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Nutrition Education of Mothers and Zinc Supplementation among Children in the Rural Community, Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Author NPN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MMKS designed the study and managed the analyses of the study. Author MJM performed data collection and managed the literature searches. Author IG supervised all the steps of study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Malnutrition among children, especially stunting is a public health problem in Cameroon. This study assesses the impact of zinc supplementation of children and nutrition education of mothers on the nutritional status of the children in the Bangang rural community.
Study Design: This was a descriptive and prospective study.
Place and Duration of Study: The study took place in the Bangang community in the Region of West Cameroon, during the period from March to December 2015.
Methodology: The children aged 6 to 48 months and mothers aged 20 to 34 years were selected after the baseline survey and enrolled. Dietary surveys were used to evaluate the frequency of foods consumed by 150 children. Zinc supplementation group of children (ZSG, n= 25) received 10 mg of zinc sulfate tablets per day for 14 days and control group (CG, n=25) was formed by children whose mothers received nutrition counseling. The nutrition education sessions organized into 4 modules were conducted quarterly for 9 months on a sample of 100 mothers. After interventions,

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impact of zinc supplementation and maternal education was assessed by determining height for age and weight for age indices, and biochemistry parameters.
Results: The results showed that zincemia of ZSG varied significantly (*P* = .0001) and not significantly (*P* = .23) for CG. After nutrition education, dietary diversity was improved; reduction of chronic malnutrition (10.9%) and increasing number of children with good nutritional status (6.6%) were observed. Increased for phosphoremia (3.6 ± 2.4 to 5.7 ± 1.8 mg/dl; *P* = .001) and albuminemia (34.8 ± 15.5 to 46.9 ± 8.9 g/l; *P* = .002) were significant which was not the case of calcemia, zincemia, magnesemia and serum iron.
Conclusion: This study showed positive impact of zinc supplementation and maternal education on the nutritional status of children.

Keywords: Zinc supplementation; nutrition education; children; Bangang; malnutrition.

1. INTRODUCTION

Malnutrition is a major global public health problem particularly in communities of developing countries [1]. Indeed, malnutrition refers a pathological state resulting from relative or absolute deficiency or excess of one or more essential nutrients [2]. Although, malnutrition can affect any age group but children are at a higher risk. It is associated with an increased morbidity and mortality [3].

Undernutrition is an underlying cause of almost half (45%) of deaths in children under five worldwide, promotes an increased susceptibility to infection and predisposes to poor physical and cognitive development [4]. Globally, 144 million (21.3%) children under-five years of age suffering from stunting, 47 million (6.9%) suffering from wasting and 38.3 million (5.8%) are overweight [5]. Africa and Asia bear the greatest share of all forms of malnutrition. In Africa, according to several Organizations, 40% children under-five were stunted, 27% wasted and 24% overweight [5]. The stunting and wasting rates are above global estimates, albeit inter-country variations. Although childhood stunting is ubiquitous round the world, it is particularly prevalent in low and middle income countries [6]. Stunting is associated with cognitive and behavioral problems, delayed mental development, poor school performance, and reduced intellectual capacity [7].

A national multiple indicator cluster survey indicated that in Cameroon, 31.7% of children under five are stunted, 14.8% are underweight and 5.2% are wasted [8]. In rural community, high prevalence of malnutrition was observed among children, particularly stunting, poor knowledge on feeding practices, low education and socio economic level of mothers [9,10].

Inadequate food intake and zinc deficiency are followed by multiple consequences among children. Zinc deficiency in children is associated with growth retardation and contributes to a large amount to childhood morbidity and premature mortality [6].

These situations are so important that, the WHO adopted a resolution on maternal, infant and young child nutrition including a global target to reduce by 40% the number of stunted under-five children by 2025 and by 2030, end all forms of malnutrition [11,12]. To participate in this wide program and improve nutritional status of the children, this study was conducted and assessed the impact of zinc supplementation of children and nutrition education of children' mothers in the Bangang rural community.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in the Bangang rural community, were previous field work was carried out. This community is located in the Region of West Cameroon, Department of Bamboutos. It has a tropical rainy grass field and savannah forest vegetation, humid climate with a population of 250 000 inhabitants.

2.2 Study Design

A cohort of 150 children (100 for nutritional education and 50 for zinc supplementation) and 100 mothers (75 for nutritional education and 25 for nutritional consultation in zinc supplementation) was recruited in the Bangang community. The ages of the children ranged from 6 to 48 months with a mean of 16 ± 6 months.

2.3 Procedures

A questionnaire was developed, tested, adjusted and validated prior the field survey. During the study, the knowledge of mothers on children nutrition, the eating habits of children, the estimated consumption of energy, nutrients of children and food availability on the market during harvesting and sowing seasons were evaluated. Subsequently, the impact of the interventions was evaluated by comparing the anthropometric parameters (height-age and weight-age indices) and biochemical parameter (serum albumin level, calcium, zinc, iron, magnesium and phosphorus) before and after these interventions.

2.4 Dietary Surveys

Food history was made during the months of March, June, September and December, representing small and great sowing and harvesting periods. The reason of choosing different months in harvesting and sowing seasons was to evaluate food access and availability in each season. The eating habits of children whose parents agreed to participate in the study were reported. In fact, mothers of those children listed all meals including drinks consumed by children each day for a week.

2.4.1 Food intake

The food diary was used to compare the food consumption between healthy children (2 < z-score below < -2) and malnourished children (z-score below -2), and to determine the amount of food consumed by children every day for a week. The amount of food consumed by each child was weighed using measurement tools (standard bowls and cups) and the balance (Kern EMB 2000 -2; Germany) to reach 2,000 g and 0,001 g accuracy. The food composition table [13,14] was used to evaluate the composition and nutrient content of food consumed by children.

2.4.2 Bangang market

The market investigation took place at Bangang market (The big market day is held 1 time per week) during the same periods as the food history. This survey was to identify all major traded food and determine their frequencies.

2.5 Nutritional Status of Children

2.5.1 Anthropometric parameters

The weight and height were measured before and after interventions. We used electronic balance (AEG PW4923, Germany) with a range of 0-150 kg with accuracy of 0.1 g for children 24 to 48 months, and the scale baby weighs (EBSA Kinlee-20, Germany) with a range of 0-20 kg with accuracy of 0.5 g for children under 24 to 6 months. The height was measured with a sliding latch (Kern MSF200, Germany) for children aged 12 to 48 months, or a length board (Kern MSA80, Germany) for children less than 12 month.

The nutritional status of children was assessed by z-score according to the WHO new Growth Standards in 2006 [15]. Stunting and underweight were defined as a z-score of height-for-age (HAZ) and weight for age (WAZ) respectively below -2. Stunting or chronic malnutrition was classified as moderate when the z-score of height for age was between -2 and -3 and severe when below -3 compared to the reference population. Underweight was classified as moderate when the z-score of weight for age was between -2 and -3 and severe when below -3 compared to the reference population.

2.5.2 Biochemical parameters

Blood samples were collected from children. Before sample collection was carried out by a qualified nurse, wearing gloves and labeling tubes with code number was mandatory. The blood was collected from children following standard techniques of blood collection between 8 and 10 AM. The collected blood was centrifuged at 3600 rpm for 10 minutes and the obtained serum was used for the determination of albumin, zinc (Zn), phosphorus (P), iron, magnesium (Mg) and calcium (Ca) by colorimetric method and the determination of their concentrations by means of a spectrophotometer (URIT-810, China).

2.6 Intervention

2.6.1 Zinc supplementation intervention

In this pilot study, a sample of 50 children with stunting was selected and all of these children had hypozincemia. Zinc supplementation group of children (ZSG, n= 25) received an oral dose of 10 mg of zinc sulfate tablets per day for 14 days. The control group (CG, n=25) was formed by children whose mothers (n=25) received nutrition counseling. In this nutrition counseling, the mothers were encouraged to give regularly the food rich in zinc (fish, meat, eggs, dairy products and vegetables) to their children. Before and after zinc supplementation, anthropometric parameters of children were taken and the serum zinc concentration was evaluated.

2.6.2 Nutrition education intervention

Nutrition education sessions organized into modules was conducted on a sample of 75 mothers of children. The information supplied was intended to facilitate a change in bad eating habits observed, improve knowledge, attitudes and skills of mothers on child nutrition. The participatory approach based on dialogue and demonstrations was adopted during this nutrition education which was held once quarterly for nine months. The first nutritional session was held in June in the Bangang Medical Center and the two others (September and December) in family homes by appointment made with mothers. During the dialogue with the mothers, we were with community workers whose role was to ease local language communication when necessary. Nutritional information was delivered individually to each mother for those who were not in the same concession and group for those who were. The duration of the interview varied from one mother to another, according to his capacity for understanding and spoken national language. The information was grouped into four modules: In the food practice module, the benefits of breast milk, the duration of the breastfeeding and the consequences of a poor diet for children were clarified. In the food diversity module, the roles of food constituents from animal and plant origin were taught. We also showed that for the harmonious development of a child, its diet must be diversified. In the food supply module, choice of child's foods on the availability of food and the cost was demonstrated. Processing and storage of food in times of plenty were encouraged. In the socio-cultural and economic aspects module, it was recommended to avoid the consumption of foods with low energy density and avoid selling all grown produce and livestock to market at the expense of consumption. Before and after nutrition education of the mothers, anthropometric parameters of children were taken and calcemia, zincemia, magnesemia, phosphoremia, serum iron and albuminemia were evaluated.

2.7 Statistical Analyses

The analyses have been done by using the statistical software SPSS 16. Descriptive statistics were used to determine the means and percentages of the various parameters. Student's t test was used to compare the means values two by two and to appreciate the significance before and after nutrition education and zinc supplementation. The Pearson chi-square test was used to analyze the difference frequencies of food consumption between healthy and malnourished children. Results were considered significant at $p < .05$.

3. RESULTS AND DISCUSSION

About 5.1%, 82.3% and 12.6% of the mothers said that they weaned their children before the age of one year, before 2 years of age and 2 years of age respectively. Approximately 18.6% of mothers did not know the value of colostrum. An hour after birth, 40.6% of mothers had not breastfed their children. Several mothers, specifically 16.2% and 7.1% gave honey and water respectively on the first day of birth of their children. The age of introduction of complementary feeding was not known by 49% of mothers. Almost 23.2% and 34.1% of mothers had learned how to nurse their children through their mothers and nurses respectively. All of these children ate the food, 42.6% once to twice a day and 57.4% three times a day.

3.1 Food Consumption

3.1.1 Frequency of consumption

The food consumption was classified in two groups of children. The frequency of meals in healthy children was 3 times a day and their diet was diversified. The frequency of meals in malnourished children with stunting ranged from 1 to 2 times a day. The Table 1 shows that, eggs (7% to 1%; P = .03), dairy products (12% to 4%; P =.04) and vegetables (24% to 12%; P=.03) consumption frequency was significantly higher in healthy children than in malnourished children. Low consumption of fruit in the two groups of children was observed (2%). The nutritional status of these children varied according to the quality of food consumed, monotony and food diversification which justify that proper nutrition helps to promote growth and harmonious development [16].

Table 2 shows the frequency of meals consumed by children during the harvest periods (greater harvest "June" and small harvest "December") and the sowing periods (small sowing "September" and large sowing "March").

Pounded Irish potatoes, Rice with peanut sauce, “Jallot” rice, “Jallot” potatoes, Corn meal with okra and Corn meal with leaves were the regular meals and had a high frequency of consumption in children in households during the different seasons (Table 2). The frequencies of consumed food in harvest periods were higher than those in sowing periods. This can be explained by monotonous eating habits which do not take into account the different seasons but rather the cultural aspect. This poor nutrition in quality could be due to a poor knowledge of children's nutrition and the education level of mothers and in long-term could be the cause of chronic malnutrition (stunting) in some children [9].

3.1.2 Estimated daily energy and nutrient intakes

The average micronutrient intakes are presented in Table 3. The daily intake of calcium and zinc did not cover the recommended needs of children in all age groups. Similarly, average daily iron intake did not cover the recommended needs of children in the age range 12 - 36 months. The coverage rates for the consumption of magnesium and phosphorus in these children were above 100% in all age groups (Table 3).

Table 4 shows that the energy consumed daily by children did not cover the recommended needs in all age groups, except for girls in age group 36 - 48 months. The most affected age groups for boys were 6 - 12 months and 24 - 36 months with coverage rates of 56.9% and 63.7% respectively. In girls, the most age groups concerned were those of 6 - 12 months and 12 - 24 months with a coverage rate of 67.3% and 67.0% respectively (Table 4).

The daily consumption in calcium, iron, zinc (Table 3) and total energy did not cover the recommended energy needs (Table 4). This poor nutrition in quantity could be due to a poor knowledge of children's nutrition and the education level of mothers. Although the level of education is not synonymous with knowledge of eating practices, it could influence the nutritional status of children due to poor understanding of the food needs and nutritional values of the foods served to children by mothers. The low coverage of energy needs is due to the insufficient quantity and quality of food consumed by children or to the consumption of food with low energy density which in the long term could lead to chronic malnutrition.

3.2 Impact of Interventions

3.2.1 Impact of nutrition education on the nutritional status of children

After nutrition education, only 70 mothers and 92 children were presented. Before nutrition education with mothers, the anthropometric and biochemical parameters of I00 children were taken. After 9 months of this intervention, only 92 children were present with age between 6 to 38 months. In Table 5 the average values of HAZ index (-2.34 ± 1.4 to -1.9 ± 2 z-score, P = .39) and WAZ index (-0.42 ± 1.5 to -0.46 ± 1.4 z-score, P = .59) were not varied significantly.

Before nutrition education, 56 (60.9%) and 36 (39.1%) children had stunting and a good nutritional status respectively and after, 46 (50%) children had stunting, 4 (4.3%) were underweight and 42 (45.5%) had good nutritional status. These results showed a reduction of chronic malnutrition by 10.9%, but also an increase of 6.6% of children with good nutritional status. The children with the most improved nutritional status were those in the 6 - 12 months age group. This would be due to the improvement of the quality of the food of the children of this age group consisting of breast milk and complementary foods or family foods. Table 5 shows significant improvement of phosphoremia (P = .001) and albuminemia (P = .002), which was not the case of the calcemia (P = .35), zincemia (P = .29), magnesemia (P = .91) and the serum iron (P = .35).

Before nutrition education on mothers, the prevalence of hypoalbuminemia, hypozincemia, hypomagnesemia, hypocalcemia, hypo-phosphoremia and deficiency of serum iron among children were 15%, 19%, 12%, 20%, 7% and 8% respectively. Micronutrients are essential and their deficiencies could be due to poor consumption of animal products and poor bioavailability of these micronutrients from plant based foods [17]. Dairy products are rich in nutrients that are essential for good bone health, including calcium, potassium and phosphorus in an easily absorbed form [18]. Heme iron is estimated to contribute 10 - 15% of total iron intake in meat eating populations, and has better bioavailability. Iron bioavailability has been estimated to be in the range of 14 - 18% for mixed diets and 5 - 12% for vegetarian diets [19]. Meat, fish and certain shellfish such as oysters are good sources of zinc [17]. Insufficient coverage of micronutrients could also lead to stunting as zinc, calcium and iron are essential for growth, behavioral and neurological development of infants [17,20]. For children, the magnesium and phosphorus coverage were greater than 100%, the serum deficiency in these micronutrients could be due to low consumption of animal products and interactions with other minerals. Meat, fish, milk and dairy products, even if they contain on the whole less magnesium and phosphorus some plants are

Table 1. Frequency of food consumption according to nutritional status

Foods intake	Scientific names of the majority food	Frequency of consumption (%)		P-value
		Healthy children	Malnourished children	
Pounded Irish potatoes	*Solanum tuberosum*	67	89	<0,0002*
Rice / peanut sauce	*Oriza sativa*	47	23	<0,0003*
‘‘Jallot’’ rice	*Oriza sativa*	29	35	0,36
‘‘Jallot’’ potatoes	*Solanum tuberosum*	28	29	0,87
Corn meal / leaves	*Zea mays/Amaranthus*	24	12	0,03*
Banana stew	*Musa sapientum*	17	20	0,58
Cocoyam / yellow sauce	*Colocasia esculenta*	16	17	0,80
Corn porridge / soybean	*Zea mays*	11	3	0,03*
Corn porridge simple	*Zea mays*	15	14	0,84
Dairy products	-	12	4	0,04*
Corn meal / okra	*Hibiscus esculenta*	18	14	0,44
Rice / beans	*Phaseolus vulgaris*	1	5	0,10
Eggs	-	7	1	0,03*
Water	-	100	100	1
Fruits (orange, papaya, avocado)	*Citrus, Carica papaya, Citrullus lanatus*	2	2	1

** Significantly different*

Table 2. Food frequency consumed by children during different survey periods (n = 100)

Foods intake	Frequency of consumption (%)				
	Periods				
	March	June	September	December	Cumulative frequencies
Pounded Irish potatoes	32	50	35	38	155
Rice / peanut sauce	14	24	22	33	123
‘‘Jallot’’ rice	13	22	26	27	88
‘‘Jallot’’ potatoes	12	19	24	21	76
Corn meal / okra	7	13	19	23	62
Corn meal / leaves	8	13	24	23	68
Banana stew	7	12	19	17	55
Cocoyam / yellow sauce	7	8	16	14	45
Dairy products	4	5	10	16	35
Corn porridge	7	5	10	8	30
Rice / tomato sauce	7	5	9	8	29
Irish potato pure	4	5	9	9	27
Rice / beans	4	5	8	7	24
Corn porridge / soybean	3	6	5	7	21
Grounded cocoyam / peanut sauce	3	2	3	4	12
Fruits	2	3	4	2	11
Patatoes / leaves	2	1	1	2	6
Yam stew	1	0	1	3	5

Table 3. Estimated and rates of coverage of micronutrient intakes in children (n = 100)

Nutrients	Ages(months)	P (mg)	Ca (mg)	Iron (mg)	Zn (mg)	Mg (mg)
Mean daily intake	6-12	466.4 ± 281.1	326 ± 72.2	10.5 ± 9.4	2.46 ± 1.2	99.6 ± 34.3
FAO/WHO daily needs		275	500	6-10	5	75
Rates of coverage		169.6	65.2	105	49.2	132.8
Mean daily intake	12-36	765.4 ± 77.9	297.4 ± 61.2	4.4 ± 1.1	3.9 ± 3.5	200.5 ± 26.2
FAO/WHO daily needs		450	700	7	7	130
Rates of coverage		170.1	42.5	62.8	55.7	123.2
Mean daily intake	36-48	782.5 ± 131.4	424.5 ±132.8	7.3 ± 1.1	6.3 ± 3.6	201.3 ± 84.6
FAO/WHO daily needs		600	900	7	7	200
Rates of coverage		130.4	47.2	104.3	90	100.7

Values expressed are mean ± standard deviation good sources of these micronutrients due to better bioavailability [21,22]. The Bangang market had all the food groups, but children's diet was unbalanced in quality and quantity. This could be due to poor knowledge of the diets of children by their parents, but also due to cultural factors. The average values of biochemical analyzes before and after nutrition education (Table 5) showed significant improvement in phosphoremia (3.6 ± 2.4 to 5.7 ± 1.8 mg/dl; *P* = .001) and albuminemia (34.8 ± 15.5 to 46.9 ± 9 g/l; *P* = .002), which was not the case of the calcemia (6.7 ± 3.1 to 7.9 ± 0.9 mg/dl; *P* = .35), magnesemia (1.99 ± 1 to 2.01 ± 0.2 mg/dl; *P* = .91) and the serum iron (96.3 ± 62.9 to 108.9 ± 22.2 mg/dl; *P* = .35). This proves that nutrition education is a means of choice for improving the nutritional status in a population because it provides the ability to simultaneously improve not only micronutrient intake but also many dietary constituents [23]. Table 5 shows also that, height for age and weight for age indices were -2.34 ± 1.4 and -0.42 ± 1.5 respectively before nutrition education and -1.9 ± 2 and -0.46 ± 1.4 respectively after nutrition education. These differences of anthropometric indices were not significant; this could be justified by the fact that diet remained monotonous during the tracking period, but also the relatively short observation time (9 months). Some children, whose diet was as diverse and adequate in quantity, could have their nutritional status improved significantly only in the long term.

3.2.2 Impact of zinc supplementation

The zincemia of supplemented children varied between 20.3 to 63.7 µg/dl before zinc supplementation and 45 and 165.1 µg/dl after this intervention (14 days). Table 6 shows the variations of serum zinc concentration according to the period of zinc supplementation. The zincemia of these children increased significantly (*P* = .0001) after zinc supplementation (Table 6). For the control group, the zincemia varied between 30.4 and 64.4 µg/dl the first day and 34 and 110 µg/dl fifteenth day after the nutrition counseling to their mothers. Table 6 also shows that the zincemia of control group was not significantly increased (*P* = .23) after the nutrition counseling. After 14 days, the zincemia of supplemented children was significantly higher (*P* = .0001) than zincemia of control group (Table 6). This impact could be explained by rapid bioavailability of supplemented zinc. The results of this study corroborate with others results which showed that supplementation with zinc alone significantly increases the serum zinc concentration in children [24]. In addition to improving serum zinc status, zinc supplementation could also prevents and reduce morbidity and mortality in young children [25]. On the other hand, zinc supplementation in drug form costs more than the consumption of foods rich in zinc which are readily available (products of animal origin). For non-supplemented children (control group), the difference between zincemia before and after the observation period was not significant. This result could be explained by a low consumption of animal products [17], but also a high consumption of grains, beans, legumes and plant-based diets which affect a bioavailability of zinc due to the presence of anti-nutritional factors such as phytates and oxalates [26,27].

The main limitation of this study was the lack of formulation of food recipes based on locally available and accessible food resources. This should maximize the intake of micronutrients and essential nutrients in children. The strength of this study was an important contribution on nutrition public health interventions to reduce children malnutrition.

Table 4. Daily intakes and coverage of energy by children (n = 100)

Age group (months)	Coverage daily energy					
	Boys (n = 46)			Girls (n = 54)		
	Daily energy intake (kcal)	Daily needs (kcal)	Coverage (%)	Daily energy intake (kcal)	Daily needs (kcal)	Coverage (%)
6 – 12	441.1 ± 189.6	775	56.9	479.5 ± 208.7	712	67.3
12 – 24	734.9 ± 336.2	950	77.4	569.4 ± 248.1	850	67.0
24 – 36	716.5 ± 197.4	1125	63.7	-	-	-
36 – 48	1048.3 ± 331.2	1250	83.9	1257.2 ± 709.2	1150	109.3

Values expressed are mean ± standard deviation

Table 5. Biochemical and anthropometric parameters according to the period of nutrition education

Nutrition education	Biochemical parameter						Anthropometric indices	
	Alb (g/l)	P (mg/dl)	Ca (mg/dl)	Zn (µg/dl)	Mg (mg/dl)	iron (µg/dl)	HAZ (z-score)	WAZ (z-score)
Before	34.8 ± 15.5	3.6 ± 2.4	6.7 ± 3.1	74,9 ± 25,8	1.99 ± 1	96.3 ± 62.9	-2,34 ± 1,4	-0,42 ± 1,5
After	46.9 ± 9	5.7 ± 1.8	7.9 ± 0.9	82,7 ± 26,6	2.01 ± 0.2	108.9 ± 22.2	-1,9 ± 2	-0,46 ± 1,4
P-value	0.002*	0.001*	0.35	0.29	0.91	0.35	0,39	0,59

*Alb: Albuminemia; P: Phosphoremia; Ca: Calcemia; Mg: Magnesemia; iron: Serum iron; HAZ: height for age; WAZ: weight for age; Values expressed are mean ± standard deviation; * Significantly different*

Table 6. Variation of zincemia according to the period of zinc supplementation (n = 50)

Children' group	n	Zincemia (µg/dl)		
		First day	15th day	P-value
Supplemented children	25	48.9 ± 12.7	92 ± 27.7	0.0001*
Control group	25	55.4 ± 9.9	60.4 ± 18.1	0.23
P-value		0.05	0.0001*	

*n: Number of children; Values expressed are mean ± standard deviation; * Significantly different*

4. CONCLUSION

The study showed a positive impact of maternal education on biochemical and anthropometric parameters of the children. Zinc supplementation may be an effective intervention to promptly raise the zincemia levels. Additionaly, our study highlights the low consumption of fruits and animal products among children from Bangang rural community.

CONSENT

Written informed consent was obtained from the parents.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the National Ethics Committee of Cameroon (N°: 177/CNE/SE/2012) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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