

Nutrition Rehabilitation of HIV-Infected and HIV-Negative Undernourished Children Utilizing Spirulina

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Key Words

Malnutrition · Rehabilitation · Spirulina (*Spirulina platensis*) · HIV-infected children · HIV-negative children · Burkina Faso

Abstract

The objective of this study was to assess the impact of an alimentary integrator composed of spirulina (*Spirulina platensis*; SP), produced at the Centre Médical St Camille of Ouagadougou, Burkina Faso, on the nutritional status of undernourished HIV-infected and HIV-negative 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. In

fact 81.8% of HIV-negative undernourished children recuperated as opposed to 63.6% of HIV-infected children (Z: 1.70 (95% CI –0.366, –0.002, p = 0.088)). Our results confirm that SP is a good food supplement for undernourished children. In particular, rehabilitation with SP also seems to correct anaemia and weight loss in HIV-infected children, and even more quickly in HIV-negative undernourished children.

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Malnutrition constitutes a public health problem all over the world, but particularly in developing countries [1]. In Africa, more than 30% of the infant mortality rate <5 years of age results directly or indirectly from malnutrition [2]. Since 1999, Burkina Faso has been confronted with protein-energetic malnutrition and 13% of the infant population is affected by emaciation, 29% by growth delay and 30% by underweight [3]. The consequences of protein-energetic malnutrition in Burkina Faso are multiple and manifest severe forms of marasma, kwashiorkor and kwashiorkor + marasma [3]. Today it is recognized

that this form of malnutrition is coupled with vitamin and mineral deficiencies [4, 5]. It creates inexorably an uncorrectable spiral between malnutrition and infectious pathologies, which often is associated with chronic diarrhoea [6]. The most frequent infection found in severe undernourished children is HIV infection, which occupies an important place in this country. In fact, an HIV prevalence of 40.5% was recognized in undernourished children [3] and compounds the prognosis of these children.

At the Centre of Education and Nutritional Rehabilitation of Ouagadougou, Burkina Faso, a supplement with spirulina (*Spirulina platensis*; SP) has been used since January 2000 to improve the nutritional status of undernourished HIV-infected and HIV-negative children. The choice of this alimentary integrator, a cyanobacterium which is easily cultivated at the temperatures of Burkina Faso, was guided by a biochemical composition in amino acids, iron, and carotenoids of the SP powder. SP has recently been introduced in the treatment of undernourished HIV-infected children because several advantages of its use have been reported in the rehabilitation of adult HIV patients [7, 8]. The SP used in this study was also analysed for its glucide and lipid composition which are influenced by environmental conditions of growth.

Subjects and Methods

Study Protocol

This research was conducted at the Centre Médical St Camille (CMSC) of Ouagadougou during 2002–2003. This Centre was created in 1974 by the religious order of St Camille and comprises a maternity unit, a health centre, an analysis laboratory for biological and biochemical examination, a centre for neonatal pathology, a greenhouse for the culture of SP and a Centre for Education and Nutritional Rehabilitation (CREN). The CREN follows on average 700 children annually.

HIV-infected and HIV-negative infants and children aged <5 years were enrolled using the CONSORT criteria [9]. Dehydrated children in shock needing rapid transfer to hospital for intensive therapy were excluded from this study. Each child admitted to the protocol study was given a progressive number and at the end, each was selected with a casual number generator program. This procedure was repeated separately for each group.

Study Patients

All the children studied were undernourished according to the z-score criteria, recommended by the WHO and the Funds of the UNICEF; their average age was 14.37 (range 12–60) months. The ages were confirmed by the birth notebooks. Many of them had diarrhoea, which was treated with nasogastric rehydration according to the CMSC protocol [6], but the nasogastric rehydration was

interrupted before inclusion into the study. 170 children (84 HIV-infected and 86 HIV-negative), randomly enrolled, followed one of four rehabilitation protocols: (A) 46 HIV-negative children were treated with the SP supplementation to traditional meals (millet, vegetable, fruit); (B) 44 HIV-infected children were given the SP supplement together with the traditional meals (millet, vegetable, fruit); (C) 40 HIV-negative children were given only traditional meals, and (D) 40 HIV-infected children of the same age range were given only traditional meals. Groups C and D represent the control groups for SP 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

The Ethical Committee of CMSC gave permission for the study. All parents provided written consent for participation of their children in the study protocol. The exclusion criteria were refusal to participate in the study, while discontinuation of participation criteria were abandonment, death and the interruption of treatment at the Centre during the study.

Anthropometric Parameters

The weight of the children was recorded once a week from the day of admission to the CREN with a 10-gram sensitivity balance. The height of children <2 years was measured by resting the child in the supine position; in those children >2 years, height was measured in the upright position. The nutritional status, evaluated by brachial perimeters, was compared to Jelliffe's classification [10], considering that it varies little for children <4 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 [11].

Evaluation of Results

Evaluation of the nutritional status of children was made according to nutritional indices. The index weight for age expressed in z-score (WAZ) or weight insufficiency translates 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 translated a chronic malnutrition provoked by an extended reduction of the food consumption and by repeated pathologic episodes. The emaciation or weight loss expressed by the index weights for height (WHZ) indicates a slighter status or weight deficit due to a decrease, or slowdown of regular growth. These tests were performed to detect significant changes within the treatment groups in order to assess whether SP is a useful supplement of feeding in rehabilitation.

Biological Analyses

The haemoglobin (Hb) level, number of leukocytes, lymphocytes and neutrophils were only measured before and after 8 weeks from the start of the study among HIV-infected and HIV-negative children treated with SP, because our prime objective was to demonstrate the efficacy of this treatment in nutritional rehabilitation of HIV-infected and HIV-negative children. Blood numeration was done using a Coulter Counter T540. Hb and haematocrit levels were measured in order to determine the anaemic status. The number of lymphocytes, neutrophils and leukocytes gives an idea of the severity of immune involvement. An anti-HIV antibody test was

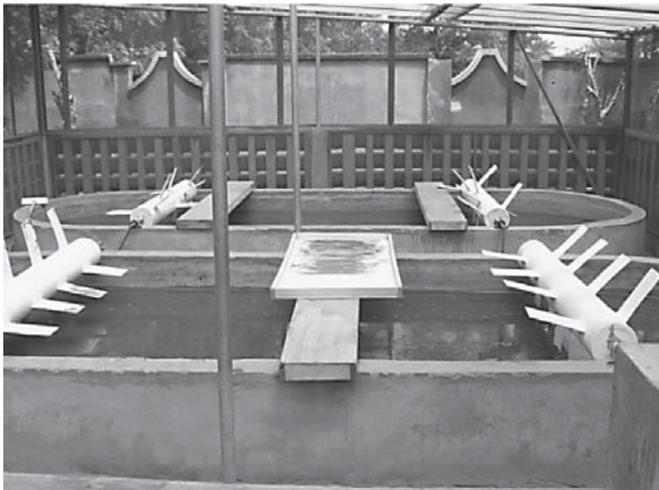


Fig. 1. Special basins for SP cultivation.

done using the BioRad Genie II Rapid Test (USA) on children whose parents freely accepted our research protocol. All of the positive samples were tested with the enzyme immunoassay technique using an Abbott IMX System (USA) in order to confirm the HIV-infected samples.

Plant Material

SP was cultivated in Burkina Faso, in artificial ponds and dried at room temperature (fig. 1). The material was stored in the dark at 4°C to prevent photodegradation.

Preparation and Administration of the SP

The mothers of the undernourished children who received SP were given weekly rations of 70 g of SP in a sachet. Every day, they had to mix 10 g of SP with a graduated container to the traditional meal of their children composed of millet flour. This mixture was made at least twice a day. It was given to children in a quantity covering their caloric requirements, and outside the suckling time in children whose mothers continued to breast-feed. The mothers were quickly instructed how to prepare mixes and feed their children at the Centre. After this preliminary phase they continued to administrate the mixture at home. Each week the mothers accompanied their children to the CREN to control weight and other anthropometric parameters.

Fatty Acid Quantification and Identification

The SP was ground and extracted 3 times with hexane. The mixture of fatty acid methyl esters was extracted with hexane and analyzed using a Hewlett-Packard gas chromatograph (model 5890) equipped with a flame ionization detector 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.

Statistical 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 determine a statistical difference. The data were prepared with Excel (Office, Microsoft) software, Epi-Info software version 6 for the anthropometric data and SPSS-10 for biological data, according to the opportunities of calculations and analysis were used. The difference between mean values before and after 8 weeks of treatment were calculated by Student's t test (for paired and unpaired data). $p < 0.05$ was considered significant.

Results

Nutritional Rehabilitation

Table 1 shows the anthropometric parameters of the HIV-infected and HIV-negative children at the beginning of the study. The impact of study participation was elevated and all children randomly chosen completed the 8 weeks of treatment.

The nutritional changes pre/post improved in all children, more significantly in the group who received SP. These changes among treatment groups are reported in table 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 SP plus traditional meals. The differences within the 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 and 20 g/day, respectively).

The HIV-negative children who were given SP plus traditional meals had a more significant ($p < 0.0003$) decrement of WHZ parameter than the HIV-infected children ($p = 0.004$). At the end of the 8 weeks of the treatment, nutritional status improved in the majority of children, but the average nutritional status of HIV-infected children was not as 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 SP group. The percentages of WHZ and WAZ are reported in table 2. The association of SP 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 only traditional meals. In HIV-negative children the gain was 42.1 and 22.19%, respectively, when SP was added to traditional meals and 17.3 and 13.53% when they were only given traditional meals.

Table 1. Anthropometric parameters of the children subjected to the study

| | A: 46 children HIV-negative with SP + traditional meals | B: 44 children HIV-infected with SP + 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 | 15.19 ± 4.35 | 14.96 ± 5.9 | p = 0.789 |
| | | p = NS | p = NS | p = NS | |
| Height, cm | 69.64 ± 1.1 | 69.72 ± 5.8 | 68.24 ± 4.5 | 69.84 ± 5.8 | p = 0.370 |
| | | p = NS | p = NS | p = NS | |
| Brachial parameter | 10.65 ± 1.1 | 10.14 ± 0.9 | 10.37 ± 1.0 | 10.40 ± 1.0 | p = 0.124 |
| | | p = NS | p = NS | p = NS | |
| Weight, kg | 6.05 ± 1.1 | 5.91 ± 1.2 | 6.10 ± 1.2 | 5.98 ± 1.1 | p = 0.882 |
| | | p = NS | p = NS | p = NS | |
| HAZ | -2.59 ± 1.5 | -2.88 ± 1.3 | -3.23 ± 1.5 | -2.64 ± 2.1 | p = 0.758 |
| | | p = NS | p = NS | p = 0.000 | |
| WHZ | -2.94 ± -0.7 | -2.87 ± 1.0 | -2.42 ± 1.02 | -2.88 ± 0.9 | p = 0.038 |
| | | p = NS | p = NS | p = NS | |
| WAZ | -3.83 ± 0.9 | -4.10 ± 0.8 | -3.99 ± 0.9 | -3.88 ± 1.0 | p = 0.503 |
| | | p = NS | p = NS | p = NS | |

HAZ = Height for age z-score; WHZ = weight for height z-score; WAZ = weight for age z-score.

Table 2. Nutritional status to the beginning (1) and at the end of the study (2)

| | A: 46 children HIV- negative with SP + traditional meals | B: 44 children HIV- infected with SP + traditional meals | C: 40 children HIV-negative with traditional meals | D: 40 children HIV-infected with traditional meals |
|--------------------|----------------------------------------------------------------|----------------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|
| WHZ1 | -2.94 ± 0.73 | -2.87 ± 1.00 | -2.42 ± 1.02 | -2.88 ± 0.95 |
| 1 → 2 | p = 0.000* | p = 0.004* | p = 0.065* | p = 0.000* |
| WHZ2 | -1.70 ± 1.62 | -2.23 ± 1.01 | -2.00 ± 0.99 | -2.58 ± 1.53 |
| WHZ2/(WHZ1 + WHZ2) | 42.1% | 22.2% | 17.3% | 10.41% |
| WAZ1 | -3.83 ± 0.91 | -4.10 ± 0.83 | -3.99 ± 0.9 | -3.88 ± 0.90 |
| 1 → 2 | p = 0.000* | p = 0.007* | p = 0.013* | p = 0.000* |
| WAZ2 | -2.98 ± 1.16 | -3.59 ± 0.91 | -3.45 ± 1.0 | -3.59 ± 1.14 |
| WAZ2/(WAZ1 + WAZ2) | 22.19% | 14.63% | 13.53% | 7.47% |

WHZ1 = Weight for height z-score at beginning of the study; WHZ2 = weight for height z-score at the end of the study; WAZ1 = weight for age z-score at the beginning of study; WAZ2 = weight for age z-score at the end of the study; * 1 → 2 Student's t test.

The biological analyses allowed some indexes which are reported in table 3. Analysis of this data shows that the best improvement was observed in the group of HIV-infected children. This response to the SP supplement was more significant than the increment of weight and height shown by WHZ and WAZ parameters. The median number of red cells and the median concentration of Hb allowed us to diagnose anaemia ($3.59 \times 10^6/\text{mm}^3$ of red cells and 8.44 g/dl of Hb) in all children. HIV-infected children were more anaemic ($3.34 \times 10^6/\text{mm}^3$) than

HIV-negative ones ($3.64 \times 10^6/\text{mm}^3$). At the end of the 8 weeks, anaemia was slightly corrected for all HIV-infected and HIV-negative children who nevertheless remained anaemic ($3.92 \times 10^6/\text{mm}^3$).

Treatment compliance was excellent and none of the children dropped out. The mothers reported that the children accepted the mixes and rarely had difficulties in feeding their children. They attended weekly appointments, but only the first and the last visit (8 weeks) were considered in the final evaluation.

Table 3. Biological parameters of the children made at the beginning (1) and at the end of the study with SP (2)

| | Children treated with SP | | | |
|--------------------------------|--------------------------|------|-----------------------|------|
| | HIV-negative (n = 46) | | HIV-infected (n = 44) | |
| | mean | SD | mean | SD |
| Red cells/mm ³ (1) | 3.64 | 0.82 | 3.34 | 0.63 |
| 1 → 2 | p = 0.000* | | p = 0.000* | |
| Red cells/ mm ³ (2) | 3.96 | 0.65 | 3.72 | 0.54 |
| Hb, g/dl (1) | 8.53 | 1.54 | 8.01 | 1.10 |
| 1 → 2 | p = 0.000* | | p = 0.000* | |
| Hb, g/dl (2) | 9.73 | 0.83 | 9.42 | 1.20 |
| Leukocytes × 1,000 (1) | 11.75 | 3.02 | 13.00 | 3.00 |
| 1 → 2 | p = NS* | | p = 0.000* | |
| Leukocytes × 1,000 (2) | 12.00 | 5.51 | 10.00 | 4.03 |
| Lymphocytes, % (1) | 38.41 | 3.59 | 39.09 | 4.48 |
| 1 → 2 | p = NS* | | p = 0.000* | |
| Lymphocytes, % (2) | 40.87 | 1.64 | 41.64 | 1.29 |
| Neutrophils, % (1) | 51.04 | 4.40 | 49.82 | 5.30 |
| 1 → 2 | p = NS* | | p = 0.000* | |
| Neutrophils, % (2) | 47.85 | 2.50 | 42.83 | 0.83 |

* Student's t test.

Table 4. CMSC's nutritional composition of 100 g cultivated SP in comparison to values of Green Flamant in the literature [12]

| | Our results, % | Green Flamant, % |
|---------------|----------------|------------------|
| 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 |

Chemical Analysis

The CMSC's composition of the cultivated SP is given in table 4. The composition values of SP of CMSC of Ouagadougou are the currently used values of the international firm Green Flamant [12]. The composition of our SP proves the good quality of the CMSC's SP. The only difference between the two is the glucide levels. The lower content in glucides of the analysed SP in our culture conditions was near to the one of Sautier and Tremolieres [13], who in 1975 found a laboratory value of 12.4% for cultivated SP. The quality of SP with the time, i.e. in the first 3 months of storage, did not show any significant changes. Some significant changes were detected for longer periods of storage, such as a decreased

protein content and an increased pH value (table 5). The lipid composition of SP growth in Burkina Faso is listed in table 6. Fatty acid content is represented by palmitic, linoleic, oleic, α -linolenic, stearic and palmitoleic acids.

Discussion

After 8 weeks of study, HIV-infected and HIV-negative children treated with SP plus traditional meals appeared improved, their weight had increased 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 might appear unethical among these severely malnourished children, but it was organized by randomly choosing a control group among children whose mothers had preliminarily accepted the protocol study. In this way, the influence of not being willing to participate in a study on caloric and nutrient intake (supplement of SP vs. traditional meals) became insignificant.

The results of this study prompted us to continue the culture of SP 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.

Table 5. Physicochemical composition of SP with the time

| Analysed sample | T0 (1st day) | T1 (1st month) | T2 (2nd month) | T3 (3rd 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/100 g | 338 | 360 | 363 | 340 | 331 |

Student's t test for paired data: T0 → T1 = p = 0.273; T0 → T2 = p = 0.310; T0 → T3 = p = 0.763; T0 → T4 = p = 0.625.

Table 6. Fatty acid composition of *S. platensis* strain from Burkina Faso

| Fatty acid | Weight % 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 |
| α-Linolenic acid, 18:3 (6, 9, 12) | 15.08 |

In the context of low intake of proteins, 10 g/day by inhabitants in Africa against 29 g in Latin America and 63 g in industrialized countries, the integration of traditional meals with SP improves the nutritional and micronutrient requirements of undernourished children [14]. Moreover, these results show that SP is also effective in haematopoiesis and especially for HIV-infected persons in accordance with the data in the international literature [8, 15]. This may be due to the iron content of SP supplement [16], which corrects anaemia due to a deficient iron intake.

Since at the beginning of this study the number of leukocytes ($13,000/\text{mm}^3$) with relative granulocytopenia was found elevated in HIV-infected children (49.8%) (table 3), the increase of lymphocyte number in HIV-infected children who received 8 weeks of SP confirms the immune modulation of this cyanobacterium. This mecha-

nism may be due to the high amount in the lipid fraction of ω-6 derivative, namely α-linolenic acid [17]. This exclusive presence of ω-6 represents a metabolic gain, since desaturase enzyme could be deficient in undernourished children [18].

The growth recovery is slower than weight recovery, and this could be determined by the diarrhoea which was present at the beginning of treatment of these children [19]. In fact, in our study regarding an 8-week period, the variations of weight were more significant owing to the liquid content dehydration associated with malnutrition. WHZ was lower in HIV-infected children in comparison with HIV-negative children (table 1) since they frequently show diarrhoea associated with HIV infection.

One could object that this study assigned SP treatment mainly to HIV-infected and non-infected children who showed more severe alterations of WHZ and WAZ values. This choice could alter the analysis of results; however, the unexpected increment in weight with the use of SP confirms the opportunity for continuing the use of this supplement in undernourished children. A previous study undertaken by Branger et al. [20] in Burkina Faso did not show a significant improvement by adding SP to traditional meals, but, as considered by the same authors, the scarce results which they obtained could be due to the quantity of SP, which was half that used in our study (5 vs. 10 g). Moreover, the present study is more conclusive than the one realized in Dakar by Sall et al. [21], in which the gain of weight was inferior, probably due also in this case to a reduced supplement in SP.

The anthropometric characteristics of the children studied varied little according to gender (table 1), but they were different according to the nutritional and serological status. This observation is the same as that of Kelly et al. [22] in undernourished HIV-infected children with persistent diarrhoea. At CMSC a prevalence of 0.82% for kwashiorkor, 95.96% for marasma and 3.22% for kwashiorkor + marasma was found, which corresponds to the effect of HIV infection of nutritional status of children in Burkina Faso [23]. Moreover, the screening of these afore-said children at CMSC confirmed 24.44% HIV-infected children. This frequency is less than the prevalence of 27% found at Bobo-Dioulasso (Burkina Faso) by Prazuck et al. [24] in 1992 and of 28.6% in Togo by Atakouma et al. [25] in 1994. The strong prevalence of kwashiorkor and/or marasma is characteristic of Sub-Saharan Africa, where maize and millet are the staple. In these countries, a high intake of linoleic acid in a diet deficient in other polyunsaturated fatty acids and in riboflavin results 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 [26]. Diet-associated inhibition of the Th1 subset is a major contributor to the high prevalence of these clinical pictures of malnutrition in Sub-Saharan areas.

Infection with HIV among undernourished children worsens the situation and highlights the problem of the costs of medical and nutritional structures. This study could suggest a preliminary solution with SP for accelerating the nutritional rehabilitation before starting an antiretroviral treatment.

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Conclusion

This study shows that malnutrition remains a public health problem in Burkina Faso. The consequence of malnutrition in association with HIV infection represents a global problem which affects morbidity as well as mortality. Awaiting for the enrolment in treatment protocols of these undernourished children with different pathologies associated to HIV, 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 paediatric malnutrition associated with HIV.

SP consists of 57.10% protein and has a high amount of ω -6 lipid component which support an efficient recovery of the precarious immune system of these children. These characteristics confirm the benefit of the SP supplement (this association gave a gain of 25 g/day in HIV-negative children). 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 malnutrition and HIV infection in African countries.

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