Comparative Cost-Effectiveness of Four Supplementary Foods in Treating Moderate Acute Malnutrition in Children 6-59 Months in Sierra Leone

Section 3: Environmental Enteric Dysfunction Sub-Study

A Report from the Food Aid Quality Review

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ABBREVIATIONS AND ACRONYMS

AAT   Alpha-I-Antitrypsin
EED   Environmental Enteric Dysfunction
EPS   EED Protein Score
GIS   Gut Inflammation Score
GAPDH Glyceraldehyde 3-Phosphate Dehydrogenase
GDS   Gut Defense Score
GSS   Gut Structure Score
SNF   Supplementary Nutritious Foods
LMER  Lactulose:Mannitol Excretion Ratio
MAL-ED Malnutrition and Enteric Disease
MAM   Moderate Acute Malnutrition
MPO   Myeloperoxidase
mRNA  Messenger Ribonucleic Acid
MUAC  Mid-Upper Arm Circumference
NEO   Neopterin
PCoA  Principle Coordinates Analysis
PERMANOVA Permutational Multivariate Analysis of Variance
PHU   Peripheral Health Unit
PPB   Project Peanut Butter
SD    Standard Deviation
WASH  Water, Sanitation, and Hygiene
TABLE OF CONTENTS

Section 3: Environmental Enteric Dysfunction Sub-study

Abstract .............................................................................................................................................................................. 4

1. Background .................................................................................................................................................................. 6

2. Methods......................................................................................................................................................................... 8

   2.1 Study Design...................................................................................................................................................... 8

   2.2 Approvals......................................................................................................................................................... 8

   2.3 Participants and Sample Size ....................................................................................................................... 8

   2.4 EED Sample Collection Methodology ......................................................................................................... 9

   2.6 Hypotheses ......................................................................................................................................................... 12

3. Statistical Analysis ................................................................................................................................................... 13

4. Results......................................................................................................................................................................... 15

   4.1 Study Population ............................................................................................................................................. 15

   4.2 Prevalence of EED .......................................................................................................................................... 15

5. Challenges and Limitations ......................................................................................................................................... 26

6. Summary of Findings .............................................................................................................................................. 27

7. Recommendations .................................................................................................................................................. 28

Acknowledgements..................................................................................................................................................... 29

References...................................................................................................................................................................... 30

Appendix 1: Description of Fecal Host mRNA Transcripts.................................................................................. 33
ABSTRACT

Introduction: Environmental enteric dysfunction (EED), an impairment of the structure and function of the small intestine, may be involved in processes that lead to undernutrition, including moderate acute malnutrition (MAM). However, little is known about the role of EED during MAM treatment with specialized nutritious foods (SNFs) or about factors associated with EED during MAM. Measuring EED in the field is also challenging, since current biomarkers measure specific domains of EED, and some of the newer measures have yet to be validated against standard measures or compared with each other.

Objectives: The objectives of the EED sub-study were to examine 1) whether EED modifies the effects of SNFs on recovery from MAM (reaching a mid-upper arm circumference greater than or equal to 12.5cm), 2) if there is an improvement in EED over four weeks of treatment with SNFs, 3) how EED biomarkers compare with each other and with measures of intestinal inflammation and damage, and 4) the association between water, sanitation, and hygiene (WASH) practices and EED, as well as 5) the association between the microbiota profile of MAM children and their varying levels of EED.

Methods: At eight of the peripheral health units of the Four Foods MAM Treatment Study in Pujehun district of Sierra Leone, EED was assessed at enrollment (n=484) using the lactulose:mannitol (L: M) test, fifteen fecal host messenger RNA (mRNA) transcripts and three fecal host proteins. Fecal host mRNA transcripts were also assessed after 4 weeks of supplementation. Factor analysis was used to develop three scores using the fecal host mRNA transcripts: Gut Inflammation Score (GIS), Gut Structure Score (GSS), and Gut Defense Score (GDS). A composite score was developed using the three fecal proteins: EED Protein Score (EPS).

Logistic regression models were built to test for effect modification by percent lactulose (%L) excreted, GIS, GDS, GSS, and EPS. Linear regression models were constructed to examine change in fecal host mRNA transcripts after 4 weeks of supplementation. Spearman correlation, sensitivity/ specificity analysis, and random forest classification were explored to assess whether the biomarkers measured the same domain of EED. Household WASH observations were conducted in a sub-set of study participants’ homes (n=40), and summary statistics of specific behaviors were calculated by EED status (%L<0.2 vs. ≥0.2). The microbiota profile of MAM children with varying levels of EED was compared using alpha (Faith’s phylogenetic diversity) and beta diversity (UniFrac distance) metrics.

Results: EED at enrollment using any of the biomarkers did not modify the effect of the study foods except for GDS (p=0.001). More children with high GDS (a sign of good gut health) recovered compared to children with lower GDS (p<0.001). There was no change in the fecal host mRNA transcripts between two time points or by study food. Among the biomarkers, only GIS and EPS were weakly but significantly correlated (r=0.22, p<0.05) with each other. None of the biomarkers predicted presence of EED as measured by lactulose:mannitol

1 AQP9, BIRC3, CDS3, CDX1, DECR1, DEFA6, HLA-DRA, IFI30, LYZ, MUC12, PIK3AP1, REG1A, REG3A, S100A8, SELL
2 Alpha-1-Antitrypsin, Neopterin, and Myeloperoxidase
Eight fecal host mRNA transcripts (AQP9, REG3A, IFI30, DECR1, BIRC3, SELL, PIK3AP1, DEFA6) identified EED (%L≥0.2) and severe EED (%L≥0.45) with 85% sensitivity and 80% specificity.

Differences were observed in household WASH behaviors between study participants with and without EED. These behaviors included children observed putting soil or animal feces in the mouth, animals observed drinking from household drinking water, and household drinking water storage containers having a lid. Participants with high GIS (more inflammation) had lower bacterial diversity compared to low or medium GIS (p=0.005), and different bacterial communities were present at varying levels of GIS (p=0.009).

**Conclusions:** MAM children who start the program with a healthier small intestine (based on GDS) are more likely to graduate from the treatment program within 12 weeks. None of the study foods performed better than the referent food in enabling MAM children with poor intestinal health to graduate from the program. Therefore, this sub-study does not support choosing any specific food for treatment of MAM based on EED status at enrollment. Most of the biomarkers examined in this sub-study measured different domains of EED. Specific WASH practices may be associated with EED, and could be targeted to address EED among MAM children.
I. BACKGROUND
Specialized nutritious foods (SNFs) have been used for many years to treat children with moderate acute malnutrition (MAM). However, the most cost-effective formulation of such foods is still to be determined, and the biological pathways by which these foods enable recovery from MAM remain poorly understood, which hampers product optimization and tailoring of complementary intervention activities. Environmental enteric dysfunction (EED), an impairment of the small intestine, might be involved in processes that lead to undernutrition, including MAM (Crane et al., 2015). EED is characterized by permeable intestinal walls, poor absorption of nutrients, and increased inflammation (Crane et al., 2015; Keusch et al., 2014). Little is known about whether EED modifies the response to SNFs during MAM treatment.

Biopsy of the small intestine is the most precise way to assess small intestinal health (Denno et al., 2014). However, this invasive method is not feasible in field conditions, nor is it appropriate for most studies. The most commonly used method to detect EED is the dual sugar or lactulose:mannitol test (L:M test). In the L:M test, a mixture of lactulose and mannitol is given orally to the subject, and the ratio of the two sugars in the subject’s urine describes the functional capacity of the small intestine (Denno et al., 2014). A study from Bangladesh among severely and moderately wasted children showed reduction in L:M ratio was positively associated with weight gain after nutritional intervention (Hossain et al., 2010). Despite being widely used, the dual-sugar test has drawbacks: there is a high participant burden (fasting requirements, collection of all urine excreted over four to five hours), and the test only describes two domains or characteristics of EED (permeability and absorption of the small intestine) (Figure1). Thus, field studies now use newer methods for characterizing EED, such as fecal host messenger ribonucleic acid (mRNA) transcripts and fecal host proteins (Arndt et al., 2016; George, et al., 2015; Kosek et al., 2013; Lin et al., 2019; Mahfuz et al., 2017; Ordiz et al., 2016).

Some studies have shown that several fecal host mRNA transcripts correlate with the L:M ratio or with the percentage lactulose excreted (٪) (Agapova et al., 2013; Ordiz et al., 2016; Ordiz, et al., 2018; Yu et al., 2016). These mRNA transcripts might be more informative than the L:M test, because they capture a range of domains to measure characteristics of EED. Stool samples are also comparatively easier to collect than all urine voided over several hours. However, prediction of EED (based on L:M ratio or ٪L) by fecal host mRNA transcripts might be age dependent (Ordiz et al., 2016; Ordiz, et al., 2018). In this sub-study, we assessed 15 fecal host mRNA transcripts that describe three domains of EED: inflammation, permeability, and defense (Appendix Table1).4

The most common fecal host protein markers of EED are alpha-1-antitrypsin (AAT), neopterin (NEO), and myeloperoxidase (MPO) (Arndt et al., 2016; Campbell et al., 2017; George et al., 2015; Kosek et al., 2013; Mahfuz et al., 2017; Morita et al., 2017). The marker AAT is a measure of intestinal barrier integrity, while NEO and MPO are measures of inflammation (Kosek et al., 2015; Kosek et al., 2013; Mahfuz et al., 2017; Morita et al., 2017).

3 Lactulose is a disaccharide, and mannitol is a sugar alcohol.
4 CDX1, HLA-DRA, MUC12, REG1A, S100A8, CD53, AQP9, BIRC3, DECR1, DEFA6, IFI30, LYZ, PIK3API, REG3A, SELL
2013). Although the evidence for the correlation between L:M ratio and the fecal protein markers is less clear, some studies have demonstrated a negative association between some of these markers and weight gain (Campbell et al., 2018; Kosek, 2017). A composite EED score is calculated comprising all three proteins, which we refer to as the EED Protein Score (EPS) (Kosek et al., 2013).

**Figure 1. Biomarkers of Environmental Enteric Dysfunction (Prendergast et al., 2015).**

Note: Biomarkers with * represent L:M test variables, with ** represent fecal host proteins, and with no asterisks represent fecal host mRNA transcripts.

While there is a need to identify field-appropriate biomarkers of EED, it is equally important to understand the factors that might contribute to EED. Observational studies have demonstrated positive associations between EED and different sub-optimal water, sanitation, and hygiene (WASH) practices, including water quality, sanitation, handwashing, animals corralled in a room where children sleep, and geophagy (soil consumption) (George et al., 2015; Lauer et al., 2018; Lin et al., 2013). Additionally, there is growing recognition that alterations of the host microbiota (microorganisms that colonize the gut) play a critical role in the health and nutrition status of young children. Studies have found differences in the microbiota of malnourished children compared to their healthy peers (Subramanian et al., 2014). There is also some evidence for variation in the prevalence of certain types of bacteria among children with low and high EED (Ordiz et al., 2017). These findings suggest that the microbiota profile of a malnourished child might indicate the level of EED detected.

The objectives of the EED sub-study were, therefore, to examine 1) whether EED modifies the effects of SNFs on recovery from MAM (mid-upper arm circumference [MUAC] greater than
or equal to 12.5 cm), 2) if there is an improvement in EED over four weeks of treatment with SNFs, 3) how EED biomarkers compare with each other and with measures of intestinal inflammation and damage, 4) the association between WASH practices and EED, and 5) the association between the microbiota profile of MAM children and their varying levels of EED. Results from the sub-study will provide information on the role of EED in children with MAM and the ability of supplementary foods to achieve growth in the presence of EED, which could be used to guide selection of SNFs in future MAM treatment programs.

2. METHODS

2.1 Study Design
The EED sub-study was nested within the *Four Foods MAM Treatment Study*. The latter was a prospective, cluster-randomized, controlled clinical and cost-effectiveness trial assessing four SNFs to treat children age 6-59 months with MAM, defined as MUAC greater than or equal to 11.5 cm and less than 12.5 cm without bipedal edema. While the main study was conducted in 29 peripheral health units (PHUs) randomly assigned to deliver one of four isoenergetic foods, the EED sub-study was conducted at eight of the study PHUs, two per arm, purposively selected based on logistical constraints. Biological samples were collected from July 2017 to August 2018 in collaboration with the Project Peanut Butter (PPB) study staff. The PHUs where the EED sub-study was conducted were:

- Corn Soy Blend Plus (CSB+) with oil: Gissiwolu and Nyandehun
- Super Cereal Plus with Amylase (SC+A): Gbongay and Wai
- Corn Soy Whey Blend (CSWB) with oil: Bandasuma and Gbaa
- Ready-to-Use Supplementary Food (RUSF): Jendema and Sengama

2.2 Approvals
The sub-study was approved by the Sierra Leone Ethics and Scientific Review Committee and the Tufts University Health Sciences Institutional Review Board. Written informed consent was obtained from caregivers of all sub-study participants.

2.3 Participants and Sample Size
To examine the effects of the study foods on recovery from MAM in the presence of EED, the total planned sample size was 404 (101 per arm). This achieves 80 percent power to detect an R-squared value of 0.2 in a multivariable regression model with eight predictors at a significance level (alpha) of 0.05, assuming a design effect of 1.2. To examine if there is an improvement in EED due to supplementary feeding, the planned sample size was 230 (approximately 58 per arm). This achieves 80 percent power to detect a mean of paired differences of 0.153 copies of the mRNA transcripts with an estimated standard deviation of differences of 0.846 and with a significance level (alpha) of 0.05 using a two-sided paired t-test, assuming a design effect of 1.2. The sample size for WASH observations, fecal host protein markers, and microbiota analysis was based on logistical and budgetary constraints. Participants enrolled at the EED sub-study sites over a three-month period were invited to participate in the WASH observations. For the fecal host protein marker analysis, the sample size was capped at 200 (approximately 50 per
arm). For the microbiota analysis, the sample size was capped at 100:25 for the three levels of EED (low, medium, high) independent of arm, and 25 healthy (non-MAM) controls.

2.4 EED Sample Collection Methodology
At the eight EED sub-study sites, caregivers of eligible children who consented to the *Four Foods MAM Treatment Study* were invited to participate in the sub-study. In this sub-study, EED was measured using three biomarkers: L:M test, fecal host mRNA transcripts, and fecal host proteins (Table 1).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sample type</th>
<th>Time point</th>
</tr>
</thead>
<tbody>
<tr>
<td>L:M test</td>
<td>Urine</td>
<td>Enrollment</td>
</tr>
<tr>
<td>Fecal host mRNA transcripts</td>
<td>Stool</td>
<td>Enrollment and after 4 weeks</td>
</tr>
<tr>
<td>Fecal host proteins</td>
<td>Stool</td>
<td>Enrollment</td>
</tr>
</tbody>
</table>

[1] L:M test: Because it was known from the beginning that the study would also be using mRNA transcripts and proteins, and because these methods provide more detailed information than the L:M test, the research team decided it was in the best interests of the children to expose them to this method only once, at baseline, to reduce respondent burden. The L:M test was administered as follows: At the health facility, a 20 ml dose containing 5 g of lactulose (McKesson, San Francisco, CA) and 1 g of mannitol (Sigma Aldrich, St. Louis, MO) was given to each participant orally, using a medicine cup or syringe, after an eight-hour overnight fast. Water was allowed as often as desired by the child prior to and after being dosed with the sugar solution. After dosing, a urine collection bag (Thermo Fisher Scientific, Waltham, MA) and a locally prepared, non-absorbent diaper were attached to the participant.

All urine excreted over the next four hours was collected in a cup with 10 mg of thimerosal (Sigma Aldrich, St. Louis, MO) to prevent bacterial degradation of the sugars. The urine was mixed with a pipette, transferred to plastic cryovials, and flash frozen in liquid nitrogen at the PHU. Participants and caregivers were provided with lunch after the three-hour mark, when breastfeeding was also allowed. The total urine volume was recorded using a graduated cylinder. Every month, samples were transferred to a -20°C freezer at the University of Makeni prior to being shipped on dry ice to Baylor College of Medicine (TX). The concentration of the sugars in the urine was analyzed by high-performance liquid chromatography (Shulman et al., 1998).
A concentrate of the two sugars in the samples collected was used to calculate the L:M test variables (Table 2). Although the lactulose:mannitol excretion ratio (LMER) or L:M ratio have historically been the markers of EED, new evidence suggests that both high or low LMER or L:M ratio could represent poor gut function and that %L might be a more accurate measure of intestinal health (Ordiz, Davitt, et al., 2018). For this reason, our primary L:M test indicator is %L, but we also conducted the analysis with LMER and L:M ratio. Severity terciles of %L were generated based on existing literature (Table 3) (Ordiz et al., 2016). Presence of EED was determined as values of %L≥0.2, which included both medium and high terciles (Yu et al., 2016).

**[2] Fecal markers:** Stool samples were collected at any point prior to, during, or after the four-hour wait period for the L:M test. Once a participant had a bowel movement, the diaper was removed, and all stool collected was mixed with a spatula and transferred into plastic cryovials without any fixative. The cryovials with stool were flash frozen in liquid nitrogen at the PHU. Every month, samples were transferred to a -80°C freezer at the University of Makeni prior to being shipped on dry ice to labs for analysis. Fifteen fecal host mRNA transcript were analyzed by digital droplet polymerase chain reaction at Washington University School of Medicine (St. Louis, MO). Fecal host proteins were analyzed by Enzyme Linked Immunosorbent Assay (kits from R&D Systems for AAT and MPO, and GenWay Biotech for NEO) at the USDA Human Nutrition Research Center on Aging at Tufts University (MA) (Agapova et al., 2013; Kosek et al., 2013; Stauber et al., 2016). Microbiota analysis was conducted by 16S ribosomal RNA sequencing at the Tufts Medical Center (MA) (Caporaso et al., 2011).

The fecal host mRNA transcript concentrations were continuous variables denoting copies of the mRNA transcripts per copy of GAPDH, the transcript to which all other transcripts were normalized. Using these concentrations, the fecal host mRNA transcript EED scores were developed via factor analysis. Three fecal host mRNA transcript EED scores that resulted from

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**Table 2. Calculation of L:M Test Variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>%Lactulose excreted (%L)</td>
<td>Concentration of lactulose in urine sample * 4 hour volume of urine</td>
</tr>
<tr>
<td></td>
<td>Concentration of lactulose in the dose</td>
</tr>
<tr>
<td>%Mannitol excreted (%M)</td>
<td>Concentration of mannitol in urine sample * 4 hour volume of urine</td>
</tr>
<tr>
<td></td>
<td>Concentration of mannitol in the dose</td>
</tr>
<tr>
<td>Lactulose: Mannitol Excretion Ratio (LMER)</td>
<td>%Lactulose excreted (%L) / %Mannitol excreted (%M)</td>
</tr>
<tr>
<td>L: M ratio</td>
<td>Concentration of lactulose in urine sample</td>
</tr>
<tr>
<td></td>
<td>Concentration of mannitol in urine sample</td>
</tr>
</tbody>
</table>

---

5 Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)
the factor analysis are referred to here as Gut Inflammation Score (GIS), Gut Structure Score (GSS), and Gut Defense Score (GDS), based on functions of the mRNA transcripts that grouped together\(^6\). The GIS tercile was constructed by dividing the continuous GIS variable into three equal groups to examine severity using this biomarker (Table 3). The process was followed for GSS and GDS. We speculate that high GIS and GSS, but low GDS are indicative of EED.

The three fecal host protein concentrations were continuous parameters. To construct the EPS, we first calculated the 25\(^{th}\) and 75\(^{th}\) percentile for each of the three proteins separately. Then a new variable was generated for each protein, which took the value 0 for <25\(^{th}\) percentile, 1 for 25\(^{th}\) - 75\(^{th}\), and 2 for >75\(^{th}\) percentile. These values were input in the formula $\text{EPS} = 2(\text{AAT category}) + 2(\text{MPO category}) + 1(\text{NEO category})$, and the resulting score ranged from 0 to 10 (Kosek et al., 2013). The EPS terciles were constructed by dividing the continuous EPS variable into three equal groups to examine severity using this biomarker (Table 3):

Table 3. Severity Cutoffs for EED Biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>%L</td>
<td>&lt;0.2</td>
<td>0.2-0.44</td>
<td>≥0.45</td>
</tr>
<tr>
<td>GIS</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>GSS</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>GDS</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>EPS</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
</tbody>
</table>

Note: T1, First tercile; T2, Second tercile; T3, Third tercile; GIS, Gut Inflammation Score; GSS, Gut Structure Score; GDS, Gut Defense score; EPS, EED Protein Score

\[3\] **WASH observations**: All caregivers of participants who consented to the EED sub-study from June to August 2018 were invited to participate in the WASH observations (n=70). Seven members of the Sierra Leone Red Cross Society, Pujehun branch, were trained for five days on consent and the observation procedures, including one full six-hour practical training. These staff conducted direct observation of the study child and the surrounding environment for six hours in each household, from 7:00 a.m. to 1:00 p.m. The Red Cross study staff used a paper-based semiquantitative form to record their observations. This form was divided into four sections: 1) mouthing (placing something in the mouth, but not eating), 2) eating, 3) defecation/urination, and 4) spot check (assessment for hand cleanliness of child and caregiver, and status of latrine, drinking water, compound, and animals).

2.5 **Data management**: PPB study staff recorded data for biological samples on paper clinic cards and then entered data from the cards into an electronic database (KoBoCollect) at the PHU. Tufts research assistants cross-checked electronic data entry against the clinic cards on a

\(6\) The mRNA transcripts AQP9, CD53, IFI30, PIK3AP1, S100A8, and SELL correlated highly with GIS; BIRC3, CDX1, AND MUC12 correlated highly with GSS, and DEFA6 and REG3A correlated highly with GDS. We hypothesize that high GIS and GSS but low GDS represent EED.
monthly basis. One research assistant entered data for the WASH observations into an electronic database (KoBoCollect) after completion of observations on all participants.

2.6 Hypotheses

1. The presence of EED at enrollment modifies the effect of the four study foods on recovery (MUAC≥12.5cm) from MAM within 12 weeks.
   Measurements: The outcome was the binary variable recovery from MAM within 12 weeks. The EED variables were terciles for %L, GIS, GSS, GDS, and EPS.

   a) One of the four study foods achieves recovery better than another in the presence of EED.
   Measurements: The outcome was the binary variable recovery from MAM within 12 weeks. The exposure was a categorical variable for study food. The effect modifiers were the continuous EED variables: %L, GIS, GSS, GDS, and EPS in separate models. An interaction term for study food and the EED indicator was included in each model to assess effect modification.

2. There is an improvement in EED after four weeks of treatment.
   Measurement: The continuous variable for concentration for each mRNA transcript at enrollment and after four weeks of treatment were compared.

   a) One of the four foods performs better than another at improving EED after four weeks of treatment.
   Measurements: The outcome was the continuous variable change in mRNA transcript concentration from enrollment to after four weeks. The exposure was a categorical variable for study food, and the covariate was the continuous variable for the mRNA transcript concentration at enrollment. Separate models were run for each transcript.

3. The EED biomarkers are correlated with each other and can correctly identify MAM children with %L≥0.2.
   Measurements: The continuous EED variables were %L, GIS, GSS, GDS, EPS, and 15 fecal host mRNA transcripts. The categorical EED variables were %L (binary), and two binary variables each for GIS, GSS, GDS, and EPS constructed by regrouping the respective terciles.

4. Household WASH practices and microbiota profile are associated with EED at baseline among MAM children 6-59 months of age.
   Measurements: For WASH, the outcome was the binary variable for EED, %L. The WASH variables were as follows: children putting soil/animal feces in mouth, animals drinking from household drinking water, clean compound, clean caregiver hands, dirt floor of dwelling, no access to latrine, and household drinking water storage container has lid. For microbiota analysis, the diversity metrics were alpha diversity (Faith’s phylogenetic diversity) and beta diversity (unweighted UniFrac distance). The diversity
metrics were examined for categorical variables of EED: %L tercile, GIS tercile, GSS tercile, GDS tercile, and MAM status.

Anthropometry and covariate data were extracted from the main dataset of the Four Foods MAM Treatment Study for analysis of the above-mentioned hypotheses.

3. Statistical Analysis
A total of 601 individuals were enrolled in the EED sub-study, with urine samples collected from 422 participants and stool samples collected from 475 participants at enrollment (Figure 2). Urine samples were not collected from 179 participants due to illness or no show. Stool samples were not collected from 126 participants due to illness, no show, or no bowel movement at PHU. All defaulters\(^7\) were excluded from statistical analysis (n=34 for urine and n=36 for stool). Twenty-nine stool samples were excluded from analysis because of low levels of GAPDH in the lab reports. Stool samples were also collected from 277 subjects after four weeks of treatment. Stool samples were not collected from 198 participants who provided a sample at enrollment because of no show, illness, transfer to severe acute malnutrition (SAM), death, or they only consented to sample collection at one time point.\(^8\) Four stool samples were excluded because of low levels of GAPDH as reported by the lab.

Fecal protein markers were examined in 190 stool samples collected at enrollment. Microbiota analysis was conducted on 78 samples. Among the healthy participants, 32 caregivers were recruited, and samples were collected from 21 participants. Samples were not collected from 11 healthy participants because of no bowel movement at the PHU.

Statistical analyses were conducted using Stata 15 software (StataCorp, College Station, TX). First, chi-square test was used to assess whether any of the EED biomarker terciles at baseline predicted recovery (binary) from MAM irrespective of study food. Then, unadjusted and adjusted logistic regression was used to examine modification of the effect of the study foods (categorical) on recovery from MAM (binary) by EED (continuous). To do this, we first ran the model with EED and study food as predictors. Then an interaction term for the EED variable and study food was included in the model. Separate models were run for each EED biomarker. Covariates for the adjusted model were selected based on biological relevance (age, gender, and previous SAM status of the child).

Paired t-test was used to assess differences in each mRNA transcript concentration (continuous) from enrollment to after four weeks of treatment irrespective of study food. Then, unadjusted and adjusted linear regression models were used to determine whether there was a difference in change in each mRNA transcript (continuous) from enrollment to after four weeks of treatment by study food (categorical). The adjusted model included age, gender, previous SAM status, and baseline concentration of the transcript. The continuous mRNA

\(^7\) Participants who missed three consecutive visits
\(^8\) Samples were collected from some participants only at enrollment because the sub-study had a definite end date that would not allow four-week sample collection for these participants.
transcripts were natural log transformed because they were not normally distributed. Statistical significance was set at p-value <0.05 for all analyses.

Since the sub-study used three biomarkers to examine EED, tests were run to determine whether the biomarkers described the same domains of EED (permeability, inflammation, defense). This was done by assessing the Spearman correlation among the biomarkers, and the ability of the mRNA scores and EPS to differentiate participants with and without EED based on the dichotomous variable for %L (<0.2 vs. ≥0.2). The standard of %L was used because it is the measure with the strongest physiologic basis as a direct measure of gut integrity. Additionally, the random forest classification model was estimated using the Stata “randomforest” module to assess how well the 15 fecal host mRNA transcripts could predict %L≥0.2 and %L≥0.45. This approach was also examined because we suspect that the relationship between the mRNA transcripts and %L might not be linear.

Summary statistics were calculated for the variables “putting soil/animal feces in mouth,” “animals drinking from household drinking water,” “clean compound,” “clean caregiver hands,” “dirt floor of dwelling,” “no access to latrine,” and “household drinking water storage container has lid.” Differences in these characteristics were presented for participants stratified by the dichotomous variable for %L (<0.2 vs. ≥0.2) (n=40).

Figure 2. Samples Collected for EED Sub-Study Analysis at Enrollment and After Four Weeks

![Diagram of sample collection](attachment:diagram.png)

Note: Boxes in gray are samples collected at enrollment and boxes in blue are samples collected after four weeks. Out of the 601 participants who consented, 70 were included in the WASH observations.
For the microbiota profile, statistical analysis was conducted using QIIME2 version 2018.8 (http://www.qiime2.org) on 78 MAM participants with varying levels of EED and on 21 healthy participants. The differences in bacterial diversity were examined at varying levels of EED (using different EED biomarkers), and between children with and without MAM. The diversity metrics were Faith’s phylogenetic diversity for alpha diversity and unweighted UniFrac distance for beta diversity. Alpha diversity measures the within-group diversity: the number of bacterial species within a group (Lozupone & Knight, 2008). The alpha diversity between groups can be tested using the Kruskal-Wallis test. A high alpha diversity score is generally considered a sign of better microbiota profile. Beta diversity measures the between-group diversity: bacterial species that are dissimilar between two groups (Lozupone & Knight, 2008). A high beta diversity represents more dissimilarities between groups. Beta diversity is first examined visually through principal coordinates analysis (PCoA) plots, and then permutational multivariate analysis of variance (PERMANOVA) can be used to statistically test differences in centroids between groups.

4. Results

4.1 Study Population
Background characteristics of study participants that contributed to at least one EED biomarker result were balanced across the arms except for gender and previous SAM status (Table 4). The participants’ mean age ± standard deviation (SD) was 13.96 ± 8.71 months, 58 percent were female, and 23 percent had transferred from SAM. Caregivers reported that 77 percent of participants were currently breastfeeding, 6 percent had experienced diarrhea in the past seven days, and 54 percent experienced severe household food insecurity. The anthropometric measurements were balanced across arms. Overall, 68 percent of sub-study participants graduated from the treatment program.

4.2 Prevalence of EED
The median (25th, 75th percentile) of %L at enrollment was 0.34 (0.21, 0.73) (Table 5); 77 percent of participants had EED (%L≥0.2) (Figure 3). The median and interquartile range of MPO was 42,173 (18,895, 88,332) ng/mL, and NEO was 940 (456, 1,874) nmol/L. These values were higher than non-tropical standards of ≤2,000 ng/mL for MPO and <70 nmol/L for NEO (Beckmann G, 2000; Ledjeff, Artner-Dworzak, Witasek, Fuchs, & Hausen, 2001; Saiki, 1998). Using these standards, 98 percent of sub-study participants had elevated MPO and 99 percent had elevated NEO. The median and interquartile range for AAT was 2,217 (1,756, 2,916) ng/mL, which was lower than the non-tropical standard of <0.27 mg/g. Using this standard, none of the study participants had elevated AAT.
Figure 3. Prevalence of EED (%L ≥ 0.2)

Table 4. Characteristics of EED Sub-Study Participants

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>CSWB w/oil</th>
<th>SC+A</th>
<th>CSB+w/oil</th>
<th>RUSF</th>
<th>P-value ¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>484</td>
<td>74</td>
<td>142</td>
<td>122</td>
<td>146</td>
<td></td>
</tr>
<tr>
<td>Enrollment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>13.96±8.71</td>
<td>12.67±8.65</td>
<td>14.15±9.13</td>
<td>13.43±7.66</td>
<td>14.86±9.12</td>
<td>0.299</td>
</tr>
<tr>
<td>Female</td>
<td>283(58)</td>
<td>46(62)</td>
<td>94(66)</td>
<td>56(46)</td>
<td>87(60)</td>
<td>0.008*</td>
</tr>
<tr>
<td>Transferred from SAM</td>
<td>112(23)</td>
<td>16(22)</td>
<td>34(24)</td>
<td>39(32)</td>
<td>23(16)</td>
<td>0.019*</td>
</tr>
<tr>
<td>Currently breastfed</td>
<td>368(77)</td>
<td>61(82)</td>
<td>106(76)</td>
<td>96(79)</td>
<td>105(72)</td>
<td>0.335</td>
</tr>
<tr>
<td>Diarrhea in past 7 days</td>
<td>31(6)</td>
<td>5(7)</td>
<td>10(7)</td>
<td>7(6)</td>
<td>9(6)</td>
<td>0.978</td>
</tr>
<tr>
<td>HFIAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.070</td>
</tr>
<tr>
<td>Food secure</td>
<td>160(33)</td>
<td>29(39)</td>
<td>38(27)</td>
<td>43(35)</td>
<td>50(34)</td>
<td></td>
</tr>
<tr>
<td>Moderately food insecure</td>
<td>64(13)</td>
<td>15(20)</td>
<td>17(12)</td>
<td>11(9)</td>
<td>21(14)</td>
<td></td>
</tr>
<tr>
<td>Severely food insecure</td>
<td>260(54)</td>
<td>30(41)</td>
<td>87(61)</td>
<td>68(56)</td>
<td>75(51)</td>
<td></td>
</tr>
<tr>
<td>Anthropometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUAC</td>
<td>11.97±0.27</td>
<td>12±0.27</td>
<td>11.95±0.26</td>
<td>11.94±0.27</td>
<td>11.99±0.27</td>
<td>0.311</td>
</tr>
<tr>
<td>LAZ</td>
<td>-2.78±1.23</td>
<td>-2.65±1.22</td>
<td>-2.75±1.24</td>
<td>-2.88±1.25</td>
<td>-2.77±1.2</td>
<td>0.646</td>
</tr>
<tr>
<td>WLZ</td>
<td>-1.82±0.76</td>
<td>-1.66±0.8</td>
<td>-1.77±0.73</td>
<td>-1.84±0.79</td>
<td>-1.93±0.72</td>
<td>0.064</td>
</tr>
<tr>
<td>WAZ</td>
<td>-2.93±0.84</td>
<td>-2.86±0.87</td>
<td>-2.85±0.81</td>
<td>-3±0.88</td>
<td>-2.99±0.8</td>
<td>0.344</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graduated</td>
<td>327(68)</td>
<td>48(65)</td>
<td>93(65)</td>
<td>78(64)</td>
<td>108(74)</td>
<td>0.262</td>
</tr>
</tbody>
</table>

*Cells represent Mean±SD or n(%)  
* p<0.05  
²ANOVA for continuous variables and chi-square test for categorical variables  
Abbreviations: SAM, severe acute malnutrition; HFIAS, household food insecurity access scale; MUAC, mid-upper arm circumference; LAZ, length-for-age z score; WLZ, weight-for-length z score; WAZ, weight-for-age z score
Hypothesis: The presence of EED at enrollment modifies the effect of the four study foods on recovery (MUAC≥12.5cm) from MAM within 12 weeks. Recovery from MAM did not vary by level of EED at enrollment as indicated by %L tercile, EPS tercile, GIS tercile, or GSS tercile. However, there was a statistically significant difference by level of the GDS tercile (Figure 4). A significantly larger proportion of participants from the high group of the GDS tercile at enrollment graduated from the program compared to participants from the medium or low group using the chi-square test (P<0.001).

We also examined the above-mentioned EED biomarkers as modifiers of the effect of the study foods on recovery from MAM within 12 weeks. Since the model with the terciles was not stable when performing diagnostic tests, we used the continuous forms of the variables. Of the five biomarkers, only GDS was a significant modifier in both unadjusted model (P=0.002) and model adjusted for age, gender, and previous SAM status (P=0.001) (Figure 5). With the exception of RUSF, we found no statistically significant differences between the study food and the comparator CSB+ with oil when controlling for multiple comparisons using the Bonferroni method; RUSF performed better than CSB+ with oil at high levels of GDS (P=0.035), which we suspect is a sign of good gut health. We did not find effect modification by LMER or L:M ratio.
### Table 5. EED Biomarkers of Sub-Study Participants at Enrollment

<table>
<thead>
<tr>
<th></th>
<th>n¶</th>
<th>Median (25th, 75th percentile)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LM test</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMER</td>
<td>388</td>
<td>0.10 (0.06, 0.15)</td>
</tr>
<tr>
<td>LM Ratio</td>
<td>388</td>
<td>0.48 (0.32, 0.73)</td>
</tr>
<tr>
<td>%Lactulose</td>
<td>388</td>
<td>0.34 (0.21, 0.62)</td>
</tr>
<tr>
<td>%Mannitol</td>
<td>388</td>
<td>3.86 (2.42, 5.65)</td>
</tr>
<tr>
<td><strong>mRNA§</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AQP9</td>
<td>398</td>
<td>0.13 (0.06, 0.31)</td>
</tr>
<tr>
<td>BIRC3</td>
<td>399</td>
<td>0.21 (0.11, 0.37)</td>
</tr>
<tr>
<td>CD53</td>
<td>405</td>
<td>0.20 (0.07, 0.55)</td>
</tr>
<tr>
<td>CDX1</td>
<td>406</td>
<td>0.05 (0.03, 0.08)</td>
</tr>
<tr>
<td>DECR1</td>
<td>400</td>
<td>0.06 (0.03, 0.09)</td>
</tr>
<tr>
<td>DEFA6</td>
<td>400</td>
<td>0.08 (0.04, 0.21)</td>
</tr>
<tr>
<td>HLA-DRA</td>
<td>404</td>
<td>0.14 (0.07, 0.25)</td>
</tr>
<tr>
<td>IFI30</td>
<td>400</td>
<td>0.31 (0.14, 0.64)</td>
</tr>
<tr>
<td>LYZ</td>
<td>410</td>
<td>0.11 (0.05, 0.21)</td>
</tr>
<tr>
<td>MUC12</td>
<td>406</td>
<td>0.43 (0.23, 0.84)</td>
</tr>
<tr>
<td>PIK3AP1</td>
<td>400</td>
<td>0.16 (0.06, 0.38)</td>
</tr>
<tr>
<td>REG1A</td>
<td>406</td>
<td>0.15 (0.06, 0.36)</td>
</tr>
<tr>
<td>REG3A</td>
<td>400</td>
<td>0.07 (0.03, 0.15)</td>
</tr>
<tr>
<td>S100A8</td>
<td>403</td>
<td>1.19 (0.53, 2.83)</td>
</tr>
<tr>
<td>SELL</td>
<td>400</td>
<td>0.06 (0.02, 0.16)</td>
</tr>
<tr>
<td>TNF</td>
<td>124</td>
<td>0.06 (0.01, 0.02)</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAT (ng/mL)</td>
<td>190</td>
<td>2,217.49 (1,756.40, 2,915.82)</td>
</tr>
<tr>
<td>MPO (ng/mL)</td>
<td>190</td>
<td>42,172.51 (18,895.35, 88,332.42)</td>
</tr>
<tr>
<td>NEO (nmol/L)</td>
<td>190</td>
<td>939.71 (456.18, 1,873.8)</td>
</tr>
<tr>
<td>EED Score</td>
<td>190</td>
<td>5 (4, 6)</td>
</tr>
</tbody>
</table>

§Copies per copy of GAPDH
¶One outlier for LYZ excluded
Figure 4. Recovery from MAM by Level of EED at Enrollment

Note: P-values are from Chi-square test: A) %L, B) EED Protein Score, C) Gut Inflammation Score, D) Gut Structure Score, E) Gut Defense Score.
**Hypothesis:** There is an improvement in EED after four weeks of treatment.

The concentration of 15 fecal host mRNA transcripts changed slightly between enrollment and after four weeks on the study foods (Figure 6). However, these changes were not large enough to detect a statistically significant difference. We also examined whether this difference varied by study food and did not find a statistically significant difference for any of the mRNA transcripts.
**Hypothesis:** The EED biomarkers are correlated with each other and can correctly identify MAM children with %L≥0.2.

The correlation among %L, GIS, GSS, GDS, and EPS was weak (Table 6). However, there was a significant correlation between GIS and EPS ($r=0.22$, $p<0.05$) and a significant but negative correlation between GIS and GSS ($r=-0.19$, $p<0.05$). The significant positive correlation between GIS and EPS suggests that both are measuring the same characteristics of EED. Since EPS is a marker of inflammation, we can infer that GIS also detects inflammation during EED.

Given that the L:M test is the most widely used EED biomarker, assessing the ability of newer biomarkers to differentiate participants with and without EED based on the L:M test is of interest. We find that GIS, GSS, GDS, and EPS poorly differentiated participants with and without EED based on %L as demonstrated by the fact that none of the biomarkers could predict the presence of EED (%L≥0.2) with high sensitivity and specificity ($>0.8$) (Table 7). These results suggest that the EED biomarkers used in this sub-study are measuring different aspects or stages of EED, as was suspected.
Using the random forest classification method, we found that eight fecal host mRNA transcripts\(^9\) were important predictors of \(\%L \geq 0.2\) (Figure 7). Furthermore, a model with these fecal host mRNA transcripts was able to identify \(\%L \geq 0.2\) with 100 percent sensitivity and 80 percent specificity. These same fecal host mRNA transcripts were also able to identify \(\%L \geq 0.45\) (severe EED) with 85 percent sensitivity and 80 percent specificity. Unlike the factor analysis-based scores that separately identify a particular domain of EED, these eight transcripts together likely capture many domains of EED.\(^{10}\)

Table 6. Spearmen Correlation Among Untransformed EED Biomarkers

<table>
<thead>
<tr>
<th></th>
<th>%L</th>
<th>GIS</th>
<th>GSS</th>
<th>GDS</th>
<th>EPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>%L</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIS</td>
<td>-0.06</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSS</td>
<td>-0.00</td>
<td>-0.19*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GDS</td>
<td>-0.05</td>
<td>0.02</td>
<td>0.02</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>EPS</td>
<td>0.08</td>
<td>0.22*</td>
<td>-0.13</td>
<td>-0.14</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^9\) AQP9, REG3A, IFI30, DECR1, BIRC3, SELL, PIK3AP1, and DEFA6

\(^{10}\) AQP9, IFI30, DECR1, SELL, and PIK3AP1 – inflammation; BIRC3 – structure; and REG3A and DEFA6 – defensins (anti-microbials)
Hypothesis: Household WASH practices related to MAM children are associated with EED.

A few differences in WASH practices among study participants with ($%L\geq0.2$, $n=32$) and without ($%L<0.2$, $n=8$) EED were observed (Figure 8). Thirty eight percent of participants without EED were observed putting soil or animal feces in their mouth compared to 63 percent of children with EED. Animals were observed drinking from household drinking water in 13 percent of homes without EED compared to 25 percent of homes with EED. Drinking water storage containers had a lid in homes of all participants without EED compared to 72 percent of homes of participants with EED.
**Hypothesis:** The microbiota profile of MAM children is associated with EED. There was a statistically significant difference in alpha diversity using Faith’s phylogenetic diversity between children of different GIS terciles (p=0.005), borderline for GDS terciles (p=0.052), but not for %L terciles, GDS terciles, or EPS terciles (Figure 9). There was also a statistically significant difference in beta diversity by unweighted UniFrac distance between children of different GIS terciles (p=0.009) (Figure 10) and GDS terciles (p=0.048), but not for %L terciles, GSS terciles, or EPS terciles. Statistical significance in alpha diversity and beta diversity between MAM children and their healthy peers was borderline (p=0.059 and p=0.051 respectively).
Figure 9. Alpha Diversity as Measured by Faith’s Phylogenetic Diversity for GIS

Note: P-value for Kruskal-Wallis test

Figure 10. Principal Coordinates Analysis Plots for GIS Terciles

Note: P-value for PERMANOVA testing if centroids of the terciles are the same
5. Challenges and Limitations

**Challenges:** The team faced a number of challenges while implementing the EED sub-study in Pujehun district, which is a resource-constrained setting. Maintaining temperature control was critical for the stool samples. Due to lack of electricity at the study site, stool samples were stored in liquid nitrogen until they could be transferred to a -80°C freezer for long-term storage. When the only liquid nitrogen machine in Sierra Leone broke down, dry ice had to be imported. Importing dry ice not only was expensive, but also made the study reliant on the often unpredictable international flight schedule. However, none of the samples was compromised due to flight delays.

Given the mobile nature of the Four Foods MAM Treatment Study and subsequently the EED sub-study, the field team spent only one day at each PHU. Therefore, the sub-study was only able to attempt sample collection one time for each participant. Collecting both stool and urine on the same day was also challenging, because attaching the diaper and urine bag at the same time obstructed the urine bag on a number of occasions.

**Limitations:** Conducting the L:M test on the same day as stool collection might have affected the AAT protein concentrations. This is because lactulose is a laxative that could have resulted in participants passing watery stool. Stool samples with high water content are expected to show low AAT values (Crossley & Elliott, 1977). Despite the rather low AAT concentrations observed in this sub-study, the values were higher than the mean AAT concentration of 597 ng/mL reported by the Malnutrition and Enteric Disease (MAL-ED) Peru birth cohort (n=303) (Kosek et al., 2013).

Although it was not possible to extend the duration of the second collection of stool samples, a longer exposure period beyond four weeks might have allowed us to see the impact of the study foods on intestinal health. It was not possible to extend the second sample collection to, for example, recovery because this outcome varied from 2 to 12 weeks among the population and we would not be able to control for endogenous differences between children who recovered faster and those who recovered more slowly. Furthermore, conducting all three biomarker assessments at both time points could have allowed us to examine a more complete picture of EED after the intervention. However, this was not possible due to logistical and budgetary constraints.
6. Summary of Findings

The prevalence of EED assessed using %L, MPO, and NEO was high among children with MAM enrolled in a supplemental feeding program in the Pujehun district of Sierra Leone. Three EED scores based on 15 fecal host mRNA transcripts were used: GIS, GSS, and GDS. The EPS, GIS, GSS, and GDS did not correlate with %L. That said, a weak but significant correlation was found between EPS and GIS, suggesting that they are both markers of inflammation, which is characteristic of EED. Previous studies have found mixed results for the association between proteins comprising EPS and L:M ratio or %L (Harper, Mutasa, Prendergast, Humphrey, & Manges, 2018). However, eight fecal host mRNA transcripts were able to identify the presence of EED and severe EED using %L with high sensitivity and specificity. This finding is in contrast to what we found with the factor analysis-based scores (GIS, GSS, and GDS), probably because these scores measure specific domains of EED separately while the eight mRNA transcripts together capture many domains of EED. These eight transcripts were not the same as those reported by a previous study that successfully identified children with severe EED (L:M ratio ≥0.45), also using the random forest classification method (Ordiz et al., 2016).

The level of EED at enrollment that was assessed using any of the four biomarkers of EED did not influence the effectiveness of the study foods except for GDS. More children with high GDS (a sign of good gut health) at enrollment recovered compared to children with lower GDS. This finding suggests that children who start the program with a healthier small intestine are more likely to recover than children with a less healthy small intestine. We might not have found a difference in the effects of the foods at different levels of EED because the foods, though variable in composition, performed comparably in terms of their effect on recovery from MAM. The fact that more children with a healthier small intestine recover was also supported by EPS, where more participants with low scores (sign of less inflammation) graduated from the treatment program compared to participants with a higher score. The difference was not statistically significant (p=0.125), possibly due to the sample size (n=189). Similar to our findings, a study from Bangladesh also reported that effectiveness of a zinc supplementation trial did not vary by presence of EED based on L:M ratio at enrollment (Long et al., 2019).

We did not find an effect of the study foods on EED using 15 fecal host mRNA transcripts. This might be because the study foods do not improve EED or because the exposure period of four weeks was not sufficient to result in significant change. However, we did see trends in certain mRNA transcripts that we would expect to change after an intervention. For example, the concentration of mRNA transcript S100A8 was, on average, lower after four weeks on the study foods, signaling reduction in inflammation. Studies that have shown improvements in %L after a nutrition intervention have had a longer exposure period, such as three months or at least eight weeks (Agapova et al., 2018; Cheng et al., 2019).

Household WASH practices were generally poor among participants and caregivers of the EED sub-study. Differences in practices between participants with and without EED (based on %L)

11 S100A8 encodes the inflammatory protein calprotectin [41].
suggest that interventions targeted at improving these behaviors might be necessary to improve EED in contexts of high risk of MAM. A study from Uganda showed that unsafe drinking water was associated with EED and poor child growth (Lauer et al., 2018). Differences in the microbiota profile of MAM children with varying levels of EED as assessed using different EED biomarkers was statistically significant for GIS terciles when examining both alpha and beta diversity. A study conducted among Malawian children without acute malnutrition found significant differences in beta diversity but not alpha diversity when assessing different degrees of EED based on the L:M ratio (Ordiz et al., 2017).

7. Recommendations

Based on results from the EED sub-study, a few recommendations can be made for future food assistance programs:

- MAM children who start the program with a healthier small intestine (based on GDS) are more likely to graduate from the treatment program within 12 weeks. Strategies to improve intestinal health through efforts in addition to SNFs (such as WASH interventions) should therefore be explored. None of the study foods performed better than CSB+ with oil in enabling MAM children with poor intestinal health to graduate from the program. This sub-study does not support choosing different foods for treatment of MAM children based on EED status at enrollment.

- WASH practices among MAM children and their caregivers were poor. Some WASH practices may be associated with EED, a marker of poor intestinal health. To address EED, it might improve effectiveness to incorporate WASH actions in MAM treatment programs and counsel caregivers on the benefits of good WASH practices.
Acknowledgements

This study would not be possible without the support of the USAID Bureau for Humanitarian Assistance (BHA) and the legacy Office of Food for Peace (FFP) and their ongoing commitment to improving Title II programming in order to address food insecurity in vulnerable populations. The time and support offered to the Food Aid Quality Review by Title II awardees, both headquarters and field staff, as well as Food for Peace Officers abroad and in Washington, D.C. and the Policy and Technical Division of FFP headquarters were also invaluable to informing this study.
References


Appendix I: Description of Fecal Host mRNA Transcripts

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Description</th>
<th>EED domains</th>
<th>Functions (Source: <a href="https://www.genecards.org">https://www.genecards.org</a>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP9</td>
<td>Aquaporin 9</td>
<td>Absorption, Immune</td>
<td>Membrane channels that allow passage of noncharged particles; might be involved in immune response</td>
</tr>
<tr>
<td></td>
<td></td>
<td>response</td>
<td></td>
</tr>
<tr>
<td>BIRC3</td>
<td>Baculoviral IAP Repeat Containing 3</td>
<td>Immune response</td>
<td>Prevents apoptosis (cell death) by binding to tumor necrosis factor receptor-associated factors</td>
</tr>
<tr>
<td>CD53</td>
<td>CD53 Molecule</td>
<td>Immune response, Cell</td>
<td>Cell surface protein involved in cell growth and development; binds integrins; lack of this protein results in immunodeficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>adhesion, Cell</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>development</td>
<td></td>
</tr>
<tr>
<td>CDX1</td>
<td>Caudal Type Homeobox 1</td>
<td>Cell differentiation</td>
<td>Regulates differentiation of intestinal cells</td>
</tr>
<tr>
<td>DECR1</td>
<td>2,4-Dienoyl-CoA Reductase 1</td>
<td>Fatty acid metabolism</td>
<td>Enzyme involved in fatty acid metabolism</td>
</tr>
<tr>
<td>DEFA6</td>
<td>Defensin, Alpha 6, Paneth Cell-Specific</td>
<td>Immune response</td>
<td>Antimicrobial and cytotoxic peptides present in neutrophils (innate immune system) and mucosal surfaces; might protect cells against HIV-1</td>
</tr>
<tr>
<td>HLA-DRA</td>
<td>Major Histocompatibility Complex, Class II, DR</td>
<td>Immune response</td>
<td>Expressed on the surface of antigen-presenting cells, binds peptides to present to T cells (adaptive immune system)</td>
</tr>
<tr>
<td></td>
<td>Alpha</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFI30</td>
<td>Lysosomal Thiol Reductase</td>
<td>Immune response</td>
<td>Enzyme that has a role in antigen processing (adaptive immune response)</td>
</tr>
<tr>
<td>LYZ</td>
<td>Lysozyme</td>
<td>Immune response</td>
<td>Antimicrobial agent associated with monocyte-macrophage system (innate immune system)</td>
</tr>
<tr>
<td>Gene symbol</td>
<td>Description</td>
<td>EED domains</td>
<td>Functions (Source: <a href="https://www.genecards.org">https://www.genecards.org</a>)</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>MUC12</td>
<td>Mucin 12</td>
<td>Permeability Cell differentiation</td>
<td>Formation of protective mucous barrier and differentiation of epithelial cells</td>
</tr>
<tr>
<td>PIK3AP1</td>
<td>Phosphoinositide-3-Kinase Adaptor Protein 1</td>
<td>Immune response</td>
<td>Adaptor involved in B cell development (adaptive immune system), links Toll-like receptors to PIK3 to prevent excessive production of cytokines.</td>
</tr>
<tr>
<td>REG1A</td>
<td>Regenerating Islet-Derived 1 Alpha</td>
<td>Repair/Injury</td>
<td>Regeneration of islet cells of the pancreas</td>
</tr>
<tr>
<td>REG3A</td>
<td>Regenerating Islet-Derived 3 Alpha</td>
<td>Immune response</td>
<td>Mediates bacterial killing; pancreatic secretory protein possibly involved in cell proliferation and/or differentiation</td>
</tr>
<tr>
<td>S100A8</td>
<td>S100 Calcium Binding Protein A8 (Calprotectin)</td>
<td>Immune response</td>
<td>Encodes the protein calprotectin, which is a marker of neutrophil activity (innate immune system)</td>
</tr>
<tr>
<td>SELL</td>
<td>Selectin L</td>
<td>Immune response Cell adhesion</td>
<td>Cell surface protein involved in binding leucocytes (innate immune response)</td>
</tr>
</tbody>
</table>