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Mark Manary:

# Milk Matters – Mælks betydning i underernæring, er det laktose eller protein?

## Milk Matters – Milk matters in malnutrition, is it the lactose or protein?

Mejeribrugets
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# Final report

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## 1. Title of the project

**Danish:** Mælks betydning i underernæring, er det laktose eller protein?

**English:** Milk matters in malnutrition, is it the lactose or protein?

## 2. Project manager

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## 3. Other project staff

None other than Dr. Manary's colleagues

## 4. Sources of funding

Danish Dairy Research Foundation
Arla Foods Ingredients

## 5. Project period

| | |
|---|---|
| **Project period with DDRF funding:** | 11/2019 – 03/2023 |
| **Revised, if necessary:** | NA |
| **Total project period, if sub-project within a larger project:** | 11/2019 – 03/2023 |
| **Revised, if necessary:** | NA |

## 6. Project summary

**Danish:**

Akut underernæring er almindelig og påvirker dagligt 45 millioner børn på verdensplan og medfører øget risiko for infektioner, hospitalsindlæggelse, død og svækket udvikling blandt dem, der overlever. Terapeutiske og supplerende fødevarer givet i hjemmet er de mest almindelige behandlinger for akut underernæring. Det er kendt, at vægtøgning og restitution er større, når disse fødevarer indeholder mælkeprodukter, oftest skummetmælkspulver.

Det vides dog ikke, hvilke indholdsstoffer i skummetmælkspulver, der medfører disse positive effekter eller hvad de bagvedliggende mekanismer er. Det primære formål med dette projekt var at gennemføre et fodringsforsøg med supplerende fødevarer blandt moderat underernærede børn i Sierra Leone for at afgøre, om laktose, mælkeprotein eller begge påvirker tarmens permeabilitet og den fækale mikrobiomkonfiguration. Et delmål var at belyse, hvordan laktose og mælkeprotein påvirker den fækale metabolomiske konfiguration hos disse moderat underernærede børn.

Fodringsundersøgelsen blev udført mellem september 2020 og december 2021 i Pujehun-distriktet i den sydlige region af Sierra Leone. Pujehun er landligt og uelektrificeret, og det meste af dets befolkning er afhængigt af subsistenslandbrug. Både moderat akut underernæring (MAM) og svær akut underernæring (SAM) er almindelige i Pujehun hos børn under 5 år, og indtagelse af animalske fødevarer såsom mælk er ualmindeligt. Børn i alderen 6-59 måneder blev tilmeldt 17 perifere sundhedscentre, hvis de havde mid-upper arm circumference (MUAC) < 12,5 cm og ≥ 11,5 cm, ingen kronisk medicinsk tilstand og ingen allergi over for jordnødder eller mælk. Deres pårørende gav samtykke. Deltagerne blev randomiseret til at modtage en af fire supplerende fødevarer, der var pakket i identiske folieposer bortset fra et farvet klistermærke, som indikerede randomiseringsgruppe. De fire fødevarer var isoenergetiske og isonitrogene og adskilte sig i deres kilder til protein og kulhydrat: mælkeprotein-mælkekulhydrat (MPMC), mælkeprotein-vegetabilsk kulhydrat (MPVC), vegetabilsk protein-mælkekulhydrat (VPMC) og vegetabilsk protein-vegetabilsk kulhydrat (VPVC). Skummetmælkspulver blev brugt til MPMC, valleproteinisolat til MPVC, vallepermeat i VPMC og soja, majs og sukker i VPVC. Børn med højere risiko MAM, defineret ved MUAC ≤ 12,0 cm, blev udvalgt til at gennemgå urin- og afføringsprøvetagning. Tarmens permeabilitet blev målt ved hjælp af en sukkerabsorptionstest, hvor der gives en kendt mængde af det ufordøjelige sukkerlaktulose, og al efterfølgende urinproduktion opsamles i mindst 4 timer. Afføring blev opsamlet frisk, lynfrosset i flydende nitrogen og opbevaret ved -80°C indtil analyse. 16S rRNA-sekvens blev brugt til at analysere mikrobiomkonfigurationen. Fækal metabolomisk analyse blev udført ved hjælp af flere ekstraktionsteknikker og fire væskechromatografi-massespektrometrimetoder.

Af de 1.067 tilmeldte børn havde 413 MUAC ≤ 12,0 cm og gennemgik urin- og afføringsopsamlinger på tidspunktet for tilmeldingen og efter 4 ugers fodring. Median deltageralder var ~11,5 måneder, og 60% var kvinder. Der var ingen forskelle i % lactulosepermeabilitet mellem de 4 fødevaregrupper efter 4 ugers supplerende fodring, hvor 70 % af deltagerne stadig havde abnorm tarmpermeabilitet. For mikrobiomresultater var mediantallet af 16S-aflæsninger 218.000 pr. prøve. Der var ingen forskelle i β-diversitet mellem fødevaregrupper. Der var ingen forskelle i α-diversitet mellem grupper, som vurderet ved Shannon-indekset. Blandt de 20 mest almindeligt identificerede fækale arter blev der ikke set signifikante forskelle mellem de fire diætgrupper. Til metabolomiske analyser blev 5.681 unikke fækale metabolomiske træk identificeret. 88 (1,5%) viste sig at være forbundet med mælk vs. vegetabilsk protein- eller kulhydratkilde. De unikke egenskaber var næsten udelukkende fytokemikalier og flavonoider, der forekommer i soja og modstår fordøjelse/absorption i den menneskelige tarm. Ingen mikrobielle metaboliske veje unikke for mælkeforbrug blev identificeret. Vægtstigningsraten blandt børn, der fik MPMC, var større end blandt børn, der indtog VPVC.

Som konklusion var der ingen evidens for, at mælkeprotein eller kulhydrat påvirkede tyndtarmspermeabiliteten, vurderet ved % lactuloseudskillelse. Den fækale 16S-konfiguration viste heller ingen forskelle mellem grupperne. Den ikke-målrettede fækale metabolomik viste forskelle i overensstemmelse med indtagelse af tildelte supplerende fødevarer, hvilket understøttede overholdelse af randomiseret gruppe, men ikke indikerede aktivering af nogen mikrobielle eller luminale metaboliske veje som en konsekvens af mælk vs. vegetabilsk protein- eller kulhydratkilde. De fækale resultater var overraskende, da kosten menes at være en primær determinant for 16S-konfigurationen.

Denne undersøgelse sætter spørgsmålstegn ved denne dominerende teori. Som forventet tog børn, der blev fodret med MPMC, hurtigere på i vægt end dem, der fik VPVC. Dette resultat styrker vigtigheden af nulfundene med hensyn til tarmpermeabilitet og fækalt mikrobiom/metabolom. Rent klinisk havde fødevarerne effekt, men de mekanistiske tests afslørede ingen klar årsag. Undersøgelsen tyder på, at både mælkeprotein og mælkesukker (laktose) er vigtige for at forbedre vægtøgningen.

**English:**

Acute malnutrition is common, affecting 45 million children worldwide at any one time, and morbid, increasing risks for infections, hospitalization, death, and impaired development among those who survive. Therapeutic and

supplementary foods given at home are the most common treatments for acute malnutrition. It is known that weight gain and recovery are greater when these foods contain milk products, most commonly skimmed milk powder. It is not known, however, which components of skimmed milk powder confer its benefits, nor how they do so. The primary aim of this project was to conduct a feeding trial with supplementary foods among moderately malnourished children in Sierra Leone to determine if lactose, dairy protein, or both affect intestinal permeability and fecal microbiome configuration. A sub-aim was to elucidate how lactose and milk protein affect the fecal metabolomic configuration in these moderately malnourished children.

The feeding study was conducted between September 2020 and December 2021 in Pujehun District in the Southern Region of Sierra Leone. Pujehun is rural and unelectrified, and most of its population relies on subsistence farming. Both moderate acute malnutrition (MAM) and severe acute malnutrition (SAM) are common in Pujehun in children under 5 years of age, and consumption of animal-source foods such as milk is uncommon. Children 6-59 months of age were enrolled across 17 Peripheal Health Units if they had mid-upper arm circumference (MUAC) < 12.5 cm and ≥ 11.5 cm, no chronic medical condition, and no allergy to peanuts or milk. Their caregivers provided consent. Participants were randomized to receive one of four supplementary foods that were packaged in identical foil sachets aside from a colored sticker which indicated randomization group. The four foods were isoenergetic and isonitrogenous, differing in their sources of protein and carbohydrate: milk protein-milk carbohydrate (MPMC), milk protein-vegetable carbohydrate (MPVC), vegetable protein-milk carbohydrate (VPMC), and vegetable protein-vegetable carbohydrate (VPVC). Skimmed milk powder was used for MPMC, whey protein isolate for MPVC, whey permeate in VPMC, and soy, maize, and sugar in VPVC. Children with higher-risk MAM, defined by MUAC ≤ 12.0 cm, were chosen to undergo urine and stool sample collection. Intestinal permeability was measured using a sugar absorption test, where a known quantity of the non-digestible sugar lactulose is given and all subsequent urine output is collected for at least 4 hours. Feces were collected fresh, flash frozen in liquid nitrogen, and stored at -80C until analysis. 16S rRNA sequence was used to analyze microbiome configuration. Fecal metabolomics analysis was done using multiple extraction techniques and four liquid chromatography-mass spectrometry methods.

Of the 1,067 children enrolled, 413 had MUAC ≤ 12.0 cm and underwent urine and stool collections at the time of enrollment and after 4 weeks of feeding. Median participant age was ~11.5 months and 60% were female. There were no differences in % lactulose permeability between the 4 food groups after 4 weeks of supplementary feeding, at which time 70% of participants still had abnormal intestinal permeability. For microbiome results, the median number of 16S reads was 218,000 per sample. There were no differences in β-diversity between food groups. There were no differences in α-diversity between groups, as assessed by Shannon index. Among the 20 most commonly identified fecal species, no significant differences were seen between the four dietary groups. For metabolomic analyses, 5,681 unique fecal metabolomic features were identified. 88 (1.5%) were found to be associated with milk vs. vegetable protein or carbohydrate source. The unique features were almost entirely phytochemicals and flavonoids that occur in soy and resist digestion/ absorption in the human gut. No microbial metabolic pathways unique to milk consumption were identified. The rate of weight gain among children receiving MPMC was greater than among children consuming VPVC.

In conclusion, there was no evidence that milk protein or carbohydrate affected small intestinal permeability, as assessed by % lactulose excretion. The fecal 16S configuration also showed no differences between groups. The untargeted fecal metabolomics showed differences consistent with consumption of assigned supplementary foods, supporting compliance to randomized group, but do not indicate activation of any microbial or luminal metaboic pathways as a consequence of milk vs. vegetable protein or carbohydrate source. The fecal results were surprising, as diet is thought to be a primary determinant of 16S configuration. This study calls into question this dominant theory. As expected, children fed MPMC gained weight faster than those fed VPVC. This result strengthens the importance of null findings with respect to intestinal permeability and fecal microbiome/metabolome: clinically, the foods exerted their effects, but the mechanistic tests revealed no clear cause. The study suggests both milk protein and milk carbohydrate are important for improving weight gain.

## 7. Project aim

**Danish:**

Det primære formål med dette projekt var at gennemføre et fodringsforsøg med supplerende fødevarer blandt moderat fejlernærede børn i Sierra Leone for at bestemme, om laktose, mælkeprotein eller begge påvirker tarmens permeabilitet og det fækale mikrobiom. Delmålene var at bestemme hvilke mælkekomponenter, protein vs. laktose, kan være ansvarlige for dets virkninger og for at belyse, hvordan laktose og mælkeprotein påvirker den fækale metabolomiske konfiguration hos moderat fejlernærede børn.

**English:**

The primary objective of this project was to conduct a feeding trial with supplementary foods among moderately malnourished children in Sierra Leone to determine if lactose, dairy protein, or both affect intestinal permeability and fecal microbiome. The sub-objectives were to determine which milk components, protein vs. lactose, might be responsible for its effects, and to elucidate how lactose and milk protein affect the fecal metabolomic configuration in moderately malnourished children.

## 8. Background for the project

At any one time, an estimated 45 million children worldwide are acutely malnourished, and the annual incidence is likely 3-5 times this number. Acute malnutrition is divided into moderate and severe types. Moderate acute malnutrition (MAM) is defined by mid-upper arm circumference (MUAC) < 12.5 cm and ≥ 11.5cm, or weight-for-length z-score (WLZ) < -2 and ≥ -3. Severe acute malnutrition (SAM) is defined by MUAC < 11.5, WLZ < -3, or the presence of nutritional edema, which is bilateral pedal pitting edema, also known as kwashiorkor. Both MAM and SAM increase short-term risks for infections, hospitalization, and mortality (11-fold), as well as impair physical and cognitive development for years thereafter. Despite advances in treatment, acute malnutrition remains the strongest risk factor for child mortality, including in hospitalized children.

Ready-to-use therapeutic food (RUTF) revolutionized the treatment of acute malnutrition by allowing treatment to take place in the child's home by their caregiver. In its most common form, RUTF is composed of peanuts, milk powder, oil, sugar, and micronutrient mix. Since its codification as the recommended treatment strategy for SAM by the World Health Organization (WHO), attempts have been made to modify the ingredients of RUTF to improve efficacy and reduce costs. One target for cost-savings has been to replace skimmed milk powder. Multiple clinical trials have demonstrated that reducing or removing milk powder from RUTF results in lower rates of weight gain and reduced recovery from SAM. For this reason, WHO and UN Codex Alimentarius guidelines specify that at least half of protein in RUTF must come from milk. These guidelines are followed by food aid producers worldwide.

Treatments for MAM have been modeled off of RUTF and, just as in SAM, it has been shown that milk products improve weight gain and recovery from MAM. Skimmed milk powder is largely milk protein and lactose and is the primary ingredient used in food aid products. It is not known whether the milk-based clinical benefits seen in malnourished children result from milk protein, lactose, or both, nor is it known how these ingredients confer their benefits.

Acute malnutrition exacts enormous metabolic stress on a child's body. To recover from malnutrition, children must re-build their damaged tissues, which can include every organ in the body from the skin, heart, and liver, to the brain and the intestines. The gut epithelium forms a primary barrier between the environment and the individual and is the site where ingested nutrients are absorbed. In malnutrition, this barrier is disrupted, allowing for translocation of pathogens into the bloodstream, and the surface area along which nutrients are absorbed in the small intestine is denuded and inflamed. This constellation of findings is called environmental enteric dysfunction (EED) and can be found in up to 75% of malnourished children. EED is known to impair physical growth. The gut epithelium rapidly regenerates in health and disease, on average every 11 days. Milk protein is considered an ideal dietary protein for children because it is rich in essential amino acids that are highly digestible. It is possible that milk protein better

facilitates the recovery of the small intestinal epithelium and its function than vegetable proteins and thereby reduces EED to allow for superior weight gain and recovery in acutely malnourished children.

There is evidence that the microbiome contributes to malnutrition. In a proof-of-concept study, researchers in Bangladesh provided a supplementary food to MAM children that had been designed to promote a healthier microbiome configuration. Despite this food containing fewer calories than its comparator, it demonstrated greater anthropometric gains. Lactose is the primary carbohydrate in milk powder, comprising 56% of its content. Lactose is a disaccharide that is digested less efficiently than other sugars in the human intestinal tract. While the duodenum and jejunum contain smaller numbers of microbes, the ileum is home to the greatest number of bacteria in the human gut. The ileum can absorb nutrients like the proximal small intestine. It is possible that lactose is feeding these ileal microbes and thereby providing fuel for the bacterial synthesis of nutrients that are key for weight gain and recovery from malnutrition or altering the metabolomic content in the intestines to produce such a result.

Treatment of children with MAM offers an opportunity to study these questions, as supplementary feeding guidelines are less strict, allowing for all-vegetable source food as a control. Determining which component of skimmed milk is responsible for its benefits in malnutrition as well as the mechanism(s) whereby such benefits are accrued would have substantial implications for food aid programming and children served. Modifications of the milk component might yield greater clinical benefits or cost-savings, which could help expand coverage.

## 9. Sub-activities in the entire project period

The sub-activities were Development and Production of Study Foods, Clinical Feeding Trial, Intestinal Permeability testing, Fecal Microbiome Analysis, Fecal Metabolomic Analysis, and Scientific Manuscript Production.

## 10. Deviations

While the COVID epidemic prolonged the study, we did not deviate from its purpose.

## 11. Project results

Sub-activity: Development of study foods

The foods were developed to test the hypotheses regarding milk protein and milk carbohydrate sources. The foods were isoenergetic and isonitrogenous and contained the same added micronutrients. All foods were peanut paste-based and made with palm or canola oil. Skimmed milk powder provided dairy protein and carbohydrate in the MPMC group. Whey protein isolate was the source of protein for MPVC while maize composed the vegetable carbohydrate. Whey permeate was the dairy carbohydrate for VPMC. Finally, the “all vegetable” food used maize, sugar, and soy. Informal acceptability testing revealed all foods were acceptable to young children in Sierra Leone.

| *Ingredient* | MPVC | VPMC | VPVC | MPMC |
|---|---|---|---|---|
| Peanuts, g/100g | 15.2 | 9.1 | 9.1 | 23.7 |
| Canola oil, g/100g | 26 | 25.5 | 26 | 5.1 |
| Palm oil, g/100g | 1 | 1 | 1 | 16.0 |
| Maize flour, g/100g | 18.4 | 0 | 10 | 0 |
| Sugar, g/100g | 23 | 9 | 16 | 23.0 |
| Soy flour, g/100g | 0 | 33 | 33 | 0 |
| Skim milk powder, g/100g | 0 | 0 | 0 | 27.3 |
| Whey protein isolate, g/100g | 11.5 | 0 | 0 | 0 |
| Whey permeate, g/100g | 0 | 17.5 | 0 | 0 |
| Vitamin pre-mix, g/100g[1] | 2.9 | 2.9 | 2.9 | 2.9 |
| Hydrogenated Soy Oil, g/ 100g | 2.0 | 2.0 | 2.0 | 2.0 |
| | | | | |
| Milk protein, g/ 100 g | 10.6 | 0 | 0 | 9.8 |
| Lactose, g / 100 g | 0.1 | 14.9 | 0 | 13.8 |
| Protein, % | 15.4 | 15.2 | 15.5 | 15.8 |
| Energy, kcal/ 100g | 550 | 548 | 551 | 552 |

Sub-activity: Clinical feeding trial

The Project Peanut Butter (PPB) team began trial preparations in 2019. These were delayed by COVID-related disruptions in 2020. 17 Peripheral Health Units (PHUs) in Pujehun District were identified as targets for the trial. The PPB team met with local chiefs for each PHU and obtained permission and support for the trial. The Sierra Leone Ethics and Scientified Review Committee application was approved. Similarly, the trial was submitted to the Washington University in St. Louis Institutional Review Board and was approved. For sample collection, the cold chain was secured through a partner for liquid nitrogen and -80°C freezer storage. PPB sustained or launched acute malnutrition treatment across the 17 study PHUs to solidify community knowledge and trust of PPB for the care of children with malnutrition. The four study foods were produced at the PPB factory in Freetown, Sierra Leone, and labeled with colored stickers indicating randomization group.

Enrollment began in September 2020 and continued through December 2021. There were no challenges with respect to recruitment, consent, or randomization. Children who met criteria for higher-risk MAM were enrolled in the sample collection mechanistic sub-study (**below**).

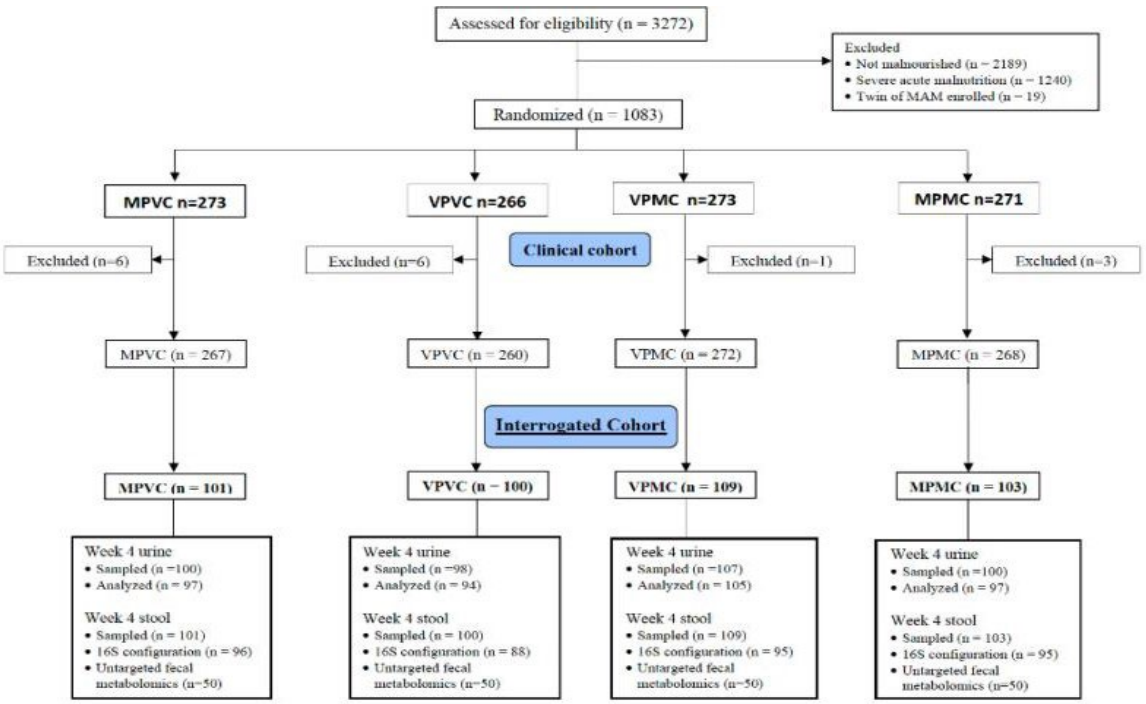


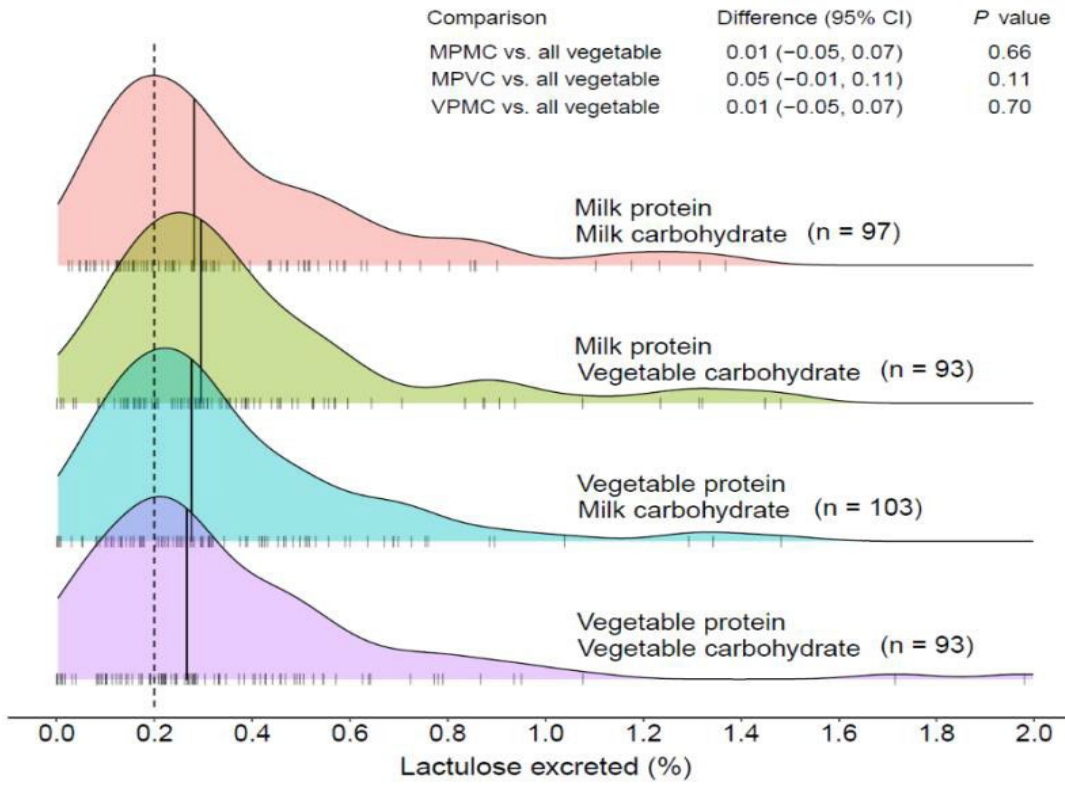

**Figure 1.** Consort Sub-Study Participant Flow Diagram. MAM-moderate acute malnutrition; MPVC- milk protein/vegetable carbohydrate; VPVC – vegetable protein/vegetable carbohydrate; VPMC – vegetable protein/milk carbohydrate; MPMC – milk protein/milk carbohydrate;

The four groups of children were similar at baseline with respect to key characteristics including age, sex, socioeconomic indicators, and anthropometrics (**table below**). MPMC yielded greater weight gain compared with VPVC (difference = 0.3 g/kg/d, 95% 0.0, 0.6, $p$ = 0.03). Otherwise, clinical outcomes were similar across the groups, including recovery, deterioration to SAM, and death, although the study was not adequately powered to detect such differences. The greater weight gain for MPMC provides internal validity for the mechanistic evaluations: if intestinal permeability, microbiome, or metabolome changes were responsible, differences should be present.

| Characteristic | MPMC (n = 271) | MPVC (n = 273) | VPMC (n = 273) | VPVC (n = 266) |
|---|---|---|---|---|
| Age, mo; median (IQR) | 11.4 (8.4, 17.0) | 11.6 (8.0, 17.0) | 11.7 (8.1, 17.6) | 11.9 (8.3, 17.2) |
| Female sex, n (%) | 158 (59) | 146 (55) | 152 (56) | 158 (61) |
| Breastfeeding, n (%) | 206 (77) | 210 (79) | 213 (78) | 192 (74) |
| Mother is primary caregiver, n (%) | 245 (91) | 249 (93) | 248 (91) | 235 (90) |
| Father in the home, n (%) | 179 (67) | 209 (78) | 202 (74) | 193 (74) |
| Number of siblings | 1.8 ± 1.7 | 1.8 ± 1.7 | 1.7 ± 1.7 | 1.8 ± 1.8 |
| Animals sleep in the home, n (%) | 39 (15) | 36 (13) | 45 (17) | 39 (15) |
| Water drawn from river or stream, n (%) | 34 (13) | 26 (9.7) | 26 (9.6) | 33 (13) |
| Anthropometry | | | | |
| MUAC, cm; median (IQR) | 11.8 (11.7, 12.1) | 11.8 (11.7, 12.1) | 11.8 (11.6, 12.0) | 11.9 (11.7, 12.1) |
| Weight, kg | 6.65 ± 0.88 | 6.74 ± 0.88 | 6.71 ± 0.92 | 6.68 ± 0.87 |
| Length, cm | 68.8 ± 5.6 | 68.9 ± 5.4 | 69.1 ± 5.7 | 68.8 ± 5.6 |
| Length-for-age z-score | -2.6 ± 1.3 | -2.6 ± 1.1 | -2.5 ± 1.2 | -2.7 ± 1.4 |
| LAZ < -2, n (%) | 188 (70) | 187 (70) | 179 (66) | 187 (72) |
| Weight-for-length z-score | -2.1 ± 0.7 | -2.0 ± 0.8 | -2.1 ± 0.8 | -2.1 ± 0.9 |
| Weight-for-age z-score | -3.0 ± 0.8 | -2.9 ± 0.8 | -2.9 ± 0.8 | -3.0 ± 0.9 |

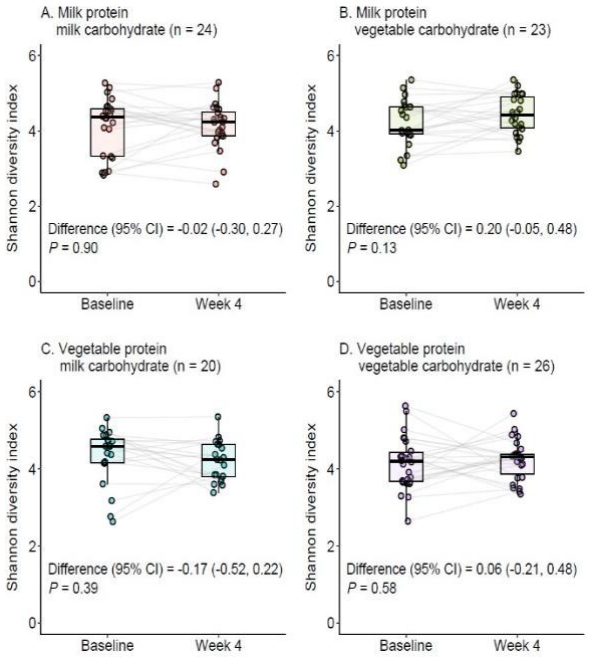
Sub-activity: Intestinal permeability testing

Lactulose absorption testing was completed after 4 weeks of feeding on approximately 95 participants per group. 98% of samples were adequate for analysis. There were no differences between food groups in % lactulose (%L) excretion (**below,** with % L displayed as probability density plots). In addition, nearly 70% of participants continued to have increased intestinal permeability despite 4 weeks of supplementary feeding, a result which aligns with prior research showing persistence of intestinal dysfunction during acute malnutrition treatment. These results suggest the benefits of milk are not exerted via greater healing in the small intestine. Clinical differences (greater weight gain) were apparent by 4 weeks, yet no signal was identified for intestinal permeability improvement in this population.

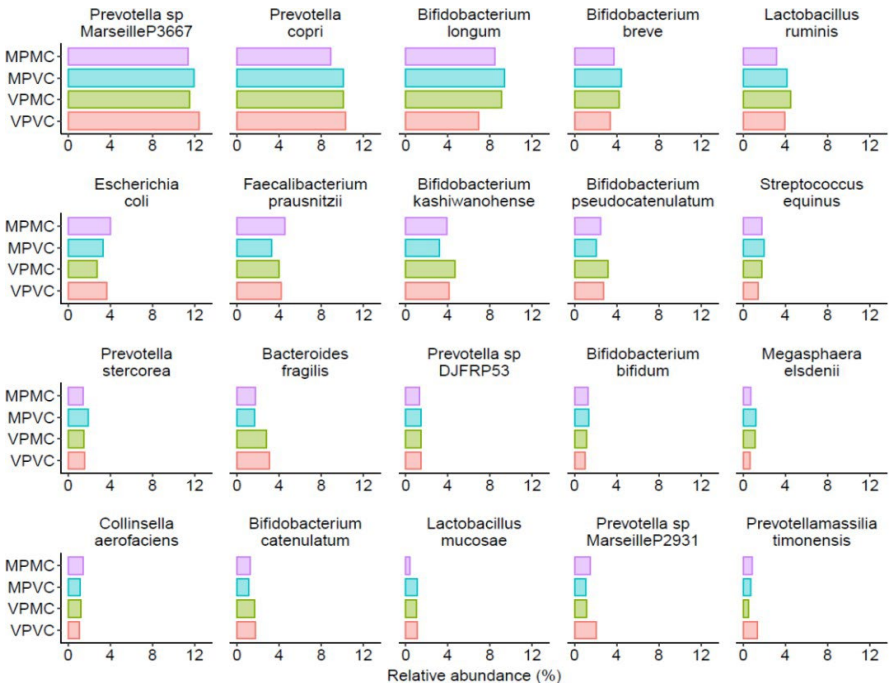


Sub-activity: Fecal microbiome analysis

The median (IQR) number of 16S reads was 218,506 (178,170, - 244,841) per fecal sample. Comparison of the β-diversity of the fecal 16S communities showed no difference for MPMC vs VPVC (pseudo-F-statistic = 0.882, $p$ = 0.51), MPVC vs VPVC (pseudo-F- statistic = 0.870, $p$ = 0.54), or VPMC vs VPVC (pseudo-F-statistic = 0.964, $p$ = 0.43). α-diversity as measured by the Shannon index was similar between the four dietary groups as well (**left part of figure**). Among the subset of participants who underwent enrollment and week 4 testing, there were no changes in α-diversity despite receipt of supplementary feeding (**right part of figure**).



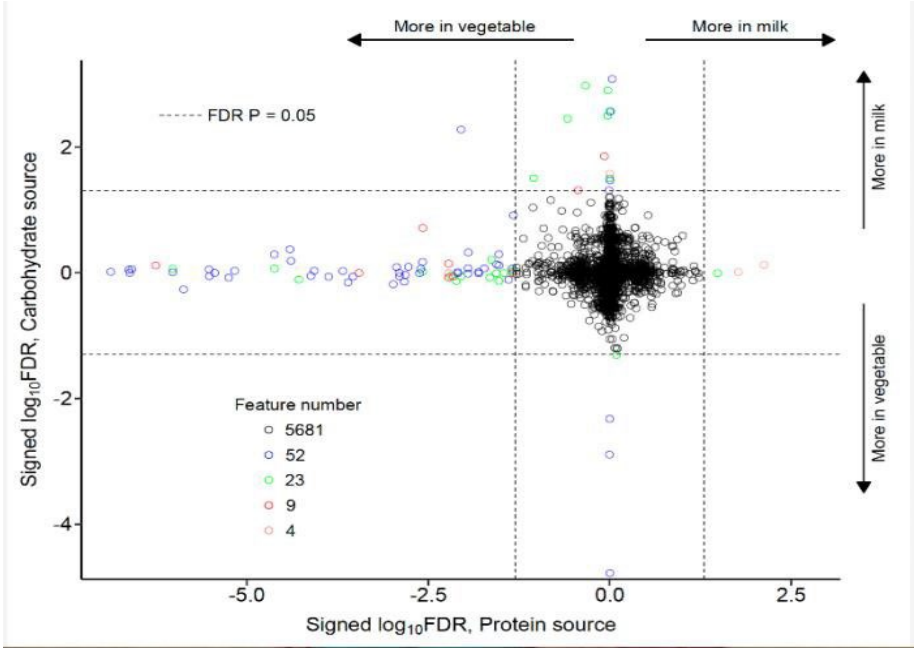

Among the 20 most prevalent fecal species, no significant differences were seen between the four dietary groups (**below**).



Despite thorough analysis using 16S methodology, no measure of microbiome composition proved different between food groups. The supplementary foods were expected to provide approximately 50% of calories consumed by a child with MAM and their composition varied by as much as 25%, contrasting milk with soy, maize, and sugar. Despite these profound differences in diet, fecal 16S microbiome diversity and species abundance levels did not vary significantly. This result agrees with another study among severely malnourished children who were fed a therapeutic food with or without 18% oat, wherein again no differences in 16S configuration were identified. Animal studies have shown that diet changes can alter the ileal microbiome. Human studies have shown there are substantial differences between the ileal and colonic microbial populations. The Milk Matters results above raise the question of whether fecal microbiome analyses can inform ileal assessments, a longstanding assumption in microbiome research. A limitation of this study is depth of sequencing (~200K). It is possible that deeper sequencing might yield further insights.

Sub-activity: Fecal metabolomic analysis

Among the 5681 unique fecal metabolic features detected, 88 (1.5%) were found to be associated with consumption of either MP or MC (**below**). Among the 21 features associated with dietary carbohydrate source, 16 were associated with MC and 5 with VC. Those features associated with MC consumption were pyrimidine nucleosides, while aryl ketones and amino acids were associated with VC consumption. Among the 75 features associated with dietary protein source, 3 were associated with MP and 72 with VP. Those features associated with MP consumption were cholestane steroids and ceramides, important components of milk lipids (table S4 in Supplemental Appendix). Those 72 features associated with VC consumption were almost all flavonoids, amino acids and heterocyclic, nitrogen-containing, aromatic ring structures. These features are indicative of ommon phytochemicals seen in a multitude in plants.




Conclusions

This study suggests that both milk protein and lactose are required to improve weight gain in acutely malnourished children. Milk products do not exert their benefits through reducing small intestinal permeability. There were no fecal 16S configuration differences between groups. This provocative result suggests that diet is not a significant determinant of the fecal microbiome. The untargeted fecal metabolomic analyses did not reveal alterations in metabolic pathways or luminal products, at least as are present in the feces. Metabolomics results support adherence to study intervention. The study's results confirm that milk matters in malnutrition, but the mechanisms whereby it exerts its effects are as yet unknown.

## 12. The relevance of the results, including relevance for the dairy industry

This study calls into question two prominent hypotheses in microbiome research: (1) diet is a significant determinant of the fecal microbiome, and (2) that fecal microbiome analyses inform understanding of potential ileal microbiome differences. There is significant research and funding dedicated to microbiome-directed foods worldwide. The results of this study should add skepticism to such ventures. The intestinal permeability results strongly argue this is not the pathway by which milk helps kids and, more broadly, show that supplementary food alone is insufficient to drive intestinal healing. %L did not improve despite 4 weeks of feeding in any of the groups. EED is common worldwide, associated with growth faltering, and has proven intractable to various interventions. Further research is needed to identify treatments that help gut healing during acute malnutrition better to promote recovery.

This study is the latest in a series that has demonstrated the power of milk in malnutrition treatment. Milk's role in SAM treatment has been solidified. As international guideline bodies meet to set recommendations for the treatment of MAM, this study will play a role, demonstrating improved weight gain with skimmed milk. The randomized, blinded trial of high-quality cuts through the confounding that often plagues retrospective analyses of diet, showing milk's role in child health and development.

In short it is both milk protein and lactose that contribute to milk's success as a **the** superior ingredient in food aid.

## 13. Communication and knowledge sharing about the project

**Papers in international journals:**

These are currently under preparation

**Easily read papers:**

None

**Student theses:**

None

**Oral presentations at scientific conferences, symposiums etc.:** Neha Adari, Mark Manary, Kevin Stephenson, Nur Shaikh, Donna Wegner. Milk Matters: Examination of the Efficacy of Lactose and Dairy Protein on Gut Health and Fecal Microbiota in Moderate Acute Malnutrition. Pediatric Summery Research Program 2022. Washington University in St. Louis, USA.

**Oral presentations at meetings:**

**Other:**

None

## 14. Contribution to master and PhD education

Nino Naskidashvil was the primary research associate running the trial in Sierra Leone. She holds undergraduate and medical degrees from the country Georgia. The Milk Matters Study will contribute to her Masters of Public Health Degree at Washington University in St. Louis.