| w | ***COMIRB Protocol*** |
| --- | --- |
| COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARDCAMPUS BOX F-490 TELEPHONE: 303-724-1055 Fax: 303-724-0990 |  |

**Protocol #:24-1030**

**Project Title:** Absorption of Zinc and Iron from Nixtamalized Biofortified Maize in School-aged Children in Guatemala (AMAIZING Study)

**Co-Principal Investigator:** Nancy F. Krebs, MD, MS; Douglas Taren, PhD; and Manolo Mazariegos, MD (INCAP)

**Version Date:** May 24, 2024

**I**. **Hypotheses and Specific Aims**

The overall goal of this study is to determine the amount of zinc and iron absorbed from tortillas made with nixtamalized biofortified maize compared to nixtamalized non-biofortified maize. Zinc and iron deficiencies are common in Guatemala, and maize is a staple food throughout the country. This study will demonstrate if utilizing nixtamalized biofortified maize has the potential to increase dietary zinc and iron intake and improve zinc and iron status when compared to diets incorporating traditional maize. The proposed study setting is in the Western Highlands of Guatemala near Tecpán, Chimaltenango, and the population to be studied is school-aged children 10-14 years old.

***Hypothesis:*** The amounts of zinc and iron absorbed from tortillas made with nixtamalized biofortified maize will be significantly greater than the amounts absorbed from nixtamalized non-biofortified maize (control).

**II**. **Background and Significance**

Zinc and iron deficiencies continue to be a major cause of early childhood morbidity and mortality globally (1). The use of biofortified crops to increase the food system’s supply of high-quality micronutrient foods has been well established. In recent years, biofortified crops have been a popular solution to addressing micronutrient deficiencies globally (2-5). One organization working to develop and expand the use of biofortified crops is Semilla Nueva in Guatemala. Through traditional crop breeding, Semilla Nueva has developed and bred a new biofortified maize that aims to improve the dietary intake of zinc and iron via maize-based foods such as tortillas. Since tortillas are a staple food in Guatemalan households, maize is ideal for biofortification. To understand the potential public health impact of incorporating nixtamalized masa, fresh dough, prepared from biofortified maize into the diet, especially in children, absorption and bioavailability studies must be undertaken.

The University of Colorado Pediatric Nutrition Lab (CU) has extensive experience using stable isotope techniques to measure zinc and iron absorption from dietary and supplementary sources (4-8). To understand the bioavailability of these minerals in the nixtamalized masa, dough made from corn flour, from biofortified maize, researchers at CU, Instituto de Nutrición de Centro América y Panamá (INCAP), and Semilla Nueva will collaborate to conduct stable isotope studies in school-aged children 10-14 years old in the Western Highlands of Guatemala, as recommended by USAID. Measurements of zinc absorption from meals for an entire day and of iron absorption from all meals over two days, in which maize is the food staple and accounts for ~70% of the energy intake are essential for understanding the quantitative benefits of breeding high zinc and iron maize for diets of children. Coupled with representative population-specific dietary data, this will allow reliable estimates of the benefits of enhancing biofortified grains to improve population-specific nutrition targets.

**III**. **Preliminary Studies/Progress Report**

See Appendix 1 for the literature review describing in vitro and in vivo studies relevant to this study.

**IV. Research** **Methods**

**A. Outcome Measure(s)**

The **primary outcomes** are total absorbed zinc (TAZ) from one day’s consumption and total absorbed iron (TAI) from two days’ consumption of nixtamalized biofortified maize versus nixtamalized control maize.

**Secondary outcomes** include the fractional absorption of zinc (FAZ) measured by dual isotope tracer ratio (9) and fractional absorption of iron (FAI) by iron incorporation in erythrocytes (4), total dietary zinc (TDZ) and total dietary iron (TDI), and biomarkers of systemic inflammation (serum CRP and AGP). Additional measures include serum hepcidin for iron status and stool collections for intestinal inflammatory markers (calprotectin and myeloperoxidase). The intestinal markers will be analyzed if additional funding is obtained and would be used as co-variates to evaluate potential impact on zinc and iron absorption (10).

1. **Description of Population to be Enrolled**

The isotope studies will take place in a rural/semi-rural setting in the Western Highlands of Guatemala near Tecpán, Chimaltenango. School aged children 10-14 years old of both sexes will be enrolled in the study. Children will be from agricultural-based communities that make homemade tortillas for daily consumption.

Children will be recruited from the schools in the surrounding area. Community primary schools usually have 400-500 children aged 6-14 yrs, of which one-third are estimated to be 10-14 yrs of age. The target area, located in Western Highlands is predominantly indigenous with stunting rates exceeding the national average of 47% (11).

There are no recent data on micronutrient status of school aged children in this area of Guatemala or similar communities. The most recently published Informe del Sistema de Vigilancia Epidemiológica de Salud y Nutrición (SIVESNU) found that anemia rates nationally for pre-school aged children was 6.1% and 7.3% in rural communities (12). Similarly, the report found that anemia rate non-pregnant women of reproductive age was 4.8%, and 4.9% in rural settings (13).

After informed consent is obtained from the parent and assent from the child, the child will be randomized to either the biofortified or control group. Families of participating children will be provided with biofortified or control maize to prepare all tortillas consumed by everyone in the family for three days prior to the start of the isotope administration.

Inclusion criteria:

* 10-14 years old school children (male and female sex)
* BMI between10-85%tile
* Typically eat homemade tortillas at each meal every day

Exclusion criteria:

* Taking iron or zinc supplements
* Hemoglobin <12.9 g/dl for children 10-11 yrs old and <13.4 g/dl for children 12-14 yrs old
	+ Per the WHO’s 2024 publication hemoglobin cut-offs for identifying anemia are 5-11 yrs: <11.5 g/dl & 12-14 yrs: <12.0 g/dl. To adjust for the elevation of Tecpán at 2254 m, 1.4 g/dl is added to the cut-off point (14).
* Girls who are having a menstrual period during the metabolic studies
1. **Study Design and Research Methods**

This is a randomized, double blinded, comparison study of biofortified vs control maize prepared as tortillas from nixtamalized masa (Figure 1). This study will enroll up to 56 school aged children living in peri-urban communities in Guatemala (28/group x 2 groups). It is anticipated that it will take 9-12 weeks to recruit all 56 children for the study. All participants will be on the study for 27 days (Figure 2). Five to 6 children will complete the isotope studies each week over a 9-12 week period (Figure 3). Parents will be financially compensated with the appropriate local wage for their and their child’s time participating in the study. The appropriate rate of reimbursement will be set by guidelines from INCAP.

Sample size:

Based on previous research (6), we anticipate that the minimum difference in TAZ between the two groups to be 0.28 mg/day with a standard deviation of 0.30 and an α = 0.05. We will enroll 26 children per a group (power > 90%). Our recruitment goal is 28 per group which includes a 10% drop-out rate based on previous research in these types of studies (6).



Figure 1. Study Consort Diagram

| **Participant Study Procedures** |
| --- |
| **Day of Study** | **-7** | **1-3** | **4** | **5** | **6** | **7-10** | **11-19** | **20** |
| Screening (anemia (Hemocue® 301), health status, anthropometrics, maize intake) | X |  |  |  |  |  |  |  |
| Enroll and randomize | X |  |  |  |  |  |  |  |
| Deworm children | X |  |  |  |  |  |  |  |
| Study Diet lead-in & monitor dietary intake (>100 gm maize/day) |  | XXX |  |  |  |  |  |  |
| Baseline stool sample |  |  | X |  |  |  |  |  |
| Baseline urine sample (Zn) |  |  | X |  |  |  |  |  |
| Baseline health history assessment |  |  | X |  |  |  |  |  |
| Study diet (>100 gm maize/day) & snacks for entire day |  |  | X | X | X |  |  |  |
| 70Zn isotope administration(orally w/ all meals) |  |  | XXX |  |  |  |  |  |
| Afternoon blood collection (CRP, AGP, ferritin, sTfR, Zn, hepcidin, hemoglobin, erythrocytes for 58Fe) |  |  | X |  |  |  |  |  |
| IV 67Zn isotope administration (immediately after blood draw using same needle) |  |  | X |  |  |  |  |  |
| 58Fe isotope administration(orally w/ all meals) |  |  |  | XXX | XXX |  |  |  |
| 24-hr weighed dietary intakes and duplicate diet collections(Dietary Zn and Fe) |  |  | X | X | X |  |  |  |
| Spot urine collections for Zn absorption study (AM & PM) |  |  |  |  |  | XX |  |  |
| NO STUDY PROCEDURES |  |  |  |  |  |  | X |  |
| Collect blood for Fe absorption study and anthropometry |  |  |  |  |  |  |  | X |

 Figure 2. Participant Study Procedures

| **Study Timeline** |
| --- |
| **Phase** | **Jul** **24** | **Aug** **24** | **Sep** **24** | **Oct** **24** | **Nov** **24** | **Dec** **24** | **Jan** **25** |
| Recruitment | X | X | X | X |  |  |  |
| Enrollment | X | X | X | X |  |  |  |
| Isotope studies | X | X | X | X |  |  |  |
| Ship samples to Colorado |  |  |  | X |  |  |  |
| Sample analyses |  |  |  |  | X | X | X |

 Figure 3. Study Timeline Gantt Chart

 Recruitment and Informed Consent:

With support from the school (see attached letter), children will be recruited by schools in the local community in Tecpán, Chimaltenango. All research procedures will be conducted in the local language. Prior to the start of the study, the research team will hold school-based community sensitization meetings with parents and flyers may be sent home with the children to inform parents about the study. Those interested in learning more about their children participating in the study will attend a more detailed session including the informed consent/ assent process. If the parents and child are interested in joining the study, oral consent and assent will be given to the research staff to complete the screening procedures (a partial waiver of consent will be obtained by the IRB to complete the screening procedures).

During the screening procedures, the research staff will collect weight (to the nearest 0.1 kg) and height (to the nearest 0.1 cm) to calculate BMI. Children will only be eligible for the study if their BMI is between the 10-85%tile. Additionally, using sterile techniques, a finger prick to obtain a pool sample of blood will be used to measure hemoglobin status using Hemocue® 301. If the results of hemoglobin testing using the blood obtained from finger prick, indicate anemia, the researchers will immediately confirm this result by taking a venous blood sample from the child. The researchers will measure hemoglobin status via the venous blood sample with the Hemocue 301. These steps to measure hemoglobin status of potential participants will be utilized to minimize the screening procedures burden for the participant (this mixed approach is a compromised decision to avoid a third blood sample in all participants). There is also a growing body of evidence that measuring hemoglobin using a venous blood sample is more accurate than using a fingerpick, so if the presence of anemia is suspected the participant and the researcher will have an accurate hemoglobin result (via venous blood) to provide appropriate recommendation of care to the participating children (15). If the anemia testing via a venous blood sample suggests the child has anemia, the researchers will refer the child to the local health clinic. The physicians at the health clinic will treat the child for anemia per their established guidelines. The researchers of the study will ensure that the child receives the appropriate treatment for anemia from the local health clinic. To be enrolled in the study, children must have a hemoglobin level ≥12.9 g/dl for children 10-11 yrs old and ≥13.4 g/dl for children 12-14 yrs old (this cut-off accounts for the high altitude of the study setting to rule out anemia) (14). Using an inclusion/exclusion criteria questionnaire, the research staff will ask the child (and their parent as appropriate) questions to assess study eligibility. If the child is deemed eligible for the study, the trained research staff will review the informed consent form with the child and parent. Written assent (child) and consent (parent) must be obtained for the child to officially enroll in the study. Children will then be randomized to one of two groups: with an equal chance of being assigned to either the biofortified or control maize group.

Deworming:

After informed consent and assent are obtained, the research staff will ascertain if child has recently been given deworming medication at school. If not, each participant will be given one oral dose of albendazole for deworming of intestinal helminth 7 days before the isotope administration. This is a standard procedure to deworm children at risk for these parasites. Helminths are a common parasite in these populations with up to 85% of children having one present (12, 13) and is known to affect mineral absorption (16-20). Per INCAP’s input, the school recently dewormed all their children. If the children have been systematically dewormed at the school, we will not need to complete this step.

Lead-in Period:

Three days leading up to the isotope studies, the households will be given the assigned study maize (biofortified or control, blinded) about equal to the amount that will be consumed in the study. The families will prepare their tortillas and other maize-based dishes with the study maize to ensure that it is well accepted and tolerated during the isotope studies. This maize will be provided by Semilla Nueva.

Baseline Sample Collection:

A community research center or designated location in the school will be established in a centrally located place for the study. The center/school will facilitate the commuting of families/children from neighboring communities, and the center/school will provide researchers with access to basic resources to collect the biological samples in the community such a clean environment with tile floor, light, ventilation, running water, electricity, toilet, biowaste disposal, and freezers. The first option will be to set the community research center in a local health facility or at the school, and the second option will be a rented facility with the conditions required to run the study.

It is estimated that children and their families will be invited to a community research center in groups of 5-6 children. All isotope and study meal administration and blood collections will occur in the community research center/school. Urine collection will occur in the home or research center under the supervision of the research staff.

In the morning of study day 4, a baseline stool sample will be collected for markers of gut inflammation. A baseline spot urine sample (~30 mL) will be collected using metal-free procedures before any studies are initiated to measure zinc isotope enrichment (10). A baseline blood sample will be taken to measure natural iron abundance in erythrocytes necessary to calculate iron absorption; procedures discussed later in this protocol. All samples will be stored at -20°C at INCAP until shipment to the University of Colorado for elemental and isotopic enrichment of Zn and Fe.

Zinc Absorption Studies:

On the isotope administration day (study day 4) 5-6 children will come to the research clinic/school early in the morning to consume all meals for the day and to administer the oral and intravenous Zn isotopes. They will be instructed not to eat or drink anything after midnight on that day, excluding water. All meals and snacks for the day will be provided and consumed under the supervision of the research team. The three major meals (breakfast, lunch, and dinner) will be extrinsically labeled with 70Zn isotope doses adjusted to be proportional to the amount of zinc in the meal and to ≤10% of total daily zinc intake (~300 µg of 70Zn). Oral isotope solutions will be administered in sips, starting mid-way through the meal, according to procedures described previously (4, 21).

Weighed duplicates of all foods consumed by each child during the isotope administration day will be collected and subsequently analyzed for total dietary intake of zinc and phytate, at CU Pediatric Nutrition and USDA Agriculture Research Service laboratories, respectively. The study diet provided to the children will include tortillas from the study maize. This will make up 55-70% of their daily calories. The study’s Registered Dietitian will calculate estimated daily caloric needs of the children based on body weight (kcal/kg/day). Additional foods provided include those that are low in zinc and iron, such as fruits and vegetables, and a small source of protein, such as beans, will be provided. Weighed duplicates of each child’s total diet will be collected and analyzed for zinc and iron content to calculate total dietary zinc and iron intake per day.

Between the labeled meals of lunch and dinner, a single dose of 67Zn (~800 ug) will be administered intravenously (IV) by the study physician using a “butterfly” infusion set in an antecubital vein (i.e., not an in-dwelling catheter) fitted with a 3-way stopcock. Prior to administering the single dose of IV 67Zn, ~5 mL of blood will be drawn for measurement of circulating biomarkers of micronutrient status, systemic inflammation (AGP and CRP), and anemia. A drop of venous blood will be immediately (within 30 seconds of the blood draw) used to measure hemoglobin levels using the Hemocue® 301. This hemoglobin measurement is required for baseline iron isotope assessment, and it will be used to verify anemia status of the children. If the child presents with anemia, the researchers will ensure that the child receives proper treatment. This sample will also be used to measure baseline iron isotope enrichment in the erythrocytes.

On the third day after isotope administration (study day 7), the research team will visit participants in their homes or participants will come to the research site to collect morning and afternoon spot urine samples (30 mL each) for 4 days, taking precautions to avoid zinc contamination from stool or other sources (e.g. careful cleaning of skin in perineum and hands). Research staff, parents, and children will receive training regarding all urine collection procedures. The CU team will provide pre-labeled collection vessels. Similar collection procedures have been used previously by both CU and INCAP teams (4, 21).

Duplicate diet and urine samples will be transported to CU, where zinc stable isotope enrichments will be used to determine total dietary zinc (TDZ) and fractional absorption of zinc (FAZ). TAZ will be calculated by TDZ x FAZ using the dual isotope tracer ratio methods described previously (9, 22). Phytate analysis of duplicate diets will be undertaken by analyses of phytic acid phosphorus by a modified ferric precipitation method at the USDA ARS lab (23). The stool sample will be sent to CU for storage and for measurement of fecal inflammatory markers pending obtaining supplementary funds (10, 24).

Iron Absorption Studies:

Similar to study day 4, on study days 5 & 6 participants will come to the research center in a fasted state. Oral administration of accurately measured concentrations of tracer solutions (58Fe, ~2000 µg divided over all meals on two days, not to exceed 10% of the natural Fe in the meal) will be initiated at approximately the mid-point of each of the three major meals on each day. The oral isotope dose will be administered in sips between bites as the children finish their meal, i.e., eat a bite, drink a sip of dose, eat a bite, drink a sip of dose until both the meal and isotope are consumed. Weighed duplicates of all foods consumed by each child during the isotope administration days will be collected and subsequently analyzed for total dietary intake of iron at CU lab and phytate at the USDA ARS lab. Only de-identified diet samples will be sent to the USDA ARS lab for phytic acid analysis.

On study day 20, the child and their parent will return to the center to have a 2 mL blood draw for erythrocytes for 58Fe. The blood draw will be completed under sterile conditions by a trained phlebotomist and study physician. Prior to the blood draw on day 20, the child’s weight will be taken for use in iron absorption calculations.

1. **Description, Risks and Justification of Procedures and Data Collection Tools**

This is considered to be a very low risk study. The investigators have extensive experience conducting stable isotope studies, using similar procedures, in very diverse populations, age groups, and settings (4, 9, 21).

There are no known risks with consuming solutions enriched in zinc and iron stable isotopes. These isotopes are naturally occurring and are already found in the body and foods. There is minimal risk with the study blood draw and isotope infusion. The researchers have previously administered over 3000 isotope doses in the pediatric population with no adverse effects.

Sometimes, the administration of albendazole (used for deworming) can cause stomach pain or nausea. The study physician will monitor the administration and will observe the children for a period after consumption.

Additionally, there are no risks with the consumption of the control or biofortified maize which are locally grown in Guatemala. The biofortified maize is cross-pollinated and grown to increase the amount of zinc and iron naturally occurring in the maize. (This is not a genetically modified food). In addition, for several years now, biofortified maize has been available for purchase and consumption in Guatemala. Many farmers opt for growing this maize, and it is common in household consumption and in the markets. For this study, all the maize will be provided by Semilla Nueva.

Finally, there is a very small risk of a loss of confidentiality with participating in any research study. The researchers will adhere to all ethical guidelines and make it a top priority to ensure that the privacy, anonymity, and confidentiality of data/information identifying study participants will be strictly maintained. All consent forms, medical information, description of treatment, and results of the laboratory tests collected during the study will be kept under lock and key. Additionally, there is no known social stigma to participating in this kind of research study.

The study will be approved by the Colorado Multiple Institutional Review Board (COMIRB) and INCAP IRB (in Guatemala) prior to implementation and registered on clinicaltrials.gov.

Currently, our research team is holding exploratory informative meetings about the study procedures with school principal, school parents association, health and civil community authorities of Chirijuyú, Tecpan where we plan to conduct the study. The response in terms of interest and support for the study has been very positive.

**E. Potential Scientific Problems**

Although the biofortified maize has about 20% more iron incorporated in the grain compared to traditional maize, the iron concentration in the biofortified maize in this study is lower than other studies that have studied biofortified grains. This smaller difference in iron content of the two study maizes may lead to the inability to detect a significant difference in total absorbed iron between the biofortified and control maize groups.

**F. Data Management, Processing, and Analysis**

All data will be collected using pen and paper during the metabolic studies and stored under lock and key at INCAP. A soft copy of the data will be stored in excel on the CU protected shared drive and/or in REDCap.

Zinc absorption studies: Data processing will include determination of FAZ by urine enrichment (dual isotope tracer ratio), according to the following equation: FAZ = enrichment (oral/intravenous) x dose (intravenous/oral) (9, 10, 25). Along with total dietary zinc intake (TDZ) derived from analysis of the duplicate diets, FAZ will be used to determine TAZ, mg/d, (TAZ = FAZ x TDZ) including amount of zinc absorbed specifically from the biofortified and control maize meals.

Iron absorption studies: For iron absorption, we will determine how much iron was incorporated into the erythrocytes on Day 20. The quantity of administered enriched 58Fe dose (mg) incorporated into erythrocytes (58Fe(inc)) on day 20 is calculated as follows (26):

 58Fe(inc) = 58Fe(enrichment) × Fe(circ)

where Fe(circ) is the quantity of total circulating Fe (mg) on day 20. Fe(circ) is estimated as:

 Fe(circ) = Volblood × Hb × 3.47

Where Volblood is calculated by assuming 70 ml blood / kg body weight. 85-90% of absorbed iron is assumed to be incorporated into erythrocytes (27):

Fractional iron absorption (FAI) = 58Fe(inc) ÷ (amount of 58Fe enriched dose × 0.85-0.9).

Total dietary intake of iron (TDI) will be calculated from weighed dietary intake on dosing days x amount of iron in the diet determined by lab analyses of duplicate diets. Total iron absorption will be calculated by multiplying the TDI x FAI.

Summary statistics (mean, standard deviation, median, interquartile ranges) will be calculated, and distributions will be examined for all proposed measured variables. Unpaired Student’s t-tests will be used to compare the means of TAZ and TAI for the 2 groups (biofortified maize vs. control maize). Associations between inflammation and absorption, as well as dietary intake and absorption will be investigated with regression analysis. Together with data from other concurrent and completed studies feeding other grains, a trivariate model will be expanded, which predicts the quantity of zinc absorbed each day as a function of dietary zinc and phytate (28). All comparisons will be considered significant at p <0.05. Simple and multiple linear regression will be used generally. All statistical analyses will be completed with STATA and GraphPad PRISM.

**G. Summarize Knowledge to be Gained**

Measurements of zinc and iron absorption from meals for an entire day in which maize is the food staple and accounts for most of the energy intake are essential for understanding the potential quantitative benefits of breeding high zinc and iron maize for use in diets of school aged children. These data will be applied to the trivariate model to extend this approach to young children, with potential to better understand zinc absorption as a function of zinc and phytate intakes. Finally, the results will provide important knowledge for government health and nutrition programs that currently support the incorporation of biofortified maize as a part of food and nutrition security programs, including the support to vulnerable farmers, who receive either maize seeds for planting or ground maize for household consumption.

 **H. References:**

1. Stevens GA, Beal T, Mbuya MN, Luo H, Neufeld LM, Addo OY, et al. Micronutrient deficiencies among preschool-aged children and women of reproductive age worldwide: a pooled analysis of individual-level data from population-representative surveys. The Lancet Global Health. 2022;10(11):e1590-e9.

2. Gupta S, Zaman M, Fatima S, Shahzad B, Brazier AKM, Moran VH, et al. The Impact of Consuming Zinc-Biofortified Wheat Flour on Haematological Indices of Zinc and Iron Status in Adolescent Girls in Rural Pakistan: A Cluster-Randomised, Double-Blind, Controlled Effectiveness Trial. Nutrients. 2022;14(8). doi: 10.3390/nu14081657.

3. Mehta S, Huey SL, Ghugre PS, Potdar RD, Venkatramanan S, Krisher JT, et al. A randomized trial of iron- and zinc-biofortified pearl millet-based complementary feeding in children aged 12 to 18 months living in urban slums. Clin Nutr. 2022;41(4):937-47. doi: 10.1016/j.clnu.2022.02.014.

4. Kodkany BS, Bellad RM, Mahantshetti NS, Westcott JE, Krebs NF, Kemp JF, et al. Biofortification of pearl millet with iron and zinc in a randomized controlled trial increases absorption of these minerals above physiologic requirements in young children. J Nutr. 2013;143(9):1489-93. doi: 10.3945/jn.113.176677.

5. Rosado JL, Hambidge KM, Miller LV, Garcia OP, Westcott J, Gonzalez K, et al. The quantity of zinc absorbed from wheat in adult women is enhanced by biofortification. J Nutr. 2009;139(10):1920-5. doi: 10.3945/jn.109.107755.

6. Chomba E, Westcott CM, Westcott JE, Mpabalwani EM, Krebs NF, Patinkin ZW, et al. Zinc absorption from biofortified maize meets the requirements of young rural Zambian children. J Nutr. 2015;145(3):514-9. doi: 10.3945/jn.114.204933.

7. Rosado JL, Díaz M, Muñoz E, Westcott JL, González KE, Krebs NF, et al. Bioavailability of zinc oxide added to corn tortilla is similar to that of zinc sulfate and is not affected by simultaneous addition of iron. Food Nutr Bull. 2012;33(4):261-6. doi: 10.1177/156482651203300406.

8. Adams CL, Hambidge M, Raboy V, Dorsch JA, Sian L, Westcott JL, et al. Zinc absorption from a low-phytic acid maize. Am J Clin Nutr. 2002;76(3):556-9. doi: 10.1093/ajcn/76.3.556.

9. Krebs NF, Westcott JE, Culbertson DL, Sian L, Miller LV, Hambidge KM. Comparison of complementary feeding strategies to meet zinc requirements of older breastfed infants. Am J Clin Nutr. 2012;96(1):30-5. doi: 10.3945/ajcn.112.036046.

10. Long JM, Mondal P, Westcott JE, Miller LV, Islam MM, Ahmed M, et al. Zinc Absorption from Micronutrient Powders Is Low in Bangladeshi Toddlers at Risk of Environmental Enteric Dysfunction and May Increase Dietary Zinc Requirements. J Nutr. 2019;149(1):98-105. doi: 10.1093/jn/nxy245.

11. USAID. Guatemala: Nutrition Profile. 2018.

12. INCAP. Informe del Sistema de Vigilancia Epidemiológica de Salud y Nutrición - SIVESNU (agosto 2018 – abril 2019)Módulo 2: Salud y nutrición infantil. 2020.

13. INCAP. Informe del Sistema de Vigilancia Epidemiológica de Salud y Nutrición - SIVESNU (agosto 2018 – abril 2019) Módulo 3: Salud y nutrición de la mujer. 2020.

14. Organization WH. Guideline on haemoglobin cutoffs to define anaemia in individuals and populations. Geneva, 2024.

15. Hackl LS, Karakochuk CD, Mazariegos DI, Jeremiah K, Obeid O, Ravi N, et al. Assessing Accuracy and Precision of Hemoglobin Determination in Venous, Capillary Pool, and Single-Drop Capillary Blood Specimens Using three Different HemoCue® Hb Models: The Multicountry Hemoglobin Measurement (HEME) Study. J Nutr. 2024. doi: 10.1016/j.tjnut.2024.03.019.

16. Gaensbauer JT, Lamb M, Calvimontes DM, Asturias EJ, Kamidani S, Contreras-Roldan IL, et al. Identification of Enteropathogens by Multiplex PCR among Rural and Urban Guatemalan Children with Acute Diarrhea. Am J Trop Med Hyg. 2019;101(3):534-40. doi: 10.4269/ajtmh.18-0962.

17. Jensen LA, Marlin JW, Dyck DD, Laubach HE. Prevalence of multi-gastrointestinal infections with helminth, protozoan and Campylobacter spp. in Guatemalan children. J Infect Dev Ctries. 2009;3(3):229-34. doi: 10.3855/jidc.41.

18. Watkins WE, Cruz JR, Pollitt E. The effects of deworming on indicators of school performance in Guatemala. Trans R Soc Trop Med Hyg. 1996;90(2):156-61. doi: 10.1016/s0035-9203(96)90121-2.

19. Watkins WE, Pollitt E. Effect of removing Ascaris on the growth of Guatemalan schoolchildren. Pediatrics. 1996;97(6 Pt 1):871-6.

20. Croke K, Atun R. The long run impact of early childhood deworming on numeracy and literacy: Evidence from Uganda. PLoS Negl Trop Dis. 2019;13(1):e0007085. doi: 10.1371/journal.pntd.0007085.

21. Ariff S, Krebs NF, Soofi S, Westcott J, Bhatti Z, Tabassum F, et al. Absorbed zinc and exchangeable zinc pool size are greater in Pakistani infants receiving traditional complementary foods with zinc-fortified micronutrient powder. J Nutr. 2014;144(1):20-6. doi: 10.3945/jn.113.178715.

22. Sheng XY, Hambidge KM, Zhu XX, Ni JX, Bailey KB, Gibson RS, et al. Major variables of zinc homeostasis in Chinese toddlers. Am J Clin Nutr. 2006;84(2):389-94. doi: 10.1093/ajcn/84.1.389.

23. Raboy V, Dickinson DB, Below FE. Variation in seed total phosphorus, phytic acid, zinc, calcium, magnesium, and protein among lines of *Glycine max* and *G. Soja*. Crop Sci. 1984;24:431-4.

24. Mondal P, Long JM, Westcott JE, Islam MM, Ahmed M, Mahfuz M, et al. Zinc Absorption and Endogenous Fecal Zinc Losses in Bangladeshi Toddlers at Risk for Environmental Enteric Dysfunction. J Pediatr Gastroenterol Nutr. 2019;68(6):874-9. doi: 10.1097/mpg.0000000000002361.

25. Friel JK, Naake VL, Jr., Miller LV, Fennessey PV, Hambidge KM. The analysis of stable isotopes in urine to determine the fractional absorption of zinc. Am J Clin Nutr. 1992;55(2):473-7. doi: 10.1093/ajcn/55.2.473.

26. Fomon SJ, Serfass RE, Nelson SE, Rogers RR, Frantz JA. Time course of and effect of dietary iron level on iron incorporation into erythrocytes by infants. J Nutr. 2000;130(3):541-5. doi: 10.1093/jn/130.3.541.

27. Assessment of Iron Bioavailability in Humans Using Stable Iron Isotope Techniques. Vienna: INTERNATIONAL ATOMIC ENERGY AGENCY, 2012.

28. Miller LV, Krebs NF, Hambidge KM. A mathematical model of zinc absorption in humans as a function of dietary zinc and phytate. J Nutr. 2007;137(1):135-41. doi: 10.1093/jn/137.1.135.