**TECHNICAL APPROACH *(modified to include a cowpea component)***

***Problem statement***

 The proposed research will help the Pulse CRSP achieve the goal delineated in the RFP as Global Theme B “To increase the utilization of bean and cowpea grain, food products and ingredients so as to expand market opportunities and improve community health and nutrition”. The topical area that the research will address is Topical Area 2: Achieving Nutritional Security for Improved Health of Target Populations. The overall goal of the research is to determine if eating beans or cowpeas will improve the nutritional and immune status of HIV+ children that are not being treated with antiretroviral drugs.

***Background and justification***

 The statistics regarding HIV are staggering! HIV has caused an estimated 25 million deaths worldwide in just 27 years [1]. There are approximately 33 million people in the world living with HIV while the current annual rate of new infections is 2.7 million and the annual death rate estimated as 2.0 million. Most children living with HIV are innocent victims – more than 90% of the infections occur during pregnancy, birth or breastfeeding. Approximately 2 million of the 33 million cases of HIV occur in children less than 15 yr. In this age group there are 370,000 new infections and 270,000 deaths per year. Ninety percent of the children less than 15 yr living with, and dying from, HIV live in sub-Saharan Africa. Furthermore, about 140,000 of these children live in Tanzania (the host country) [2].

 It is well known that insufficient intake of macronutrients or selected micronutrients leads to a decrease in immune function and an increase in infectious diseases in children [3, 4]. Infections lead to nutrient loss from the body and a vicious cycle of greater malnutrition, additional suppression of the immune system, more infections, etc., etc. Malnutrition is considered the primary cause of immunodeficiency worldwide [5]. Because malnutrition is more common in infants and children, they suffer the greatest insult to the immune system. HIV infection destroys the CD4 cells (immune cells) leading to a decreased ability to prevent other infectious diseases. Even before there is a significant decrease in CD4 cells, the immune system’s response to the virus leads to an acute-phase response that in turn causes protein catabolism and deficiencies of some micronutrients. If an HIV infected person becomes malnourished, the effects of malnutrition and HIV on the immune system are synergistic [4]. Young children with HIV are 2.5 – 4 times more likely to die than their counterparts that are not infected [1,6]. HIV is considered to be responsible for more than a third of the deaths in children under the age of five [1]. If the young child does not receive highly active antiretroviral (HAARV) treatment, many of them will die at a very young age [1] and some estimate that 60% of HIV infected children will die before they reach the age of five [7]! For these reasons, countries that can afford antiretroviral drugs choose to treat infants and children with HAARV drugs as soon as they are identified as being infected with the virus. Other countries like Tanzania cannot cope with the magnitude of the HIV problem and choose to delay treatment until the CD4 count drops below 200/µl (i.e., the same criteria for initiation of drug treatment for adults).

 The Lancet in 2008 published a series of articles dealing with severe malnutrition in children with HIV. Severe malnutrition is considered the most common cause of death in children infected with HIV [8]. Regardless of HIV status, the greater the severity of malnutrition, the less likely the child will recover. Thus, it is imperative to prevent malnutrition in HIV infected infants and children or at least identify malnutrition in the early stages to simplify treatment and to increase the odds of recovery. When HAARV drugs became widely available, the medical community assumed that viral control and antibiotics to prevent/treat opportunistic infections would eliminate nutrition concerns [9, 10]. However, we now know that malnutrition persists even though HIV infected patients are treated with HAARV drugs and antibiotics.

For severe acute malnutrition with life-threatening symptoms, highly fortified therapeutic foods designed to replace the family diet is appropriate. However, for less severe forms of malnutrition (mild or moderate wasting or stunting), less expensive forms of treating malnutrition is recommended [11]. The approaches to treat mild and moderate malnutrition have changed little in the past 30 yr [11] and the results of these approaches have been mixed. A group of international public health experts convened in 9/30/08-10/3/08 to thoroughly evaluate and make recommendations for improving treatment of moderate forms of malnutrition [12]. If a malnourished child is identified in a society that has access to a variety of nutritious foods, nutritional counseling is the appropriate intervention. If food insecurity exists and there is a general lack of nutrient dense foods, then food supplements to provide the missing nutrients are recommended [11]. Tanzania, like other countries in the region, needs to utilize supplements. It should be noted that supplements are to augment typical diets and are not a replacement for the foods that the rest of the family eats.

The co-PIs for this proposal have conducted three research projects relevant to this proposal. The general approach has been to utilize foods produced in the country or region to prepare a culturally acceptable food supplement. The only ingredient not produced within the country was the vitamin/mineral mix that was shipped from the US because there was no local supplier. The first project dealt with rehabilitation of malnourished children. The composition of the supplement is shown in the first column of Table 1. The supplement was formulated to meet the nutrient requirements of 9 – 15 mo infants and for rehabilitation of malnourished children. The supplement was fed to 188 small children that had been hospitalized for malnutrition. After correction of electrolyte imbalance, treatment for parasites, and weight stabilization, the child was discharged and they were enrolled in our study. The results are shown in Figure 1. Most of the children achieved normal weight for age within four to six weeks. Only the most severely malnourished or children with other disease complications did not achieve normal weight for age within six weeks of treatment. A longer time period is required to correct stunting; growth in height improved significantly, but many of the children hadn’t achieved normal height for age by the end of the six mo study.

The second study was with HIV infected, HAARV naïve children. One hundred and seven HIV+ orphans ranging from 2 to 15 years consumed the supplement for six months. None of the children received HAARV drugs before or during the study. The changes in weight for

Table 1: The compositions of the supplementary foods produced at Sokoine Agricultural University

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Ingredients Rehabilitation HIV

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Study, g\_\_\_\_\_\_\_\_\_\_\_\_\_Study, g\_\_\_\_\_\_\_\_\_\_\_\_\_

Maize 38.6 21.52

Beans 38.0 55.48

Fish 5.0 0.0

Soybeans 0.0 5.50

Vegetable oil 6.0 4.50

Sugar 4.0 4.00

Cassava 5.0 5.20

Salt (iodized) 0.2 0.30

Mineral/Vitamin premix 2.9 3.00

Baking soda 0.3 0.50

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

TOTAL 100.00

All ingredients except the vegetable oil and mineral-vitamin premix were, extruded, dried, and ground. The meal was then mixed with the fat and mineral-vitamin premix and 100 g packages of meal were sealed in plastic bags. One hundred grams of supplement provided the RNI for all vitamins and minerals except for calcium and phosphorus. The supplement contained only 50% of the RNI for calcium and phosphorus. The bags of supplement were provided to the subjects on a bi-weekly basis and the child’s care giver mixed the meal with boiling water to prepare “thin-porridge”.

age and body mass index for age are shown in Figures 1 and 2 on page 5. Eating the nutritional supplement allowed the HIV infected children to grow and achieve normal weights for their age (Figures 1 and 2, page 5). There have been numerous studies evaluating the potential for food supplements or individual nutrients to reverse malnutrition in HIV infected children [12 and references therein]. The previous studies had mixed success in treating malnutrition in HIV infected children. We feel that our subjects were better able to achieve normal growth than what has been reported in the literature while employing a sustainable approach. Moreover, utilizing beans and cowpeas as the main ingredient would have a very positive effect on the bean/cowpea value chain.

The nutritional promoting affects were quite remarkable, but even more remarkable was the improvement in the immune status when the bean based supplement was consumed. It is amazing (almost too amazing to be true) that the immune status of the children improved significantly simply by consuming the bean-based supplement (Figure 3, page 5). None of the children received HAARV drugs during the study. All age groups improved their immune status such that they were not put on drugs. Tanzania’s policy regarding use of anti-retroviral drugs is to administer HAARV drugs to children or adults only when their CD4 cell counts fall below 200. ***This study demonstrates the tremendous importance of providing an adequate supply of nutrients for children that are HIV+. Normal physical development was achieved and the immune system***



***improved such that there was no need to treat the children with HAARV drugs (based on Tanzanian criteria).*** Eliminating the need for HAARV drugs, even if for a short time, would significantly reduce the tremendous costs associated with treating HIV+ individuals.

The third study was with children and adolescents in Botswana that had been receiving HAARV for one year or more. Like the second study, nutritional status improved quite dramatically. Most importantly, the CD4% increased from 23% to > 27% (i.e., based on the average CD4%, the children were no longer in the “at risk” category). The second and third study show that providing a food supplement improves the immune status of HIV+ positive children with or without HAARV drugs.

There has been only one very recent report that evaluated the impact of nutritional rehabilitation on immune status of HIV+ children[13]. These researchers followed the WHO guidelines for treating severely malnourished children (i.e., expensive, hard to obtain supplements for most under developed African countries were used). Unfortunately because part of the data included in the study by Hughes et al. [13] was presented at a conference, the general consensus today is that nutritional rehabilitation of malnourished HIV infected children allows attainment of normal nutritional status, but the immune system does not respond and the CD4 counts remain low. We also noted that in severely malnourished children (weight for age z score < -3), the immune response to eating the bean supplement was attenuated; but in children with weight for age z score > -2.99, the CD4 cell count increased quite dramatically (Figure 4).

 Shortly after HIV was identified as the agent causing AIDS, it was determined that the virus caused mucosal defects throughout the GI tract. It is now recognized that HIV infection rapidly destroys gut-associated lymphoid tissue [14]. In 2006 it was proposed that HIV infection increases gut permeability allowing translocation of gut microbial antigens through the mucosal

 



barrier into underlying tissues and into the lymph and blood systems. The bacterial antigens promote immune activation, which in turn promotes viral replication and disease progression [15]. Since that time, there have been numerous publications implicating bacterial translocation as a key factor in sustaining viral replication in HAARV naïve individuals and in promoting non-responsiveness or poor response to HAARV treatment [reviewed in 14 & 16, 17-20]. Rhesus macaques infected with SIV are often used as a primate model for studying HIV pathogenesis and this model substantiates the role of increased gut permeability and bacterial translocation as key factors promoting viral replication [21].

At first glance, the etiology contributing to chemically induced colon cancer would seem to be completely unrelated to HIV. However, Dr. Bennink has shown that feeding beans will reduce chemically reduced colon cancer by 55 – 70%. Through the use of gene arrays, it was determined that feeding beans lead to a more effective repair of carcinogen induced mucosal damage. This in turn reduced activation of innate immunity and subsequent inflammation. Reduced inflammation was related to better colon mucosa integrity and a greater ability to prevent passage of bacteria and bacterial antigens into the mucosal lining. Reducing chronic low-grade inflammation in the colon mucosa was directly correlated with reduced colon tumor incidence. The connection between these two lines of research is the potential for beans (and possibly cowpeas) to maintain a better intestinal barrier to microbial products present in the lumen of the intestine. By reducing mucosal permeability, inflammation was reduced and so was chemically induced cancer. We are hypothesizing that when beans are consumed by HIV infected individuals, mucosal permeability and bacterial translocation are reduced and this attenuates the cycle that promotes viral replication and CD4 cell destruction.

***Rationale for proposed study***

 The results of the previous research are very exciting, but a follow-up study is required to determine if the improvement in CD4% was non-specific and simply due to increasing protein intake or if the improvement was specific to eating beans. Furthermore, ***the potential for cowpeas to improve the nutritional and immune status of HIV infected, HAARV naïve children has not been evaluated***. Whenever decision makers are presented with a new intervention or alternative approach, cost is an important consideration influencing whether the new intervention or alternative approach is adapted. Moreover, the scientific and health communities will not be satisfied with simply knowing a dietary intervention is purported to be beneficial; they will expect a plausible physiological/biochemical explanation before accepting that the treatment affect is real.

***Objectives***

 The objectives are to determine:

1. If HIV infected, HAARV naïve, 2 to 15 year old children and adolescents eating a bean-maize or a cowpea-maize supplement will maintain higher CD4 % than HIV infected, HAARV naïve, 2 to 15 year old children and adolescents eating a fish-maize supplement;
2. The relative costs of 5 dietary treatments compared to HAARV; and
3. If eating the bean-based and cowpea-based supplements improve the integrity of the mucosal barrier in the gut and leads to reduced gut permeability and release of pro-inflammatory cytokines.

***Approach and methods***

*Experimental design* To achieve objective 1, a randomized, prospective study with a 3 X 2 factorial design (three treatment groups and two locations) will be used. The three dietary supplements (treatment groups) that will be consumed are shown in Table 2. The supplements will be matched for protein and fat content and for the mineral/vitamin mix content. White maize will be the cereal base in three supplements and small fish will be the source of protein in one supplement while beans or cowpeas will be the protein source in the other two supplements. Nutritionally, fish protein is considered superior to bean and cowpea protein because fish protein is more digestible and it has a better amino acid balance; but, we expect the bean supplement to increase CD4% more than the fish supplement. There is no published data regarding the effect of feeding cowpeas to HIV+ children and thus we propose to determine if feeding cowpeas will improve the nutritional and immune status of HIV+ infected children. We anticipate that the

Table 2. Compositions of the three supplements to be used in the study.

|  |  |
| --- | --- |
|  | Diets |
|  | Fish | Bean | Cowpea |
|  | g/100 g |
| White maize | 70.89 | 21.93 | 21.93 |
| Phaseolus vulgaris | 0 | 58.71 | 0 |
| Vigna unguiculata | 0 | 0 | 58.71 |
| Fish\* | 11.31 | 0 | 0 |
| Sugar | 4 | 4 | 4 |
| NaCl | 0.3 | 0.3 | 0.3 |
| Mineral/Vitamin mix | 3 | 3 | 3 |
| Cassava | 6 | 7.56 | 7.56 |
| Vegetable oil | 4.5 | 4.5 | 4.5 |
| Total | 100 | 100 | 100 |
|  |
|  % protein | 14.2 | 14.2 | 14.2 |
|  % fat | 6.22 | 6.22 | 6.22 |
| \*Boiled, dried, ground and extruded sprats |

CD4 % in the fish group may increase by 25% compared to baseline while the CD4 % in the bean group will increase by ≥ 50% compared to baseline. Based on the variance in CD4 % in our previous experiments, a study with 125 subjects in each treatment and a 25% greater increase in CD4% will produce a statistically significant treatment effect (P ≤ 0.05, ANOVA test). We plan to have a minimum of 150 subjects complete each treatment so that if the difference between treatments is slightly less than 25% or if the variance is slightly greater than in the previous studies, the results will still be statistically valid.

The dropout rate in the previous two HIV studies was very small (<5%) and the majority of the dropouts occurred because subjects moved from the study area. But, we know from the previous rehabilitation trial that the fish supplement will not be as widely accepted as the product prepared from beans and maize. Likewise, we anticipate that the cowpea supplement will be less acceptable than the bean supplement but more acceptable than the fish supplement. Table 3 shows the number of subjects that we anticipate enrolling into each treatment group at each location to account for dropouts. In total 540 subjects will be enrolled so that at the end of the 18 mo treatment period, 150 subjects will complete each treatment.

*Study locations* The study sites will be Home Based Care (HBC) centers and Mother Child Reproductive Health Centers (MCRHC) in Turani (Morogoro region) and Rombo

 (Kilimanjaro region) since most HIV+ children in these areas do not have ready access to HAARV drugs and these children/adolescents will have the greatest need for an intervention.

Table 3. Experimental design and number of subjects per treatment and location

|  |  |  |  |
| --- | --- | --- | --- |
| Supplement group | Turani | Rombo | Total |
| Fish (control) | 100 | 100 | 200 |
| Beans | 80 | 80 | 160 |
| Cowpeas | 90 | 90 | 180 |
| Total  | 270 | 270 | 540 |

*Subjects* Children seen at HBC centers and MCRHC that are from 2 to 15 yr of age, are HIV+, and are not receiving HAARV drugs will be eligible for the study. The subjects will be age and gender matched and then randomly assigned to one of the three supplement groups. If a family has more than one HIV infected child between the ages of 2 to 15, all infected children will be allowed to enroll in the study and all family members will receive the same supplement treatment. Because of randomization by gender and age, some subjects in families with multiple children in the study may not have a “match” and their data will not be included in the final statistical comparisons. Pre-existing diseases other than HIV+ will be identified by a nurse at baseline and through health records maintained by the centers. Prior health status will not be a factor in randomization to treatment. At the end of the study, the data will be analyzed without and with statistical adjustment for pre-existing health indicators that may confound the results. If a subject is placed on HAARV drugs during the study, the subject will be removed from the study and any data collected from that subject will be destroyed.

*Dosage:* The supplements will provide approximately 50% of the “safe level of protein intake” as recommended by WHO-FAO. The amount of supplement that will be provided per day per person is: 75g for the 2-7 yr olds; 125g for the 8 – 11 olds; and 175g for the 12-15 yr olds. The supplements will be distributed to the subjects bi-weekly at the study sites. The supplements will be provided as pre-weighed packages that contain a one-day ration of the supplement so that portion size will not be an issue. Consumption of the supplements (compliance) will be monitored by requesting the subjects (≥ 12 yr) or their care providers (subjects < 12 yr) to complete a journal to record days on which the product was eaten, the approximate amount taken, and the frequency of intake per day. Compliance will be measured as the number of days that the subject ate the supplement, expressed as percentage of the total number of feeding days. If for some reason the subject did not eat the product, the reason must be recorded in the journal. Subjects will be required to present the completed journals to the research assistant before collection of the next supply of supplement. We found in a previous study that if we referred to the supplement as “medicine”, there was little sharing among the family members. Trained nurse counselors will be hired to assess health status of the subjects when the subjects report for their routine checkup/counseling session.

*Study Indicators* The physical and biochemical indicators for the study include weight, height, lean body mass, white blood cell number (CD3, CD4, and CD8), and estimated CD4%. viral load, C-reactive protein, soluble tumor necrosis factor receptor p55, interferon-γ levels, and ribosomal 16s (R16s, an indicator of bacterial products in blood and therefore an indicator of bacterial translocation from the gut). Inadequate or delayed weight gain and height velocity or weight loss (particularly loss of lean body tissue) are important indicators of nutritional risk in individuals with HIV, especially children. Weight and height are therefore important measures to assess both growth as well as nutritional status. More specifically, weight for height and weight for age z scores will be calculated to determine the extent of wasting and stunting respectively. **A** physical examination **of the subjects including body temperature, pulse rate, and blood pressure will be measured quarterly****.**

Nurses at the study sites will obtain an appropriate quantity of blood by venipuncture for determination of study indicators. CD4% and cell counts will be measured at baseline and then quarterly by trained personal at SUA. The difficulty in collecting, storing, and analyzing biological samples in community-based settings has long limited assessments that could be conducted in public health nutrition research in resource poor countries. The techniques for utilizing dried blood spots to conduct cutting edge methodologies has progressed to the point that blood can be collected and briefly stored in resource-poor countries and then shipped to locations that are capable of utilizing modern molecular biology techniques for analysis [22, 23]. Dried blood spots containing a known quantity of blood will be prepared from the blood sample collected by venipuncture. The dried blood spots will be stored in desiccators at -20 °C at SUA and periodically, the dried blood spots will be sent to MSU for analysis. Shipping of the blood spots will conform to CDC safety regulations and an import permit will be obtained to allow shipment of dried blood spots from Tanzania to MSU. R16s and viral load will be measured by PCR and of viral load, R16s, and the inflammatory markers (C-reactive protein, soluble tumor necrosis factor receptor p55, and interferon-γ) will be measured by multiplexed sandwich immunoassay [24]. R16s concentrations in blood obtained from the children and adolescents will reflect the extent of bacterial translocation from the gut. C-reactive protein, soluble tumor necrosis factor receptor p55, and interferon-γ in blood are markers of inflammation and are expected to parallel changes in R16s and to decrease as the CD4% increases.

*Ethics* Permission to conduct this research will be obtained from the IRB at MSU and from the Ethics Committee, National AIDS Control Program (NACP) of the Ministry of Health, Government of the United Republic of Tanzania. The parents or legal guardians of the subjects will be made aware of the study objectives and the expected benefits from the outcomes during the recruitment period. They will be required to provide verbal and signed consent before a subject is enrolled into the study. All subjects will be identified through HBC and MCRHC and will be assigned identity numbers. This number will be used to identify the subjects during their visits to the clinic and there will be no names in data entry, analyses or writing of the report.

*Relative intervention costs* The relative cost of the following interventions will be compared: 1) cooking a bean/maize supplement at the household level; 2)cooking a cowpea/maize supplement at the household level; 3) providing a precooked bean/maize supplement; 4)providing a precooked bean/maize supplement; 4) providing a precooked fish/maize supplement; and 6) HAARV treatment. Interventions 1-5 will include identical amounts of the mineral/vitamin supplement used in the clinical study.

 *Rodent studies* In a mechanistic study to augment the human feeding study in Tanzania, rats will be fed the same supplements that are provided to the children in the human feeding study. The experiments with rats will be conducted at MSU to compliment the assessment of gut permeability and bacterial translocation indices assessed in HIV+ children and adolescents in Tanzania. Gut permeability and bacterial translocation are important parameters in HIV progression. R16s, the cytokines and c-reactive protein will be measured as described above.

***Collaboration with Host Country Institution***

Drs. Bennink and Laswai have worked together for the past seven years and Drs. Bennink and Mosha have worked together for the past 11 years. We have mutual respect for each other’s contribution to the total research effort and our collaboration in the past has been excellent. We have published together and written other proposals together.

Drs. Mosha and Laswai, will come to MSU to receive certification in HACCP training so that they can train food processors in Tanzania in food safety.

The Food Science and Human Nutrition department at MSU conducts a 3 week class every summer at SUA. Dr. Mosha coordinates the study specifics and he is an adjunct professor in the Food Science and Human Nutrition department at MSU. Drs. Laswai and Bennink participate in the study abroad at SUA. Thus, the three PIs on this project continually work together on a variety of projects.

***Benchmarks***

Year 1 (9 mo)

1. Application for IRB approvals for the HIV study from MSU and Tanzania will be submitted.
2. Revisions to the applications for IRB approvals will be made and approval of the study will be obtained from the MSU and Tanzania IRB boards.
3. Application for IRB approval for animal use for objective 3 will be submitted.
4. IRB approval for animal use for objective 3 will be obtained
5. Application and approval to transport and import infected dried blood spots will be obtained from the CDC.
6. Nurses will be trained for blood collection and data recording specific for the study.
7. One hundred and thirty five subjects will be enrolled into the HIV study.
8. Three M.S. students will receive training in research.
9. Two Ph.D. students will receive training in research.
10. Ten undergraduates will receive field practical training in community nutrition and health.
11. One hundred and thirty five blood samples will be analyzed for multiple analytes.
12. Two rodent studies will be conducted.

Year 2

1. Four hundred and five additional subjects will be enrolled into the HIV study.
2. A paper presenting results from objective 1 will be given at a national/international meeting.
3. Three M.S. will complete their M.S. degrees and 3additional students will receive training in research.
4. Two Ph.D. students will receive training in research.
5. Ten undergraduates will receive field practical training in community nutrition and health.
6. Blood samples (1,755) will be analyzed for multiple analytes

Year 3

1. Blood samples (1890) will be analyzed for multiple analytes.
2. The HIV study will be completed; Data will be summarized.
3. Three papers presenting research results will be given at national/international meetings.
4. Three M.S. will complete their M.S. degrees.
5. Two Ph.D. students will receive training in research.
6. Ten undergraduates will receive field practical training in community nutrition and health.

# **HC INSTITUTIONAL CAPACITY BUILDING**

The capacity of SUA will be enhanced through support of undergraduate and graduate students during field practical training and graduate research, respectively. Field practical training is required for all undergraduates and approximately 10 students in each of the 3 years will do their training in cooperation with the HIV research sites. The study will support training for six MS students in Food Science/Nutrition at SUA; they will participate in research activities planned for the project and the research will a component of their dissertations. A Ph.D. student in Agricultural Marketing at SUA will receive training while conducting research to achieve objective 2. This project will enhance the research capabilities of the Food Science and Nutrition Department and the Dry Grain Pulse CRSP food-processing center at SUA. The project will also enhance the capacity of the HC’s institution to develop new food products.

***Long term training***

M.S. training in Food Sci. and Nutrition and Ph.D. training in Agricultural Marketing at SUA

Six students (to be named) will receive M.S degrees with research support from this project. One Ph.D. student in Agricultural Marketing will receive partial support for her Ph.D. studies. Tanzania desperately needs individuals with advanced degrees in Food Science and Nutrition and in Agricultural Marketing for positions in government bureaus such as the Ministry of Health, Tanzania Food and Nutrition Centre, and Home Based Care program and for non-government positions.

***Short term training***

Training for current faculty members This is a short-term training activity with long term dividends. Drs. Laswai and Mosha (co-PIs on the project) will receive training in Hazard Analysis Critical Control Points (HACCP). This training will certify them to train students and individuals in the private sector in HACCP. As the food processing industry is in its very early stages of development in Tanzania, HACCP training will be a key aspect of food safety.

# ***Strategies for achieving developmental impacts***

1. Disseminate information regarding how eating fortified bean and cowpea composite products will improve the immune integrity, prolong the time before HAARV would be needed and reduce progression from HIV to AIDS. The information will be presented via workshops and booklets to relevant multi-national institutions/agencies such as World Vision International, FAO, World Food Program, Christian Relief Services, USAID missions in the region, UNICEF, IDRC offices and other stakeholders who can disseminate our results across borders and support use of beans and cowpeas throughout the region.
2. Organize a workshop for policy and decision makers in the government to share results of our study, to influence policy and to sensitize decision makers at various levels of the government to incorporate some of the beneficial aspects in our study into the health care system and programs.
3. Collaborate with other researchers within the region and share results, methodologies and new technologies to improve and increase the impact of our results. More specifically, the PIII-TAMU-1 group will perform detailed analyses on the beans and cowpeas used in the feeding experiment before and after extrusion and after preparation of the gruel. A workshop will be held in the fall of 2011 at SUA for the PIs from the PIII-TAMU-1 group and the PIII-MSU-3 group (any other PIs in the region would be welcome also). The PIs will share ideas of ways to increase the incorporation of beans and cowpeas into the food supply of the respective countries. A workshop to provide HACCP training to small scale food processors is being planned and one PI from the PIII-TAMU-1 group will attend.
4. Present frequent updates regarding study outcomes to the USAID Mission in Tanzania and the Tanzanian Ministry of Health.

***Rationale for selecting Tanzania as the host country***

Tanzania fits the description of a “transformational development country” in the context outlined in the Strategic Framework for Africa. It has a stable government that is trying to address the many problems and constraints facing a resource poor country. Additionally it is one of the IEHA countries. The proposed HIV mitigation in orphans and vulnerable children is consistent with the overall PEPFAR goals and is particularly in alignment with the PEPFAR activities in Tanzania. Therefore, the monies used by this project will assist the USAID Mission in Tanzania achieve its goals.

# **CONTRIBUTION TO USAID OBJECTIVES AND INITIATIVES**

This project directly supports the central objectives of bilateral assistance set out in the USAID’s Policy Framework for Bilateral Foreign Aid. The project uses a two-pronged approach of trying to reduce poverty, hunger and improving health while promoting growth and sustainability. The first approach will involve direct interventions and social investments that address the immediate needs of the poor and hungry populations specifically, orphans and vulnerable children. The research directly links production agriculture and health as recommended by Hawkes and Ruel [25] and USAID.

The study on using nutrition to mitigate the complications of HIV/AIDS is in line with the USAID Tanzania mission priorities. We discussed the tentative research idea with the Mission in Dar es Salaam in July 09. They were supportive of the research direction we outlined. The project will also make use of the information and expertise that the Mission is willing to offer for the benefit of the project. By working with the USAID mission and the Ministry of Health, the results of the study will immediately be available to these two organizations. In addition, both Drs Mosha and Laswai work with the Tanzanian Food and Nutrition Centre. This working relationship will help promote dissemination of the results of the study.

The project will specifically address the issue of gender equity in the long term training component. The research project is consistent with the national Food and Nutrition Policy, which spells out that agricultural production, good nutrition/health and food security are issues of high importance.

The planned studies will not have any negative impacts on biodiversity and all food processors will be trained to handle the foods in accordance to the Tanzania Food Safety (2001) Act and principles of HACCP to ensure human and environmental safety.

The initiative to end Hunger in Africa (IEHA) recognizes that hunger in Africa is driven by squalid poverty and that economic growth based on increase in agriculture is the best approach to reduce poverty and hence end hunger and malnutrition. Using food to address and mitigate one of the worst health problems in Africa directly ties agricultural production to public health.

***Outreach***

Bean and cowpea products designed to improve the health of HIV+ people (with and without HAARV drugs) and rehabilitation of malnourished children will be displayed at Agricultural Exhibitions and Expositions in the Eastern, Northern and Southern zones. The bean products will also be exhibited at the Tanzania Universities Scientific Expo, the Dar es Salaam International Trade Fair, and the East Africa Industrial/Manufacturing Expo. Brochures and leaflets will be distributed to the public during the shows.

***Plans for leveraging funds***

The three PIs for this project have two projects funded by the Heinz Company Foundation. One is an iron efficacy study and is only somewhat related to the proposed research. The second project is directly related to this proposal since it deals with rehabilitation of malnourished children. The latter is not a research project, it is humanitarian relief.

**Budget Narrative**

First year

MSU:

1. Line a– (salary) $8,000 is for summer stipend for a Ph. D. student (Sharon Hooper) to assist in research for objectives 1 & 3.
2. Line d – $34,000 total. $10,125 for multiplex assays (135 assays @ $75/assay). $22,875 is for chemicals, reagents, animals, and animal per diems to perform the animal work related to objective 3. $1,000 is for costs related to shipping dried blood spots from Tanzania to MSU.
3. Line h – Indirect cost is calculated as 52% of total direct costs.

US (MSU) for HC

1. Line d – $7,000 total; $4,500 is for the purchase of spare parts for the extruder and other equipment necessary to produce the supplements. It is critical that the extruder be kept functional at all times. The parts will be purchased by Dr. Bennink and sent to SUA. The vitamin/mineral premix will be purchased ($2,500, line d) by Dr. Bennink and sent to SUA ($2,000 for shipping, line f).

SUA

1. Line a. – $ 24,825 total; Salaries for technicians to run the extruder and prepare the supplements, out of station per diem for 3 graduate students and 2 faculty while conducting research activities related to objective 1.
2. Line d – $43,623 total; $34,573 is for raw ingredients to produce the supplements, $4,050 for determination of cell counts (135 samples X $30/sample), and $5,000 for conducting the economic study to determine the relative treatment costs.
3. Line e – (degree) $8,400 for program fees for 3 M.S. students.
4. Line e – (non-degree) $10,000 total; $5,000 each for the 2 HC co-PIs to travel to MSU and receive HACCP training.
5. Line f – $3,500 transportation costs for delivering supplements to distribution sites.
6. Line j – The rate for indirect cost for the first $25,000 for HC subcontract is 26%.

Second year

MSU:

1. Line a – (salary) $8,000 is for summer stipend for a Ph. D. student (Sharon Hooper) to assist in research for objective 1.
2. Line b – $8,000 is requested for travel to meet with key decision makers regarding the potential for nutritional supplements to mitigate consequences of HIV.
3. Line d – $58,750 total; $57,000 for multiplex assays (760 assays @ $75/assay). $1,750 is for cost of shipping dried blood spots to US.
4. Line h – Indirect cost is calculated as 52% of total direct costs.

US (MSU) for HC

1. Line d – $4,900 total; 2,200 is requested for the purchase of spare parts for the extruder and other equipment necessary to produce the supplements. The parts will be purchased by Dr. Bennink and sent to SUA. The vitamin/mineral premix will be purchased ($2,700, line d) by Dr. Bennink and sent to SUA ($2,700 for shipping, line f).

SUA

1. Line a – $89,200 total; Salaries for technicians to run the extruder and prepare the supplements, out of station per diem for 6 graduate students and 2 faculty while conducting research activities related to objective 1.
2. Line b – $6,000 is requested for travel to meet with key decision makers regarding the potential for nutritional supplements to mitigate consequences of HIV and to attend one scientific meeting.
3. Line d – $80,250 total; $27,600 is for raw ingredients to produce the supplements and $52,650 is for determination of cell counts (1,755 samples X $30/sample).
4. Line e – (degree) $16 for,800 program fees for 6 M.S students.
5. Line f – $32,400 is for transportation costs for delivering supplements to distribution sites.

Third year

MSU:

1. Line a – (salary) $8,000 is for summer stipend for a Ph. D. student (Sharon Hooper) to assist in research for objective 1.
2. Line b – $10,000 is requested for travel to meet with key decision makers regarding the potential for nutritional supplements to mitigate consequences of HIVand attend scientific meetings.
3. Line d – $73,000 total; $54,375 for multiplex assays (725 assays @ $75/assay). $2,000 is for cost of shipping dried blood spots to US and $16,625 is for chemicals, reagents, animals, and animal per diems to perform the animal work related to objective 3.
4. Line h – Indirect cost is calculated as 52% of total direct costs.

US (MSU) for HC

1. Line d – $2,700 total; $1,000 for the purchase of spare parts for the extruder and other equipment necessary to produce the supplements. The parts will be purchased by Dr. Bennink and sent to SUA. The vitamin/mineral premix will be purchased ($1,700, line d) by Dr. Bennink and sent to SUA ($1,700 for shipping, line f).

SUA

1. Line a – $89,200 total; Salaries for technicians to run the extruder and prepare the supplements, out of station per diem for 3 graduate students and 2 faculty while conducting research activities related to objective 1.
2. Line b – $6,000 for travel to meet with key decision makers regarding the potential for nutritional supplements to mitigate consequences of HIV and to attend one scientific meeting.
3. Line d – $49,527 total for raw ingredients to produce the supplements and for determination of cell counts.
4. Line e – (degree) $16,800 for program fees for 6 M.S. students.
5. Line f – $32,400 is for transportation costs for delivering supplements to distribution sites.

**Split in direct costs between U.S. and HC institutions**: The split is 28.16% ($207,750) for the U.S. and 71.84% ($529,925) for SUA. The field work and blood cell counting will be done in Tanzania and the immunoassays will be done at MSU. The rodent work done at MSU is expected to demonstrate that gut permeability and bacterial translocation are important parameters in the pathogenesis of both HIV and colon cancer. Thus, the rodent studies will provide important information for Americans (cancer and HIVAIDs) and for Africans (HIVAIDs).

**Cost Share**: The $77,127 for the U.S. contribution for cost sharing is 20% of Dr. Bennink’s salary ($25,709 per year for 3 yr).

**Budget attributed towards capacity building.** It is estimated that 18.52% of the total cost of the project ($159,401) will be for building HC capacity. $42,000 is for degree training and $10,000 is for non-degree training (faculty development). The remaining $107,401 is a conservative estimate of improved capacity for research and outreach.