

Effect of *Spirulina platensis* Supplementation on Nutritional and Biochemical Parameters of Under Five Years Malnourished Children from an Orphanage in Douala, Cameroon

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Abstract: Malnutrition is the underlying cause of 50% of morbidity and mortality in the under-five age group. Its frequencies have been increasing in young Cameroon children during the past three decades (stunting $\geq 38\%$; anemia $\geq 58\%$; Zinc $\geq 69\%$ and vitamin A $\geq 38\%$)

We carried out a prospective study to assess the supplementation effect of *Spirulina platensis* on moderate and mild malnutrition on children under five years old. Seven children (4-5 years) were enrolled in this study. Children were enrolled in nutritional rehabilitation for 25 days. Each child received 8g of supplement daily, 4g in the morning and 4g in the evening. Anthropometric and haematologic parameters were measured before and after rehabilitation.

Mild and moderate malnutrition, wasting and underweight was seen in 4 children anthropometrically (no child was stunted) and haematologically in all 7 children before rehabilitation started. The children were weighted four times (First week at enrollment (W1), week 2 (W2), Week 3 (W3), Week 4 (W4)). After 25 days, a significant gain in weight was observed and varying from $17.21 \pm 2.00\text{Kg}$ (W1) to $18.45 \pm 2.07\text{Kg}$ (W2) and from $18.143 \pm 1.77\text{Kg}$ (W3) to $18.67 \pm 1.93\text{Kg}$ (W4) with their respective P- values at 0.02 (W2), 0.02 (W3) and 0.04 (W4) after rehabilitation. The following biomarkers and their constants also showed a significant variation: calcium ($73.91 \pm 16.89\text{mg/L}$ vs $88.41 \pm 14.83\text{mg/L}$ ($p = 0.03$), mean cell volume ($86.14 \pm 4.38\text{ fl}$ vs $83.86 \pm 4.83\text{ fl}$ ($p = 0.04$) and mean cell haemoglobin concentration ($32.10 \pm 0.38\text{ g/dL}$ vs $34.27 \pm 3.35\text{ g/dL}$ ($p = 0.03$) respectively before and after rehabilitation. Other biomarkers (proteins, haemoglobin, haematocrit, platelets, red blood cells, white blood cells, neutrophils, eosinophils, basophils, monocytes, lymphocytes) had also increased in non a significant manner.

The study showed that *spirulina platensis* impact positively in the fight against malnutrition by improving weight and raising the blood content of biomarkers due to its high content of proteins (60-70%) and pigments.

Keywords: Malnutrition, *Spirulina platensis*, Supplementation, Rehabilitation, Biomarkers, children.

INTRODUCTION

The world health organisation (WHO) defines malnutrition as the cellular imbalance between the supply of nutrients and energy and the body's demand to ensure growth, maintenance and specific function [1]. Malnutrition in children is the consequence of much food insecurity, which stems from poor quality, especially that which is poor in micronutrients [2] and quantity, severe and/or repeated infections or combinations of all three [3]. These conditions are linked to the standard of living and to insure maintenance of basic needs [4, 5].

According to UNICEF, 36% of children under the age of five were stunted in Cameroon, while 16% were underweight, and 7% were wasted [3].

However, in Cameroon, 12% of children in the richest quintile are stunted, highlighting that this is not an issue of food access, but caring practices and diseases control. In Cameroon under nutrition is the underlying cause for 50% of morbidity and mortality in

the under - five age group [3]. The solution for this situation however, is nutritional intervention (supplementation, fortification and diversification of foodstuffs) which helps in rehabilitating the children. For supplementation intervention to reduce malnutrition, many dietary supplements have been proposed (including *Spirulina sp.*, *Moringa oleifera*, *Glycine max* etc.). Among them, *Spirulina platensis* was found to be the best supplement, due to several advantages it possessed, notably high content of proteins, micronutrient (such as calcium, magnesium etc) pigments and so on [6]. There is today a strong need to expand the facilities available for improving the child feeding patterns especially in the developing countries through formal education on how to best harness the locally available foods to supply the much needed nutrients [3]. An adequate feeding (balanced diet) reinforce the immune system in the fight against infection [7].

In its latest guidelines regarding the choice of foods and ingredients for moderately malnourished children, the WHO concluded (for the first time) that *Spirulina* could play a role in treating children with moderate malnutrition and recommended further investigation of this subject [8]. The WHO projects that spirulina will

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become one of the most curative and prophylactic components of nutrition in the 21st century [9, 10].

Therefore, this let us to propose *Spirulina platensis* as a food supplement for the fight against malnutrition under a prospective study in Douala. In Cameroon, only one study has been done as far as spirulina is concern in the rehabilitation of Insulin resistance HIV-infected patient [11].

Type of Study

It is a prospective study carried out from 16 February to 9 March 2013 where by only seven children was enrolled. The study protocol was validated by the National Ethic Committee at Yaoundé under the research project entitle "FOOD BASE APPROACHES TO REDUCE CHILDHOOD NUTRIENT ENERGY MALNUTRITION IN CAMEROON». The approval was on the agreement that patient anonymity must be maintained, best clinical practice be ensured.

Study Area

The study took place in an orphanage "orphelinat de COMPASSION", Douala, situated at TEXACO aeroport Douala (Cameroon). It embodied (orphanage) 22 children of varied ages, which were then separated into two groups: those below 5 years and those above. Seventeen children below 5 years were selected out of 22 and the former were further separated into seven healthy and ten malnourished. Blood was collected from seven while the last three refused. Four children were anthropometrically malnourished and all seven haematologically. The purpose of choosing an orphanage is due to the fact that children are in a closed environment where they can easily be rehabilitated, control and follow-up while children in the quarter are dispersed and unstable therefore may render the study ambiguous.

MATERIAL AND METHODS

Seven children were enrolled in the study. Their anthropometric measurement (weight and height) using Salter-scale (1 - 120 Kg Cap, AMSUA at 0, 01 Kg) and tape were taken, while nutritional indices were calculated using Waterlow and Gomez formula for determination of malnutrition level. Blood sample were collected in order to determine the red blood cells counts, white blood cells count, platelets; percentage of: haematocrit, neutrophils, eosinophils, basophiles, monocytes and lymphocytes; concentration of: calcium, total proteins, haemoglobin, mean cell haemoglobin concentration, mean cell volume using URIT 810 (biométrieux) and ABX Micros 60. This was done with the aim to identify malnourished children after the first analysis. Then, those children were rehabilitated with *Spirulina platensis* in order to compensate their deficiencies.

Clinical Assessment

Anthropometric measurements included weight, height and body mass index (BMI). Body height and weight were measured without shoes and stockings using height gauge (toise) and portable Salter scale (1 - 120 Kg Cap, AMSUA at 0, 01 Kg) measure.

The measurements of height were made using height gauge (toise). The children were made to stand on a flat surface with feet parallel and with heels, buttocks and shoulders and backs of head touching the right tape. Measurements were taken to the nearest 0.1cm (see Table 1).

A portable Salter scale measure was used for weight measurement. The subjects were made to stand on the platform of the scale erect without shoes and with minimum clothing, when the pointer was at zero. Measurements were taken to the nearest 0.1kg. The

Table 1: Nutritional Indices of the 7 Children According to Waterlow's and Gomez Classification of Malnutrition before and after Rehabilitation

Children	Sex	Age (years)	Weight (Kg)		Height (cm)	Weight-for-height (%)		Weight-for-age (%)	
			BR	AR		BR	AR	BR	AR
1	M	5	15.5	16.5	106	78.61	83.67	83.33	88.70
2	M	4	17	17.5	102	97.92	100.79	106.25	109.37
3	M	5	16	16.5	109	78.92	81.38	86.02	88.70
4	M	4	15.5	16.5	108	77.16	100.12	83.33	103.13
5	F	5	20.5	21.5	114	100.45	105.35	114.52	120.11
6	F	5	19.5	20	122	89.29	91.58	108.93	111.73
7	M	4	16.5	18.5	107	90.60	101.57	103.13	115.62

BR: Before Rehabilitation, M: Male, Weight-for-height: wasting, AR: After Rehabilitation, F: Female Weight-for-age: underweight.

scale was standardized each time measurements were made (see Table 1). Anthropometric indices were calculated Gomez and Waterlow classifications of malnutrition were used. These include height-for-age, weight-for-age and weight-for-height. The measurements were made before and after rehabilitation (administration of spirulina).

Biochemical Assays

Four mL of venous blood were collected using sterile disposable 5mL syringe under aseptic technique from peripheral vein of the subjects, and place in sterile plastic. The four mL blood was separated into two aliquots; 2mL was poured into a test-tube containing anti-coagulant and the rest 2mL, into another not containing anti-coagulant for plasma and serum acquisition respectively. Collections were made before and after rehabilitation. The collected blood was taken to the nearest biochemical laboratory for treatment and conservation where it was centrifuged at the speed of 3600 rev/min at 4°C then stored at -20°C until analysis. The concentration of the following blood parameters were measured: Calcium, total proteins, red blood cells count, white blood cells count, hemoglobin, haematocrit, and blood Constance (mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), mean cell content of haemoglobin (MCCH).

Total Proteins were determined by Biuret Method that involves a Colorimetric quantitative determination of proteins giving an intensive violet-blue complex with copper salt in an alkaline medium while Iodide was introduced as an antioxidant. The intensity of the color form was proportional to the proteins concentration in the sample measured at 540nm.

Concentration of calcium was determined by colorimetric quantitative analysis. Calcium gives intensive violet coloration in an alkaline medium by forming a complex with cresolphthalein whose intensity is proportional to calcium concentration. The measurement was made with spectrophotometer read at 570nm using URIT 810 spectrometer (Biomérieux). Haemoglobin, haematocrit, Red Blood Cell (RBC), White Blood Cell (WBC), platelets, neutrophiles, eosinophiles, basophiles, monocytes, lymphocytes and blood constance were read directly from the instrument ABX Micros 60 used for this purpose.

All values obtained below the cut-off point indicated a deficiency consequently, malnutrition. The results of the two assays (before and after rehabilitation) are shown in Tables 1 and 2.

Biological Material

Spirulina was chosen in between others (*Moringa oleifera* and *Glycin max*) as material for this study and was supply by a care giver and trained mother for its production in Yaoundé. Among many food supplements proposed, notably, *Moringa oleifera*, *Glycin max* and *Spirulina platensis*, the latter was preferred due to its high content of proteins (55-70%) and various micronutrients. Moringa and soy bean had low content of proteins (5.95 and 35% respectively) compared to spirulina. Fifteen sachets of spirulina were bought for 75000CFA with each containing approximately 100g of spirulina costing 5000CFA (equivalent of 10\$ US). Before the rehabilitation started, three sachets were taken to Research Center of Food and Nutrition in Yaoundé, for the determination of protein contents. Result of the test showed that, the protein contents of the three sachets were respectively 52.66%, 53.05% and 55.84%.

Nutritional Rehabilitation with *Spirulina p.*

Preparation and Administration of *Spirulina platensis*

Malnourished children from "Orphelinat la Compassion" were supplemented with spirulina. This monitoring was done to see the effect of nutritional supplementation on rehabilitation of children for a period of 25 days.

The mother responsible for the orphanage was given in weekly base, the sachets of weekly ration of 4g x 2 (4g in the morning and 4g in the evening) x 25 days of *Spirulina platensis* in powder form to be mixed in traditional food (vegetable, fruits etc) of the malnourished children. The dose given to one child was equivalent to 200 sachets of 4g spirulina needed to appreciate absorption of nutrients while the dose for 7 children was 1400 sachets. Measurement of 4g of spirulina was done using a balance for measuring foods. The content of proteins in 4g of spirulina was 2.8g, while 8g of spirulina contain 5.6g of proteins. Eight grams (4g x2) was mixed in traditional meal or eaten solely daily, 4g in the morning and 4g in the evening to derive 5.6g of proteins.

The methodology was described in the Figure 1 described.

Duration of Administration

The duration of administration lasted for 25 days. This allowed us to know whether the supply of different

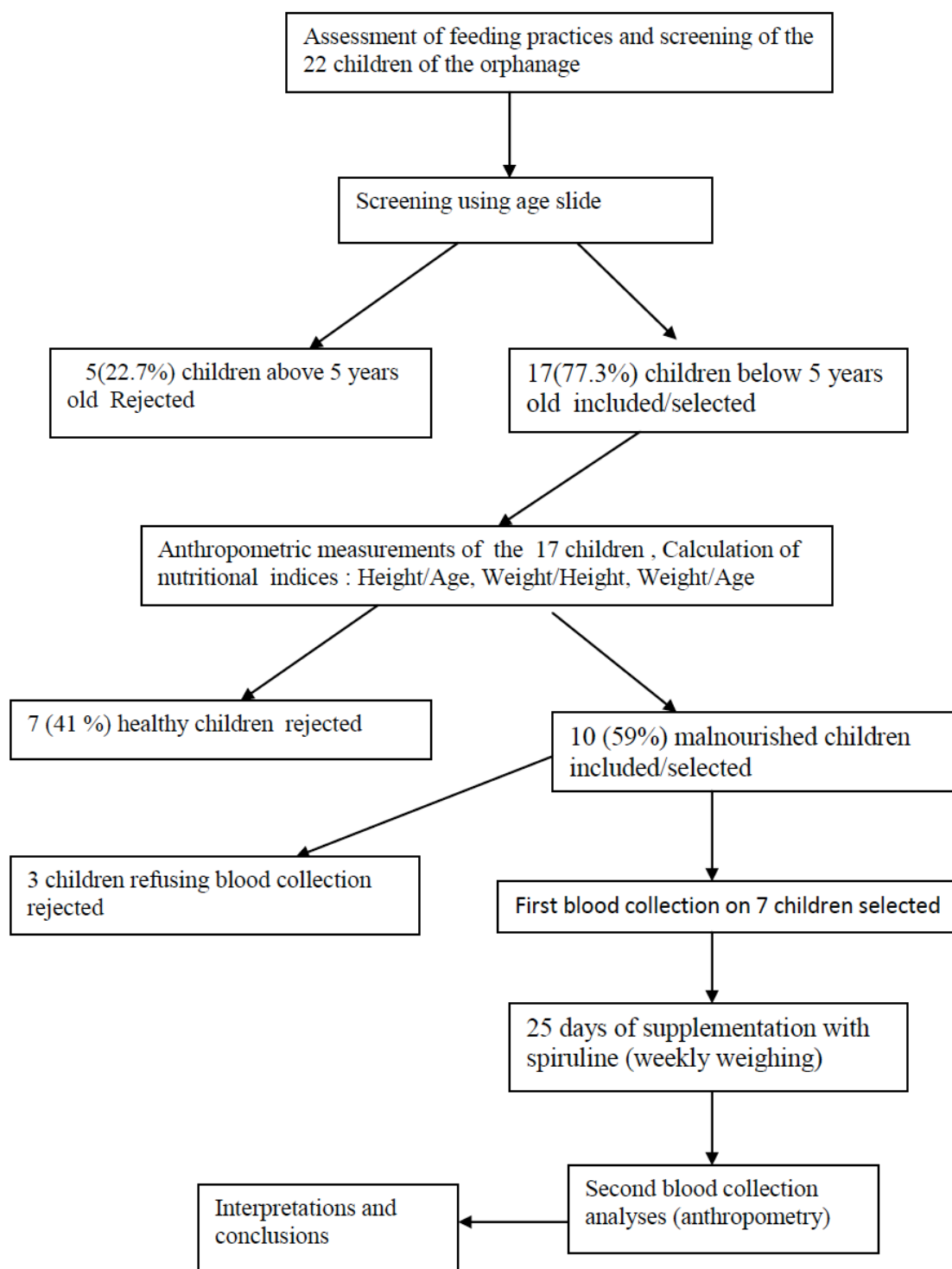


Figure 1: Schematic Protocol of Field Work.

type of nutrients has been filled despite the short duration of rehabilitation. The period taken for rehabilitation to be done was not sufficient to appreciate maximum gain in weight and absorption of deficient nutrient.

Four mL of blood were re-collected for the second time (after 25 days of rehabilitation) at the end of

supplementation to test for the presence of micronutrients already mentioned above.

Analysis and Interpretation of Data

Statistical analyses of data were performed using Statview 5.0 (SAS Inc., USA). Spearman's correlation (IC95%, p -value ≤ 0.05), Row test of Wilcoxon (Non

parametric test of appariated series) were used to analysed anthropometric and haematologic parameters. A p-value of < 0.05 was interpreted as indicating statistically significant differences.

RESULTS

After we have gone through our study which was to assess the effect of *spirulina platensis* supplementation on nutritional and biochemical parameters of under five years malnourished children in Douala, Cameroon, two results, both anthropometrical and haematological before and after rehabilitation were obtained. The results of nutritional indices calculated from anthropometric data are summarized on the Table 1 below using to the classification of Waterlow and Gomez. The result showed the occurrence of malnutrition in 4 children (children 1, 3, 4 and 6) while the remaining three children (2, 5 and 7) were normal (See Table 1) as obtained from nutritional indices before rehabilitation and biochemical assay. Three children were moderately malnourished (Children 1, 3, and 4) and only one was mildly malnourished (child 6). A child was said to be moderately malnourished if his or her value of nutritional indices lied between 70-80% in the classification of waterlow (weight-for-height: wasting) and 60-74% in Gomez classification (weight-for-age: underweight) or mildly malnourished when it is between 80-90% in the classification of waterlow (weight-for-height: wasting) and 75-89% in Gomez classification (weight-for-age: underweight) respectively. The normal cut-off for the two respective classifications are >90 and 90-110%. The results of anthropometry only give broad information of malnutrition while biochemical assay gives detail information. The latter showed the occurrence of malnutrition in all the children before the process of rehabilitation.

After administration of spirulina to the children, their nutritional indices (both weight-for-height and weight-for-age) were improved significantly and dependently with an increase in weight. From the table below Children 2, 4, 5, 6 and 7 had regained their normal weight-for-height (90%), while children 1 and 3 continue to have mild malnutrition (80-90%) despite the weight gain. The reason is that, the time taken for the process of rehabilitation was short if not all will have become normal. Children 2, 5, 6 and 7 had normal weight-for-age (90-110%) while children 4, 1 and 3 had mild malnutrition (75-89%).

Biochemical Assay after Rehabilitation

After rehabilitation, a significant increase in calcium content, Mean Cell Volume (MCV) and Mean Cell Haemoglobin Concentration (MCHC) was observed (see Table 2 below). On the other hand non significant increased of proteins, haemoglobin, haematocrit, red blood cell, white blood cell, neutrophile, eosinophile, basophile, monocytes and lymphocytes was also observed as seen in Table 2 below.

DISCUSSION

After 25 days of rehabilitation with *Spirulina platensis*, an improvement in nutritional indices and an increase in immunological markers were observed in some of the children. Previous studies also reported increasement of anthropometric indices after supplementation with spirulina [11, 12]. Similar results were obtained by Salle *et al.* in 1999 in Senegal under the study of nutritional rehabilitation [13]. Nutritional indices were recalculated after administration of the supplements to the children. The improvement obtained, may be explained by the fact that spirulina is a supplement that is rich both in micronutrients and

Table 2: Results of weight variations throughout the weeks of rehabilitation (after 25 days)

Children Weighing(Kg)	1	2	3	4	5	6	7
W_1	15.5	17	16	15.5	20.5	19.5	16.5
W_2	16.5	18.5	17	16.5	21.5	21	18
$W_2 - W_1$	1	1.5	1	1	1	1.5	1.5
W_3	16.5	17.5	17	16.5	21	20	18.5
$W_3 - W_2$	0	1	0	0	-0.5	-1	0.5
W_4	17.5	17.5	16.5	16.5	20.5	20	17.5
$W_4 - W_3$	1	0	-0.5	0	-0.5	0	-1
$W_4 - W_1$	2	0.5	0.5	1	0	0.5	1

W_1 : week 1, W_2 : week 2, W_3 : week 3, W_4 : week 4.

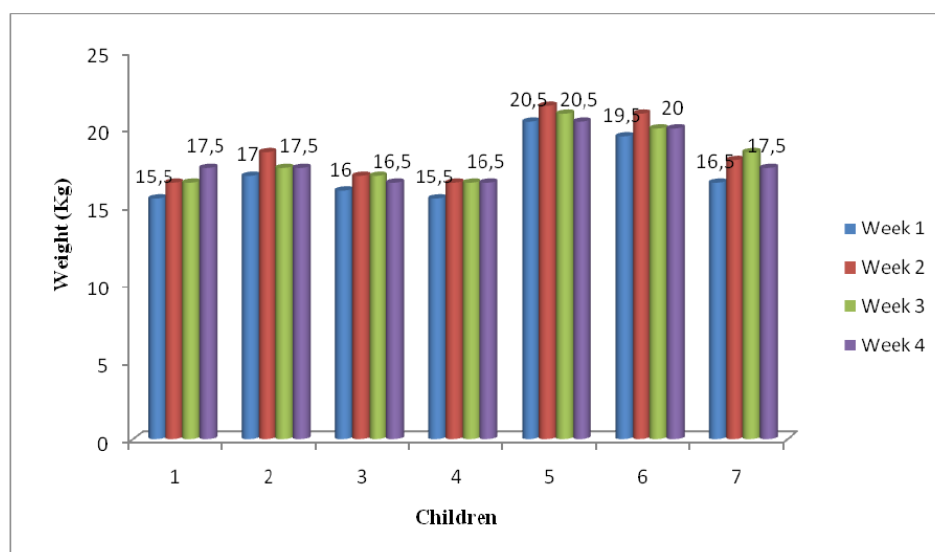


Figure 2: Average children weights for 25 days.

It is observe that from the first to the last day of the experiment, the children weights were increased.

Table 3: Mean Weights and p-value expressed as Mean Kg \pm Standard deviation

Variables	Mean \pm SD (Kg)	p-value
Weight (W_1)	17.21 \pm 1.99	-
Weight (W_2)	18.43 \pm 2.07	0.02*
Weight(W_3)	18.14 \pm 1.77	0.02*
Weight(W_4)	17.67 \pm 1.93	0.04*

Compared to the first week all the p-values were less than 0.05 indicating significant differences.

Table 4: Variables before and after Rehabilitation Expressed as Mean \pm Standard Deviation

Variables	Before rehabilitation	After réhabilitation	p-value
Red blood cell	9.29 \pm 2.69	9.19 \pm 1.75	0.79
White blood cell	3.70 \pm 0.41	3.60 \pm 0.34	0.46
Haemoglobin	10.06 \pm 0.72	10.54 \pm 0.90	0.07
Haematocrite	31.54 \pm 2.29	30.09 \pm 2.42	0.13
Platelet	421.71 \pm 86.29	353.71 \pm 97.23	0.09
Neutrophiles	49.50 \pm 22.78	57.86 \pm 7.01	> 0.99
Basophiles	0.57 \pm 1.51	0 \pm 0.00	Not available
Eosinophiles	10.57 \pm 18.30	4 \pm 1.52	0.73
Monocytes	4.36 \pm 1.03	4.43 \pm 0.79	> 0.99
Lymphocytes	35 \pm 6.83	33.71 \pm 8.32	> 0.99
Total proteins	62.61 \pm 2.08	69.03 \pm 2.25	0.06
Calcium	73.91 \pm 16.89	88.41 \pm 14.83	0.02*
MCV(VGM)	86.14 \pm 4.38	83.86 \pm 4.23	0.04*
MCCH(TGMH)	27.63 \pm 1.27	28.66 \pm 2.74	0.10
MCHC	32.10 \pm 0.38	34.27 \pm 3.35	0.03*

MCV: mean cell volume, MCCH: mean cell content in haemoglobin, MCHC: mean cell haemoglobin concentration, RBC: red blood cell, WBC: white blood cell *: significant; Values are expressed as Mean \pm Standard Deviation (SD).

macronutrient. It contains high percentage of macronutrient (especially proteins which is 60-70%) and micronutrient (especially iron, calcium and magnesium).

On the other hand, a similar study on 52 HIV-positive patients compared a group supplemented with spirulina and a group supplemented with soya beans [11]. This work showed a comparable efficacy for

spirulina and soya beans as regards weight gain, but also a significant increase in immunological markers for the group treated with spirulina, and not with the group treated with soya beans.

The gain in proteins and weight obtained in the course of this study was similar to the result observed by [11, 13]. However, the child whose total proteins experienced a decrease after rehabilitation was also had low calcium was found bonded or linked with protein during its metabolism. Studies had shown that, there are statistically significant correlations between calcium and total proteins and between calcium and albumin in normal subjects, but the correlation coefficients are low (less than 04) and because of the limited ranges of the observed values the regression lines cannot be extrapolated with confidence to low proteins concentrations [14]. Keating and colleague also reported a relation between calcium and total proteins, calcium and albumin in patients who showed a wide range of abnormalities of proteins concentration.

Individual calcium needs are affected by the rate of growth, the degree of absorption, and the availability of other nutrients, including vitamin D and phosphorus, as well as calories and proteins. Dietary substances that may decrease calcium retention include caffeine, excessive phosphorus intake, oxalic and phytic acids, and proteins [15]. When the content of calcium in a diet is poor, it affects the synthesis of red blood cells which takes place at the bone marrow. After rehabilitation, the concentration of calcium of 6 children had increased significantly as seen above. This was due to the fact that the nutrient content of *Spirulina* in terms of calcium is high (1000mg/100g of spirulina) [11]. The increase in calcium blood content simply implies that, calcium has been absorbed by the body after ingestion of spirulina. This has also gone a long way to show the high absorption and digestibility of spirulina in the body.

The levels of haematocrit (PCV) of six children out of seven were below the cut-off point level while only one child had normal cut off point value after rehabilitation. Low values of PCV (anaemia) are indicative of bacterial infections and gastrointestinal disorders, including parasitism and haemorrhage [16], or may reflect nutritional deficiencies of minerals like iron or copper [17]. This suggested that malnutrition in the Orphelinat la Compassion may also be due to frequent infections.

There was a decrease in platelets value for all the children excepting child 2(from 333 to 428mm³) where an increase is observed. This increase can be

explained by the effect of spirulina on biomarkers (platelets) where it leads to hematopoiesis including platelets [18]. No matter the decrease in platelet, the children still maintain their cut-off point of platelets and none of them was observed to have value below the cut-off level.

An increase in RBC was observed in the following child 1(from 3.55 to 3.80), child 2(from 3.24 to 3.29), and child 6 (from 3.65 to 3.71), while RBC for child 7(from 3.54 to 3.54) remained constant. *Spirulina platensis* has an increasing effect on many of the analysed biomarkers, including RBC. This was also observed in other works as long as beneficiaries were in deficit [13]. In another similar study improvement of haematopoiesis was observed (production function of a blood and oxygen transport) [18]. A deficiency in oxygen-carrying red blood is defined as iron deficiency anaemia (IDA). It is the most common cause of anaemia which is frequently related to vitamin B12 or folate deficiency. Two boys of 5 years old had abnormal RBC values (3.04 and 3.29x10⁶mm³), below the cut-off after rehabilitation which can be interpreted as anemia. Their initial values were 3.47 and 3.24 respectively. They fall sick the second week of the study. Common factors leading to IDA include insufficient iron intake or poor dietary sources of iron (e.g., vegetarian/vegan diet), early introduction of whole cow's milk [19, 20].

The increase of WBC was influenced by the spirulina intake which set off the activation of immune system defense function, as observed elsewhere [21].

The increase in neutrophils, eosinophile above the normal range of child 6, is due to reinforcement, activation of the immune system by spirulina as reported in previous studies, reason for slight elevation [21]. Many studies carried out using animals by Pascaud in 1993 and Borchers *et al.* in 2007 had also shown similar results with the reinforcement of the immune system after administration of spirulina [22, 23].

The results of basophile before and after rehabilitation of the 7 children were respectively 0.0 except for child 1, where the value before rehabilitation was 4 beyond the cut-off point. This implies that there were infections before rehabilitation, while after rehabilitation the values were reduced significantly after healing of infections as described by [24].

The increase of monocytes observed with the administration of spirulina may be due to the set off of

the immune defence's activation. This activity was demonstrated by many authors, after they have studied a polysaccharide (Immulina) compound derived from spirulina as set off of immune defences [18, 25, 26].

After administration of spirulina, we had the following values for lymphocytes: 27, 42, 42, 41, 25, 31 and 44% respectively for each child (1 to 7). The increase for and decrease observed in children was due the effect spirulina on defence and maintenance of an immune system as described previously in other studies [27, 28].

The decrease of MCV ($<80\mu\text{m}^3$) observed usually seen in microcytic anaemia due to iron deficit or during inflammatory syndrome and thalassemia. MCV defines the average volume of RBC's. Defining anaemia as micro, normo or macrocytic is the initial step in diagnosing ID. After rehabilitation we observed the following results for MCV respectively in the same order for 7 children (1 to 7): 27.6, 29.7, 34, 28.8, 26.2, 25.8 and 28.5 pg. However, the normal value of MCCH ranges between 26-36 pg. An increase in MCCH was noticed in all the children except child 6 whose value decreases from 26.2 to 25.8. The children having their MCCH values below the cut-off point may had microcytic anaemia and they also include child 4(25.9) and 5(25.8) before rehabilitation.

MCHC test reveals after rehabilitation the following results: 32.9, 32.5, 41.2, 36.1, 32.0, 32.8 and 32.4g/dL. The normal cut-off point is 31.5-37g/dL. The results showed that all values are normal despite the increase (effect of spirulina on biomarkers).

CONCLUSION

Supplementation with spirulina in less than five years old children have shown to play a beneficial role in the fight against malnutrition (protein energy malnutrition and micronutrient deficiency). Further studies, involving more subjects and for a longer duration, are needed to replicate these results and uncover the underlying mechanisms involved.

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