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# Childhood stunting and micronutrient status unaffected by RCT of micronutrient fortified drink

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## Abstract

Micronutrient supplementation is widely used to prevent stunting in children under 5 years in low- and middle-income countries (LMIC), but the impact of treatment has been disappointing, possibly due to non-compliance. Our aim was to deliver long-term micronutrient supplementation via a novel, culturally acceptable liquid food to improve linear growth in a high stunting prevalence region.

In a randomised control trial, 971 children aged 6–72 months received either 'Chispuditos®' ($n = 681$), a hot drink (atole) fortified with micronutrients (atole + MN) (9 mg/zinc, 12.5 mg/iron), or lactose-free milk ($n = 290$) for 18 months. Primary outcomes were changes in length/height-for-age (HAZ) score and the prevalence of stunting at 18-month follow-up. Adherence was monitored monthly, and 73% children in atole + MN group consumed at least half their daily zinc and iron requirement. At 18 months, there was no difference between the treatments in growth [mean change in HAZ −0.02 (95% CI −0.12, 0.08)] or stunting [atole + MN 41%, milk 41%; RR 0.99 (95% CI 0.84, 1.19)]. There were no differences in haemoglobin (HB), ferritin or zinc. No children had iron deficiency anaemia (IDA) at outcome, but zinc deficiency remained equally prevalent in both groups: atole + MN 35%, milk 35% [RR 1.02 (95% CI 0.83, 1.24)]. There was no difference in morbidity between the groups, and micronutrient status was unrelated to HAZ. Long-term micronutrient supplementation via a culturally acceptable food had no impact on stunting or morbidity, raising the question of whether large-scale micronutrient supplementation is worthwhile.

## 1 | INTRODUCTION

Global stunting prevalence in children under 5 years of age has been shown to be slowly declining, but in many countries like Guatemala, with low human development and severe inequity, it remains stubbornly high (2020 Global Nutrition Report: Action on equity to end Malnutrition, n.d.). Stunting is strongly associated with iron and zinc deficiency, which are still prevalent in low-income countries (Black et al., 2008) and all are strongly associated with increased risk of infections and overall morbidity and mortality

(Black et al., 2013). Thus, an extensive body of research has focused on addressing these problems (Lassi et al., 2020). However, the results of trials of micronutrient supplementation have been disappointing; some show positive outcomes for iron and zinc status, and mixed for weight outcomes, but most trials have shown no or only very small effects on linear growth (Lassi et al., 2020; Petry et al., 2016; Tam et al., 2020). It has been suggested that this may reflect poor adherence to the supplementation programmes (Zaidi et al., 2020).

In Guatemala, stunting affects nearly half of all children under 5 years of age with higher levels in rural and indigenous populations (2020 Global Nutrition Report: Action on equity to end Malnutrition, n.d.). Anaemia and iron deficiency are also highly prevalent, affecting up to 56% and 81%, respectively (Palacios et al., 2020). In Guatemala, breastfeeding during the first year of life is highly prevalent, though exclusivity is not universal (Colombara et al., 2015), but the transition to solid foods is marked by poor quality and quantity of complementary foods. The diet in Guatemala tends to be monotonous, plant based and high in phytates (Arsenault & Brown, 2017; Vossenaar et al., 2018), with limited intake of animal source or nutrient-dense foods, particularly bioavailable sources of iron and zinc. For decades, an approach to fighting chronic malnutrition in Guatemala has been to provide fortified food supplements, and food aid programmes are well established and valued by governments and non-governmental organisations alike. However, compliance issues and reduced evidence of long-term impact on stunting reduction are reported (Hossain et al., 2017). In Guatemala, 'atoles' (hot starch-based drinks) are culturally accepted and form part of the diet pattern. In order to increase the acceptability and thus compliance with supplementation programmes, a drink-based supplement (Chispuditos®) was developed, which was fortified with high levels of zinc iron and other vitamins and minerals, thought to be important for longitudinal growth. This novel food was administered in two different longitudinal cohorts of toddlers attending nurseries in Guatemala city over a period of 2.5 and 4 years with pre- and post-measurements of growth and findings suggesting small but promising improvements in stunting and iron status (Villanueva et al., 2015; Villanueva & Reinhart, 2013). Thus, the aim of this study was to test in a randomised control trial (RCT) the hypothesis that providing extra micronutrients via this culturally acceptable liquid food over an extended period would reduce stunting, improve zinc and iron status and reduce morbidity compared to an energy/protein-matched milk with no added micronutrients.

**Key messages**

- Multivitamin mixes including zinc are widely used in food supplementation programmes for stunting prevention.
- Meta-analyses suggest these may have effect on growth and morbidity, but many earlier studies were small and/or short term and may have had poor compliance.
- Despite long-term supplementation with a culturally acceptable fortified atole, no effect was found on growth or morbidity.

## 2 | METHODS

### 2.1 | Study design and sample size

This was an open-label RCT (Registration NCT01643187) conducted between 2012 and 2013 in the southwest region of Guatemala in 18 rural communities and one urban district, San Felipe, Retalhuleu. Participants received an atole + MN, or lactose-free milk (henceforth referred as milk), over 18 months with a 7:3 ratio of intervention to control. The original protocol did not calculate power, but post hoc calculations based on the sample size used suggest that the study was 80% powered at 0.05 level to detect a difference between the two groups at follow-up of 0.22 height z-scores using the Altman nomogram (Altman, 1980) and a reduction in stunting from 40% to 31% (Epi Info StatCalc 7.2.3.1).

### 2.2 | Participants, inclusion criteria and recruitment

All families with children aged 6–72 months of age living in the study sites were invited to take part in a nutritional screening programme coordinated by the non-governmental organisation APEVIHS (Asociación Para la Prevención y Estudio del VIH/SIDA) with approval of the local authorities. All parents were informed of the study, and if their children were in the age range of 6–72 months, they were invited to take part. Following parental signed consent, children were measured, and socio-demographic information was recorded. Criteria for inclusion in the study were then presenting a degree of malnutrition in the form of being <−1SD for weight, height or WHZ while not suffering from any severe health problem.

Participants were allocated to intervention (70%) and control groups (30%) using a computer-generated random allocation procedure stratified by community and age group.

### 2.3 | Intervention

The fortified atole (Chispuditos®) consisted of a daily dose of 18.75 g powder to be cooked with water and 18.75 g of sugar. The amount of powdered drink to be supplemented by the families was measured using a standardised plastic scoop provided to the families. The control drink consisted of 27 g of lactose-free milk (De-lactosed Nido®, Nestle, Centro America) provided as powder to be reconstituted with water (Table 1). The supplements were delivered to each child at their

**TABLE 1** Nutrient composition and amounts per portion of made-up atole + MN (Chispuditos®) and lactose-free milk (Milk)

| Nutrients | Unit | Atole + MN (Chispuditos®) Quantity | Milk[b] Quantity | RNI/day |
|---|---|---|---|---|
| Energy | Kcal | 147.8[a] | 116 | 1165 |
| Protein | g | 4 | 4.1 | 14.5 |
| Carbohydrate | g | 87[a] | 16.1 | |
| Fat | g | 1 | 3.9 | |
| Zinc | mg | 9 | 1.5 | 5.0 |
| Iron | mg | 12.5 | 1.9 | 6.9 |
| Folic acid | µg | 160 | 243 | 70 |
| Iodine | µg | 90 | N/R | 70 |
| Vitamin A | µg | 250 | 64.8 µg | 400 µg |
| Vitamin C | mg | 40 | 40 | 25 mg |
| Vitamin $B_{12}$ | µg | 0.9 | N/R | 0.4 mg |
| Vitamin $B_6$ | mg | 0.5 | 0.13 | 0.2 mg |
| Niacin | mg | 6 | 1.85 | 4 mg |
| Vitamin $B_2$ | mg | 0.5 | N/R | 0.2 mg |
| Vitamin $B_1$ | mg | 0.5 | 0.12 | 0.4 mg |
| Copper | mg | 0.3 | N/R | 0.5 mg |
| Vitamin $D_3$ | mg | 5 | 0.000945 | 0.007 mg |
| Vitamin E | mg | 5 | 0.00135 | 0.4 mg |
| Calcium | mg | 200 | 243 | 400 mg |
| Phosphorus | mg | 150 | N/R | 400 mg |
| Magnesium | mg | 40 | N/R | 80 mg |
| Selenium | µg | 17 | N/R | 10 µg |
| Manganese | µg | 0.17 µg | N/R | 16 µg |
| Pantothenic acid | mg | 1.8 mg | 0.59 | 1.7 mg |
| Biotin | µg | 8 µg | N/R | 2.4 µg |

*Note:* Parents were instructed to prepare Chispuditos® using 18.75 g of Chispuditos® and the equivalent measurement of sugar mixed with 8 oz. of water (240 ml) and boil for 8 min.
Abbreviations: N/R, not reported by the manufacturer; RNI, UK Recommended Nutrients Intake for age group 1-3 years.
[a]Energy contribution increased from 73 to 147.8 and carbohydrate from 12 to 87 with addition of 18.75 g of sugar.
[b]De-lactosed milk (Nido®, Nestle) in powder form; the amount recommended was a standardised measure of 27 g to be mixed with 8 oz. of boiled water (240 ml).

homes every 24 days for 18 months in a bag without commercial names of 450 g for Chispuditos® and 648 g of milk. Families supplied their own sugar, which is widely available. Instructions on preparation and distribution were given to the mother/career. Both drinks provided 4 g protein per day but differed slightly in energy contribution (∼30 kcal less in the control drink); the total volume instructed to be given was 8 fluid oz. (240 ml).

### 2.3.1 | Data collection

Anthropometry and blood samples were obtained at baseline and end point. Compliance and markers of morbidity were checked by staff monthly but recorded only three times for compliance and twice for morbidity. Information on socio-economic status and mothers' education were obtained at the start of the trial.

### 2.3.2 | Anthropometry

Anthropometric measures included weight, height/length and mid-upper arm circumference (MUAC). Weight was measured with a Roman-type 'paediatric scale' with a precision of 10 g and expressed from 10 to 100 g. Height/length was measured using a portable infant meter calibrated in cm. For the MUAC, the circumference of the non-dominant arm was measured with a band tape to the nearest 0.1 cm. 'WHO Anthro software' was used to calculate the z-scores of growth indicators and MUAC.

#### 2.3.3 | Blood measurements

For biomarkers, samples were drawn into trace-element-free tubes by trained staff to test for haemoglobin, serum zinc and serum ferritin. The Guatemalan National Micronutrient Survey (ENMICRON 2009–2011) procedures were used to ensure the integrity of blood samples from the field of laboratory (Ministerio de Salud Publica y Asistencia Social [MSPAS], 2010). For haemoglobin, whole blood was analysed using an auto haematology analyser (RT-7600 Rayto, Shenzhen, China), and the results were confirmed using peripheral smear and microscopy. Serum zinc (samples >20 μl) were determined using atomic emission spectrometry (MP-AES 4200, Agilent Technologies, Australia). The chemiluminescence immunoassay method was used to analyse serum ferritin with Maglumi 1000 (SNIBE, Shenzhen, China). C reactive protein (CRP) and α-acid glycoprotein AGP1 were analysed using nephelometry with a BN ProSpec Siemens® system. Lab analysis was conducted in the Institute of Central American and Panama laboratories and the University of Colorado.

#### 2.3.4 | Assessment of compliance and morbidity

Monthly home visits by researchers were conducted to check how the drink was being made up and monitor compliance in both treatment groups. On three occasions at median 27, 42 and 60 weeks from baseline, the researchers also weighed the current bag of atole + MN/milk and recorded the number of days since it had been delivered.

At the start of the intervention, children in both groups who were >24 months and that had not previously been dewormed in the last 6 months were given oral paediatric deworming treatment. At two home visits at median 36 and 79 weeks from baseline, research staff recorded if in the last 2 weeks a child had been referred to the health centre for, dehydration, haemorrhages, difficulty in breathing or diarrhoea.

### 2.4 | Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) software, Version 24. Height/length-for-age z-score (HAZ), weight-for-height z-score (WHZ) and weight-for-age z-score (WAZ) were assessed using the WHO growth reference standards (30) values < −2SD were taken for stunting, wasting and underweight, respectively. For the standardised anthropometric scores, outliers above +6 and −6 for the LAZ and WAZ z-scores were identified and removed.

For the analysis of serum ferritin, we excluded participants with inflammation, AGP ≥ 1. IDA was defined as haemoglobin concentration lower than 11 g/dl with serum ferritin concentration less than 12 μg/dl. Low zinc levels were defined according to WHO standards as serum zinc lower than 65 μg/dl for children under 10 years of age (de Benoist et al., 2007).

For the morbidity, participants were categorised as having had none, one or more than one of the diseases investigated.

For each compliance occasion, the total amount of product used (weight of bag when full − weight of bag at visit) was divided by the number of treatment days to give a weight per day. Then, the mean for each child was calculated. Where one of the three values was missing, the mean of two was taken, and where two were missing, the sole value was used. The full dose of atole + MN (18.75 g) supplied nearly twice the RNI for zinc and iron so that 10 g would fully meet the child's requirements.

#### 2.4.1 | Outcomes

This analysis of the trial data was undertaken in Glasgow 6 years after data collection was complete. All analyses were performed using the original randomised groups. Before handling the data, an analytical plan was agreed with those who had conducted the trial, specifying plausible primary and secondary outcomes, given the high rates of iron and zinc deficiency and their known effects. The primary outcomes as specified at registration were change in HAZ to and the percentage stunted at 18-month follow up. Four secondary outcomes were change in haemoglobin and zinc and prevalence of IDA and zinc deficiency at 18 months. A subgroup analysis was performed to determine if there was an association between the outcomes and potential confounders including age, anaemia (HB < 11 g/l) status at baseline and the level of compliance with the treatment. The Bonferroni correction method was applied, dividing the conventional threshold for statistical significance (0.05) by the number of statistical tests performed.

### 2.5 | Ethical considerations

Parental written consents were obtained from each eligible child at the time of enrolment. The study was approved by the ethics committee of the Esperanza University Hospital, University Francisco Marroquin, Guatemala.

## 3 | RESULTS

A total of 1238 children were screened, and 971 met the criteria and were included in the study.

Of the 971 children initially enrolled to participate in the study, 681 were allocated to receive atole + MN and 290 to receive milk. Of the total sample size, 54 children were lost to follow-up (Figure 1): 5.6% in the atole + MN group and 2.4% in the milk group ($P = 0.058$).

### 3.1 | Baseline characteristics of the participants

Overall, the two treatment groups did not differ significantly in any of the variables examined at baseline (Table 2). Stunting and anaemia

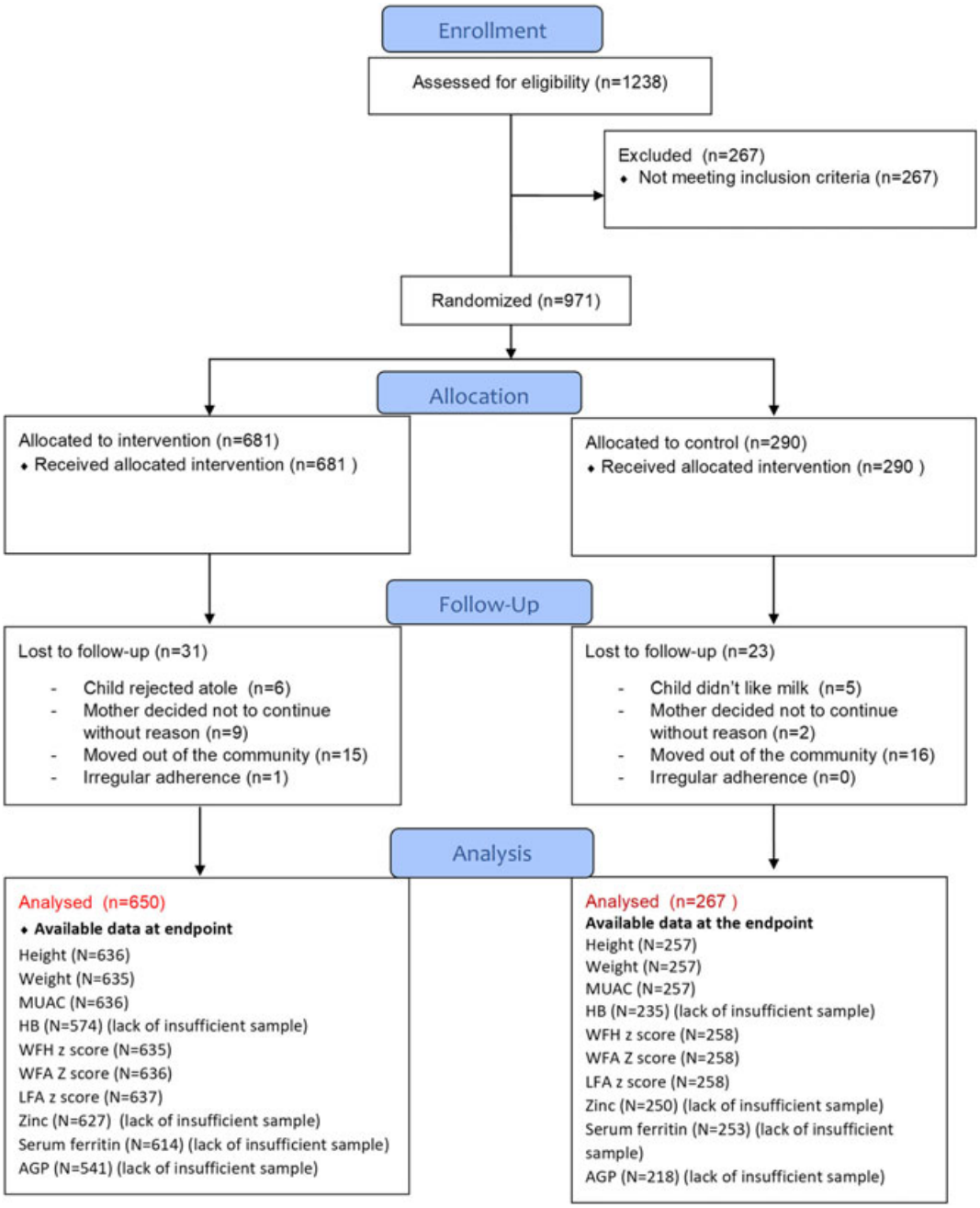


**FIGURE 1** Consort diagram of study participants' progression through the trial

were present at baseline in around half of both groups, with 10% having IDA. There were slightly higher rates of zinc deficiency in the atole + MN (31.4%) than in the milk group (25.3%) at baseline, but this was not statistically significant ($P = 0.057$).

## 3.2 | Compliance

815 children had data at each monitoring occasion, 102 for two and 33 at only one occasion, whereas 21 had none, meaning that there was at least some compliance data for 950 children. The mean (SD) intake of atole + MN per day was 15.9 (6)g overall, and 80% of that group consumed 10 g or more, thus fully meeting their RNI for iron and zinc from the supplement alone, whereas 23% consumed 20 g.

## 3.3 | Effect of micronutrient supplementation on growth, micronutrient status and morbidity

The mean change in height/length did not differ significantly between the two groups or for any other growth variables. There was an overall decrease in the prevalence of stunting at 18-month follow-up in both groups (48.4/47.9% at baseline vs. 40.6/40.7 at

**TABLE 2** Baseline characteristics of participants according to treatment groups

| Variable | Atole + MN ($n = 681$) | Milk ($n = 290$) | *P*-value |
|---|---|---|---|
| Sex, *n* (%) | | | 0.645 |
| Male | 328 (48.2) | 135 (46.6) | |
| Female | 353 (51.8) | 155 (53.4) | |
| Continuous variables, mean ± SD | | | |
| Age (months) | 37.72 ± 20.4 | 38.54 ± 20.2 | 0.566 |
| Initial body weight (kg) | 12.10 ± 3.59 | 12.28 ± 3.53 | 0.484 |
| Height/length (cm) | 86.96 ± 13.6 | 87.66 ± 13.65 | 0.468 |
| BMI (kg/m$^2$) | 15.78 ± 1.79 | 15.76 ± 1.83 | 0.885 |
| MUAC (cm) | 15.29 ± 1.44 | 15.32 ± 1.49 | 0.791 |
| Length-for =-age z-score (LAZ) | −2.02 ± 0.99 | −2.03 ± 1.04 | 0.864 |
| Weigh-for-height z-score (WHZ) | −0.21 ± 1.17 | −0.20 ± 1.25 | 0.826 |
| Weight-for-age z-score (WAZ) | −1.31 ± 1.01 | −1.30 ± 1.09 | 0.900 |
| Haemoglobin (g/dl) | 12.24 ± 1.07 | 12.28 ± 3.53 | 0.804 |
| Serum ferritin (μg/dl) | 31.17 ± 21.07 | 33.92 ± 36.44 | 0.190 |
| Serum zinc (μg/dl) | 71.43 ± 13.67 | 73.76 ± 14.15 | 0.273 |
| AGP (g/l) | 0.73 ± 0.26 | 0.72 ± 0.29 | 0.705 |
| Categorical variables, prevalence, n (%) | | | |
| Stunting (LAZ < −2SD) | 329 (48.4) | 137 (47.9) | 0.970 |
| Wasting (WHZ < −2SD) | 37 (5.5) | 19 (6.7) | 0.455 |
| Underweight (WAZ < -2SD) | 135 (19.9) | 53 (18.7) | 0.656 |
| Iron deficiency (serum ferritin < 12 μg/dl) | 86 (15.2) | 33 (13.9) | 0.644 |
| Anaemia (HB < 11 g/dl) | 63 (10) | 27 (10.1) | 0.953 |
| Iron deficiency Anaemia (HB < 11 g/dL and serum ferritin < 12 ug/dl) | 13 (2.1) | 6 (2.3) | 0.853 |
| Low zinc level, (zinc level < 65 ug/dl) | 210 (31.4) | 71 (25.3) | 0.057 |
| Inflammation (AGP ≥ 1 g/l) | 71 (11.5) | 25 (9.9) | 0.494 |

Abbreviations: AGP, alpha-1-acid-glycoprotein; HAZ, height-for-age z-score; HB, haemoglobin; LAZ, length/height-for-age z-score; SD, standard deviation; WAZ, weight-for-age z-score; WHZ, weight-for-height z-score.

follow-up for atole + MN/milk), but the relative risks were not statistically significant (Table 4).

Rates of anaemia and IDA fell markedly over time, but rates of zinc deficiency remained high (Table 4). There was no consistent difference between the two treatment groups for any micronutrient status variable evaluated (Table 3) or in the relative risk of micronutrient deficiency. There was a trend ($P = 0.09$) to less decline in serum zinc in the intervention group, but no difference in the proportion with low zinc. The milk group had significantly higher HB and were less likely to be anaemic, but there was no difference in ferritin or rates of iron deficiency. Overall, 36% of participants in the atole + MN against 26% for the milk group had at least two of the investigated diseases during the study period, but there was no difference in morbidity score between the two treatment groups (Table S1).

At baseline, micronutrient status was unrelated to height or weight in the whole cohort. At follow-up, there was a weak correlation between HAZ and serum zinc ($r = 0.08$, $P = 0.011$) and between change in HAZ and serum zinc ($r = 0.07$, $P = 0.038$). When this analysis was restricted to the atole + MN group, the effect was slightly enhanced for LAZ ($r = 0.1$, $P = 0.016$) but was no longer significant for change in LAZ.

## 3.4 | Effect of micronutrient supplementation in different subgroups

The subgroup analysis suggested no differences in relative risks for stunting, ID, anaemia and zinc deficiency between the subgroups by age at baseline or anaemia at baseline. There were too few cases of IDA at follow-up to examine subgroup effects (Table S2).

In the atole + MN group, there was a weak correlation between the amount of supplement consumed and serum zinc levels ($r = 0.08$, $P = 0.046$) and a borderline association with change in zinc ($r = 0.07$, $P = 0.084$). The 123 children consuming less than 10 g were also more likely to be zinc deficient (45%) compared with those consuming >10 g (33% *P*, $\chi^2 = 0.01$). There was no association between intake and zinc status in the milk group.

**TABLE 3** Mean change between baseline and 18-month follow-up and comparison between treatment groups (continuous outcome data)

| | Atole + MN | | Milk | | Mean change between treatment groups (mean ± SD) | | Comparison between groups[a], atole + MN vs. milk at 18 months | | |
|---|---|---|---|---|---|---|---|---|---|
| Variables | Baseline (mean ± SD) | 18 months (mean ± SD) | Baseline (mean ± SD) | 18 months (mean ± SD) | Atole + MN ($n = 681$) (mean ± SD) | Milk ($n = 290$) (mean ± SD) | Mean difference | 95% CI | *P*- value |
| Growth | | | | | | | | | |
| Height (cm) | 86.96 ± 13.6 | 102.36 ± 11.3 | 87.66 ± 13.65 | 103.32 ± 10.93 | 15.33 ± 3.93 | 15.26 ± 3.91 | 0.076 | −0.49, 0.65 | 0.793 |
| Height z | −2.01 ± 0.99 | −1.87 ± 0.94 | −2.03 ± 1.03 | −1.82 ± 0.93 | 0.17 ± 0.69 | 0.19 ± 0.61 | −0.018 | −0.12, 0.08 | 0.712 |
| Weight (kg) | 12.10 ± 3.59 | 16.47 ± 3.94 | 12.28 ± 3.53 | 16.84 ± 3.63 | 4.39 ± 1.66 | 4.51 ± 1.40 | −0.116 | −0.35, 0.11 | 0.322 |
| Weight z | −1.31 ± 1.01 | −1.17 ± 0.89 | −1.32 ± 1.01 | −1.10 ± 0.81 | 0.14 ± 0.87 | 0.18 ± 0.78 | −0.053 | −0.18, 0.07 | 0.393 |
| WHZ | −0.22 ± 1.16 | 0.01 ± 0.91 | −0.21 ± 1.16 | 0.08 ± 0.78 | 0.22 ± 1.12 | 0.30 ± 1.03 | −0.069 | −0.23, 0.09 | 0.392 |
| MUAC (cm) | 15.29 ± 1.44 | 16.79 ± 1.56 | 15.32 ± 1.49 | 16.86 ± 1.38 | 1.50 ± 1.13 | 1.52 ± 1.11 | −0.026 | −0.19, 0.14 | 0.753 |
| Micronutrient status | | | | | | | | | |
| Serum HB (g/dl) | 12.24 ± 1.07 | 12.28 ± 0.86 | 12.22 ± 1.11 | 12.40 ± 0.80 | 0.02 ± 1.13 | 0.19 ± 1.06 | −0.171 | −0.3, 0.0 | 0.048 |
| Serum ferritin (μg/l) | 31.17 ± 21.07 | 47.35 ± 36.00 | 33.92 ± 36.44 | 48.48 ± 25.58 | 15.56 ± 28.35 | 12.20 ± 42.00 | 3.371 | −2.5, 9.2 | 0.257 |
| Serum zinc (μg/dl) | 71.43 ± 13.67 | 69.99 ± 12.27 | 73.76 ± 14.22 | 70.15 ± 12.07 | −1.35 ± 16.04 | −3.43 ± 16.77 | 2.079 | −0.3, 4.5 | 0.088 |

Abbreviations: HB, haemoglobin; Z = Z-score for age and gender; WHZ, Weight-for-Height-Z-score.

[a]Group comparison using independent *t*-test.

**TABLE 4** Relative risk for outcomes at 18 months between treatment groups

| Variable at 18 months | Atole + MN/milk (%) | RR (95%CI) | *P*-value[a] |
|---|---|---|---|
| Stunting (LAZ < −2SD) | 40.6/40.7 | 0.99 (0.84, 1.19) | 0.971 |
| Wasting (WHZ < −2SD) | 5.6/6.7 | 0.84 (0.49, 1.43) | 0.519 |
| Underweight (WAZ < −2SD) | 13.2/12.0 | 1.10 (0.75, 1.62) | 0.630 |
| Anaemia (HB < 11 g/dl) | 7.3/3.6 | 2.02 (1.02, 4.14) | 0.043 |
| Iron deficiency (ID) (serum ferritin < 12 μg/dl) | 1.6/0 | N/A[b] | N/A[b] |
| IDA (HB < 11 g/dl and serum ferritin < 12 μg/l) | 0/0 | N/A[b] | N/A[b] |
| Low zinc (serum zinc < 65 μg/dl) | 35.4/34.9 | 1.02 (0.83, 1.24) | 0.878 |
| Morbidity[c] | 10.6/9.2 | 1.16 (0.75, 1.16) | 0.505 |

Abbreviations: CI, confidence interval; HB, haemoglobin; IDA, iron deficiency anaemia; LAZ, length/height-for-age z-score; N/A, not applicable; SD, standard deviation; WAZ, weight-for-age z-score; WHZ, weight-for-height z-score.

[a]The significance level was set at $P = 0.006$ (Bonferroni correction method).

[b]It was not possible to calculate the relative risk because of lack of cases in the atole + MN group.

[c]Morbidity was defined as the presence of one or more of the diseases including diarrhoea, dehydration, difficult breathing, diarrhoea with blood, bleeding and bronchitis at the end point assessment.

## 4 | DISCUSSION

This trial found that 18 months' use of a liquid food supplement fortified with micronutrients had no impact on either the growth or the micronutrient status of rural Guatemalan infants and preschool children, compared with an isoenergetic lactose-free milk. This is disappointing, as reduction in the rates of stunting and IDA was observed when the same atole + MN (Chispuditos®) was given as a supplement to urban toddlers in an uncontrolled study in nurseries in Guatemala City (Villanueva et al., 2015; Villanueva & Reinhart, 2013).

Strengths of this study were its large-scale and long follow-up and its use of a culturally acceptable food supplement. This could be drunk as part of the family meal pattern, mostly as a part of breakfast, to counteract the problems of non-compliance described in previous trials (Ramakrishnan et al., 2009; Zaidi et al., 2020). Compliance was monitored monthly, and objective measures collected at three censuses point suggested that 84% of children in the intervention received at least the RNI of iron and zinc daily, whereas 30% received more than double the RNI.

There were some limitations of this study. The drinks were not fully equivalent in energy contribution, but the difference was very small (∼30 kcal higher in the atole + MN). Ethical concerns led to a 7:3 allocation ratio, but the numbers treated still represent a larger sample than most already published trials. Participants and measurers were not blinded to the treatment received, but analysis of the blood samples was performed blind, and the absence of any observed differences makes assessment bias unlikely. In our study population, the prevalence of anaemia and IDA was lower than in some previous studies (De-Regil et al., 2011; Thompson et al., 2013), and there was no IDA in either group at follow-up. There was no trend to increased effects in those who were iron or zinc deficient at baseline, so the role of iron in this supplement had not really been tested in this trial. The age range of the children at baseline was wide, and those aged over 24 months were less likely to benefit from any intervention to prevent stunting. However, there was no suggestion of an intervention effect in the younger children (Table S3).

These findings are mainly in keeping with what is now known from other trials assessing the effect of zinc supplementation in isolation or combination. Since this trial was completed, a systematic review and meta-analysis reported similarly null findings in relation to growth, but still reported some benefits (Petry et al., 2016) Most recently, a review suggested that micronutrient supplementation alone 'slightly increased length-for-age z-scores' (Lassi et al., 2020). The latter review aimed to include grey as well as published literature, but it is of note that this trial's results were not included. We could identify only four other published studies including zinc supplementation of larger scale than this one (Barffour et al., 2019; Becquey et al., 2016; Bhandari et al., 2002; McDonald et al., 2015). Each reported at least one significant finding, but these were not consistent, and none found positive effects on growth. It is thus imperative that null trial results like these are also published, because there is risk that small effects detected in large meta-analyses simply reflect publication bias.

Despite the high prevalence of zinc deficiency at baseline, we found only limited evidence of an effect of atole + MN on serum zinc, as those randomised to that treatment had only slightly higher serum zinc, a difference that was non-significant ($P = 0.09$). No difference was seen between the randomised groups in rates of deficiency, though the children who consumed more than their RNI of zinc were less likely to be zinc deficient. In a recent meta-analysis, six of the 19 RCTs with relevant data had similarly found no significant difference in serum zinc at follow-up (Tam et al., 2020). Because zinc plays a crucial role in cell function, serum levels are maintained in a tight homeostatic range, so serum zinc levels are a poor reflection of total body zinc (Hess et al., 2007). A previous meta-analysis of 18 RCTs found great heterogeneity between studies and suggested that doubling the intake of zinc only increased the serum/plasma zinc status by 9% (Moran et al., 2012). The follow-up in this study was longer than most published studies, and it has been suggested that the increases in zinc levels due to supplementation may decline with

time (Hess et al., 2007). Thus, it is possible that total body zinc differed when serum levels did not.

Low levels of serum zinc prevalent in Guatemala have been attributed to poor zinc absorption 'poor intrauterine zinc accretion' from poor maternal status and gastrointestinal dysfunction (Krebs et al., 2014). This was thought to relate to high phytate consumption, though one trial in Guatemala found no increase in zinc absorption with reduced phytate consumption (Mazariegos et al., 2006). Thus, even if the zinc is taken, it is not necessarily absorbed.

Micronutrient fortified drinks are a relatively cheap, culturally acceptable powdered milk replacement in vulnerable communities where lactose intolerance is common and fluid milk is expensive and impractical. As such, this is a popular form of food aid that can facilitate engagement with vulnerable families. However, the hopes that such fortified drinks alone would prove to be the answer to Guatemala's exceptionally high rates of stunting have not been borne out, not only on the evidence of this trial but also from the worldwide evidence of lack of effect of such preventive supplementation on growth. The lack of effectiveness of solely nutritional programmes suggests the need to seek other explanations and possible solutions. Stunting is an intergenerational problem, and much stunting has its origins before birth, so earlier interventions focusing on adolescent nutrition need to be considered for future programmes (Martorell & Zongrone, 2012). Childhood infections are highly prevalent in poor resource settings; as well as impacting absorption from the gut, recurrent inflammation may also act directly on the growth plate (Krebs et al., 2014; Owino et al., 2016). So far, WASH programmes combined with supplementation have also failed to demonstrate strong efficacy (Null et al., 2018), but these have not been able to address the profound underlying factors that lead to poor hygiene and limited diet. It is clear from large birth cohorts from LMIC (India, the Philippines, South Africa, Guatemala, Brazil) that child stunting is a strong determinant of reduced human capital (Richter et al., 2011). Guatemala remains one of the poorest and most unequal countries in Latin America. More than half of the Guatemalan population live below the national poverty line, and about a quarter of the population lives in extreme poverty (Instituto Nacional de Estadistica [National Statistics Institute], 2017). The structural and underlying causes of malnutrition, poverty, household food insecurity and poor access to healthcare facilities that are driven by socio-demographic inequalities remain unsolved in these settings.

In conclusion, this trial observed no impact of long-term preventative supplementation with micronutrients added to a culturally accepted drink on either growth or micronutrient status in infants and young children in rural Guatemala. This suggests a need to review the usefulness and cost effectiveness of widespread supplementation programmes.

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## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## CONTRIBUTIONS

VAM designed and conducted research; AO and CMW analysed data; AO, ALG and CMW wrote the paper. ALG was responsible for the paper's final content. All authors read and approved the final manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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