Towards a food-based approach to improve iron and zinc status of rural Beninese children: enhancing mineral bioavailability from sorghum-based food

Comlan Evariste S. Mitchikpe

Promotor

Prof. dr. ir. F.J. Kok
Hoogleraar Voeding en Gezondheid
Afdeling Humane Voeding
Wageningen Universiteit

Co-promotors

Dr. ir. J.M.A. van Raaij
Universitair Hoofddocent
Afdeling Humane Voeding
Wageningen Universiteit

Dr. ir. E.A.D. Atègbo
Département de Nutrition et Sciences Alimentaires
Faculté des Sciences Agronomiques
Université d'Abomey-Calavi
République du Bénin

Dr. ir. R.A.M. Dossa
Département de Nutrition et Sciences Alimentaires
Faculté des Sciences Agronomiques
Université d'Abomey-Calavi
République du Bénin

Promotiecommissie

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Universiteit van Amsterdam

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Wageningen Universiteit

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Wageningen Universiteit

Dr. ir. P.C.H. Hollman
RIKILT – Instituut voor Voedselveiligheid, Wageningen en
Afdeling Humane Voeding, Wageningen Universiteit

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A mes parents Florence et Gaston: pour le couronnement de vos efforts
A ma femme Stella: pour ton courage et ta patience
A nos enfants Larissa et Nina

ABSTRACT

Background: Prevalence rates of stunting and anaemia are high in Beninese children: 20 to 40 % of pre-school children are stunted and more than 70 % are anaemic. Prevalence rates of iron and zinc deficiencies are also expected to be high. The poor nutritional status of Beninese children might be due to low energy and nutrient intakes and to inhibiting effects of antinutritional factors such as phytate and polyphenols on iron and zinc bioavailability from the diet.

Objective: To study growth performance and iron and zinc status of rural Beninese school-age children in relation to their actual food consumption pattern and in relation to the iron and zinc bioavailability from their foods.

Methods: Eighty children aged 6 to 8 years were randomly selected from 3 villages in northern Benin and were studied in a post-harvest and a pre-harvest season. Nutritional status was assessed by anthropometry and blood analyses, and their food consumption was assessed by an observed weighed record method. Chemical analyses were performed in 23 samples of most frequently consumed foods. The potentially inhibiting effect of phytate on iron and zinc bioavailability from these foods was estimated using phytate/iron and phytate/zinc molar ratios. The effect of various food processing methods on iron and zinc solubility and on iron bioavailability from a commonly consumed food (dibou) was studied using an in vitro digestion/Caco-2 cell model.

Results: In the post-harvest season, Z-scores for height-for-age and for weight-for-height were -1.72±0.89 and –0.89±0.62, respectively. Obviously, the children were stunted but not wasted. Seasonal variation in growth performance was only marginal. In post-harvest season haemoglobin and serum ferritin levels were 119±13 g/l and 43±31 μg/l. Haemoglobin level was significantly decreased by 7 g/l from the post- to the pre-harvest season (P<0.01).Prevalence of iron deficiency was high: 49 and 33 % in the post- and pre-harvest season, respectively. Serum zinc level was 17.9±4.6 μmol/l. So, zinc status seemed to be normal. The children consumed a plant-based diet. The observed seasonal variation in food pattern did not really result in seasonality in energy and nutrient intakes. Cereals (maize, sorghum and millet) contributed to 43 % (post-harvest season) and 55 % (pre-harvest season) of the daily energy intake. Daily energy intakes were 5.2±1.4 and 5.4±1.3 MJ in the post- and pre-harvest season, respectively. Although energy and nutrient intakes seemed to be adequate, the diet was not well balanced and was low in animal products, protein, fat and vitamin C and high in fibre. In cereals, phytate/iron and phytate/zinc molar ratios ranged from 1 to 11 and 3 to 22, respectively. Such molar ratios indicate poor iron and zinc bioavailability. Dephytinisation (using an exogenous phytase) and dehulling resulted in an increased iron and zinc solubility in dibou and in a 10 % higher iron bioavailability from dibou when compared to dibou prepared from whole grains. Pounded grains soaked for 1 and 3.5 h did not show positive effects. However, none of the effects of food processing methods on iron bioavailability reached statistical significance.

Conclusion: School-age children in northern Benin have a poor growth performance and many of them can be classified as stunted. This suggests that these children suffer from a long-term marginal food intake, quantitatively and qualitatively. This is confirmed by the food intake results which underline that the iron and zinc bioavailability is poor because of the presence of high levels of antinutritional factors as phytate and polyphenols. The low iron bioavailability is undoubtedly one of the causes of the high prevalence rate of anaemia among these children. To improve the iron and zinc status of the children, further reductions of phytate and phenolic compounds in the food might be warranted.

Contents

Chapter 1

Introduction

In developing countries, deficiencies in iron, iodine, vitamin A and zinc are still major public health problems[1-3]. Socio-economic and age categories are differently affected. Most vulnerable groups are children, adolescents, women of reproductive age and pregnant women. Infants and pre-school children are more vulnerable than school-age children because of a higher growth rate. However, school-age children also represent an important age category for various physiological and socio-economical reasons. Although the growth speed is reduced compared to pre-school children, school-age children are still in the middle of their growth process. This growth implies increased needs for specific macro- and micronutrients. Therefore the supply by the diet of appropriate macro- and micronutrients for an adequate growth and development remains very important also for school-age children. The diet of these children is also important because they have to go to school. A better nutrition is associated with increased school performances and a better school achievement[4,5]. In addition, school-age children are dependent on their parents for access to adequate foods and nutrition. So parents' knowledge and practices on food and nutrition might also affect the nutritional status of these children. Therefore there is a need to give special attention to the nutritional status of school-age children in developing countries.

In this chapter, the prevalence and consequences of iron and zinc deficiencies (paragraph 1.1) and their causes (paragraph 1.2) will be reviewed. Subsequently, strategies for controlling iron and zinc deficiencies will be discussed (paragraph 1.3). Since some strategies focus on bioavailability, methods of measuring iron and zinc bioavailability in humans will be presented (paragraph 1.4). Finally, the rationale and the main objectives of the research presented in this thesis and the outline of the thesis will be given (paragraphs 1.5 and 1.6).

1.1. Prevalence and consequences of iron and zinc deficiencies

Iron deficiency is the most widespread nutrient deficiency in the world, affecting more than 2 billion people of whom 1.2 billion suffer from iron deficiency anaemia[2,6,7]. The prevalence rate of iron deficiency anaemia among school children is estimated at 53 %. The highest prevalence rate is reported in Asia (58.4 %) followed by Africa (49.8 %). In children iron deficiency anaemia is associated with decreased physical development, impaired immune function, poor growth and decreased physical activity. It also affects cognitive function and school achievement[5,8].

In 2002, zinc deficiency was included as a major risk factor to the global and regional burden of disease, along with iron, vitamin A and iodine deficiencies[9]. There are very limited nation-wide representative data on the magnitude and severity of zinc deficiency, partly due to the lack of reliable biomarkers of zinc status[7,10,11]. However, information from food balance sheets indicated that about 48 % of the world population are at risk of zinc deficiency[12]. A moderate zinc deficiency in infants and children has been associated not only with reduced growth and development, but also with impaired immunity and increased morbidity and mortality from infectious diseases[13,14]. Zinc deficiency is also known to impair taste perception and appetite, gonad development and skin integrity, and leads to delays in cognitive development[10,15].

Various micronutrient deficiencies often coexist in the same population. Multiple micronutrient deficiencies in developing countries are now gaining increasing recognition[16-20]. Like most age categories in low income countries, school-age children suffer from such coexisting micronutrient deficiencies[6,21-24]. To alleviate such multi-micronutrient deficiencies, an appropriate initial assessment of the problem and its causes needs to be made.

In conclusion, iron and zinc deficiencies are co-existing and are major public health problems in developing countries, including Benin. However in Benin, information on the prevalence rates of iron and zinc deficiencies in children are not yet available.

1.2. Causes of micronutrient deficiencies

One of the direct causes of micronutrient malnutrition in developing countries is the inadequate supply of micronutrients. Poor micronutrient supplies may be caused by an inadequate food quantity and/or by poor dietary quality. Undernutrition is still affecting about 852 million people worldwide with most (815 million) living in developing countries[2]. In many areas in developing countries, diets are predominantly based on cereals, tubers and legumes and contain few animal products, fruits and vegetables. These type of diets are known to be high in absorption inhibitors such as phytate and polyphenols. Although probably containing high amounts of minerals, these diets are associated with poor iron and zinc bioavailability[25-29]. Therefore the risk for iron and zinc deficiencies in populations consuming such diets is high. Animal food products are good sources of highly bioavailable iron and zinc. Vitamin C rich foods and animal foods enhance non-heme iron bioavailability but are often consumed in only very small amounts in many areas in developing countries.

Infections and parasitic infestations are other direct causes of micronutrient malnutrition in developing countries. They may cause impaired absorption and utilisation of micronutrients and increase their needs because of induced losses. Malaria, acute lower respiratory tract infections, diarrhoeal diseases, measles, HIV and helminth infections are some of the most important health hazards affecting children in developing countries[6,30]. A high prevalence of infections or parasitic infestations might increase the risk of micronutrient deficiencies in populations in areas where people experience inadequate supply of micronutrients.

The consumption of plant foods as main sources of energy, iron and zinc, constitute a risk factor for iron and zinc deficiencies. Although information on food supply exist for most developing countries, data on actual iron and zinc intake and on content of antinutritional factors such as phytate and polyphenols are scarce. In Benin, such information on children are not yet documented.

1.3. Strategies for controlling iron and zinc deficiencies in developing countries

Several approaches can be used to control micronutrient deficiencies. These include food-based approaches such as increasing household food and nutrition security, adding nutrients to foods, increasing mineral and vitamin bioavailability from foods and increasing nutrient supply potential of plants or animals[18,31-33]. Supplementation and control of diseases are other approaches used to reduce micronutrient deficiencies[11,34]. Approaches and strategies used to alleviate micronutrient deficiencies and their advantages and limitations are summarised in Table 1. Depending on the type and the magnitude of micronutrient deficiencies, an appropriate choice might be made.

Increasing household food and nutrition security is an essential approach as diets are characterised as inadequate for many areas in developing countries[18]. Adding nutrients to foods can be done for a specific age category such as infants for the production of weaning or complementary foods. It is recommended when there are widespread deficiencies and when habitual foods consumed cannot meet recommended intake. Increasing minerals and vitamins bioavailability from foods using modified food preparation methods is an approach that can be easily accessible to populations in rural poor settings. That approach has also the advantage of addressing multiple micronutrient deficiencies simultaneously in a sustainable way. A recent approach being tested by plant breeders consists of increasing nutrient supply potential of plants or animals. It has also the potential of increasing yields and might be easily adopted by producers and consumers. However, investments to develop seeds might be high[35,36] and therefore beyond the economic power of most developing countries. In combination with other approaches, reducing nutrient requirements by improving sanitation and controlling infections and infestations should always be considered in malaria endemic zones and non-hygienic environments. This might increase the chance of success of other interventions by reducing micronutrients losses and by increasing their absorption and utilisation. Supplementation can achieve a rapid improvement in the nutritional status of people but it is usually limited to specific age categories because of compliance problems and high costs to produce and distribute mineral preparations. Moreover, it might not be suitable as a long-term approach because of risks of toxicity[37]. As micronutrient deficiencies often coexist in the same population, no single type of intervention can solve the micronutrient malnutrition problems on its own. Comprehensive strategies involving multiple types of interventions adapted to conditions in specific countries and regions might be required[35].

In conclusion, in countries with limited resources like developing countries, food-based approaches seem to be more appropriate for reducing micronutrient deficiencies than other approaches. However, the effectiveness of these food-based approaches is not yet well documented. To be successful, a food-based approach should preferably focus on commonly eaten food.

Table 1: Strategies, advantages and limitations of different approaches used to alleviate micronutrient deficiencies

Approaches	**Strategies**	**Advantages**	**Limitations**
Increasing household food and nutrition security	- Increase household access to foods all the year round - Promote diversification of food supply - Increase availability of food all the year round - Promote appropriate intra household food distribution	- Promotion of local foods (staple crops, livestock, wild food resources, wild animal and fish) - Compliance of population can be high - Can be sustainable - Cost can be relatively low - Can benefit the whole population - Can address multiple micronutrient deficiencies simultaneously	- Not always possible to find local foods with sufficient amount of iodine - Mineral bioavailability in plant resources may be low (iron, zinc, calcium, vitamin A) - Changing dietary practices is difficult
Addition of nutrient to foods (nutrification)	- Fortification (commercial or home fortification) - Enrichment - Restoration - Addition of micronutrient to substitute products	- Compliance of population can be high - Can be sustainable - Can benefit the whole population but also specific target groups - High benefit cost ratio	- Commercial fortification require centrally processing of foods - Selection of appropriate dietary vehicle may be difficult - Nutrient added should be in bioavailable form - Changing dietary practices is difficult - Recommended when there are widespread deficiencies (e.g. iron) - Reach rural population in more remote areas with commercial fortification can be hard - Require committed partnership between governmental organisations, food industry, trade organisations, marketing specialists, scientific community and consumers - Require government regulation to eliminate competition with unfortified products - Require financial support and long-standing sound economic basis

Approaches	Strategies	Advantages	Limitations
Increasing bioavailability of mineral and vitamins	- Reducing content of absorption inhibitors (phytate, tannins, goitrogens) using processing - Modifying matrix - Increasing consumption of absorption enhancers (ascorbic acid, organic acids) - Combined strategies	- Compliance of population can be high - Cost can be relatively low (do not need extra resources) - Can be sustainable - Can benefit the whole population but also specific target groups - Can be implemented at household level by rural populations - Can address multiple micronutrient deficiencies simultaneously	- Achieving adequate bioavailability - Changing dietary practices is difficult
Increasing nutrient supply potential of plants or animals (biofortification)	- Development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology - Increasing the mineral and vitamin content - Reducing the level of antinutrients in food staples that inhibit the bioavailability of mineral and vitamin - Increasing the level of compounds that promote the bioavailability of mineral and vitamin - Combined strategies	- Capitalisation on food staples consumed by all family members - Can reach undernourished and poor populations in remote rural areas - Can be sustainable if adopted by populations - Cost can be relatively low after seeds have been developed - Limited or no risk of toxicity	- Adoption of new crops by producers may be difficult - Investment to develop seeds is high - Access to plant material through the development of seed system may be limited - Changing knowledge, attitudes and beliefs of the role of biofortified foods to control micronutrient malnutrition may be difficult - Changing dietary practices is difficult
Reducing nutrient requirements	- Improving sanitation - Controlling malaria - Vaccination - Birth control - Deworming - Multiple method	- Reduce nutrient needs - Better utilisation of nutrient intake by the body - Can benefit the whole population - Address multiple micronutrient deficiencies simultaneously	- Repeated treatment is needed for infections - Requires funding and management - Requires coordination between different sections of the health care system

Approaches	Strategies	Advantages	Limitations
Supplementation (pharmanutrient)	- Massive dosing - Short term supplementation	- Rapid improvement in the nutritional situation of target groups - High benefit:cost ratios	- Compliance can be low - Costly to produce and provide mineral preparations - Require management system - Continuous distribution and local availability of supplements may be a problem - Not the whole population is covered (coverage often limited to sub-groups) - External support needed - Risk of toxicity, overdose or accidental ingestion high

1.4. Methods of measuring iron and zinc bioavailability in humans

Bioavailability is defined as the proportion of an ingested trace element in food that is absorbed and utilised for normal metabolic and physiological functions or storage[38]. Radioactive and stable isotope techniques have been developed to measure iron and zinc bioavailability in vivo. They are the most accurate methods used to measure iron and zinc bioavailability. Stable isotope techniques are preferred especially in children because of the absence of health risk although they are less sensitive and more expensive than radioisotopes[39]. Radioactive or stable isotope studies to measure iron and zinc bioavailability from plant foods are limited[40-42].

Iron and zinc bioavailability are also estimated using in vitro methods. In vitro techniques are relatively simple, rapid and inexpensive methods developed as alternative for human absorption studies. An inter-laboratory study involving nine laboratories concluded that dialyzable data were in reasonable agreement with human absorption data[43]. Various levels of improvement in iron and zinc solubility in plant foods modified using household strategies were described[29,44-47]. However the relevance of these improvements for human absorption is not well documented. Furthermore, not all soluble or dialyzable iron or zinc is absorbable. So there is a need to include the process of absorption in the in vitro methods. Therefore a Caco-2 cell culture model has been developed. Caco-2 cells are cell lines from human adenocarcinoma. They have been used by Glahn and coworkers to estimate iron bioavailability in various rice and maize genotypes[48-51]. Other researchers used Caco-2 cells to study mechanisms of zinc absorption and factors affecting zinc absorption[52-54].

The choice of methods for mineral bioavailability depends on several factors such as the element under investigation, aims of the study, the location, characteristics of subjects and resources and skills available.

In conclusion, although the radioisotope and stable isotope methods to assess micronutrient bioavailability are the most accurate techniques, the in vitro digestion technique including micronutrient absorption by Caco-2 cells can be considered as an appropriate alternative.

1.5. Rationale and main objectives

As mentioned before, school-age children in developing countries might be considered as a nutritionally vulnerable group. Iron and zinc deficiencies in that age category are estimated to be high. Unfortunately, the level of iron and zinc deficiencies in school-age children is not yet well documented in Benin. However, iron and zinc deficiencies are also assumed to be high in Beninese school-age children because of expected low levels in foods and because of the expected high content of antinutritional factors (i.e. phytate). Daily intakes of micronutrient in foods might be influenced by the food pattern and therefore by seasonal fluctuation in food availability. A food-based approach to improve the supply and bioavailability of iron and zinc from the most commonly eaten food may improve iron and zinc status in a sustainable way. Such food-based approach might be related to food preparation methods aiming at a reduction of the phytate or polyphenol level. Bioavailability of iron and zinc from commonly eaten food might be assessed by the in vitro digestion technique including micronutrient absorption by Caco-2 cells.

Therefore the main objectives of the present study can be formulated as:

1. to assess the growth performance and iron and zinc status of rural Beninese school-age children in two seasons with a different food availability (post- and pre-harvest season)
2. to assess the actual food pattern of these school-age children in a post- and pre-harvest season
3. to establish the actual composition of foods commonly eaten in northern Benin, with special attention to iron, zinc, and phytate contents
4. to get more insight in the bioavailability of iron and zinc from foods commonly eaten in northern Benin, in particular of dibou, a commonly eaten sorghum-based food.

The study was carried out in three villages in Natitingou in the Atacora province in northern Benin

1.6. Outline of thesis

Chapter 2 describes the nutritional status of rural Beninese school-age children in a post- as well as a pre-harvest season. Chapter 3 provides their food patterns and the resulting energy and nutrient intakes. Chapter 4 shows the macro- and micronutrient composition of the foods as well as their phytate content and Chapter 5 describes the bioavailability of iron and zinc from dibou, a commonly eaten sorghum-based food. Chapter 6 integrates the findings from the various studies and includes possible implications for nutrition policy and suggestions for future research.

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Chapter 2

Growth performance and iron and zinc status of rural Beninese school-age children in post- and pre-harvest season

Comlan E.S. Mitchikpe

Romain A.M. Dossa

Eric A.D. Ategbo

Joop M.A. van Raaij

Frans J. Kok

Submitted

ABSTRACT

Objectives: To analyse the growth performance and the iron and zinc status of school-age children in rural Benin, not only in relation to season but also to school attendance.

Subjects and methods: The study was carried out in three villages in the Atacora province in northern Benin. Eighty children aged 6 to 8 years were randomly selected. Anthropometric parameters, haemoglobin, serum ferritin, CRP and serum zinc were measured in the same children in the post-harvest season and the next pre-harvest season. Complete anthropometric datasets were available for 74 children while for blood analysis 69 children completed the study.

Results: In the post-harvest season, mean Z-scores for height-for-age and for weight-for-height were -1.72±0.89 (mean±SD) and -0.89±0.62, respectively. The Z-score for height-for-age of children attending school (-1.55±0.87) was significantly higher than that of children not attending school (-2.14±0.80) (P<0.05). In the post-harvest season, haemoglobin level was 119±13 g/l, serum ferritin level was 43±31 µg/l and serum zinc level was 17.9±4.6 µmol/l. The haemoglobin level of children attending school (121±13 g/l) was significantly higher than that of children not attending school (114±12 g/l) (P<0.05).

Conclusions: The school-age children have a poor growth performance and a poor iron status that does not differ worth mentioning between seasons. Surprisingly, zinc status seems to be adequate. The reasons behind the difference in nutritional status in relation to school attendance need further investigation.

Key words: growth performance, iron and zinc status, school-age children, seasonality

INTRODUCTION

Iron and zinc deficiencies are common in populations dependent on cereal-based diets because of the poor bioavailability of these minerals from such diets[1]. Inadequate absorption of dietary iron is indeed the main explanation for the high prevalence rate of anaemia in developing countries unless the anaemia is also caused by malaria or by infections[2] (for example by hookworm). An inadequate absorption may also apply to dietary zinc as iron and zinc bioavailability are both dependent on the same potential inhibitors in plant-based foods. The most cited inhibitors of iron and zinc bioavailability are phytate and polyphenols[3,4,5].

Iron deficiency is the result of a long-term negative iron balance. It is not the only cause of anaemia. However when anaemia is prevalent in populations, iron deficiency usually is the common cause[6]. The World Health Organisation estimated that about 40 % of the world population suffers from anaemia. Current estimates in developing countries suggest that 53 % of school children and 42 % of preschool children are anaemic[2]. Previous studies in Benin suggest a prevalence of more than 70 % among children of 6 to 59 months[7,8]. In Benin, no such information is available on school children.

Zinc deficiency is difficult to quantify but is assumed to be as widely spread as iron deficiency[9,10,11]. From national food supplies data, it is estimated that nearly half of the world's population is at risk for low zinc intake and that in Sub-Saharan Africa, 68 % of the population is at risk for low zinc intake[12]. The prevalence of stunting in preschool children and the amount of absorbable zinc in available foods are suggested as indirect indicators to estimate the risk of zinc deficiency in populations[13]. A previous study performed in northern Benin reported prevalence of stunting of 28 – 36 % among children < 5 years of age[14]. In that area, diets were predominantly plant-based and consumption of animal foods was very small. Such conditions are probably associated with a high prevalence of zinc deficiency. In addition, a seasonal pattern in growth performance of the children was reported[14]. This was probably mainly due to seasonal variation in food availability[15,16,17]. Comparable information for school age children in Benin is scarce.

Most nutritional status studies on school age children focused on children attending school and only few did concentrate on children not attending school. In Sub-Saharan Africa this latest category represents as much as 36 % of the whole age group[18]. In rural areas of Benin, the proportion of children 6 to 11 years not attending school can reach 52 %[7].

Therefore, the objective of the present study was to analyse the growth performance and the iron and zinc status of school-age children in rural Benin, not only in relation to season but also in relation to school attendance.

SUBJECTS AND METHODS

Study area

The study was carried out in three villages in the Atacora province in northern Benin. This province is characterised by a unimodal climate with only one rainy season lasting from May to October and one dry season lasting from November to April. Average annual rainfall is about 1300 mm. It is savannah open grassland. The population density is low (21 inhabitants/km²) and the economic activity mainly is subsistence farming. Cultivated food crops are maize, sorghum, millet, yam, cassava, bambara groundnuts, peanuts and beans. Early crops such as yam, sweet potato, and early varieties of beans, maize and millet are harvested two to three months before the staple cereals harvesting in November and December[15]. Cash crops like cotton, tobacco or cashew nuts are also cultivated. Small livestock and poultry are raised in some households but animals are slaughtered when there is a ceremony or sold when there is an urgent need for money. Clean water sources are very limited and a lot of households are still using water from rivers and ditches for drinking and cooking. There is no latrine and domestic wastes are disposed around the house.

Study design

The nutritional status of 6-8 years old children was assessed by anthropometry and blood analysis during November and December, so at the end of the harvest season and six months later in June and July, so at the end of the pre-harvest season. Two anthropometric measurement sessions with a time interval of five weeks were organised in each season. Blood samples were collected at the second anthropometric session and were analysed for haemoglobin, serum ferritin, C-reactive protein (CRP) and serum zinc. The anthropometric sessions and the blood sampling took place at a hospital annex in one of the villages.

Subjects

From the local register of birth, a list with names of 302 children between the ages of 6 to 8 years was obtained. Out of those 302 children, 143 could be traced and served as the sampling population. Only 143 children could be traced mainly because of complicated naming practices and changing of names. Eighty children were randomly selected and this random selection resulted in a sample of 45 boys and 35 girls. This random selection resulted also in a sample of 57 children attending primary school (35 boys and 22 girls) and 23 children not attending primary school (10 boys and 13 girls). Informed consent was obtained from the parents of the children. Complete anthropometric dataset became available for 74 children while for blood analysis 69 children completed the study. The slightly lower numbers were caused by moving to other places and by refusing follow-up blood sampling.

The baseline anthropometric characteristics including prevalence rate of stunting and wasting of the randomly selected subjects are presented in Table 1. Anthropometric results are based on the mean values from the two measurement sessions in the post-harvest season.

Table 1: Baseline anthropometric characteristics of the children (Post-harvest season)

	All	Boys	Girls	School attendance	No school attendance
N	80	45	35	57	23
Age (yrs)	7.1±0.9	7.0±0.9	7.2±0.8	7.1±0.9	7.0±0.9
Height (cm)	112.6±5.5	113.1±6.4	112.0±4.1	113.7±4.8*	110.0±6.4
Weight (kg)	18.0±2.0	18.3±2.1	17.5±1.6	18.3±1.6*	17.2±2.6
HAZ[a]	-1.72±0.89	-1.72±0.94	-1.71±0.83	-1.55±0.87*	-2.14±0.80
HAZ<-2 (%)	38	33	43	28	61
WHZ[b]	-0.89±0.62	-0.91±0.64	-0.87±0.59	-0.90±0.58	-0.86±0.72
WHZ<-2 (%)	3	4	0	2	4
MUAC[c] (cm)	15.6±0.8	15.6±0.9	15.7±0.8	15.6±0.8	15.5±0.9

Mean±SD; [a] HAZ: height-for-age Z-score; [b] WHZ: weight-for-height Z-score; [c] MUAC: mid-upper-arm circumference

* Significantly higher than in children not attending school ($P<0.05$)

Methodology

Anthropometry

Anthropometric measurements were performed according to standard procedures[19]. Body weight was measured early in the morning using a SECA platform spring weighing scale (Seca, type 725; Vogel und Halke Mess-Und-Wiege Techniek, Hamburg, Germany). The scale was placed on a horizontal surface and calibrated before each measurement session using a standard weight of 20 kg. Children were measured wearing a minimum of clothing. Readings were done to the nearest 0.5 kg.

Height was measured using a microtoise. Measurement was performed with the subject standing without shoes on a horizontal surface against a wall with heels together, chin tucked in and body stretched upwards to full extent and head in the Frankfurt plane. Heels, buttocks

and shoulders were in contact with the wall to which the microtoise was attached. Height was read to the nearest 0.1 cm.
Mid-upper arm circumference (MUAC) was measured on the left side of the body halfway between the tip of the shoulder and the elbow with the subject's arm hanging freely along the body using a flexible non-extensible tape. Readings were done to the nearest 0.1 cm.
Age, height and weight were used to derive anthropometric indexes height-for-age and weight-for-height. Z-scores for height-for-age (HAZ-score) and weight-for-height (WHZ-score) were calculated using the National Centre for Health Statistics (NCHS) reference data and Epi info software (version 2002).

Blood sample collection and handling

Fasting venous blood samples were collected by medically qualified technicians. Subjects were sitting stretching the arm in a straight line from the shoulder to the wrist and were asked to make a fist. The puncture site was cleaned with antiseptic and an elastic band was placed around the upper arm. A stainless steel needle was inserted within one minute after applying tourniquet and about 10 ml of blood was collected in the evacuated tube. During the procedure, the tourniquet was released to restore circulation.
Haemoglobin concentration was determined immediately after sample collection. The remainder of the blood sample was let to clot for 30 minutes at room temperature while tubes were covered with foil. The clotted blood samples were packed in an isotherm container with ice and transferred to the departmental hospital in Natitingou at about 30 km from the hospital annex. Subsequently the samples were centrifuged the same day at 1200 rpm for 15 minutes. Serum was transferred to freezer-proof labelled tubes of 1.5 ml, closed firmly and frozen at -20 °C until analyses.

Blood analyses

Blood analyses included haemoglobin, serum ferritin, C-reactive protein (CRP) and serum zinc determinations. Haemoglobin concentration (g/l) was determined from whole blood just after collection using a photometry analysis method: the HemoCue device[20]. The accuracy of the HemoCue was checked every day with a control cuvette provided by the manufacturer.
Serum ferritin, CRP and serum zinc were determined using kits provided by Randox Laboratories Ltd. Serum ferritin was measured by immunoturbidimetric assay using FN 2464 kit. C-reactive-protein was also measured by immunoturbidimetric assay using CRP – CP 7950 kit and serum zinc was measured by colorimetric method using the ZN2341 kit. These

serum analyses were performed in the laboratory of biochemistry of the national university hospital in Cotonou using the automatic biochem-analyser RAXT TECHNICON. To define iron deficiency, serum ferritin < 12 µg/l is generally accepted in absence of infection. Serum ferritin < 30 µg/l is suggested in populations in which prevalence of infection or inflammation is high[6,21,22]. Therefore in the current study serum ferritin < 30 µg/l is used to define iron deficiency. Iron deficiency anaemia (IDA) is defined as iron deficiency simultaneously with anaemia. This latter is defined as haemoglobin concentration below 115 g/L[23]. CRP>10 mg/l is used to indicate presence of infection or inflammatory conditions. Zinc deficiency is defined as serum zinc value below the cut off point 9.9 µmol/L[13].

Statistical analyses

Before statistical analysis, data were checked for normality. Anthropometric data of the two measurement sessions in each harvest season were averaged after verification by paired t-test of no session difference. The datasets obtained in the post- and pre-harvest seasons were compared using paired-samples t-test or Wilcoxon signed-rank test. Subgroups of children, based on gender and school attendance (attending or not attending school), were also compared using independent samples t-test or Mann-Whitney test. Correlation coefficients of Pearson and Spearman were used to study the association between blood and anthropometric parameters. All statistical tests were two tailed and p values less than 0.05 were considered statistically significant. Analyses were performed using SPSS statistical package for Windows (version 11.0).

RESULTS

The anthropometric results of the children in the post- and pre-harvest season are summarised in Table 2. Height, weight and MUAC in the pre-harvest season were significantly higher than in the post-harvest season (P<0.01). The pre-harvest Z-score for height-for-age was significantly higher than the post-harvest score (P<0.01) and the pre-harvest Z-score for weight-for-height was significantly lower than the post-harvest score (P<0.05). For the total group, prevalence of stunting was high in both seasons (34% in post-harvest and 27% in pre-harvest season), but prevalence of wasting was quite low (3% in post-harvest and 7% in pre-harvest season).

Table 2: Anthropometric characteristics of the children measured at both post-harvest season and pre-harvest season

	All	Boys	Girls	School attendance	No school attendance
N	74	41	33	54	20
Post-harvest season					
Age (yrs)	7.1±0.9	7.1±0.9	7.2±0.8	7.1±0.9	7.1±0.9
Height (cm)	112.9±5.3	113.8±6.1	111.8±3.8	113.7±4.6	110.8±6.5
Weight (kg)	18.0±2.0	18.0±2.0	17.5±1.6	18.2±1.6	17.3±2.4
HAZ	-1.69±0.83	-1.63±0.92	-1.77±0.70	-1.56±0.79‡	-2.05±0.83
WHZ	-0.92±0.62	-0.90±0.62	-0.87±0.60	-0.91±0.58	-0.96±0.71
MUAC (cm)	15.6±0.9	15.6±0.9	15.5±0.9	15.6±0.8	15.4±1.0
Pre-harvest season					
Age (years)	7.7±0.9	7.7±0.9	7.8±0.8	7.7±0.9	7.7±0.9
Height (cm)	116.4±5.2*	117.4±5.9*	115.2±4.0*	117.2±4.4*	114.3±6.7*
Weight (kg)	18.9±2.2*	19.4±2.4*	18.3±1.8*	19.2±1.7*	18.2±3.1*
HAZ	-1.59±0.83*	-1.50±0.92*	-1.69±0.72*	-1.46±0.79*,‡	-1.93±0.86*
WHZ	-1.08±0.65†	-1.11±0.68†	-1.05±0.62†	-1.07±0.61†	-1.13±0.75†
MUAC (cm)	16.2±0.8*	16.3±0.8*	16.1±0.9*	16.3±0.7*	16.1±1.1*

Mean ± SD
* Significantly higher than in post-harvest season (*P*<0.01)
† Significantly lower than in post-harvest season (*P*<0.05)
‡ Significantly higher than in children not attending school (*P*<0.05)

The height-for-age and weight-for-height Z-scores of boys were not significantly different from those obtained on girls. The height-for-age Z-score of school attending children was significantly higher than that of children not attending school, in post-harvest as well as in pre-harvest season (*P*<0.05).

The nutritional status of the children was also assessed by biochemical parameters (Table 3). The pre-harvest haemoglobin level was significantly lower than the level measured in the post-harvest season (*P*<0.01). Using the cut off point of 115 g/l, prevalence of anaemia has doubled from 33% in post-harvest season to 70% in pre-harvest season. For both seasons, haemoglobin level of school attending children was significantly higher than that of children not attending schools (*P*<0.05). All other comparisons between and within seasons did not reveal significant differences (see Table 3). For the whole group of children, prevalence of iron deficiency defined as ferritin value < 30 µg/l was 49% and 33% in the post- and pre-harvest season, respectively. Prevalence of iron deficiency anaemia defined as presence of iron deficiency simultaneously with anaemia was 10% in the post-harvest season and 16% in the pre-harvest season. Elevated CRP values were observed in both seasons suggesting an increased level of infection and inflammatory conditions. CRP values higher than 10 mg/l were observed in 20% of the children during the post-harvest season and in 30% of them during the pre-harvest season. Between serum zinc level and height-for-age Z-score positive correlation coefficients of 0.22 (P=0.06) and of 0.37 (P<0.05) were found in the post- and pre-harvest season, respectively.

Table 3: Biochemical characteristics of the subjects measured in both post-harvest season and pre-harvest season

	All	Boys	Girls	School attendance	No school attendance
Post-harvest season					
Haemoglobin (g/l)	119±13 (67)	120±12 (36)	119±14 (31)	121±13‡ (49)	114±12 (18)
Ferritin^{a} (µg/l)	51±40 (69)	51±40 (40)	52±40 (29)	51±38 (51)	53±45 (18)
Ferritin^{b} (µg/l)	43±31 (49)	41±29 (27)	46±35 (22)	42±29 (38)	46±40 (11)
Serum zinc^{a} (µmol/l)	18.4±4.7 (71)	19.0±4.9 (41)	17.5±4.4 (30)	18.5±4.9 (52)	17.9±4.3 (19)
Serum zinc^{b} (µmol/l)	17.9±4.6 (49)	18.0±4.4 (27)	17.7±5.0 (22)	17.8±4.5 (38)	18.1±5.2 (11)
Pre-harvest season					
Haemoglobin (g/l)	112±10* (67)	114±10* (36)	110±10* (31)	113±10*,‡ (49)	109±10 (18)*
Ferritin^{a} (µg/l)	48±26 (64)	46±26 (33)	50±26 (31)	45±23 (47)	55±33 (17)
Ferritin^{b} (µg/l)	44±23 (49)	44±23 (27)	45±23 (22)	42±18 (38)	53±34 (11)
Serum zinc^{a} (µmol/l)	18.6±4.1 (65)	18.8±4.8 (35)	18.3±3.2 (30)	18.4±3.9 (48)	19.1±4.7 (17)
Serum zinc^{b} (µmol/l)	18.4±3.7 (49)	18.5±4.2 (27)	18.3±3.1 (22)	18.0±3.3 (38)	20.0±4.9 (11)

Mean ± SD (n)
[a] All available data
[b] Data on the 49 subjects with measurements in both seasons
[*] Significantly lower than in post-harvest season (p<0.01)
[‡] Significantly higher than in children not attending school (p<0.05)

DISCUSSION

The objective of the present study was to analyse the growth performance and the iron and zinc status of school-age children in rural Benin, not only in relation to season but also in relation to school attendance.

Growth performance

The mean height and weight of the children were 112.6±5.5 cm and 18.0±2.0 kg, respectively (Table 1). In this study, children were clearly stunted (38 %) but not wasted (3 %). Comparable levels of stunting were reported in Beninese children < 5 years of age[7] and in school-age children in Benin and Ghana[14,24]. As expected for children of 6 to 8 years, there were no significant differences between boys and girls with respect to anthropometric parameters. However, children attending school have significantly higher heights and weights compared to children not attending school. As a consequence, children attending school have a significantly higher HAZ-score than children not attending school. Similar results were reported among school-age children in Tanzania and Ghana[24,25]. It is generally assumed that differences in nutritional status between children attending school and children not attending school are due to differences in socio-economic background. However, nearly all children not attending school have a sister or a brother who do attend school. In addition, in the same area, no socio-economic differences between households have been reported[15]. It is conceivable that parents cannot financially allow all their children attending school, and that the children attending school might be the victims of their parents' choice for already nutritionally better-off children. From post-harvest to pre-harvest season, the HAZ-score was significantly increased whereas the WHZ-score was significantly decreased (Table 2). However the absolute differences between the post- and pre-harvest season for the HAZ- and WHZ-score were only 0.10 and 0.16 units, respectively. Such small differences are probably not relevant. Similar results were suggested by Wright *et* al.[26]. The currently observed high prevalence of stunting might be the expression of a cumulative effect of poor nutrition starting at a young age maybe even before birth. Prevalence of low birth weight (birth weight < 2500 g) in the study area ranged from 21 to 28 % (Departmental Hospital of Natitingou, unpublished). The poor growth as reported in this study may have a negative impact on the school performance of the children[27,28,29]. The absence of seasonality was consistent for boys and girls and for children attending and not attending school.

Iron status

Haemoglobin level in the post-harvest season is similar or higher than values reported for schoolchildren in other African countries[25,30,31]. It is similar to values recently reported in Thai schoolchildren[32]. Serum ferritin level is also comparable to that reported in Thai school children but is slightly lower than the value reported for Kenyan children[31]. As expected for children of 6 to 8 years, there was no difference between boys and girls with respect haemoglobin and serum ferritin levels. However children attending school have significantly higher haemoglobin levels compared to children not attending school. Similar findings were also reported by Fentiman *et al.*[24]. From post-harvest to pre-harvest season, haemoglobin level was significantly decreased by 7 g/l. This resulted in an increased prevalence of anaemia from 33 to 70 %. Comparable high prevalence rates of anaemia have been reported for Beninese children of 6 to 59 months[7,8]. Serum ferritin levels were not significantly different between seasons. The commonly used cut off value of serum ferritin < 12 µg/l as an indicator of iron status might underestimate the prevalence of iron deficiency if infections or inflammations are present[33,34]. In such situation, serum ferritin < 30 µg/l is suggested[6,21,22]. Using this cut off point of serum ferritin < 30 µg/l, the prevalence of iron deficiency is estimated to 49 and 33 % in post- and pre-harvest season, respectively. These high prevalence rates are in line with that obtained in school children in Côte d'Ivoire[22]. The prevalence of iron deficiency anaemia defined as presence of anaemia simultaneously with iron deficiency was 10 and 16 % in the post- and pre-harvest season, respectively. The high prevalence of iron deficiency in the post- and pre-harvest season strongly suggests the poor iron nutrition in the children. This may partly be caused by an inadequate iron intake. However, iron absorption inhibiting dietary factors may also play a role. The increased level of anaemia with unchanged iron status from the post-harvest to the pre-harvest season might be explained by the increased prevalence of infections and malaria[35]. Our CRP data indeed suggest an increased prevalence of infections in the pre-harvest season (30 %) when compared to the post-harvest season (20 %). The poor hygiene and sanitation as observed in the villages also might explain these high prevalence rates. A similar prevalence rate of infections was reported in school children in Côte d'Ivoire[22].

Zinc status

The observed serum zinc level in the post-harvest season (17.9±4.6 µmol/l) is higher than the reported serum zinc levels for children of 3 to 9 years in the United States National Health Examination Survey II (13.0±1.8 µmol/l for boys and 13.2±2.0 µmol/l for girls)[13]. It is also higher than average values reported for 6 to 20 months Zambian children (14.8±3.1 and

15.5±2.9 µmol/l), and for Thai school children (10.1±2.2 µmol/l)[32,36]. We have no explanation for the higher values observed in the present study. There was no significant difference between boys and girls, neither between children attending and not attending school, nor between seasons. None of the children had a serum zinc level below 9.9 µmol/l. This suggests that children in the present study cannot be considered as zinc deficient. However the high prevalence of stunting and infections in both seasons suggest a high risk of zinc deficiency[13,37,38,39]. Indeed it has been suggested that even in case of zinc deficiency in diets, serum zinc is maintained at normal level and consequences still may appear in terms of growth retardation in young children[40]. Although serum zinc level does not suggest zinc deficiency, the significant association between serum zinc and HAZ-score (indicator of stunting) suggests that zinc status might be relevant to growth performance.

In conclusion, school-age children in northern Benin have a poor growth performance that did not differ worth mentioning between seasons. Iron status should also be considered as poor and also did not differ between seasons. Prevalence rate of anaemia was high but significantly higher in pre-harvest season probably because of infections. Surprisingly, zinc status seems to be adequate but more research on actual zinc intake and its bioavailability is needed. Reasons behind the difference in nutritional status of children attending school and those not attending school need to be further explored.

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Chapter 3

Seasonal variation in food pattern but not in energy and nutrient intakes of rural Beninese school-age children

Comlan E.S. Mitchikpe
Romain A.M. Dossa
Eric A.D. Ategbo
Joop M.A. van Raaij
Frans J. Kok

Submitted

ABSTRACT

Objective: To analyse the actual food pattern and the resulting energy and nutrient intakes of school-age children in rural Benin, not only in relation to season but also in relation to school attendance.

Subjects and methods*:* The study was carried out in three villages in the Atacora province in northern Benin. Eighty children aged 6 to 8 years were randomly selected. Food, energy and nutrient intakes were measured in the same children in the post-harvest season and the next pre-harvest season. Complete food consumption datasets were available for 75 children.

Results: The food pattern showed seasonal variations. Cereals and roots and tubers were the main staple foods. Contributions of animal products to the diet were very small. The food pattern was neither different for boys and girls nor for children attending and not attending school. Daily energy intakes were 5.2±1.4 and 5.4±1.3 MJ in the post- and pre-harvest season, respectively. Differences between seasons in energy and nutrient intakes were not significant except for fat and vitamin C ($P < 0.05$). Energy and nutrient intakes were different for boys and girls but unexpectedly not for children attending and not attending school.

Conclusions: Seasonal variations in the food pattern didn't result in seasonality in energy and nutrient intakes. Because the diet of the children was low in animal products, protein, fat and vitamin C and high in fibre, absorption of fat, fat-soluble vitamins, carotenoids, iron and zinc might be low. Iron and zinc bioavailability from such a diet needs further investigation.

Keywords: Food pattern, energy and nutrient intake, school-age children, seasonality, school attendance, Benin

INTRODUCTION

Inadequate dietary iron and zinc intake and consumption of poor quality diets are major causes of iron and zinc deficiencies in most developing countries[1]. In these countries, inadequate iron and zinc intake may be the consequence of seasonal food shortage and limited resources for access to foods. Seasonal food shortage occurs when food production is not sufficient to cover the population needs in the period between two harvests. To adapt to such a situation, people may change their food pattern[2]. This may result in a different energy and nutrient intake with consequences on nutritional status[3,4,5]. In northern Benin, seasonality in food pattern has been reported in previous studies[6,7]. However, its impact on the energy and nutrient intake of children is not yet documented.

A recent study performed in northern Benin revealed that school-age children were clearly stunted but not wasted and that children attending school were less stunted than children not attending school. Moreover, the iron status of the children was poor and school attendants were less anaemic than no school attendants (Mitchikpe *et al*, unpublished). These findings might be caused by inadequate dietary patterns, but food consumption data on school-age children to support these findings are lacking.

Therefore, the objective of the present study was to analyse the actual food pattern and the resulting energy and nutrient intakes of school-age children in rural Benin, not only in relation to season but also in relation to school attendance.

SUBJECTS AND METHODS

Study area

The study was carried out in three villages in the Atacora province in northern Benin. The population density is low (21 inhabitants/km²). The economic activity is dominated by farming and is strongly dependent on rainfall. In the area, the unique rainy season lasts from May to October followed by the dry season lasting from November to April. Average annual rainfall is about 1300 mm. Cultivated food crops are maize, sorghum, millet, yam, cassava, bambara groundnuts, peanuts and beans. Early crops such as yam, sweet potato, and early varieties of beans, maize and millet are harvested two to three months before the staple cereals harvesting in November and December[6]. Food consumption in the household mainly depends on the size of the food stores until the next harvest. During the post-harvest season plenty of food is available. At the beginning of the rainy season the granaries get depleted (pre-harvest season). Cash crops like cotton, tobacco or cashew nuts are also cultivated. Small livestock and poultry are raised in some households but animals are mainly slaughtered when there is a ceremony or sold when there is an urgent need for money.

Study design

Children of 6 to 8 years old were randomly selected and involved in a longitudinal study. Food intakes of the children were assessed by food consumption surveys carried out in November and December, so at the end of the harvest season and six months later in June and July, so at the end of the pre-harvest season. Food intakes were measured during three consecutive days using an observed weighed records method. Data were collected by well-trained local assistants.

Subjects and sampling procedure

From the local register of birth, a list with names of 302 children between the ages of 6 to 8 years was obtained. Out of those 302 children, 143 could be traced and served as the sampling population. Only 143 children could be traced mainly because of complicated naming practices and changing of names. Eighty out of the 143 children were randomly selected and this random selection resulted in a sample of 45 boys and 35 girls. This random selection also resulted in a sample of 57 children attending primary school (35 boys and 22 girls) and 23 children not attending primary school (10 boys and 13 girls). Informed consent was obtained from the parents of the children. Complete food consumption datasets became available on 75 children.

The baseline anthropometric characteristics (in the post-harvest season) including the prevalence rate of stunting and wasting of the randomly selected subjects are presented in Table 1.

Measurement of dietary intake

Food intake of the children was assessed using the observed weighed records method[8]. All foods were weighed and recorded before and after cooking, as well as served portions and leftovers using a digital weighing scale (Tefal type Ovelys 3) for weights up to 3 kg and a spring weighing scale (Soehnle type 1203) for weights between 3 and 10 kg. Measurements were performed by well-trained local assistants every day from 7 a.m. until subjects had eaten their last meal, usually between 7 and 9 p.m. Foods consumed when local assistants were not present were assessed by recall using local household measures. For children who used to eat together with other members of the family, mothers were asked to serve their portion on separate plates.

Table 1: Baseline anthropometric characteristics of the children (post-harvest season)

	All	Boys	Girls	School attendance	No school attendance
N	80	45	35	57	23
Age (yrs)	7.1±0.9	7.0±0.9	7.2±0.8	7.1±0.9	7.0±0.9
Height (cm)	112.6±5.5	113.1±6.4	112.0±4.1	113.7±4.8*	110.0±6.4
Weight (kg)	18.0±2.0	18.3±2.1	17.5±1.6	18.3±1.6*	17.2±2.6
HAZ[a]	-1.72±0.89	-1.72±0.94	-1.71±0.83	-1.55±0.87*	-2.14±0.80
HAZ<-2 (%)	38	33	43	28	61
WHZ[b]	-0.89±0.62	-0.91±0.64	-0.87±0.59	-0.90±0.58	-0.86±0.72
WHZ<-2 (%)	3	4	0	2	4
MUAC[c] (cm)	15.6±0.8	15.6±0.9	15.7±0.8	15.6±0.8	15.5±0.9

Mean±SD; [a] HAZ: height-for-age Z-score; [b] WHZ: weight-for-height Z-score; [c] MUAC: mid-upper-arm circumference

* Significantly higher than in children not attending school ($P < 0.05$)

Data elaboration

Energy and nutrient intakes of the children were calculated using an updated version of the Benin food composition table. The previous version of this table as used in the University of Benin was mainly derived from the FAO food composition database. To update the food composition table, samples of foods consumed by the children were collected in two local markets in the study area. There were analysed for energy, macro- and micronutrients contents (Mitchikpe *et al*, unpublished). The amount of the various food categories consumed by the children (in gram) and the energy and nutrient content of each food were computed using the computer programme Komeet 4.0[9]. The contribution of each food to the daily energy and nutrient intake of each child was calculated by dividing the energy and nutrient composition of the food consumed by the total intake. Subsequently, the contributions of a food category to the energy and nutrient intakes as obtained on the three measurement days were averaged.

Statistical analyses

Food, energy and nutrient intakes of the children consumed over three consecutive days were compared and checked for systematic day effects using ANOVA. Mean values were obtained by averaging intakes over the three consecutive days and these mean values were used for further analyses. Before statistical analyses, data were checked for normality. Datasets obtained in the post- and pre-harvest season were compared using paired-samples t-tests or Wilcoxon signed-rank tests. Subgroups of children based on gender and school attendance were also compared using independent samples t-tests or Mann-Whitney tests.

All statistical tests were two tailed and P values less than 0.05 were considered statistically significant. Analyses were performed using SPSS statistical package for Windows (version 11.0).

RESULTS

The daily intakes of foods of the children during the post- and pre-harvest season are given in Table 2. The average amount of maize consumed in the post-harvest season (104 g) was not different from that consumed in the pre-harvest season (107 g). The intakes of millet, sorghum, cassava and vegetables in the post-harvest season were significantly lower than those in the pre-harvest season ($P < 0.05$). The amount of yam consumed in the post-harvest season (298 g) was substantially higher than that consumed in the pre-harvest season (39 g) ($P < 0.05$). Although the amount of animal products consumed in the post-harvest season (16 g) was two times the amount in the pre-harvest season (8 g) the difference did not reach statistical significance. For both seasons, the intakes of the various foods were not different between boys and girls except for beans in the post-harvest season (9 g versus 14 g). Although for some foods there were differences between the intakes of children attending and not attending school, none of these differences were statistically significant.

The daily intakes of energy, protein, carbohydrate, fibre, calcium, iron and zinc in the post-harvest season were not different from those in the pre-harvest season (see Table 3). Intakes of fat and vitamin C in the post-harvest season were 15 % and 24 % lower than intakes in the pre-harvest season ($P < 0.05$). For both post- and pre-harvest seasons, boys showed higher intakes than girls for energy, protein, fibre, iron and zinc ($P < 0.05$). Intakes of energy and nutrients of children attending school were not different from those of children not attending school.

Table 2: Daily intakes of foods of the children in the post- and pre-harvest season

	All	Boys	Girls	School attendance	No school attendance
			g/day		
n	75	41	34	54	21
Post-harvest season					
Maize	104±104	96±104	114±105	115±109	76±87
Millet	30±67	33±76	26±57	26±66	41±71
Sorghum	42±70	47±75	36±65	41±70	46±71
Cassava	21±39	23±47	19±27	21±42	21±30
Yam	298±260	310±298	283±209	307±287	274±177
Beans	11±21	9±21‡	14±22	11±23	11±15
Bambara groundnuts	24±33	27±35	20±30	26±34	19±28
Peanuts	11±14	12±14	10±15	12±16	8±10
Vegetables	18±17	20±19	15±14	19±18	15±14
Fruits	9±21	10±25	8±16	10±23	7±16
Animal products	16±29	16±28	16±30	15±27	19±33
Pre-harvest season					
Maize	107±117	118±130	93±100	101±119	121±115
Millet	57±75*	64±86	49±58*	59±76*	53±72
Sorghum	75±110*	86±132	62±75	84±118*	50±82
Cassava	79±66*	74±71*	84±61*	75±69*	89±61*
Yam	39±145†	52±191†	22±53†	41±165†	34±75†
Beans	16±26	17±30	15±19	15±27	17±24
Bambara groundnuts	18±22	17±22	20±23	16±20	24±26
Peanuts	5±7†	4±6†	6±7	5±7†	5±7
Vegetables	43±30*	40±32*	46±28*	45±32*	38±23*
Fruits	11±27	17±35	3±7	11±29	9±22
Animal products	8±13	7±12	10±13	8±13	8±11

Mean±SD; standard deviations should be interpreted with caution because distributions are not normal
* Significantly higher than in the post-harvest season ($P < 0.05$)
† Significantly lower than in the post-harvest season ($P < 0.05$)
‡ Significantly different from girls ($P < 0.05$)

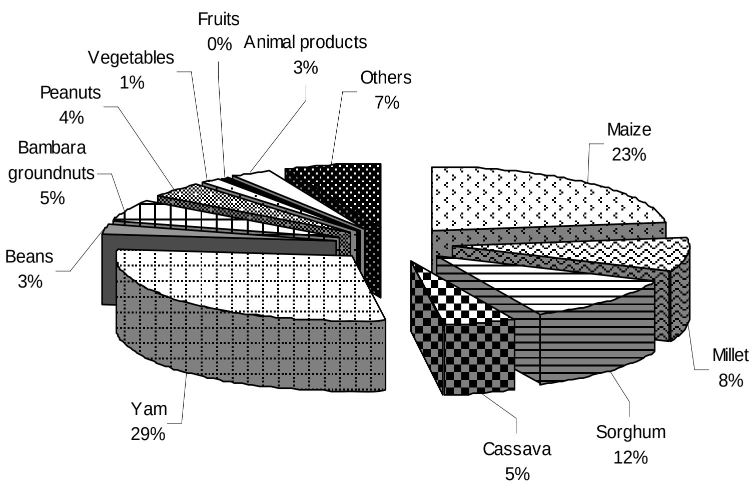
Table 3: Daily energy and nutrient intakes of the children in the post- and pre-harvest season

	All	Boys	Girls	School attendance	No school attendance
n	75	41	34	54	21
Post-harvest season					
Energy (MJ/day)	5.2±1.4	5.5±1.4‡	4.9±1.4	5.4±1.5	4.9±1.1
Energy (kJ/kg/day)	293±79	302±73	282±86	296±84	284±64
Protein (g/day)	32±10	34±9‡	29±10	33±10	29±8
Protein, % energy	10	10	10	10	10
Fat (g)	23±11	24±10	21±11	23±10	21±11
Fat, % energy	17	17	16	17	16
Carbohydrate (g/day)	245±77	257±80	230±71	251±83	229±58
Carbohydrate, % energy	73	73	74	73	74
Fibre (g/day)	55±19	60±19‡	49±17	58±20	47±13
Calcium (mg/day)	274±109	299±114‡	245±95	285±105	248±116
Iron (mg/day)	17±7	19±7‡	15±6	18±7	17±6
Zinc (mg/day)	6.6±1.9	7.2±1.9‡	6.0±1.8	6.8±2.0	6.2±1.5
Vitamin C (mg/day)	22±16	23±17	20±15	23±16	18±15
Pre-harvest season					
Energy (MJ/day)	5.4±1.3	5.8±1.3‡	4.9±1.2	5.4±1.3	5.4±1.3
Energy (kJ/kg/day)	284±68	300±73‡	264±55	281±67	292±70
Protein (g/day)	31±9	33±10‡	29±7	31±9	31±9
Protein, % energy	10	9	10	10	9
Fat (g/day)	27±11*	29±11*	25±10*	27±10*	28±13*
Fat, % energy	19	19	19	19	20
Carbohydrate (g/day)	244±69	264±69‡	221±61	245±71	242±65
Carbohydrate, % energy	71	72	71	71	71
Fibre (g/day)	55±20	59±20‡	51±18	55±20	57±18
Calcium (mg/day)	266±128	255±96†	279±160	273±140	247±94
Iron (mg/day)	18±6	20±7‡	17±6	19±6	18±8
Zinc (mg/day)	7.1±2.9	7.9±3.4‡	6.2±1.7	7.3±3.1	6.7±2.2
Vitamin C (mg/day)	29±20*	28±19	30±22*	30±22*	25±13*

Mean±SD; standard deviations should be interpreted with caution because distributions are not normal
* Significantly higher than in the post-harvest season ($P < 0.05$)
† Significantly lower than in the post-harvest season ($P < 0.05$)
‡ Significantly different from girls ($P < 0.05$)

Figures 1-4 summarise the contributions of the various foods to daily energy, protein, iron and zinc intake, respectively. Cereals (maize, millet and sorghum) contributed to 43 % (post-harvest season) and 55 % (pre-harvest season) of the daily energy intake of all children (Figure 1). Contributions to the daily energy intake by millet and cassava in the post-harvest season were significantly lower than those in the pre-harvest season ($P < 0.05$), whereas the contribution of yam was significantly higher ($P < 0.05$).

Post-harvest season



Pre-harvest season

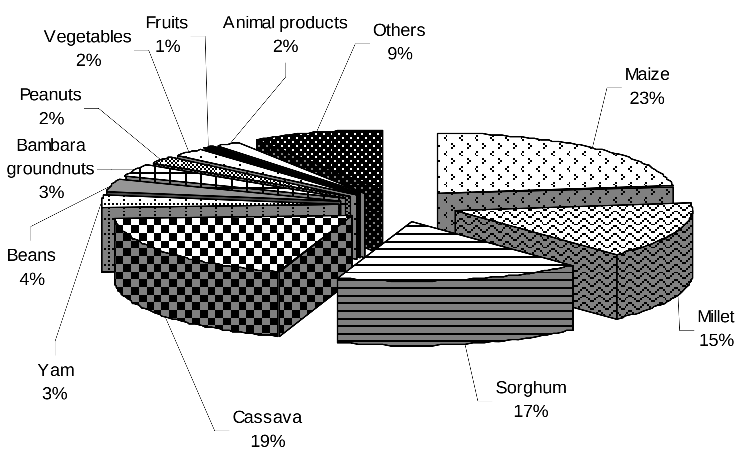
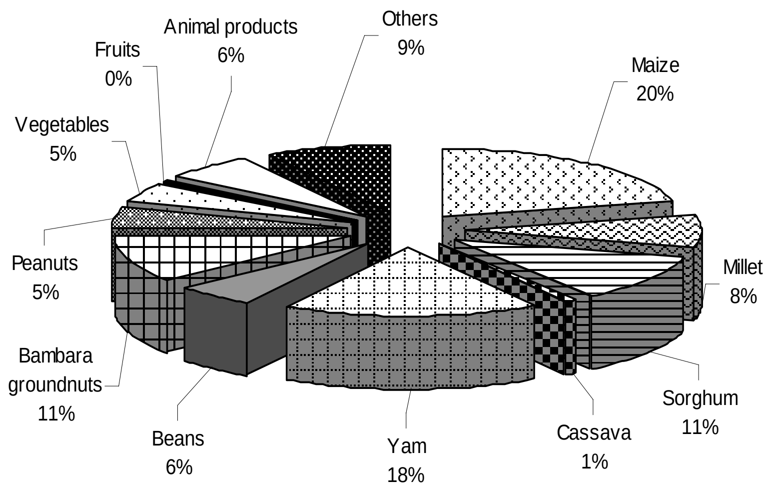


Figure 1: Contribution of foods to the daily energy intake of the children in the post- and pre-harvest season

Cereals contributed to 39 % (post-harvest season) and 53 % (pre-harvest season) of the daily protein intake (Figure 2). The contribution of millet in the post-harvest season was significantly lower than in the pre-harvest season ($P < 0.05$), whereas the contribution of yam was significantly higher ($P < 0.05$). Contributions of vegetables and animal products to daily protein intake fluctuated around 5 and 7 %.

Post-harvest season



Pre-harvest season

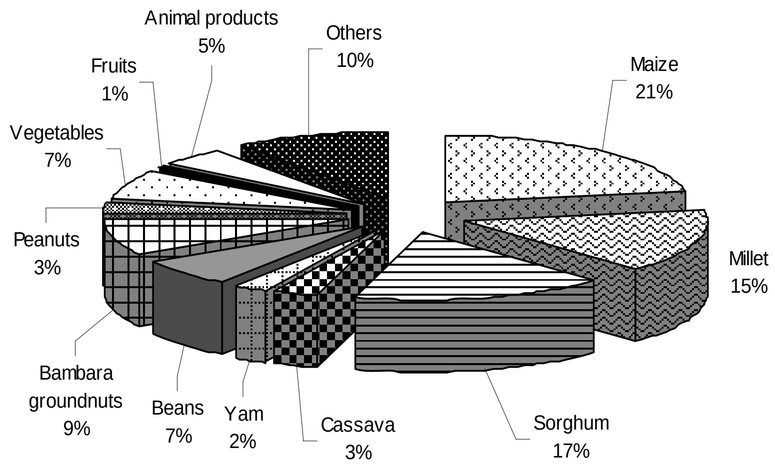
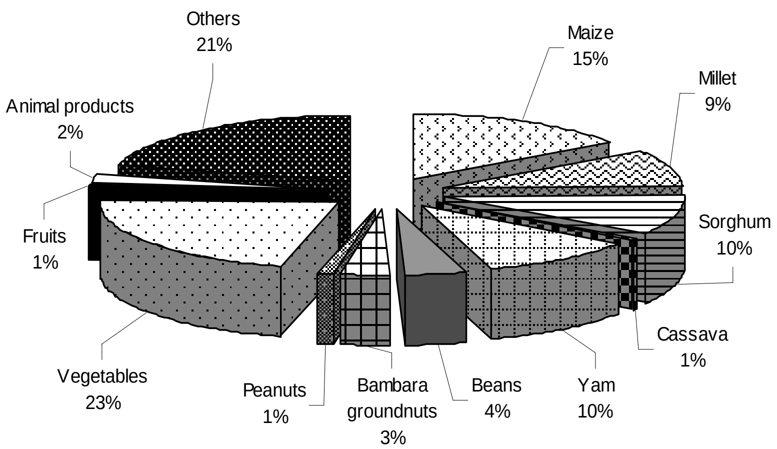


Figure 2: Contribution of foods to the daily protein intake of the children in the post- and pre-harvest season

Cereals contributed to 34 % (post-harvest season) and 50 % (pre-harvest season) of the daily iron intake (Figure 3). The contribution of millet in the post-harvest season was significantly lower than in the pre-harvest season ($P < 0.05$), whereas contributions of yam and vegetables were significantly higher ($P < 0.05$). The contribution of animal products to the daily iron intake was 1 to 2 %.

Post-harvest season



Pre-harvest season

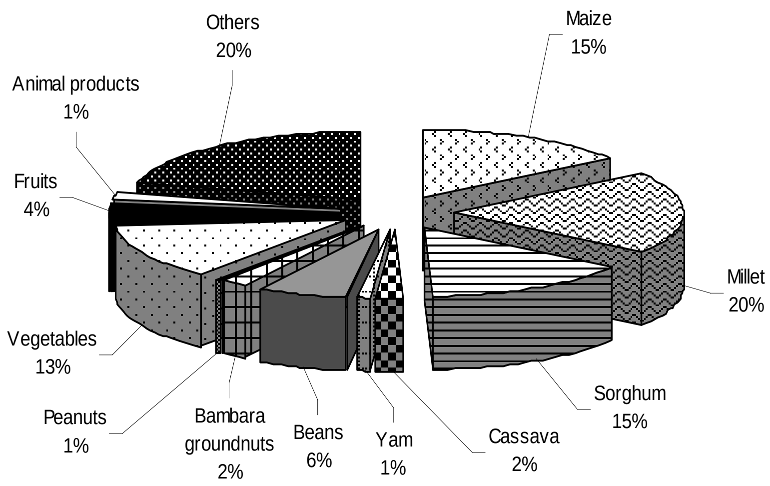
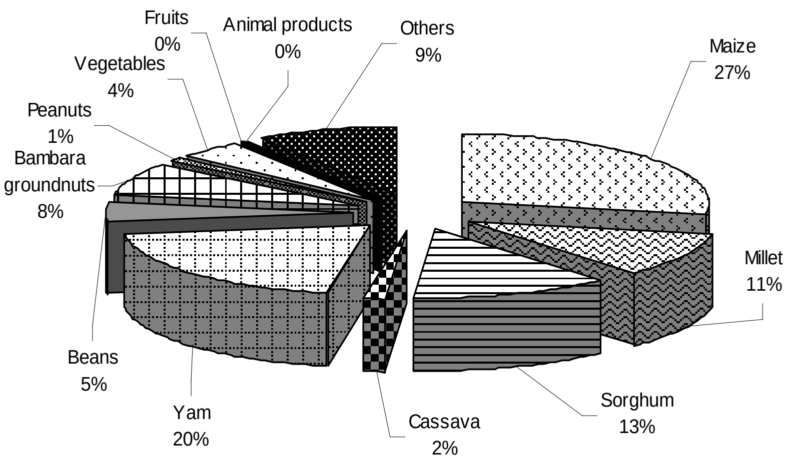


Figure 3: Contribution of foods to the daily iron intake of the children in the post- and pre-harvest season

Cereals contributed to 51 % (post-harvest season) and 70 % (pre-harvest season) of the average daily zinc intake (Figure 4). Contributions of millet and cassava in the post-harvest season were significantly lower than those in the pre-harvest season ($P < 0.05$), whereas contributions of yam and vegetables were significantly higher ($P < 0.05$).

Post-harvest season



Pre-harvest season

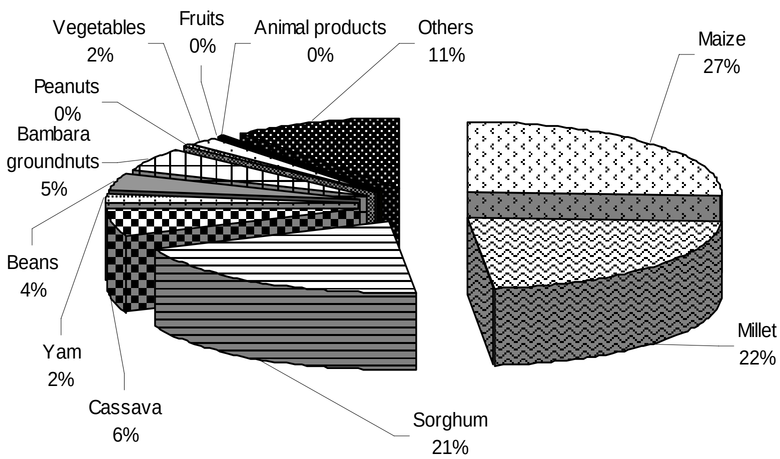


Figure 4: Contribution of foods to the daily zinc intake of the children in the post- and pre-harvest season

Table 4 provides the percentage of children not meeting their energy requirement. Data show that 49 to 59 % of boys and girls have an energy intake lower than their EAR (Estimated Average Requirement). The percentage of boys who have an intake lower than 2SD (two Standard Deviations) below the EAR (7 and 15 % in the post- and pre-harvest season, respectively) is lower than the percentage of girls (29 and 27 % in the post- and pre-harvest season, respectively). Table 5 shows the percentages of children not meeting the protein, iron and zinc requirements. For zinc intake, 24 to 32 % of the children were below the EAR and up to 79 % were below the RNI (Recommended Nutrient Intake).

Table 4: Percentage of children not meeting their energy requirement

	Boys (n = 41)		Girls (n = 34)	
	Post-harvest season	Pre-harvest season	Post-harvest season	Pre-harvest season
EAR[a] (MJ/day)	5.4	5.7	4.9	5.1
% below the EAR	54	49	53	59
EAR-2SD (MJ/day)	4.1	4.3	4.0	4.1
% below EAR-2SD	7	15	29	27

[a] Estimated average energy requirements were calculated based on the average body weight of the children and the recommended energy intake for children 7 to 8 years[29].

Table 5: Percentage of children not meeting their estimated average requirements and recommended intakes for protein, iron and zinc

	Protein[a] (g/day)		Iron[b] (mg/day)		Zinc[b] (mg/day)	
	Post-harvest season	Pre-harvest season	Post-harvest season	Pre-harvest season	Post-harvest season	Pre-harvest season
EAR	20	21	10*	10*	5.4	6
% below the EAR	4	12	7	3	24	32
RNI	25	27	18	18	8	9
% below the RNI	23	36	61	51	79	79

EAR = Estimated average requirement; RNI = Recommended Nutrient intake for iron and zinc and safe level of protein intake
[a] Protein requirements were calculated based on actual weight and age of the children and the FAO/WHO/UNU (1985) recommendations[16].
[b] Iron and zinc requirements were derived from the FAO/WHO (2002) recommendations[14].
* Median basal requirement for an intermediate bioavailability diet[14].

DISCUSSION

The objective of the present study was to analyse the actual food pattern and the resulting energy and nutrient intakes of school-age children in rural Benin, not only in relation to season but also in relation to school attendance.

The study was carried out in 6 to 8 years old children in northern Benin. These children were clearly stunted but not wasted (Table 1). Prevalence rates of stunting (38 %) and wasting (3 %) are within reported ranges for the study area[7,10]. In northern Benin, not all the school-age children will attend school. The reasons for not attending school remain to be clarified. However, both school attending and no school attending children are involved in the present study. With respect to the interpretation of our findings, it should be noticed that boys are overrepresented in the school attending group (35 boys versus 22 girls).

Food pattern

The higher consumption of millet, sorghum, cassava and vegetables in the pre-harvest season and the lower consumption of yam and peanuts indicate that the food pattern of the children had changed. Such a change in food pattern was also reported in previous studies[6,7] and might be related to the availability of foods. There were no differences between the food patterns of boys and girls, but this is not surprising as they came from the same area. The lack of difference between the food patterns of children attending and not attending school might be unexpected, but not if it is taken into account that both groups of children came from the same families.

In northern Benin, the seasonality in food pattern didn't result in remarkable changes in the food groups eaten. Cereals were the main sources of energy followed by yam in the post-harvest season and by cassava in the pre-harvest season. They all together contributed to 75 % of the energy intake of the children (Figures 1-4). As a consequence, they were also the main sources of protein, iron and zinc. Contributions of animal foods to protein and iron intakes were marginal. Similar findings were reported in previous studies[6,7]. Because of missing values in the Benin food composition table, the zinc contribution by animal products was not taken into account in the calculation of zinc intake. However, underestimation of zinc intake is probably very small as the consumption of animal products was very limited (only 2 to 3 % of energy intake). Iron and zinc bioavailability from unrefined cereal foods have been shown to be low because of phytate and polyphenols content of cereals[11,12,13]. The diet of the children is mainly based on cereals and therefore, iron and zinc bioavailability from such a diet might be low[14].

Energy and nutrient intake

In the post harvest season, the energy intake of the children was 5.2±1.4 MJ. This energy intake is higher than that reported for Kenyan school-age children from a slum area (3.6 to 4.1 MJ/day)[15], but it is in line with the EAR which is based on the actual weights of the children as shown in Table 4. By definition, 2 to 5 % of the children might be expected to have energy intakes lower than 2SD below the requirement. However, especially for girls, the percentage of low intake is substantially higher (about 25 %; see Table 4). In addition, the actual available energy from the diet of the children might be lower because of the high fibre content. A correction factor of 0.95 is suggested for diets containing large amounts of fibre[16]. Therefore the percentage of children not meeting the energy requirement might be higher. A long term shortage of energy might be one of the explaining factors for the high prevalence of stunting among the children (Table 1).

The observed protein intake of the children is higher than that reported in Kenyan school-age children (20.6 to 23.5 g/day)[15], but is in line with the requirements (Table 5). However, limiting amino-acids in cereals may be a problem in the diets as consumed by the children[17].

Contributions of protein and fat to the energy intake of the children (10 and 17 %, respectively) are low and suggest that the diet is not well balanced. For example, low fat intake may result in an increased risk of inadequate absorption of fat-soluble vitamins such as vitamin A.

Because of the low contribution of animal products in the diet, the high fibre intake is not surprising. However, this high level certainly will have consequences on nutrient absorption such as reduced absorption of fats, fat-soluble vitamins and carotenoids[18].

The iron intake of the children is higher than that reported for Namibian school children although those children were older (11 years old in average)[19]. It should be realized that the Namibian children were measured during the drought. Van't Riet *et al.*[15] have reported similar iron intakes for children attending school but lower values for children not attending school. The iron intake is in line with the requirement and suggests that it is probably adequate. By definition, half of the children with appropriate intakes will have their values below the EAR. This might mean that also the zinc intake of the children might have been adequate. However, the presence of anti-nutritional factors such as phytate and polyphenols in cereals might reduce iron and zinc bioavailability[12,13,20-22]. The relation between food intake and prevalence of iron and zinc deficiencies was shown in several studies[23-28].

Vitamin C is known to counteract the inhibiting effect of phytate in plant-based foods. The vitamin C intake of the children is lower than the recommended intake (35 mg/day)[14]. The high percentage of children below the recommended intake (73 to 85 %) suggests that vitamin C intake might be inadequate. Therefore, the potential enhancing effect of vitamin C on iron and zinc bioavailability probably is quite limited.

Seasonality

Although the food pattern was clearly different from post-harvest to pre-harvest season, seasonal differences with respect to energy and nutrient intake were not large. The only significant differences were with respect to fat and vitamin C. The increased consumption of fat and vitamin C in the pre-harvest season is due to the availability of shea butter, fruits and fresh vegetables. Because of only small changes in fat and vitamin C intake, the energy and nutrient intake of the children was not really dependent on season. The lack of seasonality in the energy and nutrient intake may be explained by adaptation strategies to food shortage developed by households[2].

School attendance

The lack of difference between the energy and nutrient intakes of school attendants and no school attendants was unexpected because of the difference in nutritional status (Table 1). The differences in stunting could not be explained by the present data, but is probably the result of past detrimental events in the life of the children.

In conclusion, the food pattern of school-age children in northern Benin showed a seasonal variation. The diet was mainly plant-based with cereals, roots and tubers as main staple foods. Although there were changes in the food pattern, the energy and nutrient intake of the children didn’t show seasonal variation. The energy and nutrient intake seems to be adequate for the whole group; however, some of the children may have inadequate intakes. Unexpectedly the energy and nutrient intakes of school attendants and no school attendants were similar and did not differ between seasons. Because the diet contains only very small amounts of animal products, and is low in protein, fat and vitamin C, and high in fibre, the absorption of nutrients such as fat, fat-soluble vitamins, carotenoids, iron and zinc might be low. Therefore, although iron and zinc intake might be adequate, their bioavailability from the diet as consumed by the children need further investigation.

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Chapter 4

Macro- and micronutrient contents of foods commonly consumed in northern Benin

Comlan E.S. Mitchikpe
Romain A.M. Dossa
Eric A.D. Ategbo
Joop M.A. van Raaij
Paul J.M. Hulshof
Frans J. Kok

Submitted

ABSTRACT

Objective: The objective was to evaluate the proximate and inorganic composition of foods commonly eaten in northern Benin and to estimate the potentially inhibiting effect of phytate on iron and zinc bioavailability.

Materials and methods: Chemical analyses were performed in 23 composite samples of most frequently consumed foodstuffs collected from retailers in local markets. The proximate composition was analysed by standard methods. Inorganic constituents and phytate were analysed using the Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) and the HPLC.

Results: Protein contents were in agreement with those from the FAO food composition table for Africa. Fat and fibre levels were higher whereas carbohydrate and energy levels were lower. Differences are mainly due to either analytical or calculation methods. Most important sources of iron and zinc in the diets in northern Benin were maize, sorghum and millet. In these cereals, iron and zinc ranged from 2.6 to 8.4 and 2.2 to 3.4 mg/100 g, respectively. Phytate (inositol hexaphosphate) ranged from 104 to 503 mg/100 g. Phytate/iron and phytate/zinc molar ratios ranged from 1 to 11 and 3 to 22, respectively, and suggest poor iron and zinc bioavailability.

Conclusion: The new proximate and inorganic compositions of foodstuffs provide a better estimate of actual energy and nutrient intakes of people in northern Benin. In order to improve iron and zinc bioavailability, reducing phytate contents of cereal foods by modifying food preparation methods looks promising and needs further investigation.

Keywords: Proximate composition, inorganic constituents, phytate, iron and zinc bioavailability, Benin.

INTRODUCTION

Deficiencies in iron and zinc are major health problems in developing countries[1,2]. A recent study performed in rural Beninese school-age children revealed a high prevalence of stunting and a poor iron status (Mitchikpe *et al*, unpublished). The nutritional status of these children might be caused not only by their actual energy and nutrient intakes but also by the poor bioavailability of minerals from their diet[3-6]. Information on the actual food intakes of the children can be obtained by a food consumption survey. However, to derive the energy and nutrient intakes of the children and to estimate iron and zinc bioavailability from their diet, a valid food composition database is needed. Such a database, which should include representative foods as eaten by the children, is not yet available.

In Benin, the food composition database used at the University of Abomey-Calavi is mainly derived from the FAO food composition table for Africa[7]. However, the FAO food composition table includes only limited information on sampling procedures and analytical methods used. In the FAO table data on fat, fibre, carbohydrate and energy are based on outdated analytical or calculation methods. Presented iron values are sometimes accompanied by question marks and no data are presented on zinc and phytate contents of the foods. The evaluation of other regional tables like the Mali and Nigeria tables also reveals scarce information on food data quality and on cooked foods, and shows only limited numbers of nutrients of the foods[8]. As for most food composition databases, the tables also do not account for the variability in inorganic contents caused by factors such as soil type, climatic conditions and duration of growth period.

Therefore, the objective of this study was to evaluate the proximate and inorganic composition of foods commonly eaten in northern Benin and to estimate the potentially inhibiting effect of phytate on iron and zinc bioavailability from these foods.

MATERIALS AND METHODS

Study area

The study was carried out in the Atacora province in the northwestern part of Benin. This province is characterized by a unimodal climate with only one rainy season lasting from May to October and one dry season lasting from November to April. Average annual rainfall is about 1300 mm. It is savannah open grassland area characterized by a low population density. The main economic activity is farming essentially dependent on rainfall. Cultivated food crops are maize (*Zea mays*), sorghum (*Sorghum bicolor*), millet (*Pennisetum glaucum*), yam (*Dioscorea spp*), cassava (*Manihot esculenta*), Bambara groundnuts (*Voandzeia* *subterranean*), peanuts (*Arachis hypogaea*) and beans (*Vigna unguiculata).* Early crops such as yams, sweet potatoes (*Ipomoea batatas*), and early varieties of beans, maize and millet are harvested two to three months before the staple cereals which are harvested in November and December[9]. During post-harvest seasons, plenty of foods are available. Stored foods are consumed until the beginning of the rainy season when food shortages occur (pre-harvest season). Small livestock and poultry are raised in some households.

Selection of foodstuffs for sampling

The selection of foodstuffs for sampling for analyses was based on food consumption surveys carried out in three villages in the study area. Food consumption surveys on children were performed during a post-harvest season and after 6 months in a pre-harvest season during 3 consecutive days using the observed weighed record method (Mitchikpe *et al,* unpublished). Energy intakes from the various food categories were calculated using the computer programme Komeet 4.0[10] and the not yet updated Beninese food composition database (Dossa *et al,* unpublished data).

A complete meal usually is composed of two main parts. The first part is the bulky portion and the main source of energy. It is usually made of cereals (maize, sorghum, millet) of roots and tubers (yam, cassava) or of legumes (bambara groundnuts, cowpea). The second part is the sauce which is a mixture of different foods, mainly vegetables (Okra leaves or Okra pods (*Hibiscus esculentus)*, sorrel (*Hibiscus sabdariffa*), etc.) and condiments (fermented African locust bean seeds called afitin or soumbala (*Parkia biglobosa*), powder of the roots of *Cochlospermum spp*. etc.) and also peanuts.

All locally cultivated foodstuffs consumed as main source of energy were sampled. Their contribution to total daily energy intake of the children amounted to 85 % in both post- and pre-harvest seasons (Table 1). The most frequently used foodstuffs for the preparation of sauces were also sampled (peanuts, vegetables, condiments and fruits). Various cultivars of sorghum, bambara groundnuts and peanuts were sampled based on differences in colour. In total 23 foodstuffs were identified for sampling. Table 1 summarises the contribution of the different foodstuffs or clusters of foodstuffs to the energy intakes of the children.

Sample collection

The 23 samples of raw foodstuffs were collected in two local markets. One market is located in the city of Natitingou and the second is located in a village at 30 kilometers from the city. Food samples were collected at the beginning of the rainy season (May 2005). Each foodstuff was purchased from 3 to 5 different retailers in both markets and mixed together based on equal amounts from each retailer, to arrive at a total amount of at least 1 kg. In total, 6 to 10 samples of each foodstuff were mixed to form the composite sample (for exact number of samples, see n in Tables 2 and 3). Composite samples were then transported to the laboratory of the Département de Nutrition et Sciences Alimentaires of the Faculté des Sciences Agronomiques of Université d'Abomey-Calavi in Benin. In the laboratory, samples were cleaned from waste and dust. They were also dry-cleaned using a piece of cloth in order to eliminate soil contamination. Wet cleaning of foods bought in market is not a common practice in the study area.

Table 1: Contribution of various foods to daily energy intake of children in northern Benin[a]

Foodstuffs	Contribution to energy intake (%)		Number of composite samples[b]
	Post-harvest season	Pre-harvest season	
Sorghum (*Surghum bicolour*)	11	16	2
Maize (*Zea mays*)	23	23	1
Millet (*Pennisetum glaucum*)	8	13	1
Yam (*Dioscorea spp.*)	24	4	1
Cassava (*Manihot esculenta*)	5	20	1
Bambara groundnuts (*Voandzeia subterranea*)	10	7	2
Cowpeas and soybean (*Vigna unguiculata ; Glycine max*)	3	2	3
Peanut (*Arachis hypogaea*)	5	2	2
Vegetables	2	2	5
Condiments and fruits	3	4	5
Animal products	2	2	0
Others	4	5	0

[a] Mitchikpe et al. (unpublished data)
[b] Number of composite samples of foodstuffs collected for chemical analyses. In total, 23 composite samples from 10 foodstuffs or clusters of foodstuffs were taken. See text for more detail on sampling procedure and see Tables 2 and 3 for details on the foodstuffs.

Sample handling and shipping

From each cleaned raw foodstuff, a representative composite sample (100 g) was taken, put in a plastic bag, labelled and heat-sealed. The 23 composite samples were then shipped to the laboratory of the Division of Human Nutrition of Wageningen University in The Netherlands.

Sample elaboration

In the laboratory, the composite samples of foodstuffs were homogenised using the Moulinette, type D56 (Moulinex, GMBH). The Moulinette was equipped with stainless-steel blades. The homogenised composite samples were divided into two portions of about 50 g each, put in air-tight containers and kept in a deep freezer at -20 ºC until analyses. One portion of each homogenised sample was used for proximate analyses and the second portion was used for inorganic constituents and phytate (inositol hexaphosphate) analyses.

Proximate composition was analysed in the laboratory of the Division of Human Nutrition of Wageningen University. Inorganic constituents and phytate contents were measured in respectively the Analytical Soil Laboratory and the Laboratory of Plant Physiology of Wageningen University.

Methodology of sample analysis

Proximate composition

Moisture and ash contents of foodstuffs were determined as described by Osborne and Voogt[11]. Total nitrogen of foodstuffs was analyzed using the automated Kjeldahl method[12]. Protein was calculated using specific Jones conversion factors (N x 5.46 for peanuts, N x 5.71 for soya, N x 5.30 for other beans, N x 5.83 for millets and N x 6.25 for maize, sorghum and other foods)[13]. Total fat content was analysed using acid hydrolysis method (AOAC method 14.019)[14]. Dietary fibre was determined using Prosky method (AOAC 985.29)[15]. Available carbohydrate, which represents the fraction of carbohydrate that can be digested by human enzymes, was estimated by difference. It was calculated by the following formula: 100 – (weight in grams [protein + fat + water + ash + fibre] in 100 g of food).

Energy content of each food was calculated from the amount of protein, fat and carbohydrate in the food using the following energy conversion factors[16]: protein, 16.7 kJ/g; fat, 37.7 kJ/g and carbohydrate, 15.7 kJ/g. Energy contents of foods are expressed in both kJ and kcal (1 kcal = 4.184 kJ).

Inorganic constituents: Ca, Fe, Zn, P, K Cu, Mn, Mg

Inorganic constituents were determined using a microwave digestion and spectrometric method as described by Novozamsky *et al*[17]. Approximately 0.4 g of dried samples of foodstuffs were weighed in a metal weighing funnel and transferred to a digestion vessel. 5.0

mL of hydrofluoric acid 40 % (w/w) and 5.0 mL nitric acid 65 % (w/w) were added to the samples and mixed. The mixtures were let to stand overnight at room temperature and then heated at 120 ºC to almost dryness. Another 5.0 mL of concentrated nitric acid 65 % (w/w) and three times 1.0 mL of hydrogen peroxide 30 % (w/w) were added to the dried plant material. The mixture was carefully swirled in order to moisten all the plant material and the digestion inner vessels were put into the polyetherimide outer vessels. The vessels were closed and placed in the digestor (MDS-2100). After the microwave digestion, vessels were allowed to cool down for 45 min and were opened in a fume hood. The digests were quantitatively transferred to a 50 mL polythene volumetric flask, made up to mark with Millipore water, mixed and then filtered over fine paper into a polythene bottle. The concentration of Ca, Fe, Zn, P, K Cu, Mn and Mg in the digests were analysed using the Inductively Coupled Plasma-Atomic Emission Spectrometer (VITA-PRO CCD Sinultaneous ICP- AES, Varian).

Phytate content

Phytate was analysed by anion exchange HPLC (Dionex DX300, ICS2500 system) according to the methodology used by Bentsink *et al*[18]. Approximately 2 – 3 mg of the homogenized sample were extracted with 1 mL 0.5N HCl containing 50 mg/L cis-aconitate used as internal standard. The mixture was boiled in a water bath at 100 ºC for 15 minutes and then centrifuged at 14000 rpm for 5 min. The supernatant was diluted 10x in Millipore water. Anions were separated on an AS11 anion exchange column (4 x 250 mm, Dionex) preceded by an AG11 guard column and eluted with NaOH. 20 µL of the diluted supernatant was analysed for anion detection with suppressed conductivity. Background conductivity was decreased using ASRS suppressor with water as a counterflow (5 mL/min). Twenty milligrams (20 mg) $Na_2HPO_4.2H_2O$ and 50 mg Na (12)-phytate (sigma P3168) were used as external standards.

Analytical quality control

All the analyses were performed in duplicate. For proximate composition, in-house control samples were used for quality control. For the assessment of ash, fat and nitrogen, babyfood (Humana Milchwerke Westfalen eG, D-4900 Herford, Germany) was used as control sample. Within and between-run CVs over a more than 10 year period are: for ash 0.9 % and 1.6 %; for fat 2.3 % and 3.9 % and for nitrogen 0.8 % and 1.2 %, respectively. For fibre the in-house control sample was breadcrumbs, with a between-run CV of 7.8 % (within-run variation was not recorded). Results from proficiency testing (FAPAS, 2006) showed z-scores as a measure for deviation from the consensus value (accuracy) for fibre -0.2; for nitrogen +0.7 and for ash 0.0. Results from proficiency tests organised by the Swedish National Food Administration (Uppsala, Sweden) showed average z-scores over a 10 year period for ash +0.52; for dry matter +0.99; for fat +1.72 and for nitrogen -0.24. For inorganic constituents' analyses, the ICP-AES was calibrated with a blank without mineral content and two different types of reference materials with known mineral content. A control standard with known concentration of elements was used to check calibration at the beginning of a run and after each series of 10 samples analysis.

RESULTS

Table 2 shows the proximate composition of 23 composite samples of foodstuffs locally cultivated and frequently consumed in northern Benin. Protein content of cereals ranged from 7 to 11 g/100 g dry matter. For legumes, they ranged from 20 to 23 g/100 g except for dried soybean (*Glycine max*) which contains 36 g/100 g. Among vegetables, condiments and fruits, fermented African locust bean and seeds of monkey-bread (*Adansonia digitata*) have the highest protein values (45 and 38 g/100 g, respectively). Fat content of cereals ranged from 4 to 6 g/100 g whereas for yam and cassava it was about 1 g/100 g. Peanut, soybean and condiments (African locust bean, seeds of monkey-bread and ackee fruit (*Bligia sapida*)) have the highest fat levels. Fibre content ranged from 11 to 26 g/100 g for cereals and from 25 to 44 g/100 g for legumes except for peanuts (8 to 10 g/100 g). Vegetables have the highest fibre levels. Roots and tubers and cereals have the highest carbohydrate content (59 to 89 g/100 g). They are followed by pulp of African locust bean (58 g/100 g), roots of *Cochlospermum spp* (46 g/100 g) and legumes (17 – 39 g/100 g).

Table 3 shows inorganic constituents of 23 composite samples of foodstuffs frequently consumed in northern Benin. Iron content of cereals ranged from 2.6 to 8.4 mg/100 g whereas zinc content ranged from 2.2 to 3.4 mg/100 g. Dried cassava has the lowest iron and zinc level (0.3 and 0.5 mg/100 g, respectively). For soybean, black cowpea and white cowpea, iron and zinc values ranged from 6.8 to 7.8 and 4.6 to 5.1 mg/100 g, respectively. Highest iron levels were measured in dried leaves (111.3 – 236.0 mg/100 g), pulp of African locust bean (268.3 mg/100 g) and roots of *Cochlospermum spp* (103.8 mg/100 g). Highest zinc levels were measured in fermented African locust bean (12.9 mg/100 g) and dried seeds of monkey-bread (7.9 mg/100 g).

Table 2: Proximate composition of 23 composite samples of foodstuffs frequently consumed in northern Benin

Foodstuffs	n[a]	Dry Matter (%)	Protein	Fat	Fibre	Carbohydrate[b]	Ash	Energy[c] kJ (kcal)/ 100 g dry matter
			g/100 g dry matter					
Cereals								
Sorghum, whole grain, white (*Surghum bicolour*)	9	90.6	10.3	4.3	19.0	64.8	1.6	1351 (323)
Sorghum, whole grain, red (*Surghum bicolour*)	9	89.9	7.4	3.9	15.6	71.4	1.7	1392 (333)
Maize, whole grain, white (*Zea mays*)	10	90.3	7.6	6.2	26.0	59.0	1.2	1285 (307)
Millet, whole grain (*Pennisetum glaucum*)	7	91.0	7.9	6.3	11.6	72.7	1.5	1509 (361)
Roots and tubers								
Yam, dried (chips) (*Dioscorea spp.*)	7	88.1	3.9	0.9	12.8	80.4	2.1	1358 (325)
Cassava, dried (chips) (*Manihot esculenta*)	6	89.8	1.1	1.0	7.6	88.5	1.8	1443 (345)
Legumes								
Bambara groundnuts, seeds, red, dried (*Voandzeia subterranea*)	8	91.9	20.0	6.0	37.0	33.6	3.4	1087 (260)
Bambara groundnuts, seeds, white, dried (*Voandzeia subterranea*)	8	92.1	21.7	7.1	29.1	38.6	3.4	1236 (295)
Soya, seeds, whole, dried (*Glycine max*)	6	92.9	36.1	13.8	25.2	19.7	5.2	1432 (342)
Cowpea, seeds, whole, dried, black (*Vigna unguiculata*)	7	87.1	20.3	1.8	43.7	30.5	3.8	885 (212)
Cowpea, seeds, whole, dried, white (*Vigna unguiculata*)	8	90.8	20.1	2.0	35.7	38.7	3.5	1018 (243)
Peanut, seeds, dried, rose (*Arachis hypogaea*)	6	93.8	21.7	46.1	8.2	21.6	2.3	2440 (583)
Peanut, seeds, dried, red (*Arachis hypogaea*)	6	94.9	22.9	47.5	9.9	17.4	2.4	2442 (584)
Vegetables								
Okra ladies-fingers, pods, dried (*Hibiscus esculentus*)	7	91.3	16.3	2.6	51.8	19.1	10.2	670 (160)
Okra ladies-fingers, leaves, dried (*Hibiscus esculentus*)	8	90.1	18.4	4.7	48.7	13.4	14.8	695 (166)
Bombax, flower calyces, dried (*Bombax buonopozense*)	7	89.1	4.3	1.8	77.1	7.9	8.9	263 (63)
Onions, leaves, dried (*Allium cepa*)	6	90.2	13.9	4.3	45.3	12.5	24.0	591 (141)
False sesame, leaves, dried (*Ceratotheca sesamoides*)	7	84.6	25.0	4.3	51.1	6.1	13.6	673 (161)
Condiments and fruits								
African locust bean, seeds, fermented (*Parkia biglobosa*)	8	65.1	44.6	34.8	15.1	1.3	4.2	2078 (497)
Monkey-bread, seed, dried (*Adansonia digitata*)	10	95.0	38.2	38.0	10.0	6.4	7.5	2169 (518)
African locust bean, fruit, pulp (*Parkia biglobosa*)	7	86.0	3.2	2.1	32.8	57.9	4.1	1040 (248)
Cochlospermum spp, root's powder (*Cochlospermum spp.*)	10	92.0	4.6	13.0	31.0	45.5	5.8	1280 (306)
Ackee fruit (*Bligia sapida*)	6	93.3	12.1	46.2	17.4	20.0	4.4	2255 (539)

[a] The analyses presented are performed in a composite sample based on 6 to 10 samples (see text)
[b] Protein, fibre and ash were analysed as described in the text. Carbohydrate was derived by substracting the sum of figures for moisture, protein, fat, ash and fibre from 100.
[c] Energy calculated as metabolizable energy from the amount of protein, fat and carbohydrate using energy conversion factors 16.7, 37.7 and 15.7 kJ/g for protein, fat and carbohydrate respectively.

Table 3: Inorganic constituents and phytate content of 23 composite samples of foodstuffs frequently consumed northern Benin

Foodstuffs	n[a]	Fe	Zn	Ca	P	K	Cu	Mn	Mg	Phytate
		mg/100 g dry matter								
Cereals										
Sorghum, whole grain, white (*Surghum bicolour*)	9	4.3	2.3	7	275	329	0.2	1.7	129	503
Sorghum, whole grain, red (*Surghum bicolour*)	9	3.8	2.2	11	330	431	0.3	1.8	138	373
Maize, whole grain, white (*Zea mays*)	10	2.6	2.2	6	276	367	0.2	0.7	105	348
Millet, whole grain (*Pennisetum glaucum*)	7	8.4	3.4	21	228	441	0.5	1.2	107	104
Roots and tubers										
Yam, dried (ships) (*Dioscorea spp.*)	7	2.3	1.2	30	68	1011	0.3	0.2	53	ND
Cassava, dried (ships) (*Manihot esculenta*)	6	0.3	0.5	54	69	899	0.3	0.9	75	ND
Legumes										
Bambara groundnuts, seeds, red, dried (*Voandzeia subterranea*)	8	1.4	2.2	38	296	1405	0.7	1.1	194	220
Bambara groundnuts, seeds, white, dried (*Voandzeia subterranea*)	8	2.1	2.6	54	335	1484	0.9	1.4	201	325
Soya, seeds, whole, dried (*Glycine max*)	6	7.4	5.1	349	616	1926	1.6	3.7	264	808
Cowpea, seeds, whole, dried, black (*Vigna unguiculata*)	7	7.8	4.6	87	448	1582	0.7	2.4	232	769
Cowpea, seeds, whole, dried, white (*Vigna unguiculata*)	8	6.8	4.9	101	413	1441	1.0	2.2	218	251
Peanut, seeds, whole, dried, rose (*Arachis hypogaea*)	6	3.9	3.9	36	358	758	1.2	2.2	208	703
Peanut, seeds, whole, dried, red (*Arachis hypogaea*)	6	2.4	3.0	46	353	834	1.1	1.7	203	483
Vegetables										
Okra ladies-fingers, pods, dried (*Hibiscus esculentus*)	7	33.7	5.1	960	616	2775	1.2	4.8	600	ND
Okra ladies-fingers, leaves, dried (*Hibiscus esculentus*)	8	111.3	5.0	3565	600	1918	1.3	17.2	1191	ND
Bombax, flower calyces, dried (*Bombax buonopozense*)	7	9.8	2.8	2200	88	1923	1.2	4.0	626	ND
Onions, leaves, dried (*Allium cepa*)	6	236.0	3.4	2009	206	3799	0.7	41.2	525	NM
False sesame, leaves, dried (*Ceratotheca sesamoides*)	7	146.2	5.0	1207	377	2125	2.6	20.7	592	NM
Condiments and fruits										
African locust bean, seeds, fermented (*Parkia biglobosa*)	8	57.2	12.9	1045	875	466	3.3	17.7	379	ND
Monkey-bread, seed, dried (*Adansonia digitata*)	10	10.3	7.9	348	1767	1029	2.6	3.4	782	4903
African locust bean, fruit, pulp (*Parkia biglobosa*)	7	268.3	0.9	123	101	1947	0.5	5.1	156	91
Cochlospermum spp, root's powder (*Cochlospermum spp.*)	10	103.8	1.0	703	48	373	0.3	5.0	404	ND
Ackee fruit (*Bligia sapida*)	6	5.5	2.2	127	189	1576	1.4	1.0	261	NM

[a] The analyses presented are performed in a composite sample based on 6 to 10 samples (see text)
ND: Not detectable
NM: Not measured

Phytate (as inositol hexaphosphate or IP6) content of foodstuffs frequently consumed in northern Benin are shown in Table 3. Phytate levels ranged from 104 to 503 mg/100 g for cereals and 220 to 808 mg/100 g for legumes. Monkey-bread seeds have the highest phytate content (4903 mg/100 g).

In order to predict the inhibitory effect of phytate on iron and zinc bioavailability from cereals, phytate/iron and phytate/zinc molar ratios were calculated (Table 4). Molar ratios of phytate to iron and phytate to zinc of maize and sorghum ranged from 8 to 11 and 16 to 22, respectively. Millet has the lowest molar ratios (1 and 3 for phytate/iron and phytate/zinc molar ratios, respectively).

Table 4: Molar ratios of phytate to iron and phytate to zinc of cereals as measured without processing[a] and as projected after various processing methods[b]

	White sorghum		Red sorghum		White maize		Millet	
	Phytate/ iron	Phytate/ zinc	Phytate/ iron	Phytate/ Zinc	Phytate/ Iron	Phytate/ zinc	Phytate/ iron	Phytate/ zinc
Measured								
Unprocessed cereals	10	22	8	17	11	16	1	3
Projected								
Soaking whole grains[c]	10	21	8	16	11	15	1	3
Soaking pounded grains[d]	5	11	4	8	6	8	1	1
Dehulling[e]	6	14	5	11	7	10	1	2
Malting[f]	2	5	2	4	3	4	< 1	1
Fermentation[g]	4	9	3	7	5	7	< 1	1

[a] Molar ratios of phytate to iron and phytate to zinc of cereal without processing are calculated by dividing the number of moles of phytate (IP6) by the number of moles of iron or zinc in 100 g dry matter (molar weights of IP6, Fe and Zn are 660,03; 55,845 and 65,39 g/mole, respectively)

[b] Molar ratios of phytate to iron and phytate to zinc of processed sorghum, maize and millet are calculated based on the assumption that initial iron and zinc content is not affected by food processing and that the following reduction in native phytate content can be achieved:

[c] *Soaking whole grain*: 4 % reduction of native phytate content[20]

[d] *Soaking pounded grains*: 51 % reduction of native phytate content[35]

[e] *Dehulling*: 35 % reduction of native phytate content[33]

[f] *Malting*: 77 % (68 – 87 %) reduction of native phytate content[34]

[g] *Fermentation*: 58 % (57 – 60 %) reduction of native phytate content[34]

DISCUSSION

The objective of this study was to evaluate the proximate and inorganic composition of foods commonly eaten in northern Benin and to estimate the potentially inhibiting effect of phytate on iron and zinc bioavailability. To achieve this objective, samples of foodstuffs were analysed for proximate composition, inorganic constituents and phytate contents.

The food pattern of the children in the study area is dominated by the consumption of foods from plant origin (Table 1). Cereals (maize, sorghum and millet) are main staple foods and together they are a main source of energy in both post- and pre-harvest seasons.

Proximate composition of foodstuffs

In the food composition database as used at the University of Abomey-Calavi, values are mainly derived from the FAO food composition table for Africa. Therefore this FAO table is used to compare the present food analysis results.

Protein content of the foodstuffs is in the same range as given in the FAO food composition table[7] whereas fat contents of cereals, roots and tubers are generally higher. Differences in fat levels can amount to 6 – 40 % for cereals and 43 – 100 % for yam and cassava. These differences might partly be explained by the acid hydrolysis method used in the present study as the method contributes to a better extraction of fat from low fat foodstuffs. Fibre values as analysed in the foodstuffs are also generally higher than those reported in the FAO table. In cereals, fibre values were 5 to 12 times higher than FAO data. Differences in fibre levels are less pronounced for legumes, yam and cassava (3 to 8 times higher than FAO data) and for vegetables (2 to 3 times higher). The high measured fibre levels are probably due to differences in methods of analysis. As far as carbohydrate is concerned, also differences are noticed. Carbohydrate values are lower than those reported in the FAO table. For cereals, carbohydrate values are 10 to 35 % lower than FAO data. For yam and cassava, values are 6 to 14 % lower and for legumes they are 30 to 48 % lower. These differences amount to 75 to 95 % for vegetables. Similar differences are observed when present results are compared to values obtained by Nordeide *et al*[19], especially for dried onion leaves (*Allium cepa*), dried Bombax flower calyces (*Bombax buonopozense*) and fermented African locust bean. Differences between carbohydrate contents in the present study and data reported by others may be explained by the calculation method used. In the present study, available carbohydrate is calculated by substracting the sum of the figures for moisture, protein, fat, ash and fibre from 100, whereas in the FAO table, fibre was not taken into account. In the present study, no account is made for alcohol since none of the foodstuffs under study is supposed to include alcohol. As a consequence of underestimation of fibre and overestimation of carbohydrate, reported energy values in the FAO table are generally higher than the available energy calculated in the present study. For cereals, FAO values are 8 to 20 % higher whereas for legumes, there are 20 to 35 % higher. More important differences are reported for dried pods of okra[7] (54 % higher) and for dried onion leaves and dried bombax flower calyces (55 and 82 % higher, respectively)[19]. Therefore using the FAO data in food consumption surveys will result in an underestimation of fat and fibre and in an overestimation of carbohydrate and energy.

Inorganic constituents of foodstuffs

Millet appears to be a good source of iron and zinc in comparison with sorghum and maize. Similar observations were made by Lestienne *et al*[20] and Ma *et al*[21], with millet showing the highest iron and zinc content followed by sorghum and maize. Iron and zinc values of millet in this study are lower than those reported by Abdalla *et al*[22]. Lower zinc levels of millet, maize and sorghum were reported by Adeyeye *et al*[23]. The contribution of millet to energy intake of the children (Table 1) shows that millet is less consumed than maize and sorghum in both post- and pre-harvest seasons. The reduced use of millet may be explained by its low storage properties compared to maize and sorghum. Iron content of maize in this study (2.6 mg/100 g) is higher than the average iron content of 8 maize cultivars (1.8 mg/100 g) reported by Nago *et al*[24] but zinc level is similar. The difference in iron levels may be due to the origin of the samples. Factors such as soil type, climatic conditions during growth and duration of growth period may influence inorganic constituents in foods. Sorghum has higher iron and similar zinc levels compared to maize. In addition, the observed increased sorghum consumption during the pre-harvest season suggests its importance in the study area. Yam and cassava were poor sources of iron and zinc (Table 3). They were used as energy source during the post- and pre-harvest season. Soybean, black cowpea and white cowpea were good sources of iron and zinc contrary to bambara groundnuts and peanuts. Similar iron and zinc contents were reported in other studies[7,20]. However because of their low digestibility, these legumes cannot be consumed as main source of energy. The highest iron contents were measured in dried onion leaves (236 mg/100 g dry matter) and pulp of African locust bean (268.3 mg/100 g). Nordeide *et al*[19] have also reported a high value for dried onion leaves (335 mg/100 g), but suggested that this can be due to soil contamination. Compared to the present study, they have reported lower values for dried fermented seeds of African locust bean (37 mg/100 g) and pulp of African locust bean (16 mg/100 g). This suggests that our samples might have been contaminated by iron from soil or other origin. Samples were purchased in markets and could not be dry-cleaned like grains or seeds. In addition, ash is sometimes added to African locust beans during preparation of fermented products[25]. This can be an additional source of iron. Baobab seeds are one of the best sources of zinc in the 23 selected foodstuffs. A high value of zinc content (5.2 mg/100 g) was also reported by Osman[26]. As expected, leafy vegetables and condiments are the most important sources of iron and zinc. But they are consumed only in very small amounts (Table 1). In summary, observed iron and zinc levels are in line with values published by others.

Phytate content of foodstuffs

Phytate content of millet, maize, sorghum, peanut, cowpea and soybean are lower than those reported in other studies[27,28]. They are also lower than those reported by Lestienne *et al*[20] except for soybean. Phytate contents of maize, sorghum, peanut and soybean are higher than those reported by Adeyeye *et al*[23], however in their study, the reported value for phytate content of millet was two times higher. Phytate contents of sorghum are in agreement with those published by Bunch and Murphy[29] and Ma *et al*[21]. The observed differences between studies might be due to choice of varieties, conditions of cultivation, time of harvesting and storage conditions. Therefore an exact description of the varieties of the crops analysed is important for making appropriate comparisons and for borrowing data from other sources.

Estimation of iron and zinc bioavailability

Phytate/iron molar ratio > 1 is an indication of poor iron bioavailability and phytate/zinc molar ratios > 15 is regarded as being associated with reduced zinc absorption and negative zinc balance[30,31]. Molar ratios of phytate to iron and of phytate to zinc of maize and sorghum ranged from 8 to 11 and 16 to 22, respectively. All these ratios are above the critical values, suggesting that the absorption of iron and zinc from maize and sorghum might be poor. Millet has the lowest molar ratios (1 and 3 for phytate/iron and phytate/zinc molar ratios, respectively) and the phytate/iron ratio indicates that iron bioavailability might be impaired. Molar ratio of phytate to iron should be ideally decreased to < 0.4 to achieve adequate iron bioavailability[32]. According to Lestienne *et al*[33], molar ratio of phytate to iron is not an adequate indicator of iron availability if the level of antinutritional factors other than phytate is high. Molar ratios of phytate to iron and phytate to zinc of millet calculated in this study are lower than those reported by Lestienne *et al*[20]. This might be due to the reported higher phytate content.

Possibilities of improving iron and zinc bioavailability

Several studies have focused on improving iron and zinc bioavailability from cereal foods by modifying food preparation methods. Reducing phytate levels by using different food processing methods have been reported: soaking whole grains, soaking pounded grains, dehulling, malting and fermentation[20,33-35]. These studies showed various levels of phytate reduction. Using this information, the projected phytate reduction and the expected improvement in iron and zinc bioavailability from cereals are summarised in Table 4. Assuming that no iron or zinc losses occur during processing (for a comparison purpose), malting and fermenting appear to be the most appropriate methods to increase iron and zinc bioavailability. However, because of sensorial changes, malting and fermenting might not be suitable for the preparation of dibou, the most commonly eaten cooked food in the study area. The choice of a food processing method to increase iron and zinc bioavailability should also take into account the acceptability of the new product and the sustainability of the method.

CONCLUSION

The proximate and mineral composition of foodstuffs analysed in the present study are more appropriate for assessing macro- and micronutrient intakes of people living in northern Benin than the previous Benin food composition database. The new food analyses might also be incorporated in other national food composition tables where similar foods are eaten. However, more data on the variability of the inorganic compositions and phytate level are needed. Phytate content of cereals predict poor iron and zinc bioavailability and therefore a negative impact on the micronutrient status. Appropriate strategies aiming to improve the bioavailability of iron and zinc from a high fibre plant-based diet as consumed in the study area are warranted.

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Chapter 5

Effect of soaking, dehulling and dephytinisation on iron and zinc solubility and on iron bioavailability from a sorghum paste (dibou) as consumed in northern Benin

Comlan E.S. Mitchikpe
Romain A.M. Dossa
Guido JEJ Hooiveld
Paul J.M. Hulshof
Robert MJ Nout
Joop M.A. van Raaij
Frans J. Kok

Submitted

ABSTRACT

Objective: To measure the effect of reducing phytate and polyphenol contents in dibou (the most commonly eaten sorghum food in northern Benin) by various food processing methods (1) on iron and zinc solubility and (2) on iron bioavailability.

Material and methods: Grains from a sorghum cultivar (Chabicouman) were purchased in a local market in northern Benin. The grains were used for 5 different preparations based on: whole grains, pounded grains soaked for 1 and 3.5 h, dephytinised grains (using exogenous phytase) and dehulled grains. Each preparation resulted in flour which was used to cook dibou. In vitro iron and zinc solubility were measured in dibou using the Glahn and the Kiers method. Iron bioavailability was measured using the in vitro digestion/Caco-2 cell model.

Results: Dehulling resulted in the highest iron and zinc losses (46 and 36 %, respectively). Highest phytate reductions were obtained by dephytinisation and dehulling (73 and 37 %, repsctively) and the highest reduction in phenolic compounds was obtained in flour by dehulling (68 %). There was a significant food processing effect on iron and zinc solubility ($P<0.01$), the highest values being obtained for dephytinisation followed by dehulling. There was no food processing effect on iron bioavailability although dephytinisation and dehulling gave 10 % higher values.

Conclusion: To increase iron bioavailability from dibou, further reduction of phytate and phenolic compounds might be warranted. More research combining varieties and food processing methods might achieve an adequate degradation of phytate and phenolic compounds for increased iron and zinc bioavailability.

Key words: Food processing methods, iron and zinc solubility, iron bioavailability, sorghum paste, Benin

INTRODUCTION

Several studies have shown relations between the source of iron and zinc in foods and their bioavailability[1-3]. It has been demonstrated that micronutrient bioavailability (in particular that of iron and zinc) from legumes and cereals might be poor because of inhibiting factors present in these foods, the most important among them being phytate and polyphenols[4-6]. Although iron and zinc bioavailability from cereals might be poor, many people have to live on cereals as main staple food with only a small consumption of meat and fish, especially in rural areas in developing countries. As demonstrated in several studies, this is the main cause of the high prevalence rates of iron and zinc deficiencies in these populations[7-10]. Improving iron and zinc bioavailability from commonly consumed cereal food products might therefore be beneficial for such populations and might contribute to a better iron and zinc status.

Previous studies performed in school-age children in northern Benin revealed a poor growth performance and a poor iron status. The food pattern was dominated by foods from plant origin with cereals as main staple foods. Iron and zinc bioavailability from the diet is estimated to be low and is probably one of the causes of the poor nutritional status of the children (Mitchikpe *et al.*, unpublished results).

Several studies did investigate the effect of reducing phytate and polyphenol contents of foods by various processing methods on iron and zinc bioavailability. Iron and zinc bioavailability was then often estimated using mineral solubility, and phytate/zinc or phytate/iron molar ratios[4, 11-15]. However, estimates of bioavailability from foods based on measurements of solubility alone have proven to be inadequate[16,17]. All soluble or dialyzable iron or zinc is not absorbable. Therefore in the present study, the recently developed Caco-2 cell model for measuring iron bioavailability[18] was used to include absorption.

Previous bioavailability studies performed in Africa mainly focussed on food eaten by children (porridges or weaning foods) but only limited data are available on foods consumed by adults as well as children. In northern Benin, Dibou is the main cereal food consumed by children and adults and therefore it represents a good vehicle for improving iron and zinc status of the population.

Therefore, the objective of the present study was to measure the effect of reducing phytate and polyphenol contents in dibou (the most commonly eaten sorghum food in northern Benin) by various food processing methods (1) on iron and zinc solubility and (2) on iron bioavailability using in vitro digestion methods with iron uptake by Caco-2 cells.

MATERIAL AND METHODS

Design of the experiment

About 20 kg of sorghum grains from a cultivar locally called Chabicouman were purchased in a local market in Natitingou in the Atacora province in northern Benin. From this batch of sorghum (see Figure 1), samples were used for 5 different preparations. Each preparation used another combination of food processing methods. Each preparation resulted in flour and 500 g of each flour were used to prepare dibou, a local sorghum paste. The dibou was prepared according to the traditional recipe. The 5 dibou were then freeze-dried and powdered. Iron bioavailability from dibou powders was measured using the in vitro digestion/Caco-2 cell model as described by Glahn *et al.*[18]. The freeze-dried dibous were also digested according to Kiers *et al.*[19]. Iron, zinc, phytate (inositol hexaphosphate or IP6) and total phenolic compounds (PC) were analysed in sorghum flours and in dibou powders. In addition, iron and zinc were analysed in digests of dibou powders obtained using Glahn method as well as Kiers method.

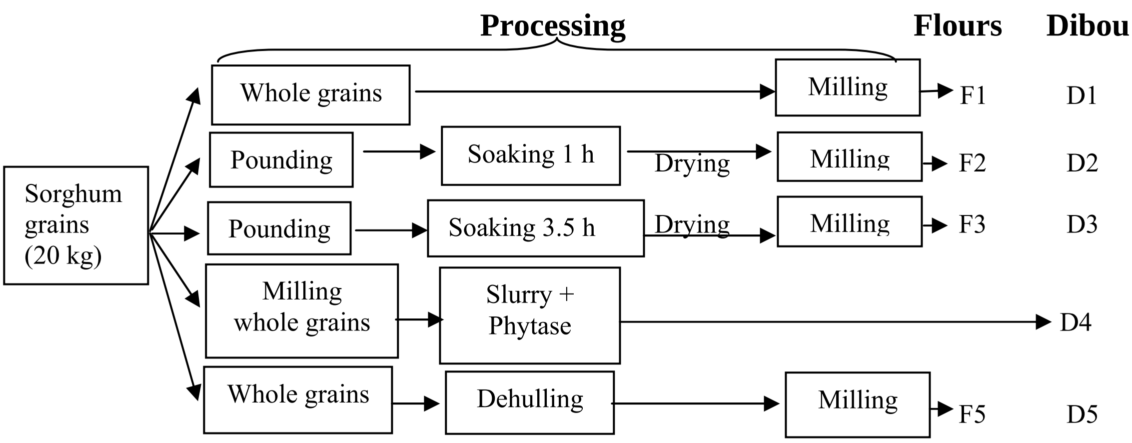

Fig. 1. Flow chart for the preparation of different dibous, F = flour ; D = dibou

Collection of sorghum grains and handling

Initially the 5 most common sorghum cultivars in the region of Natitingou were identified. Among these cultivars, Chabicouman appeared to be the most commonly used and therefore it was selected for the present study. Chabicouman is also easily recognisable because of the large size of the grains and it has the highest 1000-kernel weight compared to other cultivars[20].

About 4 kg of Chabicouman were purchased from 6 retailers in the local market in Natitingou. Equal portions from each retailer were mixed to arrive at about 20 kg of grains. The grains were transported to the Laboratory of the Département de Nutrition et Sciences Alimentaires of The Faculté des Sciences Agronomiques of the Université d'Abomey-Calavi in Benin. In the laboratory, the grains were cleaned from dust and were washed several times with tap water. The cleaned humid grains were then sun dried, homogenously mixed and handled as follows:

- 2 x 1.5 kg were sampled, put in plastic bags and heat-sealed.
- 3 kg were sampled and pounded using a wooden mortar. The pounded grains were divided into 2 portions of equal weights (2 x 1.5 kg), put in plastic bags and heat-sealed.
- 8 kg were sampled and dehulled using a dehuller: Décortiqueur PRL. The characteristics of the dehuller were as follows: capacity, 8 kg; extraction yield, 70 % in two minutes. From the dehulled grains, 1.5 kg was sampled, put in plastic bag and heat-sealed.

The 5 sealed plastic bags were transferred to the laboratory of the Division of Human Nutrition at Wageningen University in the Netherlands. In the laboratory, they were stored at 4 °C until further elaborations. Processing of sorghum flours, preparation of dibous, digestion of dibou powders according to Kiers method and analysis of PC were performed within the laboratory of Food Microbiology. Digestion of dibou powders according to Glahn method and measurement of iron bioavailability were performed in the laboratory of Nutrition, Metabolism and Genomics. Phytate was analysed in the laboratory of Plant Physiology. Iron and zinc were analysed in the Analytical Soil laboratory.

Processing of sorghum flours: from grains to flours and suspension

Before processing, each sample of the cleaned sorghum grains was homogenised using the Moulinette, type D56 (Moulinex, GMBH). The Moulinette was equipped with stainless-steel blades. The homogenised samples were subsequently used in one of the 5 preparations (see figure 1):

Preparation 1: no pounding, no soaking

A sample of whole grains was ground into fine flour using a Retsch mill with 0.5 mm screen. The flour was used to prepare dibou. This dibou was considered as reference.

Preparation 2: pounding and soaking for 1 hour

A sample of pounded grains was soaked for 1 h as follows. One part pounded sorghum grains was soaked in 4 parts of water at 30 °C and after 1 h excess water was decanted. Soaked pounded grains were dried overnight on filter paper at 45 °C in an air oven. Grains were then milled as described for preparation 1.

Preparation 3: pounding and soaking for 3.5 hours

The second sample of pounded grains was soaked for 3.5 h, dried and milled as described for preparation 2.

Preparation 4: phytate degradation using exogenous phytase

The second sample of whole grains was ground into fine flour as described for preparation 1. Plant phytase from wheat (Sigma P-1259) was purchased and used for phytate degradation. 10 g of phytase (0.03 units/mg solid) were added to a suspension obtained by adding water at 55 ºC to 500 g of sorghum flour. The pH of the suspension was adjusted to pH 5.15 and the mixture was incubated for 5 h in a water bath at 55 ºC. After the incubation period, the suspension was used to prepare dibou.

Preparation 5: dehulling

The sample of dehulled grains was milled as described for preparation 1. The obtained flour was used for the preparation of dibou.

Preparation of dibous from flours and the suspension

500 g of each flour obtained in preparations 1-3 and 5 were used to prepare dibou according to the traditional recipe. 125 g of flour were mixed with water (1:3, wt:wt). The slurry obtained was poured into boiling water while stirring (The ratio boiling water to total sorghum flour was 3:1, wt:wt). The mixture was let to boil for 5 min after which the remainder of the flour (375 g) was added gradually and mixed properly to give the thick porridge called dibou. The dephytinised sorghum suspension obtained in preparation 4 was also used to prepare dibou by pouring gradually in 1.5 L boiling water and mixing properly. Since there were 5 different preparations, also 5 different dibous were prepared. After preparations, dibous were freeze-dried, powdered, put in jar, labeled and stored at 4 ºC for further analyses.

In vitro digestion of dibou powders: preparation of digests

Two in-vitro digestion methods (Glahn and Kiers methods) were used for a comparison purpose.

a) In vitro digestion of dibou powders according to Glahn method

Samples of the 5 different dibou powders were digested for iron uptake by Caco-2 cells and also for iron and zinc solubility. The preparation of digestion solutions including pepsin, pancreatin and bile extract and the in vitro digestion procedures were performed as described by Glahn *et al.*[18].

Peptic digestion was conducted on a rotating mixer in an incubator at 37 °C. Intestinal digestions were conducted on a rocking platform shaker (Reliable Scientific, Hernando, MS) in an incubator at 37 °C with a 5 % CO_2/95 % air atmosphere maintained at constant humidity. The intestinal digestion was carried out in the upper chamber of a two-chamber system in 6-well plates, with the cell monolayer attached to the bottom surface of the lower chamber[18].

To start the peptic digestion, 0.5 g of each of the 5 dibou powders were suspended in about 8 mL of 120 mmol/L NaCl and 5 mmol/L KCl solution and homogenised. The pH of the suspension was adjusted to pH 2.0 with 5.0 mol/L HCl and the volume was brought to 10 mL with the 120 mmol/L NaCl and 5 mmol/L KCl solution. 0.5 mL of pepsin solution was added to the suspension contained in 50-mL screw-cap culture tube. The tube was capped, placed in oblique position on a rotating mixer and incubated for 60 min (20 rpm) at 37 °C. For the intestinal digestion step, the pH of the suspension treated with the pepsin solution was raised to pH 6 by dropwise addition of 1 mol/L $NaHCO_3$. Then 2.5 mL of pancreatin-bile extract mixture were added per 10 mL of original suspension. The pH was adjusted to pH 7 with NaOH, and the volume was brought to 15 mL with 120 mmol/L NaCl and 5 mmol/L KCl.

Ascorbic acid was added to each sample before the peptic digestion in order to increase the amount of absorbable iron in the lower chamber. The molar ratio of added ascorbic acid to iron was 10:1.

Digests of dibou powders obtained as described above were used for iron uptake by Caco-2 cells. For measurement of iron and zinc solubility, digests were incubated for two hours under the same conditions as described for intestinal digestion and centrifuged at 3600 g for 15 min at 4 ºC.

b) In vitro digestion of dibou powders according to Kiers method[19].

Duplicate samples of dibou powders (5 g) were suspended in 30 mL distilled water and digested under simulated gastro-intestinal conditions, using α-amylase solution (Sigma A-1031), stomach medium consisting of lipase (Amano Pharmaceuticals, Rhizopus F-AP15) and pepsin (Sigma P-6887), and pancreatic solution consisting of pancreatin (Sigma P-1750) and bile (Sigma B-3883). After digestion, the suspension was centrifuged at 3600 g for 15 min at 4 ºC. The supernatant was decanted and the pellet was washed twice in 20 mL of distilled water and centrifuged. The supernatants were pooled and filtered in a 0.45 µm pore filter. A blank was included consisting of 30 mL distilled water digested and filtered as described above. Both filtered supernatants from sample and blank were analyzed for Fe and Zn.

Samples were corrected for added reagents/water by subtracting Fe and Zn content of blank from that of supernatants from samples. The amount of Fe and Zn in supernatant was regarded as soluble minerals. Percentage of soluble mineral was calculated as:
Solubility (%) = (Fe or Zn in supernatant – Fe or Zn in blank)/ (Fe or Zn in undigested sample).

Preparation of the in vitro digestion/Caco-2 cell model: Caco-2 cell culture

Caco-2 cells were grown in a Dulbecco's modified Eagle's medium as described by Glahn *et al.*[18]. Caco-2 cells were obtained from a stock used in the laboratory of Nutrition, Metabolism and Genomics. They were used in experiments at passage 64-74. Cells were seeded at a density of 50,000 cells/cm^2 in collagen-treated 6-well plates (6-well cell culture cluster dishes, Costar, Cambridge, MA). They were grown in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY) with 10% v/v fetal calf serum (GIBCO), 25 mmol/L HEPES, 1 % antibiotic antimycotic solution (GIBCO) and 1 % non-essential amino acid. The cells were maintained at 37 °C in an incubator with a 5 % CO_2/ 95 % air atmosphere at constant humidity; the medium were changed every 2 days. Cells were used in iron uptake experiments at 12 or 13 days post seeding.

Iron uptake by Caco-2 cells

The previously prepared digests of dibou powders were used for iron uptake by the Caco-2 cells. Immediately before the intestinal digestion period, the growth medium was removed from each culture well and the cell layer was washed twice with 37 °C Minimum Essential Medium (MEM, GIBCO) at pH 7. The MEM was supplemented with 10 mmol/L PIPES (piperazine-*N*,*N*'-bis-[2-ethanesulfonic acid]), 1 % antibiotic-antimycotic solution (Sigma), insulin (5 mg/L), and L-glutamine (5 ml/500 MEM). A fresh 1.0-mL aliquot of MEM covers the cells during the experiment. A sterilized insert ring, fitted with a dialysis membrane, was inserted into the well, thus creating the two-chamber system. Then a 2.5-mL aliquot of the intestinal digest (refers to the mixture following the peptic digestion period with pancreatic enzymes and bile extract, at pH 7.0) was pipetted into the upper chamber. The plate was covered and incubated on the rocking shaker at 6 oscillations/min for 120 min.
When the intestinal digestion was terminated, the insert ring and digest were removed. The solution in the bottom chamber was allowed to remain on the cell monolayer and an additional 1 mL of MEM was added to each well. The cell culture plate was returned to the incubator for an additional 22 h. After that period, the cells were harvested as described by Glahn *et al.*[18].

Five series of experiments (5 replications) were conducted. In each series, 5 different digests of dibou powders and 1 control (a digest containing $FeSO_4$, 100 µmol/L) were compared simultaneously using a standard 6 well plates. Preparation of the digest of the control was done exactly in the same conditions as for digests of dibou powders. Duplicate measurements of cell iron uptake was performed on each sample, so 2 x 6 well plates were used for each experiment. The standard was added to each 6 well plates. Replication of an experiment was performed on separate days.

Chemical analyses

a) Analyses of sorghum flours and dibou powders

All analyses were performed in duplicate.

- Phytate: Phytate was analysed by anion exchange HPLC (ICS2500 system) according to the methodology used by Bentsink *et al.*[21]. Approximately 2 – 3 mg of dibou powder were extracted with 1 mL 0.5N HCl containing 50 mg/L cis-aconitate used as internal standard. The mixture was boiled in a water bath at 100 ºC for 15 minutes and then centrifuged at 14000 rpm for 5 min. The supernatant was diluted 10x in Millipore water. Anions were separated on an AS11-HC anion exchange column (4 x 250 mm, Dionex) preceded by an AG11-HC guard column and eluted with NaOH. 20 µL of the diluted supernatant was analysed for anion detection with suppressed conductivity. Background conductivity was decreased using an ASRS suppressor with water as a counterflow (5 mL/min). 20 mg $Na_2HPO_4.2H_2O$ and 50 mg Na (12)-phytate (sigma P3168) were used as external standards.
- Total phenolic compounds: PC was extracted from 100 mg of dibou powder by adding 1.5 mL of 1 % (v/v) HCL in methanol, followed by continuous stirring at 25 °C for 20 minutes[22]. The suspension was then centrifuged at 1300 rpm for 10 min and 1 mL of the supernatant was collected. Next, the pellet was re-extracted with 1 mL HCl-methanol as described above, and 1 mL of the supernatant was pooled with the previously obtained supernatant. PC were determined using Folin-Ciocalteu's method adapted to a 96-well plate assay[23]. 30 µL Folin-Ciocalteu's reagent was added to 10 µL of extract (50 %, v/v). After 5 minutes of incubation at 25 °C, 50 µL of a 10 % (v/v) sodium carbonate solution and water were added to the mixture to have a final volume of 200 µL. Blanks were prepared for each sample by replacing Folin-Ciocalteu's reagent with water to correct for interfering compounds in the sample. Gallic acid was used as standard ranging from 100-500 mg/L. The plate was incubated at 25 °C for 30 minutes and the absorbance was read at 760 nm. Results were expressed as gallic acid equivalent per hundred gram of dibou powder (w/w, dry matter base).

- Iron and zinc: iron and zinc content were analyzed in dibou powders using a microwave digestion and spectrometric method as described by Novozamsky *et al*.[24]. Approximately 0.4 g of dibou powders were weighed in a metal weighing funnel and transferred to PTFE digestion vessel. 5.0 mL of hydrofluoric acid 40 % (w/w) and 5.0 mL nitric acid 65 % (w/w) were added to the samples and mixed. The mixtures were allowed to stand overnight at room temperature and then heated at 120 ºC to almost dryness. Another 5.0 mL of concentrated nitric acid 65 % (w/w) and three times 1.0 mL of hydrogen peroxide 30 % (w/w) were added to the dried dibou powder. The mixture was carefully swirled in order to moisten all the dibou powder and the digestion inner vessels were put into the polyetherimide outer vessels. The vessels were closed and placed in the digestor (MDS-2100). After the microwave digestion, vessels were allowed to cool down for 45 min and opened in a fume hood. The digests were quantitatively transferred to a 50 mL polythene volumetric flask, made up to mark with Millipore water, mixed and then filtered over fine paper into a polythene bottle. Iron and zinc concentration in the digests were analysed using Inductively Coupled Plasma-Atomic Emission Spectrometer (VITA-PRO CCD Simultaneous ICP-AES, Varian).

b) Analyses of digests of dibou powders

Iron and zinc were analysed in digests of dibou powders in order to assess iron and zinc solubility. Analyses were performed using Inductively Coupled Plasma-Atomic Emission Spectrometer (VITA-PRO CCD Simultaneous ICP-AES, Varian).

c) Analyses of Caco-2 cell monolayers

Caco-2 cell protein content and ferritin formation were analysed in cell monolayers harvested in 2 mL of deionised water. The solutions were previously centrifuged at 13200 rpm for 5 min at 4 ºC.

- Caco-2 cell protein: Caco-2 cell protein was measured in cell monolayers using a semi-micro adaptation of the Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Hercules, CA).
- Ferritin: Caco-2 cell ferritin content was measured by turbidimetric immunoassay. Ferritin kit was provided by Instruchemie (Instruchemie bv, The Netherlands). 20 µL of the sonicated Caco-2 cell monolayer, harvested in 2 mL of water, were used for each ferritin measurement.

Data elaboration and Statistical analyses

Differences in iron and zinc solubility measured by Glahn and Kiers method and the effects of food processing methods, and differences in ferritin formed by Caco-2 cells from digests of dibou were analysed using ANOVA. Experiments were replicated during 5 days. Experiments on days 1 and 2 were performed in order to get use to the methodology and data are omitted from statistical analysis. P values less than 0.05 were considered statistically significant. Analyses were performed using SPSS statistical package for windows (version 11.2).

RESULTS

The iron, zinc, phytate (inositol hexaphosphate or IP6) contents and total phenolic compounds (PC) in sorghum flours and dibou powders obtained using various processing methods are summarised in Table 1. Dehulling resulted in a substantial reduction of iron (46 %) and zinc content (36 %) of dibou compared to no processing. The highest reduction of phytate content of dibou was obtained using an exogenous phytase (73 %). Dehulling resulted in a 37 % reduction of phytate content of dibou whereas pounding and soaking for 1 and 3.5 h resulted in 29 % and 25 % reduction, respectively. Dehulling resulted in 68 % reduction of PC in the sorghum flour. The preparation of dibous from sorghum flours resulted in conflicting PC values. The comparison between sorghum flours and dibou powders shows a substantial reduction of PC (65 %) when there was no processing whereas there was a substantial increase (88 %) when dehulling was used. The lowest PC value was obtained in dibou powder with no processing whereas the highest was obtained in dephytinised dibou powder.

Table 1: Iron, zinc, phytate and total phenolic compounds contents of sorghum flours and dibou powders obtained using various processing methods

	Dry matter (DM) (%)	Fe	Zn	Phytate	Total phenolic compounds (g/100g DM)
		(mg/100g DM)			
Sorghum flours					
F1 (No processing (whole grain))	92.5	7.6	2.7	1150	0.75
F2 (Pounded and soaked for 1 h)	96.9	4.6	2.4	1062	0.51
F3 (Pounded and soaked for 3.5 h)	96.7	5.4	2.4	835	0.57
F4 (Dephytinised)	NA	NA	NA	NA	NA
F5 (Dehulled)	93.4	4.4	2.1	710	0.24
Dibou powders					
D1 (No processing (whole grain))	97.6	5.7	2.5	1177	0.26
D2 (Pounded and soaked for 1 h)	97.6	4.7	2.5	838	0.44
D3 (Pounded and soaked for 3.5 h)	97.6	4.5	2.5	881	0.47
D4 (Dephytinised)	97.4	4.9	2.8	322	1.00
D5 (Dehulled)	97.2	3.1	1.6	741	0.45

NA: Unfortunately no sample was taken for analyses

Iron and zinc in vitro solubility as measured by Glahn and Kiers method are summarised in Figures 2 and 3. Iron solubility ranged from 9 to 23 % for Glahn method and from12 to 17 % for Kiers method. There was no difference between Glahn and Kiers method; however, iron solubility was different between food processing methods ($P<0.01$). The highest iron solubility was obtained by dephytinisation. Zinc solubility ranged from 7 to 30 % for Glahn method and from 5 to 13 % for Kiers method. There was a significant difference between methods ($P<0.01$). Zinc solubility according to Glahn showed differences between food processing methods ($P<0.01$) whereas according to Kiers differences didn't reach statistical significance ($P=0.17$). Like for iron, the highest zinc solubility was obtained by dephytinisation (Glahn method).

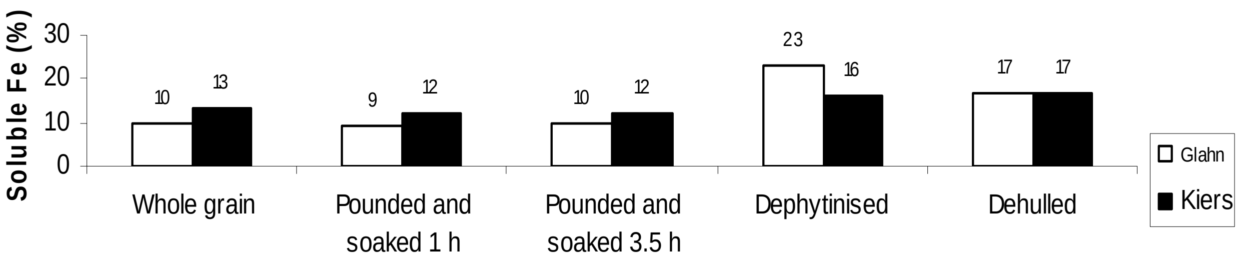

Fig. 2. In vitro solubility of iron from dibou powders using Glahn and Kiers methods

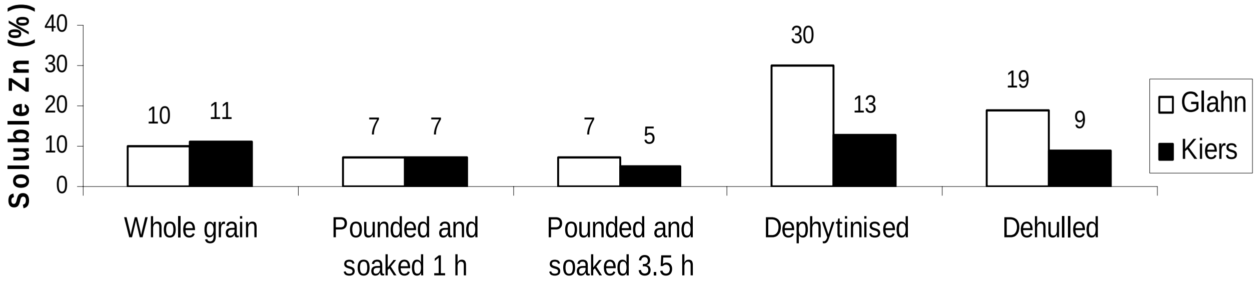

Fig. 3. In vitro solubility of zinc from dibou powders using Glahn and Kies methods

Iron bioavailability from dibous was measured using an in vitro digestion method with iron uptake by Caco-2 cells. Mean iron bioavailability data as measured by Caco-2 cell ferritin formation per milligram of cell protein are summarised in Table 2. Mean ferritin formations ranged from 95 to 122 ng mg^{-1}. Dephytinised and dehulled dibous showed the highest ferritin formation however, there was no significant difference between processing methods (P=0.61). Ferritin formation by Caco-2 cells using the control solution ($FeSO_4$, 100 µmol/L) was significantly higher than that from dibou powders (P<0.01).

Table 2: Mean iron bioavailability from dibou as measured by Caco-2 cell ferritin formation per unit of cell protein and as a percentage of reference dibou (unprocessed grain)

Dibou made of sorghum treated as below	n*	Ferritin/cell protein (ng mg^{-1})		Ferritin formation (% of reference)
		Mean	SD	
D1 (No processing (whole grain))	10	110	37	100†
D2 (Pounded and soaked for 1 h)	10	107	44	97
D3 (Pounded and soaked for 3.5 h)	12	95	38	86
D4 (Dephytinised)	12	122	45	110
D5 (Dehulled)	12	120	70	108
Control ($FeSO_4$, 100 µmol/L)	8	226‡	61	205

* Number of measurements
† Whole grain dibou is used as reference
‡ Significantly higher than other treatments (P<0.01)

DISCUSSION

The objective of the current study was to measure the effect of reducing phytate and polyphenol contents in dibou (the most commonly eaten sorghum food in northern Benin) by various food processing methods (1) on iron and zinc solubility and (2) on iron bioavailability using in vitro digestion methods with iron uptake by Caco-2 cells.

Effects of processing methods on iron, zinc, phytate and PC in flours and dibou

Dehulling (which consists in removing the outer layers of grains) had led to the highest iron and zinc losses in dibou although iron losses were more pronounced. Lower zinc losses might be due to the fact that iron and zinc are not in the same proportion in the various parts of the seeds. The proportion of iron and zinc losses by dehulling is in agreement with that reported by Lestienne *et al*. in decorticated pearl millet[11]. Soaking pounded sorghum grains for 1 and 3.5 h resulted in similar phytate reductions in dibou (29 and 25 % reductions, respectively). The observed reductions are higher than those reported by Lestienne *et al*.[12] for whole grains after soaking for 24 h. This difference in observations might be caused by differences in particle sizes. Results on the soaking of pounded grains versus whole grains suggest a passive diffusion of phytate into the soaking water. The reduction in phytate content of dibou using dehulling (37 %) is in line with that reported by Mahgoub and Elhag[15]. The highest phytate reduction was obtained by exogenous phytase (73 %) which is not surprising and is in line with results reported by Lestienne *et al*.[12].

Cooking of sorghum flours was expected to result in a reduction of assayable phenolic hydroxyl groups because of their possible polymerisation into condensed compounds as suggested in previous studies[25,26]. In the present study, a reduction of PC by cooking sorghum flour has been observed in whole grains (no processing) and in pounded grains soaked for 1 and 3.5 h. However, an increase of PC has been observed by cooking flours obtained using dephytinisation and dehulling. We have no explanation for this discrepancy between results; however, it cannot be due to the cooking method since flours were cooked in the same pot and under the same conditions. PC values of sorghum flours are in line with those reported by Dicko *et al*.[23]. The decreased PC values in dibou obtained using whole grains is also in line with results reported by Kayode *et al*.[19].

In summary, only dehulling resulted in a substantial reduction of iron and zinc contents of dibou. Moderate reductions of phytate (25 - 37 %) were obtained by the various methods (dehulling, soaking pounded grains for 1 and 3.5 h); however, the highest reduction was obtained by using an exogenous phytase. In dibou, results on PC values with respect to processing methods are inconsistent and need to be further explored.

Iron and zinc solubility in digests of dibou

The Caco-2 cell model used by Glahn *et al*. to measure in vitro iron bioavailability is preceded by an in vitro digestion of foods. In the laboratory of Food Microbiology of Wageningen University, Kiers method for an in vitro digestion of foods was recently used. This method also might be implemented as the first step in the in vitro digestion/Caco-2 cell model. Therefore, in the present study, dibou powders are digested according to both Glahn and Kiers methods.

Glahn and Kiers methods gave the highest iron solubility for dephytinisation and both showed food processing effect. Both methods also gave the highest zinc solubility for dephytinisation, but only the Glahn method showed a significant food processing effect.

Iron solubility from dibou was 17 % for dehulling and ranged from 16 to 23 % for dephytinisation and from 9 to 13 % for the other food processing methods (no processing, soaking pounded grains for 1 and 3.5 h). Zinc solubility according to Glahn method was 19 % for dehulling, 30 % for dephytinisation and ranged from 7 to 10 % for the other food processing methods. Iron and zinc solubility in dibou are in line with values reported by Kayode *et al.*[27]. The highest iron and zinc solubility obtained by dephytinisation might be due to the substantial reduction of phytate content (73 %). Iron and zinc solubility obtained by reducing phytate content of grains and PC values are in line with results reported in other studies[11,17,28].

In summary, the treatment of sorghum flour using exogenous phytase resulted in the highest phytate reduction and was followed by dehulling. 73 % reduction of the native phytate content of sorghum grains resulted in double iron and in three times zinc solubility. Reduced PC in flours by dehulling might also contribute to increase iron and zinc solubility however the effect of increase in PC values after cooking on mineral solubility needs further investigation.

Iron bioavailability from dibou

Glahn and co-workers successfully used a Caco-2 cell model to screen and rank rice and maize genotypes according to their iron bioavailability[29,30]. Therefore their approach could also be used for studying iron bioavailability from dibou varying in phytate and polyphenol contents. According to the Glahn method, iron bioavailability is approached by measuring ferritin formation by Caco-2 cells.

In the present study, a 10 % higher iron bioavailability from dibou was obtained by dephytinisation and dehulling when compared to no processing. Unfortunately, these differences didn't reach statistical significance (P=0.61). The phytate to iron molar ratio calculated by dividing the number of moles of phytate by the number of moles of iron was 17.5 for no processing, 5.6 for dephytinisation and 20.2 for dehulling. Phytate/iron molar ratio > 1 is an indication of poor iron bioavailability[31,32] and might explain the lack of food processing effect in the present study. The high ferritin formation by dehulling compared to no processing might be explained by the substantial reduction of PC value in flour (68 %).

In summary, although dephytinisation and dehulling resulted in an increased iron and zinc solubility and increased iron bioavailability, further reduction of phytate contents and in phenolic compounds might be warranted.

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Chapter 6

General discussion

Prevalence rates of iron and zinc deficiencies are high in developing countries. However, there is a paucity of data on the exact magnitude of the deficiencies. Previous studies performed in northern Benin revealed prevalence rates of stunting that ranged from 25 to 40 % in pre-school and school-age children[1] and prevalence rates of anaemia of more than 70 % among children < 5 years of age[2]. Since stunting is often associated with zinc deficiencies and anaemia with iron deficiencies, iron and zinc deficiency might be high in Beninese children. Although information on national food supplies (e.g. food balance sheets) exist for most developing countries, actual intakes of individuals on iron, zinc and antinutritional factors such as phytate and polyphenols are lacking. Also for Benin such information is not yet documented, and certainly not for children.

The main objectives of the research presented in this thesis were:

5. to assess the growth performance and iron and zinc status of rural Beninese school-age children in two seasons with a different food availability (post- and pre-harvest season)
6. to assess the actual food pattern of these school-age children in a post- and pre-harvest season
7. to establish the actual composition of foods commonly eaten in northern Benin, with special attention to iron, zinc, and phytate contents
8. to get more insight in the bioavailability of iron and zinc from foods commonly eaten in northern Benin, in particular of dibou, a commonly eaten sorghum-based food.

In order to achieve these objectives, the growth performance and the iron and zinc status (paragraph 6.1) as well as the food consumption patterns (paragraphs 6.2) of school-age children from three villages in northern Benin were assessed in a post- and a pre-harvest season. The macro- and micronutrient composition as well as the phytate content of foods commonly eaten by the children were measured (paragraphs 6.3). Finally, a commonly consumed sorghum based-food, dibou, was prepared according to the traditional recipe but the flours used were the results of five food preparation methods aiming to reduce phytate and polyphenol contents. The iron and zinc solubility and the iron bioavailability from the five different dibou were measured (paragraphs 6.4).

6.1. Nutritional status of the children

Growth performance

Anthropometrical data of the school-age children in the post-harvest season revealed that they were stunted (38 %) but not wasted (3 %). From the post-harvest to the pre-harvest season, the HAZ-score was significantly increased whereas the WHZ-score was significantly decreased (P<0.05). However the absolute differences were only 0.10 and 0.16 units for the HAZ-score and the WHZ-score, respectively and probably not relevant. Prevalence rates of stunting are in line with those reported by Ategbo about fifteen years ago[1]. This indicates that no nutritional improvement has occurred in the study area since that time, in spite of governmental and NGOs' (but apparently not very successful) efforts to improve the nutritional status of the local people. Obviously, there is still a strong need to focus on the nutritional status of children in northern Benin.

In the present study, children attending school had a significantly higher HAZ-score compared to children not attending school. However, unexpectedly, there were no significant differences in energy and nutrient intakes between both groups of children. The absence of differences in food intakes confirms that differences in anthropometrical characteristics were already present prior to the study. Since in the study area no socio-economic differences between households have been reported[3] and since nearly all children not attending school have a sister or a brother who does attend school, differences in nutritional status might not be explained by socio-economic background. Reasons behind these differences between children attending and not attending school need further investigation.

Iron status

Iron status of the children was assessed by haemoglobin and ferritin levels. Serum ferritin can be considered as an appropriate indicator of iron deficiency. Serum ferritin values < 12 µg/l always point to iron deficiency. However, serum ferritin level might be increased during infection or inflammations[4,5]. To correct for false increased values, the cut-off point was set to < 30 µg/l[6,7]. Serum transferrin receptor might be used as additional indicator but it is not appropriate for the diagnosis of iron deficiency in individuals with malaria[8]. The utility of serum ferritin will be enhanced when factors which falsely increase ferritin values can be assessed by clinical examination or by biochemical analyses such as the erythrocyte sedimentation rate or measuring C-reactive protein (CRP)[9]. In addition to these parameters, assessment of malaria and worm load would be helpful for a better distinction of anaemia with and without iron deficiency.

Biochemical analyses in blood samples were performed in the national hospital laboratory in Benin. For practical and methodological reasons an equipped laboratory in a research centre would be recommended for future research. Such a laboratory can be set up at the Département de Nutrition et Sciences Alimentaires of the Faculté des Sciences Agronomiques at Université d'Abomey-Calavi.

The prevalence of anaemia among the children has significantly increased from the post-harvest season (33 %) to the pre-harvest season (70 %). The significant decrease in haemoglobin level (7 g/l) from the post-harvest to the pre-harvest season might be due to increased levels of infections as measured by CRP. Elevated CRP values were measured in 20 and 30 % of the children in the post- and pre-harvest season, respectively. Prevalence rates of iron deficiency were 49 and 33 % in the post- and pre-harvest season, respectively, but the differences between seasons in iron status did not reach statistical significance. The iron intake of the children might be adequate; however, the high prevalence rate of iron deficiency suggests a poor iron bioavailability from the diet, probably because of inhibiting factors such as phytate and polyphenols.

Children attending school had a significantly higher haemoglobin level compared to children not attending school. This finding cannot be explained by differences in iron intake, but what the actual reasons behind this difference might be, is still to be solved.

Zinc status

Zinc status of the children was assessed by serum zinc level. There is no specific biological marker of Zn deficiency because even in the presence of zinc deficiency, zinc homeostasis might be maintained and serum zinc level might be normal[10]. However, serum/plasma Zn level is often used to assess Zn status particularly in large population studies. Assessment of hair or nail zinc concentration would be another option and would reflect long-term zinc status. In the present study we used serum zinc but we consider prevalence of stunting and adequacy of zinc intake as useful proxy indicators for zinc status.

Measured zinc levels were 17.9±4.6 µmol/l and 18.4±3.7 µmol/l in the post- and pre-harvest season, respectively. There were no significant differences between seasons and surprisingly serum zinc levels didn’t point to zinc deficiency. The food intake of the children also didn’t reveal inadequate zinc intakes. However, it goes too far to conclude that there was no zinc deficiency because of the poor growth performance. In addition, the children suffer from iron deficiency and it is known that iron and zinc deficiencies often accompany each other.

The nutritional status of school-age children in northern Benin is poor. Links between education level of a population and their social and economical development, and between education level and nutritional and health status are well established. Unfortunately, in the study area still a lot of children are not going to school. Although reasons behind attending or not attending school cannot not be elucidated by the present study, observations point out an unconscious discrimination by parents between nutritionally better-off and malnourished children. If this practice would continue, malnourished children will always remain behind. The solution is that all children should be sent to school. This can be achieved by the following actions: (1) motivate parents to send children to school. A school feeding programme might be useful. Such programme is already implemented by the international NGO Catholic Relief Service (CRS) in some schools in Natitingou, but not in the study villages. This programme needs to be improved and extended; (2) provide free access to education for all children, or at least in areas where children are not going to school because of limited resources of their parents.

In the study area, an annex hospital was available. However, access to care was limited because of lack for qualified personnel and for supplies. Qualified personnel and supplies are needed for delivering primary health care to avoid long distance travelling to people. There was no qualified nurse when we carried out the present studies and any urgent case requires transfer to the departmental hospital of Natitingou city at 30 km, whereas the road condition was poor. Moreover, in the study area, access to clean water was limited and sanitation was poor. There is a need for more infrastructures and qualified personnel in charge of primary health care and supplies for the annex hospital. Educational and health authorities in Benin recognise the right for everybody to have access to education and health services. However, actions are scarce and not evenly distributed over the country because of weak political will and limited resources. Therefore more political commitment is needed. The national and local governments, NGOs, civil societies and local people should work together.

6.2. Food intake of the children

Food consumption surveys showed that average daily diet provides three meals consisting of dibou served with a sauce of green leafy vegetables or okra and condiments. The high consumption of cereals (maize, sorghum and millet) and the consumption of small amounts of animal products (5 % of protein intake; 1 and 2 % of iron intake in the post- and pre-harvest season, respectively) suggest that iron and zinc bioavailability from the diet of the children might be poor. The consumption of animal protein by the children could not be expected to counteract effects of phytate and polyphenols in the cereal foods. In addition, the amount of consumed ascorbic acid is also not expected to improve iron and zinc bioavailability from the diet. Furthermore, the ascorbic acid content of the accompanying sauce might have been degraded because of prolonged heating practices during cooking. The food consumption data indicate that ascorbic acid intakes of the children were low and that ascorbic acid is not consumed during meal time.

The present study shows that although there was seasonality in food patterns of the children, food groups consumed were not really divers. This might be due to limited knowledge of people and to limited availability of and access to foods. Therefore raising knowledge of people and increasing incomes are important. In addition, food diversification might also be recommended. Several governmental food security projects have been implemented but were restricted to just a few vulnerable areas. Therefore a national food security programme covering all villages in Benin and monitoring and follow up of such programmes should become reality. The government, researchers, NGOs, civil societies, industries and populations should work together. The national agency for nutrition (Direction de l'Alimentation et de la Nutrition Appliquée (DANA)) should lead such an initiative and should establish a national nutrition information system which will be regularly updated.

6.3. Food composition

The FAO food composition table for Africa[11] which was used to derive the food composition database available at the University of Abomey-Calavi was critically evaluated. This evaluation revealed that there was scarce information on food data quality and there were no data on zinc and phytate content of foods. So it became necessary to perform chemical analyses in foods consumed by the children. Therefore foodstuffs most frequently consumed by the rural Beninese school-age children were sampled and analysed for their macro- and micronutrient, and phytate contents (Chapter 4).

The food composition data obtained in this study might be used by others (government, NGOs, private sectors etc.). However, updating the Benin food composition table should get more attention. Data on the composition of nutrient and antinutritional factors of conventional as well as non-conventional foods eaten in others regions in Benin are needed. A task force involving government, researchers, NGO and private sector, for the production, compilation and dissemination of food composition data, should be created.

6.4. Improving bioavailability

Iron bioavailability was assessed using an in vitro digestion method and iron uptake by Caco-2 cells. This method has been used by Glahn and co-workers to classify rice and maize genotypes[12,13] according to their iron bioavailability. Therefore we decided to use this methodology to study iron bioavailability from dibou, varying in phytate and polyphenol contents.

Effects of reducing phytate and polyphenol contents on iron and zinc availability from cereal foods were studied by several authors[14-19]. These studies showed that various processing methods can be used to achieve reductions in phytate and polyphenol contents in cereals. Among methods applicable at household level in poor rural settings, fermentation and germination were more effective. However, fermentation and germination might not be suitable for the preparation of dibou as consumed in the study area because of changes in sensory properties. Fermented dibou is consumed in southern Benin but not in the study area. Therefore introducing fermentation will need preliminary education of the population. The consumption of a fermented sorghum product (opaque sorghum beer) in the study area is promising. However, more studies on the effectiveness of using fermentation to improve iron and zinc bioavailability from dibou are needed.

Contrary to fermentation and germination, which will negatively affect the characteristics and sensory properties of dibou as consumed in the study area, soaking pounded grains and dehulling were likely to be more appropriate. The potential of soaking pounded grains and dehulling on increasing iron and zinc bioavailability was assessed by some researchers[14,16]. In the present study, we also use exogenous phytase to assess the effect of maximum phytate degradation on iron bioavailability. Although the in vitro solubility of iron and zinc has been improved by dephytinisation and dehulling, iron bioavailability was not significantly improved. This might be due to the fact that achieved reductions in phytate and polyphenol contents were not enough. In the present study, phytate/iron molar ratios of dibou using dephytinisation and dehulling were 5.4 and 18.7, respectively, and were still high[29,21]. Therefore food processing methods used in this study failed to improve iron and zinc bioavailability adequately. Therefore an optimal reduction of phytate and polyphenol contents of dibou is needed. To achieve this optimal reduction, a combination of food processing methods in the same preparation (dehulling and soaking, fermentation, dehulling and fermentation etc.) and varieties with low level of antinutritional factors might be studied. However, it should be mentioned that an optimal reduction of phytate and polyphenol contents of foods should outweigh the antioxydant properties of the antinutritional factors.

Therefore we would like to suggest: (1) Sorghum varieties with low phytate and polyphenol contents have been identified and should be selected and used in such study[22]. But in order to select such varieties, future studies are needed to assess the impact of genotype and environmental conditions on phytate, iron and zinc contents; (2) There is also a need for plant breeders to breed for low phytate and polyphenol content varieties with high iron and zinc contents[23]. Such varieties should be promoted and should replace the high phytate and polyphenol content varieties currently used in the study area.
This research should be seen as part of the national nutrition programme to improve the nutritional status of Beninese children.

The studies presented in this thesis indicate that school-age children in northern Benin have a poor growth performance and that many of them can be classified as stunted. This suggests that these children suffer from a long term marginal food intake (quantitatively and qualitatively) and inadequate sanitary conditions. This is confirmed by our food consumption surveys which also indicate that the iron and zinc bioavailability is poor because of the high levels of antinutritional factors as phytate and polyphenols. The low iron bioavailability is undoubtedly one of the causes of the high prevalence rate of anaemia among these children, with all the adverse effects on health and physical performance.

Although the situation in northern Benin might not be representative for the whole of Benin, there is not much evidence to assume that the situation in other parts of Benin would be better. Furthermore, it should be recognized that in northern Benin over the past 15 years there has hardly been any improvement in growth performance and nutritional status of the children. This should be considered as an undesirable situation.

A lot of initiatives might be started. School attendance should be further increased and sanitary conditions and nutrition and health education on schools and in households should be improved. People should become more aware that with other foods or with (slightly) modified food preparation methods healthier foods might be obtained. More research should be performed to get the most effective food-based approaches to improve the quantity and the quality of the food. Food consumption and nutritional status should be monitored, preferably throughout the whole country, in order to be able to respond timely to unwanted deteriorations, but also to evaluate the potential beneficial effects of local or national interventions. Choices should be made on the most appropriate food based approaches. For example, on the short-term food-fortification and supplementation might be appropriate, but on the long-term maybe more sustainable approaches should be implemented. For an appropriate monitoring and evaluation of food consumption a valid and complete Benin Food Composition Table is required, with commonly eaten foods and recipes from all parts of Benin.

Obviously, the nutritional and health situation of the children in (northern) Benin can only be improved if the problem is attacked by actions in which more sectors work together. The national or local governments might initiate task forces consisting of representatives of governments, non-governmental organizations and private sector, to deal with each of the above indicated initiatives.

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Summary

Prevalence rates of iron and zinc deficiencies are high in developing countries. However, there is a paucity of data on the exact magnitude of the deficiencies. Previous studies performed in northern Benin revealed prevalence rates of stunting that ranged from 25 to 40 % in pre-school and school-age children and prevalence rates of anaemia of more than 70 % among children < 5 years of age. Since stunting is often associated with zinc deficiencies and anaemia with iron deficiencies, iron and zinc deficiency might be high in Beninese children. Although information on national food supplies (e.g. food balance sheets) exist for most developing countries, actual intakes of individuals on iron, zinc and antinutritional factors such as phytate and polyphenols are lacking. Also for Benin such information is not yet documented, and certainly not for children.

The main aims of the present studies were: (1) to assess the growth performance and iron and zinc status of rural Beninese school-age children in two seasons with a different food availability (post- and pre-harvest season); (2) to assess the actual food pattern of these school-age children in a post- and pre-harvest season; and to derive from this information actual macro- and micronutrient intakes, including iron and zinc; (3) to establish the actual composition of foods commonly eaten in northern Benin, with special attention to iron, zinc, and phytate contents; and (4) to get more insight in the bioavailability of iron and zinc from foods commonly eaten in northern Benin, in particular of dibou, a commonly eaten sorghum-based food.

In **chapter 2** we addressed the issue of the assessment of the growth performance and iron and zinc status of rural Beninese school-age children in two seasons with different food availability (post- and pre-harvest season).

Eighty children aged 6 to 8 years were randomly selected from 3 villages in the Atacora province in northern Benin. Body weight, height, and mid upper arm circumference (MUAC) were measured and Z-scores for height-for-age and weight-for-height were derived. In the post-harvest season, the season with highest food availability, mean Z-scores for height-for-age and for weight-for-height were -1.72±0.89 (mean±SD) and -0.89±0.62, respectively. The children were clearly stunted but not wasted (respectively 34 and 3 % in the post-harvest season and 27 and 7 % in the pre-harvest season). A fasting venous blood sample was collected at the anthropometric sessions and used to measure haemoglobin level, serum ferritin, serum zinc and C-reactive protein. In the post-harvest season, haemoglobin level was 119±13 g/l, serum ferritin level was 43±31 µg/l and serum zinc level was 17.9±4.6 µmol/l.

The children have a poor iron status but zinc status seems to be adequate, which is surprising considering the poor growth performance. Growth performance and iron and zinc status was not significantly different between the two seasons, except for haemoglobin level (7 g/l lower in the pre-harvest season).
The Z-score for height-for-age of children attending school (-1.55±0.87) was significantly higher than that of children of the same age but not attending school (-2.14±0.80) ($P<0.05$). Also the haemoglobin level of children attending school (121±13 g/l) was significantly higher than that of children not attending school (114±12 g/l) ($P<0.05$). The reasons behind the difference in nutritional status in relation to school attendance are unclear (children attending and not attending school are often from the same families) and need further investigation.

In **chapter 3** we addressed the issue of the assessment of the actual food pattern of these school-age children in a post- and pre-harvest season. From the observed food consumption, the actual macro- and micronutrient intakes were derived, including iron and zinc. Food intake of the children was measured during three consecutive days using the observed weighed record method. Measurements were performed by well-trained local assistants from 7 a.m. until subjects eat their last meal, usually between 7 and 9 p.m. The consumed amounts of the various foods and their energy and nutrient contents were computed using the software programme Komeet and an updated version of the Benin food composition table (see further). The intakes of millet, sorghum, cassava and vegetables in the post-harvest season were significantly lower than those in the pre-harvest season (P<0.05), whereas the amount of yam consumed in the post-harvest season was substantially higher (P<0.05). Only small amounts of animal products were consumed. The observed seasonal variation in food pattern did not really result in seasonality in energy and nutrient intakes. Energy intakes of the children were 5.2±1.4 MJ/day and 5.4±1.3 MJ/day in the post- and pre-harvest season, respectively. Cereals and roots and tubers contributed to 77 % of the energy intake. Iron and zinc intakes were 17±7 and 6.6±1.9 mg/day, respectively, in the post-harvest season, and 18±6 and 7.1±2.9 mg/day, respectively, in the pre-harvest season. Energy and nutrient intakes were neither significantly different between seasons nor between children attending and not attending school. Energy and nutrient intakes seemed to be adequate. However the diet was low in animal products, protein, fat and vitamin C and was high in fibre. Therefore we hypothesised that the absorption of fat, fat-soluble vitamins, carotenoids, iron and zinc from the diet of these children might be low.

In **chapter 4** we addressed the issue of the assessment of the actual composition of foods commonly eaten in northern Benin. Special reference is given to their iron, zinc, and phytate contents.

Chemical analyses were performed in 23 composite samples of most frequently consumed foodstuffs collected from retailers in 2 local markets. The selection of foodstuffs for sampling was based on the outcomes of the food consumption surveys carried out in three villages in the study area (see before). Protein contents were in agreement with those from the FAO food composition table for Africa. Fat and fibre levels were higher whereas carbohydrate and energy levels were lower. Differences were mainly due to either analytical or calculation methods. Most important sources of iron and zinc in the diets in northern Benin were maize, sorghum and millet. In these cereals, iron and zinc ranged from 2.6 to 8.4 and 2.2 to 3.4 mg/100 g, respectively. Phytate (inositol hexaphosphate) ranged from 104 to 503 mg/100 g. Phytate/iron and phytate/zinc molar ratios ranged from 1 to 11 and 3 to 22, respectively. These molar ratios are above critical values and suggest poor iron and zinc bioavailability.

Obviously, the iron and zinc status of people might be improved when the bioavailability of these minerals from the foods is enhanced. One option to improve this bioavailability is to reduce the amount of anti-nutritional factors, such as phytate. One of the promising and sustainable food-based approaches to achieve this reduction is modification of food preparation methods (see further).

The new proximate and inorganic compositions of foodstuffs provide better estimates of the actual energy and nutrient intakes of people in northern Benin than the compositions of the foods given in the FAO food composition table for Africa. We therefore included the new data in an updated Benin food composition table for present and future use.

In **chapter 5** we addressed the issue of how the bioavailability of iron and zinc from foods can be improved by food preparation methods. In our study, we focused on dibou, a commonly eaten sorghum-based food in northern Benin.

Grains from a sorghum cultivar (Chabicouman) were purchased in a local market in northern Benin. The grains were used for 5 different preparations based on: whole grains, pounded grains soaked for 1 and 3.5 h, dephytinised grains (using exogenous phytase) and dehulled grains. Each preparation resulted in flour which was used to cook dibou. In vitro iron and zinc solubility were measured in dibou using the Glahn and the Kiers method. Iron bioavailability was measured using the Glahn in vitro digestion/Caco-2 cell model.

Dehulling resulted in the highest iron and zinc losses (46 and 36 %, respectively). Highest phytate reductions were obtained by dephytinisation and dehulling (73 and 37 %, respectively) and the highest reduction in phenolic compounds was obtained in flour by dehulling (68 %). There was a significant food processing effect on iron and zinc solubility ($P<0.01$), the highest values being obtained for dephytinisation followed by dehulling. There was no food processing effect on iron bioavailability although dephytinisation and dehulling gave 10 % higher values. Since the phytate/iron molar ratio in dibou was still high (5.6 for dibou based upon dephytinised flour and even 20.2 for dibou based upon flour after dehulling), levels of phytate and polyphenols are probably not yet sufficiently reduced.
To increase iron bioavailability from dibou, further reduction of phytate and phenolic compounds might be warranted. More research combining varieties and food processing methods might achieve an adequate degradation of phytate and phenolic compounds.

The studies presented in this thesis indicate that school-age children in northern Benin have a poor growth performance and that many of them can be classified as stunted. This suggests that these children suffer from a long term marginal food intake (quantitatively and qualitatively) and inadequate sanitary conditions. This is confirmed by our food consumption surveys which also indicate that the iron and zinc bioavailability is poor because of the high levels of antinutritional factors as phytate and polyphenols. The low iron bioavailability is undoubtedly one of the causes of the high prevalence rate of anaemia among these children, with all the adverse effects on health and physical performance.

Although the situation in northern Benin might not be representative for the whole of Benin, there is not much evidence to assume that the situation in other parts of Benin would be better. Furthermore, it should be recognized that in northern Benin over the past 15 years there has hardly been any improvement in growth performance and nutritional status of the children. This should be considered as an undesirable situation.

A lot of initiatives might be started. School attendance should be further increased and sanitary conditions and nutrition and health education on schools and in households should be improved. People should become more aware that with other foods or with (slightly) modified food preparation methods healthier foods might be obtained. More research should be performed to get the most effective food-based approaches to improve the quantity and the quality of the food. Food consumption and nutritional status should be monitored, preferably throughout the whole country, in order to be able to respond timely to unwanted deteriorations, but also to evaluate the potential beneficial effects of local or national interventions. Choices should be made on the most appropriate food based approaches. For example, on the short-term food-fortification and supplementation might be appropriate, but on the long-term maybe more sustainable approaches should be implemented. For an appropriate monitoring and evaluation of food consumption a valid and complete Benin Food Composition Table is required, with commonly eaten foods and recipes from all parts of Benin.

Obviously, the nutritional and health situation of the children in (northern) Benin can only be improved if the problem is attacked by actions in which more sectors work together. The national or local governments might initiate task forces consisting of representatives of governments, non-governmental organizations and private sector, to deal with each of the above indicated initiatives.

Résumé

Les taux de carences en fer et en zinc sont élevés dans les pays en voie de développement. Cependant, les données sur l'ampleur de ces carences sont rares. Des études antérieures réalisées au Bénin ont indiqué des taux de retard de croissance linéaire qui varient entre 25 et 40 % chez les enfants d' âge scolaire et des taux d'anémie supérieurs à 70 % chez les enfants de moins de 5 ans. Compte tenu de ces prévalences, les carences en fer et en zinc seraient également élevées au sein du groupe des enfants dans la mesure où il existe une association entre le retard de croissance linéaire et la carence en zinc d'une part et l'anémie et la carence en fer d'autre part. Malgré l'existence de systèmes d'informations sur la disponibilité alimentaire dans la plupart des pays en voie de développement, les mesures des quantités de fer et de zinc ainsi que des facteurs anti-nutritionnels (tels que les phytates et les polyphénols) consommées au niveau individuel ne sont pas disponibles. De telles informations manquent également au Bénin en général et au niveau des enfants en particulier.

Les principaux objectifs des études présentées dans cette thèse étaient (1) de mesurer la croissance et les statuts en fer et en zinc des enfants d'âge scolaire en milieu rural au nord Bénin dans deux saisons qui diffèrent par la disponibilité alimentaire (périodes post- et pré-récolte) (2) d'étudier le modèle alimentaire de ces enfants et de mesurer leurs apports en macro- et en micro-nutriments dont le fer et le zinc dans les deux saisons (3) d'établir la composition des aliments fréquemment consommés au nord Bénin avec une attention particulière au fer, au zinc et aux phytates et (4) d'analyser la biodisponibilité du fer et du zinc dans le dibou, une pâte à base de sorgho fréquemment consommés au nord Bénin.

Le **chapitre 2** est consacré à la mesure de la croissance et des statuts en fer et en zinc des enfants d'âge scolaire pendant les périodes post- et pré-récolte en milieu rural au nord Bénin.
Quatre vingt enfants âgés de 6 à 8 ans ont été sélectionnés au hasard dans trois villages du département de l'atacora. Le poids , la taille et la circonférence du bras ont été mesurés sur ces enfants et les indices taille pour âge et poids pour taille ont été calculés. Pendant la période post-récolte, les indices moyens taille pour âge et poids pour taille étaient de –1,72±0,89 (moyenne±écart-type) et de –0,89±0,62, respectivement. Les enfants avaient un retard de croissance linéaire mais n'étaient pas émaciés. Les taux de prévalence du retard de croissance linéaire et de l'émaciation étaient de 34 et 3 %, respectivement dans la période post-récolte et de 27 et 7 %, respectivement dans la période pré-récolte. Des échantillons de sang veineux ont été prélevés sur les enfants à jeun lors des séances de mesures anthropométriques. Les taux d'hémoglobine ont été mesurés dans le sang et les niveaux de

ferritin, de zinc et de protéine c-réactive ont été mesurés dans le sérum. Pendant la période post-récolte, le taux d'hémoglobine étaient de 119±13 g/l, les niveaux de ferritin et de zinc étaient de 43±31 µg/l et de 17,9±4,6 µmole/l, respectivement. Les enfants avaient un pauvre statut en fer alors que leur statut en zinc semblait adéquat. Le niveau de zinc est surprenant lorsqu'on considère le taux élevé de retard de croissance linéaire chez les enfants. La période d'étude n'a pas eu un effet important sur la croissance et les statuts en fer et en zinc des enfants, à l'exception du taux d'hémoglobine qui a diminué de 7 g/l pendant la période pré-récolte.

L'indice taille pour âge des élèves (-1,55±0,87) était supérieur à celui des enfants du même âge qui ne fréquentent pas l'école (-2,14±0,80) ($P < 0,05$). De plus, le taux d'hémoglobine des élèves (121±13 g/l) était supérieur à celui des enfants qui ne fréquentent pas (114±12 g/l) ($P < 0,05$). Les raisons qui expliquent la différence entre l'état nutritionnel des élèves et des enfants qui ne fréquentent pas l'école n'ont pas pu être élucidées par cette étude (on trouve souvent les enfants qui fréquentent et ceux qui ne fréquentent pas l'école dans le même ménage) et méritent d'autres investigations.

Le **chapitre 3** a abordé l'étude du modèle alimentaire des enfants d'âge scolaire dans les périodes post- et pré-récolte. Les apports en macro- et en micro-nutriments, dont le fer et le zinc, ont été calculés à partir des aliments consommés. La consommation alimentaire a été mesurée pendant trois jours consécutifs à l'aide de la méthode d'observation avec pesée des aliments. Les mesures ont été effectuées par des enquêteurs bien formés. Les enquêtes commençaient à 7 h le matin et se terminaient habituellement entre 19 et 21 h lorsque l'enfant finissait de consommer son dernier repas. Les quantités d'aliments consommés et leur teneur en énergie et en nutriments ont été calculées à l'aide du logiciel Komeet et de la nouvelle table de composition des aliments pour le Bénin (voir plus loin). Le mil, le sorgho, le manioc et les légumes feuilles ont été plus consommés pendant la période post-récolte comparativement à la période pré-récolte ($P < 0,05$) ; alors que la consommation d'igname pendant la période post-récolte était supérieure à celle de la période pré-récolte ($P < 0,05$). Les quantités de viandes consommées étaient négligeables. La variation saisonnière du modèle alimentaire ne s'est pas traduite par une variation saisonnière dans les apports en énergie et en nutriments. Les apports en énergie étaient de 5,2±1,4 et de 5,4±1,3 MJ/jour pendant les périodes post- et pré-récolte, respectivement. Les céréales et racines et tubercules ont contribué pour 77 % des apports en énergie. Les apports en fer et en zinc étaient de 17±7 et 6,6±1,9 mg/jour, respectivement pendant la période post-récolte et de 18±6 et 7,1±2,9 mg/jour, respectivement pendant la période pré-récolte. Les apports en énergie et en nutriments n'étaient différents ni entre les saisons, ni entre les enfants qui fréquentent et ceux qui ne fréquentent pas l'école. Ces apports semblaient être adéquats. Toutefois, l'alimentation était pauvre en produits d'origine animale, en protéine, lipide et en vitamine C et était riche en fibre. Nous avons alors émis l'hypothèse selon laquelle l'absorption des lipides, des vitamines liposolubles, des caroténoïdes et du fer et du zinc dans les aliments consommés par les enfants serait faible.

Le **chapitre 4** est consacré aux mesures de la composition des aliments fréquemment consommés au nord Bénin avec une attention particulière au fer, au zinc et aux phytates.
Les analyses chimiques ont été effectuées sur 23 échantillons composites d'aliments fréquemment consommés collectés auprès des détaillants dans deux marchés différents. La sélection des aliments pour l'échantillonnage a été fondée sur les résultats des enquêtes de consommation alimentaire réalisées dans trois villages dans la zone d'étude (voir plus tôt). Les teneurs en protéine étaient comparables à celles rapportées dans la table de composition des aliments pour l'Afrique de la FAO. Les teneurs en lipide et en fibre étaient supérieures a celles de la FAO, alors que les teneurs en glucide et en énergie étaient inférieures. Les différences avec les données de la table de composition de la FAO seraient principalement dues aux méthodes d'analyse ou aux méthodes de calcul utilisées. Les plus importantes sources de fer et de zinc dans l'alimentation au nord Bénin étaient le maïs, le sorgho et le mil. Au niveau de ces céréales, les teneurs en fer et en zinc étaient comprises entre 2,6 et 8,4 et entre 2,2 et 3,4 mg/100 g, respectivement. Les teneurs en phytates étaient comprises entre 104 et 503 mg/100 g. Les ratios molaires phytates/fer et phytates/zinc étaient estimés entre 1 et 11 et entre 3 et 22, respectivement. Ces ratios molaires sont supérieurs aux seuils critiques et suggèrent que la biodisponibilité du fer et du zinc dans les céréales est faible. Les statuts en fer et en zinc des populations pourraient s'améliorer si la biodisponibilité du fer et du zinc dans les céréales augmentait. Une des options pour améliorer la biodisponibilité du fer et du zinc dans les aliments consiste à réduire leur teneur en facteurs anti-nutritionnels tel que les phytates. Une approche alimentaire prometteuse et durable pour y arriver consiste à utiliser des méthodes de préparation améliorées (voir plus loin).
Les compositions proximales et inorganiques des aliments ont permis une meilleure estimation des apports en énergie et en nutriments des populations au nord Bénin comparativement aux données de la table de composition des aliments de la FAO. Par

conséquent, ces nouvelles données sur la composition des aliments ont été utilisées pour mettre à jour la table de composition des aliments pour le Bénin.

Le **chapitre 5** a abordé la question de savoir comment augmenter la biodisponibilité du fer et du zinc dans les aliments en utilisant des méthodes de préparation améliorées. Dans notre étude, nous avons focalisé notre attention sur le dibou, un aliment à base de sorgho fréquemment consommé au nord Bénin.

Des grains de sorgho de la variété Chabicouman ont été achetés dans un marché local au nord Bénin. Les grains ont été traités en utilisant 5 différents procédés de transformation que sont : gains entiers (pas de traitement), pilage et trempage des grains pilés pendant 1 h, pilage et trempage des grains pilés pendant 3,5 h, dégradation des phytates à l'aide d'une phytase, et décorticage. Chaque procédé de transformation a abouti à une farine qui a été utilisée pour préparer le dibou. Les solubilités in vitro du fer et du zinc ont été mesurées dans les dibou en utilisant les méthodes de Glahn et de Kiers. La biodisponibilité du fer a été mesurée à l'aide de la méthode de Glahn qui consiste en la digestion in vitro couplée avec l'absorption du fer par les cellules Caco-2.

Le décorticage s'est traduit par les pertes des teneurs en fer et en zinc les plus élevées (46 et 36 %, respectivement). Les réductions les plus élevées des teneurs en phytates ont été obtenues grâce à l'utilisation de la phytase et au décorticage (73 et 37 %, respectivement). La réduction la plus élevée des composés phénoliques a été obtenue dans la farine des grains décortiqués (68 %). Les procédés de transformation ont eu une effet significatif sur la solubilité du fer et du zinc ($P < 0,01$) ; les valeurs de solubilité les plus élevées étant obtenue pour la dégradation des phytates et pour le décorticage. Les procédés de transformation par contre n'ont pas eu d'effets significatifs sur la biodisponibilité du fer, malgré les valeurs élevées (10 %) observées pour la dégradation des phytates et le décorticage. Les niveaux de dégradation des phytates et des composés phénoliques n'étaient probablement pas suffisants dans la mesure où les ratios molaires phytates/fer étaient restés élevés (5,6 pour le dibou obtenu après dégradation des phytates et 20,2 pour le dibou obtenu après décorticage).

Afin d'augmenter la biodisponibilité du fer dans le dibou, une réduction des phytates et des composés phénoliques plus importante serait nécessaire. Ceci peut être atteint grâce à la combinaison de variétés et des méthodes de préparation.

Les études présentées dans cette thèse montrent que les enfants d'âge scolaire du nord Bénin ont une faible croissance et que plusieurs d'entre eux ont un retard de croissance linéaire. Ceci suggère que ces enfants souffrent depuis longtemps d'un apport alimentaire inadéquat (quantitativement et qualitativement) et des conditions sanitaires inappropriées. Les résultats ont été confirmés par les enquêtes de consommation alimentaire qui ont également indiqué que la biodisponibilité du fer et du zinc est faible à cause des teneurs levées des facteurs anti-nutritionnels tels que les phytates et les polyphénols. La faible biodisponibilité du fer est certainement l'une des causes du taux d'anémie élevé chez les enfants avec ses conséquences sur la santé et la croissance.

Bien que la situation au nord Bénin ne soit pas représentative pour tout le Bénin, il n'y a pas de raisons de croire qu'elle est meilleure dans d'autres régions du pays. De plus, il faut noter qu'aucune amélioration de la croissance et de la situation nutritionnelle des enfants n'a été observée durant les 15 dernières années. Ceci reste une situation déplorable.

De nombreuses initiatives doivent être prises. L'inscription des enfants à l'école, les conditions sanitaires, l'éducation en matière de nutrition et de santé dans les écoles et au niveau des ménages doivent être améliorées. Les populations doivent prendre conscience qu'on peut avoir une meilleure alimentation avec d'autres aliments ou en modifiant les méthodes de préparation. D'autres études doivent être réalisées pour identifier les approches alimentaires les plus efficaces pour améliorer la quantité et la qualité de l'alimentation. La consommation alimentaire et l'état nutritionnel doivent faire l'objet de suivi régulier, de préférence dans tout le pays, afin de pouvoir répondre à temps aux situations de détérioration indésirables. Le suivi permettra également d'évaluer les impacts des interventions sur le plan local et sur le plan national. Des choix d'approches alimentaires appropriées doivent être opérés. Par exemple la fortification et la supplémentation pourraient être envisagées pour le court terme alors que des approches plus durables peuvent être mises en oeuvre pour le long terme. Pour le suivi et l'évaluation de la consommation alimentaire, une table de composition des aliments valide et complète est nécessaire.

La situation nutritionnelle et sanitaire des enfants au (nord) Bénin ne pourra s'améliorer que si le problème est attaqué par des actions impliquant plusieurs secteurs. Le gouvernement ou les autorités locales doivent initier des groupes de travail composés des représentants, du gouvernement et des autorités locales, des organisations non-gouvernementales, et des représentants du secteur privé pour discuter chacune des initiatives proposées ci-dessus.

Samenvatting

In ontwikkelingslanden zijn de prevalentiecijfers van ijzer- en zinkdeficiënties hoog, al zijn er veelal te weinig gegevens beschikbaar om de precieze omvang vast te stellen. Voorgaande studies uit noord-Benin lieten bij kleuters en schoolkinderen prevalentiecijfers zien van *stunting* (achterblijven in lengtegroei) die varieerden van 25 tot 40%. Verder werden bij kinderen onder de vijf jaar prevalenties van anemie van meer dan 70% gevonden. Aangezien *stunting* vaak geassocieerd is met zinkdeficiënties en anemie met ijzerdeficiënties, zou er in Beninese kinderen sprake kunnen zijn van ijzer- en zinkdeficiënties. Hoewel voor de meeste ontwikkelingslanden gegevens over de nationale voedselbeschikbaarheid voorhanden zijn (bijvoorbeeld uit zogenoemde *Food Balance Sheets*), ontbreken vaak cijfers over daadwerkelijke innemingen met betrekking tot ijzer, zink en antinutritionele factoren zoals fytaat en polyfenolen. Ook voor Benin ontbreken deze voedselconsumptiegegevens, en vooral met betrekking tot kinderen.

De belangrijkste doelstellingen van de in dit proefschrift beschreven studies waren: (1) het vaststellen van de groei en van de ijzer- en zinkstatus van schoolkinderen in ruraal Benin in twee seizoen met een verschillende voedselbeschikbaarheid (na-oogstseizoen en voor-oogstseizoen); (2) het vaststellen van het daadwerkelijke voedselpatroon van deze schoolkinderen in beide seizoen, met de hieruit afgeleide daadwerkelijke innemingen aan macro- en micronutriënten, inclusief ijzer en zink; (3) het vaststellen van de daadwerkelijke samenstelling van voedingsmiddelen zoals deze in noord-Benin gewoonlijk gegeten worden, met speciale aandacht voor ijzer-, zink-, en fytaatgehaltes; en (4) het verkrijgen van meer inzicht in de biobeschikbaarheid van ijzer en zink uit in noord-Benin gebruikelijke voedingsmiddelen, in het bijzonder uit *dibou*, een veelgegeten van sorghum bereid gerecht.

In **chapter 2** hebben we ons gericht op de vaststelling van de groei en van de ijzer- en zinkstatus van rurale Beninese kinderen in de schoolleeftijd in twee seizoenen met een verschillende voedselbeschikbaarheid (na-oogstseizoen en voor-oogstseizoen).
Tachtig kinderen in de leeftijd van 6 tot 8 jaar werden ad random geselecteerd uit 3 dorpen in de Atacora provincie in het noorden van Benin. Lichaamsgewicht, lengte en bovenarmomtrek werden gemeten en z-scores voor lengte-voor-leeftijd en gewicht-voor-lengte afgeleid. In het na-oogstseizoen, dus het seizoen met de hoogste voedselbeschikbaarheid, waren de z-scores voor lengte-voor-leeftijd en gewicht-voor-leeftijd respectievelijk -1.72±0.89 (gemiddelde±SD) en -0.89±0.62. De kinderen waren duidelijk *stunted* maar niet *wasted* (mager, te licht voor hun lengte): respectievelijk 34% en 3% in het na-oogstseizoen, en 27% en 7% in het voor-

oogstseizoen. Bij de antropometrische meetsessies werden ook nuchtere veneuze bloedmonsters verzameld en werden hemoglobinewaarde, serumferritine, serumzink en C-reactive protein gemeten. In het na-oogstseizoen bedroeg de hemoglobinewaarde 119±13 g/l en was het serumferritine 43±31 µg/l en het serumzink 17.9±4.6 µmol/l. De kinderen hebben dus een een slechte ijzerstatus, maar hun zinkstatus lijkt adequaat te zijn, wat verrassend is gezien het achterblijven van hun groei. De groei-, ijzer- en zinkstatus waren niet significant verschillende tussen beide seizoenen, met uitzondering van de hemoglobinewaarde (7 g/l lager in het voor-oogstseizoen).

De z-score voor lengte-voor-leeftijd van kinderen die naar school gingen (-1.55±0.87) was significant hoger dan dat van kinderen van dezelfde leeftijd maar geen school bezochten (-2.14±0.80) ($P<0.05$). Ook de hemoglobinewaarde van kinderen die naar school gingen (121±13 g/l) was significant hoger dan dat van de andere kinderen (114±12 g/l) ($P<0.05$). De oorzaken achter dit verschil in voedingsstatus zijn nog onduidelijk en verdienen nader onderzoek (schoolgaande en niet-schoolgaande kinderen komen vreemd genoeg uit dezelfde gezinnen).

In **chapter 3** is de vaststelling van het daadwerkelijke voedingspatroon van de kinderen in een na-oogstseizoen en in een voor-oogstseizoen behandeld. Uit de waargenomen voedselconsumptie zijn de daadwerkelijke innemingen aan macro- en micronutriënten afgeleid, inclusief die van ijzer en zink. De voedselconsumptie van de kinderen is gemeten gedurende drie opeenvolgende dagen met behulp van de gewogen opschrijfmethode. De metingen zijn uitgevoerd door goed-getrainde locale assistenten van 7 uur ’s ochtends tot na de laatste maaltijd, meestal tussen 7 en 9 uur ’s avonds. De geconsumeerde hoeveelheden voedingsmiddelen als ook de hieruit afgeleide energie- en nutriënteninnemingen zijn berekend met het software programma Komeet en een herziene versie van de Beninese voedingsmiddelentabel (zie verder). De consumptie van gierst, sorghum, cassave en groenten was in het na-oogstseizoen significant lager dan in het voor-oogstseizoen ($P<0.05$), terwijl de consumptie van yam in het na-oogstseizoen substantieel hoger was ($P<0.05$). Er werden slechts geringe hoeveelheden dierlijke produkten geconsumeerd. De waargenomen seizoensvariatie in voedselpatroon vertaalde zich niet in een seizoensvariatie in energie- en nutriënteninnemingen. De energie-inneming van de kinderen bedroeg 5.2±1.4 MJ/dag en 5.4±1.3 MJ/dag in respectievelijk het na-oogstseizoen en het voor-oogstseizoen. Granen en wortelen en knollen droegen voor 77% bij aan de energie-inneming. De innemingen aan ijzer en zink waren respectievelijk 17±7 en 6.6±1.9 mg/dag in het na-oogstseizoen, en 18±6 en

7.1±2.9 mg/dag in het voor-oogstseizoen. Energie- en nutriënteninnemingen waren niet verschillend voor beide seizoenen en waren ook niet verschillend voor kinderen die wel of niet naar school gingen. De energie- en nutriënteninnemingen lijken adequaat te zijn, hoewel de dagelijkse voeding weinig dierlijke produkten bevat, laag is in eiwit, vet en vitamine C en hoog in voedingsvezel. Dit leidde tot onze hypothese dat de absorptie van vetoplosbare vitamines, carotenoïden, ijzer en zink uit de voeding van deze kinderen wel eens laag zou kunnen zijn.

In **chapter 4** is de vaststelling van de chemische samenstelling van voedingsmiddelen zoals deze in het noorden van Benin gegeten worden, besproken. Speciale aandacht is gegeven aan de ijzer-, zink- en fytaatgehaltes.

Voedselmonsters werden verzameld bij diverse handelaren op twee locale markten. De selectie van de voedingsmiddelen was gebaseerd op de uitkomsten van de voedselconsumptiesurveys welke eerder in drie dorpen in het studiegebied uitgevoerd waren (zie hiervoor). Chemische analyses werden uitgevoerd in 23 monsters. De eiwitgehaltes kwamen overeen met die in de FAO voedingsmiddelentabel voor Afrika. Vet- en vezelwaaarden waren hoger, terwijl koolhydraat- en energie-waarden lager uitvielen dan de FAO-waarden. De verschillen zijn vooral toe te schrijven aan het gebruik van betere analytische en berekeningsmethoden. De belangrijkste ijzer- en zinkbronnen in de voeding in noord-Benin waren maïs, sorghum en gierst. In deze granen varieerden ijzer- en zinkwaarden van 2.6 tot 8.4 mg/100 g voor ijzer en van 2.2 to 3.4 mg/100 g voor zink. Fytaatwaarden (inositol hexafosfaat) varieerden van 104 tot 503 mg/100 g. Fytaat/ijzer en fytaat/zinc molair ratio's varieerden respectievelijk van 1 tot 11 en van 3 to 22. Deze molair ratio's vallen boven hun kritieke waarden en suggereren derhalve een slechte ijzer- en zinkbiobeschikbaarheid.

Het is duidelijk dat de ijzer- en zinkstatus van de bevolking verbeterd kan worden indien de biobeschikbaarheid van deze mineralen uit hun voedsel verhoogd wordt. Een van de opties om deze biobeschikbaarheid te verhogen is het reduceren van de hoeveelheid antinutritionele factoren, zoals fytaat. Een van de meest belovende en duurzame voedsel-gebaseerde benaderingen om deze reductie te bewerkstelligen is de aanpassing van voedselbereidingsmethoden (zie verder).

De nieuwe macronutriënten- en mineraalsamenstellingen van voedingsmiddelen geven meer valide schattingen van de daadwerkelijke energie- en nutriënteninnemingen van de bevolking van noord-Benin dan de samenstellingen van de voedingsmiddelen uit de FAO voedingsmiddelentabel voor Afrika. De nieuwe gegevens zijn daarom voor huidig en toekomstig gebruik opgenomen in een herziene versie van de Beninese voedingsmiddelentabel.

In **chapter 5** wordt besproken op welke wijze de biobeschikbaarheid van ijzer en zink in voedingsmiddelen verbeterd kan worden met voedselbereidingsmethoden. In onze studie ging de aandacht uit naar *dibou*, een in noord-Benin veelgegeten van sorghum bereid gerecht.
Op een locale markt in noord-Benin werd graan gekocht afkomstig van een bepaalde sorghum cultivar (Chabicouman). De sorghum werd op vijf verschillende manieren in de voedselbereiding gebruikt: als hele graankorrels, als gestampte graankorrels welke gedurende 1 respectievelijk 3.5 uur geweekt zijn, als gedefytiniseerd graan (door gebruikmaking van fytase), en als gepeld (*dehulled)* graan. Elke bereidingswijze resulteerde in meel welke gebruikt werd om *dibou* van te koken. De oplosbaarheid van ijzer en zink werd in *dibou* in vitro gemeten met zowel de Glahn als de Kiers methode. De biobeschikbaarheid van ijzer werd gemeten met behulp van het Glahn in vitro verterings/Caco-2-cel model.
Het pellen van sorghum resulteerde in de grootste ijzer- en zinkverliezen (respectievelijk 46 en 36 %). De grootste fytaat reducties werden verkregen door defytinisering en door pellen (respectievelijk 73 en 37 %) en de grootste reductie in fenolverbindingen in het meel werd verkregen met pellen (68 %). Er was een significant effect van het type voedselbereiding op de ijzer- en zinkoplosbaarheid ($P<0.01$) waarbij de hoogste waarden verkregen werden met defytinisering, gevolgd door pellen. Er was weliswaar geen significant effect van de voedselbereiding op de biobeschikbaarheid van ijzer hoewel defytinisering en pellen 10% hogere waarden gaf. Aangezien de fytaat/ijzer molair ratio in *dibou* nog steeds hoog was (5.6 voor *dibou* gebaseerd op gedefytiniseerd meel en zelfs 20.2 voor *dibou* gebaseerd op meel na pellen), zijn fytaat- en polyfenolgehaltes waarschijnlijk nog niet voldoende gereduceerd. Teneinde de biobeschikbaarheid van ijzer uit *dibou* te laten toenemen zouden het fytaat en de polyfenolen verder teruggebracht moeten worden. Meer onderzoek naar combinaties van variëteiten en voedselbereidingsmethoden zou kunnen leiden tot een verdere reductie van fytaat en polyfenolen.

De in dit proefschrift gepresenteerde studies wijzen erop dat schoolkinderen in noord-Benin een inadequate groei laten zien en dat velen van hen als *stunted* geclassificeerd moeten worden. Dit suggereert dat deze kinderen waarschijnlijk niet alleen te maken hebben met slechte sanitaire voorzieningen, maar ook lijden aan een langdurige marginale voedselinneming (kwantitatief en kwalitatief). Dit wordt bevestigd door onze voedselconsumptiesurveys die er ook op wijzen dat de ijzer- en zinkbiobeschikbaarheid laag zal zijn vanwege de hoge gehaltes aan antinutritionele factoren zoals fytaat en polyfenolen. De lage biobeschikbaarheid van ijzer is ongetwijfeld een van de oorzaken van het hoge prevalentiecijfer van anemie bij deze kinderen, met alle nadelige gevolgen op gezondheid en fysieke prestaties.

Hoewel de situatie in noord-Benin wellicht niet representatief is voor heel Benin, is er ook geen reden om aan te nemen dat de situatie in andere delen van Benin beter zou zijn. Verder moet opgemerkt worden dat in noord-Benin over de laatste 15 jaar blijkbaar niet of nauwelijks verbetering gezien is in groei en voedingstoestand van de kinderen. Dit moet uiteraard als zeer onwenselijke beschouwd worden.

Er zouden diverse initiatieven gestart kunnen worden. Schoolbezoek zou verder moeten toenemen en sanitaire omstandigheden en voedings- en gezondheidsonderwijs zouden verbeterd kunnen worden, niet alleen op scholen maar ook thuis. De bevolking zou meer bewust gemaakt moeten worden van met welke andere voedingsmiddelen of met welke aangepaste voedselbereidingsmethoden een gezondere voeding verkregen kan worden.
Meer onderzoek zou ook gedaan moeten worden naar effectieve voedsel-gebaseerde benaderingen om de kwantiteit en kwaliteit van voedsel te verbeteren. Voedselconsumptie en voedingsstatus zouden gemonitoord kunnen worden, bij voorkeur door het gehele land, teneinde tijdig te kunnen reageren op ongewenste ontwikkelingen, maar ook om de potentiële gunstige effecten van locale of nationale interventies te kunnen evalueren. Men zou keuzes moeten maken voor de meest geschikte voedsel-gebaseerde benaderingen. Bijvoorbeeld, voedselverrijking en suppletie zouden op korte termijn geschikt kunnen zijn, op de lange termijn echter zouden meer duurzame benaderingen geimplementeerd moeten worden. Voor een adequate monitoring en evaluatie van voedselconsumptie is een valide en volledige Benin voedingsmiddelentabel een vereiste, met voedingsmiddelen en gerechten zoals die door heel Benin gegeten worden.

Het is duidelijk dat de voedings- en gezondheidssituatie van de kinderen in (noord) Benin alleen verbeterd kan worden als het probleem multisectoraal aangepakt wordt. De nationale en locale overheden zouden *task forces* kunnen initiëren, bestaande uit vertegenwoordigers van overheden, niet-gouvernementele organisaties, universiteiten en de private sector, en die de hierboven genoemde initiatieven ter hand kunnen nemen.

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Comlan Evariste S. Mitchikpe

Curriculum Vitae

ABOUT THE AUTHOR

Comlan Evariste Simon Mitchikpe was born on 27 October 1970 in Ouidah, in Republic of Benin. He attended primary school in Ahozon at “Ecole Primaire Public d’Ahozon” and secondary school in Cotonou at “Collège d’Enseignement Moyen Général Kouhounou/Vêdoko” and at “Collège d’Enseignement Moyen Général Père Aupiais”. He graduated from secondary school in 1990. He joined the Faculty of Agricultural Sciences of the University of Abomey-Calavi in 1991 and graduated as *Ingénieur Agronome*: *Option Nutrition et Sciences Alimentaires* in 1996. In May 1996, he joined the research team of the Department of Nutrition and Food Sciences of the Faculty of Agricultural Sciences at the University of Abomey-Calavi (DNSA/FSA/UAC) and served as a junior staff member.

In 1998 Comlan Evariste S. Mitchikpe attended the Post-graduate International Course in Food Science and Nutrition (ICFSN) at the Wageningen International Agricultural Centre.

In January 2002 he started the PhD project presented in this thesis. Field data have been collected in Benin from July 2002 to July 2005 and a final laboratory analysis has been performed in Wageningen from March to June 2006. The preparatory and final phases have been undertaken at the Division of Human Nutrition of Wageningen University.

LIST OF PUBLICATIONS

Peer-Reviewed Publications

- Bricas N, Vernier P, Atègbo E, Hounhouigan J, Mitchikpè E, Nkpénu KE et Orkwor G (1997) Le développement de la filière cossettes d'igname en Afrique de l'Ouest. *Le cahier de la recherche développement* **44**, 100 - 114.
- Slingerland, MA, Traore K, Kayode APP and Mitchikpe CES (2006) Fighting Fe deficiency malnutrition in West Africa: an interdisciplinary programme on a food chain approach. *NJAS*: *Wageningen Journal of Life Sciences* **53**, 253-279.

Other Publications

- Mitchikpè CE (1996) Physical Activity Level and food consumption of school children. A case study of Kpossidja (Abomey-Calavi), Benin. Thesis for the degree of "Ingénieur Agronome", option : Nutrition and Food Sciences. Faculty of Agricultural Sciences, National University of Benin.
- Atègbo E, Bricas N, Hounhouigan J, Mitchikpè E, Nkpénu KE, Orkwor G et Vernier P (1998) Le Développement de la filière cossettes d'igname pour l'approvisionnement des villes au Nigeria, Bénin et Togo. In *L'igname, plante séculaire et culture d'avenir . Actes du séminaire international 3-6 juin, 1997,* Berthaud J, Bricas N, Marchand JL Eds. CIRAD, Montpellier, France. pp 339-41.
- Nago CM, Fournier S, Hounhouigan J et Mitchikpè CE (1999) Qualité et stratégies commerciales dans le secteur du gari au Bénin. Communication à l'atelier international sur "Le développement des petites entreprises agro-alimentaires en Afrique de l'Ouest et du Centre : organisations locales et dispositifs d'appui", Novembre 30 – Décembre 03, 1999. Dakar, Sénégal.
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western part of Benin. Abstracts 18th International Congress of Nutrition, September 19-23, 2005, Durban, South Africa. South African Journal of Clinical Nutrition 2005; 18 suppl 1: p 409 (Abstr 6.8.25).

- Mitchikpe CES, Ategbo EAD, Raaij JMA van, Kok FJ. Nutrition status of rural Beninese school-age children in increased *vs* decreased food availability period. Abstracts 18th International Congress of Nutrition, September 19-23, 2005, Durban, South Africa. South African Journal of Clinical Nutrition 2005; 18 suppl 1: p 354 (Abstr 5.7.42).
- Mitchikpe CES, Dossa RAM, Hooiveld GJEJ, Hulshof PJM, Nout MJR, Van Raaij JMA, Kok FJ. Soaking and dehulling failed to improve zinc bioavailability from experimentally processed dibou, a thick sorghum porridge. Abstracts “Zinc Crops 2007” Conference in Istanbul, 24th – 26th May, 2007, Turkey (Accepted)

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

GRADUATE SCHOOL VLAG

Discipline specific activities

Courses

- Training in Methodology Food Consumption Surveys and in Methodology Analysis Food Consumption Data, Ouaga, Burkina Faso, 2002
- 3rd ECS AFOODS Course: Production and Use of Food Composition Data in Nutrition, Pretoria, South Africa, 2002
- Training in Chemical Analyses in Food Samples, Ouaga, Burkina Faso, 2004
- Training in Measuring Iron Bioavailability using an In-vitro Digestion/Caco-2 Cell Model, Wageningen, The Netherlands, 2006

Meetings

- Workshop: From natural resources to healthy people: food-based interventions to alleviate micronutrient deficiencies (INREF), Cotonou, Benin, 2001
- Workshop: Food-based approaches for a healthy nutrition in west Africa, Ouaga, Burkina Faso, 2003
- Workshop: Nutrition and Food Security of Households in Couffo, Lokossa, Benin, 2005
- 18th International Congress of Nutrition (IUNS), Durban, South Africa, 2005

Optionals

- PhD study tour, Division of Human Nutrition, Wageningen University, 2005
- Staff seminars, Division of Human Nutrition, Wageningen University, 2002-2006
- Preparation PhD research proposal
- Updating training in Statistical Packages and Nutrition Software (a.o. Komeet), Abomey-Calavi, Benin, 2002-2006
- Updating training Assessment Nutritional Status, Abomey-Calavi, Benin, 2002-2006

ANNEX

Interdisciplinary Research and Education Fund (INREF)

Research programme "From Natural Resources to Healthy People"

The research for this thesis has been part of the programme ***From Natural Resources to Healthy People: Food-based Interventions to Alleviate Micronutrient Deficiencies.*** This is one of the programmes sponsored by the Interdisciplinary Research and Education Fund (INREF) of Wageningen University. INREF aims to stimulate development-oriented research and education through programmes designed and implemented in partnership with research institutes in developing countries. The programmes aim to build relevant capacity in local research institutions to solve actual problems. The main partners in our programme were China Agricultural University, Beijing and the Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, both from China, the National Institute for Environment and Agricultural Research, INERA from Burkina Faso and the University of Abomey–Calavi from Benin. In total eight staff members from these institutes, including the author of this thesis, received a PhD training.

The micronutrient malnutrition problem

Chronic micronutrient deficiencies, particularly of vitamin A, iron and zinc, lead to impaired mental and physical development and decreased work output, and contribute to morbidity from infections. Pregnant women and children are vulnerable groups. Animal products are good sources of desired micronutrients, but most people in West Africa and China depend largely on sorghum and rice, respectively, for their daily food. These plant-based foods contain limited amounts of micronutrients while they also contain anti-nutritional factors such as phytic acid and polyphenols that inhibit absorption of micronutrients by humans.

Next to the nutritional quality, the production of enough food is an important problem as population growth leads to higher demands for food and more permanent cropping, both increasing pressure on natural resources. In West Africa, soil and water conservation measures are being developed to prevent soil erosion, nutrient and water losses and to maintain or even increase yields. In China, the introduction of aerobic rice systems aim to reduce water use per kg of rice, maintaining yields similar to the current flooded rice systems.

Programme strategies to improve the supply of micronutrients

The increasing demand for food stipulates that improvements in food quality cannot be accepted when they are at the expense of food quantity. Any solution should be in line with sustainable natural resource management.

The programme applied a food chain approach (figure) in sorghum and (aerobic) rice to explore synergies and trade-offs between different interventions along the chain.

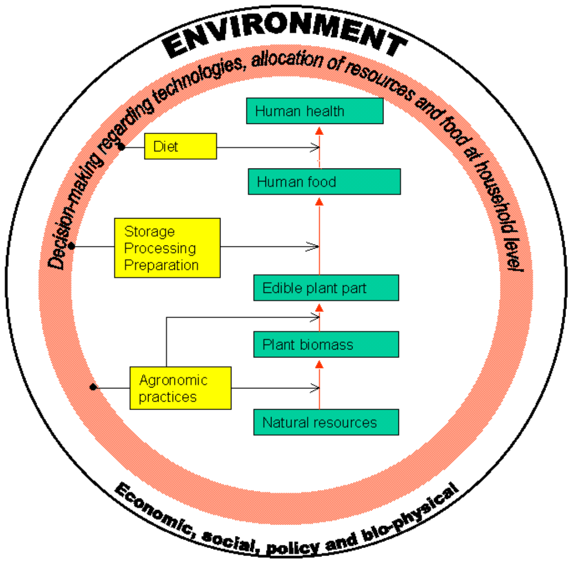

Diagram of the food chain
The food chain approach is indicated showing how external conditions like the economic and bio-physical environment set the stage for decision making at the household level. These decisions in their turn determine practices which have a direct impact on the processes at different points in the food chain. Research in the programme has been done related to each of the three types of interventions.

Agronomic practices should aim to increase uptake and allocation of micronutrients from soil to edible plant parts, while keeping accumulation of anti-nutritional factors low. Research has focussed on effects of genotype, environment & management and their interaction on micronutrient/phytic acid molar ratio in seed. This has led to recommendations on choice of genotype, fertiliser and water use.

Food processing aims to concentrate desired micronutrients in end products and inactivate anti-nutritional factors. Research focussed on effects of milling and processing on micronutrient/phytic acid molar ratio in food, leading to recommendations on optimal combinations of unit operations.

Nutrition studies aim to validate the results in humans. Research focussed on dietary composition, determination of methods to measure impact and evaluation of effects of some of the proposed changes upstream in the food chain on micronutrient uptake in vulnerable groups. This has led to insight in sources of micronutrient and anti-nutritional factors and in the potential contribution of an intervention in the staple food.

At the end of the programme an analysis will be made to determine the relative impact of the different proposed measures along the chain for the final aim: improved micronutrient nutrition of targeted vulnerable groups.