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Chapter VI

THE USE OF MISOLA AND SPIRULINE IN THE NUTRITION REHABILITATION OF UNDERNOURISHED CHILDREN OF SUB-SAHARAN AREA

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ABSTRACT

Malnutrition constitutes a public health problem throughout the world and particularly in Subsaharan area. We refer about the use of an elementar integrator composed of Spiruline (Spirulina platensis) and Misola (millet, soy, peanut) produced at the Centre Medical St Camille (CMSC) of Ouagadougou, Burkina Faso, on the nutritional status of undernourished children. 550 undernourished children of less than 5 years old were enrolled in this study. We divided the children randomly into four groups: 170 were given Misola (731 +/- 7 kcal/day), 170 were given Spiruline plus traditional meals (748 +/- 6 kcal/day), 170 were given Spiruline plus Misola (767 +/- 5 kcal/day). Forty children received only traditional meals (722 +/- 8 kcal/day) and functioned as the control group. The duration of this study was eight weeks. Other two groups of 84 HIV-infected and 86 HIV-negative undernourished children were randomly assigned to Spiruline plus traditional meal or to traditional meal as a control group. The duration of the study was 8 weeks. At the end of study anthropometric and haematological parameters allowed us to appreciate both the nutritional and biological effect of Spiruline supplement to traditional meals. The rehabilitation with Spiruline plus Misola (this association gave an energy intake of 767 +/- 5 kcal/day with a protein assumption of 33.3 +/- 1.2 g/day), both greater than Misola or Spiruline alone, seems to correct weight loss more quickly. On the contrary the rehabilitation with Spiruline shows on average a weight gain of 15 and 25 g/day in HIV-infected and HIV-negative children, respectively. Our results indicate that Spiruline plus traditional meals or Spiruline plus Misola are all a good food supplement for undernourished children, but the rehabilitation by Spiruline plus Misola seems synergically favour the nutrition rehabilitation better than the simple addition of protein and energy intake. The production of Spiruline and/or Misola must be incremented in developing countries to fight the malnutrition, considering that the costs of production could be rapidly balanced.

INTRODUCTION

Malnutrition constitutes a public health problem in all the world particularly in the developing countries [1]. In Africa, more than 30% of the deaths in children of less than five years result directly or indirectly from malnutrition [2]. Since the beginning of 20° century, Burkina Faso is confronted by protein-energetic malnutrition with 13 % of infant population affected by emaciation, 29% by growth retardation and 30% by insufficient weight [3]. The consequences of the protein-energetic malnutrition in Burkina Faso are several and manifested severe forms of marasma, kwashiorkor and kwashiorkor plus marasma [3]. To day it is recognized that this form of malnutrition is coupled with deficiencies in vitamins and in minerals [4,5]. It creates relentlessly an uncorrectable spiral between malnutrition and infectious pathologies, which often is associated with chronic diarrhoea. and compound the prognosis of these children [6]. The more frequent infection found in severe undernourished children is the HIV infection, which occupies an important place in this country. In fact, HIV prevalence of 40.5 % was recognized in undernourished children [3] and compound the prognosis of these children. In the Centre for Education and for Nutritional Rehabilitation (CREN) of Ouagadougou, Burkina Faso, Misola or Spiruline or both in association were used since 1997 to improve the nutritional status of undernourished children. The choice of these two alimentary integrators was guided by the biochemical composition of both. The Misola, a local flour traditionally produced at the CREN of the Centre Medical St. Camille (CMSC) of Ouagadougou contains millet, soy, peanut according the original formula proposed by the Association Burkinabe des Unites Misola and the powder of Spiruline, a cyanobacterium which grows easily at the high temperature of Burkina Faso, also produced at the CMSC, were recently introduced in the treatment of undernourished children for its biological activities [7]. Spiruline was utilized for its elevated content of aminoacids, iron and carotenoids. Spiruline and Misola have been recently introduced in the treatment of HIV infected undernourished children because several advantages of Spiruline in the rehabilitation of HIV adult patients have been reported [7-8]. Spiruline used in this study were also analysed for their chemical composition since their lipid composition is influenced by the environmental condition of growth.

SUBJECTS AND METHODS

This research was conducted at the CMSC of Ouagadougou. This center was created in 1974 by the religious of St Camille order and comprises a maternity, a health center, an analysis laboratory for biological and biochemical examination, a center for neonatal pathology, a greenhouse for the culture of the Spiruline and a Center for Education and for Nutritional Rehabilitation (CREN). The CREN follows in average 700 children per year.

Study Protocol

Infants and children aged <5 years were enrolled using the CONSORT criteria [8]. Each child was admitted to the protocol study given a progressive number and at the end each was selected with a casual number generator program. Dehydration resulting in shock (exclusion criteria) require rapid transfer to the Hospital for intensive therapy, while the discontinuation of participation criteria were defection, death, interruption of treatment at the Center during the study.

Study Patients

At the beginning of this study, undernourished children were anhoressic and many of them had diarrhoea, which was treated with nose-gastric rehydratation according the CMSC protocol [6]. The nasogastric rehydration was interrupted before being selected for this study. At the end 550 children were enrolled randomly in three rehabilitation rehydration protocols: A) 170 of them received an alimentation only with Misola, B) 170 were treated by supplementing Spiruline to traditional meals (millet, vegetable, fruit), C) 170 received Spiruline plus Misola. A control group of 40 undernourished children of the same age range was organized between children whose mothers did not accept the protocol study, so they were fed only with traditional meals. The vitamin and mineral deficiencies were corrected only at the end of study.

Another group of 170 children (84 HIV infected and 86 HIV negative chidren), randomly enrolled, followed one of four rehabilitation protocols: A) 44 HIV infected children were given the Spiruline supplement together with the traditional meals (millet, vegetable, fruit); B) 46 HIV negative children were also treated by the supplement of Spiruline to traditional meals (millet, vegetable, fruit); C) 40 HIV infected children of the same age range were given only with traditional meals; D) 40 HIV negative children were alimented only with traditional meals. The group C and D represent the control group for Spiruline supplement. No antiretroviral treatment was used in HIV-infected children because it was not available. The vitamin and mineral deficiencies were corrected only at the end of study.

Participation Criteria

All studied children were undernourished according to the z-score criteria, recommended by the WHO and the Funds of the United Nations Children's Emergency Fund (UNICEF). and their age was 15.29 months (6-60 months). The ages were confirmed by the birth notebooks. The Ethical Committee of CMSC gave permission for the study and all parents were informed of the aim of this study. They gave written consent for the participation of their children to the study.

Anthropometrics Parameters

The weight of the children was taken once a week since the day of their admission to the CREN with a 10 grams sensitivity balance. The height of children under 2 years is measured by resting the child on his back; those children over 2 years is measured in the upright position.

The nutritional status, evaluated by brachial perimeters was compared to the classification of Jelliffe [9], considering that it varies little for the children of less than four years.

HAZ (Height for age z-score), WHZ (Weight for height z-score) and WAZ (Weight for age z-score) parameters were calculated according to the references of the National Center for Health Statistics (NCHS) [10].

Evaluation of Results

The evaluation of nutritional status of the children has been made according to the nutritional indices. The index weight for age expressed in z-score (WAZ) or weight insufficiency indicates a global malnutrition affecting at once the linear growth and the weight increment. The index height for age expressed in z-score (HAZ) or growth delay is an index that indicated a chronic malnutrition provoked by an extended reduction of the food consumption and by repeated pathologic episodes. The emaciation or weights loss expressed by the index weights for height (WHZ) indicates a slighter status or weight deficit due to a decrease, slowdown of regular growth. These tests were performed to obtain significant changes within the treatment groups in order to detect whether Spiruline or Misola are a useful supplement of feeding in rehabilitation.

Plant Material

Spiruline was cultivated in Burkina Faso, in artificial ponds and dried at room temperature. The material was stored in the dark at 25 °C to prevent photodegradation.

Preparation and Administration of the Spiruline and the Misola

The mothers of the undernourished children who received Spiruline or Spiruline plus Misola were given weekly rations of 70 grams of Spiruline in a sachet. Every day, they had to mix 10 g of Spiruline with a graduated container to the traditional meal of their children composed of millet flour. Other mothers added Spiruline to Misola meal. These integrations were made at least two times a day. The Misola, a kind of bouillon, is a mixture of millet (60%), soy (20%), peanuts (10%), sugar (9%) and salt (1%). The preparation of the Misola or millet was carried out according to traditional habits, namely 60 grams of flour and 200 ml of water were mixed and boiled with a tiny fire, mixing for 2 o 3 minutes. This mixture was administrated to children in a quantity covering their caloric requirements, and outside the suckling moments in children whose mothers continued breast feeding. The compliance of mothers in preparing mixes and feeding them to their children was improved and they continue to administrate the mixture also at home. Each day they accompany children in the CREN for a control of weight and other anthropometric parameters.

Chemical Studies

The fatty matter content was determined by the method of the extraction of Soxhlet. The fraction « total protein» or « total nitrogen» was measured by the method of Kjedahl. The content of glucides was determined by a colorimetric dosage or method of orcinol. The lipid composition was evaluated by the analysis of fatty acid methyl esters (FAME).

Fatty Acids Quantification and Identification

The Spiruline was ground and extracted three times with hexane. The mixture of fatty acid methyl esters has been extracted with hexane and analyzed by Hewlett Packard gas-chromatograph, Model 5890, equipped with a flame ionization detector (FID) and coupled to an electronic integrator. The components were identified by using standard fatty acid methyl esters and quantified by using methyl nonadecanoate (19:0) as an internal standard.

Statistic Analysis

A power analysis was performed prior to the initiation of the study and the number of studied children was homogeneously distributed and reached the minimal number to discuss a statistical difference. The data were treated with Excel (Office, Microsoft) software, Epi-Info software V. 6 for the anthropometrics data and SPSS–10 for biological data, according to the opportunities of calculations and of analysis. The difference between mean values before and after eight week of treatment were calculated by Student T test. $P < 0.05$ was considered significant.

RESULTS

Nutritional Rehabilitation of Undernourished Children:

Table I shows the anthropometrics parameters of the children at the beginning of our study. The baseline anthropometric status was equivalent among the groups, with exception of HAZ for group B (- 2.64). No significant differences are observed in these parameters: HAZ, WHZ, WAZ and the BP.

Only males' weight was greater than females with respective significant differences: $p<0.0001$ (Table II).

Table I – Anthropometric parameters of the children subjected to the study[a]

	A 170 Children with Misola	**B** 170 Children with Spiruline plus traditional meals	**C** 170 Children with Misola plus Spiruline	**D** 40 children with traditional meals	**Variance Analysis**
Age (months)	15.39±8.3	14.96±5.9	13.86±8.5	15.19±4.35	P = 0.269
Height (cm)	67.00±8.3	69.84±5.8	69.06±8.5	68.24±4.5	P = 0.005
B.P.	11.17±1.2	10.40±1.0	11.20±1.2	10.37±1.0	P = 0.0001
Weight (kg)	6.12±1.4	5.98±1.1	5.99±1.5	6.10±1.2	P = 0.741
HAZ	-3.93±5.3	-2.64±2.1	-3.35±5.3	-3.23±1.5	P = 0.057
WHZ	-1.73±2.5	-2.88±0.9	-3.05±0.75	-2.32±1.02	P = 0.0001
WAZ	-4.01±1.0	-3.88±1.0	-4.38±0.9	-3.99±0.9	P = 0.0001

[a]HAZ = Height for age z-score; WHZ = Weight for height z-score; WAZ = Weight for age z-score, B.P = Brachial Perimeter.

Table II - Mean anthropometrics parameters of the children according to the sex at the beginning of the study

	286 Female		264 Males		All children (550)	
Parameters	Mean	Variance	Mean	Variance	Mean	Variance
Age (months)	15.64	8.08	15.01	6.87	15.30	7.41
Weight (Kg)	5.82	1.17	6.28*	1.36	6.07	1.29
Height (cm)	68.07	6.73	68.43	7.48	68.27	7.13
P.B.	10.75	1.13	10.99	1.25	10.88	1.20

Student T test * P = 0.0001.

The nutritional changes pre/post improved in all children, more significantly in the group who received Misola plus Spiruline. These changes among treatment groups are reported in Figure 1. This improvement corresponds to an increment of weight which was on the average of 20 g/day in the Misola group, 25 g/day in the Spiruline plus traditional meals group, 34 g/day in the Misola plus Spiruline group and 15 g/day in the control group. These pre/post differences within groups were statistically significant considering the differences in the nutritional status changes across the groups, but this difference was less significant in the control group.

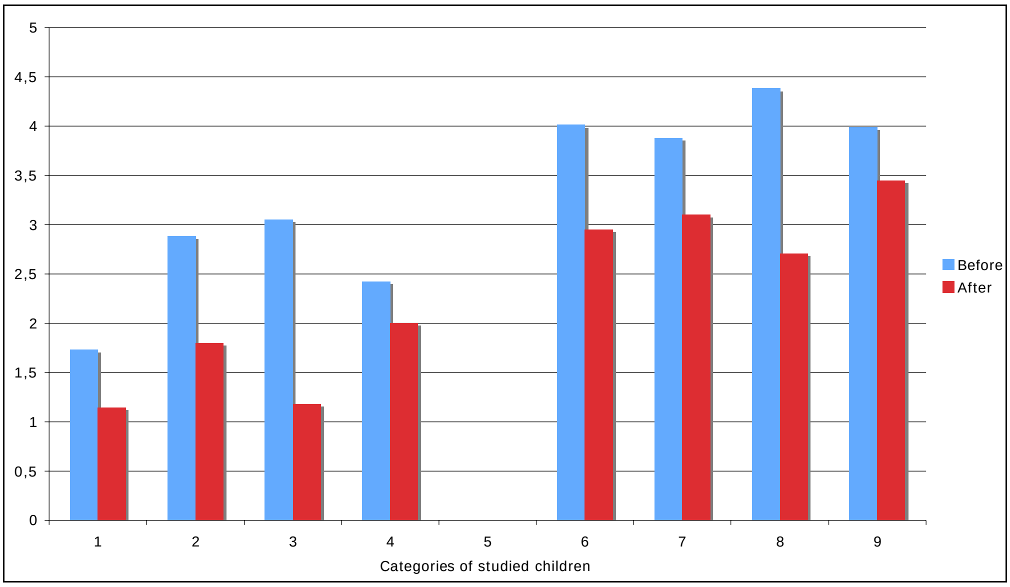

Figure 1. Nutritional status at the beginning (blue) and at the end of the study (red). WHZ = Weight for height z-score (1-4); WAZ1 = Weight for age z-score (6-9); (1, 6) 170Children with Misola; (2, 7) 170 Children with Spiruline plus traditional meals; (3, 8) 170 Children with Spiruline plus Misola;

(4,9) 40 Children with traditional meals. Student T test WHZ before à WHZ after P < 0.001; WAZ before à WAZ after P < 0.001.

Table III – Anthropometric parameters of the children subjected to the study

	A 46 Children HIV Negatives with Spiruline plus traditional meals	**B** 44 Children HIV-infected with Spiruline plus traditional meals	**C** 40 children HIV Negative with traditional meals	**D** 40 children HIV-infected with traditional meals	**Variance Analysis**
Age (months)	14.37±6.4	15.54±5.3 P = NS	15.19±4.35 P = NS	14.96±5.9 P = NS	P = 0.789
Height (cm)	69.64±1.1	69.72±5.8 P = NS	68.24±4.5 P = NS	69.84±5.8 P = NS	P = 0.370
B.P.	10.65±1.1	10.14±0.9 P = NS	10.37±1.0 P = NS	10.40±1.0 P = NS	P = 0.124
Weight (kg)	6.05±1.1	5.91±1.2 P = NS	6.10±1.2 P = NS	5.98±1.1 P = NS	P = 0.882
HAZ	-2.59±1.5	-2.88±1.3 P = NS	-3.23±1.5 P = NS	-2.64±2.1 P = 0.0001	P = 0.758
WHZ	-2.94±-0.7	-2.87±1.0 P = NS	-2.42±1.02 P = NS	-2.88±0.9 P = NS	P = 0.038
WAZ	-3.83±0.9	-4.10±0.8 P = NS	-3.99±0.9 P = NS	-3.88±1.0 P = NS	P = 0.503

[a]HAZ = Height for age z-score; WHZ = Weight for height z-score; WAZ = Weight for age z-score, B.P = Brachial Perimeter.

At the end of the eight weeks of the treatment, nutritional status normalized for the majority of children, WHZ parameter decreasing from -2.26 to -0.93; The index weight for age WAZ at the end of our study allowed to confirm that the severe malnutrition was corrected by this protocol of treatment, more significantly in the Misola plus Spiruline group. The percentage of WHZ and WAZ are reported in table III. The association of Spiruline plus Misola gave a gain of 61 % and 38 % respectively. The gain with traditional meals, Misola or Spiruline plus traditional meals was clearly of minor entity.

Nutritional rehabilitation of undernourished HIV infected children:

Table III shows the anthropometric parameters of the children HIV infected and HIV negative at the beginning of our study. The impact of study envolvement was elevated and all children randomly chosen completed the eight weeks of treatment.

The nutritional changes pre/post improved in all children, more significantly in the group who received Spiruline. These changes among treatment groups are reported in Figure 2. This improvement corresponds to an increment of weight, which was on average 15 g/day in HIV infected children and on average 25 g/day for HIV negative children both treated with Spiruline plus traditional meals. The differences within groups were statistically significant considering the differences in the nutritional status changes across the groups, but this difference was less significant in the control group (10 g and 20 g day respectively).

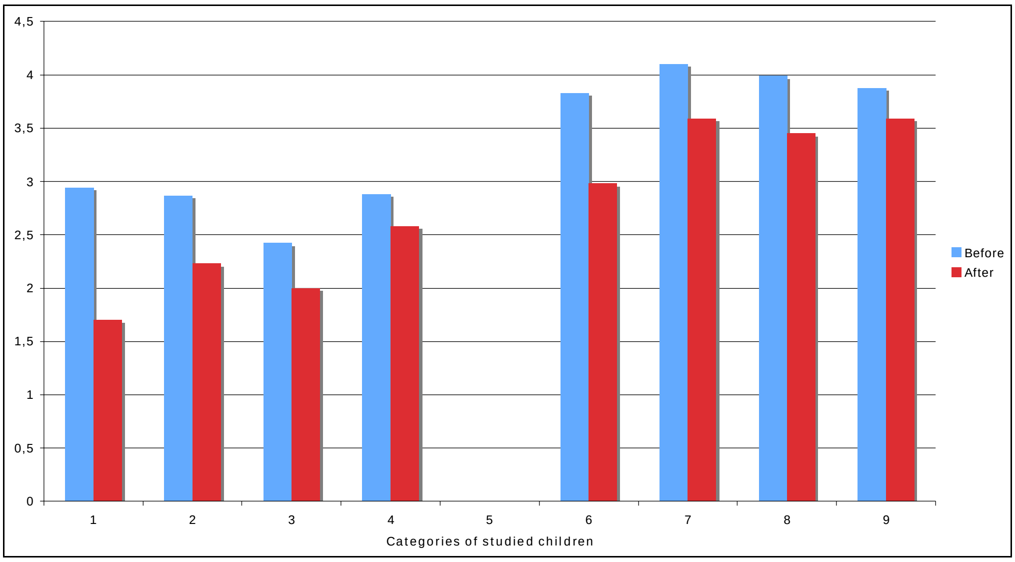

Figure 2. Nutritional status of children HIV infected and HIV negative at the beginning (blue) and at the end (red) of the study. WHZ = Weight for height z-score (1-4); WAZ1 = Weight for age z-score (6-9); (1, 6) 46 Children HIV negatives with Spiruline plus traditional meals; (2, 7) 44 Children HIV-infected with Spiruline plus traditional meals; (3, 8) 40 Children HIV negative with traditional meals; (4,9) 40 Children HIV-infected with traditional meals. Student T test WHZ before à WHZ after P < 0.01; WAZ before à WAZ after P < 0.01.

The HIV negative children who are nourished with Spiruline plus traditional meals had a more significant (p<0.0003) decrement of WHZ parameter than the HIV-infected children (p=0.0004). At the end of the eight weeks of the treatment, nutritional status normalized for the majority of children, WHZ parameter decreasing from -2.26 to -0.93; the average of nutritional status of the HIV-infected children was not so good as the average for HIV negative children. The index weight for age WAZ at the end of our study confirmed that the severe malnutrition was corrected by this protocol of treatment, more significantly in the Spiruline group. The percentage of WHZ and WAZ are reported in Figure 2. The association of Spiruline plus traditional meals gave in HIV infected children a gain of 22.2 and 14.63 % respectively, against 10.41 and 7.47 % respectively, using alone traditional meals. In HIV negative children the gain was 42.1 and 22.19 % respectively when Spiruline was added to traditional meals and 17.3 and 13.53 % when they were alimented traditionally alone.

The biological analyses allowed to obtain some indexes which are reported in the Figure 3. The analysis of this data shows that the best improvement was observed in the group of HIV-infected children. This response to the Spiruline supplement was more significant than the increment of weight and height showed by WHZ and WAZ parameters. The median number of the red cells and the median concentration of haemoglobin, allowed to diagnose an anaemia (3.59 10^6/mm^3 of red cells and 8.44 g/dl of haemoglobin) for all the children. Children who were HIV-infected were more anaemic (3.34 10^6/mm^3 and 8.01 g/dl of heamoglobin) than the children HIV negative (3.64 10^6/mm^3 and 8.53 g/dl of haemoglobin). At the end of the eight weeks, the anaemia was slightly corrected for all HIV-infected and HIV negative children who remained nevertheless anaemic (3.92 10^6 /mm^3).

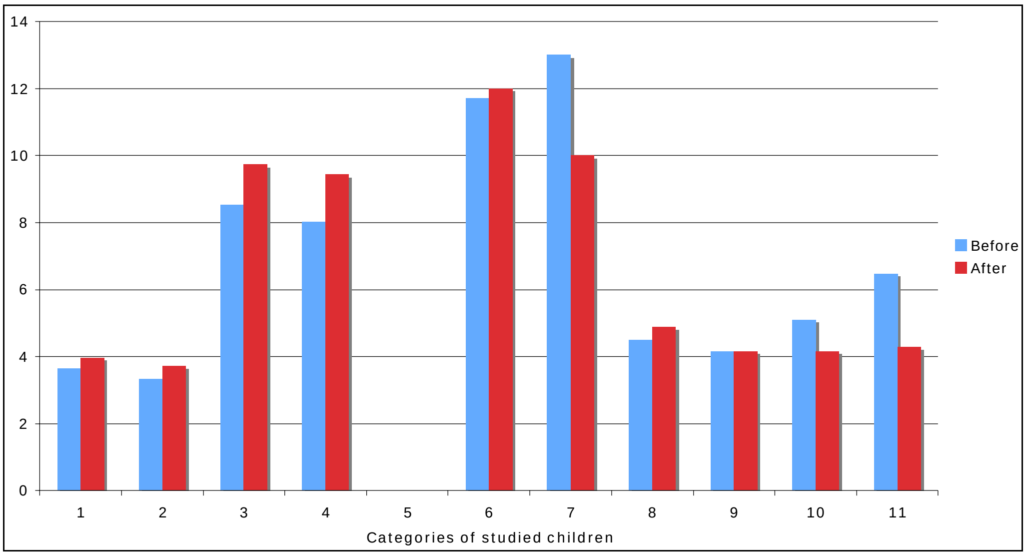

Figure 3. Biological parameters of the HIV infected and HIV negative children at the beginning (blue) and at the end (red) of the study. (1, 3, 6, 8, 10) Children HIV negative treated with Spiruline n. 46; (2, 4, 7, 9, 11) Children HIV-infected treated with Spirulinen. n. 44. (1-2) Red Cell x 10^6 mm^3; (3-4) Hb gr/dl; (6-7) Leukocytes x 10^3 mm^3; (8-9) Lymphocytes x 10^3 mm^3; (10-11) Neutrophil x 10^3 mm^3.

The compliance to treatment was excellent and no children drop out. The mothers referred that the children accepted the mixes and rarely had difficulties in feeding their children..

The compliance to treatment and participation was excellent. They come to weekly appointments, but only the first and the last visit (eight weeks) were considered in the final evaluation.

Chemical Analysis

The Misola is an infantile flour composed by millet, soy, peanuts, sugar and salt produced in the CREN of the CMSC (Ouagadougou). Table IV shows the biochemical composition for 100 grams of used Misola at the CMSC and the lipid composition of this mixture, where the fatty acid content is represented by palmitic, linoleic, oleic, γ-linolenic, stearic and palmitoleic acids.

Table IV – Biochemical composition of Misola produced at Centre Medical St Camille

Biochemical Composition	Mean Concentration
Lipid	12 %
Protein	16 %
Glucide	61 %
Calories (kcal/g)	410

Table V - The composition for 100 grams of cultivated Spiruline to the Center Medical St Camille in comparison of the given values in the literature (Green Flamant 1998)

	Our results	Green Flamant values
Water content	4.87%	3-7%
Ash	10.38%	7-13%
Vegetal Fiber	7.81%	8-10%
Lipid	6.00%	6-8%
Protein	57.10%	55-70%
Glucide	13.84%	15-25%

The composition of the cultivated Spiruline of the CMSC is given in Table V. The values of the composition of Spiruline of the CMSC of Ouagadougou are in the interval of values of the international Firm Green Flamant [11] and the physicochemical elements does not change with the time ($p>0.270$). The composition of our Spiruline proves the good quality of the Spiruline of the CMSC. The only difference is situated at the level of the value of the glucides. The lesser content in glucides of the analysed Spiruline in our culture conditions was near the one of Sautier and Tremolieres [12] that in 1975 found a value of 12.4% on the cultivated Spiruline in laboratory. The quality of the Spiruline with the time - in the first three months of storage did not show significant changes. For longer period of storage some significant changes were detected, such as a decrease of protein content and an increase of pH value (Table VI).

The lipid composition of the Spiruline growth in Burkina Faso is listed in Table VII. The fatty acid content is represented by palmitic, linoleic, oleic, γ-linolenic, stearic and palmitoleic acids.

Table VI - Physicochemical composition of the Spiruline with the time

Analysed sample	T0 (1th day)	T1 (1th month)	T2 (2nd month)	T3 (3th month)	T4 (10th month)
Protein (%)	57.10	56.22	54.69	52.28	49.22
Formic index (ml NaOH)	4.35	4.20	4.47	5.19	4.81
Total sugars (%)	12.77	16.43	19.59	18.16	16.07
Reductive sugars (%)	1.07	2.52	2.17	1.56	1.62
Fat matter (%)	6.00	7.19	6.69	5.92	7.25
Fatty acids (mg NaOH/g)	6.6	6.0	7.5	6.9	10.2
pH	6.53	6.56	6.36	6.78	7.33
Humidity (%)	4.87	4.86	5.01	4.83	4.42
Ash (%)	10.76	12.12	10.19	11.46	14.44
Phycocyanin (%)	9.76	7.46	6.12	7.32	4.46
Energetic value (kcal/100g)	338	360	363	340	331

Student T test for paired data: T0 à T1 : p = 0.273; T0 à T2 : p = 0.310; T0 à T3: p = 0.763; T0 à T4: p = 0.625.

Table VII - Fatty acid composition of *Spiruline* strain from Burkina Faso

Fatty acid	Wt % of total fatty acid
Palmitic acid, 16:0	28.04
Palmitoleic acid, 16:1 (9)	2.69
Stearic acid, 18:0	13.44
Oleic acid, 18:1 (9)	18.88
Linoleic acid, 18:2 (9, 12)	21.87
γ-Linolenic acid, 18:3 (6, 9, 12)	15.08

DISCUSSION

After eight weeks of study, children treated with Misola, Spiruline plus traditional meals and Misola plus Spiruline appear improved, their weight grew and many of them appeared less anaemic. This improvement was less significant in the control group, who received only traditional meals. The enlistment of this group could appear unethical among these severely malnourished children, but it was organized choosing a control group between children whose mothers did not accept the protocol study, so they were treated only with traditional meals.

The association between Misola plus Spiruline reached the greater gain in term of weight than the Misola or Spiruline alone. Also HIV infected and HIV negative children treated with Spiruline plus traditional meals appear, after eight weeks of study, improved, their weight grew and many of them appeared less anaemic. This improvement was less significant in the control group, who received only traditional meals. The enrolment of a control group could appear unethical among these severely malnourished children, but it was organized choosing randomly a control group between children whose mothers had preliminarily accepted the protocol study. In this way the influence of not willing to partecipate on a study on caloric and nutrient intake (supplement of Spiruline vs traditional meals) becomes insignificant.

The results of this study prompted us to continue the culture of Spiruline in the CMSC of Ouagadougou in order to utilize the biochemical composition and the beneficial action of this cyanobacterium, which may be considered as an alimentary integrator for undernourished children. In the context of weak intake of proteins, 10 g a day by inhabitant in Africa against 29 g in Latin America and 63 g in the industrialized countries, the integration of traditional meal with Spiruline and Misola plus Spiruline (57 % of protein) improve the nutritional and micronutrient requirement for undernourished children [13].

Moreover, this results show that the Spiruline is also effective for the haematopoiesis especially for the persons infected by HIV in accordance with the data of the international literature [14]. This may be due to the iron content of Spiruline supplement [15], which correct the anemia due to deficient iron intake.

Since at the beginning of this study the number of leucocytes: 12,370/mm^3) with relative granulocytopenia was found elevated in the HIV-infected children (49.8 %) (Table VI), the increase of lymphocyte number in HIV-infected children who received eight weeks of Spiruline, confirm the immune modulation of this cyanobacterium.

This mechanism may be due to the high amount in the lipid fraction of ω-6 derivative, namely γ-linolenic acid [15]. This exclusive presence of ω-6 represents a metabolic gain, since desaturase enzyme could be deficient in the undernourished children [16].

The growth recovery is more slow than the weight recovery and this could be determined by the diarrhoea, which was present at the beginning of treatment of these children [17]. In fact, in our study regarding a period of eight weeks, the variations of weight were more significant owing to the liquid content dehydratation associated to malnutrition.WHZ was smaller in HIV-infected children in comparison with HIV negative children (Table III), since they frequently show diarrhoea associated with HIV infection.

One could object that this study assigned the Spiruline treatment mainly to children with HIV-infected than non infected, who showed more severe alterations of WHZ and WAZ values.

However the percentage of increment in weight with the association of Misola plus Spiruline confirms the opportunity to continue this kind of association in undernourioshed children. Previous study made by Branger et al. [18] in Burkina Faso did not show a significant improvement adding Spiruline to traditional meal and Misola, but, as considered by the same authors, the scarce results which they obtained could be due to the quantity of Spiruline, which was half than that used in our study (5 g *vs*. 10 g). Moreover, the present study is more conclusive than the one realized in Dakar by Alling *et al*. [19], in which the gain of weight was inferior, probably due also in this case, to a reduced supplement in Spiruline.

The anthropometric characteristics varied little according to the sex (Table II), but were different according to the nutritional and serologic status. This observation is the same as the one of Kelly *et al*. [20] in undernourished HIV-infected children with persistent diarrhoea. In CMSC a prevalence of 0.82 % for the kwashiorkor, 95.96 % for the marasma and 3.22 % for the kwashiorkor plus marasma was found, which correspond to the effect of HIV infection of nutritional status of children in Burkina Faso [21]. Moreover, the screening of these aforesaid children at the Medical Center confirmed a 24.4 % of HIV-infected children. This frequency is less than the prevalence of 27.0 % found at Bobo-Dioulasso (Burkina Faso) by Prazuck *et al.* in 1992 [22] and of 28.6 % in Togo by Atakouma *et al.* in 1994 [23]. The strong prevalence the kwashiorkor and/or marasma is characteristic of sub-Saharan Africa, where maize and millet are the staple. In fact, high intake of linoleic acid in a diet deficient in other polyunsaturated fatty acids and in riboflavin results in these countries in high tissue production of prostaglandin E2, which in turn causes inhibition of the proliferation and cytokine production of Th1 cells, mediators of cellular immunity [24]. Diet-associated inhibition of the Th1 subset is a major contributor to the high prevalence of these diseases in sub-Saharan areas.

The high percentage of undernourished children in Burkina Faso highlights the problem of the charge of medical and nutritional structures, and this study could suggest a preliminary solution with Spiruline plus Misola or Spiruline plus traditional meal for accelerating in HIV infected undernourished children the nutritional rehabilitation before starting an antiretroviral treatment.

CONCLUSION

This study shows that malnutrition remains a public health problem in Burkina Faso. The consequences of malnutrition in association with HIV infection represent a global problem, which affects morbidity as well as mortality. Awaiting for the enrolment of these undernourished children in riabilitation protocols, the persons in charge at public health service and epidemiologists should work very synergically with nutritionists, bacteriologists and virologists in order to fight efficiently against malnutrition and particularly the paediatric malnutrition associated to the HIV.

The Misola which has 61 % of glycides with 410 kcal is more energetic than the Spiruline which has only 13.84 % of glycides with 338 kcal. Inversely, the Spiruline has 57.10 % of protein and the Misola has only 16 %. At the end the high amount of ω-6 lipid component support an efficient recovery of the precarious immune system of these children. These characteristics confirm the utility to supplement Misola with Spiruline (this association gave a gain of 34 g/day). According to the instructions which the mothers received, an involvement of the families of the undernourished children and of the whole community is essential to control the great prevalence of the malnutrition in African countries.

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ADDENDUM

How Fight Malnutrition

Our experience with Misola and Spiruline supplemented to traditional meal suggest to develop the production of these two integrators in the developing countries. The production of Misola may be a starting point of the nutrition rehabilitation: the procedure for the production is very simple and require only reduced economical reseourse. The production of Spirulin as a supplement in the rehabilitation of HIV positive and HIV negative children represent another miliar stone in the program of nutrition rehabilitation and the production of this blue microalgae need only simple technologies of cultivation.

History of Misola

Misola adventure has as a starting point a concrete need and not a theoretical will: the exhaustion of food supplement, provided until 1981 (the Misola flour are manufactured in Burkina Faso since 1982 starting from cereals and leguminous plants cultivated in sub-saharian areas), in the Center of Rehabilitation and Nutritional Education (C.R.E.N.) hospital of Fada Gourma.

At the beginning, in November 1982 the composition was the following one: millet, soy milk, peanuts plus the additives (iron, vitamin complex) from where the name Misola came. Taking into account multiples problems due to milk and vitamins, these ingradients have been eliminated and the current formula uses only local products i.e the millet, soy, peanuts, sugar and a little salt.

The composition of the Misola flour is based on a cereals/legumineuse association, which allows a balance of the amino acids close to that of animal proteins. The cereals and the small millet bring glucids and proteins; the leguminous plants, peanuts and soy, are rich in proteins and fat content, which makes it possible to increase the energetic value in unsatured fatty acids.

The ingredients of the flour are mixed in the following ponderal proportions: Small millet roasted 60%, Soy roasted 20%, Peanuts roasted 10%, Sugar 9%, Salt 1% . The selection of the ingredients facilitates digestion and gives to the soup a good appreciated taste even in the undernourished and anorexic children.

The use of local food (millet) allows the easy passage in future of a traditional family food. It is possible to replace the millet with corn 30% and rice 30%. The flower can be enriched with orange juice, lemon or tomato, dry fish or meat. The contribution of vitamine C and beta-carotene (provitamin A) can be done by the addition, at the end of cooking, of fresh fruit juice.

How is Flour Misola Produced

The manufacture of Misola flour comes with simple traditional hand-built techniques that allow a perfect technological control. It is simply a question of making roasted cereals, to grind them and to weigh after addition of sugar and salt. The ingredients are mixed in proportions of volumes and not in weight. This operation requires simplified technology, the use of cutlery and other kitchen articles. At beginning the ingredients must be cleaned from the contamination of insects, escrements and little stones through washing and decantation (figure 4).

A badly conserved flour or sufficiently not cleaned is responsible of gastroenteritis. To prevent from these inconvenients the following precautions are taken: washing of hands; sifting of the flour in clean dishes adapted for this purpose; cooling with the shelter before bagging; thermal welding to abolish air in the sachets; conservation of the sachets in containers hermetically closed. If it is prepared and stored under satisfactory conditions, the flour can be preserved for 6 months and sometimes more.

Rarely the bacteriological analyses carried out at the laboratory of nutrition of the CMSC revealed a high level of contamination.

Figure 4. Phases of preparation of Misola flour until the conservation in sigilled sachets.

Composition of Flour Glucids 61 grams, Lipids 11 grams, Protides 15 grams, the energy value 425 kcal (1775 kjoules /100 grams). The consumption of a soup covers half of the daily requirements out of zinc and iron for a child of more than one year. Naturally rich in vitamins B, the contribution in vitamin C and of provitamin A will be done by the addition at the end of cooking of the fresh fruit juice.

How Prepare and Utilize Cooked Misola The teaching of the preparation and the use of Misola soup is placed from the point of view of nutritional education. To propose a codified receipt: "one volume of flour for two volumes of water mixed with germinated cereal amylase to prepare an energy soup of low density" (figure 5).

Figure 5. Preparation of soup (one volume of flour for two volumes of water) and phases of cultivation of the ingredients (millet, soy and peanuts) with the active partecipation of mothers.

Guaranties on Biodiversite

The Millet, Soy, Peanuts, Corn or Rice are produced locally without use of pesticides, chemical fertilizer or others. To guarantee the quality of cereals the CMSC and the sister of CREN encourages the groups of women to cultivate the ingredients for the preparation of

Misola using the biological criteria for cereals production and applying storage methods without use of chemicals.

If the need for offering to the child a specific food is generally recognized, the economic or social difficulties ensure many mothers from making profit for their child from such a food. The manufacture of the flour in the groups of CMSC is very simple. Each mother having taken part in the cultivation of cereals and in the transformation of the agricultural matters gets benefits on the local products. The structure produces the quantity of flour necessary to her child until the following meeting. Otherwise, women can buy the flour in the pharmacy of CMSC at the price of 350 F CFA (euro 0.533) the package of 500 grams: the cost to treat a moderate malnutrition requiring one to two soups per day is 1,200 to 3,000 F CFA (euro 1.83 to 4.57) per month of expenditure.

It is perhaps one of the characteristics of the Misola to be produced within the structures of health. This collaboration constitutes a major asset to have an impact of the public health. Other than producing a special food (the flour) it helps to sensitize the mothers on the importance of nutrition for their children: the flour is a product able to give good health. Furthermore, the structure can be used for distribution of drugs and vaccines for their children. The collaboration of mothers has several other advantages: - "privatiser" the production equipment by financially interesting the women to profit from their knowledge and their permanence on the structure; - to profit from their practical direction since they can control their results - to allow a direct sensitizing of the mothers of the group to the nutrition of children. The collaboration of a O.N.G. of support allows to consider a financial assistance. This collaboration is often articulated with other shutters of a project of development and makes it possible to integrate the project in a broader context. The creation of a network among various centre of production disseminated in the country, is necessary to keep the coherence of the objective (Public Health) and the means (hand-built, female groupings). This network is often coordinated by a sister if the Structure is directed by religious or by a representant of the Management of Health and Family or by functionary of the UNICEF.

The refunding of modest investments, in particular those necessary to the construction of buildings, is not the ambition of this type of project. On the other hand, the self-financing of operating expenses and if possible the self-financing of the investments are part of the objectives to reach. In order to determine the threshold of availability one can proceed as follows: - to calculate the cost price out of all rough matters for the production of one kg flour; - to determine the reasonable selling price, which allows an accessibility of flour to a greatest number of children (30 % of increment on the price of the rough matters) in a spirit of wellfare; - to calculate the fixed loads per month (salaires, rents, water, transport, electhricity, reinvestiment, losses and others); - one can determin then the minimal production-sale necessary to the economic balance of hand-built production, assembling fixed loads by the margin of gain on each sold kg. For example in 2004 and 2005 at the CMSC of Ouagadougou the economical balance was:

2004 - Cost price of the rough matters: 462 Fs/kg; Selling price: 719 Fs/kg; . That is to say a gross margin of 257 Fs/kg; the fixed loads costant of 96,906 Fs per month, the minimum quantity to sell each month was of 1,308 sachets of different weight from 200 to 500 g, that is to say 554 kg. The gain at the end at December 2004 was 1,713,955 Fs.

2005 - Cost price of the rough matters: 508 Fs/kg; Selling price: 731 Fs/kg; . That is to say a gross margin of 223 Fs/kg; the fixed loads costant of 155,510 Fs per month, the minimum quantity to sell each month was of 1771 sachets of different weight from 200 to 500 g, that is to say 787 kg. The gain at the end at December 2004 was 2,108,270 Fs.

The need of a rigorous management imposes a certain number of measurements, in particular the behaviour of a careful account for the purchased, stocks, the production and the sales and the diffusion of informations on the efficacy of this product among the mothers.

In the CMSC the investments in rough matter was supported at beginning by C.N.H (Culture Nutrition Healthy), which funded the realization of Misola production centre in 2000.

How to Reach the Target Groups and to Improve the Nutritional State of Children

The target groups probably constitutes the most difficult problem, much more difficult than to reach the population, and this difficulty has limited up to now the extension of the project. Collaboration with the health services is essential in the prospective of commercial goal. This collaboration allows: - to conceive and develop the project in synthony with the Public Health and sensibilizy the personal of Health to the infantile nutrition (training courses); - to use the structures of distribution and sale of the drugs (pharmacy); - to start campaigns against malnutrition as there exists - to organize campaigns to the use by the mothers of the nutrition programs part of the prevention; programs of vaccination; food additional from 4 to 6 months; Furthermore the Misola flour has been proposed for the precocious weaning of the child of HIV positive mother. The dispersion of other centres for the production of Misola on the territory facilitates the distribution towards the target groups more easily accessible.

Another means of reaching the target groups is to develop and promote the "Community Village Manufacture" of flour.

It is necessary, but it is not sufficient that an infant eats when he is hungry. The food, which calms its hunger, must have a good nutritional value. It is thus important to be able to guarantee the quality of the product, the stability of its formula, the absence of toxicity, and its good conservation; in order to achieve these requirements the food must respect the international standards. Does this requirement for quality constitute a fundamental criterion to gain participation of mothers, but also to involve the national and international organizations to buy raw matters within the framework of their actions of fighting against malnutrition. It is well clear that these types of actions cannot achieve a quality control of the industrial process, with complete and systematic analyses of the batches, and eventually correction of the formula at the end of the manufacture. It is thus necessary to take a particular care in the promotion of the flour: - requirements of hygiene; - rigour of manufacture (significant points: sorting and cleaning of seeds); - rigour of measurements for the mixture of ingredients; - quality of conditioning. This constant rigour of manufacture, will be completed if possible of chemical and bacteriological analyses (composition in nutrients and aflatoxines, bacteriological controls) which constitutes the only guarantee of quality. The follow-up of the quality falls on the Misola centre of production. The biological controls fall on the Services of Nutrition and the intervention of analysis laboratory on the Ministry for Health, or on other laboratories with the O.N.G. support.

History of Spiruline

The Spiruline story starts approximately 30 years ago, when two ethnologists made research on the edges of the lake Chad, in full period of Saudi famine. They noted that one of the tribes was in full form and that there was no symptom of malnutrition. These people consumed a green pie. They brought back this green mass to the Pasteur Institute to Paris. The researchers then discovered the Spiruline. The American researchers were also interested because of the utility of the Spiruline in nutrition. Research on Spiruline lasted 10 years with the aim of identifying different species of Spiruline, their characteristics, their clinical trials, the studies of acceptability and toxicity and the installation of a system which is most productive starting from the ingredients available and a less cost. It is a small blue microalga (Cyanophiceae) (0,3 mm length), whose scientific name is "cyanobactery Arthrospira platensis" (not to confuse with the cyanobactermarinates called scientifically "Spiruline subsalsa"), which exercite photosynthesis like the plants and thrives naturally in the salted and alkaline lakes of the hot areas of the sphere. Traditional food of Aztec of Mexico and Kanembous of Chad, the Spiruline is now cultivated in large factories in the U.S.A., in India, in China, in Thailand, etc, because of the many qualities related to food and health, both for men and the animals. For example a child suffering of malnutrition can be restored by giving him a supplement (10 g) per day of Spiruline for one month. The Spiruline reinforces immunizing defences and reduces sufferings in people affected by AIDS. It makes it possible tuberculous to better support their treatment. The Spiruline is also used as active ingredient in cosmetic. In nature, the Spiruline has need "to push" only one argillaceous basin retaining a brackish and alkaline water, under a hot climate, and of some animal manure. The Spiruline is presented in the form of spirals made up of juxtaposed cells. The reproduction of the Spiruline, asexual, is done by division of the spirals. The industrial crop of the Spiruline is intensive and very technique. Its end product, dried by atomization, is lower in quality than the fresh product; moreover, the product dried manually does not plait with certain consumers who dislike the strong odour of dry fish.

Composition of Spiruline: protein 70%, i.e. 2 times more than soy and 3 times more than beef. The human body assimilates proteins of Spiruline 4 times more quickly and better than proteins of the meat and cheese. Very rich in vitamins (A. B1, B2 B12, E) and in assimilable iron, it contains also calcium, phosphorus, magnesium (in quantity comparable with cereals and the cow's milk) and γ-linolenic acid (rare in the current food).

In the CMSC, the investments of the Spiruline was supported at beginning by "Fondation Jean Paul II pour le Sahel" in 1997.

The Camillians has been first to introduce the Spiruline in Burkina Faso. At beginning two centers have been created for the cultivation of Spiruline at Koudougou and Lombila. Having seen the good ones the Guvernment of Burkina Faso has made a large plan for more than 1,372,160 euro for the recovery of the undernourished children in Burkina Faso utilizing Spiruline

Figure 6. Particular of basins for the Spiruline cultivation.

Instruction to even Cultivate the Spiruline Oneself

There are many ways of building an adequate basin, variables according to local conditions': out of plastic covers, hard clay, low walls. It is generally useful, to install a greenhouse or at least a roof on the basin allowing to protect it from the bad weather. The roof can be in white or translucent plastic, or other solutions making it possible to let pass a part of the light (see figure 6).

To cultivate Spiruline it is necessary to recreate the close culture medium of which it growth naturally. The culture medium is a controlled salt solution in water; this liquid must bring to Spiruline all the chemical elements which are necessary. The pH of the culture medium must lie between 8.0 and 11. The solutions, for a basin of 4 m^2 which may be realize in a small space are reported in Table VIII:

Table VIII - Concentration of different chemical components for the cultivation of Spiruline

Chemical Component		**Concentration (g / l)**
Sodium Hydrogen carbonate	$NaHCO_3$	8
See salt	NaCl	5
Potassium Nitrate	KNO_3	2
Magnésium Sulfate	$MgSO_4$, 7 H_2O	0,16
Ammonium Phosphate mono basic	NH_4PO_4, 12 H_2O	0,08
Urea	$CO(NH_2)_2$	0,015
Iron Sulfate	$FeSO_4$, $7H_2O$	0,005

Spiruline are the carbon whose normal source is carbon dioxide; one can increase the contribution of $C0_2$ by composting under greenhouse contiguous to the basin. The ideal temperature is between 35° and 37.

To control the level of the basin which must be at least 20 cm of water (to add water when necessary).

Agitation of the water of the basin is necessary to homogenize and ensure a good distribution of lighting among all the spirals of Spiruline. One can agitate various ways -

manually, with a brush or a wheel 4 times per day, for 2 minutes. This motor could be alimented with solar system.

To collect by skimming surface with a basin and to initially empty Spiruline use a mosquito net of 60 microns. Slight drying and conservation are worth to consume Spiruline fresh (2 times more effective), but it should be consumed 6 hours to the maximum after harvest, if not it is possible preserve it up to one year by making it dry with the sun or in a solar drier.

Use of Spiruline

Mixed with a basic food, it makes possible to overcome a malnutrition moderated with acute. By way of example, an amount of Spiruline from 1 to 5 grams per day managed during 4 weeks with an child severely undernourished from 0 to 5 years, functions like a genuine catalyst in the resumption of weight. The nutritional rehabilitation is some spectacularly improved. Many studies and clinical trials bring the proof of therapeutic values in iron deficient anaemias and the reinforcement of immunizing defenses and the lightening of the sufferings of the people affected of AIDS. A suffering child with kwashiorkor (malnutrition) can be restored by giving him one spun (10 g) per day of Spiruline for one month (figure 7).

Figure 7. Child with severe malnutrition (Kwashiorkor) before and after nutrition with Misola and supplement of Spiruline (10 g/day).

The Spiruline is a supplement micro-nutrient and it is one energizer. Beyond 5 years, one needs 2 grams of Spiruline per day. It has nutritional effects as well as therapeutic. Its food use has been suggested as therapeutic supplement in the treatment of certain pathologies: cancers, AIDS, weakeness, hypercholesterolemia, deficit of immunizing defenses, stress. 10 grams per day of Spiruline, with a cereal and vitamin C, is enough to fight malnutrition in 28 days. The Spiruline reduces the transmission of the HIV of the mother to the child.

The material used for the of the Spiruline consists of stocks of Spiruline which come from natural layers such as those of the lake Chad.