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Multiple micronutrient supplementation using spirulina platensis and infant growth, morbidity and motor development: Evidence from a randomized trial in Zambia

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Short title: Spirulina supplementation and infant growth, morbidity and motor development

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Abbreviations list

CON	control
HAZ	height-for-age z-score
MMN	multiple micronutrients
OLS	Ordinary Least Square
SP	spirulina porridge
WAZ	weight-for-age z-score
WHO	World Health Organization
SES	socio-economic status

Clinical trial registration number and name of trial registry

NCT03523182; Clinical Trial.gov

Abstract

Background: In developing countries, micronutrient deficiency in infants is associated with growth faltering, morbidity, and delayed motor development. One of the potentially low-cost and sustainable solutions is to use locally producible food for the home fortification of complementary foods.

Objective: The objectives were to test the hypothesis that locally producible spirulina platensis supplementation would achieve the following: 1) increase infant physical growth; 2) reduce morbidity; and 3) improve motor development.

Design: We randomly assigned 501 Zambian infants into a control (CON) group or a spirulina (SP) group. Children in the CON group (n=250) received a soya-maize-based porridge for 12 months, whereas those in the SP group (n=251) received the same food but with the addition of spirulina. We assessed the change in infants' anthropometric status, morbidity, and motor development over 12 months.

Results: The baseline characteristics were not significantly different between the two groups. The attrition rate (47/501) was low. The physical growth of infants in the two groups was similar at 12 months of intervention, as measured by height-for-age z-scores (HAZ), and weight-for-age z-scores (WAZ). SP infants were less likely to suffer from cough by 11 percentage point (CI: -0.23, -0.00; P<0.05); SP infants were significantly more likely to be able to walk alone at 15 months (0.96±0.19) than were CON infants (0.92±0.28).

Conclusion: Home-fortification of complementary foods using spirulina had positive effects on morbidity prevention and motor milestone acquisition.

Key words: chronic malnutrition, home-fortification, spirulina, infant growth, motor development, morbidity, Zambia

Introduction

Micronutrient deficiency in the infancy is associated with growth faltering (1), morbidity (2), and delayed motor development (3), and is common in developing countries where the food available for infants has low micronutrient density (4). A low-cost and sustainable way to address this problem is to utilize locally producible foods rich in multi-micronutrients as home supplements to complementary food. *Arthrospira platensis*, also known as spirulina, is a blue-green micro-algae indigenous to Africa (5, 6). It contains a high percentage of protein, and is rich in multiple micronutrients know to support infant growth such as beta carotene, B vitamins, and minerals such as calcium, iron, magnesium, manganese, potassium, and zinc (6-9). Other than breast milk, spirulina is the only dietary source of gamma linolenic acid, and contains various other essential fatty acids and amino acids (10, 11). The cost of producing spirulina is much lower than that of producing other comparably protein-rich foods, such as soya beans and beef (12), and therefore may potentially sustainably meet the nutritional demands of African infants.

Spirulina has been shown to be effective in treating female anemia (13-15), malnutrition in adults (16), and growth faltering in malnourished (17-20), and HIV-infected children (21). To date, however, no study has explored the association between daily spirulina supplementation and growth and development in children aged below two years. Our objective was to assess the acceptability and effects of spirulina supplementation on growth, incidence of morbidity, and level of motor development in infants in Zambia. The testable hypothesis was that spirulina supplementation for 12 months would increase infant height, reduce the incidence of morbidity, and reduce time taken to achieve motor development milestones (ability to walk unassisted). Zambia provides an appropriate setting to test this hypothesis because micronutrient deficiency and chronic malnutrition (stunting) is highly prevalent in the country (22, 23), and spirulina is locally producible.

Subjects and Methods

Study Area

This study was conducted in the Kalaba camp of the Mansa district and the Njipi camp of the Samfya district in the Luapala province of Zambia, where the proportion of stunted children remains high (56%) (22). Maize-based porridge is a common complementary food used in this area. At baseline survey, the children in these camps showed worse nutrition status compared to the national average in both height-for-age z-scores (HAZ) (-1.68) and weight-for-age z-scores (WAZ) (-1.06), while the average education status of the mothers was also lower than the national average. The proportion of stunted children was 40%, which is relatively better compared to the province average. Although most children were breastfed exclusively according to the World Health Organization (WHO) guideline (six months), the length of breastfeeding period was 10.9 months, which is less than half of the breastfeeding period recommended by the WHO.

Study Design

This study was conducted from April 2015 to April 2016 in the form of an open-labeled randomized control trial, and involved in a spirulina-fed treatment (SP) group and a control (CON) group. Initially, 547 infants under the age of 12 months from the study area were screened using the list of birth records from the local health center, and invited to participate in the trial. All infants were eligible for the study if they were: 1) between 6 and 18 month of age; and 2) were a singleton birth child. Trained local health workers contacted all the potential participants, and the infants and mothers were invited to an enrolment session. Mothers of 501 eligible infants agreed to participate in the study (**Figure 1**).

In April 2015, identification numbers were assigned to the included infants, and each infant was randomly assigned to either the SP group (n=250) or the CON group (n=251); randomization was achieved by generating a random allocation sequence using the STATA14 software (we used the runiform command) (StataCorp LLC, College Station, TX, USA). A placebo group (receiving no complementary foods) was not formulated because of ethical concerns of not providing a standard porridge to infants who were assessed as malnourished (stunted or underweight) at baseline.

The primary outcome was the HAZ as an indicator for growth, but the WAZ, morbidity, and motor development were also evaluated as the secondary outcome. We additionally collected the information on compliance and dietary habits of participants.

For assessing HAZ and WAZ, height and weight of the infants were measured by experienced field workers at baseline (April 2015) and at the endline (April 2016), and later transformed to standardized scores using the WHO Multicentre Growth Standards (15).

The five morbidity indicators evaluated were pneumonia, cough, severely high fever (probable malaria), and any other type of fever as reported by the mother, during the 12 months of study period. Pneumonia was defined as cough accompanied by short and rapid breathing and difficulty in breathing. Severe high fever was defined based on the following clinical signs: fever with rash on child's body, fever with chills, shaking, nausea, or alternating high and low body temperature. Data on these morbidity indicators were collected every month by trained local health workers who queried the caregivers of the children about the relevant clinical signs. The caregivers were also asked to recall whether they had fed the control porridge or the spirulina-fortified porridge to the children in last 7 days. The answer to the last question about feeding was used to evaluate compliance.

Ability of the infant to walk without assistance was assessed as a function of motor development, and this indicator was evaluated at baseline, 6 months, and end line by research assistants who visited the participants' homes.

Data on household socio-economic status (SES) and dietary habits were collected to check whether the randomization was done appropriately, and to control for confounders in the subsequent analysis. SES parameters such as parental demographic information and household economic activity were assessed at baseline, while dietary habits were monitored once a quarter during the study period. In addition, data on the food items that were fed to the child within the prior one week were collected to monitor changes in eating behavior. Food items were classified into seven categories: starchy staples, legumes, dairy, meat/poultry/fish/eggs, vitamin A-rich fruit and vegetables, other fruits/vegetables, and oil/fat/butter. Based on the collected data, the dietary diversity score, which ranged from 0 to 7, was calculated for each child. The score represents the number of food categories that were consumed during more than half of the week by a child.

Sample size calculation was performed based on the information from our previous study, which showed that the mean HAZ among children under the age of five in Zambia was -2.26 with a standard deviation of 0.86 in 2014 (19). We assumed the effect size of spirulina supplementation compared with control supplementation would be 0.23 [10% increase compared to the mean, or equivalent to a “small” to “medium” effect size in Cohen’s d] in the outcome. To detect this effect with a power of 0.80 and a two-sided significance level of 0.05 with an equal division between SP and CON groups would require a sample size of 438. Allowing for a 10% attrition rate, the target sample size at baseline was calculated to be 487.

Spirulina supplementation

Spirulina was produced in the USA by DIC LIFETEC Co. Ltd., Japan. We used 10 g per day of spirulina powder with a mealie meal and soya flour porridge blend. A mealie meal is made from locally available maize and has an extraction rate of about 90 per cent. **Table 1** shows the details of monthly distribution of the porridge blend to each group. Assistants delivered the porridge blend to each home every month, and advised that the participating infants should consume the porridge three times a day. Compliance was measured by the number of days that the infants had eaten the distributed porridge during the seven days preceding the assistant's home visit. Supplementation was performed for 12 months.

The intervention was designed to deliver beta-carotene (1800 μg retinol equivalent (RE)), and iron (8.3 mg) using 10 g of spirulina per day. Spirulina-fortified porridge also includes vitamin B1, B2, B3, B6, B12, vitamin E, vitamin K, calcium, phosphorus, niacin, sodium, potassium, magnesium, zinc, and copper. The detailed macronutrient and micronutrient composition of porridge in the two groups is shown in **Table 2**.

Ethical statement

The study protocol was approved by the Biomedical Research Ethics Committee of the University of Zambia. This study ensured voluntary participation and participant confidentiality throughout the study. Written informed consent was obtained from the parents of participants infants.

Analysis

Data were entered into an electronic database by trained assistants and analyzed using the STATA14 software.

In identifying the effects of spirulina intake on infant growth and morbidity, we followed the difference in differences approach. Namely, our strategy was to compare changes 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 determine the effects of spirulina intake, we pooled the observations from both baseline and end line surveys, and performed a regression of the outcome on the interaction term between the treatment status and the binary variable which takes the value of 1 if observation was collected in April 2016, and 0 if observation was collected in April 2015. We denoted the outcome of a child i in period t as $\boldsymbol{y_{it}}$ and the child's status in terms of whether he/she received spirulina as $\boldsymbol{Treatment_i}$ (i.e. 1 if an infant is in SP the group, 0 otherwise), and that in terms of whether his or her status was collected at endline as $\boldsymbol{Endline_t}$. The regression model was as follows:

$$\mathbf{y_{it}} = \boldsymbol{\alpha} + \boldsymbol{\beta Endline_t} + \boldsymbol{\gamma Endline_t} * \boldsymbol{Treatment_i} + \boldsymbol{\lambda_i} + \boldsymbol{\epsilon_{it}} - - - (\mathbf{1})$$

$\boldsymbol{\beta}$ represents the common change in the outcome of the infants in the SP and CON groups over the 12-month intervention period. Coefficient of our interest is $\boldsymbol{\gamma}$**,** which describes the treatment effects; we would expect $\boldsymbol{\gamma}$ to be positive if the provision of spirulina increases the infant's weight and height gain.

Contrary to the growth and morbidity indicators, data on the motor development parameters were available only at endline because a sub-group of participating infants were initially below 12 months of age at baseline, and it was too early to assess these indictors. In those cases, we compared the outcome in the two groups at endline by using equation (2).

$$\mathbf{y_i} = \boldsymbol{\mu} + \boldsymbol{\delta Treatment_i} + \boldsymbol{\xi X_i} + \boldsymbol{\eta_i} - - - (\mathbf{2})$$

$\boldsymbol{\delta}$ represents the treatment effects, and includes several baseline characteristics of infants, their mothers, and the households; $\boldsymbol{X_i}$, allows us to isolate the treatment effects from other confounding factors. We expect $\boldsymbol{\delta}$ to be positive if the spirulina supplementation increases the probability of walking at 12, 13, 14, and 15 months of age in the SP group, compared with that in the CON group.

The Ordinary Least Square (OLS) linear regression was used to solve equation (1) regardless of whether the outcome was a dichotomous variable or a continuous variable for the ease of interpretation of marginal effects and inclusion of the extensive set of the covariates, including individual fixed effects. Standard errors were clustered at the individual level to deal with intra-cluster correlation of standard error. Equation (2) was evaluated using the probit regression model.

In the following sections, the balance between the SP group and CON group at the start of the study was verified to show the validity of the randomization procedure. After the balance check, equations (1) and (2) were evaluated by OLS regression to assess the intention-to-treat effects of spirulina supplementation on the linear growth, morbidity, and motor milestone acquisition of the participating infants.

Results

Participant flow

Infant enrolment was conducted from January to March 2015. All 547 eligible infants were selected and invited for the intervention, but 46 did not enroll because the mother refused or we could not locate the potential participant's home due to migration. Thus we collected baseline information from 501 participants, and randomly assigned them to one of two study groups (SP and CON groups). As a result, 250 participants received the spirulina treatment and 251 received the control supplementation. Seven (1.4 %) of 501 infants passed away during the study, and the mortality rates did not differ across the groups. Another 48 (9.6%) infants did not complete the study. Twelve children were migrated out of the study site, five withdrew due to objection from the parents, and 31 withdrew due to unspecified reasons. The attrition rates were low and not statistically different between the SP and CON groups. The final data set consisted of 446 children (SP: n=222; CON: n=224).

Balance Check at Baseline

Table 3 describes the selected 11 indicators of SES and health status in the two study groups, and depicts the differences between groups at baseline. Column 1 shows the mean and standard deviation of each characteristic of infants in the SP group, whereas column 2 reports the counterpart parameters in the CON group. The last column presents the differences in the means of each characteristic between the two groups, and the p-value obtained from t-test analysis.

None of the 11 characteristics of the infants, mothers, or of the households were significantly different across the two groups. When comparing 33 observed characteristics (not shown), mothers in the SP group were slightly more likely to suffer from cough despite the random assignment of the spirulina treatment. This is likely to be derived from the small sample sizes. These characteristics are exogenous because data were collected before we started distribution of the soya porridge with/without spirulina. Thus, inclusion of these variables or individual fixed effects in the regression allowed us to control for any otherwise unobserved heterogeneity. Overall, the results from the balance test suggested that the baseline characteristics of the infants in the two groups were similar.

Given this similarity in baseline characteristics, we compared changes in growth, motor development, and morbidity during the study period between the two groups. Because infants in the two groups were similar except for spirulina treatment, the observed differences in the study outcomes could be attributed to the intake of nutrients from spirulina.

Effects on Physical Growth

As shown in the preceding section**,** at baseline, infants in the two groups did not differ in terms of height and weight (Table 3). During the 12 months of study period, infants in the SP group gained height (10.8 cm) and weight (1.9 kg) (**Figure 2**), but the infants who received the control porridge also gained height and weight (10.6 cm, 2.0 kg, respectively) (Figure 2). The degree of change in height and weight did not differ between the two groups.

In order to control for the time-invariant observed and unobserved differences among infants at baseline, we estimated equation (1) by linear regression and **Table 4** shows the results on the effects of spirulina provision on height (column 1), weight (column 2), HAZ)(column 3), and WAZ (column 4). The results are consistent with Figure 2. The results show that the infants in both SP and CON groups gained height by 11.3 cm (95% CI: 9.17, 13.36; $P<0.01$) on average (column 1). However, the insignificant coefficient of interaction term (-0.13; 95% CI: -1.23, 0.98; $P>0.10$) suggests that the change in the infant height was not statistically different between SP and CON infants even after controlling for observed and unobserved time-invariant individual characteristics. This finding was consistent even when we assessed the effects on weight (column 2), HAZ (column 3), and

WAZ (column 4). The results from subsample analysis revealed no clear heterogeneity in the treatment effects by baseline characteristics (not shown). In summary, the results suggest that, in the present study, spirulina supplementation did not significantly improve infant growth indicators as compared to control supplementation.

Effects on Morbidity

At baseline survey, the chance of presenting with a disease did not differ between the two groups except for cough-related infection, which was higher in the SP group (Table 1). After 12 months of intervention, the incidence of pneumonia in the SP group (0.15) was lower than that in the CON group (0.20) (**Figure 3**). The incidence of cough, severe high fever, and that of any fever was also lower in the SP group (Figure 3).

Table 5 shows the effects of spirulina supplementation on the incidence of pneumonia, cough, severe high fever, and fever. The results suggest that, after controlling for time-invariant characteristics including the incidence of cough at base line point, spirulina supplementation reduced the incidence of cough by 11% (95% CI: -0.23, -0.00; $P<0.05$), compared to control supplementation on average (column 2). Spirulina supplementation also showed a trend of reducing the incidence of pneumonia (column 1), severe fever (column 3), and fever (column 4) compared to control supplementation, though the effect was not significant. In summary, these results suggest that spirulina supplementation prophylactically prevented upper respiratory infection morbidity in the participating infants.

Effects on Motor Milestone Acquisition

To explore whether micronutrients from spirulina may reduce the time to acquire motor milestones among infants, we compared the proportion of children who were able to walk without assistance at 12, 13, 14, and 15 months between the two groups. The probability of walking independently at 12 months was higher (4 percentage point higher) in the SP group (0.80) compared with that in the CON group (0.76) (**Figure 4**). This difference between groups was observed even when we assessed motor milestone acquisition at 15 months (Figure 4).

To isolate treatment effects from confounders, linear regression was performed (**Table 6)**. The results show that the probability of children being able to walk by 12 months and 13 months did not differ between the two groups. This observation is possibly because, given that the mean age of infants at baseline was 11 months (Table 2), the duration of spirulina supplementation was too short to change their motor development at 12 months and at 13 months. In contrast, we observed significant differences in the motor development of the SP group at 14 months and 15 months (Table 2). The infants in the SP group were 9 percentage point and 8 percentage point more likely to walk unassisted at 14 months (CI: 0.02, 0.15; P<0.01; OR = 2.55; CI: 1.26, 5.14) and at 15 months (CI: 0.02, 0.14; P<0.01; OR = 3.12; CI: 1.37, 7.07), respectively, compared to those in the CON group. In summary, these results suggest that the fortification of porridge using spirulina improved the motor development of infants.

Discussion

Spirulina-fortified porridge supplementation increase the linear growth as large as the control porridge supplementation. Our results seem to be contrary to the findings from a study (19) in Zambia, which reported that the daily intake of fortified porridge using spirulina was associated with greater height gain compared to controls. However, this inconsistency is not surprising because in this present study, contrary to the previous study, both groups received a porridge made with a soybean-based powder and the amount of protein and energy supplied was almost equivalent between groups. Hence, infants in CON group in the present study may have shown a better increase in linear growth compared those in the previous study.

Although spirulina supplementation provides multiple micronutrients (MMN) to the infants, several randomized trials have shown that MMN supplementation has little effect on growth. Our results are consistent with the IRIS clinical studies (24-27), and other studies in Africa (28-29) and in Cambodia (30) which have shown that MMN supplementation was not associated with infant growth. These findings suggest that supplementation with MMN alone may not improve the growth of infants in some population in resource-poor settings.

Insignificant effects on growth in this study may have also been because the intervention lasted only 12 months. A previous study in Mexico provided MMN supplements to infants from 3 to 24 month of age, and found that MMN supplements increased the length of children who consumed them regularly (31). Hence, future analysis to test whether longer-than-12-months spirulina supplementation would improve infant growth significantly, is encouraged. It is also possible that the lack of positive effects on child growth could be attributed to the age of infants at baseline in the present study. In a study conducted in India, children whose mean age was 23 months (compared with 11 months in the present study) received MMN-fortified milk for 12 months, and showed a larger height and weight gain than did children who received control milk (32). Therefore, it is possible that if our intervention had targeted an older age-group of children as was the case in the Indian study, significant positive effects on child growth would have been obtained.

In this study, the infants in the SP group were 11 percentage point less likely to suffer from cough than were those in the CON group, during the 12-month study period. These data reveal significant positive effects of spirulina-fortification on respiratory infection.

Findings pertaining to the effectiveness of micronutrient supplementation on the infant morbidity by prior studies are divergent. In a study conducted in Ghana, there was no difference in the prevalence of illness, diarrhea, and pneumonia between infants who received the fortified complementary food and the infants in the placebo group (22). A different study conducted in South Africa showed that, compared with vitamin A supplementation alone, zinc supplementation with or without other micronutrients did not reduce the incidence of diarrhea and respiratory infection (33). Our results are consistent with a study conducted in Bangladesh, 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). These findings suggest that home-fortification of complementary foods with MMN reduces the incidence of morbidity in some infant populations.

The effects of spirulina on the incidence of respiratory infection may be due to simultaneous supplementation with zinc and iron. In the above mentioned study, infants received iron alone, zinc alone, or iron + zinc supplementation, and the results showed that supplementation with iron alone, and that with zinc alone had no effect on diarrhea and acute lower respiratory infection; however, when iron and zinc were given together, significant effects on disease incidence were observed (35).

The nutrient-rich nature of spirulina is optimally suited to reduce infant morbidity; however, since it contains multiple micronutrients, it is difficult to determine the role of each micronutrient in improving infant health. Hence, it is of importance to explore the mechanism through which spirulina intake may reduce the incidence of morbidity in future studies.

Spirulina supplementation reduced the time to reach the studied motor milestone (to walk independently). This finding is consistent with a study conducted in Ghana, which showed that infants who received three types of MMN-fortified complementary foods had higher chances of being able to walk independently by 12 months than did infants who received a placebo (28). Similarly, a study from Zanzibar, infants who received iron supplementation walked at an earlier age than did those who did not receive iron supplementation (36). Another randomized trial in Bangladesh found that iron and zinc supplementation with other micronutrients had beneficial effects on time to walk unassisted (37). In summary, these findings suggest that it is important to provide MMN to infants in poor nutritional settings, even if the effects on the physical growth are not directly apparent.

Existing evidence suggests that physical growth and iron deficiency predict the attainment of walking in a poorly nourished population (38, 39). Given that spirulina supplementation did not improve the growth of the treated infants compared with control infants in the present study, the effects of spirulina may be mediated by improvements in iron absorption. Although the nature of the data in the current study did not allow for examination of changes in anemic status of participating infants, it would be interesting to explore the effects of spirulina supplementation on iron deficiency in future studies.

Limitations

Our study has several limitations. The first limitation of our design was that it was not possible to conduct the study in a blinded fashion, and the mothers and the assistants who delivered the porridge were aware of the treatment allocation details. Hence, the awareness of being treated may have influenced the caregivers' attention to children in the SP group (Hawthorne effect). However, the compliance rates between the two groups in the present study did not differ, and the dietary diversity of the infants changed similarly over time in the two groups (data shown in appendix). Given such observations, it is unlikely that the Hawthorne effect significantly influenced study results.

Another concern derived from non-masking was that the distributed porridge fortified with spirulina may have been shared by other household members because the mothers knew it was nutritious. In order to explore this concern, we would ideally have had to record the dietary intake of the all household members, though it was too costly to implement in practice. As suggestive evidence, we compared the change in weight and morbidity among mothers in the two groups, and found no significant difference in these parameters between the groups. Hence, it is unlikely that the mothers in SP group would have eaten a portion of distributed porridge. However, it is still possible that the mothers may have shared the porridge with siblings of the participating infants, thus reducing the amount of porridge received by the target infants. Nevertheless, such sharing was likely to have occurred, if any, in the SP group rather than in the CON group, and the intake of porridge should have been lower in SP group than that in control group; in that case, the difference between the two groups is likely to have been underestimated, and our estimates of the effects size may provide the lower bound of true effects.

The second limitation of our study was that the intervention lasted only 12 months, and the timing of data collection allowed us to only examine the acute effects of spirulina supplementation. As discussed in the previous section, our data suggests that spirulina supplementation improved motor milestone acquisition among infants compared with control supplementation. It thus follows that spirulina intake may potentially have positive and sustained impact on intelligence, working memory, and other cognitive functions in the later period of life. In a study conducted in Peru, infants initially aged six months received micronutrient supplementation for six months, and were observed to have reduced anemia prevalence compared with those who received iron alone supplementation, though no differences in cognitive ability at the age of three were recorded between the two groups (40). In the present study, we could not assess whether spirulina supplementation had any longlasting positive effects on the cognitive ability of participating infants.

It is important to note that the low attrition rate and lack of systematic differences in the baseline characteristics between the SP and CON groups suggest that our estimates may have high generalizability to the study population.

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 morbidity and motor development. Spirulina may thus be a cost-effective home-fortification agent to improve infant health in resource-poor countries.

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KM designed research, analyzed data, wrote paper, and had primary responsibility for final content. MC conducted research and provided essential materials.

Conflict of Interest

The authors declare that they had no potential conflict of interest.

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Table 1. The amount of the porridge blend distributed by group

	Mealie Meal	Sugar	Salt	Soya	Spirulina
Control Porridge (for CON group)	4 kg	0.8 kg	0.1 kg	1 kg	
Spirulina Porridge (for SP group)	4 kg	0.8 kg	0.1 kg	1 kg	0.3kg

CON, control; SP, spirulina porridge

Table 2. Nutrient composition of the Soya and Spirulina supplements used in the study

Vitamin/ mineral/ macro nutrients	Spirulina (10g)	Soy (40g) : CON group	Spirulina 10g + Soy 40g : SP group
β-carotene(μg RE)	1800	0.32	1800.32
Vitamin B1(mg)	0.48	0.352	0.832
Vitamin B2(mg)	0.39	0.12	0.51
Vitamin B3(mg)	3.9	0.84	4.74
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
Foric acid(μg)	7.3	88	95.3
Calcium(mg)	7.05	92	99.05
Phosphorus(mg)	92.1	192	284.1
Iron(mg)	8.33	3.44	11.77
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	173.2	211.8
Protein(g)	69.4	13.2	82.6
Total fat(g)	0.82	8.68	9.5
Total carbohydrate(g)	1.27	11.52	12.79

Table 3. Baseline characteristics of the participants by intervention group

	SP (n=247)	CON (n=243)	p-value
Child characteristics			
Height (cm)	68.4±8.6	69.3±5.4	0.21
Weight at baseline (kg)	8.3±4.7	8.3±4.4	0.93
1 if a child suffered from fever during last 4 weeks	0.76±0.43	0.76±0.43	0.94
1 if a child suffered from diarrhea during last 4 weeks	0.44±0.50	0.43±0.50	0.90
Age (months)	10.6±3.7	11.2±3.8	0.11
Dietary diversity score (0-7)	3.8±1.9	3.8±2.0	0.74
Maternal characteristics			
Maternal age (year)	27.9±6.5	27.5±7.4	0.54
Maternal height (cm)	152.0±14.2	153.7±10.0	0.13
Maternal weight (kg)	49.3±7.2	49.7±8.2	0.59
Maternal education (years)	6.2±4.7	6.0±4.3	0.52
Household characteristics			
Number of children under the age 5	2.2±0.9	2.2±1.0	0.50
ln (value of household asset)	5.2±2.0	5.2±2.0	0.97
Share of households which have access to electricity	0.02±0.1	0.02±0.1	0.99

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

Groups were compared by using the t-test, and p-value is presented in the last column of the table. Value in the first column and the second column shows Mean±SD.

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)
1 if end line	11.27***	2.90***	3.58***	2.26***
	(9.17 , 13.36)	(2.08 , 3.72)	(2.89 , 4.27)	(1.77 , 2.74)
[1 if end line]*treatment	-0.42	-0.11	-0.08	-0.12
	(-1.52 , 0.69)	(-0.34 , 0.12)	(-0.30 , 0.14)	(-0.30 , 0.06)

Note: All specifications include individual fixed effects to control for time invariant individual characteristics. 95% confidence intervals are in parenthesis. *** stands for significance at 1% level, ** at 5% level, and * 10% level.

Table 5. The effects of spirulina intake on infant morbidity

Estimated association with following explanatory variables	1 if a child suffered from …. during last 12 months			
	Pneumonia	Cough	Severe high fever (Malaria)	Fever
1 if end line	-0.22*	-0.03	-0.02	-0.05
	(-0.46 , 0.02)	(-0.31 , 0.24)	(-0.24 , 0.19)	(-0.30 , 0.21)
[1 if end line]*treatment	-0.07	-0.11**	-0.03	-0.09
	(-0.17 , 0.04)	(-0.23 , -0.00)	(-0.13 , 0.06)	(-0.19 , 0.02)

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

Table 6. The effects of spirulina supplementation on the probability that a child is able to walk independently by 12-15 months

Outcome: The probability that a child can walk independently by ….	12 mo	13 mo	14 mo	15 mo
	0.07	0.08*	0.09***	0.08***
	(-0.02 , 0.16)	(-0.00 , 0.16)	(0.02 , 0.15)	(0.02 , 0.14)

Note: Estimated by probit model. Values are marginal effect and 95% confidence intervals are in parenthesis.. Each specification limits the sample only to children whose age is above the relevant age. For example, column 1 includes children who are 13 months old or older at end line point. All specifications control for individual characteristics: age in months, gender, 1 if child had suffered from malaria, measles before baseline point, and mothers' characteristics: mother's age, height, and weight. *** stands for significance at 1% level, ** at 5% level, and * 10% level.

Figure legends

Figure 1. Flow chart of study participants.

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

Figure 2. Height and weight parameters of participating infants at baseline and at the end of the study period. Mean height in cm (left) and mean weight in kg (right) of participant infants at baseline is represented in dark color and that at endline in light color, graphed by group.

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

Figure 3. Probability that children suffering from acute respiratory infection, cough, severe fever, and fever over the 12 months study period, by group. SP, maize-soya based control porridge plus the multiple micronutrient Spirulina, which provides vitamins and minerals; CON, maize-soya based control porridge supplementation.

Figure 4. Probability that children achieving the walking unassisted by 12 mo, 13 mo. 14 mo, and 15 mo, by group. SP, maize-soya based control porridge plus the multiple micronutrient Spirulina, which provides vitamins and minerals; CON, maize-soya based control porridge supplementation.

Figure 1. Study profile.

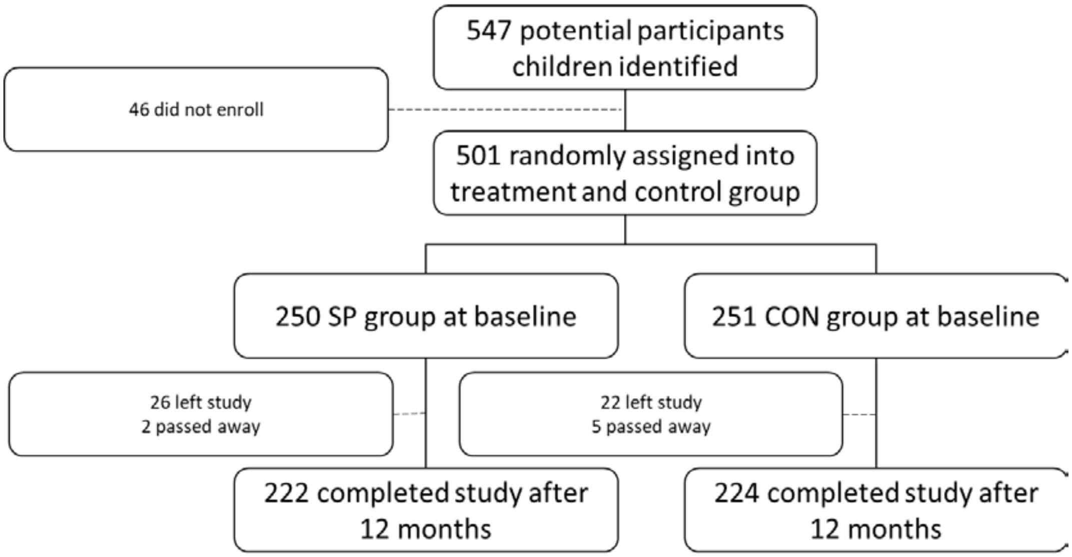

Figure 2 Height and Weight Gain of the Infants over 12 month by group

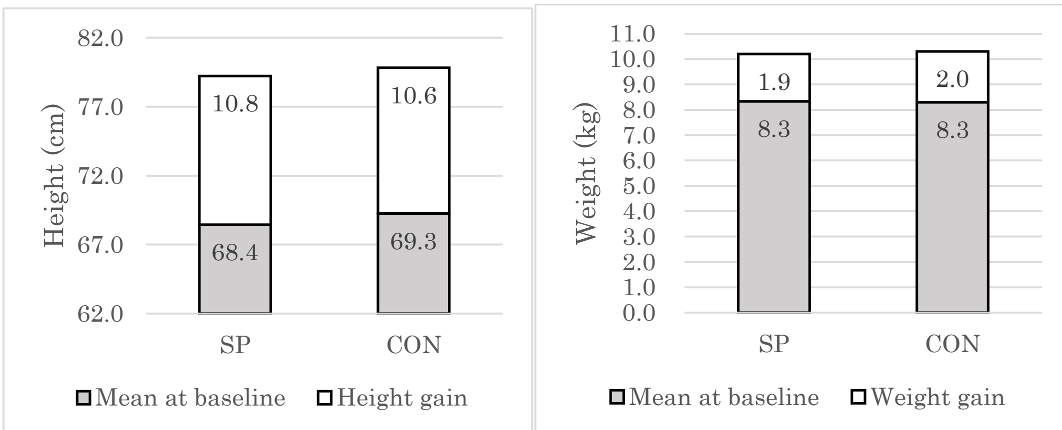

Figure 3 Share of Children suffering from pneumonia during the 12 months, and fever during the 4 weeks before the end line survey by group

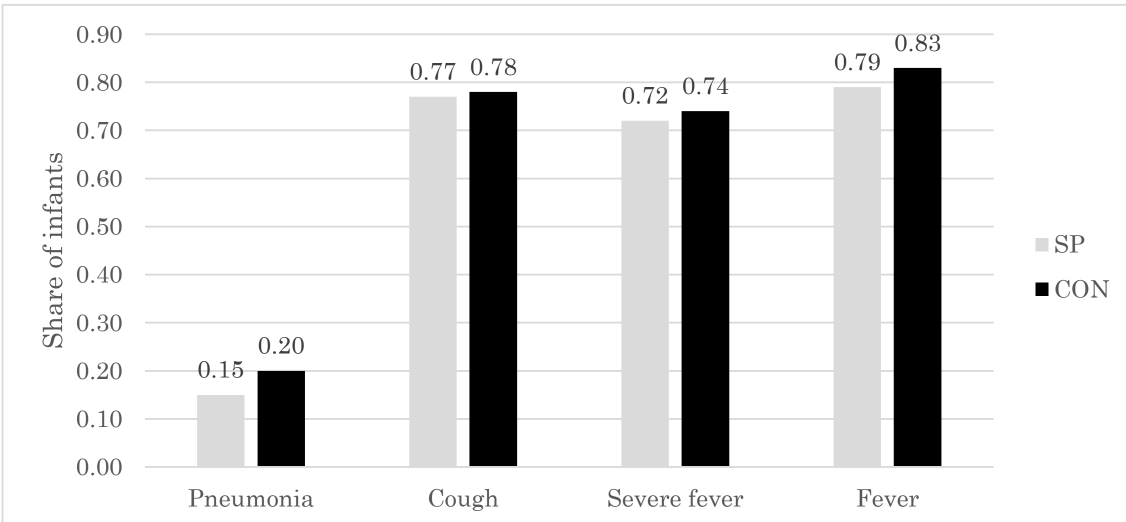

Figure 4 Share of children walking independently by 12, 13, 14 and 15 months by group