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FEASIBILITY AND ACCEPTABILITY OF A SUPPLEMENTARY FOOD WITH ADDED FISH OIL AND CHOLINE FOR UNDERNOURISHED PREGNANT WOMEN IN SIERRA LEONE

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ABSTRACT

Maternal ready-to-use supplementary foods (M-RUSF) have been shown to improve birth outcomes among undernourished pregnant women. Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and choline have been hypothesized to further improve gestational duration and cognitive development. The primary objective of this study was to determine the acceptability and feasibility of a new formulation of M-RUSF, referred to as M-RUSF+, that included fish oil, containing about 400 to 500 mg DHA and EPA, and 550 mg choline among pregnant women in the Pujehun District of Sierra Leone. Both the control and the experimental supplementary foods contained 18 g of high quality protein and 580 kcal, with generous amounts of all essential micronutrients. The ration size was 100 g/d. Both intervention and control recipes were locally produced using peanut paste by the Project Peanut Butter Factory in Freetown, Sierra Leone. The fish oil was added to the M-RUSF+ after all the grinding had been accomplished to limit degardation. The study used a 2x2 crossover design to assess the acceptability of the M-RUSF and M-RUSF+ formulations. Pregnant women were assigned to one of two sequences of foods: either M-RUSF+ followed by M-RUSF, or M-RUSF followed by M-RUSF+. Each food was given for one week. Fifty-one pregnant women were enrolled (day 0), with data collection occurring on days 3, 7, 10 and 14 after enrollment. At each visit, women returned the packets, either empty or partially consumed. Acceptability was assessed at all follow-up visits based on overall consumption, likeability, and adverse events reported. Consumption of M-RUSF and M-RUSF+ averaged 99%. The likability scores averaged 4.8 and 4.9 out of 5 for M-RUSF and M-RUSF+, respectively. For 85/90 visits during which M-RUSF+ was being consumed and 77/83 visits during which M-RUSF was being consumed, the foods were rated with a likability score of 5. Adverse events, defined as diarrhea, vomiting or rash were infrequent (<7%) and showed no significant differences. These results suggest that both M-RUSF and M-RUSF+ are acceptable and feasible for use among pregnant women in the Pujehun District, Sierra Leone.

Key words: Maternal undernutrition, supplementary food, docosahexaennoic acid (DHA), eicosapentaenoic acid (EPA), choline

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INTRODUCTION

Maternal undernutrition occurs in nearly one-quarter of pregnancies in sub-Saharan Africa and has been associated with adverse outcomes including anemia, hemorrhages, and maternal death, as well as preterm birth, low birth weight (LBW), and impaired cognitive development of the offspring [1-3]. Some nutrient deficiencies that contribute to these adverse outcomes include the omega-3 long-chain polyunsaturated fatty acids (LC-PUFA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), as well as choline. Long-chain polyunsaturated fatty acid intake in most of sub-Saharan Africa is low [4-6]. Both DHA and choline are found primarily in animal-source foods, including certain fish as well as eggs and poultry, which are limited in the diets of those who lack access to good nutrition [7]. Antenatal DHA supplementation has been found to reduce early preterm and preterm birth and increase birth weight, although studies have not targetted women with lack of access to nutrition [8-10]. Supplementation with EPA can improve mood disorders, such as ante- and postpartum depression [11, 12]. Choline supports placental and offspring neurocognitive development, and antenatal supplementation with choline has been shown to improve markers of DHA status in offspring [13-16]. Previously, consumption of a maternal ready-to-use supplementary food (M-RUSF) increased maternal weight gain and produced offspring that were heavier, longer, and had reduced neonatal mortality by 55% [17]. By including DHA, EPA, and choline in M-RUSF, these outcomes may be increased.

Successful inclusion of fish oil, a good source of DHA and EPA, and choline in a processed food requires a mixing process that does not subject the ingredients to high temperatures or shearing forces that promote oxidation. Choline and fish oil both have bitter taste and distinct flavors and their consumption is associated with taste aversion in some populations. To mitigate this taste in a processed supplementary food, the other ingredients need to have strong, pleasant flavors that will promote consumption.

This study tested the hypothesis that adding fish oil and choline to a M-RUSF (hereafter, M-RUSF+) with peanut paste is feasible and acceptable among pregnant women in Sierra Leone.

MATERIALS AND METHODS

Food Development

Both the control M-RUSF and the intervention M-RUSF+ were modeled on a RUSF developed for and successfully used in a prior clinical trial of undernourished pregnant Sierra Leonean women [17]. In accordance with World Health Organization (WHO) guidelines, M-RUSF and M-RUSF+ were designed as

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balanced energy-protein supplements and provided similar quantities of micronutrients as the UNICEF/WHO/United Nations multiple micronutrient supplement required for pregnant and lactating women (UNIMMAP) [18, 19]. Previous studies had identified a recipe containing skimmed milk powder, whey protein isolate, vegetable oil, sugar, peanut paste, and pearl millet as the preferred ingredients in Sierra Leone, and so this combination was used for both foods [20].

The addition of fish oil and choline distinguished M-RUSF+ from the control M-RUSF. Two sources of LC-PUFA, fish oil and algae, were considered. Both have previously been investigated for use and stability in peanut paste-based ready-to-use foods [21]. The amounts and forms of the LC-PUFA were based on the aims of the planned clinical efficacy trial, in anticipation of which this acceptability study was undertaken. Because the efficacy trial was designed to assess the potential effects of the M-RUSF+ on both gestational age and ante- and postpartum depression, a formulation with both DHA and EPA in a triglyceride formulation was chosen to improve bioavailability, as compared with ethyl esters [22]. Omegavie® 3030 TG was chosen as the fish oil (Polaris, Quimper, France). The origin of the fish oil was wild small blue fish, primarily anchovies and sardines. It contained equal amounts of DHA and EPA in triglyceride form, according to the manufacturer. Systematic reviews have not conclusively identified a minimum dose of antenatal LC-PUFA required for benefit [10]. There is, however, a trend toward greater prevention of preterm birth and early preterm birth with DHA and EPA doses each ≥500 mg [9]. Thus, a daily dose of fish oil containing 500 mg DHA and 500 mg EPA was chosen to be contained within each 100 g foil sachet that made up the daily dose of M-RUSF+.

Both choline bitartrate and choline chloride were considered as possible chemical forms for inclusion into M-RUSF+. Based on informal taste tests by the research staff (n = 4) of peanut pastes made with 550 mg of either choline form within the Food Laboratory of Dr. Mark Manary at Washington University in St. Louis, MO, USA (hereafter, the Food Lab), choline chloride was chosen. The source of the choline was Balchem (Montvale, NJ, USA). The US Institute of Medicine recommends 450 mg/d of choline for pregnant women, 25 mg higher than for non-pregnant adults. There is evidence, however, that choline requirements in pregnancy are greater than currently appreciated, and that supplementation with 550 – 930 mg daily leads to increased choline activity as a 1-carbon methyl donor and improves biomarkers of DHA status and trafficking [16, 22-23]. Since undernourished pregnant women in Sierra Leone are likely to have low intakes of choline, 550 mg was selected as the daily dose to be sure to be well within the active range.

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Ten preliminary ingredient compositions for M-RUSF+ with modestly differing peanut, milk, and oil content were produced at the Food Lab and assessed with informal acceptability testing among research team members (n = 4). This preliminary step was done because it was expected that fish oil would impart a fishy taste, and choline a bitterness. It was hypothesized that different ratios of peanut, milk, and oil might mask these organoleptic changes with differing success. Final ingredient ratios were chosen by concensus and used for both M-RUSF and M-RUSF+. In addition, a small amount of choline was added to the control food to further reduce differences in taste and aftertaste between M-RUSF and M-RUSF+. The final lists of ingredients for M-RUSF and M-RUSF+ are shown in Table 1.

Both foods were produced at the Project Peanut Butter Factory in Freetown, Sierra Leone. LC-PUFA are sensitive to heat, light, and oxygen, and so require care during incorporation into the peanut-based pastes. A prior study showed that DHA is best preserved when added in the final phase of production, and so this was done [21]. Choline was added at the first mixing step to maximize incorporation into the paste. Final production, labeling, quality, and safety testing of the product were done at the Freetown factory which uses Good Manufacturing Practices (GMP) and was certified in GMP in 2023 by the Pharmacy Board of Sierra Leone (Freetown). The steps of the production process are shown in Figure 1. The foods were packaged in metallized polyethylene terephthalate sachets (Huhtamaki India Limited, Khopoli, India) containing 100 g of product.

Figure 1: Maternal ready-to-use supplementary food manufacturing process

Peanut, skim milk, pearl millet, whey protein isolate, palm oil, brown sugar, multiple micronutrient premix, and choline chloride were mixed in the planetary mixer (set on sweep mode: 30 rotations/min; Single Planetary,Vertical Design, Ross Indudtries, Hauppauge, NY, USA) before entering the disk mill (AC Horn 25 micron food grinder, Dallas, TX, USA). The disk mill is where the remaining larger particles are pulverized to create a homogenous mixture. The ribbon blender (360 kg capacity, custom fabricated by Anderson-Dahlen, Minneapolis, MN, USA) mixed

the M-RUSF at low speed and prepared it for packaging in stage 4. Fish oil was added into the ribbon blender and mixed for 10 min prior to packaging. Temperatures ranged from 45–70◦C between the end of the planetary mixer and the packaging machine phases (Vertical Form Seal Package, General Packaging, Houston, TX, USA).

Ten sachets of M-RUSF and M-RUSF+ were transported to the US and used for testing for nutrient content, peroxides, anisidine, dioxin, pesticides, *Enterobacteriaceae*, and *Salmonella* by the Nestle Purina Analytical Laboratories (St. Louis, MO, USA), while aflatoxin testing was done by Trilogy Analytical Laboratory (Washington, MO, USA). Further details of testing methodology are included in Supplement 1.

Six sachets each of M-RUSF or M-RUSF+ were analyzed at the laboratories of JT Brenna at the Dell Pediatric Research Institue (Austin, TX, USA) for their fatty acid content. Fatty acid methyl esters (FAME) were prepared from each sample utilizing a one-step digestion, transmethylation, and FAME extraction procedure modified from that described previously [24,25]. Briefly, a polar aqueous reagent consisting of CH_3OH, 2,2-dimethoxypropane (DMP), and H_2SO_4 (85:11:4 by volume) and organic reagents (heptane and toluene (63:37 by volume)) were used to treat 10 mg of sample for 2 hours at 80°C. FAME in the organic layer at the top are removed with a pipet to a clean vial and evaporatored under a stream of dry N2 with gentle heating. The FAME were dissolved into heptane (100 μL) and injected into a gas chromatography-flame ionization detector (GC-FID) for quantification according to previously reported methods [26]. The GC-FID was equipped with a BPX-70 capillary column (Trajan, Pflugerville, TX, USA), used H_2 carrier gas, and a temperature program. FAME response factors were measured daily using an equal weight calibration mixture GLC-462 (Nu-chek Prep, Elysian, MN, USA*), showing responses were within 5% among FAME. Results are reported as a percentage by weight of each fatty compared to the total fatty acids.

Participants

Eligible participants were a convenience sample of pregnant women at least 16 years old, presenting to the Zimmi antenatal clinic in the Pujehun District of southern Sierra Leone. Pregnant women were excluded if they reported having a chronic debilitating illness, abnormal blood pressure or blood sugars during their pregnancy, a history of a peanut or milk allergy, or reported currently receiving supplementary food from another study.

After an explanation of the study in their local language, Mende, all women 18 yr of age or older or who were married gave informed consent either by signing their name or, for those unable to write, by affixing their thumbprint. For unmarried

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women under 18 years, participant assent was obtained, and parent or guardian informed consent was also obtained. This study was approved by the Sierra Leone Ethics and Scientific Research Committee (Ministry of Health and Sanitation, Freetown) and the Washington University Human Studies Committee (St. Louis, MO, USA). The trial was registered under the Pan African Clinical Trials Registry (# PACTR202308825529445) at https://pactr.samrc.ac.za/

Clinical Trial Design

This study was a 2x2 crossover, unblinded, controlled food acceptability trial comparing M-RUSF to M-RUSF+. Participants were assigned to the sequence in which they would receive each food in an alternating manner. Participants were not informed of the order in which the foods would be allocated. The primary outcome was the amount of food consumed, which would be reported by participants by returning unused product. Secondary outcomes included the likeability of the foods, rated on a scale from 1-5 (Figure 2), with 1 representing "dislike very much" and 5 indicating "like very much", as well as adverse events, defined as diarrhea, vomiting, and rash that would be reported by participants. Fifty participants would provide 80% power to detect a difference in consumption of 5% at a two-sided α of 0.05 (R Foundation for Statistical Computing, Vienna, Austria).

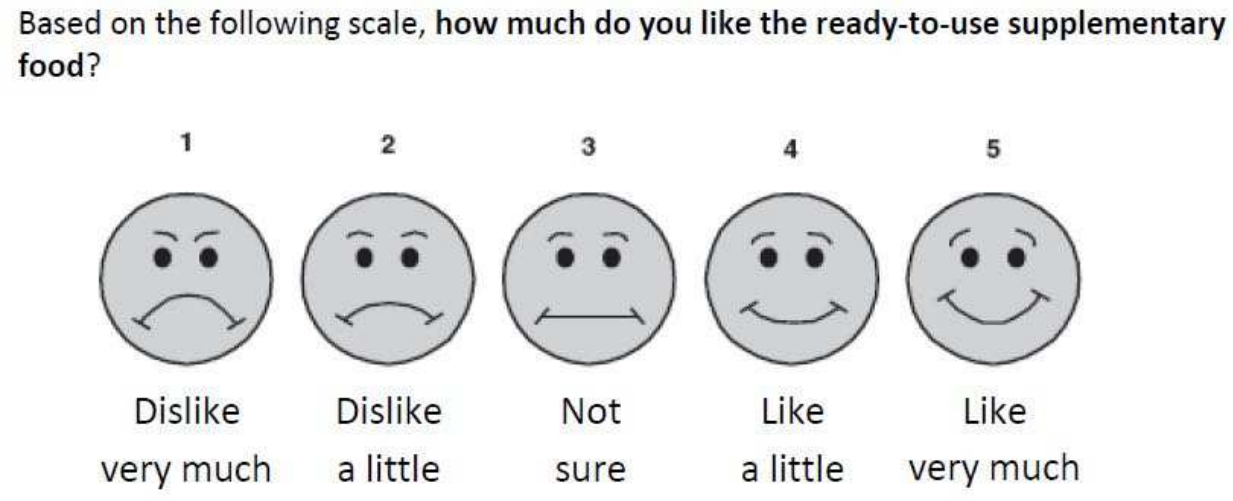

Figure 2: Likert scale for assessing likeability of the maternal ready-to-use supplementary foods

Participation

Upon enrollment, participants were given a unique identification number, answered demographic questions, and underwent measurements of height, weight, and mid-upper arm circumference (MUAC) by trained resesarch nurses. Using a sample foil sachet containing 100 g of the study food, the research nurses advised participants to massage the foil sachets thoroughly before opening them and informed participants that the foods were ready for consumption without additional

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preparation. Participants were educated that the foods could be squeezed out of the foil sachet directly into their mouths or, if they did not like to eat the food on its own, that it could be mixed with a hot porridge, most commonly made from rice in Sierra Leone. Participants were told to consume 1 sachet per day.

Each food was given for 1 wk for a total of 2 wk of feeding (Figure 3). At the beginning of the study, half of the women were given 3 sachets of M-RUSF and the other half were given 3 sachets of M-RUSF+. Participants returned for assessments of consumption, likeability, and adverse events on day 3, at which time they were given 4 sachets of the same food they had been consuming. Because of the low numeracy and literacy rates among participants, a visual Likert scale was used to complement the numbers and descriptions (Figure 2). During each assessment, empty or partially empty sachet wrappers were collected. On day 7, likeability, consumption, and adverse events assessments were repeated and the food provided was switched, with 3 more sachets given. Participants returned on day 10 for the same assessments as well as to receive 4 more sachets of food. The final study visit was on day 14 and consisted of the same assessments as prior, with no further food disbursement.

Statistical analyses

All analyses were done according to a modified intention-to-treat principle, wherein only participants who completed at least one follow-up visit were included in the outcomes analysis. Missing data were not imputed. From experience with prior acceptability trials in similar settings, it was expected that the amount of study food consumed and likeability scores would be distributed in a skewed manner. In addition, likeability scores are ordinal in nature. Thus, both variables were analyzed using the Wilcoxon signed-rank test. Adverse events were analyzed using the McNemar's chi-squared test. Significance was set at $P < 0.05$. Data were analyzed using R version 4.1.2 (R Foundation for Statistical Computing, www.r-project.org/foundation).

RESULTS AND DISCUSSION

Safety and contamination testing for M-RUSF and M-RUSF+ was done immedately after production in July 2023, which was 3 wk prior to study initiation. Neither food was contaminated with *Enterobacter* spp, *Salmonella*, aflatoxin, dioxin, or pesticides (Table 2). Nutrient content was tested 4 months after production. The supplementary food provided about 580 kcal/d and 18 g protein/d (Table 3). The micronutrient content provided 1-4 times the RDA for most vitamins and minerals that a well-nourished pregnant woman would need (Table 3). The choice for the dosage of micronutrients was based on the recipents in the planned efficacy clinical trial being expected to be moderately or severely malnourished pregnant

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women who, in addition to being protein and energy depleted, are likely depleted in all micronutrients. The micronutrients were prepared as a custom order by DSM Nutritional Products, Isando, South Africa.

The fatty acid content of M-RUSF and M-RUSF+ was very similar except for the presence of erucic acid, EPA, docosapentaenoic acid, and DHA in M-RUSF+ (Table 4). The amounts of DHA and EPA in the ration were 410 and 454 mg, respectively, with a nominal DHA:EPA ratio of 0.91. Fifty-one pregnant women were enrolled in July of 2023. The average age was 23 years. They most commonly were pregnant for the second time, and their average body mass index was 24 kg/m^2 (Table 5). Of the 51, 36 (71%) completed all 5 participant visits (Figure 3).

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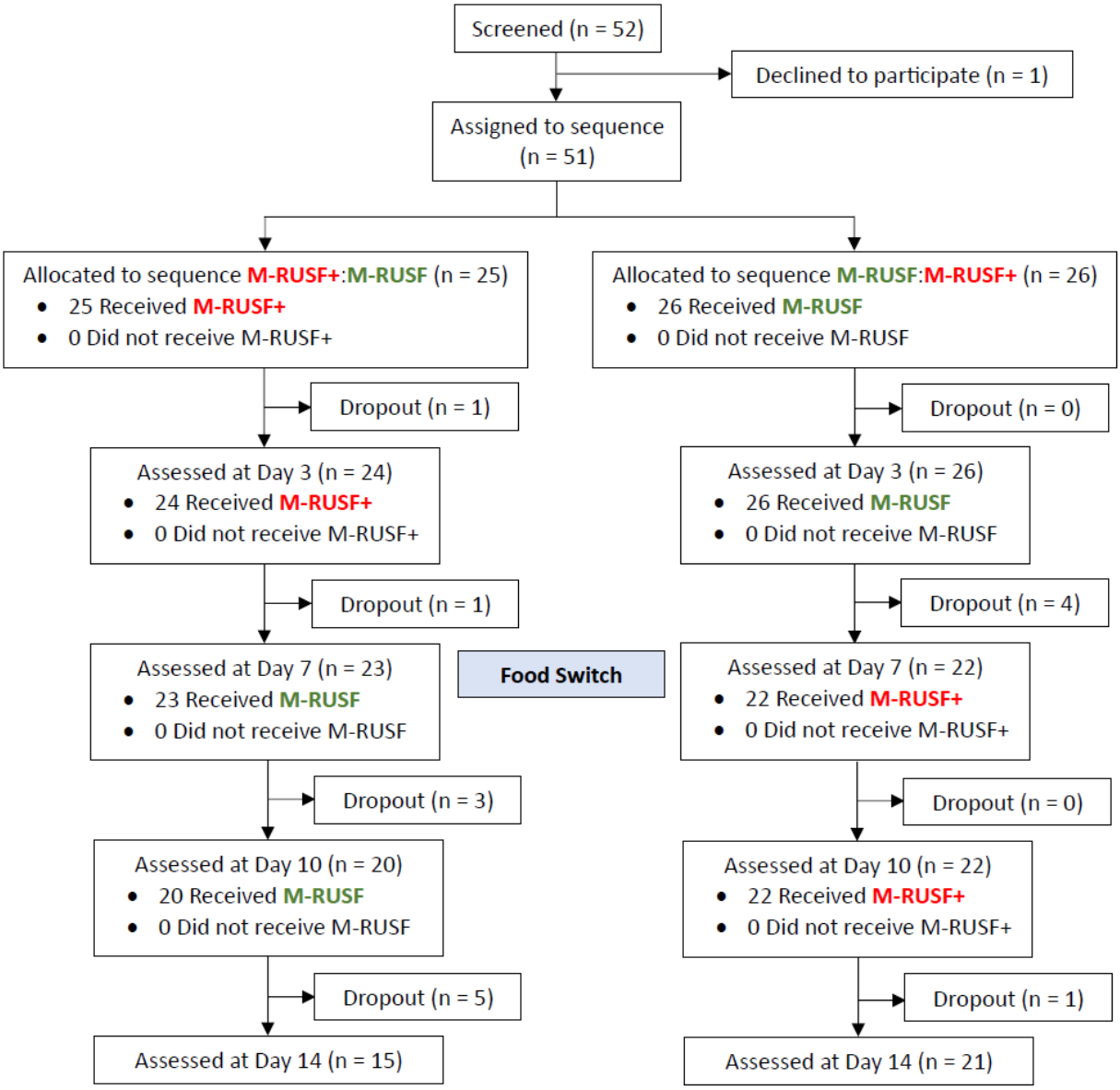

Figure 3: Study flow diagram of participation of pregnant women in supplementary feeding study

Dropouts were determined when a participant did not complete any subsequent visits. M-RUSF, maternal ready-to-use supplementary food; M-RUSF+, maternal ready-to-use supplementary food with added docosahexaenoic acid, eicosapentaenoic acid, and choline.

Nearly all participants reported consuming all the study food (Table 6). On the days when the women received M-RUSF, 96% reported consuming all the food, and on days when they received M-RUSF+, 98% of women reported consuming all the food (P = 0.20; Figure 3). The mean likeability score was 4.8 for M-RUSF and 4.9 for M-RUSF+ (P = 0.96; Table 6). For likeability, 93 and 94% of responses were 5

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on the 5-point likeability scale for M-RUSF and M-RUSF+, respectively (Figure 3). Reported symptoms of diarrhea (<7%), vomiting (<6%), and rash (0%) were rare and did not vary significantly between the two groups (Table 6).

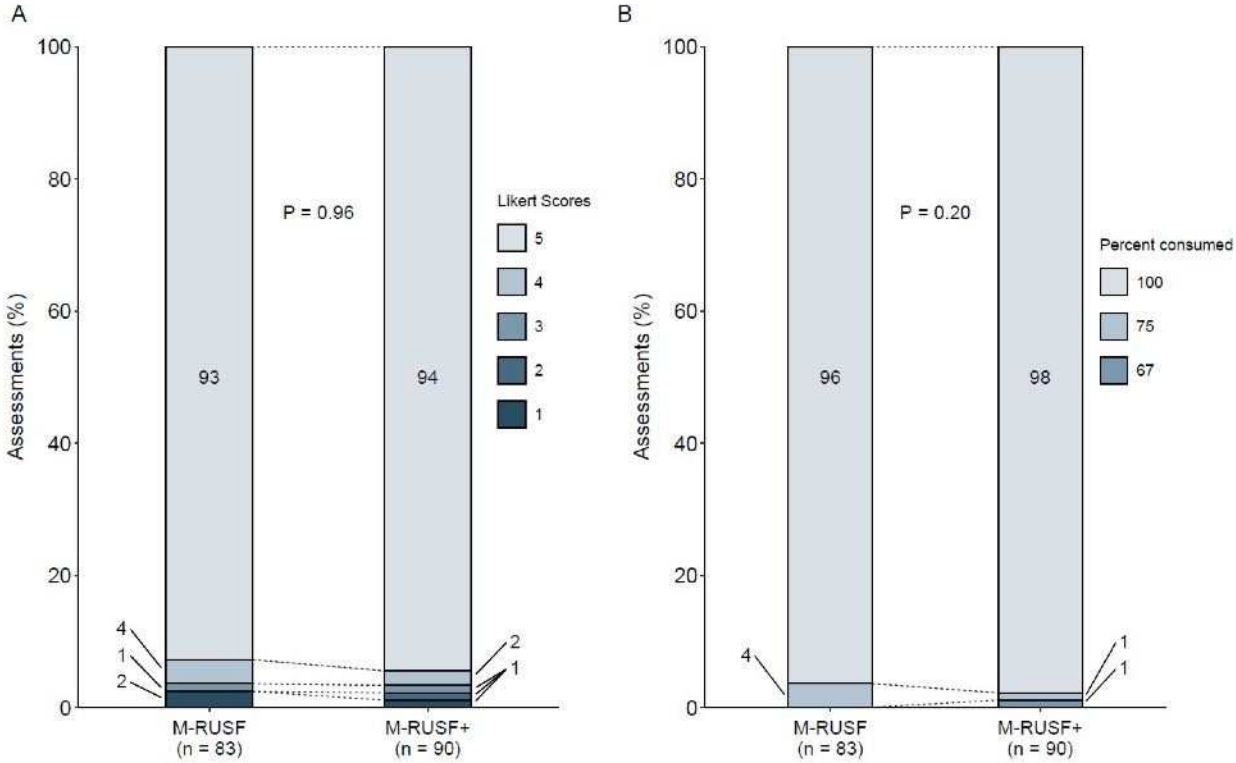

Figure 4: Likert scores (A) and food consumption (B) for the supplementary foods as reported at all study visits by pregnant Sierra Leonean women

Participants consumed both foods in a cross-over manner for 1 wk each and were asked twice during each wk about how much they liked the food. Consumption was estimated by measuring the amount remaining in the returned sachets and with a questionnaire. Four women receiving M-RUSF consumed about 75%, while 1 woman receiving M-RUSF+ consumed about 75% and 1 woman sonsumed about 67%. In the figure, the number under each barplot refers to the number of visits with data collected for that outcome. Both Likert scores and amount of food consumed were compared between the foods using the Wilcoxon signed-rank test. For Likert scores the Z value was{ -0.40 (*P*=0.69) and for consumption amounts Z value – 0.01 (*P*=0.98). This analysis indicates that likability and consumpiton amount were similar between the two foods. M-RUSF, maternal ready-to-use supplementary food; M-RUSF+, maternal ready-to-use supplementary food with added docosahexaenoic acid, eicosapentaenoic acid, and choline.

In summary, the omega-3 long chain fatty acids in the low moisture food matrix showed good stability over 4 months by meeting expected levels based on

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ingredient values. Women at the Zimmi clinic found both the M-RUSF and the M-RUSF+ products acceptable for consumption. The addition of DHA, EPA, and choline to the product did not significantly affect its acceptability in this cohort. On this basis, the planned efficacy trial can proceed without concern for the feasibility and acceptability of the novel supplementary food.

This study has multiple limitations. First, all participants were from one rural county in Sierra Leone. Salt and salt-based seasonings are popular in Pujehun District, and choline chloride may act as a salt taste enhancer, possibly increasing the likeability of the product. Fresh water fish are commonly available in Pujehun District, exposing the population to a fishy flavor profile that might reflect DHA and EPA, and potentially increasing the acceptability of M-RUSF+. Perhaps other areas where fish are not available would have lower acceptability or enjoyment of the food due to an unfamiliar flavor. Second, only 71% of participants completed all study visits. It is possible dropouts had different impressions of the foods than those who remained in the study. The rate of dropouts was higher during the M-RUSF than M-RUSF+ wk. This would suggest that any bias against M-RUSF+ was unlikely even for the dropouts. Third, assessment of consumption was done on the basis of participant report and returning of food sachets. Consumption was not directly verified and so sharing and disposal were possible. Fourth, while participants were not informed of their allocated sequence, full blinding was not verifiable, as there may have been taste and other differences detected by participants. Fifth, participants were assigned to sequence of foods in an alternating manner, rather than the sequence being randomized. The crossover design in which each participant acts as their own control should limit the degree to which this could have introduced bias, but such bias cannot be excluded. Sixth, due to the nature of the questionnaires given, the specific causes of reported adverse events could not be determined. Adverse events such as stomach pain and vomiting – although very rare within this study – may be common in pregnancy, or related to endemic diseases such as malaria or parasitic infection that are prevalent in this region. If caused by the food and occuring in the second wk, which product was at issue would be difficult to determine.

Seventh, although the Likert scales used were not novel to sensory perception or this population, it is possible that the interpretation of “Like Very Much” or “Dislike Very Much” may go beyond taste. The question specifically asked, both in writing and orally, “How much do you like the M-RUSF,” not “How much do you like the taste of the M-RUSF”. Perhaps women responded, “Like Very Much,” to indicate that they liked that the product was provided at no cost, for example, or that the product was nourishing their babies or healthy for themselves. The likeability score thus may not be based on taste alone. Accepting this limitation, however, the

results suggest that participants did consider both foods acceptable and were willing to eat them. In general, M-RUSF is well accepted by pregnant women in Sierra Leone [17]. Finally, a longer food stability study is needed to establish the shelf life of this omega-3 LC-PUFA supplement.

One potential innovation of this study is the route by which DHA and choline are usually given as a nutritional supplement. Typically, DHA and choline are provided as supplementary tablets or capsules to pregnant women, as opposed to being incorporated into a product such as was done with M-RUSF+. This incorporation is believed to be new and may enhance the functionality of DHA and choline, while providing complete nutrients in a convenient and shelf-stable package. Acceptability may have been influenced by taste perception or factors unrelated to this.

The larger scale study, Improving COgnition and GEstational duration with targeted NuTrition (COGENT; clinicaltrials.gov NCT05949190), aims to enroll 1600 participants and test the hypotheses that M-RUSF+ provided to pregnant women in Sierra Leone will extend gestational duration and improve infant cognition at 9 months of age when compared to M-RUSF. Secondary goals will be to evaluate if M-RUSF+ will reduce early and late preterm birth, increase birth length and weight, reduce neonatal mortality, and reduce ante- and postpartum depression scores when compared to M-RUSF.

In conclusion, a novel peanut paste-based maternal ready-to-use supplementary food with added DHA, EPA, and choline was feasible to produce, acceptable in a population of pregnant women from rural southern Sierra Leone, and did not appear to cause more adverse effects than a previouslty tested M-RUSF that did not contain added DHA, EPA, and choline.

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AUTHOR CONTRIBUTIONS
ASK, BG, CK, MM, SN. EK, KBS and MJM designed the study, BG, CK. MM and EK administered the intervention and collected the data, ASK, BG, CK, and MM analyzed the data, JTB analyzed the food composition, ASK, CK and MJM wrote the first draft of the manuscript, all authors edited the article. MJM is responsible for maintaining the integrity of the data.

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Table 1: Composition of the Two Supplementary Foods

Ingredient, g	M-RUSF	M-RUSF+
Pearl millet	7.3	7.5
Non-fat dry milk	21	21.5
Whey protein isolate	6.6	6.8
Palm oil	25.7	26.3
Brown sugar	17.6	18.0
Peanut	15.5	15.9
Fish oil (Polaris)	0	1.7
Choline chloride	0.02	0.80
Multiple micronutrient premix (DSM)[1]	3.9	4.0
Total	100	100

[1]The listing of the micronutrients in the premix follows in Table 3
M-RUSF, maternal ready-to-use supplementary food; M-RUSF+, maternal ready-to-use supplementary food with added docosahexaenoic acid, eicosapentaenoic acid, and choline

Table 2: Toxins and contaminants testing of both study foods

Contaminant	Amount measured	Specification from Codex Alimentarius
Aflatoxin, ppb	<5	< 10 ppb
Enterobacter spp	<10 cfu/g on 10 replicates	<10 cfu/g on 10 replicates
Salmonella	None detected in 375 g	None detected in 375 g
Peroxide value, meq/kg fat	5.31	none specified
Anisidine value	5.55	none specified
Dioxin, ng/kg	0.104	2 ng/kg
Organophosphates and chlorinated pesticides and PCBs[1]	All tests <0.0100 ppm	< 0.0100 ppm

[1]Tests for aldrin, alpha-hexachlorocyclohexane, beta-hexachlorocyclohexane, chlordane Dichlorodiphenyldichloroethylene, , dichlorodiphenyltrichloroethane, delta-hexachlorocyclohexane, diazinon, dieldrin, disulfoton, endrin, ehtion, hexachlorobenzene, heptachlor, heptachlor epoxide, lindane, malathion, methoxychlor, methyl parathion, mirex, parathion, polychlorinated biphenyls, thimet, thiodan and thrithion

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Table 3: Measured nutrient composition of both study foods

Nutrient/Component	Chemical form in micronutrient premix	Measured nutrient content of 100 g ration	Recommended Daily Allowance
Moisture, %		2.3	
Energy, Kcal		578	
Protein, g (Kjedahl method, 6.25 conversion factor)		18.0	
Fat, g		31.0	
Vitamin A, mg	palmitate	1250	770
Vitamin B1/Thiamine, mg	thiamine mononitrate	4.08	1.4
Vitamin B2/Riboflavin, mg	riboflavin	3.54	1.4
Vitamin B3/Niacin, mg	niacinamide	34.9	18
Vitamin B6, mg	pyridoxine HCl	4.06	1.9
Vitamin B12, μg	cyanocobalamin	7.2	2.6
Folic acid, μg		874	400
Vitamin C, mg	ascorbic acid	89.7	85
Vitamin D, mg	cholecalciferol	12.8	15
Vitamin E, mg	DL α-tocophenol acetate	44.7	15
Iron, mg	ferrous fumarate	30.8	27
Calcium, mg	calcium carbonate	1190	1000
Copper, mg	copper gluconate	1290	1000
Iodine, μg	potassium iodide	177	220
Magnesium, mg	magnesium oxide	336	350
Potassium, mg		5420	4700
Selenium, μg	sodium selenite	226	60

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Table 4: Fatty acid composition of maternal supplementary foods

Omega fatty acid nomenclature	Common name	Amount in M-RUSF (%)[1]	Amount in M-RUSF+ (%)[1]
10:0	Capric acid	0	0
12:0	Lauric acid	0.18 ± 0.02	0.16 ± 0.05
12:1	Dodecenoic acid	0.08 ± 0.05	0.05 ± 0.02
14:0	Myristic acid	0.44 ± 0.06	0.49 ± 0.05
14:1	Myristoleic acid	0.07 ± 0.04	0.04 ± 0.02
16:0	Palmitic acid	29.1 ± 0.2	27.9 ± 0.3
16:1	Palmitoleic acid	0.11 ± 0.04	0.17 ± 0.02
18:0	Stearic acid	4.39 ± 0.05	4.3 ± 0.1
18:1n-9	Oleic acid	54.2 ± 0.4	52.7 ± 0.4
18:1n-7	Vaccenic acid	0	0
18:2n-6	Linoleic acid	9.1 ± 0.1	8.7 ± 0.1
18:3n-6	Gamma-linoleic acid	0	0
18:3n-3	Alpha-linolenic acid	0.35 ± 0.31	0.34 ± 0.40
20:0	Arachidic acid	0.44 ± 0	0.47 ± 0.02
20:1	Gadoleic acid	0.59 ± 0.01	0.62 ± 0.01
20:2n-6	Eicosadienoic acid	0	0
20:3n-6	Dihomo-gamma-linoleic acid	0	0
20:4n-6	Arachidonic acid	0	0.03 ± 0.03
20:3n-3	Eicosatrienoic acid	0	0
22:0	Behenic acid	0.49 ± 0.01	0.50 ± 0.01
22:1	Erucic acid	0	0.12 ± 0.10
20:5n-3	Eicosapentaenoic acid	0	1.46 ± 0.03
22:2	Docosadienoic acid	0	0
22:4n-6	Adrenic acid	0.08 ± 0.02	0.09 ± 0.07
24:0	Lignoceric acid	0.37 ± 0.01	0.41 ± 0.01
24:1	Nervonic acid	0	0
22:5n-3	Docosapentaenoic acid	0	0.18 ± 0
22:6n-3	Docosahexanoic acid	0	1.32 ± 0.03

[1]Values are percentage of total ± SD

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Table 5: ***Baseline characteristics of women that were enrolled in the study***[1]

Characteristics	Participants (n = 51)
Age, years	23 ± 4
Weight, kg	61 ± 9
Mid-upper arm circumference, cm	27 ± 3
Height, cm	158 ± 6
Body-mass index	24 ± 3
Number of Children in Household	3.5 ± 2.2
Number of Previous Pregnancies	1.8 ± 2.0
Education attainment, n (%)	
None	22 (43)
Grades 1-3	3 (6)
Grades 4-6	5 (9)
Grades 7-8	1 (2)
Junior secondary	7 (13)
Senior secondary	13 (25)
Tertiary	1 (2)
House electrified, n (%)	2 (4)
Own cell phone, n (%)	19 (37)
Use bed net, n (%)	36 (71)

[1]Values are mean ± SD unless otherwise indicated

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Table 6: Outcomes of acceptability trial of new maternal ready-to-use supplementary food[1]

Outcome	Control Visits = 83[2]	Intervention Visits = 90[2]	*P* value[3]
Amount consumed, %, mean ± SD	99 ± 5	99 ± 4	0.20
Consumed all food	80 (96)	88 (98)	
Consumed ≥75% of food	83 (100)	89 (99)	
Likeability, mean ± SD	4.8 ± 0.7	4.9 ± 0.6	0.96
Score of 5	77 (93)	85 (94)	
Score of 4	3 (3.6)	2 (2.2)	
Score of ≤3	3 (3.6)	3 (3.4)	
Reported symptoms			
Diarrhea	4 (4.8)	6 (6.7)	0.76
Vomiting	4 (4.8)	5 (5.6)	1.0
Rash	0 (0)	0 (0)	NA

[1] Values presented as n (%) unless otherwise specified
[2] 51 individuals were enrolled. Visit numbers differ between groups due to drop-outs prior to study completion
[3] For amount consumed and likeability, *P* values were computed using the Wilcoxon signed-rank test. For reported symptoms, *P* values were computed using McNemar's chi-squared test

REFERENCES

1. **Bloomfield FH** How is maternal nutrition related to preterm birth? *Annu Rev Nutr*. **31(1)**: 235-261. https://doi.org/10.1146/annurev-nutr-072610-145141

2. **Desyibelew HD and AF Dadi** Burden and determinants of malnutrition among pregnant women in Africa: A systematic review and meta-analysis. *PLoS One*. 2019; **14(9)**: e0221712. https://doi.org10.1371/journal.pone.0221712

3. **Bussink-Voorend D, Bussink AP, Falama AM and J Stekelenburg** Health indicators of pregnant women in tonkolili district, rural Sierra Leone. *Int J Environ Res Public Health*. 2020; **17(11)**: 3918. https://doi.org/10.3390/ijerph17113918

4. **Delarue J** Fatty acids: A role for Africa? *Pro Nutr Soc*. 2019; **78(4)**: 532-539. https://doi.org/10.1017/s0029665119000508

5. **Petrova S, Dimitrov P, Willett WC and H Campos** The global availability of n-3 fatty acids. *Public Health Nutr*. 2011; **14(7)**: 1157-64. https://doi.org/10.1017/S1368980010003678

6. **Forsyth S, Gautier S and N Salem Jr**. Global estimates of dietary intake of docosahexaenoic acid and arachidonic acid in developing and developed countries. *Ann Nutr Metab*. 2016; **68(4)**: 258-67. https://doi.org/10.1159/000446855

7. **Adesogan AT, Havelaar AH, McKune SL, Eilitta M and GE Dahl** Animal source foods: Sustainability problem or malnutrition and sustainability solution? Perspective matters. *Glob Food Secur.* 2020; **25:** 100325**.** https://doi.org/10.1016/j.gfs.2019.100325

8. **Carlson SE, Gajewski BJ, Valentine CJ, Kerling EH, Weiner, CP, Cackovic M, Buhimschi CS, Rogers LK, Sands SA, Brown AR, Mudaranthakam DP, Crawford SA and EA DeFranco** Higher dose docosahexaenoic acid supplementation during pregnancy and early preterm birth: A randomised, double-blind, adaptive-design superiority trial. *EClinicalMedicine*. 2021; **36**: 100905. https://doi.org/10.1016/j.eclinm.2021.100905

9. **Best KP, Gibson RA and M Makrides** ISSFAL statement number 7 – Omega-3 fatty acids during pregnancy to reduce preterm birth. *PLEFA*. 2022; **186**: 102495. https://doi.org/10.1016/j.plefa.2022.102495

10. **Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF and M Makrides** Omega-3 fatty acid addition during pregnancy. *Cochrane Database Syst Rev*. 2018; **2018(11).** https://doi.org/10.1002/14651858.cd003402.pub3

11. **Hallahan B, Ryan T, Hibbeln JR, Murray IT, Glynn S, Ramsden CE, SanGiovanni JP and JM Davis** Efficacy of omega-3 highly unsaturated fatty acids in the treatment of depression. *Br J Psychiatry*. 2016; **209(3)**: 192-201. https://doi.org/10.1192/bjp.bp.114.160242

12. **Sarris J, Murphy J, Mischoulon D, Papakostas GI, Fava M, Berk M and CH Ng** Adjunctive nutraceuticals for depression: A systematic review and meta-analyses. *Am J Psychiatry*. 2016; **173(6)**: 575-87. https://doi.org/10.1176/appi.ajp.2016.15091228

13. **Caudill MA, Strupp BJ, Muscalu L, Nevins JEH and RL Canfield** Maternal choline supplementation during the third trimester of pregnancy improves infant information processing speed: A randomized, double-blind, controlled feeding study. *FASEB J.* 2018; **32(4)**: 2172-2180. https://doi.org/10.1096/fj.201700692rr

14. **Blusztajn JK, Slack BE and TJ Mellott** Neuroprotective actions of dietary choline. *Nutrients*. 2017; **9(8):** 9080815. https://doi.org/10.3390/nu9080815

15. **Wallace TC, Blusztajn JK, Caudill MA, Klatt KC and SH Zeisel** Choline: The neurocognitive essential nutrient of interest to obstetricians and gynecologists. *J Diet Suppl*. 2020; **17(6)**: 733-752. https://doi.org/10.1080/19390211.2019.1639875

16. **Klatt KC, Mcdougall MQ, Malysheva OV, Taesuwan S, Loinard-Gonzalez AAP, Nevins JEH, Beckman K, Bhawal R, Anderson E, Zhang S, Bender E, Jackson KH, King DJ, Dyer RA, Devapatia S, Vidavalur R, Brenna JT and MA Caudill** Prenatal choline supplementation improves biomarkers of maternal docosahexaenoic acid status among pregnant participants consuming supplemental DHA: a randomized controlled trial. *Am J Clin Nutr*. 2022; **116(3)**: 820-832. https://doi.org/10.1093/ajcn/nqac147

17. **Hendrixson DT, Smith K, Lasowski P, Callaghan-Gillespie M, Weber J, Papathakis P, Iversen PO, Koroma AS and MJ Manary** A novel intervention combining supplementary food and infection control measures to improve birth outcomes in undernourished pregnant women in Sierra Leone: A randomized, controlled clinical effectiveness trial. *PLOS Medicine*. 2021; **18(9)**: e1003618. https://doi.org/10.1371/journal.pmed.1003618

18. **WHO**. Recommendations on antenatal care for a positive pregnancy experience. 2016. WHO Guidelines Approved by the Guidelines Review Committee. WHO/RHR/16.12.

19. **Multiple Micronutrient Supplement Technical Advisory Group (MMS-TAG).** Micronutrient Forum (MNF). Expert consensus on an open-access United Nations international multiple micronutrient antenatal preparation-multiple micronutrient supplement product specification. *Ann N Y Acad Sci*. 2020; **1470(1)**: 3-13. https://doi.org/10.1111/nyas.14322

20. **Hendrixson DT, Koroma AS, Callaghan-Gillespie M, Weber J, Papathakis P and MJ Manary** Use of a novel supplementary food and measures to control inflammation in malnourished pregnant women in Sierra Leone to improve birth outcomes: study protocol for a prospective, randomized, controlled clinical effectiveness trial. *BMC Nutrition*. 2018; **4(1).** https://doi.org/10.1186/s40795-018-0218-y

21. **James G, Stephenson K, Callaghan-Gillespie M, Kamara M, Park HG, Brenna T and MJ Manary** Docosahexaenoic acid stability in Ready-to-Use Therapeutic Food. *Foods*. 2023; **12(2)**: 308. https://doi.org/10.3390/foods12020308

22. **Ghasemifard S, Turchini GM and AJ Sinclair** Omega-3 long chain fatty acid "bioavailability": a review of evidence and methodological considerations. *Prog Lipid Res*. 2014; **56**: 92-108. https://doi.org/10.1016/j.plipres.2014.09.001

23. **Yan J, Jiang X, West AA, Perry CA, Malysheva OV, Devapatla S, Pressman E, Vermeylen F, Stabler SP, Allen RH and MA Caudill** Maternal choline intake modulates maternal and fetal biomarkers of choline metabolism in humans. *Am J Clin Nutr*. 2012; **95(5)**: 1060-71. https://doi.org/10.3945/ajcn.111.022772

24. **Garces R and M Mancha** One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Anal. Biochem.* 1993; **211(1):** 139–143. https://doi.org/10.1006/abio.1993.1244

25. **Zhou Y, Nijland M, Miller M, Ford S, Nathanielsz PW and JT Brenna** The influence of maternal early to mid-gestation nutrient restriction on long chain polyunsaturated fatty acids in fetal sheep. *Lipids* 2008; **43(6)**: 525–531. https://doi.org/10.1007/s11745-008-3186-1

26. **Stephenson K, Callaghan-Gillespie M, Maleta K, Nkhoma M, George M, Park HG, Lee R, Humphries-Cuff I, Lacombe RJS, Wegner DR, Canfield RL, Brenna JT and MJ Manary** Low linoleic acid foods with added DHA given to Malawian children with severe acute malnutrition improve cognition: A randomized, triple-blinded, controlled clinical trial. *Am. J. Clin. Nutr.* 2022; **115(5)**: 1322–1333. https://doi.org/10.1093/ajcn/nqab363