared among the three groups. EE will be assessed using a urine dual-sugar absorption test and by quantifying human intestinal mRNA for inflammatory messages and the intestinal microbiota characterized by deep sequencing of fecal DNA to enumerate the host microbial populations and their metabolic capacity. The second randomized, controlled trial will enroll 12–35-month-old children and follow them for twelve months; each subject will receive dietary interventions, either legume-based or control. Anthropometric, host inflammatory, and gut microbiota analyses will be conducted similar to the first study. By amalgamating the power of the clinical trial and advanced biological analyses, we will elucidate the potential of legumes to have a major impact on child health in sub-Saharan Africa.

Project Statement and Justification
Approximately 45 percent of all deaths worldwide among children under the age of five (i.e., 3.1 million deaths annually), are directly or indirectly related to undernutrition. Additionally, stunting permanently affects an additional 165 million children worldwide and reduces the affected individual's physical, immunological, and cognitive capacity throughout his or her lifetime. Stunting is estimated to account for 21 percent of all disability adjusted life years (DALYs) in children. Both stunting and wasting are causally related to the dietary intake and gut health in children younger than three years of age.

In developing, impoverished settings, a nearly ubiquitous gut inflammatory condition known as environmental enteropathy (EE) 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 early in life. While subclinical, EE predisposes children to more clinically manifest forms of malnutrition: wasting and stunting. Given the significant contribution of malnutrition to childhood morbidity and mortality, meaningful progress on reducing EE is needed to establish a lasting foundation for progress against global hunger.

In traditional sub-Saharan African societies, complementary foods are dominated by monotonous, protein-poor, and micronutrient-poor starches such as maize, cassava, and sorghum. Alternative, yet culturally acceptable, complementary foods that can provide a better and more palatable balance of nutrients may promote a decrease in EE and improved growth. Legumes provide just such an opportunity, since their protein content is significantly higher than cereals and they are rich in dietary fiber, starch, minerals, vitamins, and antioxidants. Common beans and cowpeas, for example, have three- to four-fold more protein per gram than corn. The zinc content in legumes is also relatively high and might further decrease the progression of EE, as has been demonstrated recently in a prospective randomized trial. Legumes make an excellent complementary food for children weaning from exclusive breastfeeding and with appropriate preparation are quite digestible and well tolerated. Successful legume–maize blends have, in fact, already been developed in the past and demonstrated favorable acceptability profiles in children younger than one year of age; they were also nutritionally sound as a weaning supplement. Cowpea is also attractive for study, as it grows well in the African context, is culturally accepted, and is a hardy, drought-tolerant, crop. Cowpea also has significant anti-inflammatory effects, mediated by specific phenolic profiles and antioxidant activity.

Human and animal studies of the effect of legumes on the intestinal microbiome are limited. A recent study comparing the gut microbiota in children from rural Burkina Faso who consumed a diet rich in legumes with European children showed a relative lack of potentially pathogenic Enterobacteriaceae in the African children, conceivably protecting these children from severe gut inflammation and bacterial translocation.

Since EE is a chronic inflammatory condition, interventions with anti-inflammatory effects might also improve gut health. A growing body of evidence suggests that a diet enriched in legumes decreases inflammation markers correlated to illnesses with inflammatory components such as colorectal cancer and cardiovascular disease. Legumes could therefore serve as a complementary food in this high-risk population, with key measurable endpoints and biomarkers, including markers of EE and growth parameters.

Objectives

1. Evaluate changes in childhood anthropometry (height-for-age and weight-for-height z scores), biomarkers of EE (lactulose:rhamnose and a panel of human mRNA messages correlated with 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 6–11-month-old rural Malawian children.
2. Evaluate changes in child growth (height-for-age and weight-for-height z scores), biomarkers of EE (lactulose:rhamnose and a panel of human mRNA messages correlated with 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 adding either cowpeas or common beans to the diet of 12–35-month-old rural Malawian children.
3. Analyze changes in the developing intestinal microbiome among both age cohorts and all three intervention cohorts (cowpeas, common beans, standard feeding) to inform an understanding of the role of the microbiota in early childhood growth and gut health.

Approaches and Methods
This project will conduct two randomized, controlled clinical trials to investigate the effect of legume consumption on infant and toddler growth and gut health; assessment will be conducted every three months. Both trials are prompted by the overarching hypothesis that children provided with a legume supplement will have greater linear growth and an improvement in biomarkers of EE compared to those who receive standard food supplements.

Each study will randomize infants and toddlers at high risk for EE and stunting to a sustained intervention of cowpea, common bean, or standard maize supplements and assess the outcomes of interest every 3 months. The outcomes will include anthropometric measurements, clinical symptoms, biomarkers of EE and gut inflammation, and population characteristics of the microbiota. To detect a difference in change in length of 1.1 cm, which corresponds to a change in height-for-age Z score (HAZ) of 0.45 units at 12 months of age, 79 children are needed in each group.

The first trial will evaluate changes in childhood anthropometry (height-for-age and weight-for-height z scores), biomarkers of EE (lactulose:rhamnose and a panel of human mRNA messages correlated with 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 6-11-month-old rural Malawian children.

The second trial will evaluate changes in child growth (height-for-age and weight-for-height z scores), biomarkers of EE (lactulose:rhamnose and a panel of human mRNA messages correlated with 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 adding either cowpeas or common beans to the diet of 12-35 month-old rural Malawian children.

Anticipated Achievements and Outputs
- Development of legume recipes for specific aims with LUANAR colleagues
- Acceptability testing of legume recipes in infants and children
- Continuous enrollment, randomization, intervention delivery, and specimen collection in Mitondo for both groups of children
- Specimen processing and data analysis
- Manuscript preparation and submission
- Evaluation of future directions and implications of findings with key local and international stakeholders

Projected Developmental Outcomes
An understanding of whether children provided with a grain legume supplement will have greater linear growth and an improvement in biomarkers of EE compared to those who receive standard food supplements.

Contributions to Institutional Capacity Building
The studies will also facilitate the training of two doctoral-candidate nutrition students from the University of Malawi and two food science master's-level students from University of Malawi–Bunda College of Agriculture.