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Multiple micronutrient supplementation using spirulina platensis during the first 1000 days is positively associated with development in preschool-aged children: a follow up of a randomized trial in Zambia

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Abstract

Early childhood development relies on various micronutrients. We recently reported that home fortification of complementary foods using spirulina reduced the time to attain motor milestone in Zambian infants. The objective of this study is to estimate the long-term associations between spirulina supplementation during the first 1000 days and child gross motor development, fine motor development, language, and personal-social skill at preschool age. We used longitudinal data from a randomized trial conducted in Zambia. In 2015, 501 infants (age, 6–18 months) were provided daily supplements of maize-soy-based porridge with spirulina (SP) and without spirulina (CON). Supplementation period lasted for 16 months. In January 2018, children who participated in the initial trial were resurveyed (CON: 182 children; SP: 188 children; now aged 36–48 months). We assessed the infants' gross motor development, fine motor development, language, and personal-social skill using a modified version of Malawi Development Assessment Tool. The initial clinical trial registration number was NCT03523182 (ClinicalTrial.gov). Children in the SP group had higher scores on gross and fine motor development, language, and social skill than those in the CON group. Home fortification of complementary foods using spirulina during the first 1000 days improved development

among Zambian at preschool age.

Keywords: malnutrition, home-fortification, motor development, language skill, personal-social skill, Zambia, spirulina, the first 1000 days

Introduction

About 250 million children in developing counties are at risk of failing to meet development potential by the age of 5, and most of them live in Africa and South Asia [1–2]. Delayed development during childhood, especially in the first 1,000 days, is associated with lower school performance, labor productivity, and lower income in later life [2–3]. Such poorer socioeconomic status (SES) in adulthood results in the risk of delayed development of offspring in the next generations, thus forming a vicious cycle. Therefore, the first 2 years of life provide a critical opportunity for intervention to break such intergenerational transmission of poor development and poverty.

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 microalgae from the *Oscillatoriaceae* family, which is indigenous to Africa [4]. It contains key macronutrients, including a high percentage of protein, and is rich in multiple micronutrients (MMN) that are known to support brain growth and development, such as beta-carotene; B vitamins; and minerals, including calcium, iron, magnesium, manganese, potassium, and zinc [5–10]. The cost of producing spirulina is much lower than that of producing other comparably protein-rich foods, such as soya beans or beef [11], and therefore may potentially be a sustainable method for meeting the nutritional demands of infants in low- to middle-income countries.

Postnatal supplementation of spirulina has shown to reduce growth faltering the children under the age of 5 in Zambia [12], Burkina Faso [13–14], and Palestine [15]. We recently reported that the 12-month spirulina supplementation of infants reduced the incidence of upper respiratory infection and improved motor development [16]. However, no study has ever tested if such positive effects of MMN supplementation are persistent or more evident after the non-intervention period.

This study aimed to assess the long-term effect of home fortification of complementary foods using spirulina on motor and mental development of preschool children in Zambia after a non-intervention period of 1.5 years. The testable hypothesis of the present study is fourfold; children who received spirulina in the initial trial had higher scores on gross motor, fine motor development, receptive and expressive language skill, and personal-social skills at follow-up after a non-intervention period of 18 months. We previously reported that spirulina supplementation in the initial trial reduced upper respiratory infection and time to walk unassisted [16]. The present study provides the results of the follow-up survey and the effects of the intervention on child development (secondary endpoint). The results of the primary endpoint (i.e., growth) and other outcomes (i.e., morbidity) were reported separately [17].

Materials and Methods

To evaluate the long-term effects of spirulina supplementation, the present study presented the findings from the follow-up survey. The initial trial was conducted in the Luapula Province of Zambia from May 2015 to April 2016 using an open-labeled randomized control trial (ClinicalTrials.gov registration#: NCT03523182) that included a spirulina-fed treatment (SP) group and a control (CON) group. About 501 infants, aged between 6 and 18 months, were randomly fed with either (a) maize-soy-based porridge with spirulina (SP) or (b) maize-soy-based porridge without spirulina (CON). The initial supplementation period was 12 months. However, after the study protocol was approved, we reserved the funding for another 4 months of supplementation and decided to continue supplementation from May 2016 to August 2016 with informed consent from the participants. Inclusion criteria were: 1) between 6 and 18 months of age, 2) a singleton birth child, 3) residence in the study area, and 4) informed consent from at least one caregiver. Exclusion criteria included: 1) presence of severe illness warranting hospitalization on the enrolment session day, and 2) enrolment in any other clinical trial. Nutrient composition of the supplements are presented in the Supplementary Table 1.

In January 2018, trained local health workers contacted all potential participants, and the households with infants who participated in the initial trial were located and invited to participate in the follow-up survey. Children were 36-48 months old at the time of the follow-up survey. A total of 370 (SP: 188; CON: 180) children attended the survey (Figure 1).

Fig 1. Flow chart of study participants.

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

The attrition rates were low and not statistically different between the SP and CON groups. The propensity of attrition was not systematically associated with any of the baseline characteristics of the participants (Supplementary Table S2). The final dataset consisted of 370 children (SP: 188; CON: 182). All 370 observations available in the follow-up survey were included to conduct intention-to-treat treatment analysis using the full analysis set.

Measurement

Assessment of child development included the evaluation of gross motor development, fine motor development, language skills, and personal-social skills. Motor development of children was assessed by adding the gross and fine motor subscale scores. Mental development was assessed by adding the language and personal-social subscale scores. Trained assistants, who were blinded to the study groups, visited the household and read all questions out loud to mothers or main caregivers.

The two primary outcomes of the present study were motor development and mental development, which were evaluated using Malawi Developmental Assessment Tool (MDAT), with minor adoptions [18]. We assessed the children's locomotor skill (6 items), eye-hand coordination (13 items), language skill (11 items), and personal-social skill development (8 items) (S1 Questionnaire). Each item was directly tested by a trained evaluator and scored as 1 if the child had performed one task or 0. The raw score of each subscale was calculated by summing the scores of that domain. The performance of the

MDAT has been validated in several developing countries [18].

Furthermore, to isolate the treatment effects from the confounders in subsequent analysis, data on household SES and dietary habits were collected. SES parameters, such as parental demographic characteristics and household economic activity, were evaluated using a questionnaire-based interview. Dietary habits were assessed by obtaining data on food items that were fed to the child one week prior to the survey through a questionnaire-based interview (Supplementary Material S1). Summary statistics at base line and follow up of the developmental samples are shown in **Table 1**.

Table 1. Baseline and follow up characteristics of children by intervention group.

	SP (n=188)	CON (n=180)
Child characteristics		
Age at follow up (months)	43.0±4.5	43.5±5.0
Child female (%)	52.1	46.5
Stunting at base line (%)	42.1	43.0
Underweight at base line (%)	19.3	24.6
Dietary diversity score (0-7)	5.2±1.0	5.2±1.0
Maternal characteristics		
Maternal age at base line (year)	28.1±6.5	27.6±7.5
Maternal height at base line (cm)	152.5±12.8	154.1±10.6
Maternal weight at base line (kg)	49.7±7.3	49.4±8.5
Maternal education at base line (years)	6.1±4.7	5.9±4.3

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

For the follow up study, hypothesized that children in SP group will attain better scores on motor and mental development at preschool age than those in CON group. We estimated the effect size that can 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 lost to follow up were 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 equal division between 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.

Ethical statement

The study protocol for 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. Informed consent was obtained from the parents of participating infants.

Role of funding source

The funder played no role in study design, data collection, data analysis, data interpretation, or writing the report.

Statistical analysis

Data were entered into an electronic database by trained assistants and were analyzed using STATA15 software (StataCorp LLC, College Station, TX, USA). Raw scores of development measures were converted into z-scores (with 0 as mean and 1 as standard deviation) using the sample mean and standard deviation of the study sample.

To identify the effects of spirulina intake on child development, we used linear regression model to analyze the child development outcome. The results of the regression model were adjusted for child’s age in months, sex, camp of residence, and mother’s weight, and we presented the Cohen’s *d* effect size for each outcome.

We examined the effect modification on the motor and mental development scores based on the extent of malnutrition at baseline (using binary measure which distinguishes children who were moderately or severely stunted from those who were not stunted based on whether height for z-score was below or above −2 standard deviation compared with the World Health Organization Multicentre Growth Standards [19]) and dietary diversity score at baseline (using binary measure which distinguishes children with less diverse food consumption from those with diverse food consumption based on whether the score is below or above median). All observations available in the follow-up survey were included to conduct intention-to-treat treatment analysis using the full analysis set. On all the analysis, a P-value of <0.05 (two-sided test) were considered significant.

Results

To explore whether micronutrients from spirulina may improve the development even after a non-intervention period of 18 months, we compared the development score between the two groups using the modified version of MDAT. The raw score of motor development subscale was higher (by 0.47 points) in the SP group (4.68) than that in the CON group (4.21) (Figure 2). This difference between the two groups was observed even after assessing fine motor development, language skills, and personal-social skills.

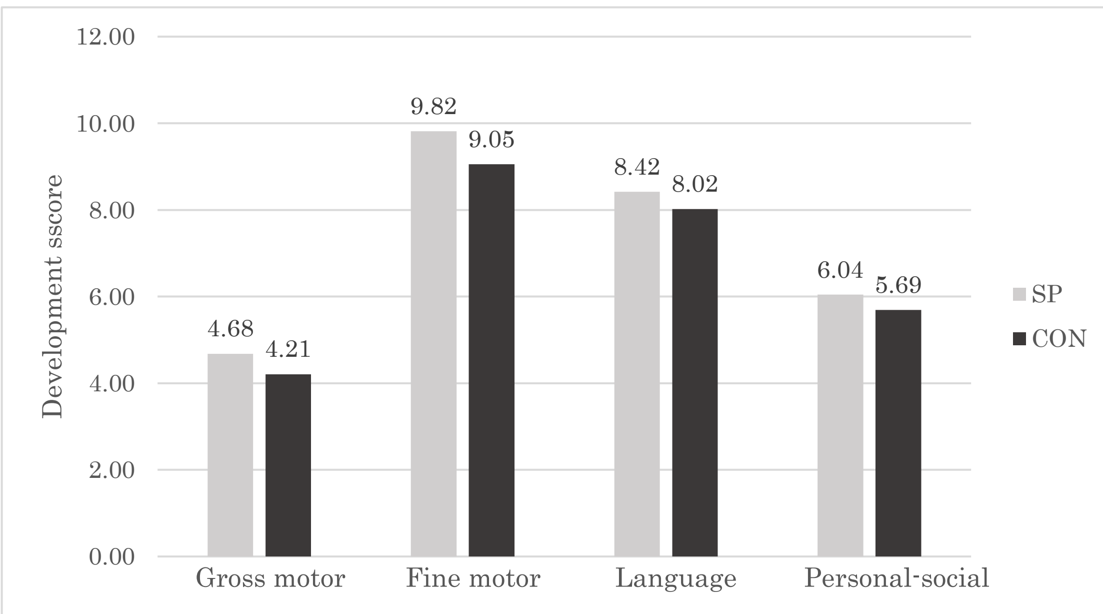

Figure 2. Gross motor development, fine motor development, language skill, and personal-social skill score of children in each group. SP, maize-soya-based control porridge plus multiple micronutrient spirulina supplementation, which provides vitamins and minerals; CON, maize-soya-based control porridge supplementation. Gross motor scale, 0–6; fine motor, 0–12 language skill, 0–11; and personal-social skill, 0–8.

To isolate treatment effects from confounders, linear regression was performed (Table 2). The results showed the significant differences in all domains of child development between the SP group and CON group (Table 2). Spirulina supplementation improved the children’s gross motor and fine motor skills (P<0.01) as well as the language and personal-social skills (P<0.05). Overall, the infants in the SP group obtained higher score in motor (Cohen’s *d* effect size: 0.42; 95% confidence interval (CI): 0.22, 0.63; P<0.01) and mental development (Cohen’s *d* effect size: 0.32; 95% CI: 0.11, 0.52; P<0.01) than those in the CON group. A heterogeneity analysis based on children’s height at baseline was conducted. Results showed that the across group difference in the motor development and mental development score were higher for children who were moderately or severely stunted at baseline (HAZ<−2) than non-stunted children (HAZ>−2). Similarly, the effects size on motor development was larger for children whose dietary diversity score at baseline was below median.

Table 2. The effects of spirulina supplementation on the child’s development score

Outcome: standardized z score	Motor development	Mental development	Gross motor development	Fine motor development	Language skill	Personal-social skill

All children (N=346)						
Effect size (mean)	0.42***	0.32***	0.33***	0.41***	0.23**	0.26**
95% CI	(0.22, 0.63)	(0.11, 0.52)	(0.12, 0.54)	(0.21, 0.62)	(0.03, 0.43)	(0.04, 0.47)
Children with HAZ<−0.2 at baseline (N=141)						
Effect size (mean)	0.58***	0.36**	0.54***	0.53***	0.20	0.40**
95% CI	(0.25, 0.90)	(0.02, 0.69)	(0.21, 0.88)	(0.20, 0.87)	(−0.13, 0.53)	(0.03, 0.77)
Children with HAZ>−0.2 at baseline (N=184)						
Effect size (mean)	0.36**	0.23	0.17	0.36**	0.13	0.23
95% CI	(0.07, 0.64)	(−0.07, 0.52)	(−0.11, 0.46)	(0.08, 0.65)	(−0.15, 0.41)	(−0.08, 0.53)
Children with dietary diversity score<median at baseline (N=128)						
Effect size (mean)	0.73***	0.34**	0.59***	0.72***	0.26*	0.25
95% CI	(0.38, 1.08)	(0.01, 0.68)	(0.22, 0.97)	(0.40, 1.05)	(−0.04, 0.56)	(−0.11, 0.61)
Children with dietary diversity score>median at baseline (N=209)						
Effect size (mean)	0.22*	0.30**	0.23*	0.16	0.21	0.29**
95% CI	(−0.04, 0.49)	(0.03, 0.58)	(−0.04, 0.49)	(−0.11, 0.43)	(−0.07, 0.49)	(0.01, 0.57)

Note: 95% confidence intervals are in parenthesis. Motor development scale, 0–18; mental development, 0–19; gross motor, 0–6; fine motor, 0–12; language skill, 0–11; and personal-social skill, 0–8. Effect sizes were calculated with adjusted mean. All specifications control for individual characteristics: age in months, gender, and mothers' characteristics: age and weight. *** Significance at 1% level, **Significance at 5% level, and *Significance 10% level

Discussion

The multiple micronutrient supplementation using spirulina in the first 2 years of life showed positive and lasting effects on child development even after 18 months of non-intervention period. We found consistent effects on all domains (gross motor, fine motor, language skill, and personal-social skill) of child development.

The observed effect size on motor development in the present study is comparable to or larger than those reported in the existing effectiveness trial in children in developing countries. In a study conducted in India, multiple micronutrient powder (MNP) was provided to households with children aged 6–18 months, and the intervention improved motor development in children aged 18 months with an effect size of 0.12 [20]. Another study in Bangladesh, which involved the administration of MNP to infants from 6 to 24 months of age, also reported that the MNP supplementation had a positive impact on motor development at 24 months, with an effect size of 0.15 [21]. Consistently, a cluster randomized trial in Burkina Faso showed that provision of lipid-based nutrient supplements (LNS) to children from 9 month to 18 months of age increased the motor development score by 0.34 standard deviation [22]. Although different assessment tools were used, it is noteworthy that our intervention improved motor development more than or at least as large as LNS intervention in Burkina Faso, in which malaria and diarrhea treatments were added to the enhanced nutrition intervention. Another study in Pakistan consistently found the positive effects of MNP on motor development at 12-month, but failed to detect the significant effects of MNP on motor development at 24-month of age

[23]. The possible explanation for this is that the effectiveness of MMN supplementation depends on the initial nutrition condition of the infants. Indeed, the proportion of moderately or severely stunted children at enrolment was lower in Pakistan (22%) than in Zambia (42%).

Consistently, the improving effects of spirulina supplementation on motor development was evident in stunted children (HAZ<−0.2), and children whose dietary diversity score at baseline was below median. By contrast, smaller effects were found in children who were not malnourished or had better dietary diversity at baseline. This result suggests that the MMN supplementation had larger effects on infants who were already disadvantaged and with higher risk of delayed motor development at enrolment. This result is consistent with those of other existing studies evaluating the effectiveness of MNP supplementation during the first 1000 days of child development in Burkina Faso, Chile, Bangladesh, and Indonesia [21, 22, 24, 25]. In summary, this is the first study to present that the supplementation of MMN using locally producible foods, spirulina, improved motor development even after an 18-month non-intervention period.

The present study showed the positive effects of MMN supplementation using spirulina on expressive and receptive language development in children aged 36–48 months. Consistent with the results of the present study, a recent cluster randomized trial in Bangladesh, Pakistan, and Burkina Faso showed that LNS and MNP supplementation had positive effects on expressive language development in infants aged 18–24 months. A recent review reported that neurodevelopment related to language development occurs in the first 2–3 years of life [26, 27], and thus the effects of supplementation becomes evident in children aged 2 years or older. The results of the present study suggest that such effects may remain persistent even at pre-school age after a 1.5-year non-intervention period.

Our data also showed that infants in the SP group attained higher score (SD: 0.26) on personal-social skill subscale than those in the CON group. This finding is consistent with the results of the trial conducted in Burkina Faso and India, but contrary to those in Pakistan and Bangladesh. Again, the difference in the initial nutrient deficiency of the infants might play a role in this discrepancy. Indeed, the positive effects were only observed among children who were moderately or severely stunted at enrolment in the present study.

Our study had several limitations. First, we could not conduct the study in a blinded manner, and the mothers and assistants who delivered the porridge were aware of the treatment allocation details. However, group allocation was masked to the data collectors. Second, we assessed and reported the effectiveness of spirulina supplementation after an 18-month non-intervention period, and data from the endline survey and follow-up survey were inadequate, which may be another limitation. To validate the effectiveness of spirulina supplementation, analysis using an inter-follow up survey at 9 months of non-intervention period, with limited child development measure, is presented in Supplementary Table S3, and the results were consistent with the findings at 18 months. This further supports that spirulina supplementation consistently improved the child development during the post-intervention period.

Lastly, the attrition was comparable to the other existing studies in this region, but may also be a limitation [28, 29]. Such attrition is, however, unlikely to have resulted in bias in the results of the study. Our analysis showed that the baseline characteristics of the infants who were lost to follow-up were not statistically different from the rest of the infants (Supplementary Table S1). This suggests that attrition occurred as if randomly and is unlikely to change the distribution of the baseline sample. Therefore, infants in the SP group and CON group in the full analysis set remained comparable.

In summary, based on the findings of the present study, we conclude that fortification of complementary food with spirulina during the first 2 years of life had long-term beneficial effects on child development. Therefore, spirulina may be a cost-effective home-fortification agent to improve human capital in resource-poor countries. To validate whether the improving effects of spirulina supplementation on child development remains evident in school age, adolescents, and even after entering the labor market, the further research by following up this cohort is encouraged.

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Competing interests

The authors have declared that no competing interests exist.

Author Contributions

Conceptualization: Kazuya Masuda.

Data curation: Kazuya Masuda, Maureen Chitundu.

Formal analysis: Kazuya Masuda.

Funding acquisition: Maureen Chitundu.

Investigation: Kazuya Masuda.

Methodology: Kazuya Masuda.

Project administration: Maureen Chitundu.

Resources: Kazuya Masuda, Maureen Chitundu.

Software: Kazuya Masuda.

Supervision: Kazuya Masuda, Maureen Chitundu.

Validation: Kazuya Masuda.

Visualization: Kazuya Masuda.

Writing – original draft: Kazuya Masuda.

Writing – review & editing: Kazuya Masuda, Maureen Chitundu.

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Supporting information

Supplementary Table S1. Nutrient composition of distributed spirulina and soy

Supplementary Table S2. Correlate of attrition with household characteristics at baseline

Supplementary Table S3. Effects of supplementation on child development at 9 month of non-intervention period

Protocol S1. Trial protocol.

Supplementary material S1. Detail of the dietary diversity measures

Questionnaire S1. Questionnaire.

Checklist S1. CONSORT checklist.

Figure 1 CONSORT 2010 Flow Diagram

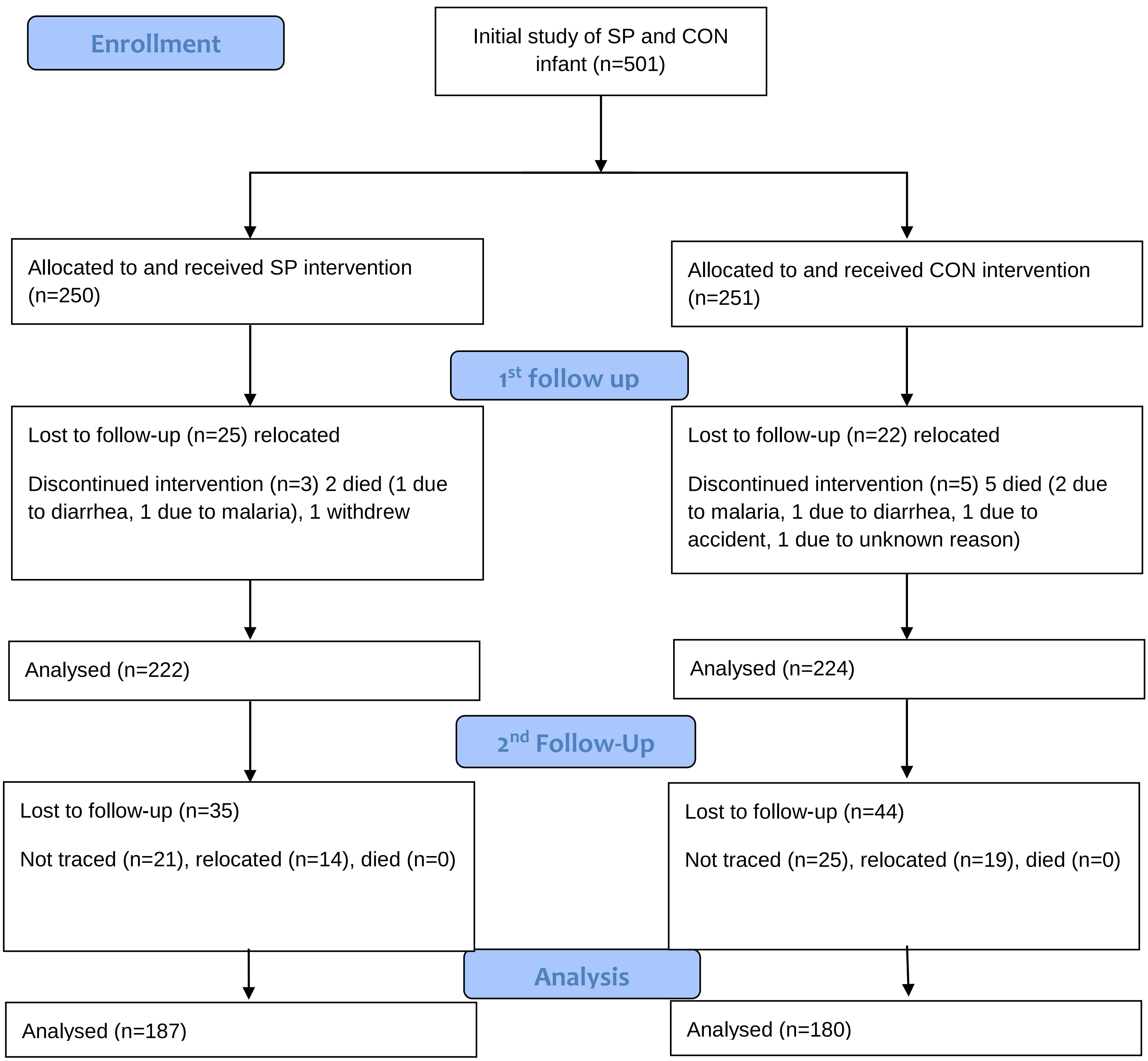
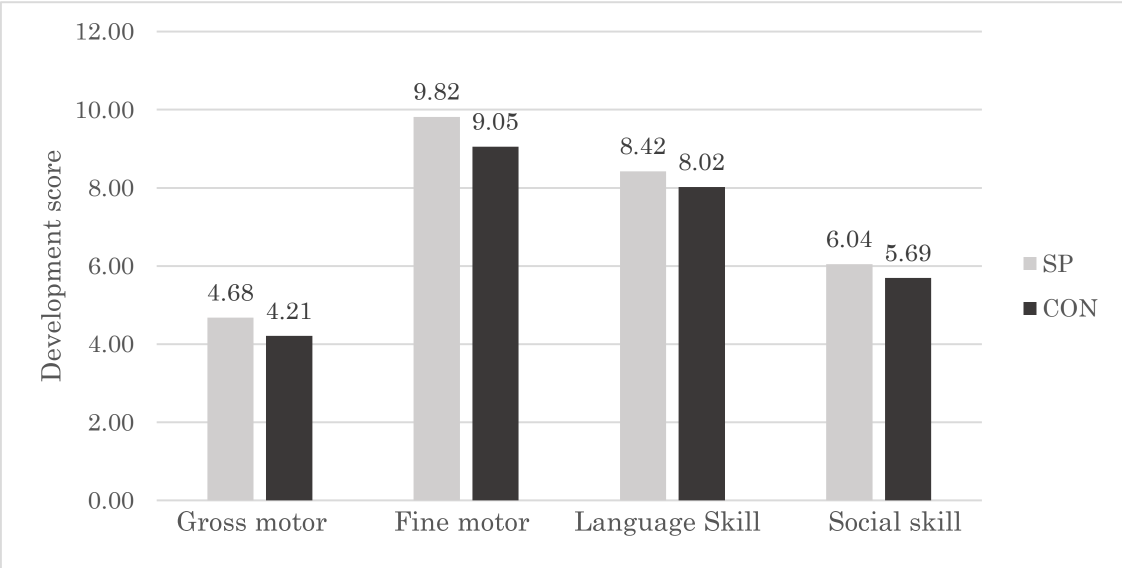


Figure2



12.00
10.00
8.00
6.00
4.00
2.00
0.00
Development score
9.82
9.05
8.42
8.02
6.04
5.69
4.68
4.21
Gross motor
Fine motor
Language Skill
Social skill
SP
CON

Supplementary Table S1

Nutrient composition of the soya and spirulina 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 (μg 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

Note: The detailed macronutrient and micronutrient composition of the porridge in the two groups was compiled based primarily on food composition data from the Zambia

Food Composition Tables published by the Zambian National Food and Nutrition Commission [1], adding data from standard tables of food composition in Japan and FAO, and Siva et al. [2, 3, 4],

References

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S2 Table. Correlate of attrition with household characteristics at baseline

Outcome	1 if attrited between baseline and follow up	
Age of child in months	0.00	(-0.01, 0.01)
1 if female	0.04	(-0.04, 0.12)
Number of household member	0.02	(-0.01, 0.04)
Number of under 5 member	-0.01	(-0.06, 0.04)
ln(household consumption)	-0.01	(-0.05, 0.03)
ln(household expenditure)	0.01	(-0.03, 0.05)
1 if lives in Mansa	0.05	(-0.04, 0.13)
1 if received any government assistance in last 12 months	-0.09	(-0.28, 0.10)
1 if with diarrhea	0.05	(-0.03, 0.13)
1 if with fever	-0.03	(-0.13, 0.07)
1 if with cough	-0.02	(-0.12, 0.08)
Mother's age	-0.00	(-0.01, 0.00)
Mother's years of education	0.00	(-0.01, 0.01)
1 if in treatment group	-0.04	(-0.12, 0.04)

Note: Values are estimated regression coefficients with 95% Cis in parenthesis.
*** stands for significance at 1% level, ** at 5% level, and * 10% level.

S3 Table. The effects of spirulina supplementation on child development at 9 month of non-intervention period.

Outcome: Standardized z score measuring….	Gross motor development	Personal-social skill
All children (N=323)		
Effect size (mean)	0.51***	0.27**
95% CI	(0.29, 0.73)	(0.05, 0.49)
Children with HAZ<-0.2 at baseline (N=136)		
Effect size (mean)	0.66***	0.53***
95% CI	(0.30, 1.02)	(0.17, 0.89)
Children with HAZ>-0.2 at baseline (N=174)		
Effect size (mean)	0.39***	0.01
95% CI	(0.11, 0.67)	(-0.28, 0.30)
Children with dietary diversity score<median at baseline (N=119)		
Effect size (mean)	0.80***	0.76***
95% CI	(0.40, 1.21)	(0.38, 1.14)
Children with dietary diversity score>median at baseline (N=196)		
Effect size (mean)	0.32**	-0.02
95% CI	(0.06, 0.57)	(-0.30, 0.26)

Note: 95% confidence intervals are in parenthesis. Motor development scale ranges from 0 to 7, and personal-social skill from 0 to 6. Effect sizes are calculated with adjusted mean. All specifications control for individual characteristics: age in months, gender, and mothers' characteristics: mother's age, and weight. *** stands for significance at 1% level, ** at 5% level, and * 10% level.