

**IMPACT OF AN INTEGRATED NUTRITION INTERVENTION ON  
NUTRIENT INTAKES, MORBIDITY AND GROWTH OF RURAL  
BURKINABE PRESCHOOL CHILDREN**

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## ABSTRACT

Iron deficiency and undernutrition often co-exist among children and the prevalence in sub-Saharan African is high. In Burkina Faso, 92% of children under the age of five years were anaemic and 39% were stunted in 2003 according to Demographics and health survey. Risk factors include inadequate dietary intakes and infections that often occur concomitantly. Orphans and vulnerable children who are already at increased risk for poor psycho-emotional development due to their social status are particularly susceptible to malnutrition. Therefore, there is a challenge to develop effective interventions that address the multiple risk factors of nutritional deficiencies in these children. An integrated nutrition intervention including dietary modification and changes in hygiene practices was implemented targeting a group of preschool orphans and vulnerable children living in group foster homes. The amount of iron-rich foods particularly goat meat and condiments as well as citrus fruits were increased in the diet. The hygiene component of the intervention focused on handwashing, eating in individual plates, food storage and stool disposal. All members of the foster homes at large benefitted from the activities of the intervention; however, measurements and impact evaluation focused on preschool children (12 - 72 months). Energy and nutrient intakes, anthropometric indices and morbidity including diarrhoea, fever, vomiting and respiratory infections were assessed at baseline and after 18 weeks. Mean bioavailable iron intake was increased from 0.4 to 0.9 mg/d. The intervention resulted in a decrease in the prevalence of inadequate intakes for energy, proteins and most micronutrients. The intervention increased the meat-fish-poultry contribution to energy and iron intakes from 1.6 to 3.5% and from 5.2 to 7.9%, respectively. There was also a decrease in the incidence of infection (from 73 to 9%,  $p < 0.01$ ) and overall morbidity (from 39 to 15%,  $p < 0.05$ ) most likely due to deworming and improvements in hygiene behaviours. Contrary to baseline, helminths such as *Hymelolepis nana*, *Strongyloïdes stercoralis* and *Necator americanus* that often cause blood loss were rarely found at the end of the intervention. The overall changes resulted in improvements in height-for-age z-score (-1.63 to -1.27,  $p < 0.01$ ) and weight-for-age z-score (-1.22 to -1.06,  $p < 0.05$ ). In conclusion, an intervention that targets bioavailable iron and hygiene behaviours improved child growth, iron status and overall morbidity in areas of high incidence of infection.

**Key words:** dietary intervention, growth, morbidity, nutrient

## INTRODUCTION

Iron deficiency impairs behavioural and cognitive development, growth and immune function of children. The main cause of iron deficiency in sub-Saharan Africa is inadequate bioavailable iron intakes that often occur concomitantly with other nutrient deficiencies and infection. In Burkina Faso, 92% of Burkinabe preschool children were anaemic in 2003 [1]. Stunting rate was also high in the same group of children. Orphans and vulnerable children are particularly at increased risk of poor nutrition. A previous study suggested that iron deficiency were highly prevalent among a group of Burkinabe preschool orphans and vulnerable children, and that about 62% of the cases of anaemia were associated with undernutrition, infection and morbid conditions [2]. Therefore, there is a challenge to develop effective interventions that simultaneously address the multiple risk factors of nutritional deficiencies in these children who are already at increased risk for psycho-emotional development. Dietary enhancement with a focus on bioavailable iron is assumed to be the most sustainable means of controlling iron deficiency anaemia. However, few studies have investigated the impact of dietary enhancement on iron status in developing countries. Some community-based interventions targeting bioavailable iron and zinc increased bioavailable iron and the overall nutrient intakes of children [3-4]. Therefore, dietary enhancement has the potential to improve iron status and might enhance the growth of children, although results of intervention studies are conflicting. Some authors reported growth improvement [5], while other studies have failed to detect changes in growth indices [6].

Community-based trials have shown that changing hygiene behaviours could decrease the incidence of childhood diarrhoea and infections including respiratory infections [7], factors which have been associated with iron deficiency and growth failure [8-9]. One study suggested that such an improvement in hygiene behaviours could lead to better growth [10]. It was hypothesized that an intervention that simultaneously addresses bioavailable iron and hygiene behaviours could improve iron status as well as growth and general health of preschool children in Burkina Faso, where infection significantly contributes to anaemia. The present study aimed to assess the impact of simultaneous enhancement of dietary iron and improvement in hygiene behaviour on nutrient intakes and adequacy, growth and morbidity of these children.

## MATERIALS AND METHODS

### Study design and participants

The study included preschool children living in group foster homes in Central Burkina Faso. The group foster home program is a humanitarian initiative that provides to orphaned, abandoned and other vulnerable children a familial environment and basic needs (food, shelter, clothing, education, and healthcare). In group foster home approach, unrelated children who are in need of care are reunited to form a family (alternative family) headed by a woman hired from the community and allocated an individual house. Details of the study population have been described elsewhere [11]. All members of the group foster homes at large benefit from the activities of the intervention. However, measurements and impact evaluation focused on preschool

children (12 - 72 months) of both sexes who were admitted in foster homes more than one month before the beginning of the study. A child was excluded if s/he presented severe anaemia ( $Hb \leq 80$  g/L), severe undernutrition (weight-for-height, height-for-age or weight-for-age z-score  $< -3$ ) or human immunodeficiency virus (HIV). Overall, 58 children living in 11 group foster homes and aged 12-71 months were recruited. Nine children were excluded for the following reasons: 2 were severely anaemic, 2 others were severely undernourished and 5 took iron/folate supplements within the previous two months. Further fourteen children were not included in the intervention trial because they were not permanently living in the foster homes. Of the 35 children who participated in the 18-week intervention, 34 completed the study; however, follow-up blood data were missing for one child.

Informed written consents were obtained from the managerial staff of the group foster homes. Verbal consent for the child's participation in the study was obtained from his/her caregiver. The study protocol was approved by the National Ethics Committee for Health Research (Burkina Faso) and Laval University Ethics Committee for Research (Canada).

## Measurements

### *Dietary assessment*

Energy and nutrient intakes of children were assessed based on the household food consumption assessment approach using consumption units (CU) because in this region, all family members ate together from a communal dish [12]. Dietary intakes of each foster family were assessed using 3 non-consecutive days weighed food records. To determine a child's food intake for a meal, the overall consumption for a family was divided by the total amount of CU ( $\sum UC_i$ ) of all individuals participating in that meal and multiplied by the child's CU ( $CU_i$ ). Consumption units used was obtained from the Department of Agriculture of Morocco (CU) as follows:  $CU = 1.0$  for each male older than 14 y;  $CU = 0.8$  for each female older than 10 y; and  $CU = 0.3 + [0.05 * \text{age (in y)}]$  for each male aged 14 y or younger and each female aged 10 y or younger [13]. Dietary inadequacy was expressed as the proportion of children whose intakes were below the Estimated average requirements (EAR) issued by the Institute of Medicine [13].

Several algorithms for predicting iron absorption have been developed, but they lack agreement and none has been developed in African settings [14]. In addition, the phytate content of most local foods consumed in Burkina Faso is not available. Therefore, for the baseline estimation of bioavailable iron, an absorption rate of 3.5% which is the average between WHO/FAO recommendations (5%) and estimation from a Moroccan study (2%) was used [12, 15]. In post-intervention, when sufficient amounts of iron absorption enhancers were present in the diet, a 5% bioavailability was used.

### *Nutritional status and morbidity*

Age was estimated from the reported date of birth obtained during the demographic assessment. When the birthday was unknown, the 15<sup>th</sup> day of the month was used and



when the month of birth was unknown, the midpoint of the year of birth was used. Weight was measured to the nearest 50 g on a medical scale (Lifesource Precision Health Scale model UC-321 PL, A & D Medical, Milpitas, California, USA) and height to the nearest 0.1 cm using a stadiometer (Seca Bodymeter 206, Hamburg, Germany) for children wearing light clothes and no shoes. Z-scores for weight-for-height/length (WHZ), height/length-for-age (HAZ) and weight-for-age (WAZ) were calculated using the WHO new growth standards [16]. Malaria parasite density was determined by counting *Plasmodium* parasites from Giemsa-stained thick blood smears. Intestinal parasites were determined using direct microscopic examination by Kato-Katz simplified method.

During the intervention period, each caregiver was asked to record daily, in a morbidity journal, whether the child experienced morbid conditions such as fever, diarrhoea, vomiting or respiratory tract infections. The incidence of each condition was determined as the proportion of children who experienced this condition per month.

## **Intervention**

### *Dietary enhancement and modification*

Data from a preliminary survey that aimed to collect information on dietary patterns and food availability were used together with baseline iron intakes to plan meals using the Institute of Medicine framework for planning diets in assisted-living facilities [17]. Estimation of the target bioavailable iron intake and dietary planning assuming a 10% prevalence of inadequacy are described elsewhere [11]. Briefly, to reach the target iron intake, caregivers were advised: 1) to increase during selected meals (5 times/week), the consumption of goat meat and vitamin C rich foods in order to enhance iron absorption, 2) to include in Saturday's breakfast a goat liver broth that could also improve vitamin A intakes in addition to providing a high bioavailable iron and, 3) to increase iron rich condiments (Soumbala, smoked fish, dried yeast) in sauces (6 times/week). It was also recommended giving an additional cereal-based porridge as a snack in order to increase energy intakes.

### *Hygiene intervention*

The intervention included sanitation activities that focused on hand-washing, eating habits, defecation and stool disposal behaviours to reduce the exposure to infection. Caregivers were invited to quickly eliminate children's feces and to wash their hands carefully with soap before food processing and after toilet activities. Children were trained to wash their hands with soap before meals. Also, since all family members ate in a communal dish that might increase the risk of parasitic contamination, a dish was provided for each child and individual eating was implemented. All children and their caregiver were provided with an antihelminthic medication before the beginning of the intervention. Finally, group meetings were held monthly with all caregivers to discuss progress and potential gaps in the implementation of prescribed activities. Caregivers were also met individually when necessary.

## Data analyses

Food consumption data were processed in order to determine the nutrient intakes using Nutrifig<sup>(TM)</sup> software, version 2.0. This software was developed at the Food Sciences and Nutrition Department (Laval University) based on the 2005 Canadian Nutrient File. Nutrifig was modified to include Burkinabe foods from the Food composition table for Mali [18]. The WHO Anthro 2005 software (WHO Anthro 2005, World Health Organization, Geneva, Switzerland) was used to process anthropometric data. Statistical analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC, USA).

Continuous variables were checked for normality. Non-normal variables such as serum ferritin were log-transformed and log transformed values were checked for normality. Pre- and post-intervention z-scores and, energy and nutrient intakes were compared using paired t-tests. Baseline and follow-up prevalence of wasting, stunting and underweight as well as the incidence of morbid factors were compared using McNemar t-test for paired proportions. To test the hypotheses, it was assumed that a p-value less than 0.05 was "statistically significant" and a p-value ranging between 0.05 and 0.10 was considered "marginally significant".

## RESULTS

### Dietary patterns

Children consumed an average of three meals daily. Breakfast consisted of bread, couscous (millet or red sorghum) with yoghurt or porridge (millet or maize based) and sugar. The most common staple dishes for lunch and dinner were "to" and different recipes made with rice (fatty rice, rice with navy beans or white rice with sauces). "To", a thick dough obtained by cooking sorghum or maize flour in a mixture of water and acidic juices, was consumed seven times per week. It was served with different sauces prepared from various ingredients. The eight items used alternatively as a base for sauces were: dried okra (*Hibiscus esculentus*), sorrel leaves (*Hibiscus sabdariffa*), bean leaves, baobab leaves (*Adansonia digitata*), tossa jute (*Corchorus olitorius*), Kapok tree calyces (*Ceiba pentandra*), *Centrosema pubescens* leaves and sesame seeds (*Sesamum indicum*). Condiments such as salt, potash (extract of wood ashes), tomatoes and onions were always included in the sauces. Other ingredients were often added to sauces: sea butter, red palm oil, peanut oil, peanut butter, local yeast, garlic, squash, parsley, celery, eggplant, cabbage and green pepper. Animal foods were less frequently consumed. Smoked fish was often added to sauces in small amounts as condiments. Goat meat (about 14 g/d/child) was consumed twice per week, but was not sufficient to enhance non-heme iron absorption.

### Impact of the intervention on nutrient intakes and adequacy

The energy and nutrient intakes of children are shown in Table 1. The mean ( $\pm$ SD) daily energy intake at baseline was  $1052 \pm 275$  kcal. The total iron intake was 10.8 mg/d and 96% was nonheme iron. Mean bioavailable iron was estimated at 0.4 mg/d and more than three-quarters of the children were below the EAR at baseline. Mean intakes for vitamin A, B<sub>6</sub>, B<sub>12</sub> and C were lower than the EAR. In post-intervention, intakes of energy, protein and iron were higher compared to baseline. Also, vitamins

A, B<sub>12</sub> and C and zinc intakes increased, while mean intakes of vitamin B<sub>6</sub>, fiber as well as the fiber: iron and fiber: zinc ratios decreased (Table 1).

As indicated in Figure 1, energy was provided at baseline by cereals (73.7%), legumes (12.6%), oil/fat (6.1%) and dairy products (3.4%). The main sources of iron (Figure 2) included cereals (57.3%), legumes (17.8%), vegetables and fruits (16.8%), spices and condiments (2.4%) and milk (0.5%). Meat-fish-poultry contributed only 1.6% and 5.4% of total energy and iron intakes, respectively. In post-intervention, the cereal contribution to energy and iron intakes increased, although not significantly. The intervention increased the meat-fish-poultry contribution to energy and iron intakes by 119% (from 1.6 to 3.5%) and 52% (from 5.2 to 7.9%), respectively (Figures 1 and 2). Vegetables and fruits contribution did not change for energy but decreased for iron, whereas condiment contribution to iron intakes increased.

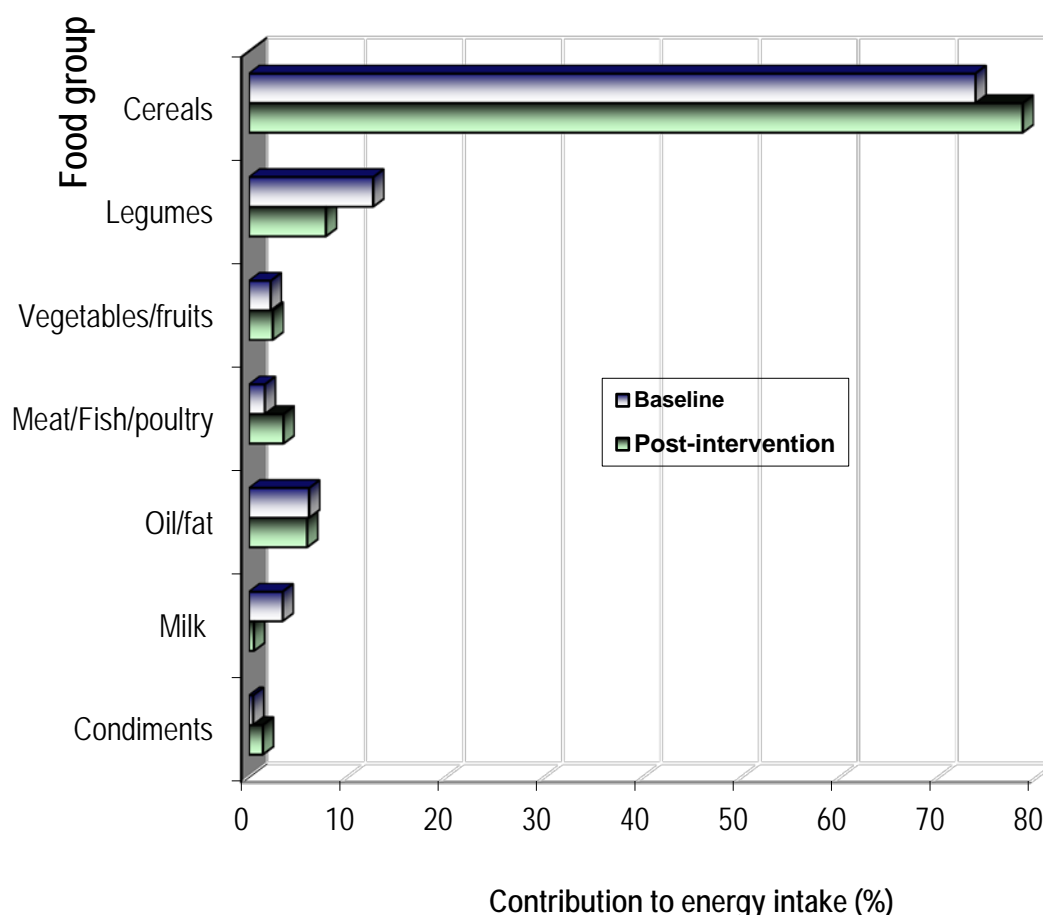


Figure 1: Food group contribution (%) to energy intake following 18 weeks of intervention

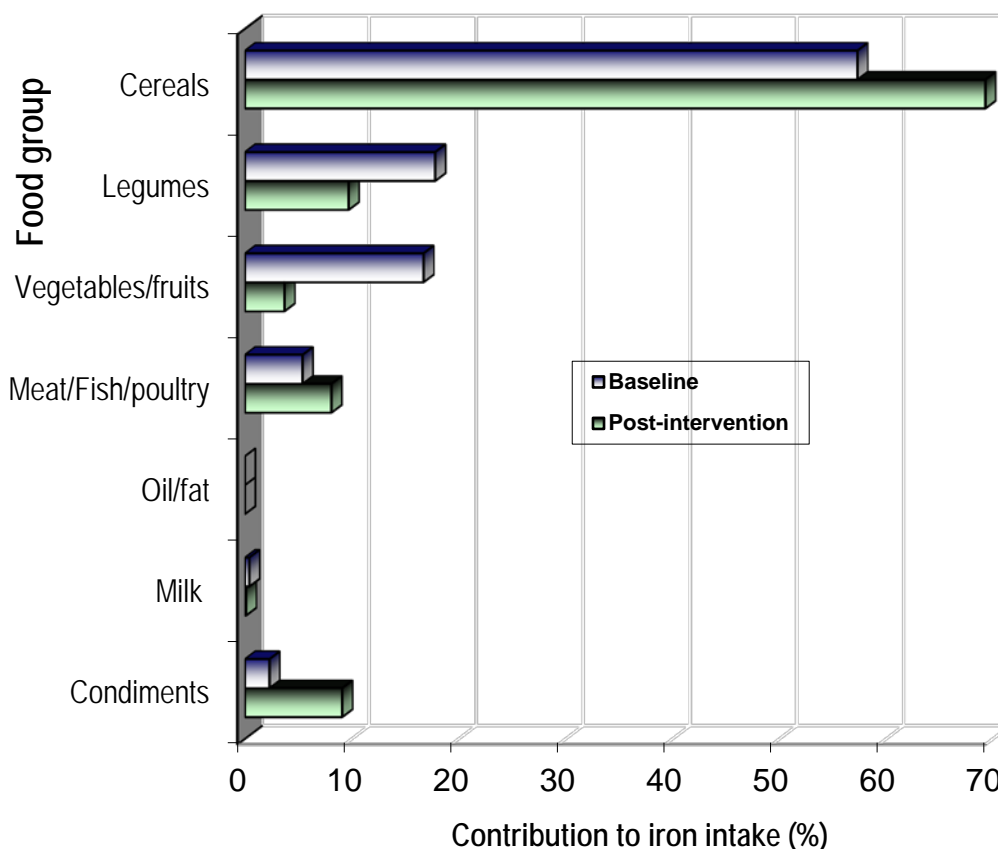


Figure 2: Food group contribution (%) to iron intake following 18 weeks of intervention

### Impact on morbid factors

The prevalence of intestinal parasites was reduced from 73 to 9% (Table 2). Contrary to data obtained at baseline, helminths such as *Hymelolepis nana*, *Strongyloides stercoralis* and *Necator americanus*, which often cause blood loss, were rarely found at the end of the intervention. Malaria prevalence remained low and comparable to the baseline value (6% vs 3%,  $p=0.56$ ). Frequencies of overall morbidity symptoms reported by caregivers including fever, diarrhoea, vomiting, respiratory infection decreased by 54%.

### Impact on nutritional status

Anthropometric characteristics are presented in Table 3. After 18 weeks of intervention, children gained about 4 cm in height and 1 (one) kg in weight. Compared to baseline, HAZ (-1.63 vs -1.27,  $p<0.01$ ) and WAZ (-1.22 vs -1.06,  $p<0.05$ ) were significantly higher in post-intervention, while the change in WHZ was not significant (-0.41 vs -0.35,  $p=0.01$ ). Prevalence of stunting and underweight



decreased. Of the eleven children with baseline HAZ < -2, five (45%) recovered from stunting after 18 weeks. Three children who were underweight became non-underweight and two wasted children became non-wasted.

The impact of the intervention on iron status indicators is presented and discussed elsewhere [24]. Briefly, mean hemoglobin increased from 98.7 to 103.8 g/L ( $p=0.006$ ). Total iron binding capacity (TIBC) decreased from 107.2 to 91.5 ( $p=0.05$ ) while mean transferrin saturation increased from 12.6% to 17.4% ( $p=0.06$ ) suggesting a marginal improvement. There was a slight reduction in iron deficiency from 94% to 79%, but this reduction was not significant ( $p= 0.23$ ).

## DISCUSSION

The nutrient intakes of children participating in the current study were mainly provided by plant based-foods. In targeting bioavailable iron by raising the consumption of meat and vitamin C- rich foods, the overall nutrient intakes increased. Such changes combined with improvement in hygiene behaviours lowered morbidity variables, incidence of infection and prevalence of stunting in post-intervention.

In the present study, cereal-based foods were the main source of energy and nutrients, followed by legumes. The food consumption patterns concerning the main food groups for children were comparable to patterns observed in the overall population of Burkina Faso as reported by the FAO. Indeed, according to the FAO Statistics Division, dietary energy is provided by cereal products (74%), legumes (9%), oil/fat (5%), sugar and sweeteners (2%) and meat products (2%) [19]. The results suggest that food group consumption patterns of preschool children are similar to those of adults in this area of Burkina Faso. Some studies conducted elsewhere reported similar dietary patterns [2, 20-21]. Markedly inadequate intakes in this study were observed for vitamins A, C, B<sub>6</sub>, B<sub>12</sub>, folate and absorbable iron.

Eighteen weeks after the beginning of the intervention, the mean nutrient intakes increased and children were at lower risk of inadequate intakes for most nutrients, except for vitamin B<sub>6</sub> and fiber. The improvement of the diet quality was also reflected by increases in the contribution of animal source foods to energy and iron intakes. Although it was not possible to quantify micronutrient bioavailability, the decrease in fiber: iron and fiber: zinc ratios might be indicative of the improvement in iron and zinc absorption. Further, during the intervention, potash, local yeast and “soubbala” were most often used in the daily preparation of sauces. Since “soubbala” is a fermented product, high bioavailability of its iron content is expected. An appropriate combination of ingredients including dried fish, potash and “soubbala” in rural Burkinabe children dishes could increase the *in vitro* digestibility of iron and zinc in sauces by 2 and 3 times, respectively [22].

In the current study, nutrient intakes provided by cereal products were higher in post intervention, although the difference was not significant ( $p>0.05$ ). Such observation is in line with findings from Malawi where it was found that increasing animal source foods did not displace intakes of other food groups [5].

Anthropometric variables suggested poor nutritional status of children prior to nutrition intervention, with stunting being the most prevalent form of malnutrition. The intervention in this study slightly improved HAZ and WAZ and induced reversal of stunting after 18 weeks. Other studies including school-age children have reported increased growth for a comparable follow-up period [23-24]. However, a study in Benin did not find any improvement in growth, 3 and 10 months after providing preschoolers with albendazole and iron supplements without enhancing the quality of food [21].

Because stunting results from the effects of several risk factors, the improvement in nutritional status is likely due to the cumulative effects of increased energy and overall nutrient intakes, de-worming and sanitation improvement.

As mentioned above, energy and nutrient intakes increased during the intervention and prevalence of inadequate intakes was reduced for most nutrients. Since, plant proteins have low digestibility and efficiency ratio and lack some essential amino acids such as lysine, methionine and cysteine [25], the increased consumption of animal source foods might also have contributed to accelerated growth.

The present study has certain limitations that need to be taken into account when interpreting the results. The first limitation is the lack of a control group. Undoubtedly, a randomized control trial is the best choice for evaluating the impact of a nutrition intervention. However, in this particular situation, it was determined that a randomized controlled design was not appropriate because the overall outcome of the intervention was the improvement of iron status of a group of orphans and vulnerable children who were already at risk of poor development. Since the third Demographic and health survey of Burkina Faso reported a prevalence of anaemia higher than 92% among preschool children in this region, it would have been unethical to include a placebo group due to the irreversible adverse effects of iron deficiency in this age group [26-27]. An iron supplement group was also envisaged but not retained because the safety of routine supplementation in malaria endemic areas was uncertain at the time the intervention was implemented [28].

Caution should also be used in interpreting these data because of the small sample of the study participants that was drawn from a specific and finite population, reducing the statistical power and generalization of the findings. All the same, it is worthwhile to save even a single child with this kind of intervention.

Another limitation of the study is the group eating approach used to assess dietary intakes that do not take into account factors such as health status, infection and food preferences that affect dietary intakes. However, the researchers still believe that this is the most suitable approach to evaluate dietary intakes of several children eating together with their hands from a communal dish. The use of a food frequency questionnaire and a 24 h dietary recall would also have been inappropriate in this context due to the low education level of caregivers. Indeed, it was difficult for caregivers to remember and differentiate the consumption of each child when dietary

assessment was required for more than one child under their care. An attempt has been made to minimize biases in capturing familial intakes using weighed food records over three non-consecutive days including one week-end day and two week days.

Despite these limitations, it was evident that the intervention increased energy, protein and some key micronutrient intakes. There was also a reduction in the prevalence of morbid factors such as infection, fever and diarrhoea. A slight improvement in HAZ was also observed with more significant impact on the most undernourished children. This study suggests that enhancing bioavailable iron intakes through a food-based intervention increased in combination with adequate improvement in hygiene behaviours can be a relevant option for improving child growth and overall morbidity.

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**Table 1: Daily nutrient intakes and inadequacy at baseline and post-intervention<sup>1</sup> (n=33)**

	Nutrient intakes (mean ± SD)		Nutrient inadequacy (% < EAR <sup>2</sup> )	
	Baseline	Post-intervention	Baseline	Post-intervention
<b>Macronutrients</b>				
Carbohydrate (g)	191 ± 30	213 ± 34 **	0	0
Protein (g)	32 ± 30	39 ± 27 **	0	0
Total fat (g)	15 ± 14.3	17 ± 3.3 *	-	-
Fiber (g)	47 ± 27	18 ± 2 **	0	51.5‡
<b>Micronutrients</b>				
Iron				
Total iron (mg)	10.8 ± 2.6	17.3 ± 3.5**	0	0
Absorbable iron (mg)	0.38 ± 0.10	0.9 ± 0.17**	78.8	6.1**
Zinc (mg)	4.9 ± 2.7	6.7 ± 1.3**	0	0
Vitamin A (µg ER)	64 ± 28	541 ± 125**	100	3.0 **
Vitamin C (mg)	14.2 ± 2.4	42.7 ± 7.4 **	63.6	6.1 **
Vitamin B <sub>6</sub> (mg)	0.38 ± 0.17	0.34 ± 0.10 **	57.6	87.9 *
Vitamin B <sub>12</sub> (µg)	0.55 ± 0.23	3.23 ± 0.21 **	84.9	0 ‡
Folates (µg)	73 ± 16.0	154 ± 57 **	100	18.2**
Fiber : iron ratio	4.4 ± 1.16	1.1 ± 0.08 **	-	-
Fiber : zinc ratio	9.5 ± 2.47	2.8 ± 0.14 **	-	-

\*: p < 0.05, \*\*: P < 0.01

‡: Statistics were not computed if there was only one percentage or data were missing for both periods

<sup>1</sup>: The duration of the nutrition intervention was 18 weeks

<sup>2</sup>: Nutrient intakes were compared to the Estimated average requirement (EAR) according to the Dietary reference intakes [13].



**Table 2: Trends in incidence of infection and morbid conditions (n=33)**

	Baseline	Post-intervention <sup>1</sup>
<b>Intestinal parasites</b> (prevalence, %)	<b>72.7</b>	9.1**
<i>Entamoeba histolytica</i>	45.5	< 1 †
<i>Entamoeba coli</i>	18.2	3.0
<i>Giardia intestinalis</i>	6.1	12.1
<i>Trichomonas intestinalis</i>	12.1	< 1 †
<i>Hymelolepis nana</i>	15.2	< 1 †
<i>Strongyloides stercoralis</i>	3.0	< 1 †
<i>Necator americanus</i>	3.0	< 1 †
Malaria ( <i>Plasmodium falciparum</i> )	6.1	3.0
<b>Morbid conditions reported by caregivers</b>	39.4	18.2*
(% over one month)		
Fever	33.3	15.2 ‡
Respiratory tract infections	18.2	6.1 ‡
Diarrhoea	12.1	3.0
Vomiting	3.0	6.1

<sup>1</sup>: The duration of the nutrition intervention was 18 weeks

\*\* $p < 0.01$ , \* $p < 0.05$ , ‡ $p < 0.10$

†: All children examined were free of this parasite

**Table 3: Anthropometric characteristics at baseline and post-intervention<sup>1</sup>**  
 (n=33)

	Baseline	Post-intervention	Difference
Age (y)	3.54 ± 1.3	3.96 ± 1.3	0.42
Weight (kg)	13.2 ± 2.6	14.2 ± 2.5	1 **
Height (cm)	92.8 ± 9.5	97.1 ± 9.1	4.3 **
Weight-for-height z-score (WHZ)	-0.41 ± 0.98	-0.35 ± 0.79	0.06
Wasted children (WHZ < -2, %)	6.1	3.0	3.1
Height-for-age z-score (HAZ)	-1.63 ± 0.82	-1.27 ± 0.83	0.36 **
Stunted children (HAZ < -2, %)	33.3	18.2	15.1
Weight-for-age z-score (WAZ)	-1.22 ± 0.72	-1.06 ± 0.68	0.16 *
Underweight (WAZ < -2, %)	18.2	9.1	9.1 ‡

<sup>1</sup>: The duration of the nutrition intervention was 18 weeks

\*\* : p < 0.01, \* : p < 0.05, ‡ : p < 0.10

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