


Feed the Future Innovation Lab for Collaborative Research on Grain Legumes

Legumes and Growth

SO3.1 :: Improving the Nutrition of the Poor, Especially Young Women and Children Through Grain Legume Consumption

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Undernutrition is a Major Cause of Child Mortality

Maternal and Child Nutrition 1

Maternal and child undernutrition and overweight in low-income and middle-income countries

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	Attributable deaths with UN prevalences*	Proportion of total deaths of children younger than 5 years	Attributable deaths with NIMS prevalences†	Proportion of total deaths of children younger than 5 years
Fetal growth restriction (<1 month)	817 000	11·8%	817 000	11·8%
Stunting (1–59 months)	1 017 000*	14·7%	1 179 000†	17·0%
Underweight (1–59 months)	999 000*	14·4%	1 180 000†	17·0%
Wasting (1–59 months)	875 000*	12·6%	800 000†	11·5%
Severe wasting (1–59 months)	516 000*	7·4%	540 000†	7·8%
Zinc deficiency (12–59 months)	116 000	1·7%	116 000	1·7%
Vitamin A deficiency (6–59 months)	157 000	2·3%	157 000	2·3%
Suboptimum breastfeeding (0–23 months)	804 000	11·6%	804 000	11·6%
Joint effects of fetal growth restriction and suboptimum breastfeeding in neonates	1 348 000	19·4%	1 348 000	19·4%
Joint effects of fetal growth restriction, suboptimum breastfeeding, stunting, wasting, and vitamin A and zinc deficiencies (<5 years)	3 097 000	44·7%	3 149 000	45·4%

Data are to the nearest thousand. *Prevalence estimates from the UN. †Prevalence estimates from Nutrition Impact Model Study (NIMS).

Table 2: Global deaths in children younger than 5 years attributed to nutritional disorders

Lancet 2013; 382: 427

Stunting is Common and Its Effects are Lifelong

- Stunting between 12-36 months of age predicts poorer cognitive performance and lower school grades in middle childhood
- In contrast to Asia and Latin America, the number of stunted children is actually *increasing* in Africa

	Stunting (HAZ <-2)			
	UN[155]		NIMS[158]	
	Proportion	Number (millions)	Proportion	Number (millions)
Africa	35·6% (33·3–38·0)	56·3 (52·5–60·0)	35·5% (34·4–36·6)	56·6 (54·3–57·8)
Asia	26·8% (23·2–30·5)	95·8 (82·8–108·8)	29·5% (26·4–31·3)	103·5 (92·5–109·8)
Latin America and the Caribbean	13·4% (9·4–17·7)	7·1 (4·8–9·4)	14·6% (13·6–15·5)	7·8 (7·3–8·2)
Oceania	35·5% (16·0–61·4)	0·5 (0·2–0·8)	34·7% (27·8–39·5)	0·4 (0·3–0·5)
LMICs	28% (25·6–30·4)	159·7 (145·9–173·4)	29·9% (27·9–31·0)	168·3 (157·3–174·6)
High-income countries	7·2% (4·1–12·6)	5·1 (2·9–8·9)	..	..
Global	25·7% (23·5–27·9)	164·8 (150·8–178·8)	..	..

Lancet 2013; 382: 427

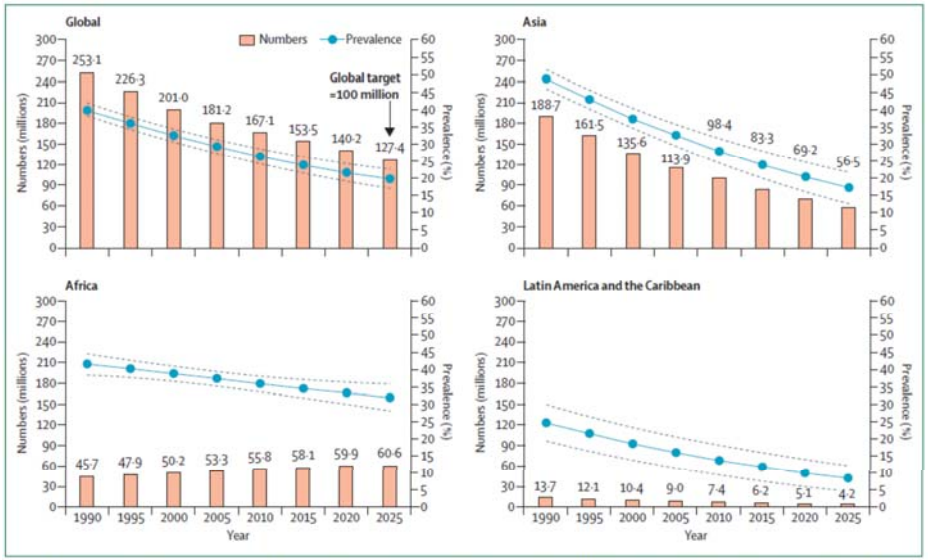
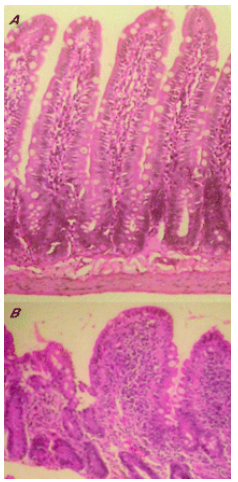


Figure 4: Trends in prevalence and numbers of children with stunted growth (HAZ <-2), by selected UN regions and globally, 1990–2010, and projected to 2025 on the basis of UN prevalence estimates
HAZ=height-for-age *Z* score. Data from UNICEF, WHO, World Bank.[154]

Lancet 2013; 382: 427

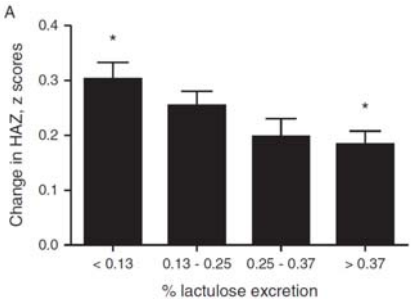
Environmental Enteropathy (EE)

- Asymptomatic morphologic changes in the gut of inhabitants of the tropics
  - Reduction in villous height
  - Broadening of villi
- T-cell infiltration of lamina propria and epithelium
  - Increased numbers of CD3, CD4, and CD8 cells
  - CD25 cells decreased 25-fold
- Most commonly measured by dual sugar absorption tests
  - Drink non-metabolized sugars of different molecular weights and measure their relative concentrations in the urine



EE is Associated with Stunting

- Prospective study of 418 apparently well 2-5 year-old children in rural Malawi
- Surveyed household, sanitation facilities, diet, food security status, biomarker of enteropathy (L:M test)
- Measured linear growth and used linear regression modelling to identify which factors predicted growth faltering
- Among several factors identified, lower L:M (less EE) was associated with more growth and lower lactulose excretion (less permeable gut) was associated with more growth



JPGN 2012; 55: 747

EE is Associated with Stunting

- In 2-15 month old Gambian infants, up to 43% of observed growth faltering can be explained on the basis of long-term intestinal lesions

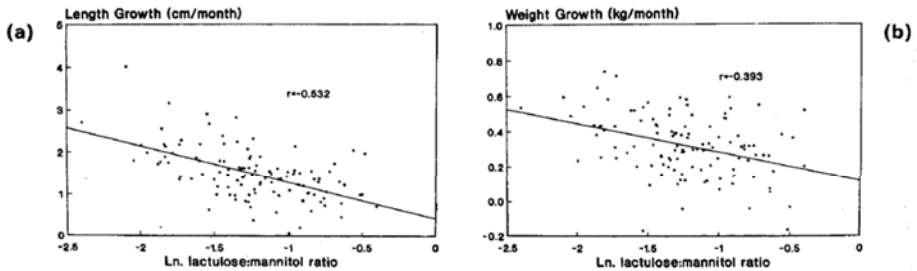
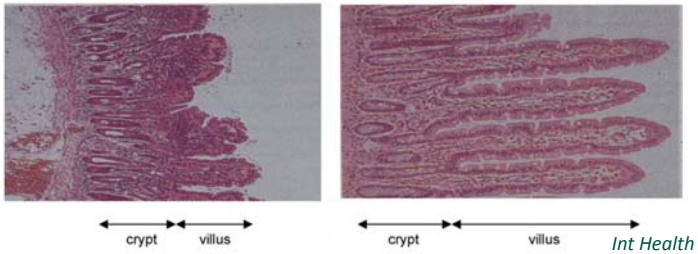


Fig 2—The relation between intestinal permeability (expressed as $\log_e$ lactulose:mannitol ratio) and mean monthly (a) length and (b) weight growth of 119 rural Gambian infants.
Significance of regression coefficients, $p < 0.001$.

Lancet 1991; 338: 907

EE and Nutrient Absorption

- Children with significant EE have reduced absorption of fat (≈5%), carbohydrates (≈5%), protein (≈15%), vitamin B_{12}
- Overall wastage of >5% of energy intake



Int Health 2010; 2: 172

Etiology of EE

- Widely thought to be associated with poor hygiene → associated with use of less clean water in daily life
- Actually more likely to be multifactorial because of the ubiquitous nature of the condition
- *EE and poor nutrition co-exist and exacerbate each other, creating a negative synergism*

Potential Consequences of Healing EE

- Lower risk of physical and cognitive stunting
- Better growth and development
- Less frequent acute infections
- Less episodes of acute malnutrition
- Better health overall
- Improved adult capacity
- Overall improvement in economic development

Prior Attempts at Healing EE

- Probiotics and antibiotics don't work
- Reduce exposure to coliforms with promotion of access to clean water and sanitation
- Glutamine shown to improve barrier function
- Micronutrient supplementation shows some improvement
  - Histology of blunted villi in EE similar to micronutrient-deprived mice
- Deworming with albendazole or high-dose zinc slows EE progression
- Poly-unsaturated fatty acid supplementation not effective
  - Used for anti-inflammatory effects in Crohn's

AJCN 2005; 82: 1040
Am J Gastro 2009; 104: 2326
Lancet 2009; 374: 1032
BMC Gastro 2014; 14: 15
Clinics 2014; 69: 225
Clin Gastro Hep 2014; in press

How Might Legumes Fit In?

- Prior interventions against EE have shown marginal benefits, at best
- Diets enriched in legumes decrease markers of inflammation
  - Increased legume intake inversely correlated with illnesses with inflammatory components such as colorectal cancer and cardiovascular disease
- May serve as a major source of protein and micronutrients in populations where carbohydrate consumption predominates in complementary feeding

J Nutr 2012; 142: 334
Nitric Oxide 1997; 1: 476
Lipids 2010; 45: 765
Eur J Clin Nutr 2011; 65: 415
Dry Beans and Pulses 2012
JPGN 2007; 44: 487

Anti-Inflammatory Effects of Beans

- Swedish brown beans decrease IL-6 and IL-18 in humans
- Non-digestible portion of common beans down-regulates signaling pathways that lead to inflammation in a mouse model of colon cancer
- When fermented by normal gut flora, the non-digestible fraction from common beans produces short-chain fatty acids that inhibit colon cancer HT-29 cell growth
- Non-digestible fraction of common beans protects against chemically-induced crypt inflammation in rat colons

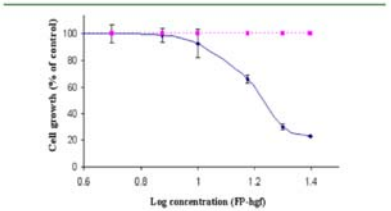


Figure 1. Concentration–response curve of FP-hgf on HT-29 colon carcinoma cells after incubation (24 h). The effect of FP-hgf (■) was normalized to the blank control (FP-hgf without cells) and to the nontreated control (0%/mL$_a$ 100%) cells (◆). IC_{50} was calculated from the antilog of the *x*-axis value at the inflection point of the sigmoid curve fit generated for the treatment (JMP V. 5.0). Values are the mean of three independent experiments with standard errors depicted by vertical bars.

PLoS ONE 2013; 8: e59985
Br J Nutr 2012; 108S1: S145
J Agr Food Chem 2012; 60: 12443
Food Funct 2010; 1: 294
J Agr Food Chem 2008; 56: 8737

The Potential of Cowpeas

- Grows well in Africa, is culturally accepted, and is a hardy, drought-tolerant crop
- Anti-inflammatory effects mediated by specific phenolic profiles and antioxidant activity
- Phenolic compounds are also active following cooking and simulated enzymatic digestion

Soil and Crop Sciences 2012
Int J Food Sci Tech 2013; 48: 2638

SO3.1 :: Hypothesis

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.

SO3.1 :: Methods

- Two large prospective, randomized controlled clinical trials testing complementary feeding with legumes in rural Malawi
  1. Children 6-11 months old
  2. Children 12-35 months old
- Apparently healthy children (no acute malnutrition) from two agrarian village clusters who are at high risk of EE and stunting

SO3.1 :: Outcomes

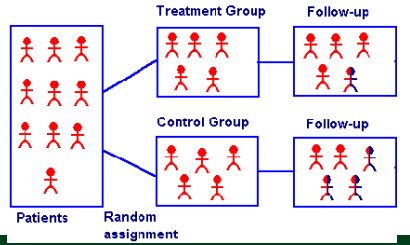
- Primary Outcomes
  - Change in height-for-age Z-scores (HAZ)
    - Anthropometric marker for stunting
  - Change in traditional biomarker of EE
    - Dual-sugar absorption test :: lactulose-rhamnose (L:R) test
- Secondary Outcomes
  - Change in novel biomarkers of EE
    - mRNA of inflammatory cytokines and interleukins in fecal specimens
  - Changes in intestinal microbiome
    - Sequencing of bacterial genomic DNA from fecal samples to determine changes in population taxonomy and their collective metabolic capacity

SO3.1 :: Methods

- Develop 2-4 culturally-appropriate complementary food recipes for common bean and 2-4 recipes for cowpeas appropriate for each age group
- Collaboration with faculty and students at Lilongwe University of Agriculture and Natural Resources (LUANAR)
  - Lead :: Vernon Kabambe, PhD
  - Capacity-building :: Food Science and Technology MSc students; equipment needed for development of recipes and nutrient testing
    - Tereza Ngoma, Ulemu Kankwatira
- Acceptability testing for 2 weeks to select recipes for use in clinical trials

SO3.1 :: Study Design

- Capacity-building :: PhD students from University of Malawi College of Medicine to supervise and implement clinical trials after training in clinical research design and in malnutrition from the senior investigators
- Randomized clinical trial of all children in a rural village cluster who are of the age of interest
  1. Rolling enrollment of children as they turn 6 months of age
  2. Rolling enrollment of children in the 12-23 month age range



SO3.1 :: Study Design

- Baseline measurements :: anthropometry (height, weight, mid-upper arm circumference), dietary surveys, health history, biomarkers for EE (dual-sugar absorption test), stool samples for intestinal microbiome analysis
  - Capacity-building :: Employment and training of students or recent graduates of the College of Medicine's Medical Laboratory Technology program



SO3.1 :: Study Design

- 2-4 recipes for each supplement
- Randomization to common bean, cowpea, or control maize supplementation
- Caretakers educated about recipes and provided with enough food supplementation for child and immediate family (to account for sharing)
  1. 200 kcal/d for children 6-9 mos old or 300 kcal/d for children 9-11 mos old
  2. 15% of estimated caloric intake

SO3.1 :: Study Design

- Prolonged supplementation
  1. 6-11 month group :: 6 months
  2. 12-23 month group :: 12 months
- Biweekly follow-up, including assessments of adverse events, anthropometry, screening for acute infections (fever, cough, diarrhea)
- Capacity-building :: Training and financial support for village health workers; supplementation of supplies (e.g., HIV test kits) and medications (e.g., antimalarials) available at village health centers

SO3.1 :: Study Design

- Dietary intake assessments every 6 weeks
- Return to clinic every 12 weeks for post-intervention assessments, including dual-sugar absorption test for EE, stool collection, anthropometry
  1. 12 and 24 week assessments
  2. 12, 24, 36, 48 week assessments
- Capacity-building :: after clinical study, PhD students to spend 12 months in St. Louis
  - Laboratory training :: specimen preparation, DNA extraction, PCR amplification, analysis of EE mRNA stool samples, liquid chromatography methods for dual-sugar absorption test
  - Classroom training :: clinical trials, statistics, scientific writing, global health, nutrition coursework as desired for their future research plans

SO3.1 :: TIMELINE	Year 1	Year 2	Year 3	Year 4
IRB approvals				
Produce manual of operations				
Staff recruitment				
Develop legume recipes with LUANAR				
Acceptability testing of legume recipes				
Identification and training of Study 1 PhD student				
Community mobilization and engagement for Study 1				
Enrollment, randomization, intervention delivery, specimen collection in Study 1				
Identification and training of Study 2 PhD student				
Community mobilization and engagement for Study 2				
Specimen processing and analysis for Study 1				
Enrollment, randomization, intervention delivery, specimen collection in Study 2				
Specimen processing and data analysis for Study 2				
Manuscript preparation and submission				
Evaluation of future directions with key stakeholders				

Alignment with Feed the Future Objectives

- Local crop-based approaches, such as with legumes, provide opportunity for sustainability as legumes are already grown in much of the developing world
- EE causes ≈1/3 of stunting worldwide
  - Multifactorial etiology
  - Dietary intervention utilizing a ubiquitous, nutrition foodstuff is thus an attractive and durable intervention
- USAID Mission in Lilongwe, Malawi, has expressed interest in associating with this study and providing funding for pilot studies if findings from this work warrant subsequent investigation

Gender Equity

- In practical terms, child health remains primarily a women's issue in sub-Saharan Africa
- Farming and food selection also remain primarily the purview of women
- If legumes are shown to improve EE and child growth, it will primarily be up to women to implement on their subsistence farms
- Improvement in child mortality leads to improvements in women's empowerment and autonomy in family planning
- Ultimately, improvements in child mortality and morbidity may lead to decreased fertility and increased opportunities for women's education and income potential

J Pol Econ 1991; 99: 582