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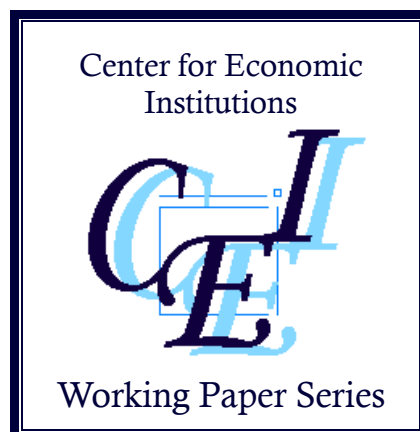
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"Post-intervention morbidity and growth among Zambian children who received multiple micronutrient supplementation using spirulina platensis: evidence from a randomized trial in Zambia"

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Post-intervention morbidity and growth among Zambian
children who received multiple micronutrient
supplementation using spirulina platensis: evidence from a
randomized trial in Zambia

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Short title: Post intervention morbidity and growth of infants who received Spirulina

Abstract

In infants, micronutrient deficiency is known to be associated with growth faltering and morbidity. We recently reported that a 12-month intervention of home fortification of complementary foods using spirulina reduced upper respiratory infections but did not affect the linear growth of Zambian infants. The intervention, originally designed to run for 12 months, was extended by 4 months. This study aimed to evaluate whether a reduction in the morbidity seen with 12-month spirulina supplementation remained persistent after the 16-month intervention, and over the subsequent 1.5-year nonintervention period. The secondary objective was to evaluate if any differences in the growth indicator emerged long-term. We used longitudinal data from a randomized trial conducted in Luapula province, Zambia. A total of 501 infants aged 6-18 months were randomly given daily supplements of maize-soya based porridge with spirulina (SP) or without spirulina (CON). In 2016 and 2018, we collected information on the change in infants' anthropometric status and morbidity (probable pneumonia, cough, probable malaria, and fever). The registration number of the initial clinical trial is NCT03523182 (Clinical Trial.gov). Children in the SP group were 13% less likely to contract an upper respiratory infection after the 16-month intervention. After the 18-month nonintervention

period, children in the SP group were 14% (95% CI: 2%, 25%; $P<0.05$) and 23% (95% CI: 11%, 36%; $P<0.01$) less likely to contract severe and mild upper respiratory infections, respectively. We found no association between SP supplementation and linear growth and weight, as measured by height for age z score and weight for age z score. Home-fortification of complementary foods using spirulina during infancy is likely to have positive and lasting impacts on upper respiratory morbidity prevention.

Keywords: malnutrition, home-fortification, infant growth, morbidity, Zambia

Introduction

Worldwide, micronutrient deficiency is one of the most prevalent nutritional problems and is more evident in the developing world where there is a low micronutrient density in the food available for infants [1]. Existing studies have shown an association with growth faltering [2] and morbidity [3]. A cost-effective measure to tackle this problem is to use locally producible foods rich in multiple-micronutrients (MMN) as home fortification of complementary food. *Arthrospira platensis* (Spirulina) is a blue-green micro-alga, from the *Oscillatoriaceae* family, indigenous to Africa [4, 5]. It contains a high percentage of protein

and is rich in MMN which promotes child linear growth [6-9]. It also involves various other essential fatty acids and amino acids [10].

Past studies have shown that spirulina improved female anemia [11-13], nutritional status in adults [14], and linear growth in children who were malnourished [15-18] or contract HIV [19]. Recently, we have also reported that early childhood supplementation of spirulina reduced respiratory infection after 12 months of supplementation [20] and improved child development even after the 18-month nonintervention period [21]. Nevertheless, no study has explored the long-term relationship between daily spirulina supplementation in infancy and morbidity and growth in children. Therefore, our aim was to study the long-term effects of spirulina supplementation during the first critical 1000 days on the incidence of morbidity and growth in pre-school aged children in Zambia. The hypothesis to be tested was that spirulina supplementation for 16 months during the first critical 1000 days would improve height gain or reduce the probability that a child contracts morbidity after 18 month of non-intervention period. To test this hypothesis, Zambia is ideal setting because micronutrient deficiency and stunting is highly prevalent [22, 23] and it can produce spirulina locally.

Materials and Methods

Area of Study

The original study was conducted between May 2015 and April 2016 in the Luapula province of Zambia, where 56% of children is stunted [22]. Common complementary food in this region is maize-based porridge. The study team secured funding for a 4-month extension at the end of the original trial, and the supplementation continued after obtaining consent from all participants.

Study design

The inclusion criteria were: 1) children aged from 6 to 18 months at enrolment, 2) a singleton birth child, 3) living in the study area, and 4) caregiver provided informed consent. The exclusion criteria included: 1) presence of severe illness on the enrolment session day and 2) entry to any other trial. All potential participants were contacted by trained local health workers and invited to an enrollment session. Caregivers of 501 infants agreed to take part in the study (Fig 1). The trial registration on clinicaltrials.gov was delayed due to an oversight, and the authors confirm that all ongoing and related trials for this intervention are registered.

Fig 1. Study participants.

SP, maize-soya based control porridge and the multiple micronutrient Spirulina; CON, maize-soya based control porridge supplementation.

In April 2015, the study team assigned identification numbers to participants, and the principal investigator assigned each infant to the SP group (n = 250) or the CON group (n = 251) at random; randomization was carried out by generating a random allocation sequence using the STATA14 software and the runiform command (StataCorp LLC, College Station, TX, USA). Due to ethical concerns regarding not providing a standard porridge to infants who were evaluated as malnourished at baseline, we did not create a placebo group.

Household survey data were collected at four time points: baseline (April 2015), endline (April 2016), 1st follow up (October 2016), and 2nd follow up (January 2018). The present study reports the results based on baseline, 1st follow up, and 2nd follow up. We have previously published the results from endline survey. Participants were located and invited to the follow up surveys in October 2016 and January 2018, when children were 18-30 and 36-48 months old, respectively. Infants in the SP group were provided with a maize-soya based porridge with 10 g of spirulina, and those in the CON group received the same porridge but without spirulina. Cold porridge blend was distributed by assistants to each home once a

month, and cold spirulina powder was supplemented to the porridge blend for the treatment group alone.

Assistants conducted the blending and the two blends have different color and flavor. Although authors hoped to provide a matching placebo for the control blend to blind the assignment of treatment, we could not mask due to the deep color and taste of spirulina. To monitor compliance, the number of days that each infant consumed the distributed porridge during the week preceding the assistant's home visit was recorded. Supplementation was performed for 16 months. Table 1 presents the detailed macronutrient and micronutrient composition of the porridge in the SP and CON groups.

Table 1. Composition of the supplements used in this study.

Vitamin/mineral/macro nutrients	Spirulina (10 g)	Soy (40 g): Control	
		group	Spirulina 10 g + Soy 40 g: Treatment group
β -carotene ($\mu\text{g RE}$)	1800	22	1822
Vitamin B1 (mg)	0.48	0.284	0.764

Vitamin B2 (mg)	0.39	0.1	0.49
Vitamin B3 (mg)	3.9	0.8	4.7
Vitamin B6 (mg)	0.09	0.184	0.274
Vitamin E (mg)	1.06	9.12	10.18
Vitamin K (µg)	222	13.6	235.6
Folic acid (µg)	7.3	88	95.3
Calcium (mg)	7.05	73.2	80.25
Phosphorus (mg)	92.1	216.4	308.5
Iron (mg)	8.33	2.44	10.77
Sodium (mg)	21	0.4	21.4
Potassium (mg)	152	720	872
Magnesium (mg)	27.8	92	119.8
Zinc (mg)	0.104	1.8	1.904
Copper (mg)	0.026	0.388	0.414
Calories	38.6	162	200.6
Protein (g)	69.4	13.48	82.88

Total fat (g)	0.82	7.16	7.98
Total carbohydrate (g)	1.27	13.56	14.83

Source: The Zambia Food Composition Tables published by the Zambian National Food and Nutrition Commission [24], adding data from the standard tables of food composition in Japan and FAO, and Siva et al. [25-27].

The primary outcomes were morbidity and the height for age z score (HAZ) as an indicator for growth; secondary outcomes were the weight for age z score (WAZ) and motor development. Effects on motor development have been reported separately [21]. We also collected information on compliance and dietary habits of participants.

To assess the HAZ and WAZ, experienced assistants measured infants following the World Health Organization guideline to obtain height and weight [29]. Using the WHO Multicentre Growth Standards [30], we standardized these measures. We used a digital weighting balance (SECA Portable flat scale 803, USA) to weigh, and recorded to the nearest 0.1 kg. Field worker used a stadiometer (SECA Portable stadiometer 217, USA) equipped with a headpiece for mothers and fathers to measure height. For children below 24 months and those with a height less than 87 cm, the length board (SECA Portable

infantometer 417, USA) was used, and recorded to the nearest 0.1 cm.

Five morbidity indicators during the preceding 12 months of the interview date were assessed: severe respiratory infection (probable pneumonia), cough, severe high fever (probable malaria), and any other type of fever as reported by the mother. Severe respiratory infection was defined as a cough accompanied by short and rapid breathing and difficulty in breathing [31]. We define severe high fever using the following clinical signs: fever with rash on the child's body, fever with chills, shaking, nausea, or alternating high and low body temperature [32]. Trained local health workers collected data on these morbidity indicators.

For the follow up study, we estimated the effect size that could be detected by the present study based on the number of the full analysis set sample in the initial trial ($n = 446$) and assumed that the loss to follow up was not more than 20%. As a result, we expected that at least 365 children aged between 36 and 48 months would attend the follow up survey. With this sample size and a power of 0.80 and a two-sided significance level of 0.05, and with an identical division between the SP and CON groups, we expected to be able to detect a difference of more than 0.29 SD between SP group and CON group.

Table 2 describes the 11 selected indicators of socioeconomic status and health status

in the two study groups in the follow up sample.

Table 2. Baseline characteristics of children by intervention group.

	SP	CON
	(n = 187)	(n = 180)
<i>Child characteristics</i>		
Age at follow up (months)	43.0±4.5	43.5±5.0
Female (%)	52.1	46.5
Stunting at baseline (%)	42.1	43.0
Underweight at baseline (%)	19.5	24.3
Wasting at baseline (%)	9.7	10.7
Dietary diversity score (0-7)	5.2±1.0	5.2±1.0
HIV positive at baseline (%)	3.1	2.5
Child exclusively breastfed for 6 months (%)	89.5	90.1
Length of exclusive breastfeeding (months)	5.9±0.5	5.8±0.5

Maternal characteristics

Maternal age at baseline (year)	28.1±6.5	27.6±7.5
Maternal height at baseline (cm)	152.5±12.8	154.1±10.6
Maternal weight at baseline (kg)	49.7±7.3	49.4±8.5
Maternal education at baseline (years)	6.1±4.7	5.9±4.3

Household characteristics

Farmer (%)	61.7	69.1
Number of household members at baseline (person)	5.8±2.1	5.7±2.5
Number of household members under the age 5 at baseline (person)	2.2±0.9	2.2±1.1
Households which have access to electricity at baseline (%)	1.1	1.1

Note: CON, control porridge group; SP, spirulina porridge group. CON group received porridge with soya. SP group received the same distribution plus spirulina.

The values in the first and second columns show mean±SD.

Statistical analysis

STATA15 software (StataCorp LLC, College Station, TX, USA) was used data analysis.

To identify the effects of spirulina intake on infant growth and morbidity, our strategy was to compare change in the children's growth and morbidity in the households who received spirulina over the study period to that in households that did not receive spirulina. To evaluate the effect of spirulina intake, we pooled the observations from both baseline and follow up surveys and performed a regression of the outcome on the interaction term between the treatment status and the binary variable; if the observation was collected in January 2018 (October 2017) this binary variable takes value of 1 if the observation was collected in April 2016, otherwise 0. We denoted the outcome of a child i in period t as y_{it} and the child's status in terms of whether he/she received spirulina as ***Treatment_i*** (i.e., 1 if an infant was in SP the group, 0 otherwise), and whether his or her status was collected at follow up survey as ***Follow up_t***. The regression model was as follows:

$$y_{it} = \alpha + \beta \text{Follow up}_t + \gamma \text{Follow up}_t * \text{Treatment}_i + \lambda_i + \epsilon_{it} \text{ --- (1)}$$

β indicates the common change in the outcome of the infants in the SP and CON groups over the 16-month intervention period and 18-month nonintervention period. The coefficient of primary interest is γ , which shows the effects of spirulina supplementation; we would expect γ to be negative if the provision of spirulina had reduced the infant's morbidity.

Equation (1) was estimated by The Ordinary Least Square (OLS) linear regression when we evaluate growth outcome, and by probit regression when we evaluate morbidity to control for the extensive set of covariates, including individual fixed effects. Standard errors were clustered at the individual level to deal with intra-cluster correlation of standard error. All observations available in the follow up survey were included to conduct intention-to-treat treatment analysis using the full analysis set.

Ethical statement

The study protocol for the initial trial was approved by the Biomedical Research Ethics Committee of the University of Zambia on March 5th, 2015. This study ensured voluntary participation and participant confidentiality throughout the study. The parents of participating infants provided informed consent.

Results

Participant flow

We enroll children between January and March in 2015. All eligible children (n=547) were invited for the intervention; however, 46 did not enroll in the study because the mother declined or we could not locate the potential participant's home due to migration. Thus, we collected baseline information from 501 participants, and assigned them to either SP (n=251) or CON group (n=250). During the original trial and extension period, 36 children were lost to follow up. Therefore, in October 2016, 202/251 (80.5%) in the SP group and 210/250 (84%) in the CON group were assessed. In January 2018, 187/251 (74.5%) in the SP group and 180/250 (72%) in the CON group were located and assessed.

SP and CON groups had the similar attrition rates , and the probability of attrition was not correlated with any of the baseline characteristics of the participants (Table S2). The final data set consisted of 367 children (SP: n = 187; CON: n = 180). All 367 observations available in the follow up survey were included to conduct intention-to-treat treatment analysis using the full analysis set.

Effects on morbidity

At baseline survey, morbidity rates did not differ between the two groups. After 16 months of supplementation, SP group contracts probable pneumonia (10%) as often as the CON group (9%) (Figure 2a). The prevalence of severe high fever was also similar; however, that of cough and any other fever was lower in the SP group (Figure 2a). After the 18-month nonintervention period, the incidence of severe upper respiratory infection was smaller in the SP group (6%) than that in the CON group (14%) (Figure 2b). Furthermore, the incidence of the all other types of morbidity was also lower in the SP group than in the CON group.

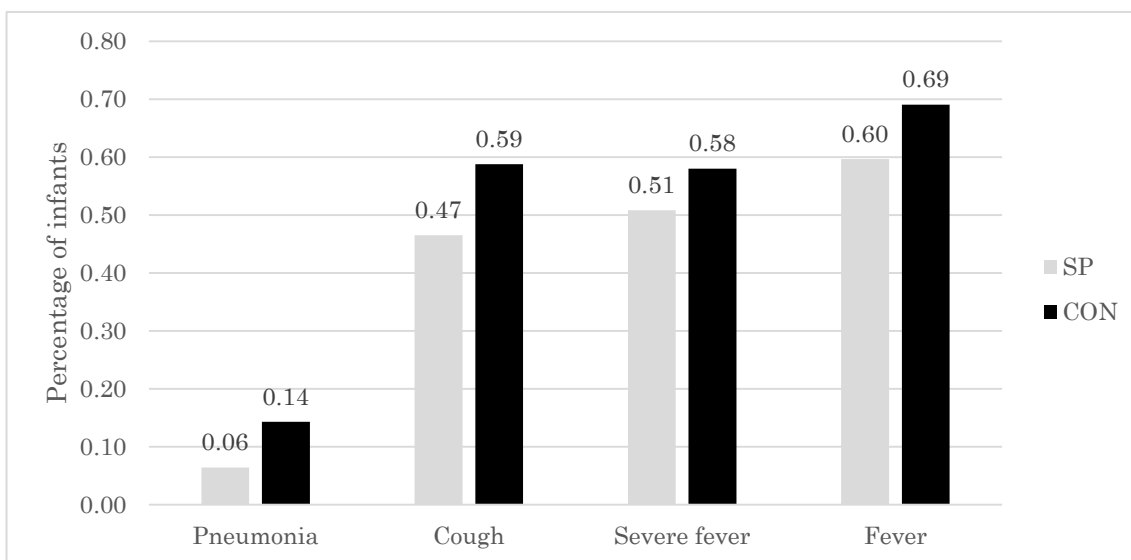


Fig 2a. Percentage of infants with morbidity during the last 12 months, by group. SP,

maize-soya based control porridge and the multiple micronutrient spirulina, which provides

vitamins and minerals; CON, maize-soya based control porridge supplementation.

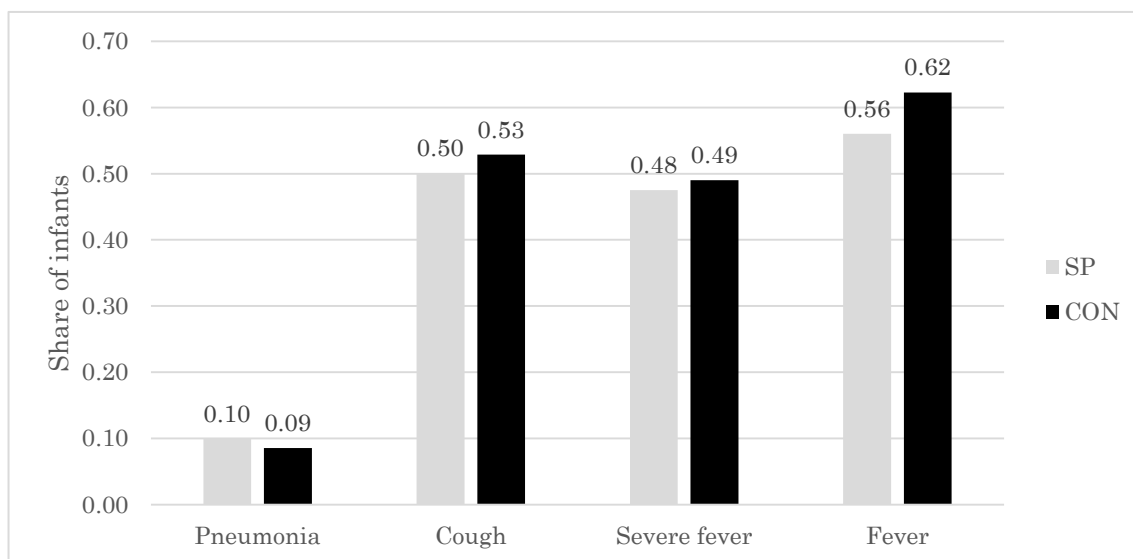


Fig 2b. Percentage of infants with morbidity during the last 12 months, by group. SP, maize-soya based control porridge and the multiple micronutrient spirulina, which provides vitamins and minerals; CON, maize-soya based control porridge supplementation.

Table 3 shows the effects of spirulina supplementation on the incidence of probable pneumonia, cough, severe high fever, and fever after 16 months of supplementation (upper panel) and after the 18-month nonintervention period (lower panel). The results suggest that, after controlling for time-invariant characteristics spirulina supplementation reduced the incidence of cough after 16 months of supplementation by 13 percentage point (95% CI: -

0.24, -0.01; $P < 0.05$) compared to control supplementation. SP supplementation for 16 months had no effect on acute respiratory infection, fever and severe high fever. After the 18-month nonintervention period, the SP group was less likely to contract pneumonia and respiratory infection by 14% (95% CI: -0.25, -0.02; $P < 0.05$) and 23% (95% CI: -0.36, -0.11; $P < 0.01$), respectively. In summary, these results suggest that spirulina supplementation had positive, sustained, and even growing protective effects on upper respiratory infection morbidity in the participating infants.

Table 3. The effect of spirulina intake on infant morbidity.

Outcome: 1 if child suffered from ... during last 12 months	Acute		Severe high	
	respiratory	Cough	fever (Malaria)	Fever
Estimated association with the following explanatory variables	infection			
[1 if 1 st follow up]*treatment	0.01	-0.13**	-0.01	-0.08
	(-0.09, 0.11)	(-0.24, -0.01)	(-0.15, 0.13)	(-0.21, 0.05)

[1 if 2 nd follow up]*treatment	-0.14**	-0.23***	-0.04	-0.11
	(-0.25, -0.02)	(-0.36, -0.11)	(-0.18, 0.10)	(-0.24, 0.03)

Note: All specifications include individual fixed effects and dummy variables for child age in months. The 95% confidence intervals are given in the parentheses. *** stands for significance at 1% level, ** at 5% level, and * 10% level.

Effects on physical growth

At baseline, infants in the two groups had similar height and weight. During the 16 month study period, infants in the SP group gained height (14.2 cm) and weight (2.7 kg); however, infants who received the control porridge gained similar height and weight (13.7 cm, 3.0 kg, respectively) (Figure 3a). After the 18-month nonintervention period, infants in the SP group gained height (22.6 cm) and weight (5.3 kg); again, infants who received the control porridge gained similar height and weight (21.9 cm, 5.2 kg, respectively) (Figure 3b).

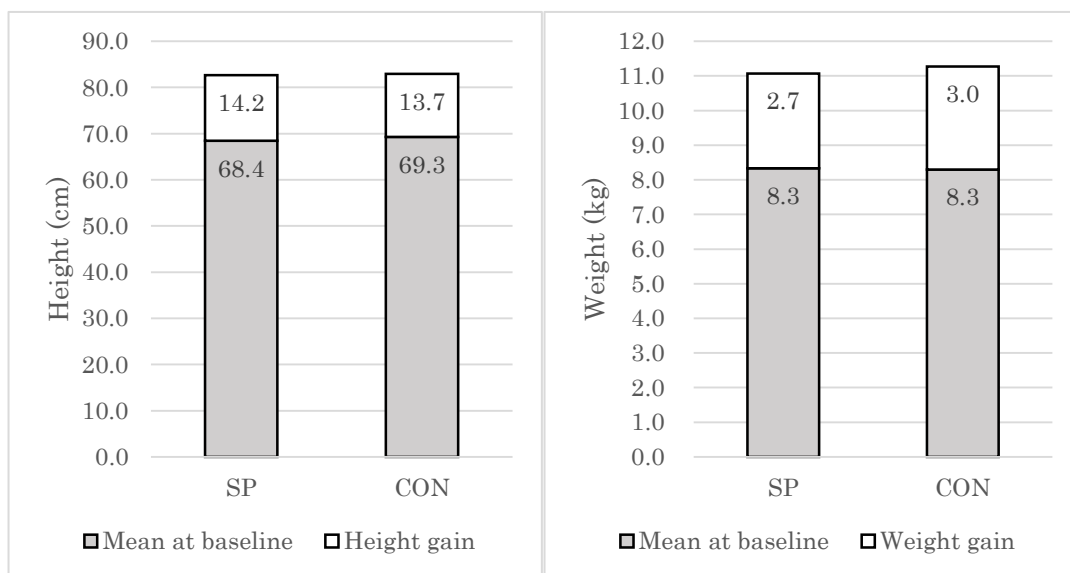


Fig 3a. Height and weight gain of infants over 16 months by group. Mean height in cm (left) and mean weight in kg (right) of participating infants at baseline and in October 2016 is represented in dark and light colors, respectively, and graphed by group.

SP, maize-soya based control porridge plus the multiple micronutrient spirulina; CON, maize-soya based control porridge supplementation.

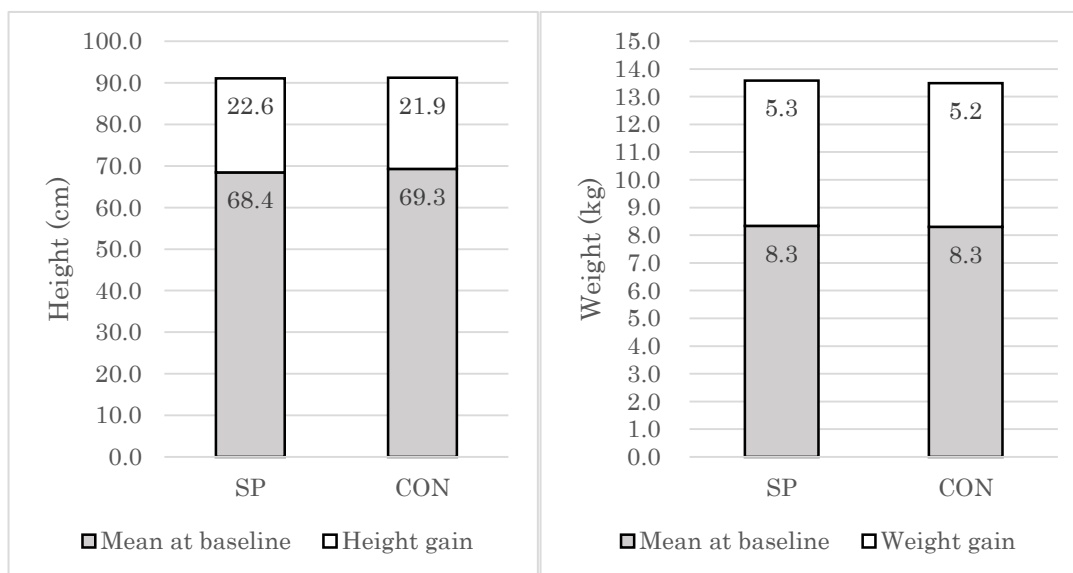


Fig 3b. Height and weight gain of infants after the 18-month nonintervention period by group. Mean height in cm (left) and mean weight in kg (right) of participating infants at baseline and in January 2018 is represented in dark and light colors, respectively, and graphed by group.

SP, maize-soya based control porridge plus the multiple micronutrient spirulina; CON, maize-soya based control porridge supplementation.

In order to control for the time-invariant observed and unobserved characteristics at baseline, we estimated equation (1) by linear regression. Table 4 shows the effects of spirulina provision on height, weight, HAZ, and WAZ after 16 months of supplementation (upper panel) and after the 18-month nonintervention period (lower panel). The estimated

coefficient of the interaction term (-0.10; 95% CI: -1.01, 0.82; $P > 0.10$) suggests that the change in infant height was not different between infants in the SP and CON groups even after controlling for individual fixed effects. No effect was seen even when children were 36-48 months old (lower panel). In summary, the results suggest that 16-month spirulina supplementation did not significantly improve infant growth indicators as compared to control supplementation both just after the termination of the intervention and after an 18-month nonintervention period.

Table 4. The effects of spirulina supplementation on infant growth.

Estimated association with following explanatory variables	Height	Weight	Height for Age Z- score (HAZ)	Weight for Age Z-score (WAZ)
<i>2016 October survey</i>				
[1 if 1st follow up]*treatment	-0.28	-0.11	-0.10	-0.11
	(-1.39, 0.83)	(-0.45, 0.22)	(-0.38, 0.17)	(-0.33, 0.11)
<i>2018 January survey</i>				

[1 if 2nd follow up]*treatment	-0.10	-0.17	-0.09	-0.14
	(-1.01, 0.82)	(-0.41, 0.07)	(-0.39, 0.20)	(-0.37, 0.09)

Note: All specifications include individual fixed effects to control for time invariant individual characteristics.

The 95% confidence intervals are given in parentheses. *** stands for significance at 1% level, ** at 5% level, and * 10% level.

Discussion

We have recently reported that the spirulina supplementation to infants during the first 1000 days reduces the infant's risk of respiratory morbidity after 12 months of supplementation, whereas we found no impact on the child's linear growth (stunting). In the present study, we aimed to evaluate the long-term association between early childhood spirulina supplementation and morbidity and growth of children by collecting information from a follow up survey in Zambia. We observed a clear discrepancy in terms of the post-intervention incidence of morbidity between the SP and CON groups. Indeed, children in the SP group were less likely to have respiratory morbidity (47% in the SP group; 59% in the CON group). Furthermore, those in the SP group contracted severe respiratory infection less often than those in the CON group, which was not evident in the previous study. The superior

respiratory morbidity reduction in addition to the availability of spirulina due to local production makes spirulina supplementation an attractive measure for home fortification of complementary foods for populations at risk of micronutrient deficiency.

These findings are consistent with those of a study conducted in South Africa, which showed that lipid-based nutrient supplements from age 6-12 months reduced the incidence of fever and respiratory infection [33]. Our results are also consistent with another study conducted in India, which showed that milk fortified with zinc, iron, and other micronutrients reduced the incidence of severe illness, diarrhea, and acute respiratory infection compared to control milk [34]. In summary, home-fortification of complementary foods with MMN can reduce the incidence of morbidity in some infant populations and, for the first time, we showed that such protective effects of MMN supplementation can persist even 18 months after the intervention period. In contrast, a study conducted in Ghana using lipid-based nutrient supplementation found little impact on the prevalence of illness, diarrhea, and pneumonia of infants [35]. Similarly, the series of IRIS studies in Peru [36] and Indonesia [37] used MMN supplementation and showed little protective effects on infant morbidity. Since spirulina involves various macro and micronutrients, which potentially optimizes its efficacy to reduce child morbidity, the data from our study does not allow us to determine the

role of each nutrient in preventing respiratory infection. Further trials should explore the mechanism through which spirulina intake reduces morbidity to uncover the generalizability of our findings.

In contrast, during the 18-month nonintervention period, children in the SP group had similar increases in height and weight as children in the CON group. The change in growth indicator from baseline (April 2016) to follow up survey (January 2018) did not differ between the SP group and the CON group. Due to the ethical concern of providing no treatment for children who were assessed as malnourished at baseline survey, one limitation of our design was the lack of a pure control group who would not receive porridge with/without spirulina. For this reason, we can only confer the relative benefit of SP supplementation compared to CON supplementation. In the present study, we found no difference in terms of height and weight gain between the SP group and the CON group. It is, however, highly possible that these supplementations improved growth of children compared to a pure comparison group, because CON supplementation should have had some impact on the growth outcome. Additional trials should examine this hypothesis in the future.

A second possible limitation is the lack of comprehensive data on the dietary intake of participants during the nonintervention period. The length of exclusive breast feeding was

comparable between the SP and CON groups at baseline, and dietary quality measures were also comparable at baseline, after 16 months of supplementation, and at follow up survey, however, dietary information between these surveys were missing. Lastly, although the attrition rate was comparable to other existing studies in sub Saharan Africa, this may also be a limitation [38, 39]. Such attrition is, however, unlikely to bias the estimates of our study. The results show that the baseline characteristics of the children and mothers who dropped out were not different from those of the remaining infants (Supplementary Table S2). This suggests that attrition occurred exogenously and is unlikely to change the distribution of the baseline sample. Therefore, the sample in the SP group and CON group in the full analysis set should be similar.

In summary, based on the findings of the present study, we conclude that fortification of complementary infant food with spirulina had beneficial effects on infant upper respiratory infection morbidity long-term. Thus, spirulina may be a cost-effective home-fortification agent to improve infant health in resource-poor countries.

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Ethical reference number

IRB00001131 of IORG0000774

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CONSORT

TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram

