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A Randomized Controlled Trial of Dietary Rice Bran Intake on Microbiota Diversity, Enteric Dysfunction, and Fecal Secretory IgA in Malian and Nicaraguan Infants

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ABSTRACT

Background: Malnutrition and diarrhea are leading causes of death in children aged <5 y. Rice bran is a nutrient-dense prebiotic available globally.

Objectives: The objective of this secondary analysis was to evaluate the effects of daily rice bran supplementation on environmental enteric dysfunction (EED) markers, total fecal secretory IgA (sIgA), and microbiota in infants at high risk of malnutrition.

Methods: Six-month-old Malian and Nicaraguan infants were randomly assigned to control or daily rice bran supplementation cohorts (1 to 5 g/d). Feces were collected monthly for 6 mo to evaluate fecal sIgA, markers of EED, and microbiota diversity. Statistical methods included linear mixed models, generalized mixed models, Spearman correlation, and Wilcoxon rank-sum tests.

Results: Six-month-old Malian infants had significantly elevated sIgA (4.0× higher, $P < 0.001$), fecal myeloperoxidase (31.6× higher, $P < 0.001$), fecal α1-antitrypsin (1.8× higher, $P = 0.006$), and lower fecal neopterin (0.13× higher, $P < 0.001$) than the age-matched Nicaraguan infants. In the Nicaraguan rice bran cohort from 6 to 12 mo of age, there was a significant decrease in sIgA concentrations (0.4×, $P < 0.05$) and a correlation between sIgA and the EED marker α1-antitrypsin (0.523, $P < 0.0001$) at 12 mo of age. In Malian infants, daily rice bran ingestion resulted in decreased EED scores (0.71×, $P = 0.02$) and a stable sIgA concentration over time. The rice bran group of Malian infants also had correlation between sIgA and the EED marker neopterin (0.544, $P < 0.001$) at 12 mo of age and a significant ($P < 0.05$) increase in microbiota α-diversity at a younger age (9 mo with rice bran compared with 10 mo in control group), which supports earlier microbiota maturation.

Conclusions: These results support rice bran as a functional food ingredient targeting gut mucosa in children at high-risk of malnutrition. *J Nutr* 2022;152:1792–1800.

Keywords: rice bran, prebiotic, environmental enteric dysfunction, fecal secretory IgA, Mali, Nicaragua

Introduction

Diarrheal disease is the second leading cause of death and a major causative factor of malnutrition in children aged <5 y worldwide (1). Low- and middle-income countries are disproportionately affected (2). Malnutrition predisposes to decreased epithelial barrier function and microbial dysbiosis, which can increase the risk of diarrhea and worsen malnutrition (3, 4). Malnutrition-associated impairments to anthropometric child growth can have lifelong health and cognitive implications (5).

Children at risk of malnutrition can also experience environmental enteric dysfunction (EED), a subclinical condition characterized by increased inflammation and permeability of the small intestine (6). EED contributes to undernutrition and malnutrition, and has been associated with a decreased response to vaccination (7, 8). Although the cause of EED has not been fully elucidated, many of the associated risk factors are similar

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to those that cause diarrhea, such as repeated exposure to enteric pathogens and microbial dysbiosis (9, 10).

Rice is a major source of calories worldwide. One of the major by-products of rice milling is rice bran (8–12%), which is often used as animal feed or treated as waste (11, 12). Rice bran includes many bioactive components that have positive health benefits and anti-inflammatory effects (12–14). We published the findings of a longitudinal phase I study in infants from Nicaragua and Mali who were supplemented with rice bran as an intervention for healthy children at risk of malnutrition, diarrhea, and EED (15). This study showed that daily rice bran supplementation was safe and well tolerated. Furthermore, rice bran supplementation increased anthropometric weight- and length-for-age *z*-scores in infants from both countries. These findings suggest that dietary rice bran consumption in low- and middle-income countries could be effective for prevention of malnutrition and stunting.

To further assess the effects of rice bran supplementation on the intestinal mucosa and local immune system of these children from 6 to 12 mo of age, we measured the concentration of the predominant humoral antibody at mucosal surfaces, secretory IgA (sIgA). sIgA helps to protect the mucosa from pathogens, regulates mucosal inflammation and tolerance, and plays an important role in modulating the intestinal microbiota (16, 17). A wide range of sIgA concentrations have been found in undernourished/malnourished children, yet total fecal sIgA concentrations in children at risk of EED have not been previously studied (18).

The objective of this secondary analysis was to evaluate the total fecal sIgA of Malian and Nicaraguan infants with and without dietary rice bran supplementation. Total fecal sIgA was correlated with the fecal EED markers neopterin (NEO), myeloperoxidase (MPO), calprotectin (CAL), and α1-antitrypsin (AAT) as well as gut microbiota diversity to assess the effect of rice bran supplementation on the gut mucosa.

Methods

Study design and sample collection

Details of this phase I rice bran intervention trial have been previously described (15) (NCT02557373 and NCT02615886). Heat-stabilized rice bran was fed daily for 6 mo with dose escalation by age (1 g/d at 6 mo of age, 2 g/d for 7 mo of age, 2–3 g/d for 8 mo of age, 3 g/d for 9 mo of age, 4 g/d for 10 mo of age, and 5 g/d for 11 mo of age; trial completed at 12 mo of age) to infants from León, Nicaragua and Dioro, Mali. Fifty healthy 6-mo-old infants were enrolled and randomly assigned into control (no intervention) and rice bran intervention groups at each site. Three participants withdrew from Nicaragua ($n = 47$) and 2 children withdrew from Mali ($n = 48$). The ethical reviews and approvals were provided by the Internal Review Board of the Colorado State University Research Integrity and the Compliance Review office (protocol ID# 14-5233 H Nicaragua, 15-5744 H Mali). For Mali, approvals were from Institut National de Recherche en Santé Publique (FWA 00000892; occurred between October 2015 and May 2016). The Nicaragua intervention occurred between March 2015 and October 2015 with approvals from Universidad Nacional Autónoma de Nicaragua—León, University of North Carolina at Chapel Hill, and Virginia Polytechnic Institute and State University. Both studies were conducted with concern for the ethical treatment of participants, and informed consent from the parents/guardians of all participating infants was obtained prior to the start of the trial. Rice bran procurement and further details on the clinical trial participants are available in **Supplemental Methods**.

Feces were collected from diapers at 6, 8, and 12 mo of age for the Nicaraguan control group. The Nicaraguan rice bran group and all Malian infants had fecal samples collected monthly. One Nicaraguan infant from the rice bran group did not have a sufficient amount of feces collected, and was omitted for this secondary sIgA analysis. Additionally, fecal samples were collected after each incidence of diarrhea. Fecal samples were mixed with PBS plus 1% glycerol, homogenized, and then frozen at −80°C. Environmental and household factors were markedly different between the 2 countries and are summarized in **Supplemental Table 1**.

Stool EED marker analysis

The EED biomarkers were evaluated via ELISA on the frozen processed feces as previously described (15). A detailed description is available in Supplemental Methods. EED scores were calculated for both groups based on the published 4-component score index (19).

Total fecal sIgA ELISA

Frozen fecal samples were mixed with commercially available ProteaseArrest protease inhibitor cocktail (G-biosciences) and homogenized. Supernatants were run in duplicate at a starting dilution of 1:500–1000 in sample buffer (1% BSA in PBS and 0.05% Tween20) followed by eleven 1:2 dilutions in sample buffer on Greiner Bio-One high-binding microplates coated overnight with mouse antisecretory component (IgA) clone GA-1 (Sigma-Aldrich). Total fecal sIgA was measured via ELISA based on a previously published method (20). Total fecal sIgA was calculated by averaging the dilution factors that fell within the range of a standard curve (0 to 100 ng/mL) of purified secretory human IgA from colostrum (Sigma-Aldrich) run on each plate.

Fecal total sIgA and EED marker statistical analysis

Statistical analysis was performed using SAS 9.4 (SAS Institute). Total fecal sIgA and EED marker comparisons between countries at 6 mo were performed using Wilcoxon rank-sum test. All other statistical analyses were performed separately by country. Intracountry covariance was analyzed using χ^2 test. For total fecal sIgA and EED score, a mixed model analysis was done separately for each response variable and country. Fixed effects included treatment and age plus treatment × age interaction. Participants were included as a random effect to account for repeated measures. Pairwise comparisons were considered regardless of *F*-test results. Dunnett adjusted pairwise comparisons were used to compare the 7-, 8-, 9-, 10-, 11-, and 12-mo timepoints compared with 6 mo. Residual diagnostic plots were used to confirm model assumptions of normality and equal variance. For total fecal sIgA, log transformation was used to satisfy model assumptions. Wilcoxon rank-sum test was used to compare diarrhea episodes between cohorts using the total number of diarrhea episodes per child. To examine association between presence/absence of ≥1 diarrhea episode (within a given month) compared with total fecal sIgA, a mixed logistic regression was used. Specifically, diarrhea was used as a binary response. Predictors included log transformed total fecal sIgA and treatment. Spearman correlations were calculated for total fecal sIgA and each EED marker separately at each month timepoint. *P*-value determinations for statistical significance were set at <0.05 for all analyses.

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Supplemental Methods, Supplemental Table 1, and Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.

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Abbreviations used: AAT, α1-antitrypsin; CAL, calprotectin; EED, environmental enteric dysfunction; MPO, myeloperoxidase; NEO, neopterin; OTU, operational taxonomic unit; sIgA, secretory IgA.

Microbiota analysis

Infant stool samples were processed and sequenced as described by Zambrana et al. (15). A detailed description is available in Supplemental Methods. Pairwise comparisons of α-diversity across groups were performed for the Malian infants at 9–11 mo of age. This analysis was done using the nonparametric Wilcoxon rank-sum test and then adjusted for multiple comparisons using the Benjamini–Hochberg procedure (21, 22). Nonparametric testing was performed after the results of Shapiro–Wilk normality testing that indicated nonnormal distributions (23). The analysis was carried out in a similar manner to that published by Parker et al. (24). Spearman correlations were calculated for fecal sIgA compared with Chao1 and Shannon diversity indexes. A z test was used to compare correlations between treatment groups (using the Fisher z-transformation). The Bonferroni method was used to account for multiple testing for between-group comparisons.

We utilized metagenomeSeq to compare the log fold change between the different treatments (25). Data were first normalized using cumulative sum scaling (25). Taxa included those with ≥1 sequence read within ≥10 of the samples observed to guard against sparsity. We used the zero inflated Gaussian model for the analysis. Log fold changes were compared using empirical Bayes moderated t statistics calculated by means of the function eBayes and false discovery rate–adjusted P values. Log fold changes were considered significant when >2 and with a P value <0.01. Correlation between operational taxonomic units (OTUs) and total fecal sIgA concentrations was determined by a nonparametric Spearman correlation without correction for multiple testing. The statistical package used was R statistical software release 3.4.4 (26).

Results

Nicaraguan and Malian infant fecal sIgA and EED marker concentrations at age 6 mo

Malian and Nicaraguan infants' fecal samples at 6 mo of age were evaluated for total fecal sIgA and the fecal EED markers MPO, NEO, CAL, and AAT. The 6-mo-old infants from Mali had a significantly elevated total fecal sIgA concentration (4.0× higher, $P < 0.001$) compared with Nicaraguan children (**Figure 1**A). Additionally, the EED markers MPO (31.6× higher, $P < 0.001$) and AAT (1.8× higher, $P = 0.006$) were significantly elevated in the infants from Mali (Figure 1B). NEO was significantly elevated (7.7× higher, $P < 0.001$) in Nicaraguan infants. There were no significant differences detected in CAL concentrations between Malian and Nicaraguan infants. There was no effect of sex on total fecal sIgA concentrations and EED markers. Evaluation of intracountry covariance for mode of delivery, breastfeeding status, sanitation system, and household animals showed no differences between control and rice bran groups. Supplemental Table 1 indicates that differences do exist between Nicaragua and Mali participants with respect to mode of delivery, breastfeeding status, sanitation system, and water source. These are environmental conditions that might influence mucosal sIgA responses in infants.

Total fecal sIgA and EED scores in Malian and Nicaraguan infants with rice bran supplementation

For total fecal sIgA in Malian infants, we found evidence of a main effect of age ($F = 3.33$, $P = 0.004$) but not main effect of treatment ($F = 0.47$, $P = 0.495$) or age × treatment interaction ($F = 0.47$, $P = 0.834$). Sex was not found to have an effect when considered for covariance. In the Malian infants, total fecal sIgA for the control cohort was significantly elevated at months 9, 10, and 11 from 6 mo of age (**Figure 2**A). Analysis of the total fecal sIgA between the control and rice bran groups from 6 to 12 mo did not show statistically significant differences between the 2 cohorts (OR for rice bran compared with control = 0.5028, $P = 0.1561$). In the Nicaraguan infants, the total fecal sIgA from 6 to 12 mo of age had decreased in the rice bran cohort (0.4×, $P < 0.05$) (Figure 2A). There were no significant changes in sIgA over time in the control group, and no differences between the control and rice bran groups.

EED scores were calculated to determine the effect of rice bran supplementation on EED risk. For the EED scores in Malian infants, we found evidence of a main effect of age ($F = 4.62$, $P = 0.004$), but not main effect of treatment ($F = 0.35$, $P = 0.556$) or age × treatment interaction ($F = 1.11$, $P = 0.345$). In the Malian rice bran cohort, there was a significant decrease in EED scores from 6 to 12 mo of age (Figure 2B). For EED scores in Nicaraguan infants, we did not find evidence of a main effect of age ($F = 0.41$, $P = 0.665$), main effect of treatment ($F = 0.90$, $P = 0.349$), or age × treatment interaction ($F = 0.16$, $P = 0.857$). In the Nicaraguan infants, there were no significant differences in EED scores over time. No correlation was detected between total fecal sIgA concentration and EED score. There was a modest positive correlation between total fecal sIgA concentration and the EED markers NEO, MPO, CAL, and AAT for Mali, and MPO and AAT for Nicaraguan infants over time (**Table 1**).

Over the course of the 6-mo study, children from Mali had a total of 28 diarrhea episodes reported compared with 9 from Nicaragua (Figure 2C). For Malian infants, there was no difference in the total number of diarrhea episodes between the treatments (control median = 0, rice bran median = 0.5, $P = 0.106$). We found a weak positive association between presence/absence of ≥1 diarrhea episode (within a given month) compared with total fecal sIgA in Mali (OR corresponding to log sIgA = 1.387, $P = 0.068$). For total fecal sIgA in Nicaraguan infants, we found evidence of a main effect of age ($F = 3.11$, $P = 0.049$), but no main effect of treatment ($F = 0.05$, $P = 0.820$) or age × treatment interaction ($F = 1.74$, $P = 0.181$). Due to the limited number of total diarrheal events for the Nicaraguan infants, formal analysis of diarrheal episodes was not performed.

Microbiota diversity and composition in Malian infants with rice bran supplementation

The total fecal sIgA concentration of the Malian control cohort was increased at 9, 10, and 11 mo of age compared with 6 mo of age, and therefore measurements of microbiota α-diversity (Shannon diversity and Chao1) during 9–11 mo of age compared with 6 mo of age were assessed for both the control and rice bran groups. Age was the strongest driver of elevated α-diversity indexes for the Malian infants. Notably, both Shannon diversity and Chao1 were significantly increased at 9 mo of age in the rice bran group compared with 10 mo of age in the control group (**Figure 3**). There were no differences in α-diversity between the rice bran and control groups. Spearman correlations between total fecal sIgA and α-diversity indices for Malian infants in both groups by months of age are shown in **Table 2**, and with statistically significant differences in this correlation at 10 mo of age ($P = 0.009$).

The microbiota composition was compared between the control and rice bran groups at 6, 9, 10, and 11 mo of age and there were significant differences at the genus and species taxonomic level (**Supplemental Figure 1**, **Figure 4**). Nine OTUs were significantly different between the control and rice bran groups at 6 mo. Rice bran supplementation resulted in a generalized increase in the number of OTUs over time compared with the control. Evaluation of OTUs between the

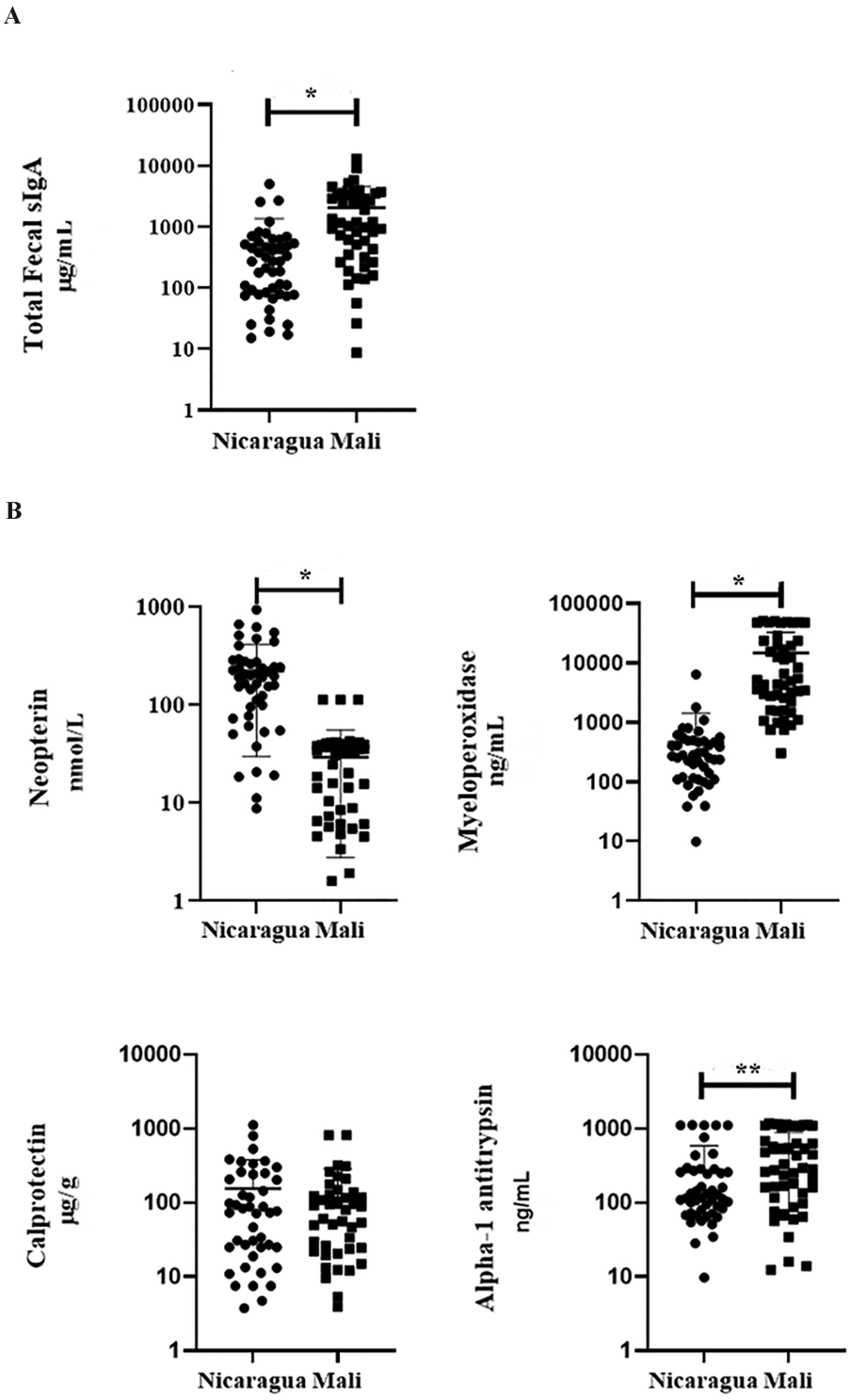

FIGURE 1 Total fecal sIgA (A) and EED markers (B) in 6-mo-old Malian ($n = 48$) and Nicaraguan ($n = 46$) infants. Values presented are mean ± SD. *,**Significant difference between the 2 cohorts: *$P < 0.05$, **$P < 0.001$. Data on the y-axis are $\log_{10}$ transformed. EED, environmental enteric dysfunction; sIgA, secretory IgA.

control and rice bran groups identified several bacterial genera and species that differed, such as increases in *Lactobacillus* spp. and *Veillonella* spp. Association between total fecal sIgA and the OTUs that were significantly different between control and rice bran are reported in **Supplemental Figure 2**. No bacterial genera or species were associated with total fecal sIgA concentrations over multiple timepoints or between the 2 cohorts.

Discussion

A secondary analysis was performed to evaluate total fecal sIgA in Malian and Nicaraguan infants with and without dietary rice bran supplementation. In the Nicaraguan infants, there was a decrease in total fecal sIgA from 6 to 12 mo in the rice bran group and no changes in total fecal sIgA over time in the control group. Dietary rice bran intake in

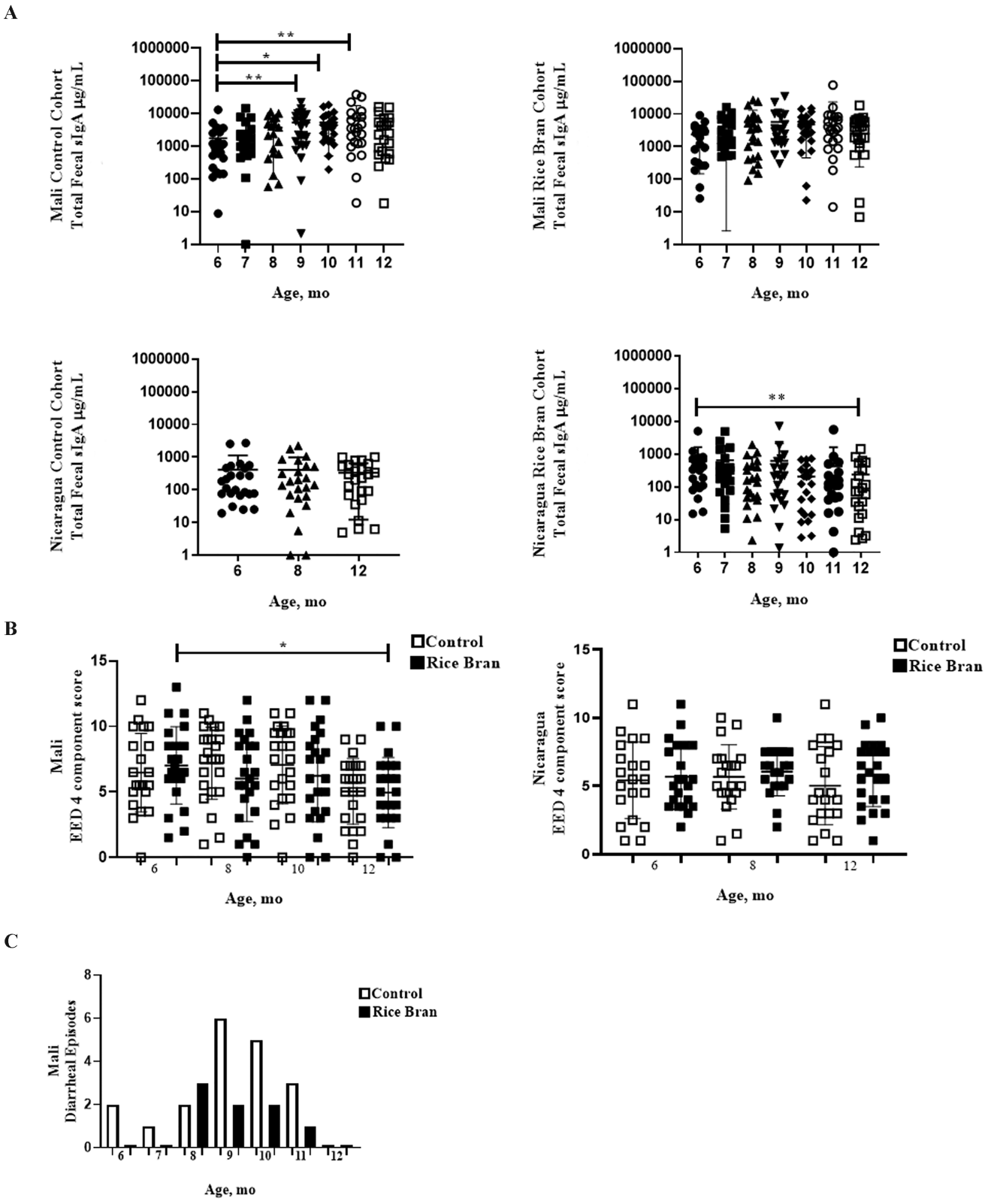

FIGURE 2 Total fecal sIgA (A) and 4-component EED scores (B) in Malian and Nicaraguan infants from 6 to 12 mo of age. Values presented are mean ± SD. Mali control cohort ($n = 24$), Mali rice bran cohort ($n = 24$), Nicaragua control cohort ($n = 24$), Nicaragua rice bran cohort ($n = 22$). Data on the y-axis are $\log_{10}$ transformed. Total number of diarrheal episodes in Mali children (C) are shown for both the control ($n = 20$) and rice bran ($n = 8$) groups. *,**Significantly different comparison: *$P < 0.05$, **$P < 0.001$. EED, environmental enteric dysfunction; sIgA, secretory IgA.

Mali resulted in decreased EED scores over time, unchanged total fecal sIgA concentrations over time, and 1-mo earlier age onset of increased microbiota α-diversity when compared with the Mali control group. A major finding from this analysis is that total fecal sIgA correlated with the fecal EED markers NEO, AAT, CAL, and MPO in Malian infants, and with MPO and AAT in the Nicaraguan infants. The association of total fecal sIgA with EED markers suggests that total fecal sIgA

TABLE 1 Spearman correlation for total fecal sIgA with markers of EED at 6, 8, 10, and 12 mo of age for all Malian ($n = 48$) and Nicaraguan ($n = 47$) infants[1]

	6 mo		8 mo		10 mo		12 mo	
EED biomarkers	Rho	*P* value	Rho	*P* value	Rho	*P* value	Rho	*P* value
Mali								
NEO	0.263	0.074	**0.458**	**0.002**	0.230	0.129	**0.544**	**<0.001**
MPO	0.263	0.163	0.175	0.267	**0.505**	**<0.001**	−0.0937	0.536
CAL	0.107	0.475	0.135	0.393	**0.371**	**0.012**	−0.157	0.298
AAT	**0.296**	**0.044**	0.117	0.460	**0.3367**	**0.026**	0.153	0.309
Nicaragua								
NEO	**0.290**	**0.048**	−0.075	0.615	NA	NA	0.233	0.133
MPO	0.283	0.054	**0.408**	**0.004**	NA	NA	0.267	0.083
CAL	0.110	0.463	−0.093	0.533	NA	NA	−0.011	0.944
AAT	0.271	0.065	**0.401**	**0.005**	NA	NA	**0.523**	**<0.001**

[1] AAT, α1-antitrypsin; CAL, calprotectin; EED, environmental enteric dysfunction; MPO, myeloperoxidase; NEO, neopterin; sIgA, total secretory IgA.

is elevated after increased intestinal inflammation and barrier dysfunction (19). Global variation for EED markers in children has been reported from studies spanning multiple countries, and provided important context for the significance of the associations observed herein for total fecal sIgA with EED markers (27–29).

Total fecal sIgA concentrations in individuals at risk of EED have not been previously published. Serum IgA has shown no

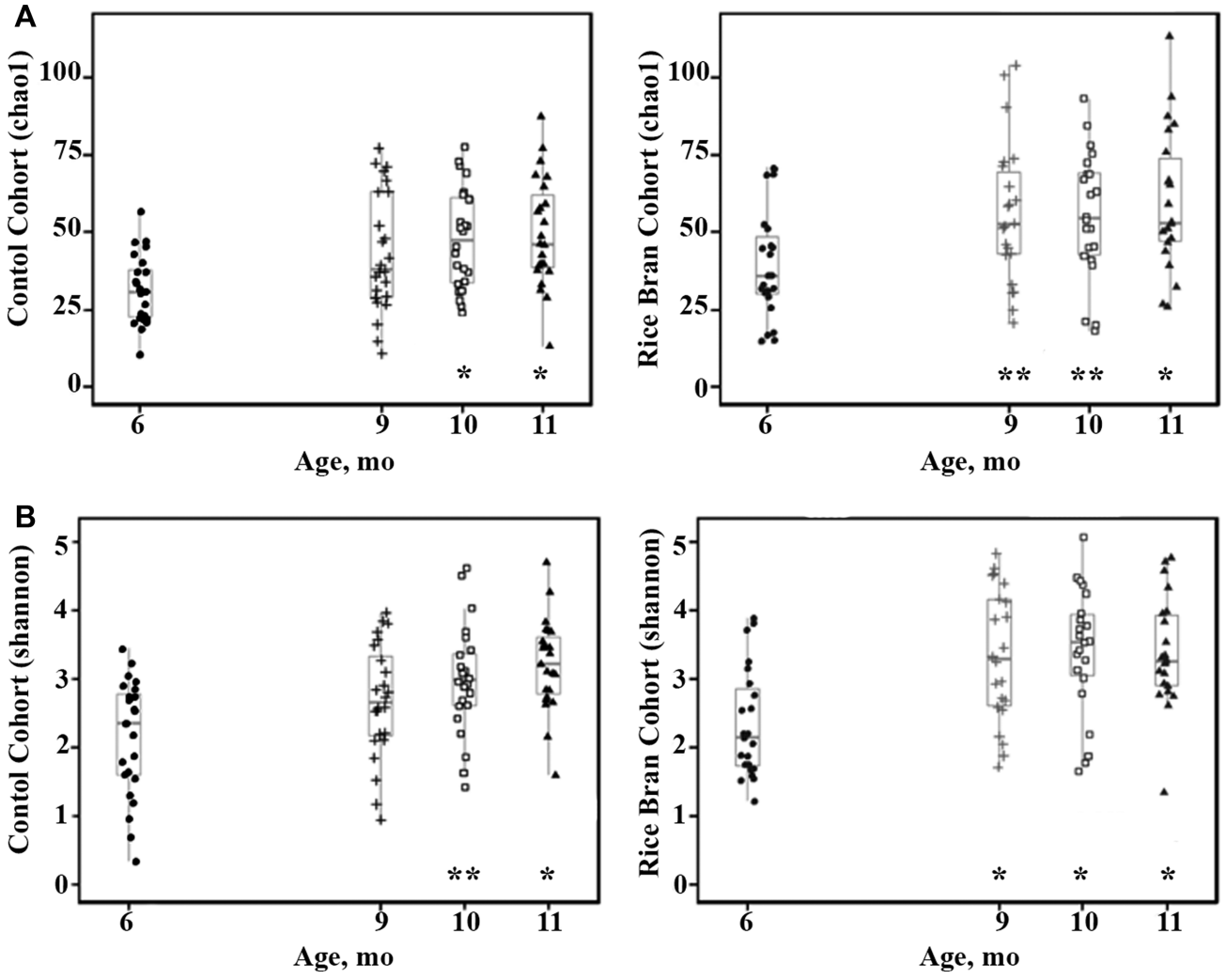

FIGURE 3 Fecal microbiota α-diversity index shown for Chao1 (A) and Shannon diversity (B) for the control and rice bran groups in Mali at 6, 9, 10, and 11 mo of age. Mali control cohort ($n = 24$), Mali rice bran cohort ($n = 24$), $^{*}P < 0.001$, $^{**}P < 0.05$. boxplots represent Q1, median, Q3. Q1 = Quartile 1 = 25th percentile. Q3 = Quartile 3 = 75th percentile. The Chao and Shannon index are comparing the 9, 10 and 11 months to 6 months of age.

TABLE 2 Spearman correlation between total fecal sIgA and α-diversity indices for Malian infants ($n = 48$) by months of age[1]

	Chao1					Shannon diversity				
	Control		Rice bran		Control vs. rice bran	Control		Rice bran		Control vs. rice bran
Age, mo	Rho	*P* value	Rho	*P* value	*P* value (Bon)	Rho	*P* value	Rho	*P* value	*P* value (Bon)
6	−0.038	0.865	0.098	0.663	1.000	−0.056	0.799	0.139	0.536	1.000
7	0.239	0.310	0.243	0.289	1.000	0.305	0.191	0.044	0.849	1.000
8	0.302	0.209	0.002	0.993	1.000	0.023	0.926	−0.137	0.532	1.000
9	−0.077	0.722	0.040	0.858	1.000	0.087	0.686	−0.027	0.904	1.000
10	0.226	0.299	−0.243	0.289	0.988	0.542	0.008	−0.413	0.063	**0.009**
11	0.278	0.200	−0.362	0.090	0.250	0.538	0.008	−0.120	0.587	0.158
12	−0.527	0.012	0.250	0.250	0.061	−0.220	0.326	0.130	0.553	1.000

[1]Bon, Bonferroni correction; sIgA, total secretory IgA.

association with fecal EED markers, and decreased vaccine-specific serum IgA has been reported in children with elevated EED biomarkers (19, 30, 31). Additionally, a large spectrum of IgA concentrations was reported for malnutrition, which can co-occur with EED (18). These variations are likely due to the complexity of the intestinal mucosal environment that is influenced by decreased barrier function, the presence or absence of enteric pathogens, and vitamin deficiencies. For example, hypovitaminosis A has been shown to result in decreased IgA concentrations (32). Conflicting findings between

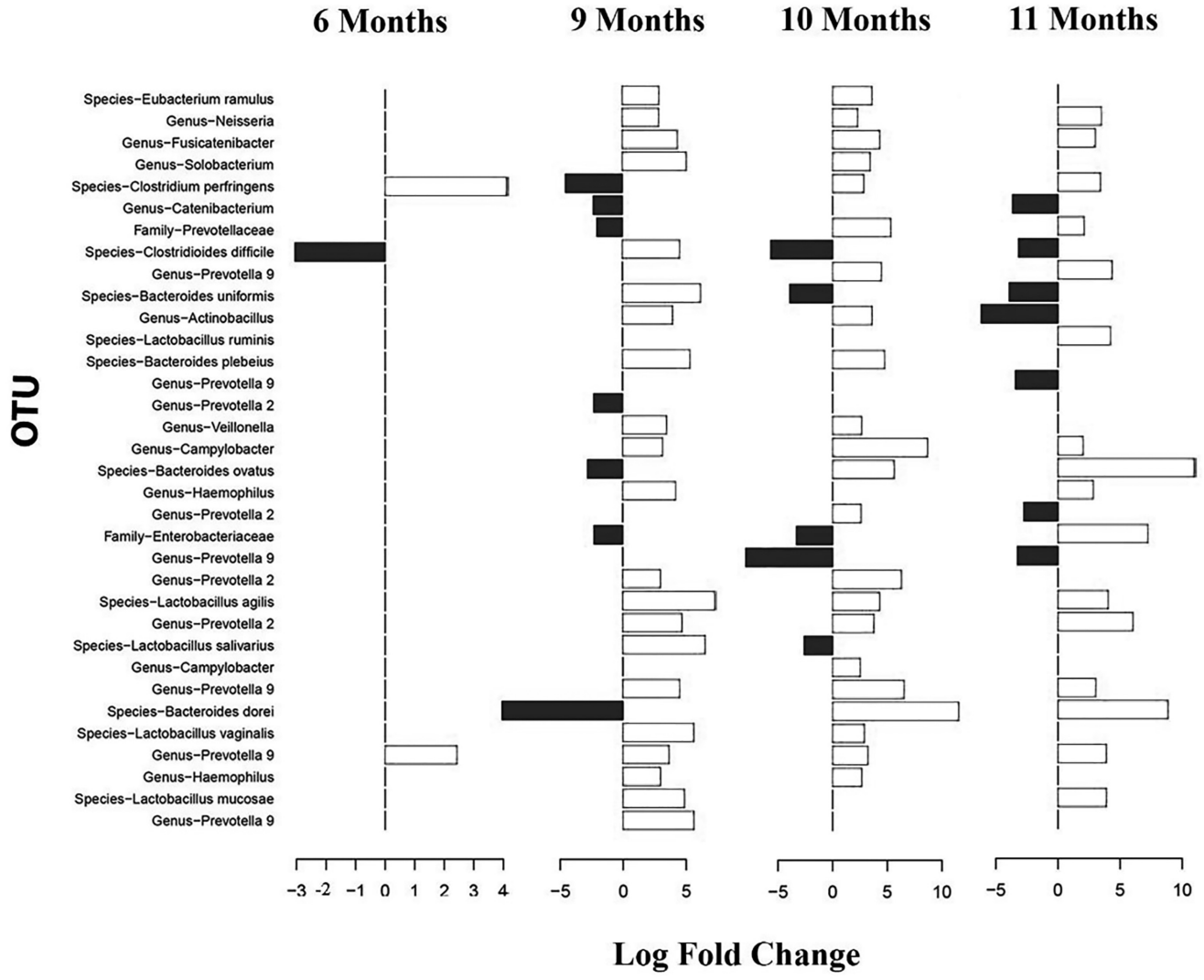

FIGURE 4 Malian infant log-fold gut microbiota differences between control and rice bran cohorts at 6, 9, 10, and 11 mo of age. White bars indicate the operational taxonomic units (OTUs) that were increased in rice bran group and black bars indicate the OTUs increased in the control group. Changes were considered significant if the log-fold change was >2 with a $P < 0.01$. The 34 genera and species are depicted out of 122 that were identified as significantly different over time (see Supplemental Figure 1 for complete list).

serum IgA and total fecal sIgA are not surprising given that fecal sIgA and serum IgA often do not correlate (33). In this study, differences in breastfeeding status, mode of delivery, and type of water source in Nicaragua and Mali might also play roles as covariates in the total fecal sIgA concentrations at 6 mo of age (Supplemental Table 1). All Malian children received vitamin A supplementation at enrollment. No other nutritional supplementation other than rice bran was provided, and it is unknown whether vitamin deficiencies could have also affected the differences observed between Mali and Nicaragua. The results reported herein for an association between total fecal sIgA with EED biomarkers hint at potential mechanisms.

The role of sIgA in maintaining mucosal homeostasis could explain the positive correlation between NEO, MPO, and AAT. In mice predisposed to colitis, sIgA increases as a compensatory mechanism to increased epithelial permeability (34). Decreased mucosal barrier function and elevated sIgA can help to restore the gut barrier by coating bacteria, which decreases local inflammation and maintains IL-10, an important cytokine for epithelial regeneration and integrity (35). Given that AAT is a measurement of epithelial permeability, it is possible that as AAT concentrations increase, sIgA could also increase to help restore mucosal barrier integrity. AAT was associated with total fecal sIgA concentrations in infants from both country cohorts even though the intestinal exposure to pathogens and diet is markedly different (36). NEO is released from macrophages and dendritic cells in response to T-cell secretion of IFN-γ (37). IFN-γ can also increase epithelial expression of the poly-Ig receptor responsible for IgA secretion into the intestinal lumen (38). The correlation between NEO and sIgA could be due to upstream regulation by IFN-γ. MPO is released from neutrophils making it an excellent marker of acute inflammation. MPO and AAT have been shown to be increased in children with enteric pathogens, suggesting a possible mechanism for the correlation observed herein (39). The association of total fecal sIgA with EED biomarkers suggests that the elevation of total fecal sIgA in the Malian control group could be related to restoration of the mucosal barrier in response to increased inflammation and/or permeability.

The microbiota changes from birth and matures into an adult-like composition around 2–5 y of age (40). The gut microbiota educates the developing mucosal immune system, which in turn helps to shape composition (41, 42). Many allergic and intestinal inflammatory diseases, as well as EED and malnutrition, are associated with an altered microbiota composition and function. Infant microbiota maturation involves increases in α-diversity (43, 44). In our cohorts, we found that age was the strongest predictor of the microbiota α-diversity. The Malian rice bran group α-diversity increased 1 mo earlier than the control group, suggesting a delayed maturation or regression in the control children. Diarrhea has been associated with decreases in α-diversity and regression in microbiota maturation (45, 46). The control infants' higher episodes of diarrhea might be responsible for the 1-mo delayed α-diversity increase. Total fecal sIgA concentration was associated with the diversity indexes in the control group and could be related to microbiota maturation differences between the control and rice bran groups over time. There are significant fold changes in bacterial species from Mali reported herein that have been associated with dysbiosis and chronic disease, including *Fusicatenibacter* spp., *Prevotella* spp., *Bacteroides* spp., and *Ruminococcus* spp. (47–49). Understanding the relation between total fecal sIgA, diversity indexes, and differences in bacterial species is challenging because interaction of the microbiota with the mucosal immune system is complex, involving not only direct interactions but also metabolite cross-feeding (50). Further evaluation of metabolites between cohorts and associations with OTUs could help to explain the correlation between total fecal sIgA and specific bacteria (51).

Study limitations are inherent in observational findings, secondary endpoint analyses, and multiple response variable testing. Further functional studies merit attention for these multiple, integrated secondary outcomes. Notably, this daily rice bran supplementation was not blinded for the guardian/infant participants and might have introduced bias in feeding regimens. Confounding variables that were not controlled for during implementation of the clinical trial included seasonal variations in food security among the households enrolled and differences in drinking water pathogens/pollutants between the 2 countries.

In conclusion, rice bran is a well-tolerated and promising food supplement that positively affects mucosal health and microbiota diversity. Long-term investigations of rice bran intake in children are needed to understand changes to EED, total fecal sIgA, and malnutrition risk. There are programs to increase rice production in Mali and Nicaragua, and the additional utilization of rice bran for complementary feeding in children could have major impacts for combating EED, food insecurity, and malnutrition.

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Conflicts of interest

There are no conflicts of interest to disclose.

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