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HIGHER FIBER COMPLEMENTARY FOOD ALTERS FECAL MICROBIOTA COMPOSITION AND NORMALIZES STOOL FORM IN MALAWIAN CHILDREN: A RANDOMIZED TRIAL

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ABSTRACT

Dietary fiber favorably modulates gut microbiota and may be protective against diarrhea in sub-Saharan Africa where rates in infants and young children are high. Soybean hull is high in fiber and accessible in rural Africa; however, its use in complementary feeding has not been evaluated. The objective of this study was to determine the acceptability and feasibility of a soybean, soy hull fiber, and maize (SFM) blend food; the primary outcome was compliance to the feeding protocol. Secondary outcomes were stool form and frequency, fecal microbiota composition, growth and dietary intake. In a parallel, single-blind study, children 6-36 months of age from the Lilongwe district of Malawi were randomized to receive daily SFM (n=69) or maize only (n=10) porridge (*phala*) for 6 months. Anthropometrics were measured monthly, and compliance, stool frequency, and stool form, weekly. At baseline, 3-month, and 6-month (study end) time points, dietary intake (24-h recall) was assessed, and fecal samples were collected. Fecal DNA was analyzed by Real-Time polymerase chain reaction (PCR) for microbes of interest and 16S rRNA gene amplicon sequencing. Mothers accessed the acceptability and feasibility of the study foods at study end. Mothers reported excellent compliance to feeding the SFM porridge, rated it more acceptable than maize, and noted improved appetite, weight, and stool consistency of their children. Stool frequency at baseline (2±1 stools/d) was unchanged with intervention; however, there were significantly fewer diarrhea-type stools reported during study months 4-6 *vs*. 1-3 for the SFM group, whereas no improvement was seen for the maize group. At study end, the fecal abundance of *Akkermansia muciniphila* was enriched in children receiving the SFM, compared to maize ($p<0.05$), and a trend for increased *Faecalibacterium prausnitzii* ($p=0.07$) was seen. A comparison of fecal microbiota composition using linear discriminant analysis effect size (LEfSe) showed notable differences in numerous taxa in the SFM group compared to baseline, whereas the maize comparator exhibited fewer changes. Fiber intake was higher for the SFM group, compared to maize at 6 months (13.7±3.8 vs. 8.4±4.5 g/day, $p<0.01$). Weight-for-height and BMI-for-age Z-scores were significantly higher for the SFM group. In young Malawian children, feeding a blend of soybean, soy hulls and maize reduced diarrhea-type stools and increased the abundance of *Akkermansia muciniphila,* a bacterial species involved in maintaining intestinal health, and thus may provide a feasible means of improving wellness in children in resource-poor settings through the modulation of microbiota composition.

Key words: complementary food, soybean, microbiome, fiber, diarrhea, stool, undernutrition, diet

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INTRODUCTION

The prevalence of undernutrition and diarrhea in young children in Malawi is high. Data from the Malawi Demographic and Health Survey indicate that 22% of children under 5 years of age experienced a diarrheal episode in the two weeks prior to the survey and 37% suffered from chronic malnutrition [1]. Dietary interventions, which are locally available, may provide lasting solutions for the prevention and treatment of undernutrition and diarrhea, and in the long-run, may improve the overall health of young children in resource-poor settings. Attention to date has focused on the provision of adequate protein and energy for growth as well as marginal micronutrients such as vitamin A [2]. In contrast, dietary fiber has been considered to be less important or potentially detrimental to nutritional status [3]. The diets of African children are generally thought to be high in fiber [4]. However, complementary food introduced to infants in rural Malawi and elsewhere in sub-Saharan Africa is often a maize-based thin (*phala*) or stiff (*nsima*) porridge, eaten very sparingly with vegetables and legumes, providing little dietary fiber. Soybean is encouraged in the diets of young children, particularly as a source of protein for child growth. However, in Malawi, processing of soybean at the household level involves the removal of the hull [5]. Thus, much of the dietary fiber, approximately 7 g per 100 g of whole soybean [6], is discarded. Although the origins of this practice may originate from concerns regarding mineral bioavailability, soybean hulls are low in phytate [7], and thus provide a potentially safe and readily available source of dietary fiber.

Although dietary fiber has long been recognized as promoting regular bowel habits, little research has focused on its role in the prevention and management of diarrhea in children. Specifically, two small trials have shown decreased duration of diarrhea with soy fiber [8, 9]. Dietary fiber is the primary substrate of gut microbiota, promoting *saccharolytic* fermentation and the production of short-chain fatty acids, such as butyrate, essential for colonic health [10]. Diets providing diverse sources of fiber may be critical for the development of favorable microbiota composition profiles [11]. In contrast, providing lower fiber, complementary foods to young children, combined with minimal diet diversity, may not promote an optimal microbial environment, particularly upon weaning and the loss of human milk oligosaccharides and their inherent benefits [12]. This, in combination with high exposure to environmental pathogens, commonplace in low-resource, rural settings [13], may contribute to intestinal dysbiosis, diarrhea, and compromised intestinal integrity, which, in turn, may negatively impact energy and nutrient absorption and utilization. Provision of complementary foods with additional dietary fiber may promote a stable, diverse microbiota in young children. The objective of this study was to assess the feasibility and acceptability of a soybean, soy hull fiber, and maize (SFM) blend porridge in children aged 6 to 36 months living in Lilongwe district of rural Malawi. The primary outcome was compliance to the SFM feeding protocol compared to maize only. Secondary outcomes were stool frequency, stool form, fecal microbiota profile, growth and dietary intake. It was hypothesized that the SFM porridge would be feasible and acceptable, and as a complex source of dietary fiber, would modulate microbiota, thereby improving stool form and frequency.

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MATERIALS AND METHODS

Study Design

A 6-month, parallel, single-blinded, intervention study was carried out in the Lilongwe district of Malawi from June to December 2018. Children aged 6 to 36 months and their mothers (or caregivers) (n = 79) were recruited. Baseline data on all outcomes were collected over a 2-week period and then participants were randomized to receive an SFM or maize flour, intended for porridge preparation, for a 6-month period. During weekly visits, mothers reported feeding compliance and their child's stool frequency, stool form, appetite, physical activity, and medication intake. The children's weight, height, and mid-upper arm circumference (MUAC) were assessed monthly. At baseline, 3-month and 6-month time points, mothers completed an interactive 24-h dietary recall of their child's diet and collected one stool sample. Feasibility and acceptability were assessed by compliance to feeding the SFM *vs*. control, specifically asking mothers if the weekly allotment of study food was successfully fed to her child and confirmed by empty flour packages. Additionally, at study end, a questionnaire was administered to mothers regarding the acceptability of the complementary foods and a focus group of mothers was conducted, using a questionnaire guide to assess their perceptions related to the overall acceptability and feasibility of feeding the SFM porridge to their child. The College of Medicine Research Ethics Committee (COMREC) of the University of Malawi and the Institutional Review Board at the University of Florida approved the study (IRB 201700644). All mother/caregiver participants provided verbal and written informed consent for themselves and their children. A thumbprint was used *in lieu* of a signature for those participants unable to sign their name. The trial is registered on clinicaltrials.gov (NCT03385590).

Participant Recruitment and Randomization

Mother-child pairs in a rural hospital catchment area in Lilongwe district of Malawi were recruited for the study through flyers and word of mouth in June 2018. The screening was conducted to exclude severely malnourished children with a MUAC less than 11.5 cm, weight-for-height of less than -3 Z-score, or the presence of edema; these children were referred to the hospital for further screening, treatment, or admission. Inclusion criteria included children, 6 to 36 months of age, with a mother/caregiver willing to undertake study activities. Additional exclusion criteria included known allergies to soy or nuts, currently taking medications for diarrhea or antibiotics, > 3 diarrheal episodes in the week before the consenting appointment, or participation in another clinical trial. The study coordinator enrolled participants and assigned interventions, randomized by a sealed envelope method, to SFM or maize (standard care in the region) at a ratio of 7:1. Assignment to the maize-only intervention was minimized due to risk, given that the frequent consumption of maize-based foods is associated with malnutrition [14].

Study Foods

Maize and soybeans were purchased locally and cleaned. Soybeans were boiled in water for 45 minutes, and sun-dried on mats. For the SFM flour, maize was mixed with the cooked, dried whole soybeans at the ratio of 4:1, following the recommendation in the Maternal, Infant and Young Child Nutrition in Malawi: Community Nutrition

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Workers Recipe Book [15]. The maize and soybean-maize blends were milled. Additional soy hull fiber (FI-1 Solvaira Specialties Inc., North Tonawanda, New York) (3 g/serving) was added to the soybean-maize flour. The study flours were provided weekly to mothers in 560 g packages, sufficient for 2, 40 g servings per day for the participating child. Mothers/caregivers were blinded to which flour, SFM or maize-only, they received; the flours were similar in color. The nutrient composition of flour formulation per 40 g serving was 20.4 g carbohydrate, 5.0 g of protein, 2.8 g of fat, 6.3 g of fiber for the SFM (estimated to provide about 94-100% of protein and 50% of energy recommendations for complementary feeding), and 25.4 g carbohydrate, 3.2 g protein, 1.8 g fat, and 3.8 g of fiber for the maize, providing 139 kcal and 138 kcal, respectively. Mothers were given measuring cups and instructed to prepare porridges, using one-part flour (SFM or maize-only) to five-parts water, and simmer for 15-20 minutes prior to feeding their child. No restrictions were placed on the addition of vegetables, legumes, or animal foods to the study porridges or otherwise to the children's diets.

Outcome Assessments

Dietary intake was assessed at baseline, 3-months and 6-months by interactive 24-hr recall. The Kenyan and Zambian Food Composition tables obtained from the Food and Agriculture Organization of the United Nations (FAO) International Network of Food Data Systems were used to analyze the nutrient composition. The United States Department of Agriculture Nutrient Database was used for a few foods that were not in the FAO database. Children's weight was measured using a UNICEF Uniscale; their height was measured by a portable height/length measuring board. MUAC was measured to the nearest 0.1 cm using a non-flexible, plastic insert tape on the child's left upper arm. All anthropometric data were measured in duplicate. A weekly questionnaire was administered to mothers to assess stool frequency, stool form, and appetite of the participating children; medication use and change in physical activity were also queried. The 5-point Modified Bristol Stool Scale for children (1 and 2 = slow transit, 3 = normal, 4 and 5 = fast transit) was used to assess stool form as a proxy of transit time [16]. Appetite was assessed using a rating from 0 = very poor to 6 = very good.

Acceptability of Study Foods

A questionnaire was administered to mothers at the end of the study to assess the acceptability of the porridges prepared from the flours provided. The criteria included appearance, flavor, texture/consistency, aroma, and overall acceptability, and were rated using a 5-point hedonic rating scale from 1 (disliked strongly) to 5 (liked strongly). A focus group discussion (n=14) was conducted immediately following the study to further assess the mother's perceptions on the long-term acceptability and feasibility. Example questions included, “Would you feed your child this porridge each and every day?” and “Would you be interested to learn how the porridge [flour] is made?” The discussion was recorded, and the recording was transcribed in Chichewa and translated to English. Themes were developed and supporting statements from the mothers were matched to the themes.

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Fecal Microbiota Analysis

Stool samples were collected the day of the study visit in plastic Whirl-Pak® specimen bags by mothers, manually mixed within the bag, sampled into 5 mL tubes, and stored at -20°C until transported for storage at -80°C. The QIAmp Fast DNA Stool Mini Kit (Qiagen) was used to extract total fecal DNA using modifications previously described [17]. Real-Time polymerase chain reaction (qPCR) was used to determine the relative abundance of the genera, *Bifidobacterium* and *Roseburia,* and species*, Akkermansia muciniphila* and *Faecalibacterium prausnitzii,* using methods previously reported [18], and with the additional primer set for *Roseburia* [19]. Community-wide taxonomic profiling by 16S amplicon sequencing was conducted on the bacterial DNA extracted from the fecal samples, using methods as previously described [18]. Linear discriminant analysis effect size (LEfSe) was used to compare the fecal microbiome profiles of the children at baseline and after 6 months of maize and SFM interventions [20]. Microbial diversity and evenness were assessed using Pielou, Faith, Observed operational taxonomic units (OTUs), and Shannon indices.

Statistical Approach

All data are presented in mean ± standard deviation (SD) unless otherwise indicated. As the data were not normally distributed, the Wilcoxon Rank Sum and Kruskal-Wallis tests were used to test for differences in nutrient intake between groups. Appetite and anthropometric data were analyzed by repeated measures, generalized linear mixed models. Fisher's Exact Test was used to test for differences in the 3-month comparisons of stool form and frequency. Acceptance analysis was by Wilcoxon rank sums test. Growth, appetite, nutrient intake, stool form and stool frequency were assessed as intent-to-treat, whereas microbiome outcomes and growth were assessed per protocol. Alpha was set at 0.05. As a feasibility study, a power calculation was precluded.

RESULTS AND DISCUSSION

The demographic characteristics of the 79 mother/child pairs who participated in the study are given in Table 1. Figure 1 shows the recruitment, randomization and study flow. No serious adverse events were reported.

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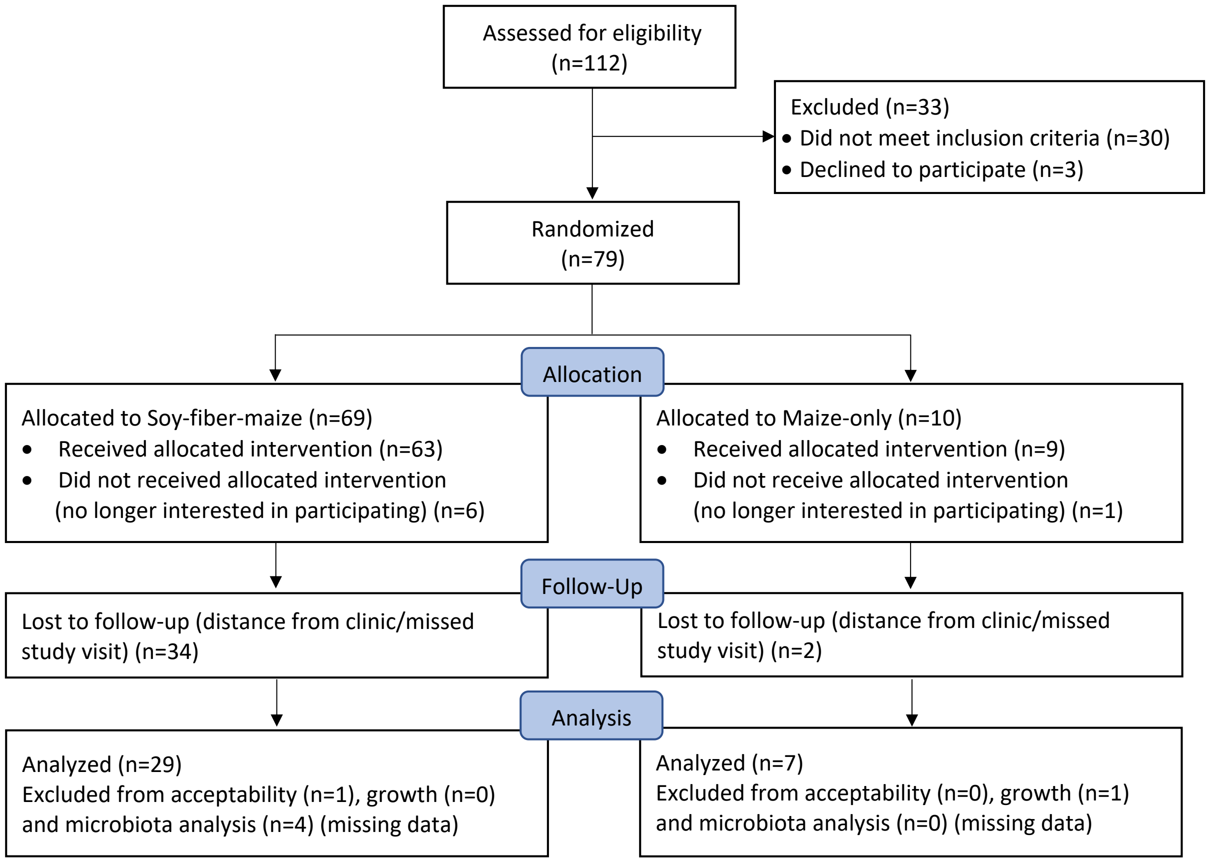

Figure 1: Recruitment, randomization and study flow of participants

Acceptability and Feasibility of SFM Porridge

No mother/child pairs withdrew from the study due to reported dissatisfaction with the SFM, side effects or adverse events, which suggests that long-term feeding of SFM is acceptable and feasible. Instead, the high loss to follow-up was thought to be due to the distance needed for mother/child pairs to travel to the clinic for weekly appointments. Future studies should plan for more local distribution of study foods. At the end of the study, mothers (SFM, n = 28; maize-only, n = 7) completed the porridge acceptability questionnaire. The SFM and maize did not differ in texture/consistency (5.0 ± 0.6; 4.8 ± 0.3, $p = 0.08$). Mothers rated the appearance of SFM higher than maize (4.9 ± 0.4; 3.7 ± 1.1, $p < 0.001$), as well as taste (4.8 ± 0.6; 3.9 ± 1.1, $p < 0.0001$), aroma (4.9 ± 0.4; 3.7 ± 1.2, $p < 0.0001$), and the overall acceptability (4.9 ± 0.3; 4.0 ± 0.8, $p < 0.0001$). Mothers attending the final study visit were invited to attend the focus group discussion. Fourteen mothers participated in a focus group discussion to discuss the feasibility of the porridge provided. The soy-fiber-maize porridge was most liked in terms of appearance, taste, aroma, and overall acceptability. The focus group discussion suggested that mothers believed it was feasible to use SFM blend porridge. Qualitative data analysis indicated central themes across the members. Mothers perceived improved appetite, weight, and stool consistency for their infants and children receiving the SFM blend. Representative comments included: *"before this porridge my child will eat a little amount but once she started receiving this porridge she started to eat more and better," "before this porridge, the weight of my baby was not increasing*

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as much, [when] she started receiving this porridge her weight has increased," and *"for my child, diarrhea was normal such that I stopped taking her for medical attention because we thought that that's how things are supposed to be."*

Dietary Intake

The dietary intake from the 24-h recalls (data not shown) indicated that children frequently consumed maize gruel (*phala*) and stiff porridge (*nsima*) at baseline. The common relish included okra leaves, dried pumpkin leaves, beans, cowpeas, fish broth and leafy vegetable broth. Throughout the study, the children did not consume meat, poultry or fish, although a small number of participants consumed fish broth/soup at baseline. At the end of the study, some children consumed insects, such as termites. Most children did not consume legumes, with the exception of bean broth, which increased at study end in both groups. Children did not consume fruit at baseline or the 3-month time points, but at study end, some children consumed mangoes. Table 2 provides the nutrient intake of children at baseline, 3- and 6-month time points. At the 6-month time point, the intakes of fiber, magnesium, and vitamin B12 were significantly higher in the SFM, compared to maize group, with a trend for higher protein ($p = 0.05$). At baseline, 80% of the children were breastfed, which decreased to 42% at the 3-month timepoint and 24% by the end of the study, although none of the children in the maize group were breastfed at study end. For the breastfed children, the mean daily breastfeeding frequency was 3.4 ± 1.1 at baseline, 2.4 ± 0.9 at 3 months, and 1.6 ± 0.5 times per day at 6 months.

Energy intake from non-breastmilk food sources was higher than that previously reported for Malawian infants, and frequency of breastfeeding was much lower [21], which may have been reflective of the older average age of the participants in the present study. Improved nutritional outcomes have been shown with higher diet diversity [22]. Maize porridges and broth were the most frequently reported food at baseline and throughout the study, with few reports of fruits, vegetables and animal-sourced foods, suggesting the diets of the participating children lacked diversity, a finding that has been previously reported in Malawian children, particularly during the lean season [23]. Soybean-maize blends provide more protein than maize-only complementary foods, so they have been used to prevent and treat malnutrition; however, the SFM porridge provided in the present study was not meant as a sole source of nutrition and was nutritionally inferior to specialty-formulated foods specifically intended for the treatment of malnutrition [24].

Appetite ratings are shown in Table 3. The SFM group showed significantly improved appetite at 3 and 6 months, compared to baseline ($p < 0.0001$). At 6-months, appetite in the SFM group was improved, compared to 3-months ($p < 0.0001$). Appetite was higher for SFM, compared to maize at baseline ($p < 0.001$) and at 3 months ($p < 0.0001$), whereas no difference between the groups was seen at 6 months ($p = 0.5$).

Anthropometric Characteristics

Mean Z-scores (SFM, n = 29; maize, n = 6) for weight-for-height (WHZ), weight-for-age (WAZ), and height-for-age (HAZ) are shown in Figure 2. Weight-for-height Z-score was higher for SFM compared to maize from month 2 through 6. The BMI Z-

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scores and MUAC-for-age are shown in Figure 3. BMI Z-scores for SFM were higher, compared to the maize from month 2 through to the study end. No differences were seen for MUAC-for-age between the groups. The prevalence of stunting was high in both the SFM and the maize groups and worsened with time. In contrast to the present study finding, Stephenson *et al*. [25] showed that complementary foods containing cowpea and common bean, providing 4–5 g indigestible carbohydrate/day, lessened growth faltering. However, Leroy *et al*. [26], in an analysis of data from six demographic and health surveys reported continued deterioration in mean height between the ages of 2 and 5 years. Given the limitations of using HAZ Z-scores to monitor changes in linear growth [26], stunting in the present study may have been underestimated.

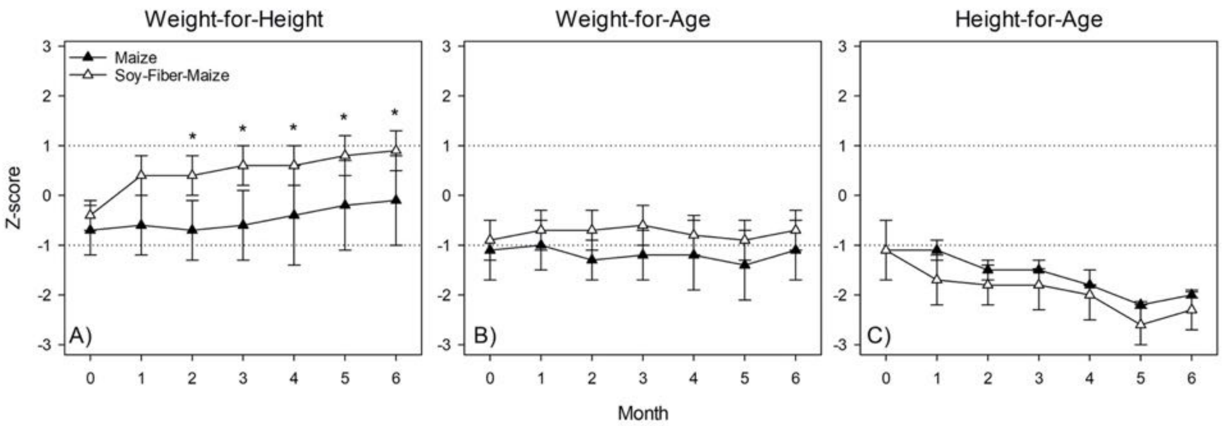

Figure 2: Z-scores (mean ± SE) A) weight-for-height, B) weight-for-age, C) height-for-age over time for the SFM blend and the maize groups (SFM = 29; Maize = 6). *Indicates significance at p<0.01

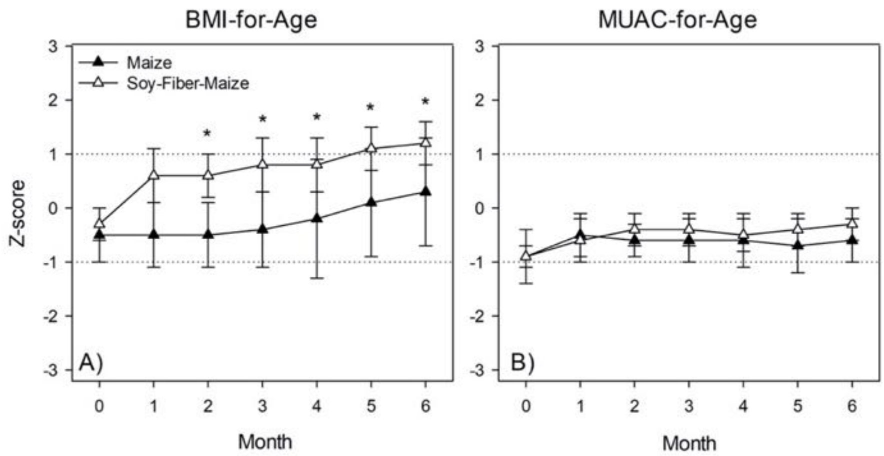

Figure 3: Z-scores (mean ± SE) A) BMI-for-age Z-scores, and B) MUAC-for-age for the soy-fiber-maize (SFM) and maize groups. (SFM = 29; Maize = 6) *Indicates significance at p<0.05

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Stool Frequency and Form

Stool frequencies at baseline were 2.3 ± 1.0 stools/day and 1.8 ± 0.8 for the maize and SFM groups, respectively, and were unchanged throughout the study (data not shown). The maize group demonstrated higher stool frequency, compared to the SFM group, during the entire study period (2.1 ± 1.6 vs. 1.8 ± 1.3, $p = 0.02$). Stool frequency, as a lone indicator, was not suggestive of diarrhea in most of the children; however, this frequency was higher than the 1.2 evacuations per week of young children in a Western country [27]. The percentages of fast-transit stool forms over time are shown in Figure 4. At baseline, both groups experienced a high rate of fast-transit stool form, suggestive of diarrhea, whereas none in either group reported slow transit stools. There were significantly fewer fast-transit, diarrhea-type stools (and increased normal transit form) during the second half of the study (months 4-6) for the SFM group, compared to the first half of the study (months 1-3), while no improvements were seen for the maize group.

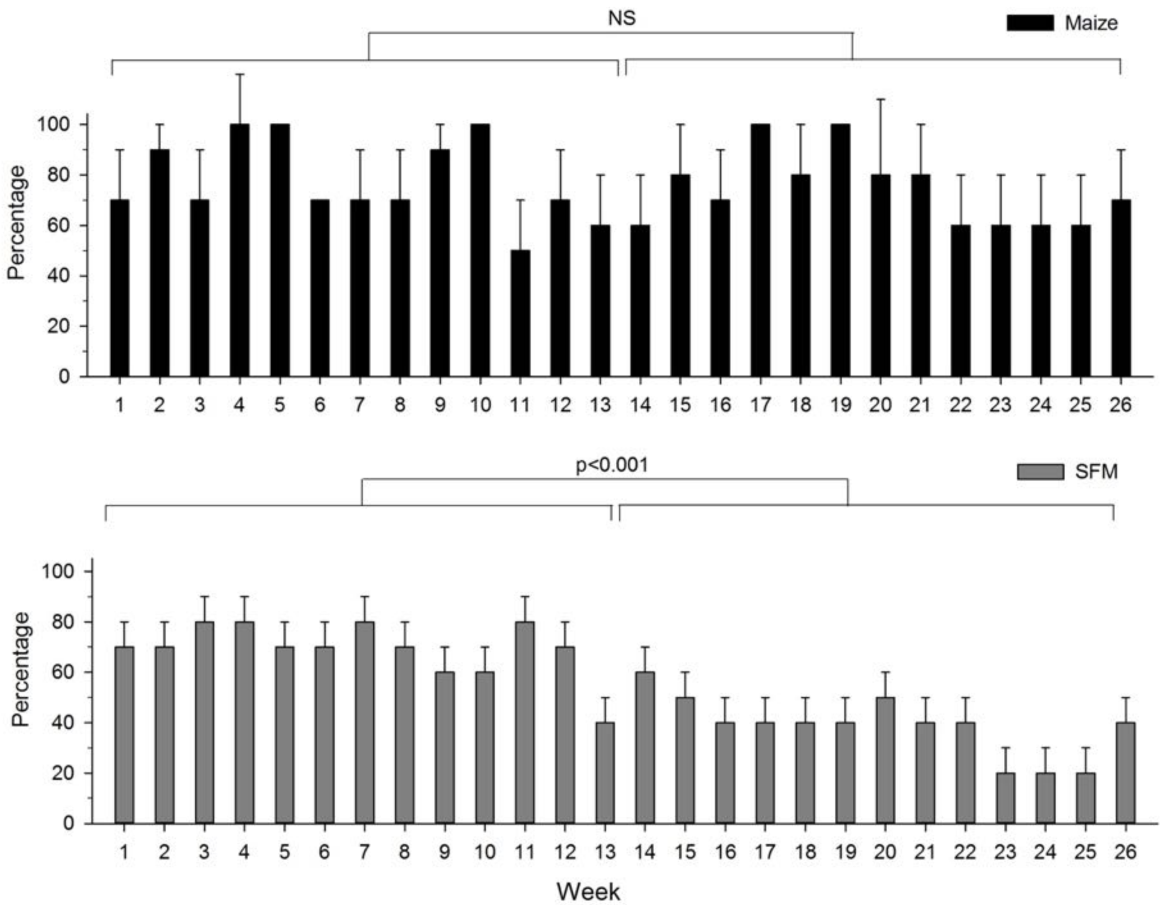

Figure 4: Percentage of fast-transit stool form (Modified Bristol Stool Form Scale for Children ratings 4 or 5) for the maize and soy-fiber-maize (SFM) groups by week over the duration of the 6-month study (intent-to-treat) (mean ± SE)

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Fecal Microbiome Analysis

Akkermansia muciniphila, *Faecalibacterium prausnitzii*, *Bifidobacterium* and *Roseburia,* considered beneficial organisms, were quantified in the fecal samples. For the qPCR analysis, participants with samples at all 3 timepoints (baseline, 3 months and 6 months) were compared (SFM: n = 19; maize: n = 6). There were no differences in relative quantification of *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Bifidobacterium* and *Roseburia* at 3 months, compared to baseline, between SFM and maize groups (Figure 5). The change from baseline at the 6-month timepoint, showed the relative quantification of *Akkermansia muciniphila* was higher in SFM, compared to maize (p = 0.03), and a trend for higher *Faecalibacterium prausnitzii* (p = 0.07) was seen (Figure 5). *Bifidobacterium* (p = 0.9) and *Roseburia* (p = 0.3) were not different between the two groups at any timepoint.

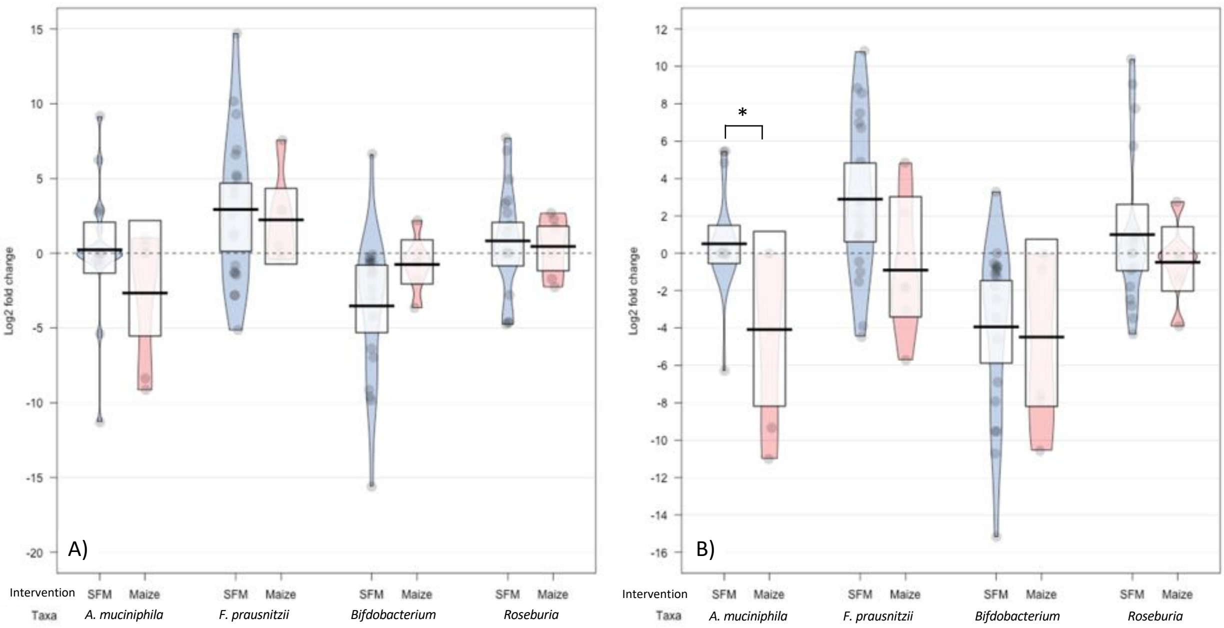

Figure 5: Pirateplot of the relative fold change from baseline of *Akkermansia muciniphila, Faecalibacterium prausnitzii, Bifidobacterium*, and *Roseburia* for soy-fiber-maize (SFM) (n = 19) and maize (n = 6) at A) 3 months and B) 6 months. *Indicates significance at p < 0.05

Diversity, the variability of microbes present, was assessed as an indicator of health. Figure 6 displays the differences from baseline at 3 and 6 months for the Pielou, Faith, observed OTUs and the Shannon alpha diversity indices. There were no significant differences.

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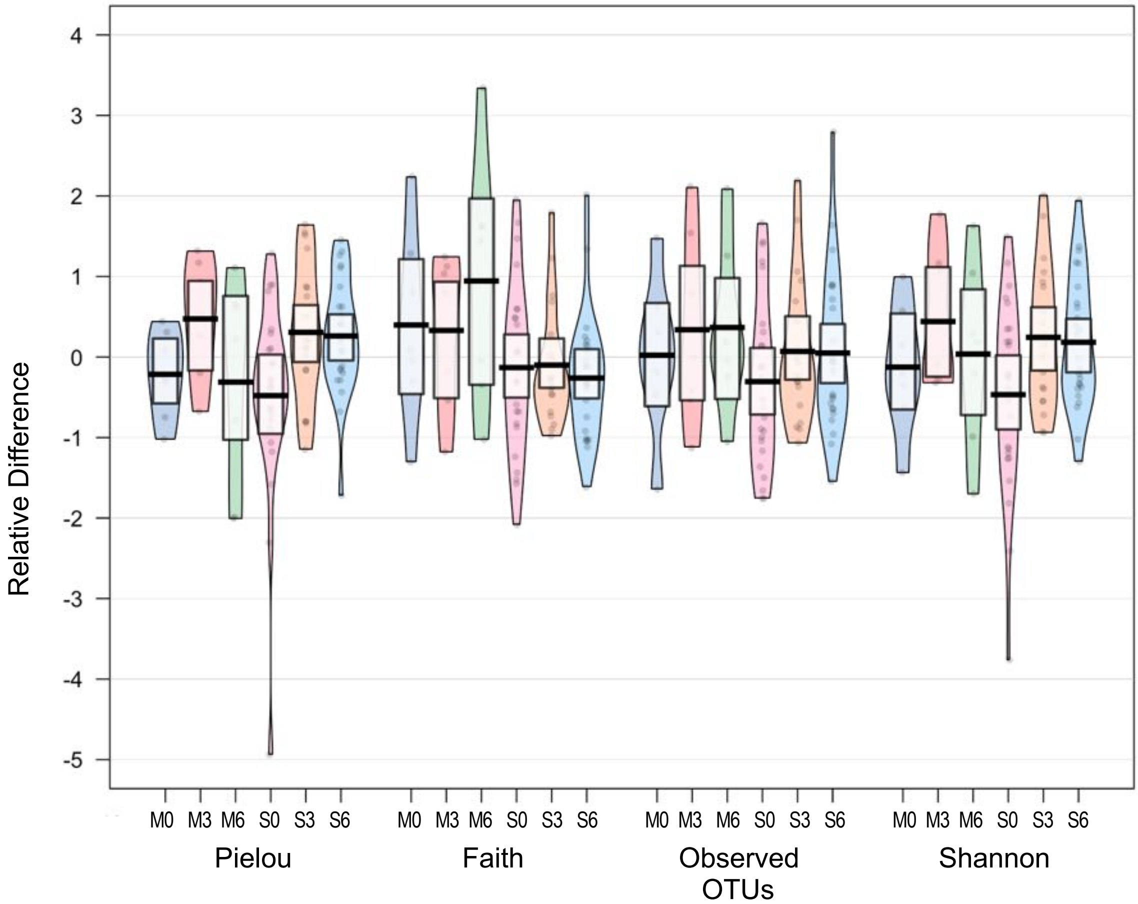

Figure 6: Pirateplot comparison of the relative difference of the diversity indices for the maize (n = 7) and soy-fiber-maize groups (n = 25) at baseline (maize, M0; soy-fiber-maize, S0), 3 months (maize, M3; soy-fiber-maize, S3), and 6 months (maize, M6; soy-fiber-maize, S6). No significant differences by Wilcoxon rank sum test with continuity correction, OTUs, Operational taxonomic units

Linear discriminant analysis effect size (LEfSe), which determines the features most likely to explain differences between groups, was used to compare the relative bacterial proportions at baseline to the 6-month time point (Figure 7). For the maize group, the genera *Enterococcus* and *Parabacteroides* and the families Odoribacteraceae and Enterobacteriaceae were enriched at 6 months, whereas only Clostridiaceae were enriched at baseline. For the SFM, *Bifidobacterium* (and all higher taxonomic levels) were enriched at baseline, as well as *Campylobacter*, *Clostridium*, *Delfitia*, *Acidaminococcus*, and *Atopbium* among other taxa. At the 6-month time point, enrichment was seen in many genera including *Slackia*, *Ruminococcus*, *Lactococcus*, *Weissella*, *Coprococcus*, *Faecalibacterium*, and *Gemmiger*, and at the phylum level, Firmicutes was increased.

The abundance of *A. muciniphila* was maintained with the provision of SFM, whereas a decrease was seen with maize. *A. muciniphila,* a mucin-degrading bacterium associated

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with the intestinal mucus layer, is considered beneficial as thinning of this layer and diminished abundance of *A. muciniphila* is thought to negatively influence intestinal permeability [28]. The qPCR data suggested a trend for an increase in *F. prausnitzii* with the provision of the SFM porridge. In addition, the LEfSe analysis showed that this genus was enriched at 6 months, compared to baseline. Lower abundance of *F. prausnitzii* has been reported in children with acute rotavirus and non-rotavirus diarrhea [29]. Additionally, enrichment of *F. prausnitzii* was seen at discharge (recovery) in severe acute malnutrition, compared to levels seen at admission [30]. An increase in *F. prausnitzii* is considered favorable, given that, in healthy individuals, it is one of the most abundant genera and low numbers have been associated with various intestinal disorders [31].

Notably, an increase in Firmicutes was seen with the SFM, a phylum containing many beneficial bacteria capable of fermenting dietary fiber [32]. Recently, it was suggested that the high dietary fiber in the pulse-based foods may promote gut health in children, compared to the traditional soybean-maize blends [33]. However, whole soybean or added soy hull fiber may also support the microbiota and its activity, and thus gut health.

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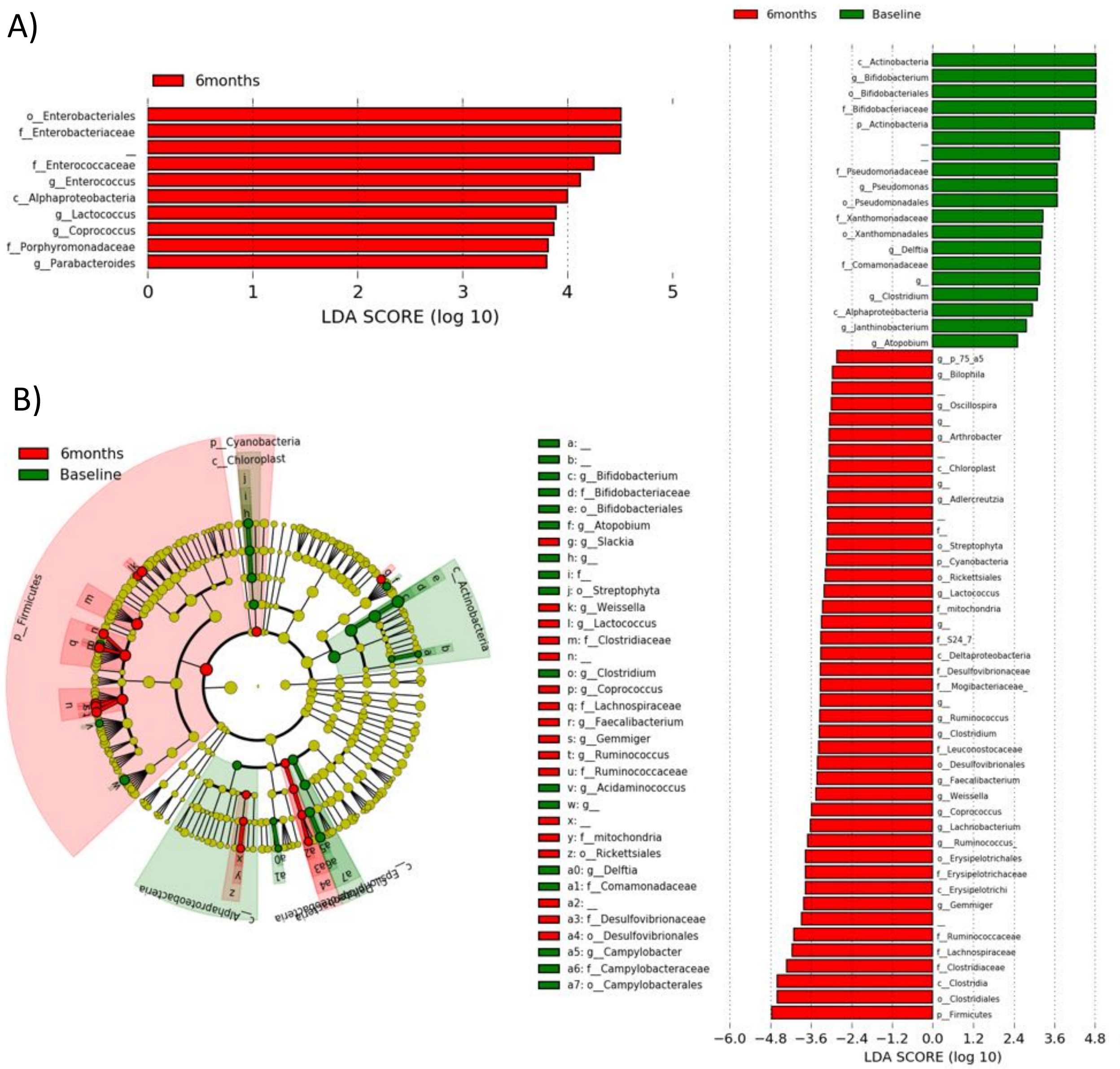

Figure 7: LDA (linear discriminant analysis) effect size (LEfSe) comparing the relative bacterial proportions at baseline to 6 months for the A) maize (n = 7) and B) soy-fiber-maize (n = 25) groups (g: genus; f: family, o: order; c: class, p: phylum)

Ordiz *et al*. [34] supplemented resistant starch in the diets of stunted 3-5 y old children, which resulted in a decrease in alpha diversity and no improvement in intestinal inflammation. In contrast, the SFM provided more diverse substrate for fermentation (fiber, resistant starch, and plant protein) and would be expected to impact microbiota, and this effect was demonstrated in the study. More recently, Ordiz *et al*. [35] examined the microbiota effects of dietary supplementation of legumes in breastfed Malawian infants aged 6 to 12 months but found no changes in the microbiome profiles. This finding may be due to the continued provision of human milk oligosaccharides that would be expected to drive the microbiota profile in the breastfed

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children. In contrast, the higher fiber SFM porridge provided in the present study altered the microbiome profile in the children, many of whom were weaned by study end. Weaning and the loss of human milk oligosaccharides explains the lower *Bifidobacterium* observed at 6 months – replaced by beneficial bacteria capable of degrading plant polysaccharides, such as *Faecalibacterium prausnitzii*.

Limitations

This study had limitations. At study initiation, withdrawals were due to mothers' concerns with stool collections. Failure to follow up was thought to be due to the significant travel time needed to attend study visits. The study coordinator was responsible for preparation and packaging of study flours as well as distribution, and thus was not blinded to the interventions. However, mothers were blinded to the intervention and the analysis of fecal microbiota composition and all statistical analysis were blinded. Antibiotic use was reported, the most common being a combination of sulfamethoxazole and trimethoprim, and thus may have impacted the microbiota outcomes.

CONCLUSION

The results of this trial confirm the acceptability and feasibility of a porridge blend of soybean, maize, and soy hull fiber. Stool frequency was not changed with the SFM intervention; however, a significant normalization of stool form was reported, specifically the reduction in fast-transit stool form. The SFM porridge supported weight gain but not adequate linear growth, and thus additional nutrient fortification may be warranted. The SFM group increased dietary fiber intake, which may have contributed to the modification of microbiota composition, particularly the enhancement of beneficial microorganisms, such as *Faecalibacterium prausnitzii*, capable of degrading plant polysaccharides. Given the prevalence of diarrhea and malnutrition in children living in sub-Saharan Africa, provision of whole soybean and perhaps additional soy hull supplementation, may provide a low-cost, sustainable solution and support improved child wellness. However, larger, multicentered randomized trials are needed to confirm the results of this preliminary trial, prior to community implementation. In addition, the feasibility and microbial safety of local production of soy hull fiber requires exploration.

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Table 1: Demographic characteristics of mother/caregiver and child pairs

Characteristics	Maize	Soy-Fiber-Maize
Children		
Female, n (%)	5 (50)	37 (54)
Male, n (%)	5 (50)	32 (46)
Age, months (mean ± SD)	20.4 ± 9.0	17.1 ± 9.6
Marital status of Mothers/Caregivers		
Single	2 (20)	14 (20)
Married	7 (70)	50 (73)
Divorced	1 (10)	4 (6)
Widow	0 (0)	1 (1)
Education level of Mothers/Caregivers		
None	1 (10)	9 (13)
Junior primary school	5 (50)	13 (19)
Senior primary school	2 (20)	35 (51)
Junior secondary school	2 (10)	8 (12)
Senior secondary school	0 (0)	4 (6)
Number of under-five in household		
1	2 (20)	29 (42)
2	4 (40)	32 (47)
3	3 (30)	7 (10)
4	1 (10)	1 (1)
Hunger period in the year		
Yes	9 (90)	54 (78)
No	1 (10)	15 (22)

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Table 2: Daily nutrient intake from non-breast food sources of the Malawian children

	Baseline	3 months		6 months	
Mean ± SD	(n=79)	Maize (n=5)	SFM (n=29)	Maize (n=7)	SFM (n=28)
Energy (kcal)	307±248	327±60	521±153**	418±268	489±148
Carbohydrate (g)	59±51	56±10	92±26**	64±34	85±24
Protein (g)	7±6	8±1	16±4**	9±6	14±4
Fat (g)	4±5	6±2	10±5	12±13	9±5
Fiber (g)	5.0±6.5	7.6±1.4	14.6±5.2**	8.4±4.5	13.7±3.8*
Calcium (mg)	27±32	73±13	78±25	81±39	78±21
Iron (mg)	2.1±2.1	2.3±0.5	4.4±6.1*	3.1±2.7	3.5±1.6
Magnesium (mg)	36±47	68±11	128±44**	69±36	118±34*
Phosphorus (mg)	127±134	342±49	320±160	353±199	311±113
Potassium (mg)	139±178	238±29	236±174	305±136	316±152
Sodium (mg)	50±100	91±119	77±109	210±345	115±148
Zinc (mg)	0.7±0.7	1.9±0.3	1.9±2.5	1.9±1.0	1.4±0.6
Vitamin A (µg)	54± 220	16±22	9±28	140±186	239±241
Thiamine (mg)	0.1±0.1	0.2±0.03	0.2±0.1	0.2±0.9	0.2±0.1
Riboflavin (mg)	0.2±0.2	0.2±0.01	0.1±0.1*	0.3±0.3	0.2±0.1
Niacin (mg)	1.4±4.3	1.5±0.3	1.6±0.8	1.6±0.8	1.8±0.7
Folate (mcg)	20±22	43±7	44±25	59±31	72±39
Vitamin B12 (µg)	0.11±0.8	0.2±0.05	0.3±1.7**	0.2±0.1	0.0±0.0*
Vitamin C (mg)	7.2±30.8	1.1±1.6	4.1±6.3	13.1±18.9	23.3±22.8

SFM, soy-fiber-maize

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Table 3: Appetite ratings of children consuming soy-fiber-maize or maize porridges at baseline, 3 months and 6 months

	Maize %			Soy-Fiber-Maize %		
	Baseline (n=10)	3-months (n=5)	6-months (n=7)	Baseline (n=69)	3-months (n=27)	6-months (n=28)
Very poor	0	0	0	0	0	0
Poor	29	15	4	14	2	0
Average	57	45	4	26	5	3
Good	14	27	55	54	62	20
Very good	0	13	37	6	31	77

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