Title:

Rice bran supplementation modulates growth, microbiome and metabolome in weaning infants: a clinical trial in Nicaragua and Mali

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One Sentence Summary:

Dietary rice bran supplementation during infant weaning from 6-12 months of age improved growth outcomes, modulated environmental enteric dysfunction biomarkers, and supported metabolism by the gut microbiome.

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Abstract:

Rice bran supplementation provides nutrients, prebiotics and phytochemicals that enhance gut immunity, reduce enteric pathogens in mice and diarrhea in neonatal pigs, and warranted attention for improvement of environmental enteric dysfunction (EED) in children at risk. EED is a condition that drives childhood stunting via intestinal dysbiosis and impaired nutrient metabolism. This study investigated effects of rice bran supplementation on growth, EED biomarkers, gut microbiome and metabolome in weaning infants from 6 to 12 months old in Nicaragua and Mali. Healthy infants were randomized to a control group or rice bran group that received daily supplementation at increasing doses each month. Stool microbiomes were characterized using 16S rDNA amplicon sequencing. Stool metabolomes were analyzed using ultra-high-performance liquid-chromatography tandem mass-spectrometry. Statistical comparisons were completed at 6, 8, and 12 months of age. Daily consumption of rice bran was safe and feasible for infant growth, decreasing alpha-1 antitrypsin levels, and modulating gut microbiome and metabolome when compared to control. Rice bran merits investigation as a practical intervention strategy that could decrease EED prevalence and risk for children from low- and middle-income countries where rice is grown as a staple food, and bran is used as animal feed or wasted.

Introduction

The prevalence of malnutrition in low and middle-income countries (LMIC) has negative consequences on growth of children during the first five years of life and has lifelong health consequences [1,2]. There is an increased risk of death among children under 5 years of age due to underweight, stunting, or wasting conditions [2,3]. Risk factors for undernutrition may include, but are not limited to: low birth weight, inadequate breastfeeding, improper complementary feeding, and recurrent infections [3,4]. Diarrheal diseases are also some of the primary causes of undernutrition in children under five years of age [1,3,4].

Environmental enteric dysfunction (EED) is an acquired subclinical condition of the small intestine among LMIC children [5-10]. Chronic exposure to enteric pathogens early in life is one likely contributor to EED [11]. The altered gastrointestinal functions in EED include intestinal nutrient malabsorption and increased intestinal permeability that leads to protein loss [6,7]. Infant weaning has been identified as a critical window for intervention [12]. Previous intervention efforts in young children have targeted micronutrient deficiencies, such as Vitamin A, Zn and Fe [13-16], oral rehydration salts for treating diarrhea [17], antimicrobial use [18,19], and community hygiene improvements [20].

Rice bran is a nutrient dense food with bioactive phytochemicals shown to prevent enteric pathogens and diarrheal disease in mice and pigs [21-24], and favorably promotes gut health in adults [25,26]. The effect of rice bran supplementation on host resistance to enteric infections and enhanced gut mucosal immunity was demonstrated for *Salmonella enterica*

Typhimurium [23,24], rotavirus [27-29], and norovirus [21]. Rice bran merits attention because it is widely available for consumption globally [30], and particularly in LMIC regions where EED is prevalent [31].

Stool EED biomarkers, gut microbiome [32] and metabolome analysis [15,33] became important surrogate markers for analysis as intestinal tissue from infants is not easily accessible to evaluate. Stool myeloperoxidase (MPO) [34], calprotectin (CAL) [35], and neopterin (NEO) [36] are indicators of inflammation; and alpha-1-antitrypsin (AAT) [34] is an indicator of barrier lumen disruption. Chronic, elevated concentrations of all four biomarkers have been associated with poor linear growth in infants up to 24 months old [10,34-36], and as the gut microbiome is maturing over the first 3 years of life [37,38]. Gut microbiome composition and metabolism is influenced by delivery mode, environment, and nutrition [39,40]. Recent studies have demonstrated that malnutrition and immature microbiomes of infants are only partially, and temporarily improved by some nutritional interventions [15,41]. The nutritional composition and metabolic profile of rice bran, which comprises a large suite of bioactive molecules, showed benefits in animal studies that provided important rationale for investigation of dietary feasibility in weaning infants and for improving growth in LMIC children.

The major objective of this study was to investigate effects of dietary rice bran supplementation during infant weaning on growth, EED biomarkers, gut microbiome and metabolome from six to twelve months of age in Nicaragua and Mali. The findings support that daily consumption of rice bran for six months is tolerable, safe and feasible for children during weaning and was associated with improved growth and decreased gut permeability via favorable modulation of the gut microbiome and metabolome.

Results

Rice bran supplementation is safe and feasible for weaning infants

Daily rice bran consumption was completed in a randomized controlled trial with infants from 6 to 12 months of age. To study the effect of daily rice bran supplementation on the gut, monthly stool samples from 47 Nicaraguan and 48 Malian children were collected (average of 7 samples per child, total of 567 samples). The flow and number of infants from study recruitment to study completion is shown in **Fig. 1**. Baseline participant characteristics for Nicaragua and Mali are shown in **Table 1.** We collected information on demographic factors and infants' household characteristics. In Nicaragua, 54.2% of infants were born via caesarean section in the control group and 30.4% in the rice bran group. All participants from Mali were delivered vaginally. For breastfeeding status, 96% of the control group and 83% in the rice bran group were consuming breast milk at six months old in Nicaragua and all children in the Mali group were consuming breast milk at the beginning and throughout the study. Of the 95 infants enrolled, 52 received antibiotics with 87 total antibiotic courses in the six-month period. Most courses consisted of systemic antibiotics given orally, with some delivered by injection for respiratory, skin, ear or diarrheal infections.

Dietary compliance to rice bran was averaged per month during the 6-month intervention with no adverse events related to rice bran intake at the increasing doses over time. Compliance to the rice bran intervention in Nicaragua was 90% and in Mali was > 99%. The feasibility and tolerability for infants to consume rice bran at increasing doses (1-5g/day) over the six month study period was demonstrated when mothers fed rice bran

168 powder alone or reported consumption with drinking water, staple grain porridges (i.e.
169 millet, sorghum, and white rice), soups, milk, fruits, juices, eggs, and fish when available.

170 **Rice bran supplementation increases infant growth**

171 Anthropometric data was collected using standardized procedures across study sites
172 at 6, 8, and 12 months of age for each child and included length-for-age Z-score (LAZ),
173 weight-for-age Z-score (WAZ) and weight-for-length Z-score (WLZ). **Table 2** reports the
174 z-scores analyzed by repeated measures and adjusted by treatment (rice bran) and age (6-8
175 months and 8-12 months) by country. Significant differences were observed for
176 anthropometric measures between treatment, and ages in both cohorts. In Nicaraguan
177 infants consuming rice bran, LAZ was significant over time (1.18 at 6-8 months, p-value=
178 0.0000; 0.35 at 8-12 months, p-value= 0.0002) and for WLZ at 6-8 months (p-value=
179 0.0000), with no changes detected in WAZ. Malian infants consuming rice bran had
180 significant growth results for WAZ (6-8 months, p-value= 0.0001, 8-12 months, p-value=
181 0.0175) and WLZ (6-8 months, p-value= 0.0141, 8-12 months, p-value= 0.0134). **Fig. 2**
182 displays LAZ, WAZ and WLZ over time and by country. The significant increase in LAZ
183 at 8 months and 12 months in Nicaraguan infants that consumed rice bran was compared to
184 control group (**Fig. 2A**, $p<0.01$). No significant differences were observed for WAZ and
185 WLZ with this control group comparison (**Fig. 2B** and **2C**).

186 **Rice bran modulation of environmental enteric dysfunction biomarkers**

187 Four EED stool biomarkers were selected for analysis at 6, 8 and 12 months of age
188 using ELISA (see materials and methods). A significant decrease in AAT was observed at
189 12 months of age (p=0.0368) in Nicaragua infants that consumed rice bran compared to
190 control (**Table 3**). No significant differences were detected in NEO, MPO and CAL

191 between treatment groups, however a slight decrease in median concentrations of all stool
192 EED biomarkers were observed in the rice bran group compared to control in both
193 countries that can be applied for future study sample size and power calculations.

194 **Rice bran modulates gut microbial communities in Nicaraguan and Malian infants**

195 The microbiome was characterized and compared for 48 Malian and 47 Nicaraguan
196 infants at 8 and 12 months of age in the rice bran and control study groups. DNA was
197 isolated from stool samples and the V4 hypervariable region of the 16S rRNA gene was
198 sequenced utilizing the Earth Microbiome Project protocol [42-46]. Sequences were
199 preprocessed for quality assurance and classified into operational taxonomic units (OTUs)
200 (see materials and methods), and the results were integrated to construct family and genus-
201 level composition profiles for all 192 samples from both countries. No major differences
202 were detected in alpha diversity indices (Observed, Shannon, InvSimpson and Richness)
203 calculated for rice bran group and control group at 8 and 12 months (**Table S1**). Beta
204 diversity analysis, depicted in the Nonmetric Multidimensional Scaling (NMDS) plot based
205 on the Bray-Curtis distance measure, indicated complete country-level separation in the
206 overall gut microbial community composition (**Fig. 3A**). This provided rationale for
207 separating analyses for the microbiome and metabolite profiles by country.

208 **Fig. 3B** shows the NMDS plot separated by country. This figure highlights
209 differences in the microbiome between two time periods, 8 and 12 months, which is more
210 pronounced in the Malian samples. These figures show overlap of microbiomes within each
211 time period indicating putative similarity between the microbial community structures
212 during growth periods. This overlap was observed to a greater level at 8 months of age as
213 compared to 12 months that may illustrate microbial adaptation to new exposures [31].

Fig. 4 illustrates the taxa (on the OTU level) with at least 2 log-fold change between the rice bran and control groups per country and per age group. These taxa were ordered based on significance, measured by the FDR-adjusted p-value, from bottom (most significant) to top (details are provided in **Table S2** for Nicaragua and **Table S3** for Mali). Given the measurable differences in growth between groups by 8 months of age, the effect of daily dietary supplementation of rice bran on the infant microbiome was compared to the control group at 8 months.

In Nicaragua, we identified 145 OTUs that were significantly different between control and rice bran across the samples (Table S2), 74 of which showed greater than or equal to 2 log fold differences between rice bran and control groups at 8 or 12 months (Fig. 4A, adjusted p-value <0.05). Seven of these 74 OTUs overlapped between the ages 8 and 12 months. In Mali, 42 bacterial OTUs were identified to significantly differ between control and rice bran across samples, and 19 showed more than 2 log fold changes between rice bran and control at 8 or 12 months (Fig. 4B, adjusted p-value < 0.05) with only three overlapping between the two age groups. Next we explored the country specific changes in genus level taxa that were responsive to rice bran intake. For Nicaraguan infants (**Table S2**), the notable rice bran responsive taxa which increased at 8 months of age compared to control group were Lachnospiraceae-unclassified-Otu0280 (log-FC 5.84, adjusted p-value 3.95E-08) Bifidobacterium-unclassified-Otu0314 (log-FC 2.04, adjusted p-value 1.38E-6), Ruminococcaceae-unclassified-Otu0238 (log-FC 2.01, adjusted p-value 0.00097), Veillonella (11different OTUs, log-FC > 2.0, adjusted p-value < 0.05), and Bacteroides (log-FC > 2.0, adjusted p-value < 0.05). The fold difference for genus level taxa that were lower in relative percent abundance for rice bran group were Bacteroides-Otu0192 (log-FC

-3.08, adjusted p-value 2.34E-07), Parabacteroides-Otu0086 (log-FC -2.34, adjusted p-value 0.0074), Lachnospiraceae-unclassified-Otu0174 (log-FC -2.27, adjusted p-value 0.0033), Lactobacillus-Out0053 (log-FC -3.85, adjusted p-value 1.09E-05), Oscillibacter (log-FC -2.49, adjusted p-value 0.0029) and Ruminococcaceae_2 (log-FC -2.69, adjusted p-value 1.98E-05).

In Mali infants, (**Table S3**), there were 2-fold increased differences observed for rice bran fed infants in Lactobacillus-Out0356 (log-FC 3.2, adjusted p-value 1.35E-09) and decreased for Lachnospiraceae-unclassified-Otu0010 (log-FC -2.3, adjusted p-value 0.016). There were also distinctions among taxa between the infant gut microbiomes that was observed by age and country with respect to rice bran intake. At 12 months of age, the Nicaragua rice bran group had increased Paraprevotella (log-FC 6.2, adjusted p-value 4.27E-08), Phascolarctobacterium (log-FC 6.12, adjusted p-value 1.60E-08) Veillonella (log-FC 3.35, adjusted p-value 3.30E-07) and Bifidobacterium (log-FC 2.6, adjusted p-value 1.44E-05). Lower abundant taxa in rice bran infants at 12 months from Nicaragua were Lachnospiraceae_ND3007_group (log-FC -2.0, adjusted p-value 0.00029) and Alisonella (log-FC -4.0, adjusted p-value 1.60E-08). Malian rice bran fed infants at 12 months of age showed increased Lactobacillus_Otu0053 (log-FC 2.7, adjusted p-value 0.0098) and Alloprevotella (log-FC 3.6, adjusted p-value 0.00034). The significantly decreased fold difference in taxa of Malian infants between rice bran and control groups at 12 months were Bifidobacteriaceae_unclassified_Otu0265 (log-FC -2.6, adjusted p-value 3.95E-05) and Clostridium_sensu_stricto_1_Otu0076, and Terrisoporobacter (log-FC -2.1, adjusted p-value 7.03E-06).

There were 12 OTUs identified as rice bran responsive from this microbial community analysis that showed changes in both Mali and Nicaragua at either 8 or 12 months of age when compared to control. The highest area of overlap occurred for both Lactobacillaceae_Lactobacillus_Otu0024 and Lactobacillus_Otu0053 (**Table S4**). Other taxa that overlapped between countries with respect to rice bran intake included three distinct Bifidobacterium, Faecalibacterium, and Lachnospiriaceae.

Metabolomics identified rice bran and microbial digested rice bran small molecules in stool of weaning infants

A total of 309 stool samples were collected from this 6-month prospective study to evaluate effects of rice bran supplementation compared to control infants from Nicaragua and Mali. ANOVA contrasts and Welch's two-sample t-test were used to identify biochemicals that differed significantly between experimental groups at the 8-month time point. Stool metabolite analysis of children at 8 months of age in Nicaraguan and Malian infants resulted in the detection of 1449 biochemicals, of which 1016 metabolites had confirmed names and 433 compounds were of unknown structural identity (see **Table S5**). **Table 4** lists the fold differences calculated from the relative abundances of each stool metabolite between study diet groups at 8 months of age, whereby infants had been consuming rice bran daily for 2 months. There are 39 (Nicaragua) and 44 (Mali) stool metabolites with significant fold differences between children consuming rice bran compared to control. There were also 33 and 31 significantly different metabolites between groups that were classified as unknown for Nicaraguan and Malian infants respectively, (data shown in **Table S5**). Significant fold differences occurred for 15 amino acids, 2 peptides, 3 carbohydrates, 9 lipids, 1 cofactor and vitamin, and 9 xenobiotics (six of these

282 considered as food components/plant-derived) in children consuming rice bran compared to
283 control in Nicaragua. In Mali at 8 months of age, there were 6 amino acids, 1 energy, 14
284 lipids, 6 cofactor and vitamins, 5 nucleotides and 12 xenobiotics (7 classified as food
285 components/ plant-derived) that showed significant differences in children consuming rice
286 bran compared to control (**Table 4**).

287 There were 62 stool metabolites from the Nicaraguan cohort at 8 months that
288 showed significantly lower relative abundances (comparing fold differences between rice
289 bran compared to control), and there were 10 significant stool metabolites with increased
290 fold differences in abundance between groups. The stool metabolites of food and nutritional
291 importance to highlight that resulted from increasing rice bran intake come from the
292 tryptophan metabolism (indolepropionate), monoacylglycerol (1-linolenoylglycerol) and
293 diacylglycerol metabolism (linoleoyl-linolenoyl-glycerol) pathways.

294 In contrast to Nicaragua, there were 54 distinct stool metabolites from the Mali
295 cohort that had increased abundance and significant fold differences at 8 months between
296 rice bran and control infants. Selected stool metabolites for gut health and nutrition
297 relevance, as well as for originating from rice bran food metabolites, included Alpha-
298 tocotrienol (vitamin E component), Pyridoxine (vitamin B6), Ferulic acid 4-sulfate, tyrosol,
299 and N-acetyl sphingosine (**Table 4**). An estimated false discovery rate (q-value) was
300 calculated to account for the multiple comparisons across metabolites that are typical of
301 metabolomic-based studies.

302

303 **Discussion**

This study demonstrated that rice bran supplementation was feasible, well tolerated, and safe for weaning infants with strong compliance to daily dietary intake in both LMIC countries. Rice bran supplementation in the diet supported growth of Nicaraguan and Malian infants with differences detected between groups by 8 months of age and improved gut permeability at 12 months. Nicaraguan infants fed rice bran had increased LAZ compared to the control, and in Mali, there was increased WAZ. Based on documented country-wide averages using the WHO scoring index [12], the 95 healthy infants enrolled in this study had slightly higher WAZ, LAZ and WLZ scores.

There is a growing body of scientific evidence for a strong relationship between EED and growth deficits in children [7,10,47]. EED biomarkers were selected in this study because high concentrations of stool AAT and MPO were associated with decreased growth in children [47], and Naylor et al. found that elevated AAT levels were associated with decreased oral rotavirus vaccine response [48]. Rice bran was shown to reduce AAT in neonatal pigs challenged with human Rotavirus infection [22] and is of translational importance herein as rice bran intake improved AAT levels in Nicaraguan infants (**Table 2**). The lack of significant differences in levels of EED biomarkers for Mali may be due to the higher number of overall diarrheal episodes, the level of variability within individuals, and breastfeeding over time and across groups, yet these findings did concur with concentrations reported in the MAL-ED cohort [34]. EED biomarkers merit continuous review for relevance with growth outcomes due to extensive global variability in concentrations reported across studies [6,49-51].

A study limitation and possible confounder of rice bran modulation to infant gut measures was that the percentage of exclusively breastfed infants was lower and delivery

mode varied in the Nicaragua cohort (<50%) between groups, which was in contrast to the Mali site that had 100% of children that were breastfed and had vaginal delivery. These are key considerations to evaluating microbiomes of children with EED that have been characterized as less mature [52], and with varied structure by geographical location [53], diet deficiencies [54-58], environmental exposures [53,59,60] and host factors [59,61]. Thus, we did expect changes in both the stool microbiome and metabolome that occurred over time with growth, and notably the microbial taxa and metabolites associated with the improved growth in rice bran groups also differed at 8 months in both countries.

Given that dietary rice bran intake has been previously shown to promote beneficial stool microbial communities, such as native gut probiotics in mice [23,62], pigs [21,22,29] and adults [25,63,64], we first evaluated significant genus level taxa differences between rice bran and control fed infants at 8 months and 12 months of age from both countries. In Nicaragua, Lactobacillus, Lachnospiraceae, Bifidobacterium, Ruminococcaceae and Veillonella were identified as responsive following rice bran consumption compared to an age and control matched group. These taxa have recognized saccharolytic mechanisms of action [65-67], are known to produce and promote crossfeeding of short chain fatty acids [68,69], as well as provide competitive inhibition of pathogen colonization [70,71]. The microbial enrichment of these communities as associated with infant growth outcomes has implications for assessing how gut microbes metabolize rice bran components. In Mali, the increased relative abundance of Lactobacillus in rice bran fed infants is also highly consistent with prior studies in young animals, and should be considered alongside evidence that human milk oligosaccharides also promote Lactobacillus in breastfed infants [72].

Stool metabolites from infants fed rice bran showed significant fold differences amongst several essential amino acids, cofactors and vitamins, lipids, phytochemicals, and in energy metabolism pathways compared to the control groups. Stool metabolites originating from rice bran were identified in both Nicaragua and Malian infants, providing additional confirmation of compliance to the dietary intervention [73]. As predicted, we observed and reported exceptionally distinct profiles for both the stool microbiome and metabolome composition of infants between Mali and Nicaragua at all ages [40,53,74] and therefore separately discussed the response to rice bran supplementation by region. For example, the nearly 5-fold increased stool detection of indolepropionate in rice bran infants compared to control from Nicaragua represents a tryptophan metabolite produced by the gut microbiota that may influence the developing immune system and intestinal homeostasis [75,76]. Increased levels of N-acetylmethionine (nutritionally and metabolically equal to L-methionine) and N-formylmethionine in Malian infants also represented rice bran derived amino acids required for normal growth and development [77]. There are several cofactors and vitamins from rice bran supplementation, such as alpha and gamma-tocotrienol and pyridoxine (vitamin B6) that merit attention for demonstrating multiple health benefits such as synthesis of amino acids and neurotransmitter precursors, as well as preventing anemia and skin problems [73,78,79]. Additional microbial digested food components in the stool metabolome that come from rice bran were ferulic acid 4-sulfate, indoleacetylaspartate, and Tyrosol. A study limitation is that we had only supplemented for a 6 month window and according to WHO, growth assessment should be standardized and compared globally over the first two years of life [80,81] . We put forth that rice bran

372 metabolism by host and gut microbes between 12-36 months of age should be captured for
373 the continuous assessment and influence on growth velocity during childhood [80].

374 Furthermore, the increased separation noted between 8 and 12 months in this study
375 showed modifications in gut microbial communities and metabolites by rice bran intake,
376 and suggests there will be long-term impact on the overall microbiome composition as it
377 continues to develop and mature [82]. The overall dietary pattern differences between mothers
378 and infants weaning practices in each country were also considered a major source of
379 variation as dietary diversity of gut microbe-food interactions were clearly observed in the
380 stool metabolite profiling (see Table S5). Nevertheless, rice bran consumption was well
381 tolerated at increasing dose supplementation amounts during the first 6 months of weaning
382 without side effects or adverse interactions.

383 This was the first randomized controlled trial of rice bran supplementation in LMIC
384 infants and provides compelling rationale for continued follow-up investigation of rice bran
385 supplementation for reducing risk of malnutrition, as well as for eliciting changes during
386 child growth that protect against enteric pathogens and diarrhea. The dose and feasibility
387 outcomes from this study support development of rice bran based complementary weaning
388 foods. Our findings also suggest that double blinded-controlled trial study designs with
389 larger infant cohorts are warranted for long-term outcomes to be assessed until five years of
390 age. Incorporating rice bran from local rice production and processing facilities should be a
391 priority in subsequent trial designs, and with the goal of supporting the development of
392 sustainable and affordable food products for weaning infants, particularly those residing in
393 in LMIC where food and nutritional security remain.

394

Materials and Methods

Study design

A 6-month, prospective, randomized-controlled, dose escalation dietary intervention was conducted in a cohort of weaning infants residing in León, Nicaragua and in the community of Dioro, Mali, West Africa. Nicaraguan infants were recruited from public health rosters provided by the local Health Ministry from Perla Maria and Sutiava health sectors, and Malian infants were recruited from the Dioro Community Health Center. To be eligible, infants were screened between 4-5 months of age for health status, and then followed weekly for diarrhea episodes. Participants were excluded if they had experienced diarrhea or received antibiotic treatment within the previous month; had known allergies, or immune-compromising conditions (e.g. parasitic or malarial infections); had previously been hospitalized; and/or enrolled in a malnutrition treatment program. In Nicaragua, all eligible participants received 3 doses of the rotavirus vaccine per regular administration through the Immunization Program [83]. Rotavirus vaccination was not yet administrated to the Mali cohort. All Malian participants received vitamin A supplementation upon enrollment. Dietary intervention with rice bran started when infants were 6 months of age because WHO guidelines promote exclusive breastfeeding for the first six months of life [81,84].

The required ethical board reviews and approvals were completed for Mali and Nicaragua as provided by the Internal Review Board (IRB) of the Colorado State University Research Integrity and the Compliance Review office. In Mali, the Institut National de Recherche en Santé Publique (National Institute of Research in Public Health, FWA 00000892) approved the intervention, which occurred between October 2015 and

May of 2016 and registered at clinicaltrial.gov as (NCT02557373) on 23 September 2015. Ethical review and approvals for the Nicaraguan intervention that occurred between March 2015 and October 2015 were provided by the IRBs of the Universidad Nacional Autónoma de Nicaragua – León, University of North Carolina at Chapel Hill, and Virginia Polytechnic Institute and State University and registered at clinicaltrial.gov on 26 November 2105 as (NCT02615886). Written informed consent was obtained from the infant's parent or responsible guardian prior to any data collection. Infant participants that met the eligibility criteria were randomized within each health sector, and sex (Nicaragua) and geographic location of household and sex (Mali) to either rice bran or control group (see **Fig. S1** for enrollment details). Randomization was completed using sequential enrollment for each site independently. Participants were randomized by CSU, enrolled and assigned to groups by study coordinators in each country. Complete study protocol is available online (http://csu-cvmbs.colostate.edu/academics/erhs/Pages/elizabeth-ryan-lab-global-health.aspx).

Rice bran packaging for consumption

The United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Dale Bumpers National Rice Research Center provided rice bran that was polished from the U.S. variety, Calrose. Rice bran is prone to fat oxidation and heat-stabilization was performed to increase shelf-life by heating the bran at 100 degrees Celsius for five minutes to inactivate the lipase/lipoxygenase enzymes that cause rancidity [85]. The rice bran was sifted to remove any debris (rice husk, rice grain). Packaging of the rice bran was completed by Western Innovations, Inc. (Denver, CO) where 22 kg of rice bran was

441 weighed into 1g increments, separated into water-proof sachets, and heat-sealed to ensure
442 the rice bran would be administered with accurate doses to infants.
443 Fourteen sachets (1g/sachet) were filled into a 4" x 3" x 2" box that was labeled for
444 study participants and included nutrient information. These boxes were stored in a cool,
445 dark, dry place until they were provided to study participants.
446 **Nicaragua and Mali intervention**
447 The study team (doctor, nurse and study coordinator) in Nicaragua and the
448 community health workers (CHWs) in Mali provided a 2-week supply of rice bran at each
449 routine home visit and instructed the participant's parent or guardian to add the daily
450 amount of rice bran to the participant's food. At 6-7 months of age, participants in the rice
451 bran group consumed 1g of rice bran/day (1 sachet). Between the ages of 7-8 months,
452 participants consumed 2g of rice bran/day (2 sachets). At 8-10 months of age, participants
453 consumed 3g of rice bran/day (3 sachets). The amount increased to 4g of rice bran/day (4
454 sachets) from 10-11 months, and 5g of rice bran/day (5 sachets) from 11-12 months of age,
455 respectively. The rice bran was added to appropriate weaning foods, such as rice cereal,
456 yogurt, fruit and natural juices, vegetables, and soups. At the beginning of the intervention
457 (six months of age), infant´s parents or guardians were instructed and monitored daily for
458 one week by study personnel to ensure that guardians knew how to administer and record
459 the amount of rice bran consumed. Compliance to the rice bran intervention was calculated
460 from records that had the dose/amount of rice bran consumed circled in daily increments of
461 none (0%), half (50%), or all (100%). The study team also collected any unused boxes or
462 sachets during these visits. Participants in the control group did not receive any rice bran
463 and there were no reports of brown rice intake during the 6-month study duration.

464 In Nicaragua**,** study personnel visited all infants weekly. In Mali, the CHWs visited
465 each participant's household daily for the duration of the 6-month study to assess
466 compliance and diarrhea episodes. If a participant had a diarrhea episode, the study team
467 would collect a stool sample, and collect information that included the diarrhea onset date,
468 how long the episode lasted, numbers of bowel movements within 24 hours, any associated
469 signs and symptoms (e.g. nausea, vomiting, fever), if any other family members had
470 diarrhea, and if any treatment was provided (e.g. antibiotics, rehydration).
471 The study team in Nicaragua collected data for control group participants at 6, 8,
472 and 12 months old, and rice bran group every month. The anthropometric measures
473 (weight and length) were collected via a portable stadiometer and weighing balance. Mali
474 participants visited the Community Health Clinic every month. Length was measured in
475 supine position using a reclining length-board. Length was collected to the nearest
476 centimeter and weight to the nearest 0.1 kg. Anthropometric measures were calculated for
477 LAZ, WAZ, and WLZ scores following the World Health Organizations (WHO) child
478 growth standards using the WHO Anthro software (version 3.2.2) [86].
479 Diapers were provided to all study participants. Stool was collected directly from
480 soiled diapers. Freshly collected stool was diluted 20-fold and homogenized in a sterile
481 pre-reduced anaerobic saline - 0.1 M potassium phosphate buffer (pH 7.2) containing 20%
482 glycerol (vol/vol). Four aliquot suspensions were prepared in 15 mL falcon tubes,
483 transported on dry ice to the UNAN-León-Center of Infectious Diseases Laboratories (and
484 liquid nitrogen in Bamako ,Mali), immediately transferred to a -80ºC freezer, shipped in a
485 liquid nitrogen chilled dry shipping dewar to Colorado State University, where they were
486 relocated into a -80ºC freezer prior to analysis.

A study questionnaire was completed by the participant's caretaker (e.g. mother, father, or grandparent) to assess for duration of breastfeeding, types of and timing of introductions to complementary foods, as well as antibiotic use. The breastfeeding questions included whether or not the child was receiving breast milk, and/or had the child been receiving received formula. The complementary feeding history included a list of common Nicaraguan and Malian foods that are normally introduced to infants during weaning. Infant´s parents or guardians recorded how often the infant consumed each of the eleven foods. The questionnaire also recorded if a participant had received treatment with antibiotics since the last visit, the reason for taking the antibiotic, the name of the antibiotic, as well as the length of time the participant had been taking the antibiotic. A household survey was also completed at the beginning of the trial to collect mother's education level, drinking water source, household flooring type, and animals present in the household. Analysis of breastfeeding and formula feeding patterns, complementary feeding practices, and associations with nutritional status at 6-months old (i.e. baseline) were previously reported for Nicaragua [87]. Monthly visits to the Mali community health clinic provided monitoring for malnutrition and severe adverse events; no adverse events were reported in the rice bran intervention group. There was one participant death reported in the control group (respiratory infection) and another withdrew to receive malnutrition treatment in the second month of the study. Diarrheal episodes were recorded, and a sample was collected in both countries using the same protocol.

Stool analysis for EED markers

Stool biomarkers were selected to report gut inflammation and epithelial integrity as indicators of EED. These included neopterin (NEO), myeloperoxidase (MPO),

calprotectin, (CAL) and alpha-1 antitrypsin (AAT) [88]. Suspended stool samples from 6, 8, and 12-month collections were centrifuged at 3,000 RPM to remove debris, following agitation, and the remaining supernatant was used for Enzyme-Linked-Immunosorbant-Assay (ELISA) determination of EED biomarker concentrations. Laboratory analysis protocols included in commercial kits were followed. Concentrations of CAL were determined at a 1:360 final dilution factor (Eagle Biosciences- Nashua, NH. Ref: CAL35-K01). Samples were diluted to 1:100 for determination of NEO concentrations (GenWay Biotech Inc- San Diego, CA, USA). MPO concentrations were determined at a 1:500 dilution factor (Immundiagnostik AG- Bensheim, Germany). Samples were diluted to 1:12,500 for determination of AAT concentrations (Immuchrom GMBH- Heppenheim, Germany), and dilution factors accounted for stool suspension ratios (20-fold). Final concentrations were determined from averages of replicate assays and duplicate optical density readings, and interpolated using Graphpad 6.0 according to standards measured on each 96-well plate.

Stool microbiome analysis for Nicaragua and Mali

The infant stool was collected at 6, 8 and 12 months of age from diapers and placed in a 1:19 ratio with Phosphate Buffered Saline + Glycerol solution. Diarrhea samples were collected using the same protocol. Suspended stool samples were vortexed before centrifuging at 3000 RPM to separate the stool debris. The remaining supernatant was used for Enzyme-Linked-Immunosorbant-Assay (ELISA) determination of EED biomarkers whereas; DNA was extracted for 16S microbial analysis from the stool pellet. DNA extraction was conducted using MoBio PowerSoil Kit (Reference number 12888, MoBio Laboratories Inc., Solana Beach, CA). PCR amplification of 390 bp amplicons was done in

533 50 μl reaction using Fischer Hot Start Master Mix and EMP standard protocols [42-46]. SPRI
534 magnetic beads were used to purify DNA, and flourimetric quantification of Sybr Green
535 tags was used to confirm adequate concentration of DNA. The pooled library was created
536 with 50 ng DNA per sample and quantified using Kapa Kit (Kapa Biosystems). The pooled
537 library was run on Illumina-MiSeq with 15% PhiX mock library to reduce discrepancies in
538 read clustering, using the Illumina V2 500 cycle kit (2 x 250/250 paired-end reads).

539 **Microbiome data processing and analysis**

540 Sequence data were processed using mothur [89] version 1.39.5 and using a custom
541 pipeline that provides an adjustment on the developers' standard operating procedure (SOP)
542 for OTU calling and taxonomic classification of MiSeq data first presented in Kozich, et
543 al., 2013 [90]. For alignment and classification within this SOP we used the SILVA database
544 [91] version 128. Clustering, for OTU identification, was performed using VSEARCH using
545 the distance based greedy clustering (DGC) option as implanted in mothur and utilizing
546 0.97 sequence similarity cutoff. We also used a cutoff of one read that was subtracted from
547 all OTU read counts to guard against overestimation of sample richness. Rarefaction curves
548 were generated using the package vegan [92] as implemented in R version 3.4.4 [93] to assess
549 diversity and suitability of depth of coverage per sample. The resulting OTU table was
550 utilized in further data analyses as follows.

551 Exploring the data: Bar-graphs for relative abundance data based on the resulting
552 OTU table were generated using the ggplot2 [94] package in R. These plots were generated
553 for the data at the genus and the family levels and meant to describe the microbial
554 community structure per sampled infant and per time point under each of the treatment
555 levels.

Data were normalized using cumulative sum scaling (CSS) [95] prior to beta diversity and log-fold change analyses. Nonmetric Multidimensional Scaling (NMDS) [96] was used on the OTU level to assess possible trends and clustering in the microbial community structure comparing the two countries, the treatment conditions and the two time points, using the vegan package and utilizing Bray-Curtis dissimilarity [96]. Data were separated per country and the metagenomeSeq [97] package in R [93] was used to fit a zero inflated normal (ZIN) model to test for log-fold change differences between the rice bran treatment and control per age group. Benjamini and Hochberg's [98] false discovery rate (FDR) method was used to correct for multiple testing and compute the adjusted p-values used to determine significance of differences in the log-fold change of OTU abundance .

Stool metabolomics analysis

Stool samples were sent to Metabolon Inc. (Durham, NC, USA) for non-targeted metabolite profiling via ultrahigh-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). All samples were accessioned into the Metabolon Library Information Management Systems (LIMS) and prepared using the automated MicroLab Star® system (Hamilton Company, Switzerland). Eight to ten recovery standards were added prior to the first step in the extraction process for quality control purposes. Extraction was performed using 80% ice-cold methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation to remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix. Each stool extract was divided into five fractions: two for analysis by two separate reverse phase UPLC-

579 MS/MS methods with positive ion mode electrospray ionization, 1 for analysis by reverse
580 phase UPLC-MS/MS methods with negative ion mode electrospray ionization, 1 for
581 hydrophilic interaction liquid chromatography UPLC-MS/MS with negative ion mode
582 electrospray ionization, and 1 sample for backup. All samples were placed briefly on
583 Concentration Evaporator (TurboVap® Zymark) to remove organic solvent. UPLC-
584 MS/MS methods utilized a Waters ACQUITY ultra-performance liquid chromatography
585 and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced
586 with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated
587 at 35,000 mass resolution. Raw data was extracted, peak-identified and processed for
588 quality control using Metabolon's hardware and software.

589 **Statistical analysis**

590 Statistical analyses for anthropometric measures (length, weight, LAZ, WAZ, and WLZ)
591 and stool EED biomarkers were completed using SAS 9.4 (Cary, NC, USA). The sample
592 size was calculated for achieving greater than 85% power and based on expected changes in
593 selected stool metabolites following dietary rice bran consumption for one month[25].
594 Normality was evaluated by visual inspection. For anthropometric variables, two-sample t-
595 tests were used to compare means for the 2 treatment groups (rice bran and control)
596 separately at birth and 6 months (prior to start of treatment). A repeated measures analysis
597 was performed for each response variable separately using SAS Proc Mixed. Specifically,
598 treatment (rice bran or control) and age (6, 8 or 12 months), and treatment-age interaction
599 were included in the model as fixed effects. The participant was included as a random
600 effect to account for repeated measures. At each age, treatment groups were compared
601 using contrasts of the model. A similar repeated measures analysis was used EED

602 biomarkers, but log transformation was used to satisfy model assumptions. For stool
603 metabolites, Welch's two-sample t-test was used to analyze statistical significance between
604 groups' stool metabolites, after participating in the 6-month dietary trial. A p-value of
605 ≤0.05 was used for statistical significance. An estimated false discovery rate (q-value) was
606 calculated to account for the multiple comparisons across metabolites that are typical of
607 metabolomic-based studies.

608

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917

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Table 1. Baseline infant participant characteristics from Nicaragua and Mali.

Variable	Nicaragua			Mali		
	Control (n=24)	Rice bran (n=23)	p-value[3]	Control (n=24)	Rice bran (n=24)	p-value[3]
Sex (%)						
Male	14 (58.0)	12 (52.0)	0.6711	12 (50)	12 (50)	1
Female	10 (42.0)	11 (48.0)		12 (50)	12 (50)	
Water source (%)						
Indoor municipal	24 (100)	23 (100)	--	0 (0)	0 (0)	--
Untreated ground water	0 (0)	0 (0)	--	24 (100)	24 (100)	--
Delivery type (%)						
Vagina	11 (45.8)	16 (69.6)	0.099	100 (100)	100 (100)	--
Caesarean	13 (54.2)	7 (30.4)		0 (0)	0 (0)	
Sanitation System						
None	0(0)	1(4.3)	--	0(0)	0(0)	--
Community latrine	0(0)	0(0)	--	21(87.5)	19(79.2)	--
Latrine	4(16.7)	9(39.1)	--	3(12.5)	5(20.8)	--
Indoor toilet	20(83.3)	13(56.5)	--	0(0)	0(0)	--
Mother education (%)						
None	1(4.2)	0(0)	--	12(50)	11(46)	--
Some primary	3(12.5)	7(30.4)	--	4(17)	7(29)	--
Completed primary	3(12.5)	2(8.7)	--	6(25)	1(4)	--
Some secondary	8(33.3)	5(21.7)	--	1(4)	2(8)	--
Completed secondary	4(16.7)	5(21.7)	--	1(4)	3(13)	--
University	5(20.8)	4(17.4)	--	0(0)	0(0)	--
Breastfeeding Status (%)						
6 months	23 (95.8)	19 (82.6)	0.1415	24 (100)	24 (100)	--
Antibiotic Use (6-12 months)						
# Infants antibiotic use	14 (58.3)	11 (47.8)	--	14 (58.3)	13 (54.2)	--
Antibiotic courses	21 (58.3)	15 (41.6)	--	26 (51.0)	25 (49.0)	--
Household Animals[1]						
Poultry	3(12.5)	9(37.5)	0.3583	21(88)	21(88)	0.4091
Livestock	2(8.3)	2(8.7)		21(88)	17(71)	
Domesticated pets	17(70.8)	16(69.6)		5(21)	1(4)	
None	7(29.2)	5(21.7)		1(4)	2(8)	
Anthropometry[2,4]						
Weight at Birth (kg)	3.17±0.39	2.94±0.38	0.0505	3.07±0.45	3.29±0.49	0.1146
Weight 6 months (kg)	8.09 ±1.10	7.93 ±0.89	0.5833	7.02±0.88	7.14±0.99	0.4832
Length Birth (cm)	50.67±1.93	49.55±3.03	0.1472	49.77±1.56	50.50±2.04	0.1789
Length 6 months (cm)	66.38 ±2.10	66.26 ±2.90	0.8787	65.57±2.56	66.56±3.12	0.2960
LAZ 0 months (cm)	0.87±0.93	0.37±1.60	0.2045	0.12±0.88	0.45±1.18	0.3289
LAZ 6 months (cm)	-0.03±0.82	0.07±1.29	0.7325	-0.30±1.70	-0.15±1.46	0.7427
WAZ 0 months (cm)	-0.30±0.85	-0.82±0.89	**0.0497**	-0.48±1.04	0.02±1.06	0.1389
WAZ 6 months (cm)	0.33±1.09	0.27±0.98	0.8412	-0.65±1.29	-0.64±1.09	0.9827
WLZ 0 months (cm)	-1.54±1.41	-2.07±1.63	0.2585	-0.92±1.31	-0.39±1.49	0.2571
WLZ 6 months (cm)	0.53±1.25	0.41±0.96	0.7107	-0.51±0.95	-0.58±1.05	0.7963

[1]More than one category may be represented per household. [2]Mean ± standard deviation. [3]p-value:Chi-squared test.
[4]Anthropometric p-values calculated by two-sample t-test.

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Table 2. Anthropometric measures adjusted by treatment and age in Nicaraguan and Malian Infants

Indicator	Nicaragua				Mali			
	Control		Rice Bran		Control		Rice Bran	
	n=24[1]	p-value[2]	n=23[1]	p-value[2]	n=24[1]	p-value[2]	n=24[1]	p-value[2]
Length-for-age Z-score								
Months								
6	-0.03 (0.17)	0.8689	0.07 (0.27)	**0.0000**	-0.30 (0.35)	0.9165	-0.15 (0.30)	0.1111
8	-0.13 (0.14)		1.18 (0.26)		-0.20 (0.23)		0.19 (0.25)	
12	-0.73 (0.18)	**0.0098**	0.35 (0.21)	**0.0002**	-0.58 (0.22)	0.1609	0.01 (0.22)	0.6229
Weight-for-age Z-score								
Months								
6	0.33 (0.22)	0.5648	0.27 (0.20)	0.3335	-0.65 (0.26)	0.4575	-0.64 (0.22)	**0.0001**
8	0.22 (0.23)		0.11 (0.20)		-0.44 (0.23)		-0.02 (0.21)	
12	-0.03 (0.20)	0.0558	0.11 (0.18)	0.9919	-1.10 (0.24)	**0.0001**	-0.44 (0.21)	**0.0175**
Weight-for-length Z-score								
Months								
6	0.53 (0.26)	0.8997	0.41 (0.20)	**0.0000**	-0.51 (0.19)	0.8419	-0.58 (0.21)	**0.0141**
8	0.44 (0.28)		-0.54 (0.22)		-0.36 (0.21)		-0.05 (0.20)	
12	0.41 (0.22)	0.9892	-0.06 (0.18)	0.0569	-1.18 (0.24)	**0.0000**	-0.59 (0.22)	**0.0134**

[1]Mean (SEM) [2]Ajusted p-value by repeated measures for each treatment and time point (6-8 and 8-12 months) LAZ: Length for Age Z-score, WAZ: Weight for Age Z-score, WLZ: Weight for Length Z-score.

Table 3. Environmental enteric dysfunction (EED) biomarkers in stool at 6, 8, and 12 months of age for Nicaraguan and Malian infants.

	Nicaragua			Mali		
EED Biomarker	**Control[1] (n=24)**	**Rice Bran[1] (n=23)**	**p-value[2]**	**Control[1] (n=24)**	**Rice Bran[1] (n=24)**	**p-value[2]**
Neopterin (nmol/L)						
6	150.8 (182.2)	208.6 (131.7)	0.7220	20.2 (31.0)	34.6 (28.0)	0.5929
8	222.3 (241.1)	144.8 (196.9)	0.5771	36.2 (27.6)	28.9 (28.5)	0.4314
12	137.5 (285.2)	182.4 (230.4)	0.8727	12.0 (33.0)	36.0 (32.6)	0.9880
Myeloperoxidase (ng/ml)						
6	277.0 (374.5)	237.5 (376.5)	0.0847	3970.6 (17776.6)	5400.9 (20794.8)	0.6139
8	331.1 (312.3)	266.3 (236.4)	0.8454	15838.7 (20511.9)	7451.0 (12972.7)	0.3763
12	182.0 (324.8)	158.5 (376.7)	0.3345	4846.4 (11266.9)	3153.9 (14095.2)	0.5437
Calprotectin (*μ*g/g)						
6	32.2 (108.7)	88.0 (281.1)	0.2394	51.7 (101.2)	53.3 (106.4)	0.7136
8	24.7 (87.5)	58.2 (213.2)	0.8023	130.0 (769.7)	68.7 (126.8)	0.0639
12	24.0 (74.4)	50.5 (133.9)	0.2629	35.0 (70.2)	20.9 (56.0)	0.2103
Alpha-1 Antitrypsin (ng/ml)						
6	130.1 (177.7)	109.5 (217.4)	0.7199	247.5 (499.6)	463.1 (891.8)	0.0999
8	152.0 (115.4)	73.5 (122.4)	0.1221	619.2 (759.0)	579.9 (899.9)	0.2237
12	130.9 (129.8)	70.8 (87.8)	**0.0368**	663.7 (580.5)	453.2 (807.5)	0.4796

[1]Median (IQR)

[2]p-value by repeated measures comparing treatments at each time point.

Table 4. Stool metabolites significantly modulated by rice bran supplementation compared to control for Nicaragua & Mali infants at 8 months of age.

Metabolic Pathway	Metabolite[1]	HMDB[2]	Nicaragua		Mali	
			Fold Diff[3]	p-value	Fold Diff[3]	p-value
Cofactors and Vitamins						
Nicotinate and Nicotinamide Metabolism	Quinolinate	HMDB00232	0.88	0.7272	0.44	0.0313
	Nicotinate	HMDB01488	1.15	0.3958	1.6	0.0053
Tocopherol Metabolism	**alpha-tocotrienol**	HMDB06327	0.69	0.3922	3.01	0.0168
	gamma-tocotrienol	HMDB12958	0.67	0.1909	2.53	0.0044
	gamma-CEHC glucuronide*		0.41	0.0035	0.96	0.8862
Vitamin B6 Metabolism	**pyridoxine (Vitamin B6)**	HMDB02075	1.58	0.3051	4.65	0.0011
	Pyridoxate	HMDB00017	0.86	0.5441	2.34	0.0014
Xenobiotics						
Benzoate Metabolism	**4-hydroxybenzoate**	HMDB00500	0.88	0.6260	1.8	0.0272
	methyl-4-hydroxybenzoate	HMDB32572	0.57	0.0299	0.9	0.7061
	3-(4-hydroxyphenyl)propionate	HMDB02199	1.08	0.8898	6.94	0.0007
Xanthine Metabolism	**Theophylline**	HMDB01889	0.86	0.6266	0.44	0.0091
Food Component/Plant	**Indoleacetylaspartate**	HMDB38666	1.1	0.7543	2.14	0.0134
	Vanillate	HMDB00484	0.82	0.5775	2.27	0.0246
	deoxymugineic acid		0.6	0.2733	4.5	0.0021
	dihydroferulic acid		0.63	0.4522	6.99	0.0032
	Ferulate	HMDB00954	1.24	0.6403	3.5	0.0100
	ferulic acid 4-sulfate	HMDB29200	1.14	0.8494	4.88	0.0274
	ferulylglycine (1)		0.4	0.0256	2.16	0.0665
	Rosmarinate	HMDB03572	0.57	0.0206	1.28	0.3008
	Tyrosol	HMDB04284	1.01	0.9832	1.98	0.0412
	Diosmetin	HMDB29676	0.29	0.0192	1.25	0.6872
	daidzein sulfate (2)		0.39	0.0123	1.38	0.4129
	daidzein sulfate (1)		0.35	0.0023	1.04	0.9083
	Salicylate	HMDB01895	1.78	0.0970	4.67	0.0000
	N-propionylmethionine		1.09	0.8631	3.6	0.0111
	malonylgenistin		0.51	0.0023	0.99	0.9670
Drug - Analgesics, Anesthetics	4-acetamidophenylglucuronide	HMDB10316	0.99	0.0484	1	1.0000
	2-methoxyacetaminophen glucuronide*		0.77	0.0049	1	1.0000
Amino Acid						
Glycine, Serine and Threonine Metabolism	**Glycine**	HMDB00123	0.66	0.0062	1.2	0.2384
	dimethylglycine	HMDB00092	0.69	0.2880	0.42	0.0153
Lysine Metabolism	N6-formyllysine		0.6	0.1944	2.37	0.0385
Phenylalanine Metabolism	**phenylpyruvate**	HMDB00205	0.59	0.0332	1.18	0.5305
	phenyllactate (PLA)	HMDB00779	0.44	0.0223	1.49	0.2855
Tyrosine Metabolism	**4-hydroxyphenylpyruvate**	HMDB00707	0.55	0.0210	0.92	0.7455
	vanillic alcohol sulfate		0.7	0.2572	2.01	0.0378
Tryptophan Metabolism	**kynurenate**	HMDB00715	0.48	0.0079	1.13	0.6565

	N-formylanthranilic acid	HMDB04089	1.2	0.5416	0.51	0.0321
	indolepropionate	HMDB02302	4.67	0.0189	1.33	0.6727
Leucine, Isoleucine and Valine Metabolism	**alpha-hydroxyisocaproate**	HMDB00746	0.45	0.0325	1.05	0.8904
	alpha-hydroxyisovalerate	HMDB00407	0.47	0.0335	1.08	0.8323
	3-methyl-2-oxobutyrate	HMDB00019	0.52	0.0299	1.4	0.2751
	2-hydroxy-3-methylvalerate	HMDB00317	0.46	0.0224	1.14	0.7078
Methionine, Cysteine, SAM and Taurine Metabolism	**N-acetylmethionine**	HMDB11745	0.89	0.8154	3.24	0.0214
	N-formylmethionine	HMDB01015	0.56	0.2035	2.84	0.0294
	cysteine	HMDB00574	0.66	0.0498	1.29	0.2445
	hypotaurine	HMDB00965	0.52	0.0439	0.9	0.7512
Urea cycle; Arginine and Proline Metabolism	**dimethylarginine (SDMA + ADMA)**	HMDB01539	0.53	0.0064	0.92	0.7445
Glutathione Metabolism	**5-oxoproline**	HMDB00267	0.5	0.0085	1.27	0.3819
	2-hydroxybutyrate/2-hydroxyisobutyrate		0.45	0.0153	0.73	0.3554
Peptide						
Gamma-glutamyl Amino Acid	**gamma-glutamylglutamine**	HMDB11738	0.49	0.0278	1.05	0.8844
	gamma-glutamyl-epsilon-lysine	HMDB03869	0.49	0.0137	0.84	0.5553
Carbohydrate						
Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	**pyruvate**	HMDB00243	0.46	0.0237	1.26	0.5202
Disaccharides and Oligosaccharides	3-sialyllactose	HMDB00825	0.8	0.0484	1	1.0000
	Lewis a trisaccharide		1.36	0.0443	0.99	0.9520
Energy						
TCA Cycle	**alpha-ketoglutarate**	HMDB00208	0.65	0.1785	1.96	0.0484
Lipid						
Fatty Acid, Dicarboxylate	pimelate (C7-DC)	HMDB00857	0.93	0.7811	2.13	0.0078
Fatty Acid Metabolism (Acyl Choline)	palmitoloelycholine		1.17	0.1301	0.74	0.0070
	linoleoylcholine*		1.85	0.0459	1.5	0.2118
Fatty Acid, Monohydroxy	**8-hydroxyoctanoate**	HMDB61914	0.75	0.1576	1.61	0.0224
Fatty Acid, Dihydroxy	**12,13-DiHOME**	HMDB04705	0.89	0.7839	2.78	0.0246
	9,10-DiHOME	HMDB04704	0.9	0.7816	3.75	0.0012
Monoacylglycerol	1-linolenoylglycerol (18:3)	HMDB11569	2.36	0.0457	1.01	0.9871
Diacylglycerol	linoleoyl-linolenoyl-glycerol (18:2/18:3) [1]*	HMDB07249	2.04	0.0285	1.18	0.6201
	linolenoyl-linolenoyl-glycerol (18:3/18:3) [2]*	HMDB07278	2.29	0.0404	0.76	0.5121
	linoleoyl-docosahexaenoyl-glycerol (18:2/22:6) [1]*		1.03	0.8045	0.66	0.0006
	linoleoyl-docosahexaenoyl-glycerol (18:2/22:6) [2]*	HMDB07266	0.98	0.9291	0.59	0.0165
Sphingolipid Metabolism	**N-acetylsphingosine**	HMDB04950	1.11	0.7987	2.44	0.0387
Mevalonate Metabolism	**3-hydroxy-3-methylglutarate**	HMDB00355	0.74	0.4206	2.77	0.0098
Sterol	**beta-sitosterol**	HMDB00852	0.77	0.3699	2.89	0.0006
	stigmasterol	HMDB00937	1.14	0.6191	2.01	0.0159
	campesterol	HMDB02869	0.86	0.6689	2.1	0.0415

Androgenic Steroids	**5alpha-androstan-3alpha,17alpha-diol disulfate**		0.56	0.0199	0.63	0.0625
	androstenediol (3beta,17beta) disulfate (2)	HMDB03818	0.71	0.2185	0.54	0.0324
Primary Bile Acid Metabolism	**glycocholate**	HMDB00138	0.39	0.0483	1.37	0.5237
	glycochenodeoxycholate	HMDB00637	0.43	0.0434	0.64	0.3052
	glycochenodeoxycholate glucuronide (2)		0.37	0.0195	0.78	0.5717
	glycochenodeoxycholate sulfate		0.57	0.2283	0.35	0.0284
Secondary Bile Acid Metabolism	7alpha-hydroxycholestenone	HMDB01993	0.64	0.0411	0.88	0.5716
Nucleotide						
Purine Metabolism, Adenine containing	N6-dimethylallyladenine		0.94	0.8249	0.28	0.0000
Purine Metabolism, Guanine containing	**Guanine**	HMDB00132	0.47	0.0876	3.22	0.0112
Pyrimidine Metabolism, Orotate containing	**N-carbamoylaspartate**	HMDB00828	1.14	0.5814	0.59	0.0326
Pyrimidine Metabolism, Uracil containing	**uridine-2',3'-cyclic monophosphate**	HMDB11640	1.27	0.3081	1.75	0.0243
Pyrimidine Metabolism, Thymine containing	**3-aminoisobutyrate**	HMDB03911	1.23	0.6961	0.27	0.0175

[1]Table displays metabolites with statistically significant differences between rice bran and control group in stool. Bold metabolites are present in the rice bran (Calrose) that the children consumed.
[2]HMDB refers to the Human Metabolome Database.
[3]Fold differences (Fold Diff) between study groups was calculated by dividing the scaled relative abundance of rice bran vs control.

Figures:

Fig. 1. Study recruitment and participation based on CONSORT statement guidelines for clinical trials conducted in Nicaragua and Mali (NCT02615886, NCT0255737315). 95 infants from León, Nicaragua and Dioro, Mali enrolled after meeting eligibility criteria, randomized by sex and location to one of two study arms. The number of diarrhea episodes and reasons for withdrawal were reported for each child.

Fig. 2. Anthropometric Z-scores for Nicaraguan and Malian infants in rice bran and control groups at 6, 8 and 12 months. A). Significant LAZ ($p<0.05$) at 8 and 12 months in the rice bran group compared to control for Nicaraguan infants. B). No WAZ significant changes between rice bran and control group in Nicaraguan and Malian infants. C). WLZ at 8 months was significantly lower for the rice bran group compared to control in Nicaragua.

Fig.3. Rice bran and control infant stool microbiome at 8 and 12 months of age in Nicaragua and Mali. Nonmetric Multidimensional Scaling (NMDS) for A). Nicaragua and Mali all samples B). Control groups and rice bran groups at 8 and 12 months. NMDS was used on the OTU level to assess possible trends and clustering in the microbial community structure per treatment and time point. C). Bacterial taxa at phylum and family level in Nicaragua (top) and Mali (bottom). Bar-graphs show phylum and family relative abundance based on the resulting OTU table generated using the ggplot2 package in R. These plots were generated for the data at the phylum and the family levels and meant to describe the

1009 microbial community structure per sampled group and per time point (8 months and 12
1010 months) under each of the treatment levels (control and rice bran).
1011
1012 **Fig. 4. Microbiome differences between Nicaragua and Mali at 8 and 12 months**
1013 **between rice bran and control groups.** Fold differences in relative percentage of OTUs
1014 different between control and rice bran groups at 8 months and 12 months. A) Nicaragua,
1015 and B) Mali. OTUs with fold difference more than 2 are shown for infants at 8 months
1016 (left) and 12 months (right). Fold difference for OTUs with FDR less than 0.05 is shown
1017 with the most significant OTUs on the bottom of each graph.
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Figure 1.

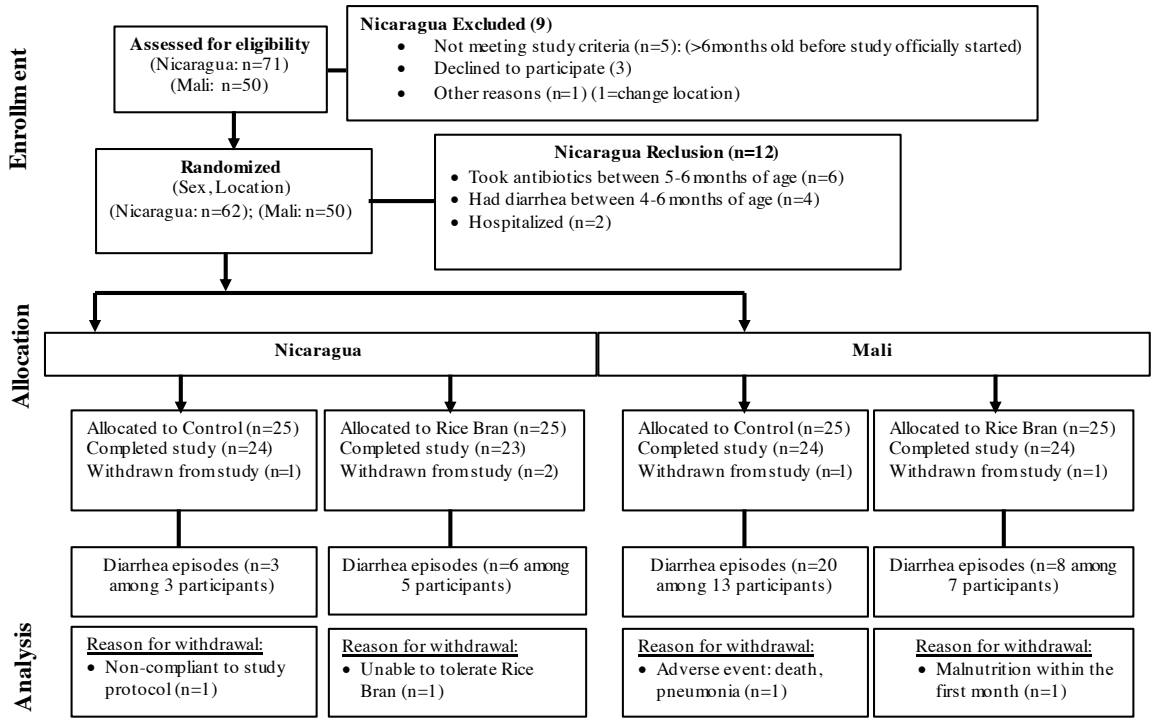

Figure 2

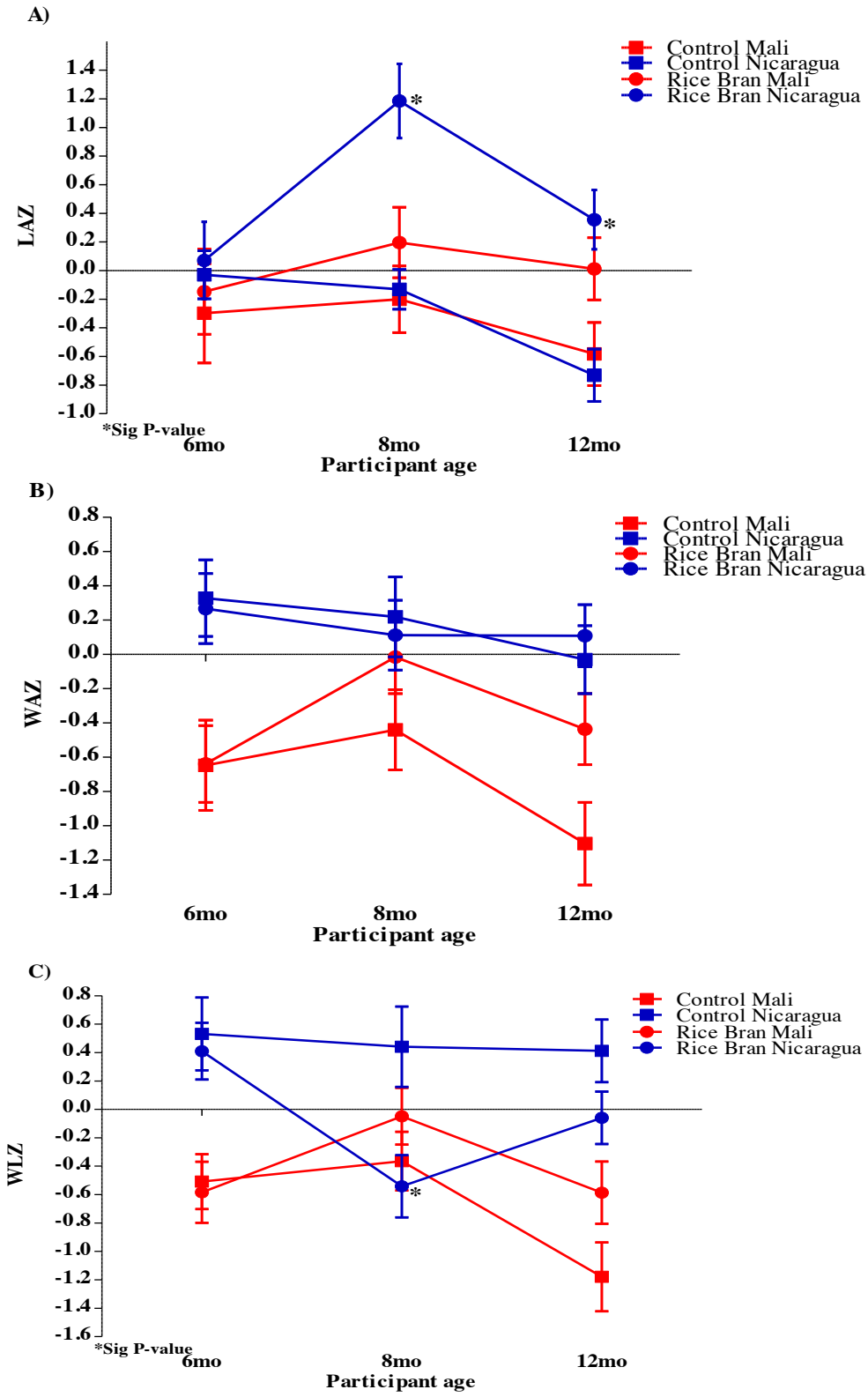

Figure 3

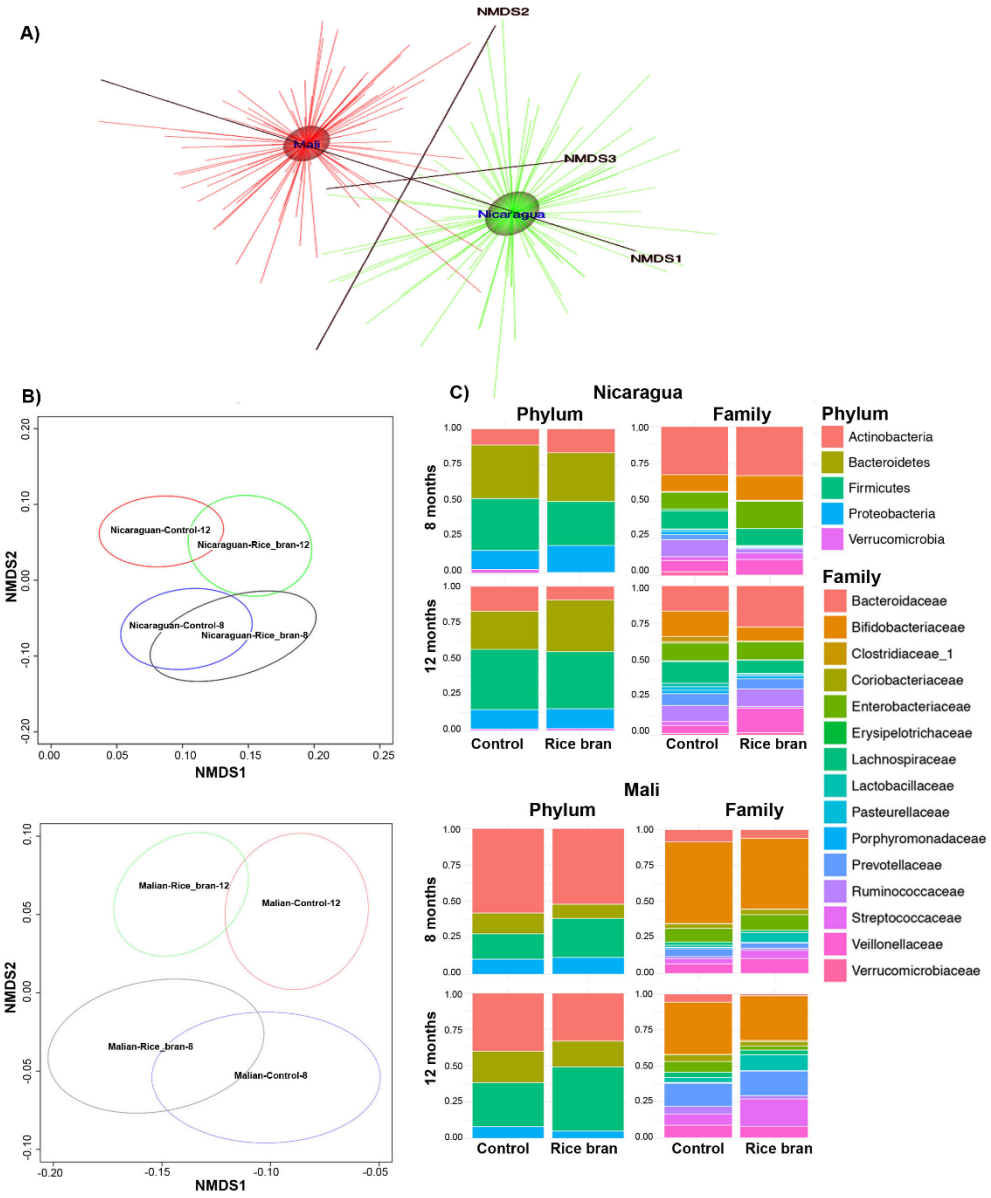

Figure 4.