Protocol version 1.4

29 July 2021

Statistical Analysis Plan (SAP)

Double-Blind Cluster Randomised Controlled Trial of Chicken Liver and Eggshell Crackers in Pregnant and Lactating Mothers in a Disadvantaged Setting in Indonesia in 2021

GENERAL INFORMATION

Research Project	:	*Wellcome Trust Grant* - 216447/Z/19/Z
Intervention Product Identity	:	UNS84605 (MEC (*micronutrient-enriched crackers)* and Placebo)
Title	:	Double-Blind Cluster Randomised Controlled Trial of Chicken Liver and Eggshell Crackers in Pregnant and Lactating Mothers in a Disadvantaged Setting in Indonesia in 2021
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ClinicalTrials.gov identifier	:	NCT04564222
Version	:	1.4
Date	:	29 July, 2021

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1. Introduction

The objective of this project is to conduct a Double-Blind Cluster Randomised Clinical Trial (RCT) to assess the impact of daily maternal consumption of MEC on infant birth length and linear growth. Previous interventions have primarily focused on birth weight. This Statistical Analysis Plan (SAP) aims to offer a comprehensive description of the study endpoints and the corresponding analysis methods.

2. Study design

2.1 Brief Description

The design of this study is a double-blind two-phase cluster randomized controlled trial to examine the effect of daily consumption of locally produced MEC (intervention group) compared to placebo crackers (control groups) in two time intervals: 1) from the second trimester of pregnancy (8-14 weeks) to delivery only; and 2) when the intervention is continued for up to 5 months post-partum, with the primary outcomes being birth length, attained linear growth, and growth velocity of the infant.

A total of 28 clusters (villages) will be randomised to either intervention or control using a randomized sequence. Selected pregnant women from these villages will receive daily interventions in the form of 7 x 75 gram micronutrient-enriched food products, made from basic ingredients of liver and chicken eggshells (Micronutrient-enriched Crackers/MEC) or placebo. The research team will give 100 grams of the same product as the respondents to the family of the respondents, so the respondents will not need to share the intervention product with the other family members. MEC or placebo will be produced locally and be identical in size, color, and packaging. MEC or placebo packages will be coded and only the production manager will know the allocated codes for the MEC or placebo packages. The production manager will be instructed to not share the code with any of the investigators involved in the trial until the primary outcomes of the study have been analyzed statistically or as requested by the Ethics Committee and/or Data Safety Monitoring Board (DSMB).

2.2 Place and Time

This study will include 28 villages (Table 2) in 3 subdistricts, namely Tanjungsari, Pamulihan, and Sukasari, in Sumedang District, West Java. The timing of the research will be from July 2020 - July 2023.

2.3 Intervention Product

This study will use micronutrient-enriched crackers (MEC) and placebo as the intervention products. The intervention products are made from the ingredients that can be seen in Table 1.

Table 1. MEC and Placebo Recipes

MEC			Placebo		
Ingredient	**Weight (g)**	**Percentage (%)**	**Ingredient**	**Weight (g)**	**Percentage (%)**
Tapioca flour	47	8.6	Tapioca flour	40	9.4
Chicken liver	160	29.3	MOCAF	35	8.2
Drinking water	10	1.8	Drinking water	38	8.9
Wheat flour	200	36.6	Wheat flour	165	38.8
Salt	3	0.5	Salt	2	0.5
Garlic	18	3.3	Garlic	18	4.2
Mushroom bouillon powder	4	0.7	Chicken stock powder	4	0.9
Margarine	50	9.2	Margarine	40	9.4
Chicken egg	30	5.5	Chicken egg	30	7.1
Green onion	15	2.7	Green onion	15	3.5
Coriander leaf	23	4.2	Celery	23	5.4
Eggshell powder	8	1.5	*Pangium edule* dried seed	15	3.5
Bay leaf	1	0.2	**Dough weight**	**425**	**100**
Ginger	1	0.2	**Cooked weight**	**300**	**-**
Lemon juice	2	0.4			
Lemongrass	2	0.4			
Alpinia galangal	1	0.2			
Lime leaf	1	0.2			
Dough weight	**546**	**100**			
Cooked weight	**450**	**-**			

2.4 Population and Sample

2.4.1 Inclusion and Exclusion Criteria

1) Inclusion Criteria
   a. Pregnant women aged 19-35 years old;
   b. Gestational age 7-13 weeks at the time of the screening process and be willing to take part in an intervention study at 8-14 weeks gestation;
   c. Permanent residents who will stay in the research area for the following 1 year.
2) Exclusion Criteria
   a. Mothers who have chronic diseases such as hypertension (blood pressure ≥ 140/90 mmHg) or under treatment for hypertension, diabetes (non-fasting blood sugar when >200 mg/dL) or under treatment for diabetes, hypercholesterolemia (blood total cholesterol >240 mg/dL), hyperuricemia (blood uric acid > 6 mg/dL), have had a history of tuberculosis or under treatment for tuberculosis or under treatment for other chronic diseases that requires a long treatment, such as cancer, heart disease, epilepsy, etc.;
   b. Have a history of preeclampsia/eclampsia and gestational diabetes in their previous pregnancy;
   c. Have a risk of chronic energy deficiency (mid-upper arm circumference < 23.5 cm);
   d. Have severe anemia (hemoglobin < 70 g/L);
   e. Have a history of allergy to chicken liver and/or eggs.
3) Drop-out criteria
   a. Preeclampsia, eclampsia;
   b. Dead infant/stillbirth;
   c. Twin delivery;
   d. Infants are born with congenital anomalies;
   e. Infants are not breastfed.
4) Other criteria

   If an infant is born with Low Birth Weight (LBW) or is premature, then the infant will not be included in the measurement of breast milk volume and

assessment of exclusive breastfeeding status with Deuterium Oxide Dose-to-Mother Technique. However, the growth and data collection such as anthropometry and ages and stages questionnaires (ASQ-3) will still be carried out.

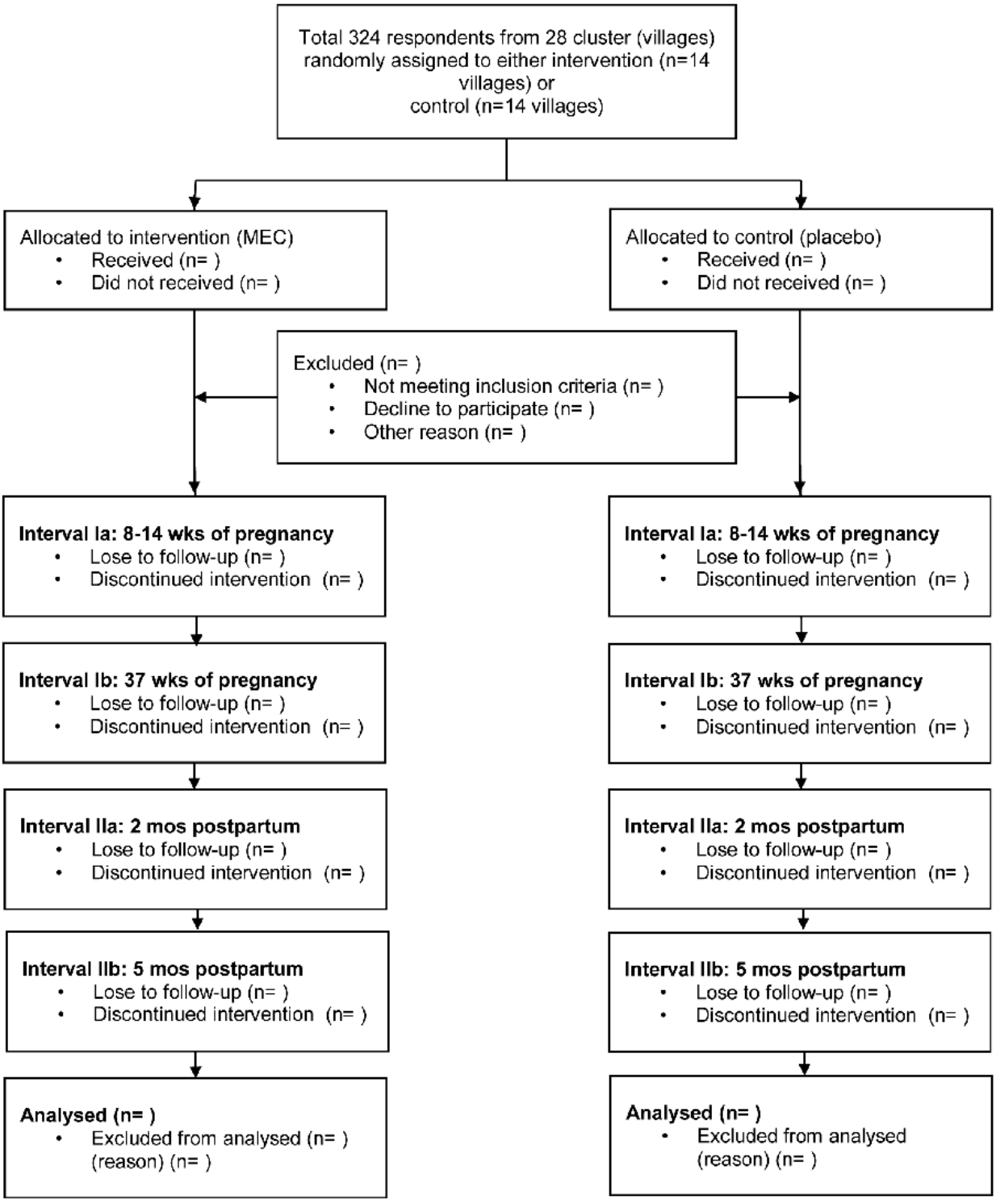

Figure 1 Study designs. MEC: Micronutrient-enriched Crackers.

2.4.2 Population and Sample size calculation

The population of this study are the people who live in the area of Pamulihan, Sukasari and Tanjungsari Sub-Districts, Sumedang District, West Java. All parameters used in calculating sample sizes can be seen in Table 1. Design effects, cluster/village, are estimated using previous data collected in the same area.

1) Phase 1: To detect a 1 cm difference in birth length between the intervention and control groups (this is approximately 0.5 SD from the WHO Growth Chart (2007)), with power of 0.90, statistical significance of 0.05, and design effect of 1.1 due to village clusters, a minimum sample size of 228 is required (114 in each group) (to be presented in tabular form).
2) Phase 2: Based on an average growth velocity of 3-3.2 cm/month, and to detect differences in growth velocity of 0.70 cm between the intervention and control groups, with power of 0.80, statistical significance of 0.05, assuming a standard deviation of 2.2 cm/month, a within-person correlation of 0.73 and a design effect of 1.1 due to village clusters, a sample size of 162 would be required (81 in each group) (to be presented in tabular form).

As the primary outcomes for Phase 1 require a sample size of 228, then 11 participants from 22 clusters will be required. However, we aim to recruit 11 participants from 28 clusters, giving a sample size of 308. This will allow for exclusion due to stillborn or premature birth (~10%, i.e., n~15 in each group) and drop-out or loss-to-follow-up of approximately 15%, so the total sample size is 324. For Phase 2, if 231 participants are retained, a further 1-5% may fail to breastfeed their infants, giving a final sample size of 219-228 to assess Phase 2 outcomes. This sample size will also permit the detection of the differences in the secondary outcomes also specified below (Table 2), in addition to the primary outcomes.

Table 2. Parameters used in calculating sample sizes for secondary outcomes

Measure	Observed mean	Observed SD	Difference to detect	ICC	*Design effect*	Sample size**
Birth length (cm)[a]	49.7	2.2	1.0	0.010	1.10	114
Infant length at 5 months (cm)[a]	63.0	2.2	1.0	0.010	1.10	114
Birth weight (g)[a]	3013	514	200	0.010	1.10	116
Haemoglobin at 3rd trimester (g/dL)[b]	12.6	1.2	0.5	0.048	1.48	132
Volume of breast milk at 5 months post-partum (mL/day)[c]	787	148	70	0.010	1.10	80
Micronutrient concentrations of breast milk at 5 months post-partum (Vit A, µg/L)[c]	634	281	130	0.036	1.36	102
Maternal diet at 5 months post-partum (Vit A, µg RAE/d)[d]	478	244	110	0.010	1.10	87
Maternal micronutrient status at 5 months post-partum (Ferritin, µg/L)[e]	35.8	29.7	13.0	0.010	1.10	91
Infant micronutrient status at aged 5 months (Ferritin, µg/L)[e]	46.7	35.6	15.0	0.010	1.10	99

*ICC: intra-class correlation

*Design effect: based on the average size of cluster= 11

**Required sample size per group with 80% power and alpha= 0.05

[a]Data from observation by Diana A, Mallard SR, Haszard JJ, Purnamasari DM, Nurulazmi I, Herliani PD, et al. Consumption of fortified infant foods reduces dietary diversity but has a positive effect on subsequent growth in infants from Sumedang district, Indonesia. PLoS One. 2017 Apr: 12(4):1-17;

[b]Data from observation by Diana A, Haszard JJ, Purnamasari DM, Nurulazmi I, Luftimas DE, Rahmannia S, et al. Iron, zinc, vitamin A, and selenium status in a cohort of Indonesian infants after adjusting for inflammation using several different approaches. Br J Nutr. 2017 Nov: 118(10):830-839;

[c]Data from observation by Daniels L, Gibson RS, Diana A, Haszard JJ, Rahmannia S, Luftimas DE, et al. Micronutrient intakes of lactating mothers and their association with breastmilk concentrations and micronutrient adequacy of exclusively breastfed Indonesian infants. Am J Clin Nutr. 2019 Aug: 110(2):391-400;

[d]Data from observation by Rahmannia S, Diana A, Luftimas DE, Gurnida DA, Herawati DMD, Houghton LA, et al. Poor dietary diversity and low adequacy of micronutrient intakes among rural Indonesian lactating women from Sumedang district, West Java. PLoS One. 2019 Jul: 14(7):1-13;

[e]Unpublished data

3. Study Goals and Objectives

This will be the first RCT in Indonesia to examine the effect of daily maternal consumption of MEC on birth length and infant linear growth; earlier interventions have examined only birth weight.

3.1 Study Goals

1) To determine whether consumption of micronutrient enriched MEC products from 8-14 weeks' gestation to delivery improves birth length in the intervention compared to the placebo group (Phase 1);
2) To determine if the daily consumption of micronutrient enriched crackers (MEC) by pregnant mothers from 8-14 weeks' gestation to 5 months post-partum improves attained linear growth of infants at age 5 months and linear growth velocity of infants from birth to age 5 months in the intervention compared to the placebo group (Phase 2).

3.2 Secondary Outcomes

1) To determine the birth weight (measured within 24 hours after delivery);
2) To determine the maternal hemoglobin levels at 35-36 weeks' gestational age and 5 months post-partum (± 1 week);
3) To determine the maternal food intake at 35-36 weeks' gestational age, and 2 months post-partum (± 1 week), and 5 months post-partum (± 1 week);
4) To determine the breast milk volume and micronutrient breast milk concentrations at 5 months' post-partum (± 1 week);
5) To determine the infant breast milk micronutrient intake, based on breast milk volume and micronutrient breast milk concentrations at 5 months' post-partum (± 1 week);
6) To determine maternal micronutrient status (ferritin, transferrin serum, RBP, zinc, and selenium of mother at 35-36 weeks' gestational age and 5 months' post-partum (± 1 week), after being adjusted with the inflammation status (C-reactive protein and alpha-1-acid glycoprotein);

7) To determine infant micronutrient status (ferritin, transferrin serum, retinol-binding protein RBP, zinc, and selenium at 5 months old (± 1 week), after being adjusted with the inflammation status (C-reactive protein and alpha-1-acid glycoprotein);
8) To determine the infant development at 2 and 5 months old (± 1 week) in the intervention and control groups;
9) To determine the morbidity incidence rate of the mothers and infants in the intervention and control groups.

4. Data Management and Analyses

4.1 Data Management

Enumerators will daily double-check the accuracy and completeness of data collected. All data will be doubly entered and checked daily for identical entries. Data cleaning will be conducted weekly by the data manager to ensure values are within the acceptable/plausible ranges and identify any missing values. Any errors will be flagged and re-checked with hard copy data.

4.2 Statistical Analysis

Primary analyses will be by intention to treat. The mean number of crackers consumed overall and compliance rates will be examined. Only live born infants will be included in all analyses. Intervention groups will be compared using mixed effect regression models using cluster (village) as a random effect. Main outcomes for analyses for phase 1 are birth length and for phase 2 are attained and incremental linear growth at 5 months post-partum. Analyses for phase 2 will be conducted with adjustment for weight-for-age and breastfeeding practice (exclusively/partially breastfeeding), where appropriate. Model assumptions will be checked using standard procedures and variables log transformed when necessary. Guidelines for reporting will be followed, as outlined in the Consolidated Standards of Reporting Trials (CONSORT) statement: extension to cluster randomised trials.

Given the short period of data collection and the anticipation that no highly potential harm/risks will be expected, the study will continue until the end of data collection without data interim analysis. As the data will not be analysed until the whole study is complete, there is no requirement to power the study for multiple looks. The research team will report

any undesirable events to the DSMB and the Ethics Committee. DSMB may call for an interim analysis if it is required. Termination of the clinical trial will be carried out based on the recommendation from the DSMB and/or Ethics Committee.