Evaluation of Spirulina as a Daily Nutritional Supplement in School Children.

A Randomised Crossover Supplementation Study in Three "Pour un Sourire d'enfant" (PSE) Schools in Cambodia

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Final report

1 June 2016

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Abbreviations

AE	Adverse Event
CI	Chief Investigator
HB	Haemoglobin
HT	Haematocrit
MCV	Mean Cell Volume
MCVC	Mean Cell Haemoglobin Concentration
SRA	Survey Research Associate (Monitor)
PI	Principal Investigator
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SP	Spirulina

Foreword

The study was conducted in PSE School with the collaboration and inputs from PSE health team: Dr Sarath Sorn, Sokheang chan, Sarapich Pin and their collaborators. The design was drawn by Hubert Barennes with inputs from the team of Antenna in Cambodia. The management of the study was conducted by Laetitia Houdart and Thea Song from Antenna Cambodia under a regular supervision of HB. The study received a grant from Antenna Technologies Suisse to cover the expenses of biological parameters. No specific budget was available for the management of the study. HB received a compensation for final analysis and report. It was planned that HB would be the only responsible for the analysis and conclusion of this report in order to have an external and if possible scientific, analysis of spirulina supplementation. So, opinions expressed in this report may or may not represent the opinion of the founder.

Acknowledgements

We would like to thank the children, teachers and health staff at PSE for their kind participation (Dr Sarath Sorn, Sokheang chan, Sarapich Pin and their collaborators,), Mrs Laetitia Houdard and Thea Song from Antenna Cambodia for their dedication in managing the survey, Jean-François Biela for his enthusiasm for initiating this project, Pascal Godon for his friendly and continuous support, Caroline de Courville and Caroline Theoule for participating to the follow-up of the study, Antenna Technologies Suisse for their financial support to the biological measurements (Denis von der Weid), as well as Antenna Technologies France. We also express our thanks to the lab team from Pasteur Institute and its director (Dr. A. Kergueler) for conducting the blood sample analysis. We thank Mr A. Lailou from UNICEF for kindly lending anthropometric measurement tools. We thank Wafik Ghommidh for his dedication to retrieved missing data. We thank Renaud d'Avout d'Auerstaedt for his kind and helpful edit.

Summary

An eight-week daily supplementation of two grams of Spirulina (SP) was evaluated in school children from deprived settings under the care of "Pour un sourire d'enfant " (PSE) using a crossover study design and randomised allocation to either SP or control group (Group 1 and Group 0 respectively). A switch was eventually made after a two months rest period: Group 0 then received SP treatment and Group 1 received control treatment. Anthropometric measurements (height and weight) were conducted before the first treatment, after the first treatment, and at the end of the study. An attempt was made to ensure double-blind standards for the study: everyone involved was kept unaware of which type of supplementation the children were receiving, which explains the use of group names 0 and 1. Biological samples were collected before the first treatment, after the first treatment, and at the end of the study. These were analysed by the Pasteur Institute for blood cell count, ferritin and C Reactive Protein (CRP). Following the biological results, all children received a deworming treatment by PSE health team after the final first supplementation.

A total of 194 children from three PSE centres were included in the survey (65 in Group 0 and 128 in Group 1 respectively). Of 194, 36 (18.5%) left the PSE school during the course of the study or did not complete the eight-week period, leaving 158 children for analysis (41 in Group 0 and 88 in Group 1 respectively). Lost to follow-up subjects were more numerous in one centre (Phrom Russey; 33.9% vs. 10.0 and 15.5% for the two other centres) with no clear explanation. Only 129 children were sampled for initial blood analysis on three tests: red blood count, ferritin and CRP. Intermediate anthropometric data were only obtained nine months after the end of the study, in March 2016.

Children characteristics (71 female, 87 male; average age of 5.1 years; 23 diagnosed with severe malnutrition) were similar in both groups except for anaemia, which was more prevalent in Group 1 at the start of survey (haemoglobin: 12.1 $g.l^{-1}$, 95% CI 11.9–12.4; vs. 11.8 $g.l^{-1}$, 95% CI: 11.6–12.0; p=0.05). Forty (31.0%) had microcytic anaemia and 15 (11.6%) had a mild iron deficiency anaemia, none (0.0%) had moderate iron deficiency anemia. Rate of eosinophilia was high before the treatment (108; 83.7% had over 500 eosinophiles per mm^3).

Acceptability was good and few health events occurred during the first cure and only minor side effects were reported during supplementation 1.

Children taken as their own control (matched analysis) increased their height and weight (1.0 kg and 4.3 cm respectively) in both groups during first supplementation and second supplementation. No anthropometric difference between groups was observed at the second and third measurements.

No difference was observed between groups for haemoglobin and other biological parameters at the second and third measurements.

There was a significant decrease of haemoglobin in Group 1 and a non-significant decrease in Group 0 before and after the first supplementation. There was then a significant increase in both groups before and after the second supplementation. Overall, there was no change of haemoglobin before and after the two supplementations.

Ferritin decreased in both groups after the first supplementation and increased after the third supplementation in both groups. There was a constant decrease in the eosiniphilic rate, falling from 12.4% to 8.1 % over the course of the study. No difference was observed between groups so this was probably related with global improvement of hygiene and the deworming treatment that took place before the second supplementation.

Conclusion

The study was conducted with limited means in the context of underprivileged children cared by PSE. The limited means and context was partially compensated by an ambitious design (randomisation, crossover, double-blinding). This explains some of the difficulties to conduct and analyse the results of the study (moderate rate of lost to follow up mostly in one centre, reporting of events, delays for obtaining data, absence of precise age, paucity of biological parameters used). The crossover and attempt of double blinding were well respected which allows drawing some conclusions.

Overall, tolerance and acceptability of SP supplementation was good in children of about five years of age attending PSE schools. The drop-out rate which was to be expected in the context was below 20% and does not seem related to the children' supplementation. Very few health events were recorded during the first and second supplementation which was an obvious benefit for all PSE children. Hence no impact on the prevention of sickness could be attributed to SP supplementation. This point suggests conduct studies on larger sample if only health events are to be the only parameters for evaluation of SP supplementation. It also suggests using less basic parameters or increase the number of clinical and specific biological parameters, other parameters

such as improvement of attention at school, school performance, learning capacity, resistance to sickness during seasonal disease or disease outbreaks etc.., and explore the sustainability of long term health benefits. In addition the doses of SP, interval of observation and probably the initial condition and heath status of children can be discussed.

Over the course of the supplementation periods at PSE schools, general benefits were observed. Children significantly increased their weight and height and reduced their level of eosinophils. They improved their anaemia after SP supplementation during the first supplementation but no difference was observed between groups when the control group received SP or before and after the start of the study. Overall, in this analysis, no difference between groups and between the types of supplementation could be related to supplementation. In the view of limited parameters that could be tested, of the doses of supplementation used (~100 gr Spiruline), and of the target group (underprivileged children over 5 years daily cared by an NGO), the study cannot conclude to a specific short term benefit of the dosage used of SP supplementation though it should be noted that overall all children experienced improved physiological status during the survey, acceptability seemed good and no harm was reported.

A good point is that in the context of limited means, a crossover with quasi double-blinding could be performed. SP Doses, parameters to be evaluated, interval between supplementation and tests, long term benefits, different children conditions and improved study condition including people specifically dedicated to the study and sufficient funds should be considered in further researches.

Introduction

How Did the Study Come About?

A few years ago, as I was a researcher/teacher at IFMT Vientiane Laos, I met with Pascal Godon from the NGO Antenna Technologies France. He was seeking to develop spirulina for humanitarian purposes in Laos and I connected him to my good friend Doctor Philippe Schmidt. In 2014, working in Cambodia, I then met Jean-François Biela (JFB), who was in charge of Antenna Cambodia, at a meeting for NGOs in Phnom Penh. Antenna was then initiating production of spirulina for humanitarian purposes in Cambodia. We discussed the issue of providing a more scientific approach to the supplementation that was being provided to childrenin a few schools in Cambodia. We then monitored, analysed and reported on the SP supplementation in preadolescent school girls in a "Toutes à l'École" programme and to children of various ages at ASPECA. This gave me the opportunity to experience how SP supplementation was distributed on the field. I then trained Mr Thea from Antenna to conduct correct antropometric measurements. After discussion with JF B we eventually accepted to develop a controlled study on SP supplementation, acting upon the interest of PSE's medical team (Dr Sarath and his collaborators). Finally, having an enthusiastic team and partners who accepted to work for this idea, and despite the departure of JFB, we refined the study model with Laetitia Houdart and Antenna Technologies. There was no specific budget, which was one major problem, but the Fondation Antenna Technologies (based in Switerland) kindly accepted to donate 10,000 euros to finance the basic biological analyses to be conducted by the Institut Pasteur and PSE accepted to finance the spirulina to be used for supplementing the children. Two 5th year pharmacy students from Lyon (France) volunteered for an internship under my direction at Pasteur Cambodia. They would participate in the study for a period of three months in 2014 and three months in 2015, alongside Mr Thea. They also contributed to processing the data. For various reasons, I could not get any intern to help us for the last three months of the study. This explained some difficulties that we faced after the departure of the pharmacy students.

Rationale

Use of spirulina (SP) as a nutritional supplement for children is quite extensive. Reports are numerous but scientific publication reporting the results of well-conducted trial are rather infrequent due to numerous reasons. These include small sample size, lack of randomization, various methodological failures, as well as the lack of funds or research capacity. A brief search on the NHS Pubmed database with the query "spirulina children" yielded only seven references, including a review by Housseini et al., and trials by Simpore et al. in Burkina Faso, while other papers were retrieved were unrelated to SP. However, the Pubmed database does not include reports from the Archive de Pédiatrie nor Médecine d'Afrique Noire, which are probably not in compliance with Pubmed's requirements for peer review.

Another reason to study SP is related to its immunological and antiviral properties that could be useful in countries where both malnutrition and HIV are prevalent.

The Health Benefits of Spirulina

In this section, we will briefly review some of the reported effects of SP. For more information, it is possible to read the following reports produced by Berli D, Documented health properties Antenna; *Gershwin ME, Belay A (2007)* and a detailed review of its nutritional activity has been done in the following reports: *Falquet J, and Hurni JP (2006) Falquet J (1996)* ; Hug and D von der Weird La spiruline dans la lutte contre la malnutrition. 2011. [1–5]

Spirulina (*Arthrospira platensis*) is a free-floating filamentous microalgae (cyanobacteria). The filaments have a spiral shape and are 0.1 mm long. Spirulina naturally grows in alkaline water bodies in subtropical and tropical areas [6,7]. It has been consumed as food for centuries in Central Africa and Chad. SP grows in high pH environment where no deleterious bacteria and viruses can develop.

SP is now widely used as nutraceutical or food supplement. *SP* is well recognized due to its high protein content (60–70% by dry weight) and its richness in vitamins, minerals, essential fatty acids and other nutrients that are very easily assimilated by the human body due to lack of cellulose walls. The most significant are: β-carotene (provitamin A), iron, vitamin B_{12} and gamma-linolenic acid (GLA). Such micronutrients enable the body to grow properly and to maintain its vital functions.
SP has bio-modulatory and immunomodulatory functions. Different SP preparations influence immune system through increased phagocytic activity of macrophages, the stimulation of the production of antibodies and cytokines, increased accumulation of NK cells into tissue and activation as well as improved mobilization of T and B cells.

SP has also been shown to perform a regulatory role on lipid and carbohydrate metabolism by exhibiting glucose and lipid profile correcting activity in animal models and in diabetic patients.

SP preparations have been found to be *in vitro* active against several enveloped viruses including herpes virus, cytomegalovirus, influenza virus and HIV. SP preparations are also capable of inhibiting carcinogenesis due to anti-oxidant properties that protect tissues and also reduce toxic effects on the liver, the kidney and the testes. [8,9]

Additional benefits have been reported, including in preventing or managing hypercholesterolemia, hyperglycerolemia, certain inflammatory diseases, allergies, cancer, viral infections, cardiovascular diseases, diabetes and other metabolic diseases. SP has also been reported as mitigating the impact of environmental toxins and drug-induced toxicity.. [10–17]

The potential of SP for HIV has been supported by an early phase trial on a limited number of patients in 2012 by Teas and al [18]. A randomized survey of 160 HIV-infected adults consuming a daily dose of 10 g of SP showed no difference between the SP group and the control group except with regard to protidemia and creatinemia, which were both higher in the group receiving SP supplement. From a clinical standpoint, results were less clear-cut since the Karnofsky score was better in the group receiving SP than in the group receiving the placebo at 3 months but not at 6 months and fewer patients presented pneumonia at 6 months. Further study over a longer period will be needed to determine if SP is useful and to evaluate if higher doses can have beneficial nutritional and immunitary effects without presenting any adverse effects, in particular in the renal system. [19]

More recently, a prospective single-blind, randomized, multicenter study was conducted over 12 months on 320 HIV-1 ARV-naive participants in Cameroon. Among the 169 ART-naive participants enrolled in the study, females were over-represented (67.1%). The significant increase of CD4 cell count (596.32–614.92cells/µL) and significant decrease of viral load levels (74.7 x 10^3-30.87 x 10^3 copies/mL) of the patients who received a supplementation of *Arthrospira platensis* was found after 6 months of treatment. Haemoglobin level was also significantly higher in the same group while the fasting blood glucose concentration decreased after 12 months compared to control. A daily supplementation with *Arthrospira platensis* to diet combined with a reasonable balanced diet has significantly increased the CD4 cells and reduced the viral load after 6 months.

In 2011 a randomized trial assessed the efficacy of two affordable and accessible nutritional supplements, *SP platensis* versus soybeans, among malnourished HIV-infected adults. [20] Fifty-two patients were enrolled. After 12 weeks, weight and BMI significantly improved in both groups. At the end of the trial however, FFM was significantly higher in the SP group (42.2 vs. 39.0 kg, $P = 0.01$). The haemoglobin level rose significantly in both groups ($P < 0.001$ for each group) with no difference between groups ($P = 0.77$). Serum albumin level did not increase significantly within groups ($P < 0.90$ vs. $P < 0.82$) with no difference between groups ($P = 0.39$). The increase in CD4 cell count within groups was significant ($P < 0.01$ in both groups), with a significantly higher CD4 count in the SP group than in the soybean group at the end of the study

(P = 0.02). Within each group, HIV viral load was significantly reduced at the end of the study (P < 0.001 and P = 0.04 for SP and soybeans groups respectively). In this preliminary study, it was therefore established that both SP and soybeans improve on nutritional status of malnourished HIV-infected patients but in terms of quality of nutritional improvement, subjects on SP were better off than subjects on soybeans. It was also demonstrated that nutritional rehabilitation improves immune status with a major drop in viral load. Further investigations on the antiviral effects of SP and its clinical implications remain strongly needed

Spirulina and HIV-Infected Children

Very few studies focussed on the consumption of SP by HIV-infected children. A recent systematic review of nutritional supplementation in HIV-infected children in resource-limited settings was recently conducted by Mchenry et al in 2015 and did not find evidence supporting the positive impact of SP. This review was limited to four studies using SP however and the authors therefore concluded that more studies are necessary. [21]

Another review, by Grobler L et al. and published by Cochrane, concluded that "In general supplementation with specific macronutrients such as amino acids, whey protein concentration or SP did not significantly alter clinical, anthropometric or immunological outcomes compared with placebo in HIV-infected adults and children." Only one study of SP supplementation was used (by Simpore – see below), out of three trials concerning macronutrient supplementation.[16] The review concluded that it is, "however, promising to see more studies being conducted in low-income countries, and particularly in children, where macronutrient supplementation both preantiretroviral treatment and in conjunction with antiretroviral treatment might prove to be beneficial."

Spirulina, Human Nutrition and Child Malnutrition

Among large number of SP species, three have been intensively investigated: *Arthrospira maxima*, *Arthrospira fusiformis* and *Arthrospira platensis.*

Early studies were mainly focused on the nutritional value of Spirulina as a food source.

Its impact in children with malnutrition was reviewed by Hosseini et al in 2013 [22]. Of 31 references, 3 randomized control trials and 4 non-controlled trials showed a postive impact on weight, height, albumin, prealbumin, protein and haemoglobin after SP supplementation.

SP is claimed to be the richest whole-food source of vitamin B_{12} (despite its corrinoid forms, analogs and pseudo vitamin B_{12}) and provitamin A (β-carotene). Only 20 g of this microalgae would fulfil nutritional requirements in vitamins B_1 (thiamine), B_2 (riboflavin) and B_3 (niacin). The high levels of several micronutrients, especially minerals (iron 0.58–1.8, calcium 1.3–14, phosphorus 6.7–9.0 and potassium 6.4–15.4 g/kg), in SP are due to the absorption of these elements during the growth of the alga, which makes it a suitable nutritional supplement.

Absorption of SP iron is 60% higher than ferrous sulphate, commonly used in iron supplements. [23]

Safety and Recommendations

In general, SP is considered a generally recognized as safe (GRAS) nontoxic dietary supplement at normal levels of consumption.[17,24,25] Few side effects have been reported from the ingestion of SP including headache, stomach ache, flushing of the face and muscle pain [9]. *Spirulina spp.* should be avoided by phenylketonuria patients and patients with autoimmune diseases (due to its immunomodulatory activity). [26,27]

The US Food and Drug Administration (FDA) certified SP as safe to eat with beneficial effects on health [28]. The Dietary Supplements Information Expert Committee (DSI-EC) of the United States Pharmacopeial Convention assigned a Class A safety rating for *Arthrospira maxima* and *Arthrospira platensis*, thereby permitting its use as ingredient for dietary supplements.[28] The Food and Agriculture Organization (FAO), a UN organization, declared that SP is an easily digestible high protein product with high levels of nutrients.

Recent reports on potential contamination by toxic agents on commercial SP have been recently published in Germany, Italy and Korea [29–31]. The Korean report analysed the lead (Pb) and cadmium (Cd) content in functional health foods (FHF). A total of 672 samples were collected from 2,347 people (1,015 adults, 557 teenager and 775 infants and young children) living in Korea. The study found that mean values of the two heavy metals were highest in SP and yeast products (0.940 mg kg^{-1} for Pb in SP products and 0.115 mg kg^{-1} for Cd in yeast products). These levels are well below the maximum levels that are legal in the EU however (lead 5.0 mg/kg and cadmium 0.5 mg/kg) according to Apssa report : www.ceva.fr/eng/content/download/3560/22750/file/r%25C3%25A9glementation%2520algues%2520MAJ%25202014.pdf.

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SP is used as a nutritional supplement worldwide. However, reviews shows that data is still lacking, especially in terms of comparative surveys and limited sample size. The impact of SP on growth and on the prevention of the occurrence of current children disease (such as diarrhea, fever, virus, pneumopathia) has been rarely been assessed.

There is a quite extensive use of SP as a nutritional supplement for children. Reports are numerous but scientific publications reporting on properly conducted trials are rather infrequent at present.

This survey is part of "Pour un sourire d'enfant" (PSE)'s strategy to improve nutritional status of Cambodian children through better nutrition, both thanks to nutritional supplements and regular nutrition. After conducting preliminary one arm studies to children of various Cambodian institution (Toutes à l'école, ASPECA), we decided to try to conduct a crossover study of daily supplementation in PSE children. The objectives were to investigate tolerance to SP supplementation (based on a daily report by PSE teachers), the impact on weight and growth, and the impact on basic biological parameters (the parameters being basic due to the limited budget on hand)

What Was Known of SP Daily Supplementation of Fragile Children?

Li et al did a randomised trial with 2, 4 and 0 g of daily SP that concluded that SP is a good dietary source of β-carotene, which may effectively increase the total-body vitamin A stores of Chinese school-age children. **[32]**

Three groups supplemented with 4 g (containing 4·18 μg β-carotene), 2 g (containing 2·54 μg β-carotene) or 0 g spirulina 5 d/week for 10 weeks, respectively in china school children and whowed: After the 10-week intervention, serum β-carotene concentrations of children with 2 or 4 g spirulina supplement increased by 0·160 and 0·389 μmmol/l, respectively. Total-body vitamin A stores increased significantly, with a median increase of 0·160 mmol in children taking 2 g spirulina and of 0·279 mmol in children taking 4 g spirulina. Spirulina is a good dietary source of β-carotene, which may effectively increase the total-body vitamin A stores of Chinese school-age children.

Dia et al conducted a prospective non-comparative supplementation of SP with a daily dose of 2 g of SP mixed with 10 g of honey during 60 days to evaluate the effects of SP on academic performance of school children in the municipality of Dakar, Senegal. They compared school

performance of schoolchildren from public elementary schools (before supplements, during and after). The average of second quarter marks before supplementation was 5.17 out of 10 IC= [4.99-5.35] and the same for the third quarter after two months of supplementation was 5.78 out of 10 IC= [5.59-5.97]. The mean difference between pupils' marks at the third and the second was 0.59 ($p<0.0001$). After two months of supplemental feeding, the academic performance of the children was improved.

However, in absence of a comparative group it is not possible to conclude that the improved performance obtained is related to SP.

Two interesting studies were conducted in Burkina Faso. [32–34]

Simpore et al. evaluated the nutritional rehabilitation of undernourished children using spirulina and Misola. They found that in addition to Misola, 5 g/day of SP was more effective than Misola or SP alone for malnourished children from Burkina Faso over the course of eight weeks.

Simpore and al. also evaluated the impact of SP supplementation on HIV-infected children.

"We compared two groups of children: 84 were HIV-infected and 86 were HIV-negative. The duration of the study was 8 weeks. Anthropometric and haematological parameters allowed us to appreciate both the nutritional and biological effect of SP supplement to traditional meals. Rehabilitation with SP shows on average a weight gain of 15 and 25 g/day in HIV-infected and HIV-negative children, respectively. The level of anaemia decreased during the study in all children, but recuperation was less efficient among HIV-infected children."

Objectives and Outcomes of the Study

Primary Objective
The primary objective is to assess the usefulness of daily SP supplements on health events for disadvantaged children attending school, whatever their level of malnutrition.

Secondary Objectives
There was a total of seven secondary objectives:

1. Acceptability of SP: Evaluate the percentage of refusal during the first week of supplementation and at the end of the study. Evaluate adherence to SP and children self-appreciation at the end of study.

2. Assess the best strategy to overcome the first week of supplementation (SP has a strong taste that some children may not like at first).
3. Evaluate the occurrence of minor side effects: nausea, vomiting, gastric pain, headache, insomnia.
4. Assess the nutritional impacts on growth of children (height and weight) of less than six years of age.
5. Estimate the impact on anaemia and inflammation in children before and after supplementation.
6. Appreciate the acceptability of the supplementation and overall satisfaction of the teachers in charge of the children
7. Appreciate the long-term tolerance of children to SP supplementation.

Primary endpoints

Comparison of health events during the study in both diet groups

Secondary End Points

Global adherence to school evaluate by daily reports on the monitoring sheet.

Percentage of children with health events,

Nutritional baseline on children attending PSE Schools

Changes in nutritional status based on weight for height, height for age, weight for age scores at enrolment, end of supplementation and 2 months after the end of supplementation, according to WHO standards and using UNICEF tools.

Primary acceptability: taste, nausea, rejection, vomiting, as measured by self-reporting and daily monitoring (percentage of vomiting episodes or nausea during first week and last week).

Safety and tolerance: occurence, number and type of adverse related effects.

Comparative trend of anaemia, C Reactive protein, ferritin and soluble transferrin receptor (sTfR) between groups and before and after supplementation.

Survey Design

This is a control group survey of nutritional supplements with a crossover match. Each child had alternatively received one of the two regimens: either SP + regular diet or regular diet + chocolate sprinkles (the appearance of which is very close to SP sprinkles) during a 2 months period. The study had two phases.

Supplementation process

Phase 1: The first group of children received 1 teaspoon of SP during the first hour of morning classes from their teacher. This amounts to 2 g per day, every day of the week excluding weekends and government holidays during 8 weeks. SP was provided in addition to the regular diet of the children. All events such as nausea, vomiting, allergic, headache, and fever were recorded daily on a standardised form.

The second group received the regular diet and chocolate sprinkles as a supplement.

After the 2 month treatment, the children had a weekly check-up during 3 months.

Phase 2: A cross-over was then applied: children who had previously received only their regular diet received SP with their regular diet, and vice-versa, during 2 months.

After these 2 months, the children had a close follow-up again during 3 months.

Duration of each phase: 5 months, including 8 weeks supplementation and 3 months of follow-up.

Total duration: 10 months.

Randomisation

Neither the teachers not the children had any idea of the allocation within groups. The choice of distribution was blind and randomized as described below.

The intake of SP + regular diet or only regular diet + chocolate sprinkles as a supplement was directly observed (DOT), daily during 8 weeks. Then children underwent passive reporting during 3 months and were to be actively monitored once a week.

Information was recorded daily on standardized forms. Data sheets were recorded daily during the first 2 weeks by a survey assistant together with the class teacher, then twice weekly. In case of side effects, children were to be sent to the nursery and, if appropriate, to the PSE doctor and the supplementation was either discontinued or decreased (see adverse event section). In case of

disease, the diagnosis and treatment were to be provided by PSE according to PSE and National health guidelines. In case of absence from school due to disease, a visit was to be made by a medical member of the study to assess the status of the child and the need for further treatment, and this was reported to the medical head of the PSE health centre.

Participant Enrolment

Survey participants

Children aged 5 to 6, attending school at PSEand having the informed consent and approval of their parents/guardians.

Inclusion Criteria
Children and parents/guardians willing and able to give informed consent for participation in the survey.
No recent gastroinstestinal episodes, no fever, no diarrhea, no vomiting or stomachache in the week before the survey.
No known allergy to SP.

Exclusion Criteria

The participant was excluded from the survey if anyof the following applied:

- Significant renal or hepatic impairment or any child who has to be under a specific treatment whatever the cause.
- Chronic disease
- Known allergy to SP
- Mental disorder or incapacity to attend school class
- Fever, diarrhoea, vomiting or stomach ache before enrolment or in the last weeks
- Participants who had participated in another research survey involving an investigational product in the past 12 weeks.
- Incapacity to attend school at PSE during the next 6 months (expected migration, etc.).

Survey Procedures: recruitment, screening and eligibility assessment

Participants were children attending one of the three schools and complying with the eligibility criteria. A first screening was done by the PSE health centre regarding age and recent disease history. Once the list of children was obtained, an interview using a standardized questionnaire was done to ensure that inclusion criteria were respected and that informed consent had been provided. Then children underwent an anthropometric assessment.

Informed Consent and Ethics

All parents/guardians/caregivers were informed by the school manager and were provided an information file in Khmer. The participant information file and informed consent sheet were presented to the participants and availed the following details: the exact nature of the survey; what it would involved for the participant; the implications and constraints of the protocol; the known side-effects and risks involved in taking part in the study. It was clearly stated that the participant was free to withdraw from the survey at any time for any reason without prejudice to future care and with no obligation to give the reason for withdrawal (see the information file in the Annex).

An information session in Khmer was then conducted at PSE with PSE teachers and staff prior to enrolment to ensure that:

- They had understood the survey design and objectives; they understand the monitoring They had the possibility to ask questions and receive answers;
- They had provided informed consent to children and families;

Children withdrawing from the study were to be offered similar access to PSE health services.

The reason for withdrawal was to be recorded in the follow-up form. If the participant withdrawal was due to an adverse event, the study assistant was to arrange for follow-up visits or telephone calls until the adverse event was to be resolved or stabilised. It is to be noted that no such event actually took place during the course of the study.

The protocol, informed consent form, participant information sheet were submitted to and received agreement from Cambodian Research Ethics Committee (REC).

Study Procedure

First of all, all the children attending the class underwent a nutritional survey and an individual health assessment to check their compliance to the inclusion criteria, as conducted by a nurse and

his/her assistant. All files were checked by the Antenna team and when necessary, further advice was requested to the PSE physician and nurses, who work daily in the centres. Medical data and medical history of the children was checked by PSE health centre medical staff.

Children who fulfilled the enrolment criteria were listed and given a unique identification code in addition to a school code. This list was sealed and kept by the school manager (who was not taking part in the study process), by the manager of Antenna (also not a part of the study process), as well as by the scientific advisor.

Randomisation, Blinding

A randomised list using computer generated numbers by blocks of four was prepared before the survey and done with no contact with the children. The randomisation list was done prior to the onset of the survey. The outcome of this randomisation process was the attribution of each child to either Group A or Group B with a study number. Each study number was written on an individual envelope and inside the envelope, Group A or Group B was specified. The envelope was prepared by an external assistant who wasn't involved in the distribution of the envelopes, and then sealed. A number corresponding to the identification code and class number of the child was then recorded on the envelope following the randomisation list. As a result, no connection between these codes and the name of the children was possible for the people preparing the envelopes.

Individual envelopes were given to each class according to the number of participants. The envelopes were opened at the beginning of the survey by the teachers under the supervision of the team, and children were thus allocated to Group A or Group B.

The randomisation list was safely locked away in the office of a person who did not take part in the study. In the event were the code had to be broken, the person in charge of keeping the code was to be asked to provide the information individually (for example, on a specific individual) without disclosing the whole set.

Preparing the Supplements

Antenna team and a young pharmacist prepared the supplements in sealed plastic pouches and attributed either the letter A or the letter B, defining inclusion in either Group A or Group B, as well as writing down on each pouch both the date of preparation of the supplements and their expiry date. Only one group of bags contained SP. No other mention to the product was made on

the pouches. These pouches were prepared under the control of the promoter prior to the onset of survey.

Since it was not possible to obtain identically matching placebo to SP, it was first suggested that children in the control group could receive a zymafluor supplement, which has an positive impact on the prevention of tooth decay. Eventually, we found diet chocolate sprinkles that were locally available and has a similar appearance to the SP sprinkles we were using as a supplement.

Baseline Assessments

Baseline nutritional (using the UNICEF scale and WHO standards) and health assessments were conducted prior to the survey, one month after the first treatment and one month after the second treatment.

On the same dates, a 4 mL blood sample was taken by a nurse from Pasteur Institute and divided into one pediatric EDTA and one 2 mL dry tube. Sampling was done by a lab nurse with experience with children. The samples were stored in the Institut Pasteur lab and assayed for blood cell count, haemoglobin(HB), ferritin, and CRP. The budget was not sufficient to sample for transferrin receptor (sTfR).

Haemoglobin and haematocrit (HT) levels were measured in order to determine the anaemic status. The number of lymphocytes, neutrophils and leukocytes would provide a good idea of the immune system of the children.

The biological analysis was similar to that of Simpore et al in Burkina Faso but did not include HIV testing or CD4 counts.

The children at PSE were foreseen to be impacted by malnutrition at different stages, and mostly stunting. As a result, we expected to find many cases of anaemia, low immunity and increased susceptibility to disease. Malnutrition induces various effects on biologic factors, which can either increase or decrease. For this reason, we decided to follow blood cell count, ferritin and C-reactive protein (CRP) concentration during this study. All of these factors are complementary and were chosen in order to provide us with an overall vision of the children's biological state depending on their nutritional status.

Most of the children were suspected to have nutritional anaemia. The determination of plasma ferritin reflects the tissues reserves that are mobilized. The measure of the concentration of

ferritin assesses iron stores and thus serves for early detection of iron deficiency as well as allowing the detection of building up of iron reserves during treatment with iron supplementation. Threshold values are different in children under 5 years of age (normal values > 120 microgram/L) and patients over 5 years of age (normal values > 15 microgram/L). However, it is to be noted that patients with infectious or inflammatory reaction have an increased ferritin rate, which can lead to a misdiagnosis of iron deficiency.

Children suffering from malnutrition frequently harbour infections. For this reason, it is important to dose CRP (C-reactive protein), which is a protein synthesized by the liver and found in the blood plasma. The level of CRP rises in response to an inflammatory reaction: it is an acute phase protein. Furthermore this analysis will inform us if the child presents an infection. This will help understand the immunostimulant, antiviral and antibacterial properties of SP.

Subsequent Visits

Information was recorded daily on standardized forms. Data sheets were monitored daily during the first two weeks by a survey assistant, then twice weekly.

In case of a health event (fever, pneumonia or the such) and in case of side effects, children were to be sent to the nursery and attended by the physician of PSE, who would provide a diagnosis and further treatment. In the event of intolerance to the product or side effects, the supplementation was to be either discontinued or given in smaller doses.

In the event a child would be absent from school, a home visit was to be conducted by PSE health staff and the child was to be followed up in accordance to the diagnosis made at the PSE health centre. Unfortunately, due to limited staff ability, this was not carried out according to plan.

An anthropometric and health survey was conducted at the end of supplementation, and 2 months after.

Description of the Supplements and Storage

As mentioned before, the SP was packaged in zipped plastic pouches of 100g each, each pouch carrying the total treatment for each child. A measuring spoon with a capacity of ~2 grams of SP was placed with each pouch. The pouches were kept in a black pail and covered in order to protect the SP from light and humidity.

Adherence to supplementation was monitored by DOT and daily follow-up. Files were not available for second treatment.

Definitions

Anaemia was defined as mild if haemoglobin was below 11 g/l and/or haematocrit below 33%, in children aged 6 months to 5 years; 11.5 g/l and 33.5% for children aged 5–11 years, as per WHO/UNICEF/UNU, 1997.

Anaemia was defined as moderate if haemoglobin was between 70–99, or 80–109 for children aged 5–11 years.

CRP was defined as normal if < 6 mg/l.

Eosinophil levels were defined as high if above 500 per mm^3

Data Analysis

Data was entered using either Epidata or Excel (for the second part of survey and for biological analysis). WHO antro software was used to determine anthropometric z-scores (either weight/height (WHZ) ≤2). The index using age was removed when we found out that nearly all birthdays were mistakenly set on January 1st. Analyses were conducted using Stata8. Children were their own control (before and after treatment) and Student test for matched analyses was used. For comparison between groups (Control and SP1) and Student test for unmatched variable was used to compare means. Fischer and Chi scores were used to compare frequency. Results are presented with means and 95% confidence interval or frequency and percentage. P<0.05 was deemed as statically significant.

Results

All parents and guardians approached accepted the enrolment of the preselected children in the study. A total of 194 children from three PSE centres were included in the survey–65 starting with the Control supplement and the remainder using the SP supplement (129). To avoid misunderstandings, the groups were defined as Group 1 being the group receiving spirulina during the first treatment and then switched to placebo and Group 0 as being the group receiving placebo first then SP during the second treatment. Of 194, 36 children (18.5%) left PSE or did not

complete the cure, leaving 158 children for analysis. Only 1 child had a precise birth date in file, and 71 no date at all, so only weight for height index and BMI were used to measure growth. 129 children were sampled for initial blood analysis, 120 for second blood analysis and 127 for third analysis.

At time of final report, only the set of health events for the first supplementation was fully available (main objective). The second set of health event was retrieved only for OBK center but did not show noticeable events. Acceptability was good and no refusals were reported by the teachers (Point 1 of secondary objective). Points 2, 6 and 7 were not supported by hard data and were not analysed. During informal interviews, teachers did not report any special effort to convince the children to take their supplements. The acceptability and satisfaction forms were not given back to the investigation team. It was not possible to conduct a quantitative satisfaction assessment on the children. Children lost to follow-up were also not visited (in some cases due to distance) and some left PSE with nospecific or known reason, so these lost to follow-up are apparently unrelated to the supplementation.

Health Events During Treatment

Overall the number of health events reported was very low and no comparison between groups could be made. Health events included 3 reports of vomiting, 3 of fever, 1 of headache and intestinal pain, and 19 children complained of stomach ache without differences between the two supplementation groups: 6 (11.7%) in Group 0 and 13 (13.5%) in Group 1, p=0.7.

The initial characteristics of children are shown in the following table. It does not show any difference of lost to follow-up between groups. The reason for lost to follow-up seems to be unrelated to the study but to the children condition. Some of the children simply left school without giving further notice.

Table 1. Participants and Lost to Follow-Up During PSE Spirulina Supplementation

	Group 0 (n=65)	(%)	Group 1 (n=129)	(%)	p	Total	
Participants	52	80	106	82.1	0.7	158	81.4
Lost to follow-up	13	20	23	17.8		36	18.5
Initial PSE centres						194	
OBK	26	40	54	41.8		80	41.2
Prek Tiol	21	32.3	37	28.6		58	29.9
Phnom Russei	18	27.6	38	29.4		56	28.8
Final PSE centres						158	
OBK	23	44.23	49	46.23	0.1	72	45.57
Prek Tiol	17	32.69	32	30.19		49	31.01
Phnom Russei	12	23.08	25	23.58		37	23.42
Lost to follow-up*					0.02		
OBK	3	11.5	5	9.3		8	10.0
Prek Tiol	4	19.0	5	13.5		9	15.5
Phnom Russei	6	33.3	13	34.2		19	33.9

There was correlation between supplementation groups and lost to follow-up. However, there was significantly more lost to follow-up in Phnom Russei classes (33.9%) compared to others (10 % and 15%, p=0.02).

The initial characteristics of 158 children are shown on table 2 and 3. Age, gender, and anthropometric measurements did not statistically differ at enrolment in both groups (p was over 0.05).

Table2. Initial Characteristics of the 158 Children not lost to follow

	Group 0 (n=70)	(%)	Group 1 (n=86)	(%)	p	Total	
Female	24	34.29	28	32.56	0.82	52	33.33
Male	46	65.71	58	67.44		104	66.67
Age (n=153)	5	(4.8–5.2)	5.2	(4.7–5.6)	0.6	5.1	(4.8–5.4)
PSE centre							
OBK	23	44.2	49	46	n/a	71	
Phrom Russey	17	32.6	32	30.1	n/a	49	31
Prek tiol	12	23	25	23.5	n/a	37	23.4

Anthropometry

Anthropometric measurements were taken on 15 October 2014, March 2015 and June 2015.

Table 3 Initial Measures of Included Children Before Supplementation (Full Sample)

	Group 0			Group 1			
	N=65	95%CI		N=129	95%CI		P
Weight (kg)	14.4	13.8	15.0	14.7	14.3	15.1	0.3
Height (cm)	100.7	98.8	102.6	101.3	100.0	102.5	0.6
WHZ	-1.1	-1.3	-0.8	-0.9	-1.1	-0.8	0.3
BMI1	14.1	13.8	14.4	14.3	14.1	14.5	0.3
Severe malnutrition *	11	16.9		12	9.3		0.1

* Weight for height Z score: WHZ<= -2; BMI Body mass index

Measures at the End of First Supplementation

A total of 148 children were present at the end of first supplementation for anthropometric measurements (Weight2 and Height2, BMI2). Mean weight2 was: 15.0 (95%CI: 14.7–15.4);

Mean height2 was: 102.3 (95%CI: 101.2–103.5); mean BMI2 was: 14.3 (95%CI: 4.1–14.5). There was no difference between the two groups (Table 4).

Table 4. Anthropometric Measures after the First Treatment

	Group 0			Group 1			
	N=51	95%CI		N=97	95%CI		P
Weight2 (kg)	14.8	14.1	15.5	15.1	14.7	15.6	0.3
Height2 (cm)	101.7	99.6	103.9	102.7	101.3	104.0	0.4
BMI2	14.2	13.9	14.6	14.3	14.1	14.6	0.6

Increase of height and weight were highly significant for both groups taking each child as his own control during the first treatment (p<0.001). Difference for BMI was not significant (Table 5).

Table 5. Changes in Anthropometric Measures Before and After the First Treatment

	Obs	Mean	[95% Conf.	Interval]	p
Group 0					
weight1	51	14.2	13.5	14.8	<0.001
Weight2	51	14.9	14.2	15.5	
Difference in weight	51	-0.7	-0.9	-0.5	
height1*	51	99.8	97.8	101.8	<0.001
Height2**	51	101.8	99.6	103.9	
Difference in height	51	-2.0	-2.4	-1.6	
BMI1*	51	14.2	13.8	14.5	0.08
BMI2 **	51	14.3	13.9	14.6	
Difference in BMI	51	-0.1	-0.3	0.0	
Group 1					
weight1*	98	14.4	14.0	14.8	<0.001
Weight2**	98	15.2	14.8	15.6	
Difference in weight	98	-0.8	-0.9	-0.6	
height1*	96	100.4	99.0	101.9	<0.001
Height2**	96	102.7	101.3	104.0	
Difference in weight	96	-2.2	-2.5	-2.0	
BMI1*	96	14.3	14.0	14.5	0.1
BMI2**	96	14.4	14.1	14.6	
Difference in BMI	96	-0.1	-0.3	0.0	

*First measurement October 2014; **Second and intermediary measurement March 2015

Measures Before and at the End of the Second Treatment

Before and after the second supplementation, a total of 142 and 144 children were present for anthropometric measurements. For children taken as their own control, there was a significant increase in weight and height and a significant decrease in BMI ($p<0.001$) (Table 6). Mean weight2 was: 15.0 (95% CI: 14.7–15.4); Mean height2 was: 102.3 (95% CI: 101.2–103.5); mean BMI2 was: 14.3 (95% CI: 4.1–14.5). These differences were also observed within each groups (data not shown).

Table 6. Changes in Anthropometric Measures Before and After the Second Treatment

	Obs	Mean	[95% Conf.	Interval]	p
Weight2*	144	15,0	14,7	15,4	
WeighT3**	144	15,3	15,0	15,7	<0,001
Difference in weight	144	0,3	0,2	0,5	
Height2*	142	102,1	101,0	103,3	<0,001
HeighT3**	142	104,3	103,1	105,4	p
Difference in height	142	2,1	1,9	2,4	
BMI2 *	142	14,3	14,1	14,5	<0,001
BMI3**	142	14,0	13,9	14,2	p
Difference in BMI	142	-0,3	-0,4	-0,2	

*Second and intermediary measurement March 2015; **Third and final measurement June 2015;

The following figures show the changes in anthropometric measures before, during and after the two treatments. It shows a continuous increase of weight and height (Figures 1 and 2). Changes of BMI first plateau in group 1 and increased in group 0, then decreases were observed in both groups.

Figure 1. Changes in Weight During Crossover Supplementation of Spirulina

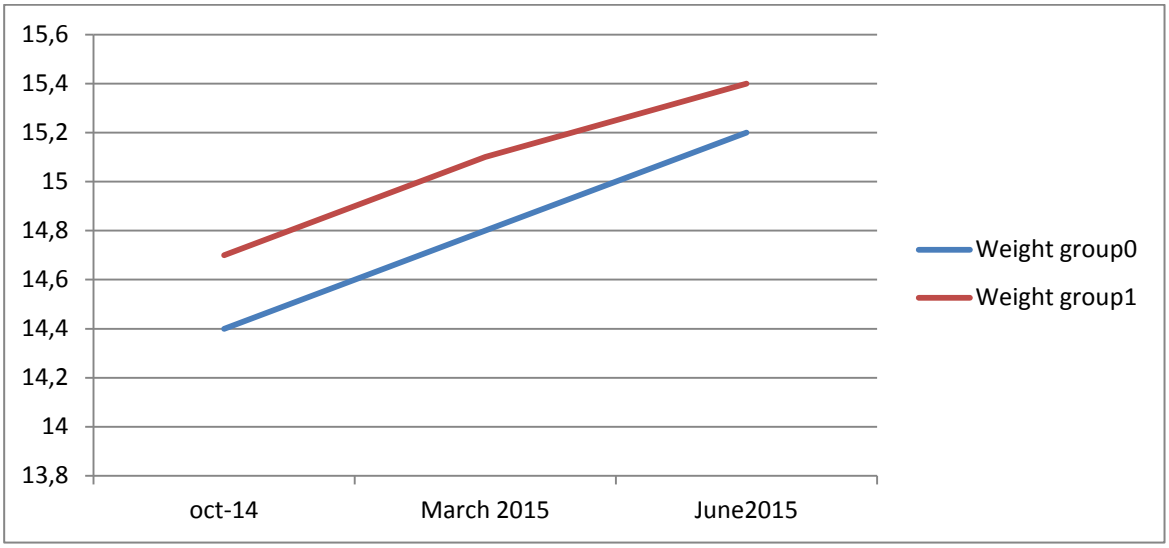


Figure 2 Changes in Height During Crossover Supplementation of Spirulina

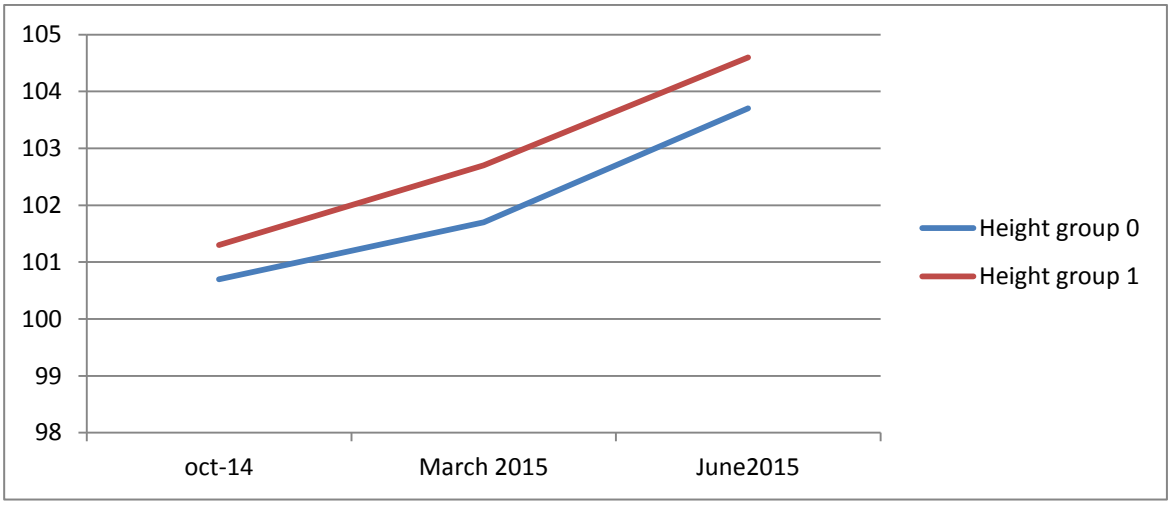
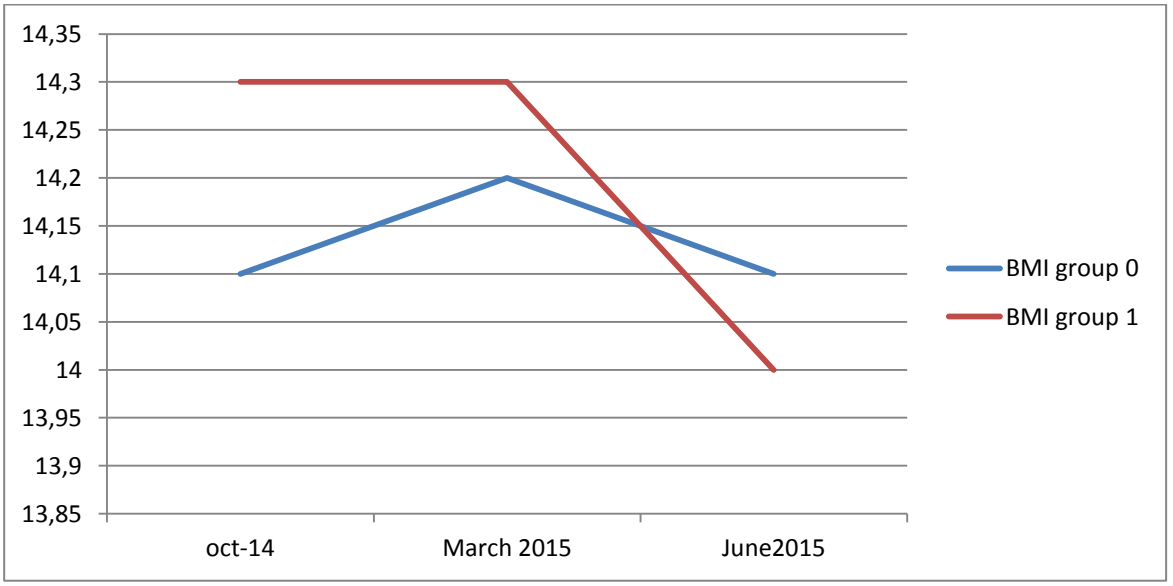


Figure 3 Changes in BMI During Crossover Supplementation of Spirulina



Before and after the survey (treament 1 and treatment2) children increased their height and weight (1.0kg and 4.3 cm, respectively) in both groups. As a result it was not possible to relate anthropometic improvements with the type of supplementation nor the stage of supplementation.

Table 7 Changes Before and After Supplementation for Children who Participated in the Full Treatment (n=158)

	Group 0			Group 1			
	N =52	95%CI		N=106	95%CI		P
Sex (n=158)							
Female (71)	24	46.1		47	44.3		0.4
Male (87)	28	58.8		59	55.6		
Initial measures							
Initial height (n=156)	101.4	98.2	102.6	101.3	99.9	102.8	0.4
Initial Weight (n=156)	14.3	13.7	15	14.7	14.2	15.1	0.4
Initial BMI	14.2	13.8	14.5	14.2	14	14.5	0.4
Final measures							
FWeight (n=144)	15.2	14.5	15.8	15.4	15.0	16.1	0.5
FHeight (n=142)	103.7	101.6	105.8	104.6	103.2	106.0	0.4
FBMI	14.1	13.7	14.4	14.0	13.8	14.3	0.9
Weight gain	1.1	0.9	1.3	1.0	0.9	1.2	0.4
Height gain	4.2	4.0	4.5	4.3	4.1	4.6	0.6
BMI gain	-0.1	-0.3	0.0	-0.2	-0.4	0.0	0.7

Biological Characteristics

Table 8 shows the baseline situation.

Table 8. Baseline Biological Characteristics

	Group 0 n=41			Group 1 n=88			
Variable	**Mean**	**95% CI**		**Mean**	**95% CI**		**P**
Haemoglobin (g/dL)	12.18	11.95	12.41	11.87	11.68	12.06	0.05
Haematocrit (%)	36.66	36.08	37.23	35.81	35.34	36.28	0.03
Anaemia Hb <11 /dL	2	4.8		13	14.7		0.1
Leukocytes	10729	9855	11602	10481	9931	11032	0.6
Red Cells	4.95	4.84	5.05	4.87	4.79	4.96	0,3
Lymphocytes/dL	4367	3945	4789	4277	4083	4470	0.6
Lymphocytes %	41.2	38.1	44.3	42	40.2	43.7	0.6
Neutrophil %	44.7	41.1	48.3	43.0	41.0	45.17	0.4
Eosinophil %	12.7	10.6	14.8	12.2	10.5	14.0	0.7
Mean cell volume (MCV) (fL)	74.41	72.57	76.26	73.77	72.36	75.19	0.5
MCV ≤ 70*	9	(21.95%)		31	(35.23%)		0.4
Platelets × 10^9 /L	431	386	476	415	394	437	0.4
Mean corpuscular hemoglobin (MCH)	24.72	24.02	25.43	24.49	23.94	25.05	0.6
Mean cell haemoglobin	33.2	33.0	33.4	33.1	32.9	33.2	0,4
Ferritin µg/L**	70.22	57.47	82.97	66.52	59.62	73.41	0.5
Ferritin <10 µg/L	0			0			
CRP***(mg/L)	1.77	1.09	2.45	1.62	1.18	2.06	0.7
CRP above 6	1	2.4		6	6.7		NS

Mean and 95% CI interval, Frequency and %; Leukocytes = white blood cell count

*defining a microcytic, ** Ferritin normal range: H=30-280µg/L; F=20-120µg/L

*** Acute Phase Reactants protein of the inflammatory phase (CRP)

A total of 40 children (31%) had microcytic anaemia, which, associated with low haemoglobin, infers an iron deficiency anaemia (or a chronic infection if associated with elevated levels of Acute Phase Reactants protein). No children had low ferritin (< 10 µg/L) and 9 had ferritin levels exceeding 120 µg/L**.** Levels of acute Phase Reactants protein reactive was low (<2) and did not show any change all along the survey (dat not shown).

Very few children (7) had elevated levels of Acute Phase Reactants protein above 5 mg/L, which indicates the presence of iron deficiency anaemia rather than inflammation.

The level of eosinophilia was high in both groups at the start of treatment. Only one child had a low number of platelets (78,000). His condition improved during first treatment and normalized at the end of study (225,000).

Overall Results between Groups; Anaemia

Compared to Group 0, Group 1 had significantly lower haemoglobin and haematocrit at the start of the supplementation but no difference between groups were observed at the second and third measurement.

Table 9 Comparison of Mean Haemoglobin Between Groups During the SP Treatment (unmatched)(measure at time1, 2, 3)

	Group 0			Group 1			
	n=41			n=88			
	Mean	95% CI		Mean	95% CI		P
Haemoglobin1	**12.10**	**11.95**	**12.41**	**11.80**	**11.68**	**12.06**	**0.05**
Haematocrit1	**36.6**	**36.08**	**37.23**		**35.34**	**36.28**	**0.03**
Anaemia: Hb <11 /dL	2	4.8		13	14.7		0.1
Ferritin	70.20	57.47	82.97	66.50	59.62	73.41	0.5
Eosinophil 1 %	12.73	10.63	14.83	12.30	10.56	14.03	0.7
Eosinophile /mm^3	1414	1099	1730	1382	1120	1644	0.8
	n=38			n=82			
Haemoglobin2	11.8	11.54	12.09	11.7	11.57	11.98	0.80
Haematocrit 2	35.3	34.65	36.07	35.2	34.75	35.83	0.8
Anaemia 2	5	13.1		15	18.2		0.4
Ferritin 2	60.84	51.10	70.59	57.43	50.24	64.61	0.5
Eosinophil 2	9.13	7.17	11.10	9.13	7.60	10.67	0.7
	n=40			n=87			
Haemoglobin3	12.1	11.94	12.39	12.0	11.85	12.24	0.40
Haematocrit 3	36.4	35.86	37.01	36.0	35.53	36.56	0.3
Anaemia 3	1	2.5		10	11.4		0.09
Ferritin 3	72.03	56.74	87.31	69.45	55.42	83.48	0.8
Eosinophil 3	8.38	6.65	10.10	8.10	6.99	9.22	0.9

The number of children with anaemia did not differ before and after the first treatment. It differed during the second treatment with children under SP being less frequently anemic.

Ferritin decreased in both groups after the first treatment and increased after the third treatment in both groups.

Figure 4 Changes in Haemoglobin During Treatment

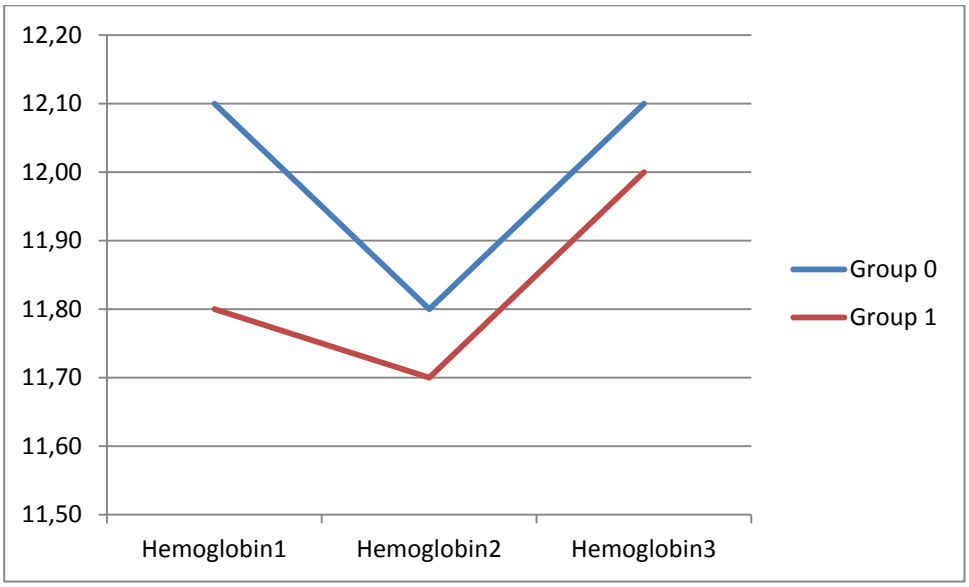


Figure 5Changes in the Level of Eosinophils During Treatment

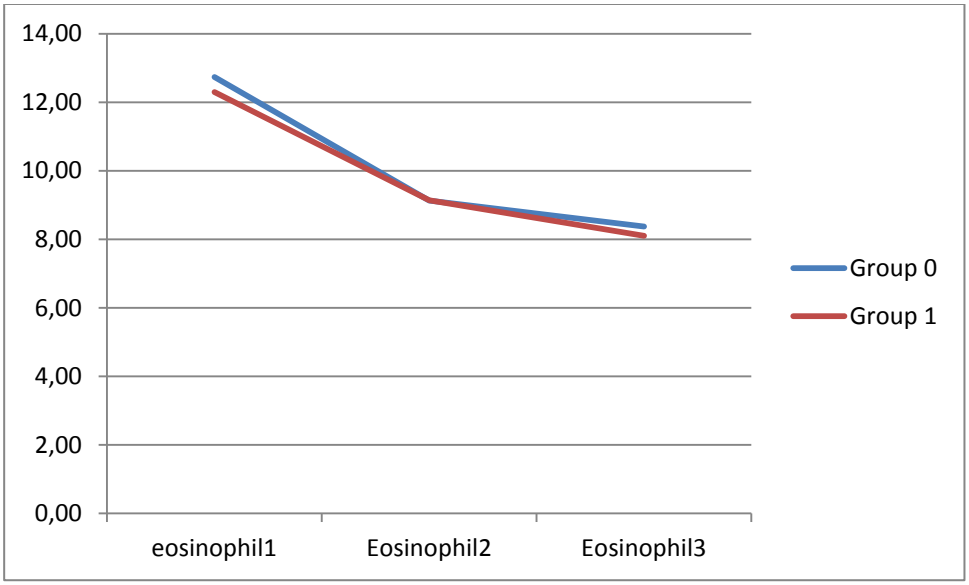


Figure 6 Changes in Ferritin During Treatment

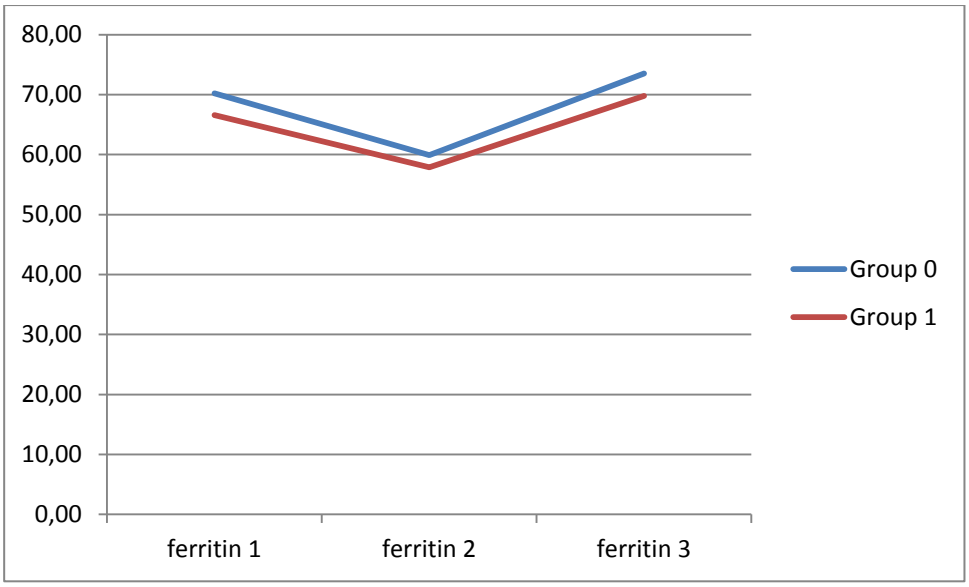


Table 10. Changes in Mean Haemoglobin During the Spirulina Treatment in Both Supplementation Groups (Treament 1 and Treatment 2 – Each Individual Being its own Control)

		Group 0						Group 1			
Treatment 1	**Obs**	**Mean**	**95% Conf.**	**Interval**	**p**		**Obs**	**Mean**	**95% Conf.**	**Interval**	**p**
Before and after first treatment											
hg1	**36**	**12.18**	**11.93**	**12.42**	**p=0.006**		79	11.87	11.68	12.07	p=0.09
hb2	**36**	**11.85**	**11.59**	**12.12**			79	11.76	11.54	11.97	
Before and after second treatment											
hg2	**31**	**11.75**	**11.43**	**12.06**	**p<0.001**		**72**	**11.78**	**11.55**	**12.00**	**p<0.001**
hb3	31	12.12	11.87	12.36			72	12.05	11.83	12.26	
Before first and after third treatment											
hg1	32	12.15	11.88	12.42	0.80		74	11.93	11.73	12.13	0.1
hg3	32	12.13	11.87	12.38			74	12.03	11.82	12.25	

Hg: haemoglobin

Before and after the first supplementation there was a significant decrease in haemoglobin in Group 1 and a non-significant decrease in Group 0. There was then a significant increase before and after second supplementation in both groups. Overall, there was no change of haemoglobin before and after the overall treatment.

Similar observations were made for haematocrit (data not shown).

Table 11. Changes in Ferritin During the SP Treatment in the Two Supplemented Groups (Treatment 1 and Treatment 2)

		Group 0						Group 1			
First treatment	Obs	Mean	95% Conf.Interval		p		Obs	Mean	95% Conf.Interval		p
Before and after first treatment											
Ferritin1	**36**	72.61	58.56	86.66	0.03		79	66.60	59.24	73.96	0.002
Ferritin2	**36**	61.25	51.12	71.38			79	57.90	50.57	65.23	
Before and after second treatment											
Ferritin2	**31**	59.90	48.18	71.63	0.09		**72**	57.90	50.18	65.62	0.2
Ferritin3	31	71.48	52.50	90.46			72	67.85	51.47	84.22	
Before first and after third treatment											
Ferritin 1	32	70.22	54.31	86.13	0.4		74	67.57	59.82	75.32	0.4
Ferritin3	32	73.53	55.38	91.68			74	69.78	53.45	86.12	

After the first treatment, ferritin decreased markedly in both groups.

After the second treatment, ferritin increased in both groups.

Overall, there was no difference between ferritin status before and after the second treatment.

Table 12. Changes in the Prevalence of Microcytic Anaemia Over the Course of the Study for Children who Participated to All Three Measurements

	Group 0		Group 1		p
Before the study (n=129)	N=41	%	N=88	%	
Microcytic anaemia	9	22.50	30	35.71	0.1
After first treatment (n=120)					
	N=38		N=82		
	8	22.86	25	32.05	0.3
After second treatment (n=129)					
	N=40		N=87		
	7	18.92	22	27.16	0.3

Before the start of the study, children in Group 1 had a higher prevalence of microcytic anaemia than children in Group 0, though this difference was not statiscally robust. The rate of microcytic anaemia in Group 1 decreased over the course of the two treatments. There was no difference in changes within groups or between groups. Also, no differences in variations were found between the two groups and within the groups when considering only children who underwent the three measurements.

Table 13. Changes in Microcytic Anemia

	Group 0	Group 1	Total	P
Total	29	70	99	
Microcyte1				
No	23	44	67	0.1
%	79.31	62.86	67.68	
YES	6	26	32	
%	20.69	37.14	32.32	
Microcyte 2				
No	23	47	70	0.5
%	79.31	67.14	70.71	
YES	6	23	29	
%	20.69	32.86	29.29	
Microcyte 3				
No	24	48	72	0.1
%	82.76	68.57	72.73	
YES	5	22	27	
%	17.24	31.43	27.27	

Changes in the Level of Eosinophils

The percentage of oesinophils was high and similar in both groups before the start of the study. This is usually correlated with parasitic infections. So, at the end of first treatment, and in agreement with the medical team of PSE, all children received a deworming treatment during three days.

The rate of eosinophils then constantly decreased during the two treatments (from 12.4% to 8.2%; $p<0.001$). In fact, it started to decrease after the first treatment in both groups and this decrease continued after deworming and the second supplementation.

Table 14 . Changes in the Prevalence of Eosinophilia During the Treatment

Initial rate of eosinophilia					
Group	Obs	Mean	95% CI		p
0	41	12.73	10.63	14.83	0.70
1	88	12.30	10.56	14.03	
Overall	129	12.43	11.09	13.78	
After first supplementation					
0	38	9.13	7.17	11.10	0.70
1	82	9.13	7.60	10.67	
Overall	120	9.13	7.93	10.34	
After second supplementation					
0	40	8.38	6.65	10.10	0.90
1	87	8.10	6.99	9.22	
Overall	127	8.19	7.27	9.11	

Since the decrease was observed continuously and in both groups, this variation could not attributed to SP supplementation and is probably due to an improvement of global hygiene and the anti parasitic treatment

Discussion

This survey provides some of the results of an eight week supplementation trial of 2 grams of daily spirulina in children attending PSE schools in the Phnom Penh area. The study did not escape the difficulties of field studies lacking resources despite a motivated team and an interesting design.

Children were matched with a control group and were randomly allocated supplements. After a cooling off period of two months we proceeded to crossover matching: children who had received SP then received a placebo, while the former control group then received a 2 g daily dose of SP. This design was chosen to allow each child to be his/her own control. It also limited requirements in terms of sample size.

Tolerance to SP and potential side effects of the supplement could only be evaluated during the first treatment. Only minor and temporary discomforts were observed, without any difference between groups. It can therefore be concluded that overall tolerance to SP supplementation was good.

Due to a lack of financial means, we had to choose basic proxy to evaluate the supplementation: three set of anthropometric measures (height and weight) and only three biological criteria could be assayed.

It was not possible to dedicate a health professional to monitoring the study and the children on a full-time basis. As a result an important number of difficulties emerged and the drawbacks seriously incapacitated the study. Despite a tiresome and lengthy analysis, results are therefore weak and their scope is much more limited that what was initially expected.

The level of participation to measurements was a crucial issue for both anthropometric and biological measures. In the context of underprivileged children, it was expected a high rate of lost to follow up. Lost to follow-up was below 20% (on average 18.5% for the whole study) and did not differ between the groups. When considering the centres participating to the treatment, the level of lost to follow-up was much higher in Phnom Russei (reaching 34%) than in other groups. This suggests some problems in this centre. Lost to follow up was similar in groups which limited the impact on the quality of the study though it led to a loss of detail and complicated our analysis. We have no explanation on why there was so much lost to follow-up in children from Phnom Russei but this centre compromised the whole study.

Height and weight were carefully measured three times: before the survey, 1 week after the second treatment and 2 weeks after the third treatment. A high level of professionalism was expected from the staff in charge of these measurements. The same person took all measurements (Mr. Thea from Antenna), and he was well trained and carefully monitored to ensure the provision of reliable data. Overall the level of wrong measures was most probably very low, contrary to what happened the year before in a previous study with Aspeca.

Finally, height increased by an average of 4 cm and weight by an average of 1 kg over the course of the study, which is a very good result. It was not possible to link this a possible effect of SP supplementation since effect was observed in both arms. Similarly, we could not show if children under the first SP supplementation were starting earlier weight and height growth. The final result suggests a global efficacy of the overall care of children at PSE, which probably goes far beyond SP supplementation.

A similar hypothesis tends to be supported by the changes in biological data, which also did not carry a difference between the two supplementation groups after the first and second supplementation. However, interestingly children in Group 1 who initially had lower haemoglobin/haematocrit than Group 0, recovered to a similar level by the end of first supplementation before deworming treatment was started and kept this similarity even after the end of the supplementation. This could be related with the content of SP supplement, which corrects iron deficiency anaemia when caused by a lack of iron intake.

We could form the hypothesis that SP gave a boost to children of Group 1 and that boost was maintained well over the first treatment. A limitation of this assertion is the fact that children were probably all suffering from parasitic infestation, as suggested by high and similar levels of oesinophilia before the treatment. Both groups started to have a regular decrease of their oesinophilia and no specific difference was observed when on SP supplementation compared to when on placebo supplementation. This infers a general (but not specific) efficacy of PSE care including the deworming treatment that was given after the first supplementation to all children.

What can Explain the Results? And perspectives

Better results of SP supplementation have been observed in severely malnourished children. Here children were moderately malnourished, only a few had moderate anaemia, no children

had severe anaemia nor were severely malnourished, and all were fed by PSE schools. We have no information on the daily nutritional intake of the children. SP was given at a dose of 2 g per day, 5 days a week, as per Antenn's standard recommendations for school children. This daily dose is inferior to the 5 g used by various studies in children [35,36] and Picard in Hug et al[1]. Better results have also been observed with doses of up to 10 g per day, but in different contexts (severe malnutrition, HIV-infected children). [33]

A good point is that in the context of limited means a crossover with quasi double blinding could be performed. SP Doses, parameters to be evaluated, interval between supplementation and tests, long term benefits, different children conditions and improved study condition including people specifically dedicated to the study and sufficient funds should be considered in further researches.

Limitations of the Study

In addition to the previous comment, another limitation was the absence of knowledge on the daily nutritional inputs of children. We did not have the composition in micronutrients of the Cambodian SP used in the survey. The nutritional profile of SP can vary widely for a variety of reasons.

The iron content of SP was analysed before the survey in July 2014 however and the iron content was found to be satisfactory, at 666 ppm (See Annex, personal communication from Vincent Guigon).

Conclusion

The study was conducted with limited means in the context of underprivileged children cared by PSE. The limited means and context was partially compensated by an ambitious design (randomisation, crossover, double-blinding). This explains some of the difficulties to conduct and analyse the results of the study (moderate rate of lost to follow up mostly in one centre, reporting of events, delays for obtaining data, absence of precise age, paucity of biological parameters used). The crossover and attempt of double blinding were well respected which allows drawing some conclusions.

Overall, tolerance and acceptability of SP supplementation was good in children of about five years of age attending PSE schools. The drop-out rate which was to be expected in the context was below 20% and does not seem related to the children' supplementation. Very few health events were recorded during the first and second supplementation which was an obvious benefit for all PSE children. Hence no impact on the prevention of sickness could be attributed to SP supplementation. This point suggests conduct studies on larger sample if only health events are to be the only parameters for evaluation of SP supplementation. It also suggests using less basic parameters or increase the number of clinical and specific biological parameters, other parameters such as improvement of attention at school, learning capacity, resistance to sickness during seasonal disease or disease outbreaks etc.., and explore the sustainability of long term health benefits. In addition the doses of SP, interval of observation and probably the initial condition and heath status of children can be discussed.

Over the course of the supplementation periods at PSE schools, general benefits were observed. Children significantly increased their weight and height and reduced their level of eosinophils. They improved their anaemia after SP supplementation during the first supplementation but no difference was observed between groups when the control group received SP or before and after the start of the study. Overall, in this analysis, no difference between groups and between the types of supplementation could be related to supplementation. In the view of limited parameters that could be tested, of the doses of supplementation used (~100 gr Spiruline), and of the target group (underprivileged children over 5 years daily cared by an NGO), the study cannot conclude to a specific short term benefit of the dosage used of SP supplementation though it should be noted that overall all children experienced improved physiological status during the survey, acceptability seemed good and no harm was reported.

Conflicts of Interest

Investigators declare no potential conflicts of interest. More information are provided in foreword.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, the host organisation, and members of the Research Ethics Committee, unless authorised to do so.

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APPENDIX A: Consent Form – English Version

Study on dietary supplements in children below six years of age attending school in Cambodia

Study Promoter: Antenna Technologies France

Principal investigator: Hubert Barennes

Information form for the parent(s) or guardian(s) of children from the patients group.

Version 1.0

Dear parent(s) or guardian(s),

The child under your care, whom we will refer as "your child", is been invited to participate to the above-mentioned research study.

WHAT IS A NUTRITIONAL STUDY?

A nutritional study is a way to find out new information about the effect of a food supplement on health. Children do not participate in a research study if you, parents or guardian do not want him or her to be included.

WHAT IS PSE'S MISSION IN TERMS OF NUTRITION?

The NGO PSE sends to school more than 6,500 children every year. On top of this education mission, PSE also emphasizes health and nutrition. So its mission goes further than simply education and PSE strives to take care of the well-being of the children that attend its schools by providing access to good and balanced food and to proper health care.

The quality of the food intake between age 0 and age 6 is key to ensure proper mental and physical development of the children.

For this reason, PSE decided to launch this research program (along with Antenna) and to provide extra nutrients to the children in the PSE centers.

WHAT IS A NATURAL DIETARY SUPPLEMENT?

A dietary supplement is a concentrated source of nutrients with nutritional and physiological effect whose purpose is to supplement the normal diet.

PSE has decided to provide a fully natural dietary supplement to the children of its centres in order to improve their nutrient intake and to enable their bodies to grow properly and maintain their vital functions.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to observe if the regular consumption of a natural food supplement during 2 months will have a significant impact on their height and weight. This food supplement is produced in Cambodia under strict quality control. To be able to find out the effect of this food supplement we will compare a supplemented regular diet and the unsupplemented regular diet in children. The children will receive the supplements during 2 months alternatively after an interval of 3 months between the two stages.

The supplement is a natural product made in Cambodia and that can be sprinkled on food. It has been consumed for centuries in many countries and is generally very appreciated for its nutrional value. It is recognized by the Food and Drug Administration in the US (FDA) as safe to eat with beneficial effects on health.

Considering the importance of nutrition for the children below 6 years of age, as previously mentioned, the study will be conducted in Cambodia and will include 215 children from PSE of less than 6 years of age.

WHY DO WE CONDUCT THIS STUDY?

Though the supplements have been used for many centuries, there are not much scientific data available in the region.

WHY HAS MY CHILD BEEN OFFERED TO PARTICIPATE TO THE STUDY?

The doctor in charge of your child at PSE suggested that he/she participates to the study because he/she has 0 to 6 years of age and has no medical contraindication for the study. Consuming a dietary supplement could improve his/her health.

DOES YOUR CHILD HAVE TO PARTICIPATE?

Your child will participate only if you agree with it. The team in charge of the study will explain the study procedures. You may ask all the questions you have. You may refuse that your child participates to the study without any consequence on his/her follow-up in PSE centre.

If you, parent or guardian, accept that he/she participates to the study, you will first sign a consent form before any procedure. You may withdraw consent or discontinue his/her participation at any time without penalty, loss of benefits, or prejudice to the quality of care and the treatment which your child will receive.

If you wish to participate but do not want to sign and just want to give oral agreement, you may accept the participation of your child if two people bear witness to your oral agreement and if they are ready to sign the form.

WHAT WILL BE DONE TO MY CHILD IF I ACCEPT HIS/HER PARTICIPATION TO THE STUDY?

If your child participates to the study, once you have signed the informed consent form, He will received either food supplement A or B daily during 2 months, then after 3 months he/she will receive the other food supplement. There will be two parts in the study: part 1 and part 2, both following the exact same stages.

The study will follow the following stages:

Part 1.

⟐ First stage: Before the study MEASUREMENTS

There will be a physical examination (weight, height) at the PSE centre by PSE and Antenna employees. A health interview will be conducted to check that your child has no recent or chronic disease or allergy that would prevent him/her to participate. During all the study, the children will be monitored and in case there is any health event, they will be cared for by the medical staff of PSE at the health centre.

In addition to the weight and height measurements, blood samples will be collected from your child in order to measure the quantity of iron present in his/her blood cells as well as to find any trace of inflammation.

⟐ Second stage: During 2 months. GROUP A OR B

In order to compare the effect of the food supplement on the children, two groups of children will be formed, following a a randomized list that was prepared before the study. This list will randomly allocate children to group A or group B with no input from the team.

One group of 161 randomly-selected children, called “Test Group”, will receive a daily spoon of food supplement under the control of their teacher and the team staff during a period of two months (during week days).

A second group of 54 children, called “Group 0”, will receive their regular diet but no genuine food supplement. A similar product having no effect with be given daily under the control of the teacher and the team staff.

Children will be monitored daily. Data regarding the ingestion of the supplement and health events will be collected daily by the team, following a suggestion by Antenna Technologies. This will be done under the close supervision of epidemiologist-pediatrician Dr. Barennes, who accepted to help Antenna in this study and will act as scientific coordinator of the study.

⟐ Third stage: At the end of 2 months: MEASUREMENTS

Physical examination (including weight, height measurements) will be done at the PSE centre by PSE and Antenna employees after these three months of observation.

Another blood test will be done.

⟐ Fourth stage: 3 months: OBSERVATION OF THE EFFECTS

After the first treatment, children will be followed daily at school during a period of three months. The aim of this is to see if there is any medically-significant event happening.

⟐ Fifth stage: Second part MEASUREMENTS

Physical examination (including weight, height measurements) will be done at the PSE centre by PSE and Antenna employees after these three months of observation.

Another blood test will be done.

Part 2.

Children will go through the same stages as in Part 1 but this time they will receive the supplement they did not receive previously.

⟐ Sixth stage: GROUP B OR A

In order to see the effect of food supplement on the children, a second treatment will be given. This time the groupes will be switched in order to make sure that all the children have received food supplement.

Datas will be collected daily following the same protocol as Phase Two.

⟐ Seventh stage: OBSERVATION OF THE EFFECTS

After the second treatment, children will be followed daily at school during a period of three months. The aim of this is to see if there is any medically-significant event happening.

⟐ Eight phase: MEASUREMENTS

Physical examination (including weight, height measurements) will be done at the PSE centre by PSE and Antenna employees after these three months of observation.

Another blood test will be done.

The total length of the research study will be ten months and there will be five blood tests done on your child.

WHAT DO I HAVE TO DO DURING THE STUDY?

Food supplements will be provided to the children at PSE center. Therefore, you do not need to take care of anything at that level.

However, you must make sure that your child goes to the PSE center every day of the week (excluding weekends and on government holidays). If your child gets sick, you have to inform the doctor at the PSE center. If the child is sick and has to stay at home, someone from the team will come and visit you to see the child (if you approve of it).

You have to inform the team and/or the doctor if you want your child to stop participating to the study.

WHAT ARE THE POSSIBLE DISADVANTAGES FOR MY CHILD IF HE/SHE PARTICIPATES?

Food supplements are usually well tolerated but they may taste differently to what the child is use to eat. As a result the child may initially feel some nausea, distate, or even vomiting. To avoid this, very small quantities of the supplements will be given during the first week. After one week, the children will typically be habituated to the consumption of the supplements. Some adverse effects have been described such as red spots on the skin, diarrhea, stomach aches, headaches, constipation, vomiting…

If any of these problems arises, the trained nurses and doctors of PSE center will make sure that the child is fine. The distribution of the food supplement might stop if necessary, and could be resumed once the symptoms have disappeared. In this event, the administration of the supplement would resume gradually under the close supervision of the PSE doctors.

WHAT ARE THE POSSIBLE BENEFITS FOR MY CHILD IF HE/SHE PARTICIPATES?

The food supplement will provide essential nutrients that enable the body to grow properly, to maintain its vital functions, and also to provide energy to the body. Therefore, by taking the food supplement, your child may benefit from improved physical and mental development.

Children will have a daily check-up by a dedicated team and will benefit from this medical attention.

The check-up and follow-up will not induce extra-cost for the patient or his/her family.

WHAT HAPPENS WHEN THE RESEARCH STUDY STOPS?

After the study's end, the child will continue to be followed by PSE doctors and nurses.

WHAT IF THERE IS A PROBLEM?

In case of problem, you have to report it to PSE doctor or nurse.

The necessary measures will be taken, including, if necessary, a pause or complete stop of the supplementation, as well as treatment by PSE doctors in case of a disease occurring during this period, following national recommandations.

WILL MY CHILD'S PARTICIPATION TO THIS STUDY BE KEPT CONFIDENTIAL?

A code will be assigned to each participant and all the data and measures will be stored without any personal identification.

During the study, your child's research records may be reviewed by research study staff or other authorized persons only. These individuals will keep any personal information strictly confidential.

When the result of this study will be presented or published, the name of your child will never be mentioned.

EXPENSES AND COMPENSATION:

You will not support any cost related to this study: the costs of the measures and product provided (food supplements) will be covered by the study.

Your participation is entirely voluntary and you will not receive any financial compensation.

DATA COLLECTION AND STORAGE

All the data will be anonymously collected in a database located in Cambodia for storage and analysis. This database will be maintained in compliance with Cambodian regulations.

CONTACT FOR FURTHER INFORMATION

From now on and during the whole course of your child's participation to the study, if you have any question regarding the study, you have the possibility of contacting Dr Sokheang CHAN (Tel: 0 12-75-44-59) or Dr Sarath (012-736-630).

The intake of the food supplement

Food supplements A and B will be provided to the children with a spoon.

Each child will get his/her own spoon and PSE nurses will give them the food supplement every morning with water, in order to make it easier to swallow.

Research study on the impact of a food supplement on the health of children in Cambodia

Study Promoter: Antenna Technologies and ANRS

Principal investigators: Hubert Barennes

Informed Consent Form for the parent(s) or guardian(s)

for children of the patients group Version 1.0

I, ❑ parent)

❑ guardian*) of the child named …….....,

certify that I have read the Information sheet of the study ANTENNA 01 and that Dr Sarath has explained the study in detail. I have been free to ask additional questions and received answers.

I understand the benefits and risks of the participation to this study. I am aware that it is a research project and that a nutritional supplement will be provided to my child.

I understand that participation of my child to this study is voluntary and that I may refuse his/her participation or may discontinue his/her participation at any time without penalty, loss of benefits, or prejudice to the quality of care which he/she will receive.

I accept that anonymous data about my child will be collected, processed, and computerized.

❑ *Furthermore, as the guardian, I certify that I usually assume responsibility for the child's custody, care, and maintenance as

❑ a family member (specify: .....)

❑ other (specify: .....)

❑ the child's caregiver formally mandated by the Director of an institution/orphanage**

and that, to my knowledge, both parents of the child are dead.

Parents or Guardian(s)	Doctor
Name:	Name:
Date:	Date:
Signature	Signature

Witness (if parent or guardian cannot read)

Name:

Date:

Signature

Investigator's name: Date 2014 Place: PSE

Individual questionnaire

Questions on the recent and past medical story of the child, preliminary information for parents/guardians/relatives, and questions to be asked to the physician in charge of the child or his/her medical file.

Good morning. Can you tell me if the child:

1. Has any medical symptoms today? Is he/she not feeling well? Is he/she exhibiting any fever, diarrhea, itching, skin problem, sore throat, etc.?
    a. If yes, can you tell me for how long and if he/she is taking a treatment for this condition?
2. Has the child already had symptoms such as allergy, itching, allergic skin reaction or skin rash?
    a. If yes, can you tell me for how long and if he/she is taking a treatment for this condition?
3. Has the child exhibited these symptoms in the past week?
    a. If yes, can you tell me for how long and if he/she is taking a treatment for this condition?
4. Is the child take any continuous treatment? Is the child presently taking any pills for his/her health?
5. Did he/you receive the information on nutritional supplements?
6. Will the child stay in school in the next six months?
7. Did you receive the consent form? Did you read it and understand it? Do you have questions? Can we explain anything to you?

Questions to the physician in charge of the follow-up of the child or elements to be obtained through him in the child's medical file

1. Does the child have any chronic disease (such as a renal or hepatic impairment, or any other condition)? Y/N
2. Does the child take specific drugs? Y/N
3. Have you ever noticed any allergy in the child?
4. Was the child sick over the last week? the past month? can you give us the diagnosis?
5. Finally, do you think that there is any contraindication for the child to receive a nutritional supplement?

Global Data Set

Investigator's name: Date 2014 Place: PSE

School number	Class room	Given name	Family name	Chronic disease. 1.Interview of the parents 2. Medical file	Health events last week Interview	Known Allergia. To What	Presence of Symptoms*	Chronic disease? 0=no 1=yes Check med File With Physician	Take continuous Treatment? 0=no 1=yes	Informed **	Oral/written consent.

*Fever (F), Diarrhea (D), Nausea (N), Vomit (V), Stomach ache (SA), Tiredness (T), Skin disease (SD), Itching (I), Sore throat (ST) Others (detail)

**0=no, 1.Child Inf2.Parents informed3.Guardian informed 4. Relatives informed

***0=no, 1.Child 2.Parents 3.Guardians 4.People in charge

Antropometric Data set

Scale seca UNICEF Yes/NO Checked? Yes/NO Toise UNICEF: Yes/NO

School number	Class room	Given name	Family name	Birth date	Age (year/ months)	weight1 ex: 12.4 kg	weight 2	height1 Ex 125.2 cm	height2

Summary of the Biological Activity of Spirulina

Biological properties	Specific effects	Bioactive component	Reference
Anticancer	Repaired of damaged DNA	Polysaccharides	[13]
	Selective inhibition of Cyclo-oxygenase 2	C-phycocyanin	[14]
	Induction of G1 cell cycle arrest, mitochondria mediated apoptosis in MCF-7 human breast carcinoma	Selenium-enriched spirulina	[15]
Antiviral	Blocking virus adsborbtion and penetration into vero cells	Calcium spirulan (sulfated polysaccharide)	[16,17]
	Inhibition of the DNA polymerase activity	Phospholipids	[18]
	Inhibition of enterovirus 71-induced cytotoxic effect, viral plaque formation and viral induced apoptosis	Protein bound pigment allophycocyanin	[19]

Antibacterial		Fatty acids e.g. GLA	[20,21]
Metalloprotective	Inhibiting lipid peroxidation, scavenging free radicals, enhancement of the activity of GHS peroxidase and superoxyde dismutase	Antioxidant compenent	[22,23]
Antioxidant	Metal chelating activity, free radical scavenging activity	Carotenoids, vitamin E, phycocyanin and chlorophyll	[24,25]
Immunostimulant			[26,27]

RAPPORT D'ANALYSES

Numéro laboratoire : 20143516
Produit : spiruline du Cambodge

Fabricant : NC
Codage : 14972

AQMC
Ecoparc
135 rue de la Garriguette
34130 Saint-Aunès

TRACABILITE

Conditionnement :	sachet prélèvement	Nb unités :	1
Marque :	NC	Poids :	NC
N° lot :	NC	Date fab. :	NC
Code C.E.E. :	NC	Date emb. :	NC
N° emballage :	NC	D.L.C. :	NC
Prélevé par :	vos soins	Date prélèvement :	NC
Lieu prélèvement :	NC	Heure prélèvement :	NC
Date réception :	01/07/2014		

*NC non communiqué

ANALYSE	UNITE	METHODE	VALEUR	CONFORMITE
		issue de		
Fer	mg/100g	Arr. 08/09/1977	66,6	
Vitamine A	µg/100g	NF EN 12823-1	1,0	+/-30%

Alès le 21/07/14

T. de Laborde
Directeur du Laboratoire