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Research Article

SUCCESSFUL USE OF SPIRULINA IN COMBATTING CHILDHOOD UNDERNUTRITION: A COMMUNITY INTERVENTION STUDYAS PART OF A MISSION PROJECT

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ABSTRACT

Background: Provision of food supplements alone has not sufficed to combat child undernutrition. Spirulina is an ancient blue-green algae, with excellent nutrient properties.
Objective: To evaluate impact of Spirulina to combat malnutrition in a "mission mode" and a non-randomized study.
Methods: A 'community-based mission', was launched wherein, 30,716 malnourished children (6 months-6 years) were fed 1-2 grams of Spirulina for 180 days to supplement Home+ICDS food in threetaluks of Bellary district in Karnataka. Third party evaluation done with non-randomized four arm community intervention study, of 1003 children, with 1:1:1:1 allocation to 1 & 2 grams of Spirulina; multivitamin and ONLY food control.
Results: Weight for age z-score data a significant 44% reduction in malnutrition in mission data was validated by study data. With 47% and 68% reduction among children who received 1 and 2 grams of Spirulina, respectively, as compared to little change in two control groups ($p<0.05$).In 2 grams Spirulina arm, an increase in mean weight of 1.25 grams/kg/day ($p<0.01$); and maximum weight gain of 7.3 grams/kg/day, implies pre/probiotic effect of Spirulina, consistent with recent data on etio-pathogenesis of child undernutrition. A significant cognitive improvement; alleviation of iron and Vitamin A deficiency was noted.
Conclusion: This is largest pilot 'mission' using Spirulina for malnutrition conducted globally to our knowledge. Spirulina offers promising opportunity to ameliorate child undernutrition across the country as it is swift in effect, simple, without adverse effects, cost effective and easy to scale up, with an impact on the etio-pathogenesis of undernutrition.

INTRODUCTION

Childhood undernutrition is a difficult, urgent, and an overwhelming global health issue. Out of 677 million children under 5 worldwide, 23% are stunted and another 7.7% are wasted.[1] Child undernutrition rates in India are among the highest in the world, with nearly one-half of all children under three years of age being underweight or stunted. India is home to over 40 million stunted children and 17 million wasted children among under-five.[2]

Undernutrition has substantial negative cascading impact on human potential and sustainable development in a given population, by contributing to morbidity, mortality, decreased learning capacity and low productivity.[3] Even with recent improvements, India's stunting problem represents the largest loss of human potential in any country in human history. Despite the fact that the Integrated Child Development Scheme (ICDS) program is one of the largest such supplementation program globally, it has had little or no impact on the rates of undernutrition in the country.[4]

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Thus epidemiologic studies have emphasized that undernutrition cannot be ascribed to food insecurity alone and reflects the intersection of multiple factors that operate within and across generations.[5] One such factor is micronutrient deficiency. More recent data have shown that immune-pathological states associated with childhood undernutrition are caused by defects in gut-microbiota maturation, abnormal nutrient processing by the microbiota, and aberrant nutrient sensing by immune cells orchestrated by the gut-microbiota.[4]

Spirulina *platensis*, a simple blue-green algae, has been used extensively to combat malnutrition, especially in Africa, and has been endorsed as the 'best food' by the United Nations.[6] An ancient cyano-bacterium, which from millions of years has populated the earth's atmosphere with oxygen, Spirulina, is a super-abundant source of protein and almost all micronutrients. The 60-70% protein it contains has the least environmental footprint in terms of land, water, and soil use as compared to other sources of protein.[7] Moreover, it is readily digestible and bio-available. It was the food of the ancient Aztecs and NASA hails it as the food of the future.[8] However, despite its extensive use, the field level research data on Spirulina use is limited.

'Mission mode' projects have clearly defined objectives, scopes, and implementation timelines and milestones, as well as measurable outcomes and service levels.

Our objective was to verify if we can ameliorate childhood malnutrition using Spirulina in a "mission-mode".[9], as well as to conduct research. As part of the 'mission-mode' we partnered with the local government, ground level NGOs, and third party research institute, to conduct unbiased research. The research institute conducted a Community Intervention Study, whose objective was to compare the effects of Spirulina on childhood malnutrition in a four-arm, non-randomized open-label study.

MATERIALS AND METHODS

There are two components to the present work: a) the field level work as part of the "mission against malnutrition" b) the community intervention study as a component of former to substantiate the results of the mission.

'Mission Against Malnutrition': In April 2014, the Corporate Social Responsibility Wing of a large Conglomerate in India, launched a community based "Mission Against Malnutrition", along with its partners; wherein, 30,716 moderate and severely malnourished children (between 6 months to 6 years), were fed Spirulina Fortified Sugar for 180 days, in Sandur, Hospet and Kudligitaluks of Bellary district of Karnataka (Figure 1). With focused advocacy, a community wide uptake without untoward side effects was witnessed.

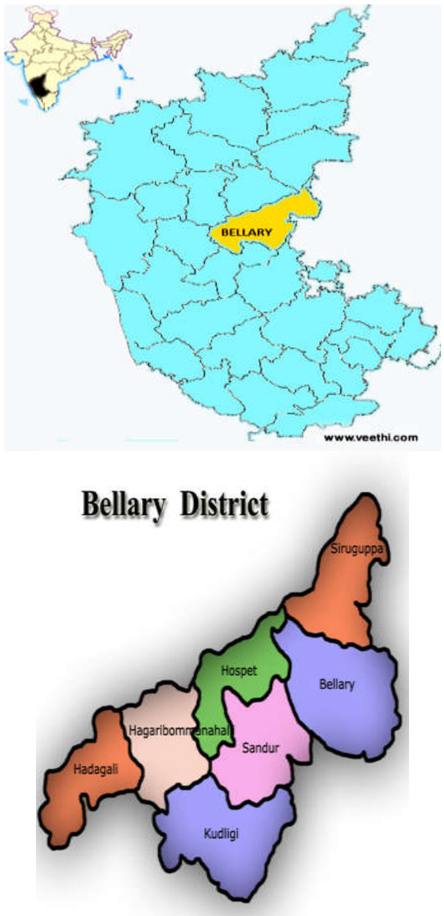

Figure 1 Map of Karnataka and Bellary District and the 3 Intervention Taluks: Sandur, Hospet, Kudligi

The aim of the mission was to reduce and eliminate malnutrition with SPIRULINA among target population. Since the first 1000 days of conception are extremely important to eliminate malnutrition, 15,000 pregnant and lactating mothers were also included in the intervention.

The following were the components of the Mission

1. Recruitment and training of local field workers for the project.
2. Sensitization with street plays, talks, posters, wall murals, and other focused information, education and communication (IEC) activities among the community regarding malnutrition and its impact on child health.
3. Identifying malnourished children through the WHO growth charts maintained by the Anganwadi workers.
4. Measuring height and weight using locally adopted WHO standards.[10]
5. Administering Sugar Fortified Spirulina 5 gram (1 gram of Spirulina) to moderately acute malnourished children and 10 grams (2 grams of Spirulina) to severely acute malnourished children using standard measuring spoons.
6. Spirulina supplement delivery at the homes of children, to accommodate compliance and prevent any discrimination at the Anganwadis.
7. Measurement of follow up weight at month 3 and month 6.
8. Data collection, compilation and analysis in Excel. We relied on the government data for the first year of the

mission, wherein, we noted a definite impact. We then began to collect our own data for the mission.

To measure the impact of the use of Spirulina alone, we did not attempt to change any other factor such as the food intake or hygiene in the target population.

The Community Intervention Study: The aim of the clinical trial as a community intervention study was to generate enough scientific evidence to substantiate the effects of Spirulina on malnutrition as part of this 'mission'. To not bias the results of the study, it was conducted by a third-party research organization.

We conducted an open-label non-randomized four arm study, with children between 6 months and 6 years being allocated in a 1:1:1:1 fashion into the following four groups:
Arm A: ICDS food+ Spirulina 1 gram
Arm B: ICDS food+ Spirulina 2 gram
Arm C: ICDS food+ Multivitamin Supplement
Arm D: ICDS food ONLY (Control group)

Study Population and Sampling: The sample size was fixed considering a prevalence of malnutrition at 40%, and based on conservative estimate of a 20% improvement in malnutrition with Spirulina, and an alpha error of 0.05 and 80% power of the study, with an attrition rate of 20% the final sample size of 1000, was calculated.

Since the bio-chemical and cognitive tests are time and resource intensive studies, we conducted a sub study of 75 children in each arm. Thus overall a multi-phasic sampling design was opted for the study.

About 750 children between 6 months to 6 years, as identified from the Anganwadiregisters were recruited from the SandurTaluk, from 5 Panchayats and 26 villages for arms A, B and C. Similarly, the Arm D 250 children were recruited from geographically adjacent KudligiTaluk, from 1 Panchayat, 13 villages (see Figure 1)considering similar sociodemographic milieu. A quick socio-demographic survey was conducted in all selected villages before the commencement of baseline assessments with the purpose of group matching of intervention and control arms as per known socio-demographic variable.

A list of all moderate and severe malnourished children was made from all the anganwadi centers of the selected villages to prepare a sampling frame. From this frame,1003 children were selected by adopting Probability Proportion to Size (PPS), and then Simple Random Sampling method. However, on our assessment there were a fraction of these children which fell into normal category, suggesting that the government data were not completely reliable. 75 children in each arm for biochemical and cognitive development assessment were selected by simple random sampling.

Ethical clearance was obtained and written informed consent was obtained from the study participants' parents or guardians. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Indian Institute of Health Management Research, (IIHMR) Jaipur, Ethics Committee(IORG 0007355). Written informed consent was obtained from the study participants' parents or guardians.

The only exclusion criteria was if the parent or guardian did not give informed consent.

Measurements

Evaluation was done at baseline, at the end of six months of intervention, and then at month 12, six months post-intervention.

Anthropometric Measurements: Weight-for-age is a composite index of height-for-age and weight-for-height. This is considered for both acute and chronic malnutrition.[11]

Weight was measured using adult analogue weighing machines for children who could stand, baby weighing scales for those who could not. Research personnel were trained to calibrate the scale with known weight and to zero the scale before each measurement. Measurements with inch tape for height and mid-arm-circumference were done. Infantometer was used for length measurement for children who could not stand.

Single person was assigned to measure weight and height both before and after intervention to reduce the inter-observer bias. Also two readings were taken each time and average of two taken to reduce intra-observer bias.Single and same weighing machines were used after calibration and zero error correction, throughout the study to reduce equipment/instrument bias.

Biochemical Tests: Following parameters were evaluated, Vitamin A, Serum zinc, Serum iron, Serum B12, Serum Alkaline phosphatase, Serum Albumin, and Serum Total Protein.

Whole blood was collected in vacutainers by venipuncture, as per pediatric and neonatal blood sampling guidance provided by WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy.[12] Serum was separated from the whole blood within six-eight hours and stored at 4^{o}C until taken for analysis.

Cognitive development: The cognitive development (psychometric assessment) was assessed using the Indian adaptation by Dr. A. J. Malin, of the Vineland Social Maturity Scale (VSMS).[13,14]

The field level research personnel were trained by a qualified psychologist to administer the VSMS test in local language. The children were observed for social adaptation behavior, and the parent were interviewed to supplement the rest of the VSMS scales.

Statistical analysis

Z-scores

All data corresponding to weight-for-age, height-for-age and weight-for-height, showing underweight, stunting and wasting respectively, along with mid arm circumferences were estimated using z-scores.

At this time, only 'weight for age' measurement is being done by the government as per ICDS standards. Thus, in keeping with the government norms, we have only reported the weight-for-age data at the follow-up intervals. Thus, children were classified as 'normal'; 'moderately under-weight/malnourished'; and 'severely underweight/malnourished' based on the z-scores. Once classified as thus, the categorical data with inter-group comparisons were done using Chi-square tests with Bonferroni correction to correct for multiple comparisons. Descriptive statistics were used to describe the data variables and the difference in the rates of outcome variables were tested using tests of significance like unpaired t test, paired t test and chi-square test

Variables, which were significantly different between groups at baseline, were adjusted for. *P-value* was fixed as 0.05 or lower for significance.

Data was analyzed using SPSS Version 24.

Spirulina supplement: Spirulina for the children was provided as sugar coated granules that were mixed with flavorings and for the women as tablets. A standard measuring spoon of 5 grams (which provided 1 gram of Spirulina) was provided along with each Spirulina granules container. Spirulina powder was supplied by a local Spirulina producer. The quality control of each batch of raw Spirulina powder was done by a NABL accredited laboratory in Bangalore. Certification was for protein, lipids, minerals and vitamin content, and bacteria and heavy metals for safety. Each batch of Spirulina granules was also tested for safety before being packaged. Field workers visited the participant's home every week and counseled regarding adherence. At the end of every month, empty bottles of Spirulina were collected as a proof of adherence.

Multivitamin Supplement: The supplement given toArm C children was "Bevon". The doses were as follows:

Age	*Dosage*	*Time of administration*	*Type*
6 months to 1 year	1ml	B.D	Drops
1 year to 3 years	2.5 ml	B.D	Syrup
Above 3 years	5 ml	B.D	Syrup

RESULTS

'Mission Against Malnutrition': A total of 30,716, children were identified as SAM/MAM from the Anaganwadi registers and received Spirulina over the period of 3 years of the mission. However, only after the first year, we have collected data as we relied on government data prior to that. The field level data on the 'Mission Against Malnutrition' is described in Figure 2. Thus, out of the 28,007 children's data we observed, there were 23,958 (84.5%) who had follow up at 6 months. Of these, 10,775 children (44.4%) recovered from malnutrition and were classified as normal at the end of 6 months. Another 8.2 %, who were classified as SAM at baseline, were classified as MAM. Only 252 (1.1%) of children who were MAM at baseline deteriorated to SAM. We had a loss to follow up rate of 15.5%, which included families that migrated out of the area.

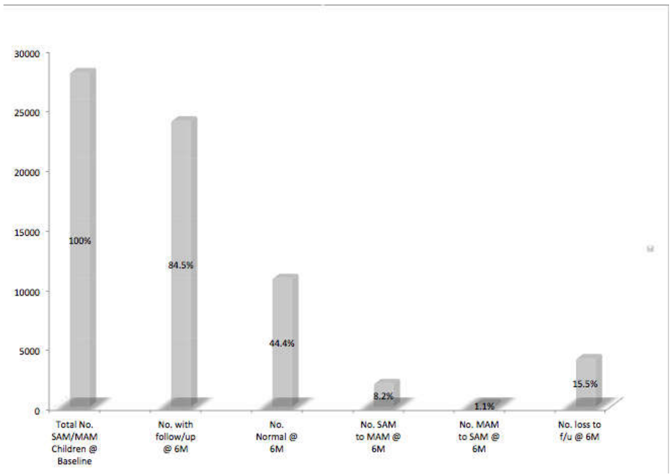

Figure 2 Field Level Data on the 'Mission Against Malnutrition'

SAM: Severely Acute Malnourished; MAM: Moderately Acute Malnourished, 6M: 6 Months

Findings from the Community Intervention Study

Baseline Characteristics and Nutritional status of Children

The children are well matched for age and sex across the four arms of the study as presented in **Table 1a.**

Weight-for-Age: During the baseline analysis more than 50% of the children below six years was under-weight for their age in all the intervention arms (A, B & C), the corresponding figure for the control arm D was at 40%. This difference was statistically significant (p <0.05). However, we did adjust for this potential confounder and controlling for it showed no statistical, or operational difference in the results.

Height-for-Age: In the Study area more than one third of the children were stunted at baseline. Since stunting is a chronic form of malnutrition, we did not expect it to be ameliorated with just six months of intervention.

Mid-Upper-Arm-Circumference: At baseline around 70% of under five children in all arms were found to be malnourished as per MUAC criteria.

Table 1a Baseline Anthropometric Characteristics of Children in the Four Study Arms

Variables	**Arm A**		**Arm B**		**Arm C**		**Arm D**		**p value**
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	
Age									
< I year	18	7.2	21	8.5	21	8.4	19	7.5	ns
1 - 4 year	187	74.5	184	74.2	186	73.8	176	69.8	
> 4 years	46	18.3	43	17.3	45	17.9	57	22.6	
Gender									
Male	101	40.2	124	50.0	108	42.9	108	42.9	ns
Female	150	59.8	124	50.0	144	57.1	144	57.1	
Nutritional status of Children									
Weight for Age									
Normal	116	46.2	107	43.1	127	50.6	151	59.9	P < 0.05
Moderately Under weight	69	27.5	71	28.6	64	25.5	65	25.8	
Severely Under weight	66	26.3	70	28.2	60	23.9	36	14.3	
Height for Age									
Normal	162	64.5	144	58.3	181	71.8	174	69.0	ns
Mod/Severely Stunted	90	35.5	104	41.7	71	28.2	78	31.0	
Mid Arm Circumference*									
Normal	66	27.8	65	27.4	71	29.6	72	29.3	ns

Between -1SD and -2SD	116	48.9	104	43.9	121	50.4	118	48.0
Beyond -2SD	55	23.2	68	28.7	48	20.0	56	22.8

Socio-demographic Characteristics

Presented in **Table 1b**. Being a community-based study, there are some missing data in the data collection.

Based on the baseline analysis, the range of children in the family was between one to two in all arms. Nuclear family was predominating in all the arms with more than 60%, except in arm A in which 53% of them belong to joint family. More than 80% were Hindus in all the four arms. In the study about more than 60% belonged to scheduled caste or scheduled tribe. Illiteracy predominated as educational status of the parents and consisted of two fifths of both mothers and fathers in all the four arms. Majority of the fathers were unskilled workers that consisted of more than 60% in all the arms. More than 80% in arm A, C, D and around 60% in arm B the mothers were either housewives or unemployed. The economic category in all arms belonged to lower and lower middle class as per the BG Prasad Scale classification.[15] More than 80 percent of the respondents have their own house in all the arms except arm 'A' that had 63%. In all arms more than 90% had a 'Kachcha' or 'Semi Pucca' houses.

Table 1b Baseline Socio Demographic Data in the Four Arms

Variables	Arm A		Arm B		Arm C		Arm D		p value
	N	%	N	%	N	%	N	%	
Family type*									
Nuclear	103	41.5	177	72.2	188	74.9	166	67.2	*ns*
Joint	147	58.5	70	27.8	64	25.1	86	32.8	
Religion*									
Hindu	232	92.7	246	99.2	205	81.3	237	94.0	*ns*
Muslim/Others	17	7.3	2	0.8	47	18.7	13	6.0	
Caste Category*									
SC/ST	127	65.8	172	70.5	126	61.5	169	70.7	*ns*
OBC	12	6.2	26	10.7	19	9.3	15	6.3	
Others	54	28.0	46	18.9	60	29.3	55	23.0	
Education of father*									
Illiterate	108	46.4	138	56.8	96	41.2	*106*	*44.0*	*ns*
Any Literacy	125	53.6	105	43.2	137	58.8	*135*	*56.0*	
Education of mother*									
Illiterate	116	47.3	150	61.0	89	35.3	*123*	*49.2*	*ns*
Any Literacy	129	52.7	96	39.0	163	64.7	*127*	*50.8*	
Occupation of father*									
Skilled labourer	50	20.1	21	8.5	84	34.0	30	12.0	*ns*
Unskilled labourer	190	76.3	224	90.7	162	65.6	219	87.6	
Unemployed	9	3.6	2	0.8	1	0.4	1	0.4	
Occupation of mother									
Skilled/Unskilled labourer	36	14.6	97	39.3	36	14.3	40	16.1	*ns*
House wife	211	85.4	150	60.7	216	85.7	208	83.9	
Economic Status*									
Below Poverty line	233	91.0	237	97.9	216	91.5	228	95.8	*ns*
Above Poverty line	23	9.0	5	2.1	20	8.5	10	4.2	
Ownership of house*									
Yes	156	62.9	244	99.2	208	82.5	235	94.8	*ns*
No	92	37.1	2	0.8	44	17.5	13	5.2	
Type of house *									
Kuchcha	110	44.5	54	21.8	72	33.8	43	18.4	*ns*
Semi pucca	123	49.8	192	77.4	122	57.3	190	81.2	
Pucca	14	5.7	2	0.8	19	8.9	1	0.4	

All data are subjected to chi square test, p value considered significant at < 0.05. ns: not significant; * Missing Data

For monitoring of acute undernutrition, one relies on weight gain change, measured as grams/kg/day. Table 2 represents the mean, minimum and maximum changes in grams/kg/day in weight of the children in the four arms of the study. There were significant differences (p<0.05) in mean weight gain change (grams/kg/day) in different arms (A, B, C & D) during baseline to 6 months and baseline to 12 months. During baseline to 6 months, maximum weight gain change was observed in arm B (7.26gm/kg/day). The mean changes were 1.378 in Arm B; 0.936 in Arm A; 1.058 in Arm C; and 0.452 in Arm D. Similarly during baseline to 12 months, also maximum weight gain change was observed in arm B (3.791 gm/kg/day). The mean changes were 0.687 in Arm B; 0.540 in Arm A; 0.439 in Arm C; and 0.244 in Arm D. All these changes were statistically significant.

Post-Hoc analysis (Bonferroni) was done to find out the exact statistical differences between different arms (Table 3). Duringbaseline to 12 months the maximum mean weight gain change was observed in arm B (0.687+0.659), which was significantly (p<0.05) higher than mean weight gain changes in arm A (0.540+0.638), arm C (0.439+0.497) and arm D (0.244+0.276). Mean weight gain change in arm A and C was also significantly (p<0.05) higher thanmean weight gain changes in control arm (arm D).

Table 2 Weight Change in Grams/Kg/Day in all the Four Arms of the Study

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	F value (ANOVA) P value Between Groups
						Lower Bound	Upper Bound			
Baseline to 6 Months	Arm A	246	0.936	1.080	0.069	0.800	1.071	-1.320	4.690	*34.75 p < 0.01*
	Arm B	224	1.378	1.211	0.081	1.219	1.539	-0.770	7.260	
	Arm C	225	1.058	1.030	0.069	0.923	1.194	-1.580	5.230	
	Arm D	239	0.452	0.540	0.035	0.383	0.521	-1.020	2.920	
	Total	**934**	**0.948**	**1.047**	**0.034**	**0.880**	**1.015**	**-1.580**	**7.263**	
Baseline to 12 Months	Arm A	231	0.5400	0.638	0.042	0.457	0.622	-0.874	3.580	*26.04 p < 0.01*
	Arm B	219	0.687	0.659	0.045	0.600	0.775	-0.788	3.791	
	Arm C	223	0.439	0.497	0.033	0.374	0.505	-0.563	2.299	
	Arm D	220	0.244	0.276	0.019	0.208	0.281	-0.448	1.333	
	Total	**893**	**0.478**	**0.563**	**0.019**	**0.441**	**0.515**	**-0.874**	**3.791**	

All data are subjected to one-way ANOVA, p value considered significant at < 0.01.

Table 3 Post Hoc Analysis (Bonferroni) Baseline to 12 months

Arm one	Arm two	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-.14764*	0.051	0.023	-0.282	-0.013
A	C	0.10047	0.051	0.288	-0.034	0.235
A	D	.29522*	0.051	<0.01	0.161	0.430
B	C	.24811*	0.051	<0.01	0.112	0.384
B	D	.44286*	0.052	<0.01	0.307	0.579
C	D	.19474*	0.051	0.001	0.059	0.331

*. The mean difference is significant at the 0.05 level.

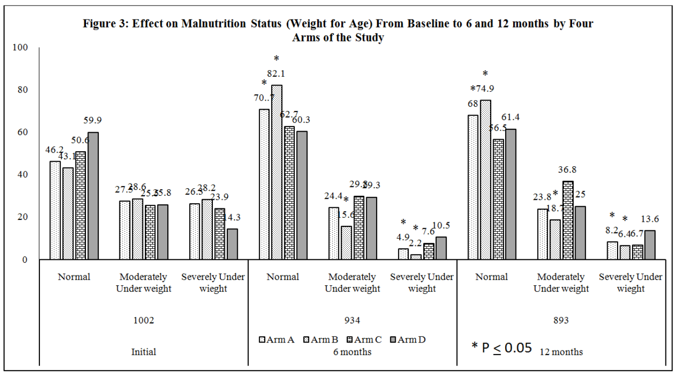

Figure 3 describes the undernutrition status, as defined by weight for age, in the four arms of the study change from baseline to 6 months and 12 months.

There is reclassification of children from moderately undernourished category to normal, and children from severely undernourished to normal and moderately undernourished categories.

Table 4 Biochemical Assessment Baseline to 6 Months by Fours Arms of the Study

Biochemical assessment	Arm A (n=76)				Arm B (n=75)				Arm C (n=77)				Arm D (n=75)				P value
	Initial		6 months		Initial		6 months		Initial		6 months		Initial		6 months		
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	
Vitamin A																	
Deficiency	30	39.5	15	19.5	33	47.1	12	17.1	15	19.5	15	19.2	11	14.9	11	14.9	P < 0.05
Normal	46	60.5	62	80.5	37	52.9	58	82.9	62	80.5	63	80.8	63	85.1	63	85.1	
Iron																	
Deficiency	24	31.6	10	13.2	25	33.3	6	8.0	16	20.8	9	11.7	15	20.0	8	10.7	P < 0.05
Normal	52	68.4	66	86.8	50	66.7	69	92.0	61	79.2	68	88.3	60	80.0	67	89.3	
Zinc																	
Deficiency	0	0.0	0	0.0	0	0.0	3	4.1	0	0.0	2	2.6	2	2.7	2	2.6	ns
Normal	62	100.0	62	100.0	74	100.0	71	95.9	77	100.0	76	97.4	73	97.3	74	97.4	
Total Protein																	
Deficiency	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	ns
Normal	76	100.0	76	100.0	75	100.0	75	100.0	77	100.0	77	100.0	77	100.0	75	100.0	
Albumin																	
Deficiency	1	1.3	0	0.0	1	1.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	ns
Normal	75	98.7	76	100.0	74	98.7	75	100.0	77	100.0	77	100.0	75	100.0	75	100.0	
Alkaline Phosphatase																	
Deficiency	0	0.0	0	0.0	0	0.0	0	0.0	1	1.3	0	0.0	0	0.0	0	0.0	ns
Normal	76	100.0	76	100.0	75	100.0	75	100.0	76	98.7	77	100.0	77	100.0	75	100.0	
Vitamin B 12																	
Deficiency	5	6.6	11	14.5	4	5.3	0	0.0	2	2.6	0	0.0	4	5.3	37	49.3	ns
Normal	71	93.4	65	85.5	71	94.7	75	100.0	75	97.4	77	100.0	71	94.7	38	50.7	

All data are subjected to chi square test
ns: not significant

Around 60 percent of moderately malnourished children in arm A, 87 percent in arm B, and 41 percent in arm C showed improvement in nutritional status by moving into normal categories from baseline to 6 months (after 6months of nutritional supplementation), and at 12 months (6 months after the withdrawal of intervention), these figures were 58 percent, 73 percent and 42 percent respectively. In control arm D, 17 percent improvement was observed at 6 months and continued to be the same in the 12 months. These changes were statistically significant in Arms A and B.

The severely malnourished children in all the intervention arms showed improvement by shifting either to normal, or moderate categories. At six months, around 88 percent of severely malnourished children in arm A, 86 percent in arm B had shown improvement.

There were no differences by sex or age groups in the rates of decline in malnutrition (data not shown). There also were no significant changes in height between the four arms as perhaps the intervention was only for six months (data not shown).

Figure 4 represents percentage decline in malnutrition i.e., total number of children who were moderately or severely under-nourished becoming normal at the end of 6 months.

A statistically significant decline inundernutrition, was observed in both the intervention Arms A and B. In arm B this decline was maximum (39%), followed by arm A (25%) and arm C (12%). Slight decline (1%) has been observed in control arm from baseline. This translated into an overall reduction in 46%, 68%, 24% and 2.5% in Arms A, B, C and D respectively which was statistically significant.

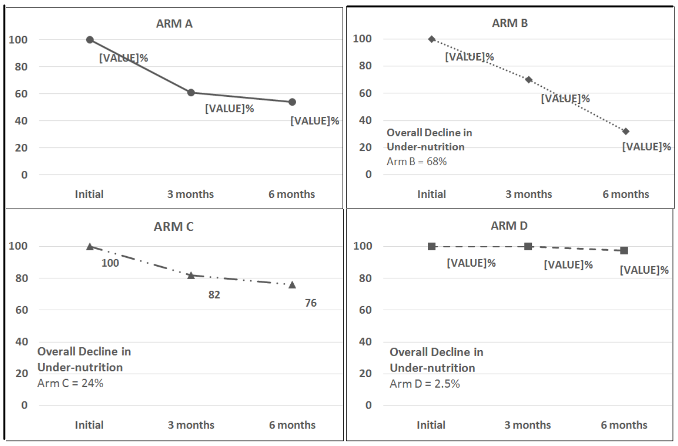

Figure 4 Percentage Decline in Undernutrition Status at 6 months by Four Arms

Table 4 represents the biochemical assessment of the substudy of 300 children

Nearly about 40% of children in arms A and B and nearly 15% of children in arms C and D were deficient in Vitamin A at baseline. Spirulina supplementation could reduce this deficiency to more than half in both arm A (19.2%) and arm B (17.1%) which were both significant (p<0.05). The improvement was slightly better in arm B as compared to arm A. There was no change in the deficiency status in the arms C and D. At baselineiron deficiency was 32% and 33% in Arm A and B respectively. There was a statistical significant reduction in the number of children to 13% in Arm A and 8% in Arm B (both significant at p< 0.05) in 6 months after intervention. In Arm C and D, there were no significant changes in the number of children with Vitamin A and Iron deficiency, except for changes in Iron deficiency reduced by 50%.

The rest of the changes in biochemical parameters were not significant.

Table 5 represents the cognitive assessment of the 300 children in the substudy by the four arms. The Vineland Social Maturity Scale was used at baseline and at 6 months, by field workers trained by a psychologist, and the responses for the sake of current analysis was categorized as IQ levels below average, average and above average. As noted in arms A and B there were 34 and 36% below average children at baseline which were reduced more than 6 fold to 5.6 and 4.6% in the two arms respectively. In arm C there was halving of the number from baseline and in arm D there was an increase in the number of children with below average IQ. Similarly, the children with average IQ were 32 and 30% in arm A and B resepcetively at baseline which increased to 55% and 43% at 6 months. And children who were above average IQ at baseline were 34 % and 34% in arms A and B which increased to 39% and 52% respectively at 6 months.
Comparitively there were little changes in arm C and deliterious changes in arm D. All these changes were statistically significant.

Moreover, without much additional calorific supplementation (just 38 kCal) we documented a mean weight gain in the 2 gram Spirulina arm of 1.25 grams/kg/day, as compared to the 500 kCal supplementation with RUTF given for 28 days with a weight gain of only 1.37 grams/kg/day.[19] This suggests that Spirulina has an impact on weight gain through a mechanism other than calorie input. Also, a maximum weight gain of >7 grams/kg/day which is very significant considering that there were no significant extra calories being fed to these children.

A significant cognitive improvement and alleviation of biochemical micronutrient deficiency was noted among children who received Spirulina.

We did not conduct a randomized study due to the ethical concerns of giving Spirulina with known benefits for combating malnutrition, to one child versus placebo to another in a community setting. The four groups in the study were however well matched socio-demographically, and statistically we adjusted for the variables, which were significantly different, thus making our data robust.

Table 5 Cognitive Assessment Baseline to 6 months by the Four Arms of the Study

Cognitive assessment	Arm A				Arm B				Arm C				Arm D				p value
	Initial		6 months		Initial		6 months		Initial		6 months		Initial		6 months		
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	
Below average	29	34.1	10	5.6	32	35.6	6	4.6	22	27.8	16	10.5	25	32.9	40	50.6	P < 0.05
Average	27	31.8	99	55.0	27	30.0	56	43.1	28	35.4	78	51.0	22	28.9	16	20.3	P < 0.05
Above average	29	34.1	71	39.4	31	34.4	68	52.3	29	36.7	59	38.6	29	38.2	23	29.1	P < 0.05

All data are subjected to chi square test

DISCUSSION

There are a several studies which demonstrate the role of Spirulina in reduction of undernutrition rates both in children and adults.[16,17,18] In this community based "Mission Against Malnutrition", we have corroborated a significant impact among 30,716 moderate and severe under-nourished children (between 6 months to 6 years), and another 15,000 pregnant and lactating women being fed 1-2 grams of Spirulina for 180 days, in three Taluksof Bellary district of Karnataka. With focused advocacy, a community wide uptake without untoward side effects was witnessed. A dramatic> 40% reduction in severe/moderately under-nourished children was documented which was validated by third party evaluation in this non-randomized study, of more than 1000 children. This, to our knowledge, is the largest such 'mission' undertaken with Spirulina for management of undernutrition.

In the community intervention study, a 46% and 68% reduction in undernutrition was evidenced among children who received 1 and 2 grams of Spirulina, respectively for 6 months, as compared to little change in the control group, which was relatively sustained post 6 months after stopping Spirulina. Despite the fact that at baseline, there was statistically significant greater number of under-nourished children in the Spirulina arms as compared to the control arms, it favored the analysis, as the results showed a significantly higher rate of response in the two Spirulina arms despite having more under-nourished children.

A higher rate of response among those who received 2 grams as compared to those who received 1 gram suggests a dose-response relationship, which suggests causality for reversal of undernutrition with Spirulina. In addition, we did not interfere with the existing food intake of the under-nourished children as per the ICDS programme. Therefore, the only intervention provided was "Spirulina supplementation". Thus, in the control arm, there was little or no-change in the rates of undernutrition at 6 and 12 months follow-up. This is consistent, in terms of outcomes, found by Lokshin et.al, on child nutrition status in villages with ICDS centers. [4] Overall, considering that the most significant changes in weight were in 2 grams category, we recommend 2 gram of Spirulina supplementation for both moderately and severely under-nourished children.

Our biochemical data are consistent with that from other investigators. Spirulina increases iron and hemoglobin levels as shown by other studies.[20,21,22,23]. Data pertaining to India shows that, in spite of iron supplementation, 50% women and 70%children under 5years of age continue to be anemic. The rates of anemia and its consequences have not changed significantly among women between the NFHS-3[24] and the NFHS-4[25]. Even though the provision of iron and folic acid coverage is reportedly more than adequate. Thus Spirulina is an important tool to combat anemia.

We also witnessed a statistically significant increase in Vitamin A levels, in the two Spirulina arms, as has been demonstrated before in other studies with Spirulina. In a Chinese study of

228 school children, a significant increase in total body Vitamin-A stores were demonstrated using stable isotopes.[26] Similarly, in 1991, a large non-randomized study of 10,000 children was conducted in rural Tamil Nadu, funded by the Government of India. Children less than 5 years were supplemented with 1 gram of Spirulina for 12-18 months. A dramatic 80% reduction in clinical signs of Vitamin A deficiency as manifested by reduction in Bitot's spots, and xerosis was seen. Statistically significant increases in beta-carotene and retinol levels were also seen.[27]

The weight gain in the multivitamin arm suggests the role of micronutrient deficiency correction in undernutrition. However, even though the mean increase in weight in terms of gram/kg/day was significant, this did not translate into significant changes in the z-score data for change from undernutrition categories to normal. Moreover, we did not see significant changes in the bio-chemical data in the multivitamin arm.

Thus, we hypothesize that undernutrition is not due to food-insecurity alone, as has been pointed out by other researchers.[28] Moreover, we found a weight gain of up to 7 grams/kg/day among children fed just 2 grams of Spirulina. Thus, the amelioration of macro-nutrient-deficiency cannot be explained just by supplementation of micronutrients. There are clearly some other factors. Since we did not interfere with the food intake, we hypothesized that Spirulina helped to increase the bioavailability of the food that was consumed. We also hypothesized that Spirulina improved the gut-immunity to break the vicious cycle of enteral infection and undernutrition. This can all be explained by the pre/probiotic effect of Spirulina to change the micro-flora of the gut favorably. As has now been well described in the literature, the profound deleterious changes in the gut-microbiota of under-nourished children, which get partially corrected by supplementation with RUTFs.[26] Importantly, there is in-vitro and in-vivo data to demonstrate the prebiotic properties of Spirulina.[29] Moreover, in collaboration with Dr. YogeshShouche at National Center for Cell Science, Pune, in preliminary study, we have shown in just 45 days, favorable changes in gut-microbiome, in 15 volunteers given 4 grams of Spirulina.[30] These observations have profound implications, for amelioration of undernutrition.

In the double-blind placebo controlled trial of live 'probiotics' in management of SAM conducted in Malawi in 2009, a trend towards improved outpatient mortality was noted even though the general results were negative. Latter was due to several reasons such as inclusion of more than half of study population who were HIV positive, and more children with complicated SAM with severe re-feeding syndrome.[31] Moreover, this suggests that feeding live-probiotics, may be both prohibitive due to costs and effects of bacteria, which are not indigenous to the population due to their dietary practice.

Recently, Jeffery Gordon's team, is developing gut microbial community-targeted therapeutics to treat under nutrition in infants and children living in low-income countries, and obesity in Westernized countries.[32] Even though, processed Spirulina is not live, it appears that it forms an important 'pre-biotic' which sustains the growth of favorable bacteria as noted in our preliminary study.[28] Thus, Spirulina may prove to be a much simpler, cheaper, and safer alternative to pro-biotics and can be considered an excellent microbiota directed food (MDF).[30]

We found a significant improvement in the cognitive function among the children who received the higher dose of Spirulina. We used Vineland Social Maturity Scale which is a comprehensive field level tool to assess social adaptive behavior of preschool and early school children which correlates well with the IQ levels.[12,13] We witnessed an increase in the Social Quotient levels in the two Spirulina arms as compared to little or no change in the control arm and multivitamin arm, within 6 months. Sachdeva *et al.*, reported similar increase in intellectual levels schoolgirls given one gram of Spirulina for just five weeks.[20]This strengthens the prebiotic hypothesis of Spirulina, as there is a well-established gut-brain axis, which modulates cognition.[33,34] This finding has significant implications in improving the human potential and productivity. We believe that Spirulina supplementation should begin with pregnancy as the first 1000 days since conception are extremely important for the long-term physical and mental development of the child.[35,36] Even though we did not have a formal quantitative data collection among the pregnant and lactating mothers, we did not see any untoward effects among the women who received Spirulina, but have affirmations through several video testimonials and interviews as to the benefit of Spirulina among women (Figure 5). Moreover, as demonstrated by our biochemical data, Spirulina helps alleviate iron deficiency anemia, which is the largest scourge for adverse fetal and maternal outcomes of pregnancy. In addition, iron fortification may affect the gut-microbiome adversely compounding the problem of undernutrition.[37] Thus, there needs to be more studies to substantiate the role of Spirulina, during pregnancy, for both maternal and child health.

Figure 5 Lactating mother's diet was supplemented with 2 gm of Spirulina/day. Malnourished baby images taken before and after the Spirulina supplements were given for 3 months. Baby was enrolled at the age of 3 months (September, 2015) and was severely malnourished as shown in the first image. Second image showing clear weight gain after 3 months (November, 2015)

One has to consider cost-effectiveness of Spirulina supplementation for it to be scaled-up across the country. So specifically, for severely acute malnutrition (SAM), Spirulina at 10 USD per beneficiary for 6 months (including cost of growth, production, transport of Spirulina and manpower for mission-mode of delivery) is much more cost-effective as a community based intervention as compared to the currently available institutionally delivered ready-to-use-foods (RUTF). Even if RUTF were to be delivered in a community setting at homes, it would still be five times as expensive at 55 USD per child per treatment.[38,39,40] Thus Spirulina is a cost-effective alternative to the currently existing strategies to combat SAM in the country. In addition, to make the model of Spirulina supplementation sustainable, local women, thus promoting sustainable living, women empowerment and combating malnutrition locally and nationally.

As an efficient energy source, Spirulina requires less energy input per kilo than soy, corn or beef including solar and generated energy.[7] Its energy efficiency (food energy output/kg/energy input/kg) is 5 times higher than soy, 2 times higher than corn and over 100 times higher than grain-fed beef.[7]What is more, the cultivation of Spirulina will help mitigate the fallout of climate change because it is a powerful carbon sink. Thus, the simple blue green algae, Spirulina, can help towards removing the root causes in inequity in sustainable development, integrating economic, social and environmental dimensions while combatting undernutrition at local and national levels.

There are limitations to the community intervention study. It is a community based non-randomized interventional study done in the rural setting in India; it has its own weightage while generalizing the study findings to similar settings in India. However, the field level data from the 'mission' mirrored the study data in a much larger population. The acceptability and low attrition rates also speak for replicable nature of our findings. The fact that the SAM children did not have to undergo the 'appetite test', as the intervention involved giving only two scoops of a sugar-fortified Spirulina, makes it a very acceptable community-based intervention for SAM.[41]

Thus, ourthree-year pilot experience, leadsus to believe that Spirulina definitely offers a promising opportunity to ameliorate undernutrition among children across the country and globally, as it is cost effective, swift in impact, sustainable, simple, without adverse effects and easy to scale up in a mission mode.

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