Efficacy of iron fortified cowpea flour in improving iron status of schoolchildren in malaria endemic rural Ghana

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This research was conducted under the auspices of the Graduate School VLAG (Advanced studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences).

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Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. dr. M.J. Kropff,
in the presence of the
Thesis committee appointed by the Academic Board
to be defended in public
on Friday 24 May 2013
at 11 a.m. in the Aula.

Abdul-Razak ABIZARI

Efficacy of iron fortified cowpea flour in improving iron status of schoolchildren in malaria endemic rural Ghana
191 pages.
PhD Thesis, Wageningen University, Wageningen, The Netherlands (2013)
With references, with summaries in English and Dutch

ISBN 978-94-6173-526-3

ABSTRACT

Children in sub-Saharan Africa are more likely to have survived the critical first 1000 days of life carrying along unresolved micronutrient deficiencies into the school-age. Iron-deficiency is the most prevalent micronutrient problem affecting school-age children in sub-Saharan Africa and yet the most difficult to resolve. It is necessary to ensure an adequate iron intake through the diet of school-age children and school-based feeding intervention may be a way to improve iron intake of schoolchildren. Such a feeding intervention would be more sustainable if it relies on locally produced food(s) with the potential to support food sovereignty. In this context, this thesis investigated whether foods based on cowpeas, an indigenous legume crop originating from Africa, can be used in a school feeding setting to improve iron status of school-age children in Ghana.

The investigations in this thesis comprised cross-sectional dietary and iron status assessment of schoolchildren (n=383), cowpea acceptability among schoolchildren (n=120 mother-child pairs), chemical analysis of cowpea landraces (n=14), an *in vivo* iron bioavailability among young women (n=16) and a randomized cowpea intervention trial (n=241) conducted mainly in Tolon-Kumbungu district of Ghana.

The results indicated that iron-deficiency and iron-deficiency anaemia affect 8 and 7 out of every 10 schoolchildren respectively. It also showed that the probability of adequate dietary iron intake is 0.32 but much larger (~0.90) if schoolchildren benefitted from a school feeding programme. Mothers/caregivers intended to give cowpeas to their schoolchildren 2–3 times per week. The positive attitudes of mothers towards cowpea predicted their intention to give them to their schoolchildren but they were worried about the cost, long cooking time and the discomfort their children may suffer after consuming cowpeas. The chemical analysis showed that cowpeas contain appreciable amounts of iron (4.9–8.2 mg/ 100 g d.w) and zinc (2.7–4.1 mg/100 g d.w) but also high amounts of inhibitory phytate (477–1110 mg/100 g d.w) and

polyphenol (327–1055 mg/100 g d.w). Polyphenol concentration in particular was higher ($P<0.05$) in coloured compared to white landraces. Iron bioavailability from red and white cowpeas were 1.4 and 1.7%, respectively, in NaFeEDTA-fortified meals and 0.89 and 1.2%, respectively, in FeSO4-fortified meals. Compared with FeSO4, fortification with NaFeEDTA increased the amount of iron absorbed from white and red cowpea meals by 0.05 and 0.08 mg ($P < 0.05$) respectively. Irrespective of the fortificant used, there was no significant difference in the amount of iron absorbed from the 2 varieties of cowpea. Finally the results from the intervention trial showed that fortification of whole cowpea flour with NaFeEDTA resulted in improvement of haemoglobin ($P<0.05$), serum ferritin ($P<0.001$) and body iron stores ($P<0.001$), and reduction in transferrin receptor concentration ($P<0.001$). Fortification also resulted in 30% and 47% reduction in the prevalence of iron-deficiency (ID) and iron-deficiency anaemia (IDA) ($P<0.05$), respectively.

Overall, this thesis has shown that in a malarious region with high iron-deficiency like (northern) Ghana, iron status of schoolchildren can be improved through the consumption of cowpeas within a school feeding programme. The improvement in iron status is however unlikely to result from the usual/conventional consumption of cowpeas but through fortification of whole cowpea flour with a highly bioavailable iron compound. This thesis has also shown that the most suitable iron compound for such whole cowpea flour fortification is NaFeEDTA irrespective of whether the cowpea has high or low concentration of polyphenols.

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Chapter 1

General Introduction

INTRODUCTION

The first 1000 days of life, from conception through the second year of life, are considered critical for survival of children [1]. However, survival does not guarantee optimal nutritional status. In sub-Saharan Africa children are more likely to have survived this critical stage carrying along unresolved micronutrient deficiencies into the school-age [2]. In addition, the growth spurts during the school-age period also impose high nutrient requirements [3, 4] which tend not to be met by dietary intakes. Also, among older school girls, the onset of menstruation further increases iron requirement. Iron, iodine [5] and vitamin A deficiencies are therefore public health problems affecting school-age children in sub-Saharan Africa [6, 7] of which iron-deficiency (ID) is the most prevalent and yet the most difficult to resolve [8]. As low intake of bioavailable micronutrients is considered as the main cause of the deficiencies, it is necessary to ensure an adequate micronutrient intake through the diet. However, if in school, the dietary intake of school-age children is less closely supervised by parents and this may affect micronutrient intake. Most interventions at household and community levels are preferentially targeted at infants, young children and pregnant women [2]. School-based feeding interventions may therefore be a way to improve micronutrient intake of school-aged children [9]. Such a feeding intervention would be more sustainable if it embeds local food(s) with the potential to support food sovereignty. In this context, this thesis investigates whether foods based on cowpeas, an indigenous legume crop originating from Africa [10], can be used in a school feeding setting to improve iron status of school-aged children in Ghana.

Prevalence and consequences of iron-deficiency

Iron is an important component of proteins such as haemoglobin and myoglobin where it is required for the transport of oxygen, and of tissue enzymes where it is involved in energy production and immune function [11, 12]. Iron is mainly ingested through food; either as haem iron (from animal sources) or non-haem iron (largely from plant sources) [13]. In the absence of active excretion of iron from the body, iron status is maintained by regulating dietary iron absorption in the proximal small intestine [13]. Iron deficiency will occur if dietary iron intake is low and/or if bioavailability of the dietary iron is poor beyond the body's ability to upregulate iron absorption to meet needs [14]. In its severe form ID causes anaemia [8]. Estimates suggest that ID may be responsible for one-half of anaemia prevalence [15]. In countries with a low human development index, about 40% of school-age children are anaemic [16]. In the absence of nationally representative data, estimates from two individual studies suggest that anaemia prevalence among schoolchildren in Ghana may range from 38% to 70% [17, 18]. The consequences of anaemia due to ID among school-age children include reduced physical work capacity, increased susceptibility to infectious diseases and impaired cognitive performance [2, 19]. In randomized controlled studies iron repletion has been shown to benefit cognition among schoolchildren [20], decrease fatigue among women [21] and decrease morbidity among school-age children [22].

Iron-deficiency anaemia in sub-Saharan Africa is a public health problem among schoolchildren and a school-based feeding intervention can contribute to iron repletion.

Bioavailability of dietary iron and its measurement

Bioavailability of dietary iron is the percentage of ingested iron that becomes available for metabolic functions [23]. It is partly influenced by an individual's iron status [24] and dietary factors. Iron bioavailability is upregulated when iron status is low [24]. Dietary factors are related to the source of dietary iron (haem or non-haem), enhancers (e.g. ascorbic acid) and inhibitors (e.g. phytic acid and polyphenols) of absorption present in the diet [6]. The bioavailability of non-haem iron, largely present in plant-based diets, is generally low (<10%) [25] and may imply that large quantities of staples need to be consumed in order to meet iron requirements. Haem iron bioavailability ranges between 3 – 7 times that of non-haem iron and does not depend on the composition of the food [26], but the main source of haem iron (animal source foods) is rarely part of the usual diets in sub-Saharan Africa [27, 28]. Similarly the intake of dietary enhancers of iron absorption is low, whereas the intakes of dietary iron absorption inhibitors, mainly phytates and polyphenols, are rather high [27].

Habitual intake of diets rich in iron inhibiting compounds coupled with low intake of animal source foods may contribute to iron-deficiency.

Phytic acid

Phytate (the salt of phytic acid) is a major storage form of phosphorous in the kernels of cereals, legumes and nuts. In cereals it is mainly located in the aleurone layer, pericarp and germ [29] while in legumes it can be found in the cotyledon [30]. Phytic acid (myo-inositolkishexaphosphate, IP6) is the most abundant derivative of myo-inositol and thus exerts significant influence on iron absorption. Other less abundant derivatives are tetraphosphates and pentaphosphates [30]. Phytic acid is negatively charged under physiologic conditions [31] and thus has affinity for cations such as iron. It is able to form insoluble complexes with iron thereby decreasing its absorption. In a single meal, Hurrell et al. [32] showed that even a small amount of phytic acid inhibits iron absorption. More so phytic acid inhibition is dose dependent [33, 34]. Phytic acid-to-iron molar ratio is an important determinant of iron absorption. Hurrell [35] suggests that phytic acid-to-iron molar ratio of <0.4:1 is preferable for iron absorption. The high concentration of phytic acid in whole cereal flour has been blamed for the poor efficacy of fortified whole cereal flours [36]. Ascorbic acid has been shown to overcome the inhibitory effect of phytic acid in maize bran [34]. Evidence is growing that NaFeEDTA is also able to overcome the inhibitory effect of phytic acid [37-39]. The EDTA moiety in NaFeEDTA chelates iron at a low pH and to some extent protects it from binding with ligands such as phytic acid in the stomach and subsequently releases iron for uptake in the duodenum and jejunum [40].

Polyphenols

Polyphenols are found in plants as a wide range of aromatic compounds with at least one hydroxyl moiety. They do not influence primary functions such as photosynthesis, reproduction or growth but play an important role in protecting the plant against pathogens [(41)] and ultraviolet radiation [(42)]. They are found in a wide range of crops including those widely consumed in sub-Saharan Africa such as cowpeas and sorghum [(43, 44)]. The structure of certain polyphenols makes them form complexes with iron and influence its absorption from food. Polyphenols with catechol or galloyl groups are potent inhibitors of iron absorption and are able to complex iron at high pH [(45, 46)]. The inhibitory action of polyphenols has been shown to be dose dependent [(45-47)]. However polyphenols in various foods and beverages seem to exert different influences on iron absorption. Hurrell et al. [(46)] showed that black tea polyphenols were more inhibitory than those of herbal tea, cocoa and wine. In common beans, it has been shown that coloured varieties have higher concentration of polyphenols than white varieties [(43, 48)]. Luckily the inhibitory effect of polyphenols can be reduced by other components of food when consumed in concomitance. Siegenberg et al. [(34)] showed that 50 mg ascorbic acid can restore iron absorption from a meal containing >100 mg tannic acid. EDTA, also a known enhancer of iron absorption, however seems unable to overcome the inhibitory effect of tea polyphenols on iron [(40, 49)]. Given the wide range of polyphenols and their varying ability to inhibit iron absorption, it is unknown whether EDTA is also unable to overcome iron inhibition of polyphenols in other foods.

It remains to be shown whether EDTA can overcome the co-inhibitory effect of cowpea polyphenols and phytic acid.

Measuring iron bioavailability

The bioavailability of the native non-haem iron in food can be measured quantitatively using an intrinsic or extrinsic technique [(50)]. In the intrinsic technique, the tagged iron is absorbed into the seed or grain biosynthetically (stem injection or hydroponic culture) whereas in the extrinsic technique the tag is added to food at the point of consumption [50, 51]. The extrinsic tag technique has found wider use because it is easier to apply and compares favourably with the intrinsic tag technique [51]. Dietary non-haem iron present in a single meal joins a common pool, from which all non-haem iron show uniform bioavailability [52]. The extrinsic tag technique, validated across a range of foods, relies on this common pool in order to estimate the bioavailability of non-haem iron [53]. The labelling material could be a radioisotope or a stable isotope of iron; minute quantity of radioisotope is required compared to stable isotopes. The success of the technique relies on uniform mixing of the food with the isotopic tag at the site of absorption and both the tag and dietary non-haem iron should be sufficiently soluble to exchange well with the pool [52]. For this reason soluble iron compounds such as ferrous sulphate are used to carry the iron tag. Two weeks after ingestion, the tagged iron incorporates into the red blood cell and can be measured as the bioavailability of the single meal's native iron based on estimation of blood volume [54].

This extrinsic tag technique offers the opportunity to test for promising fortificants and/or likely fortification vehicles; an important exploratory step in designing iron fortification.

Indicators of iron status

Serum ferritin (SF) concentration is the most useful measure of iron status [55]. Low concentrations of serum ferritin always indicate depletion of storage iron and concentrations <15 µg/L are predictive for low iron storage [56]. Because serum ferritin is also an acute phase protein, it is elevated in the presence of inflammation therefore high SF concentration with inflammation may mask insufficient iron stores [57]. It is recommended that markers of inflammation such as C-reactive protein (CRP) and α_1-acid glycoprotein (AGP) are measured alongside SF in order to isolate the influence of inflammation on iron status or adjust SF levels for inflammation [58, 59]. In settings where infection and infestations are high serum ferritin is not a reliable measure of iron status. In such instances soluble transferrin receptor (sTfR) is a good alternative because its levels are not influenced by infection [58] and can therefore distinguish iron deficiency anaemia from anaemia of chronic disease [60, 61]. The TfR is a protein on the surface of cells and it regulates the uptake of transferrin into cells. The concentration of its soluble form in serum is a sensitive marker of tissue iron requirement [62]. Even though sTfR is not influenced by inflammation, its concentration may be increased by malaria among young children [63, 64] affecting its specificity. It is suggested that malaria may not influence sTfR among older children in endemic areas [65] likely because the age-acquired immunity may decrease parasite density and reduce haemolysis [66].

At the community level in sub-Saharan Africa, SF and sTfR are not routinely applicable as measures of iron status because of infrastructural and cost implications [58]. As such haemoglobin concentration is often used as proxy measure of iron status in population level surveys, but this has the tendency to overestimate anaemia due to ID in settings where anaemia may also be caused by malaria, helminths, haemoglobinopathies or other micronutrient deficiencies [16]. The recommendation is that in settings like sub-Saharan Africa iron status should be measured using combination of these indicators rather than a single measure [58]. *The high infection and infestation burden among children in sub-Saharan Africa make accurate measurement of iron status both difficult and expensive.*

The search for sustainable strategies to control iron-deficiency

Supplementation is recommended when diet alone is unable to restore iron status within a short time span [67]. As such pharmacologic doses of iron are used. Studies have shown that iron supplementation is both able to prevent and treat iron-deficiency [68]. This makes supplementation suitable for use in developing countries. However, cost implications are very high and successful implementation requires behaviour modification to improve adherence [8].

As our understanding of the host-pathogen competition for iron deepens, there are growing concerns over the safety of iron supplements in areas with endemic malaria, especially among pre-school children. Supplementation iron is likely to increase malaria morbidity and increase the risk of hospitalization especially among young children who are iron-replete [69]. WHO therefore advises against blanket iron supplementation in malaria endemic areas like Ghana and recommends screening for iron status and improved malaria surveillance [70]. The recommendation makes population-wide iron supplementation for vulnerable children in malaria endemic areas less feasible owing to logistic barriers [71].

Currently food-based approaches represent the most desirable and sustainable way of preventing iron-deficiency. They are designed to increase iron intake through the diet. The food-based approaches include dietary diversification, biofortification and fortification.

Diets in developing countries are quite monotonous [27] because populations are largely agrarian and subsist on their own food production. **Dietary diversification** is therefore an important strategy for developing countries. It aims at improving iron intake through the promotion of production and utilization (throughout the year) of iron-rich traditional foods that are already utilized or underutilized [72]. Dietary diversification does not only improve iron intake but also intakes of other important micronutrients [72]. Evidence suggest that dietary diversity could be effective in preventing iron deficiency but some of the effective interventions involve animal source of iron and protein such as meat which are generally expensive for the rural poor [73]. Meat is thought to improve non-haem iron absorption through what is referred to as “meat factor”; iron binding peptides which protect iron from complex formation with phytic acid and polyphenols [74]. Promoting plant-based foods to improve iron status should be done carefully as some studies indicated that although high in iron, consumption was not effective in improving iron status probably due to low bioavailability of the iron [75].

Biofortification is the process of increasing the concentration and/or bioavailability of essential elements in the edible part of the plant by traditional plant breeding or genetic engineering [(76)]. Biofortified crops have the potential to supply iron to rural poor farming households since they subsist on their own crop production [(77)]. The interest in biofortification is growing steadily. Even though the initial capital investment in research is high, it promises to be a safe, cheap and more sustainable strategy in the long term [(78)]. The first step in conventional biofortification for iron is to determine whether sufficient genetic variation exists to identify breeding parents. The variation in the native iron content in the landraces of some staple crops like cowpeas may suggest the possibility to selectively breed for higher iron content [(78)]. Presently a variety of common beans with high iron contents are ready for release [(79)]. *Location-specific acceptability of such biofortified crops however needs to be addressed to guarantee success in dissemination and consumption*.

Where the risk of iron-deficiency is high, universal iron **fortification** may be recommended [(6)]. At the population level, fortification of processed staples with iron is practical, sustainable, and cost effective in the long term though technically challenging [(6, 80)]. Fortifying foods with iron is technically more difficult because iron reacts with food substances to give undesirable colour and taste changes [(6, 81)]. Iron fortification is considered safer than supplementation but it is yet to show success in Africa partly for the following reasons: (i) difficulty in finding centrally processed foods that reach large sections of the vulnerable population; (ii) iron compounds with low bioavailability are often used; (iii) fortification levels usually inadequate to improve iron status [(25)]. Malaria has also been linked to the lack of success of fortification in Africa. Cercamondi et al. [(44)] have suggested that the protracted course of asymptomatic malaria in endemic areas could decrease fortification iron absorption by about 40% and may blunt the efficacy of fortification programmes. Staple cereal flours are often the target vehicles for fortification because large sections of populations consume them in sufficient amounts on regular basis [6]. However commonly recommended iron fortificants such as elemental iron, ferrous sulphate and ferrous fumarate are not well absorbed in whole cereal flours because of high phytic acid concentration [81]. Evidence is growing that NaFeEDTA is the most suitable compound for whole cereal or high extraction flour fortification [37, 39] and has been endorsed for use by WHO and partner organizations [82]. Compared to ferrous sulphate which usually serves as a reference fortificant, iron absorption from NaFeEDTA is 2–4 times higher in phytic acid containing cereal flours [40]. NaFeEDTA is slowly water-soluble and may cause less undesirable colour changes compared to ferrous sulphate [81] and is stable during processing and storage [83] . The EDTA compound binds to iron in acidic medium of the stomach and to some extent prevents it from binding with iron inhibition compounds such as phytic acid [40].

Much is known about the fortification of whole cereal flours and which fortificant is desirable, but little is known about the fortification of whole legume flour such as cowpea flour (rich in both phytic acid and polyphenols).

The TELFUN Project: Food sovereignty and the Ghana School Feeding Programme

TELFUN (*Tailoring Food Sciences to Endogenous Patterns of Local Food Supply for Future Nutrition*) is an interdisciplinary multi-centre research programme funded by the Interdisciplinary Research and Education Fund (INREF) of Wageningen University, The Netherlands. The four-year tenure project started in 2007 with twelve PhD students from three parts of the world: four each from West Africa (Ghana/Benin), Asia (India) and South America (Ecuador). In each of the sites the researchers were drawn from four scientific disciplines: plant breeding, food technology, human nutrition and social science, working as an interdisciplinary team.

The TELFUN research programme is interested in answering the question: how do technological practises, developed from within food networks, enhance food-sovereignty and the nutritional status of people? This question comprises two related concepts: "food sovereignty" and "local food networks". Food sovereignty has been described as the state where "all community residents obtain a safe, culturally acceptable, nutritionally adequate diet through a sustainable food system that maximizes community self-reliance and social justice" [(84)]. The Food Sovereignty concept aims at empowering smallholder farmers to remain productive even as they are faced with competition from imports as a result of global open market. Food Sovereignty is composed of four main intricately related components; right to safe, nutritious and culturally acceptable food, access to productive resources, agro-ecologically sound production and access to market [(85)]. Local food networks involve a collection of key actors who work within a locality to preserve the functional, ecological, cultural and political meanings of local foods [(86)]. The programme posits that the classic agro-industrial and the Green Revolution models of food production have succeeded in disconnecting the production-processing-utilization continuum in local food networks. This disconnection has been worsened by globalised and liberalised markets. Consequently the actors involved in preserving local food networks are getting marginalized.

For TELFUN, understanding and improving traditional legume networks within their centres of origin together with the key actors within each network have the potential to establish the reconnection that is necessary to improve food sovereignty [(87)]. As such, the specific legumes chosen as platforms to promote food sovereignty in Ghana/Benin, India and Ecuador respectively were: cowpea (*Vigna unguiculata* (L.) Walp), mungbean (*Vigna radiata* (L.) Wilczek) and lupine (*Lupinus mutabilis* Sweet). These legumes have major nutritional importance in developing countries because they have high protein contents of good biological value [(3, 88)] and provide significant quantities of vitamins and minerals like iron [(89)].

Targeting interventions to populations at high risk is important for more efficient delivery of limited national resources. The nutrition component of the Ghana/Benin TELFUN programme was investigated within the Ghana school feeding programme. Because of increasing enrolment in schools and existing infrastructure, the school is an important platform to implement targeted cost-effective nutrition interventions. Such interventions may keep more children in school and increase the number of years spent in school thus improving educational alongside nutritional outcomes [(90)]. The United Nations Hunger Taskforce (UNHTF) and the Comprehensive Africa Agricultural Development Programme (CAADP) of the New Partnership for Africa's Development (NEPAD) have both highlighted the important role of school feeding programmes in reducing hunger and increasing local food production [(91, 92)]. They believe that linking the school to local food production is an important strategy towards achieving the millennium development goals on reducing hunger and improving education.

The Ghana School Feeding Programme (GSFP) is aimed at reducing poverty among rural farming households through the provision of one warm and nutritious meal to school children across the country especially in needy rural communities [(93)]. The envisaged mechanism is that, the need to feed schoolchildren will trigger local farmers to produce more food and in return make more income since the feeding programme would have created a ready market. As such, the GSFP is believed to be fulfilling the schoolchildren's right to safe and nutritious food and also help to develop and sustain local markets thus supporting food sovereignty.

Cowpea in the lives of the rural poor

Cowpea (*Vigna unguiculata* (L.) Walp) is a popular food legume originating from Africa (**Figure 1.1**) [(10)]. West and Central Africa hold the largest share of worldwide cowpea production [(89)]. Cowpea is a drought tolerant crop that matures on as little as 300 mm of rain making it suitable for the arid and semi-arid conditions and also reduce farmers' exposure to yield risk (94, 95). Cowpea crop is shade tolerant and fixes nitrogen into the soil for that matter the rural poor farmers intercrop it with or grow it in rotation with maize, sorghum or millet (94). Because cowpeas mature early, they serve to bridge a hunger gap from periods when grain reserves from the previous harvest are depleted and farmers have yet to harvest the current year's crops (95).

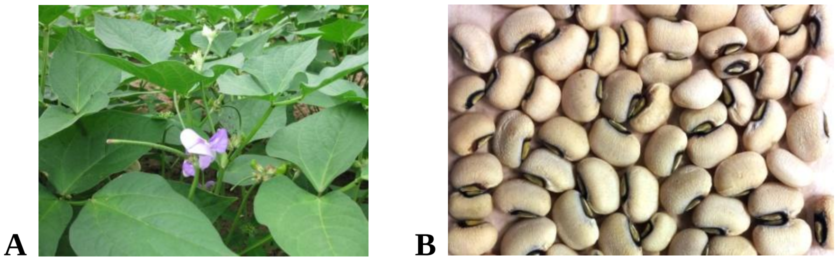

Figure 1.1. Cowpea plant with pod (A) and unpigmented cowpea seeds (B)

Cowpeas are a good source of energy, proteins, vitamins, dietary fibre and minerals such as iron and zinc (3, 89, 96). Cowpea is a cheaper source of high quality protein compared to animal proteins and it is often referred to as the "poor man's meat" (97). The high lysine content of cowpeas makes them an excellent enhancer of protein quality when combined with cereal grain proteins (89). Cowpeas are an excellent source of thiamine, niacin and also contain reasonable amounts of other water soluble vitamins. By virtue of their unique chemical composition, cowpeas offer a great potential in meeting the nutritional requirements of poor populations. They are prepared for consumption in grain, split and ground forms (89). The ground cowpea is less susceptible to post-harvest pest damage and can be used in different dishes. The fresh leaves of cowpea are also used for soup or mixed with other grains (98).

Beyond the agronomic, economic and nutritional significance of cowpeas in the lives of the rural poor, they are also useful in traditional medicine. Although not scientifically proven, the white coated cowpeas are believed to have anti-inflammatory properties and when applied topically could relieve breast engorgement among women [99]. Despite the unique nutrient profile, including appreciable levels of iron, cowpeas have been reported to contain high levels of phytate and polyphenols which inhibit iron absorption [43, 100].

Being a well-accepted and embedded local food, cowpea has the potential to contribute to food sovereignty and improve nutritional status of schoolchildren when used in a school feeding programme.

Rationale of this thesis

The rapid growth among school-age children imposes high nutrient requirements which tend not to be met by dietary intakes thereby causing micronutrient deficiencies. Iron-deficiency is a public health problem among schoolchildren in Ghana. Among older school girls, soon to be mothers, the onset of menstruation further increases iron requirement thus making iron a critical nutrient. Iron-deficiency affects educability of children and has long term negative impact on productivity and economic development. Repletion of iron among school-age children and women has been shown to benefit cognition, fatigue and morbidity.

The fastest iron repletion strategy may be to give regular iron supplements to schoolchildren but this approach is deemed unsustainable and unsafe for rural children in malaria endemic areas. Biofortification is a viable option but is currently still in the developmental phase. School feeding programmes may provide the platform to diversify the consumption of iron-rich foods but these may have to come as fortified products. Currently there is no mandatory fortification policy in Ghana and therefore the few fortified products do not reach the rural poor and thus may highlight the need to target fortification to reach vulnerable groups. In West Africa, it is difficult to find appropriate vehicles for such fortification. Cowpea may have the potential as a vehicle but little is known about fortification of whole cowpea flour.

Cowpea is a local well-accepted food legume and has popular flour-based recipes in Ghana. Cowpea is an attractive vehicle especially because of its potential in contributing to food sovereignty. In this thesis the potential of cowpea as a vehicle to contribute to solving the iron-deficiency problem among schoolchildren in Ghana is investigated within the context of food sovereignty.

Aim and outline of the thesis

The main aim of this research is to improve iron status of schoolchildren in Ghana through the consumption of cowpeas within a school feeding programme.

To achieve this aim, five related objectives were formulated as follows:

1. To assess the adequacy of nutrient intake, iron status and nutritional status of schoolchildren participating in a school feeding programme;
2. To assess the iron and zinc content of available cowpea landraces in northern Ghana;
3. To determine factors that predict the intention of mothers to give cowpeas to their school-aged children;
4. To assess the bioavailability of native iron in cowpeas while testing the relative performance of FeSO4 and NaFeEDTA as fortificants for whole cowpea flour;
5. To measure the efficacy of fortified whole cowpea flour in improving iron status of Ghanaian schoolchildren.

The outline of this thesis follows the temporal arrangement of the objectives (linkages shown in **Figure 1.2**). First, the status quo with respect to nutrient and iron intake among schoolchildren in the research area was assessed (**chapter 2**). Also, the outcome of the analysis of cowpea landraces and the factors that influence mothers to give them to schoolchildren are presented in **chapter 3**. Based on the understanding of the status quo (objectives 1–3) objective 4 was formulated to investigate whether the bioavailability of iron in cowpea is influenced by its colour and which fortificant may be suitable for cowpea flour fortification. The results are presented in **chapter 4**. The conclusion and recommendation of chapter 4 were tested in a feeding trial in which schoolchildren consumed fortified whole cowpea flour meal for seven months. The results are presented in **chapter 5.** A synthesis of the main findings of this thesis, internal and external validity as well as public health implications of the findings is presented in **chapter 6.**

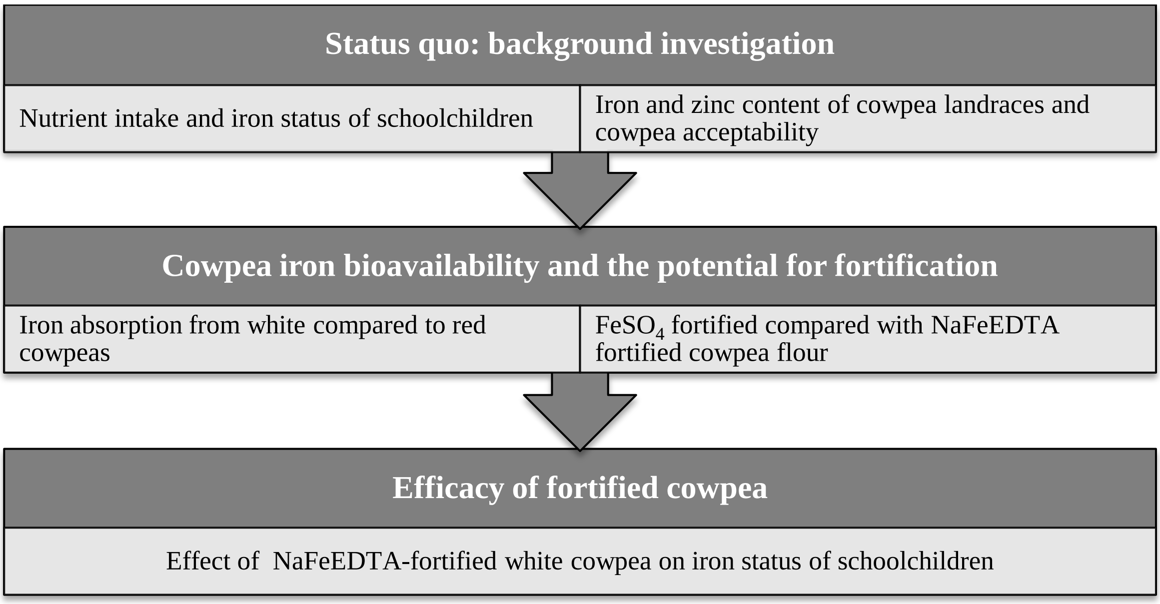

Figure 1.2. Framework of the temporal linkages in studying the efficacy of fortified whole cowpea to reduce iron-deficiency among schoolchildren.

Study area

The entry point for selection of study site in the research project described in this thesis relates to cowpeas and school feeding – both focal interests for the TELFUN programme in Ghana as described in chapter 1. Tolon-Kumbungu district was selected because it was among the 3 leading producers of cowpeas in the region and also the pilot district for the Government-sponsored school feeding programme in Northern Region of Ghana. The district is located to the west of the regional capital, Tamale, between latitudes 9^0 15^1 and 10^0 02^1

North and longitudes 0^0 53^1 and 1^0 25^1 West (**Figure 1.3**). As of January 2009 the estimated population was 249, 691 [101].

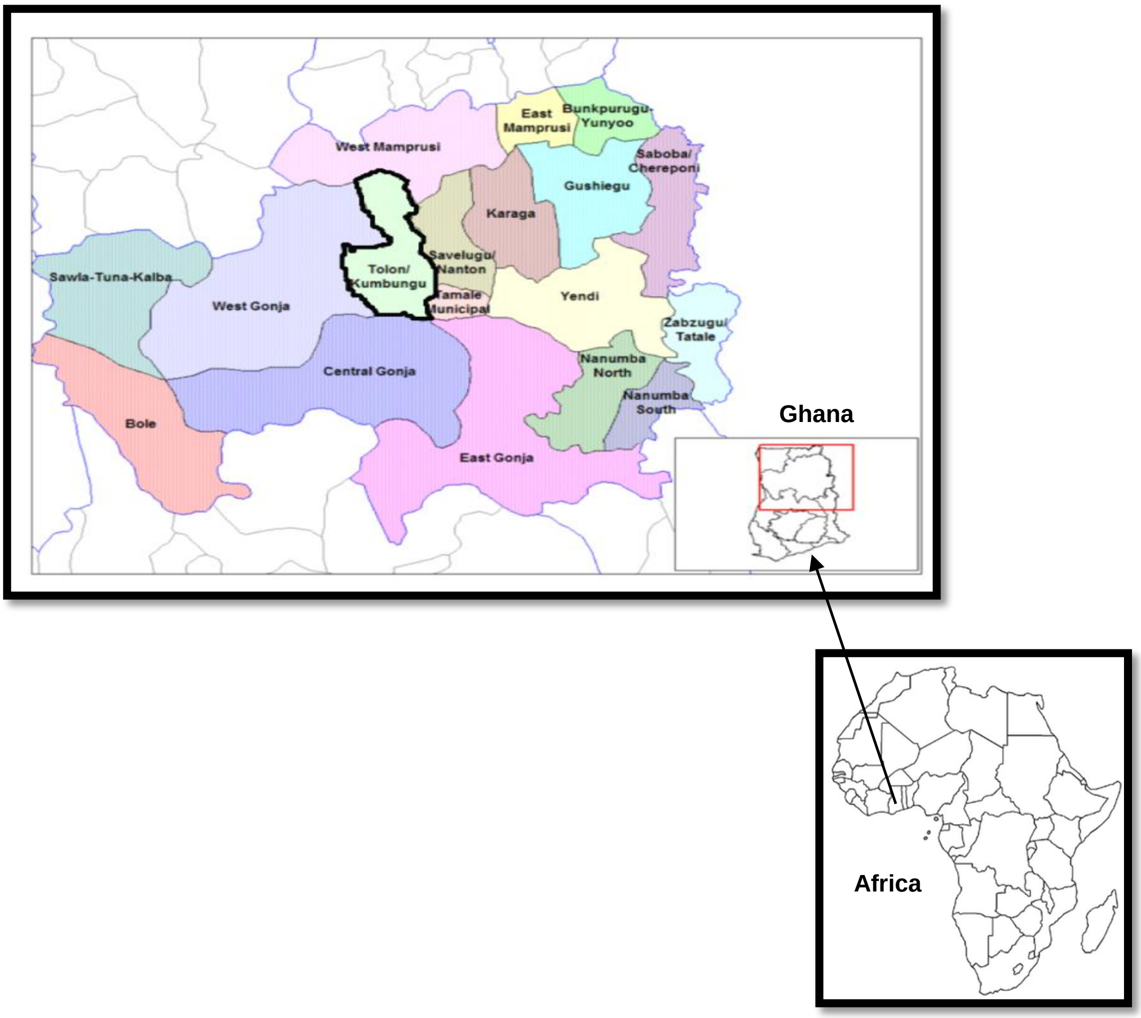

Figure 1.3. Map of northern Ghana, West Africa, showing Tolon-Kumbungu district in the middle with dark boundaries.

The research area is located within the Guinea Savannah agro-ecological zone and has two distinct seasons; rainy season (April–September) and a dry season (December–March) characterized by relatively high day temperatures (35–40 ^{0}C). Annual rainfall ranges between 900–1100 mm. The people of this area are largely rain-fed subsistence farmers and predominantly Dagombas by tribe and Muslims by religion. Malaria is hyperendemic with little seasonal variation in this area and is the main cause of morbidity among children. Staple diets are generally monotonous and based on cereals and legumes such as maize, millet, sorghum, rice, cowpeas and groundnuts.

The cowpea iron bioavailability study in chapter 4 was conducted in Wageningen, The Netherlands, largely because the resource (human and financial) requirements for such a study were not available in the research area and establishing them was beyond the capacity of the project.

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Chapter 2

School feeding contributes to micronutrient adequacy and does not replace home meals of Ghanaian schoolchildren

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Submitted for publication

ABSTRACT

Without gains in nutritional outcomes it is unlikely that school feeding programmes (SFPs) could improve cognition and academic performance of schoolchildren despite the improvements in school enrolment. We compared the adequacy of nutrient intake, iron status and nutritional status of SFP participants and non-SFP participants in a cross-sectional survey involving 383 schoolchildren (5–13-year-old). Quantitative 24-hour recall and weighed food record, repeated in 20% subsample, were used to estimate energy and nutrient intake. Haemoglobin concentration (Hb), serum ferritin (SF) and transferrin receptor (sTfR), and anthropometric measurements were used to reflect iron and nutritional status respectively. Energy and nutrient intakes, and their adequacies ($P<0.001$) as well as iron status (Hb, $P<0.001$; sTfR, $P<0.05$) were significantly better among SFP than non-SFP. SFP was associated with about 10 percentage points lower anaemia prevalence ($P=0.06$). However the prevalence of iron-deficiency and iron-deficiency anaemia were not different. Whereas BMI-for-age Z-scores were significantly higher among non-SFP ($P=0.008$), the prevalence of stunting was significantly lower among SFP participants ($P<0.04$). We conclude that school feeding contributed to energy and nutrient intake and their adequacies which probably improved iron status and reduced stunting prevalence. The results also suggest an important role of targeted flour fortification in achieving micronutrient adequacies within SFPs.

INTRODUCTION

Chronic malnutrition is highly prevalent in sub-Saharan Africa especially among rural poor households [1] and it is exacerbated by morbidity and inadequate dietary intake [2]. Infants and young children are most affected by the physical and mental deficits. These deficits are carried over into the school-age period where they greatly retard cognitive function, educability and future productivity [1]. Most interventions at the household and community levels are, however, preferentially targeted at the first 1,000 days of life [1, 3]. “The school” may serve as an avenue for targeted interventions such as school feeding programmes (SFPs). However in settings where school enrolment and attendance are low, targeting interventions at school children may still be problematic.

In Africa and other developing continents, SFPs have therefore been instituted primarily as food-for-education (FFE) in resource-poor settings but also as means of improving nutritional status through improved energy and nutrient intake [4]. Following the formulation of the United Nations Millennium Development Goals (MDGs), SFPs have received renewed interest for their potential contribution to achieving MDGs 1 and 2.

In line with the recommendations of the United Nations Hunger Taskforce (UNHTF), the paradigm in school feeding programmes shifted towards linking local food production to consumption at school with the aim of improving access to market for poor rural farmers and also improve the local economy of beneficiary communities [5]. The shift in paradigm received support from African Governments through the Comprehensive Africa Agricultural Development Programme (CAADP) of the New Partnership for Africa’s Development (NEPAD) thereby putting school feeding programmes on the political agenda of Africa [6]. Since 2005, the Government of Ghana has piloted and up-scaled the Ghana School Feeding Programme (GSFP) through which schoolchildren are provided one nutritious meal per school-day to encourage educational participation (enrolment, attendance and retention) and also improve nutrient intake and nutritional status [7].

However, comprehensive reviews of empirical research [3, 4, 8] and programme evaluation reports [9-11] have shown that whereas SFPs have shown positive impact on educational participation, their impact on nutritional outcomes have been rather unclear [3, 4, 8-11]. More so, in the Lancet series on Maternal and Child Undernutrition, school feeding programmes targeting children older than 2 years have been described as interventions that are unlikely to improve nutritional status [12].

Without evidence of positive impact on nutritional outcomes it is unlikely for SFPs to improve cognition and academic performance despite the demonstrable improvements in educational participation. Our aim therefore was to assess the adequacy of nutrient intake, iron status and nutritional status of schoolchildren participating in a school feeding programme in northern Ghana relative to non-participating children.

SUBJECTS AND METHODS

Study design

This was a cross-sectional study involving the quantitative measurement of energy and nutrient intakes, and iron - and nutritional status of schoolchildren in school feeding and non-school feeding schools. The study was conducted over a period of one month (1st week November – 2nd week December 2008). Ethical clearance was given by the Institutional Review Board of Noguchi Memorial Institute for Medical Research, University of Ghana (NMIMR-IRB 022/08-09). We also sought permission from the district administration, district education office, head teachers and local authorities in each community. After information sessions, parents/caregivers who volunteered to participate gave informed written consent.

Study area

This research was conducted in 4 of the 132 primary schools in Tolon-Kumbungu district (TKDA, 2012) of Northern Region, Ghana. The 4 rural primary schools (from four communities) were approximately 50 km away from the main city in the region, Tamale. Two of the schools (Tibung and Kpalgung primary) were the pilot schools for the Government-supported school feeding programme (SFP) in the Tolon-Kumbungu district which started in October 2005. The two other schools (Wayamba and Jegbo primary), who qualified to benefit from SFP but were not yet enrolled, were selected as control schools based on similarity with the pilot schools in the following characteristics: number of children enrolled in school, school infrastructure, size of community, absence of market, water and sanitation facilities, and proximity to each other (within 5 km radius). The research area is within the Guinea Savannah vegetation zone, having a typical unimodal rainy season (April – September) and one dry season (December – March) characterized by relatively high temperatures (35 – 40^{o}C). People in this area are mostly subsistence farmers [13]. Malaria is hyperendemic with little seasonal variation in this area [14] and is the main cause of morbidity among children [13]. Malaria transmission peaks towards the end of the rainy season (October and November) [15].

Subjects and sampling

Subjects

Schoolchildren (5 – 13-year-old) in classes 1 – 3 from the four schools in Tolon-Kumbungu district were included if they were enrolled in school for at least one academic year at the time of survey. Mothers or alternate caregivers were interviewed to obtain the dietary intake of the children because they prepared and served meals in the households.

Sample size and Sampling procedure

Due to paucity of literature on usual nutrient intake among schoolchildren in the study area, anaemia prevalence (proxy for iron status) among schoolchildren was used for sample size determination. A sample size of 200 per group was needed to estimate a 10% difference in anaemia prevalence between school feeding and non-school feeding schools with 95% confidence and a power of 90% taking into account 10% attrition.

A total of 383 school children were recruited for the study; 196 from the school feeding group and 187 from the non-school feeding group. In each of the four schools, children were randomly selected from a sampling frame of pupils in lower primary (classes 1 – 3). The sampling frame was constructed separately for each school by pooling together the registers of lower primary. If two or more children were selected from one household, one of them was randomly selected by lottery to participate in the study.

Data collection and measurements

Household questionnaire

A semi-structured survey instrument was used to collect information on the socio-demographic characteristics of children and their households. Parents/caregivers were asked to indicate whether the schoolchild was ill within the two weeks preceding the survey. The instrument also included the standardized and validated [(16)] FANTA (Food and Nutrition Technical Assistance) Household Hunger Scale (HHS). The HHS is a 3-item by 3 frequencies of occurrence scale and was used for the assessment of the food supply situation of participating households [(17)]. The survey instrument was translated into the local language (*Dagbani*) and pretested by trained research assistants before use in this survey. The standard reference period of 30 days was used for the HHS assessment [(17)].

24-h recall method

A quantitative 24-hour recall (24-hR), repeated in 20% subsample, was collected by six trained research assistants (first degree nutrition graduates), who spoke the local language and had knowledge of the research area. A minimum of two days were allowed between repeated recalls to avoid dependency of intake on two consecutive days, especially caused by the consumption of leftover foods [18]. Weekend days were excluded. Days of the week and interviewers were randomly allocated to children to account for differences between days and interviewers; and interviewers were not allowed to interview the same household twice. All 24-hR were completed within the same post-harvest season.

A standard multiple pass procedure was used for all 24-hR [18]. First, mothers/caregivers were asked to mention all foods and beverages the children consumed during the preceding 24-hours (wakeup-to-wakeup) including anything consumed outside home. After probing for likely forgotten foods with the help of the index child [19], they were then asked to give detailed description of foods and beverages consumed, including ingredients and cooking methods for mixed dishes, place and time of consumption. The amount of each food, beverage and ingredients of mixed dishes was weighed or, when not available, estimated in household measures or their monetary equivalent. Weight of foods and ingredients of mixed dish were measured using a digital kitchen scale (Soehnle Plateau, model 65086); precise to 2 g with a maximum capacity of 10 kg. Conversion factors from household measures and monetary values to weight were determined afterwards. The total volume of all foods and mixed dishes cooked, volume consumed by child and leftover from child's food were determined to derive the proportion of total prepared food consumed by the child.

Communal eating was a common practice in this area therefore the number of children who shared meals with the index child was obtained and used as a divisor to obtain an estimated quantity of food consumed by the index child. In such situations, equal sharing of food was assumed. The weight of the various ingredients consumed by the child was obtained by multiplying the weight of ingredients used in cooking the food by the proportion of total prepared food consumed by index child.

Weighed school lunch

For the children from the two schools participating in the school feeding programme, lunch was consumed at school. Therefore, weighed food records (WFR) were taken from Monday to Friday to assess the food and nutrient intake from the school lunch. For the 20% of the children who had a repeated 24-hR, a second weighed food record was taken on a non-consecutive day. WFRs were taken on days preceding the scheduled 24-hR for each child. All raw ingredients used in preparing the school lunch for a particular day were weighed using a digital kitchen scale (HD-801 model, Zhejiang, China); precise to 1 g and a maximum capacity of 3 kg. Bulk food ingredients were weighed with a platform scale (Camry FD-250, Guangdong, China); precise to 500 g and a maximum capacity of 250 kg. The weight of the total food cooked, the quantity served to each child and the leftover for each child (when applicable) was determined to derive the proportion consumed by the child from the total dish. For SFP all other meals not consumed in school were considered as home-consumption but for non-SFP all meals were considered home-consumption.

Anthropometric measurements

Weight and height measurements were done following standard procedures [20, 21]. Weight of children was measured precise to 0.1 kg with an electronic scale (UNIscale; Seca GmbH, Hamburg, Germany). A known weight (20 kg) was used to calibrate the scale on each measurement day. A microtoise (Bodymeter 208; Seca GmbH, Hamburg, Germany) was used to measure the height of children precise to 0.1 cm. For both weight and height, an average of two measurements was taken. Ages of children were determined using the date of birth (from a verifiable document) and the date of measurement. In the absence of verifiable documents, parents/caregivers estimated age based on another child’s records or event on the traditional calendar.

Blood samples collection

From each child, 6 ml of venous blood was drawn through venepuncture. One-third (2 ml) of the whole blood was put into EDTA coated vacutainers (Becton-Dickinson diagnostics, Belgium) and were used for the determination of haemoglobin concentration on the same day. The remaining 4ml of blood was stored in a plain tube without anticoagulant at ambient temperature. Serum was separated at room temperature at 500 x *g* for 10 min (Hettich GmbH, Germany) and stored at -80^{0}C (Thermo Fisher Scientific, Asheville, USA). Serum samples were transported on dry ice to Germany via The Netherlands for analysis of serum ferritin (SF), soluble transferrin receptor (sTfR) and C-reactive protein (CRP).

Data Analysis

Household hunger score

Following the standard coding, each of the 3 items in the HHS was coded 0, 1 or 2 corresponding to hunger frequencies of “never”, “rarely or sometimes” and “often”. This yielded total scores ranging from 0 – 6 based on which households were categorised into 3 standard groups: 1=little/no household hunger (HHS $\leq$ 1); 2=moderate household hunger (HHS 2 – 3); 3=severe household hunger if HHS was 4 – 6 [17].

Food composition and nutrient intake calculation

Calculation of the nutrient intake was based on a food composition database primarily created using nutrient values from the West African Food Composition Table (WAFCT) [22]. In case of missing foods (21 out 138 foods), the following food composition tables were used in the order mentioned: Mali food composition table [23], the United States Department of Agriculture National Nutrient database for standard reference [24] and the Ghana food composition table [25]. When foods were taken from the Ghana food composition table, missing nutrients (vitamins and some minerals) were updated with close substitutes from the WAFCT. Phytate values were taken from the International Minilist (IML) [26]. For Corn Soya

Blend (CSB plus) consumed in SFP, nutrient content was obtained from WFP [27]. Where appropriate, yield [22] and nutrient retention factors [24, 28] were applied to account for nutrient losses during cooking before computing nutrient intakes. The Atwater general factors for carbohydrate, protein and fat and the recommended metabolizable energy for dietary fibre in ordinary diet (8.4 kJ/g) were used in calculating energy [29]. Total vitamin A (RAE) was calculated as the sum of retinol and 1/12 β-carotene [22]. The food consumption data was analysed using the VBS Food Calculation System, version 4 (BaS Nutrition Software, The Netherlands). Using the National Research Council's method the observed intakes from the 24-hR were adjusted for day-to-day variation to get the estimated usual intake for the children [30]. Individual foods were categorised into 13 food groups [22]. Thirty-one (8%) children with implausible dietary intake (energy intake > 20,920 kJ) were not included in the dietary analysis.

Energy and nutrient adequacies

Estimated energy requirements (EER) was calculated separately for each child by multiplying the EER·kg bodyweight $^{-1}$·day $^{-1}$ by the child's weight assuming a moderate PAL [31]. Similarly, gender and age specific safe level of protein intake·kg bodyweight $^{-1}$·day $^{-1}$ were multiplied by weight of children to obtain safe level of protein intake for each child[32]. To assess the prevalence of adequate or inadequate intake, each child's adjusted energy and protein intakes were compared to their respective calculated requirements.

The probabilities of adequacy (PA) for vitamins A, C, B_{12} and folate, zinc and calcium were calculated using their respective estimated average requirements (EARs) and distributions [33-35]. Because the distribution of iron requirement is skewed we used probability of adequacy values derived by Institute of Medicine [33] but adjusted for 5% bioavailability to reflect the inhibitory nature of the predominantly cereal-based diet in rural northern Ghana. Similarly, the EAR for zinc was adjusted for low (15%) bioavailability [36]. Mean probability of adequacy (MPA), a summary measure of micronutrient adequacy, was computed from PAs of all seven micronutrients reported in this paper.

Anthropometry

Anthropometric Z-scores were calculated using WHO AnthroPlus (version 1.0.3). Anthropometric indices (weight-for-age, height-for-age and BMI-for-age Z-scores) were transformed into anthropometric indicators using a cut-off value of <-2 SD [20, 21].

Biochemical analysis

The cyanmethaemoglobin method (using colorimeter) was used to measure haemoglobin concentration of schoolchildren [37]. Measurements of serum parameters (ferritin, sTfR and CRP) were done in an accredited laboratory (Labor Centrum Nordhorn, Nordhorn, Germany). Ferritin was measured using ElectroChemiLuminescence Immunoassay on a Roche E170 clinical analyser (Roche diagnostics) with an intra and inter assay variation of 2-5%. Soluble transferrin receptors were measured with Ramco-ELISA kit (Ramco Laboratories Inc.) with an intra and inter assay variation ranging from 5-8%. Turbidimetry was used to measure CRP on a Beckman Coulter Synchron clinical analyser (Beckman Coulter, Miami, FL, USA) with combined intra and inter assay variation ranging from 1.6 – 3.5%.

Anaemia was defined as Hb <115 g/l for children <12 years and <120 g/l for children ≥12 years. Iron deficiency (ID) was defined as SF <15 µg/l and/or sTfR >8.5 mg/l (Ramco Laboratories Inc.) and iron-deficiency anaemia (IDA) as concurrent anaemia with ID. Inflammation was defined as CRP >10 mg/l. Body iron was calculated using Cook's formula [38].

Statistical Analysis

Data entry was done using Epi Info for windows version 3.2.1 (CDC). Data cleaning and analysis was done in SPSS version 18.0 (Armonk, NY, USA) and SAS version 9.2 (SAS

Institute, Cary, NC, USA). The distribution of data were checked by visual examination of Q-Q plots and normal-curve-fitted histograms, and also tested for normality with Kolmogorov-Smirnov test. Nutrient and iron status variables that were not normally distributed were log-transformed and the transformed variables were used in subsequent analysis. ANOVA was used to generate within-person day-to-day variance component that was used to adjust energy and nutrient intake.

ANCOVA was used to test differences in mean adjusted nutrient intake, haemoglobin concentration as well as serum iron parameters between the two groups while controlling for age, household size, household hunger score, and nutritional status (BAZ). Descriptive statistics were computed for background and household characteristics of children, and Chi-squared test was used to test differences in proportions of household and parent characteristics between the two groups. Differences in prevalence of recent illness, anaemia, inflammation, ID, IDA and inadequate nutrient intakes between the two groups were checked using Cox-regression with robust variance (covsandwich) and constant time to event [39, 40]. Where appropriate, child and household characteristics were included in the regression model as covariates. In all analysis, a *P*-value of < 0.05 was the default value for an outcome to be considered statistically significant.

RESULTS

Background characteristics of schoolchildren

Characteristic of the survey area, more than 55% of the children in both groups of school were boys. The average age of the children in both groups was 8.5 (SD 2) years however, children in SFP were on average 8 months older than their counterparts in non-SFP (*P*<0.01). The proportion of children who were reported ill within the two weeks preceding the survey was not significantly different between groups (*P*>0.05). Household size was larger for SFP group than non-SFP group (P<0.001). More than half of the children in both groups were from polygamous households. The proportion of households who reported moderate or severe hunger did not differ between the two groups (P>0.05). In both groups, majority of parents/caregivers were illiterate and engaged in farming as their main occupation (**Table 1**).

Table 1. Background characteristics of children in school feeding and non-school feeding schools in northern Ghana

	SFP		non-SFP		P
	Mean	SD	Mean	SD	
n	194		180		
Boys (%)	55.2		57.8		NS
Age of child (years)	9.0	2.1	8.4	2.0	0.007
Sick two weeks preceding survey (%)	26.9		32.8		NS
Household size (*n*)	8.0	3.0	7.0	2.0	<0.001
Household type (%)					NS
Monogamous	36.8		39.4		
Polygamous	56.5		52.2		
Other	6.7		8.4		
Household hunger category (%)					NS
Moderate	30.5		31.7		
Severe	4.7		7.8		
Mother of index child					NS
Education (literate) (%)	5.6		3.4		
Occupation (%)					NS
Farmer	65.6		57.8		
Trader	22.6		23.8		
Other	11.8		18.4		
Father of index child					NS
Education (literate) (%)	21.5		16.0		
Occupation (%)					NS
Farmer	90.7		91.2		
Trader	2.6		2.8		
Other	6.7		6.0		

Food consumption pattern at home and school

At home, the three main meals served to children in both groups consisted of maize porridge (*koko*) with or without sugar served as breakfast and *tuo zaafi (TZ)* – a thick/stiff maize porridge – served as lunch and dinner with varying vegetable soups. At the time of survey (post-harvest), the most dominant soup consumed by more than 50% of the children consisted of dried powdered okra with or without groundnut paste/groundnut flour. When available, green leaves such as amaranth, *hibiscus sabdariffa* and baobab leaves (fresh and dried) were also used to prepare the soups accompanying the TZ. A key ingredient of the soup, among all households, was powdered *amani* (small dried whole fish (anchovies), eaten with bones).

School lunch, for the SFP participants, was more varied and based on a menu. The menu was generally planned around three main food items; rice, cowpea and multiple micronutrient fortified corn-soy blend (CSB+ from WFP). Eggs, meat and fish were served at least once per week whiles oranges were served twice per week.

Energy, nutrient intake and their adequacies among schoolchildren

Median energy, macronutrients (except dietary fibre) and selected minerals and vitamins intakes were higher among children participating in the school feeding programme than non-participating children (P<0.001) and remained higher after controlling for child and household covariates. Whereas the contribution of fat to total energy intake was significantly higher among SFP (P<0.001), the contribution of carbohydrate to total energy intake was significantly higher among non-SFP (P<0.001). The proportion of total protein intake from animal sources, a measure of protein quality, was larger among SFP than non-SFP (5% v. 3%; P<0.001). However, the proportion of total iron intake from animal sources (meat, fish and poultry) was not different (P>0.05) between the two groups of schoolchildren (**Table 2**).

The proportion of SFP children with energy intakes below the requirement is significantly smaller than the proportion among the non-SFP (4.7% v. 21.8%; P<0.001). For protein however, none of the children in both groups had intakes below the requirement. The probabilities of nutrient adequacy for iron, zinc, calcium, vitamins A and C, and folate were significantly higher (P<0.001) for SFP than non-SFP with a mean probability of adequacy of 0.74 (SD 0.09) for SFP compared to 0.32 (SD 0.11) for non-SFP (**Table 3**).

Table 2. Energy, nutrient and phytate intakes of children in school feeding and non-school feeding schools in northern Ghana[1]

Variable	SFP	non-SFP	*P*
n	174	178	
Energy (kJ)	9493 (8669, 10652)	7590 (6895, 8289)	<0.001
Macronutrients			
Protein (g)	68 (66, 71)	54 (46, 62)	<0.001
% of energy	12 (11, 13)	12 (11, 13)	0.22
% from animal[3]	5 (2, 10)	3 (2, 5)	<0.001
Fat (g)	49 (49, 49)	32 (32, 33)	<0.001
% of energy	20 (18, 21)	16 (15, 17)	<0.001
Carbohydrate (g)	365 (319, 422)	294 (270, 319)	<0.001
% of energy	64 (61, 67)	64 (63, 66)	<0.01
Dietary fibre (g)	43.1 (36.0, 51.3)	40.4 (36.0, 45.1)	<0.01
Minerals			
Iron (mg)	28.3 (28.2, 28.4)	23.4 (20.5, 26.1)	<0.001
% from animal[4]	0.7 (0.4, 1.3)	0.6 (0.4, 1.2)	0.268
Zinc (mg)	13.1 (13.0, 13.1)	8.6 (7.7, 9.8)	<0.001
Calcium (mg)	399 (398, 400)	287 (237, 322)	<0.001
Vitamins			
Vitamin C (mg)	47.1 (47.1, 47.3)	8.6 (8.6, 8.7)	<0.001
Vitamin A (μg RAE)	493.0 (492.2, 494.8)	64.3 (40.9, 102.2)	<0.001
Vitamin B-12 (μg)	1.0 (1.0, 1.0)	0.1 (0.1, 0.1)	<0.001
Folate (μg)	245.8 (236.2, 255.8)	134.0 (114.8, 173.6)	<0.001
Phytate (mg)	3065 (2526, 3730)	3083 (2730, 3355)	0.95

[1] Values are medians (IQR) . [2] Adjusted for within-person day-to-day variation based on a 20% subsample of repeated recalls. [3] Meat, fish, eggs and milk. [4] Meat and fish

Table 3. Proportion of children in SFP and non-SFP below EAR for energy and protein, and the probabilities of adequacy for selected micronutrients[1]

Variable	SFP		non-SFP		
	Mean	SD	Mean	SD	*P*
n	174		178		
Prevalence of inadequate intake (%)					
Energy	4.7		21.8		<0.001
Protein	0		0		
Probability of micronutrient adequacy					
Iron	0.94	0.02	0.72	0.23	<0.001
Zinc	0.65	0.29	0.32	0.32	<0.001
Calcium	0.001	0.0	0.001	0.0	<0.001
Vitamin C	1.0	0.00	0.00	0.00	<0.001
Vitamin A	0.87	0.12	0.02	0.09	<0.001
Vitamin B-12	1.0	0.0	1.0	0.0	NA[2]
Folate	0.70	0.31	0.19	0.35	<0.001
Mean probability of adequacy[3]	0.74	0.09	0.32	0.11	<0.001

[1] EAR, estimated average requirement. [3] Computed from the PA values of micronutrients.

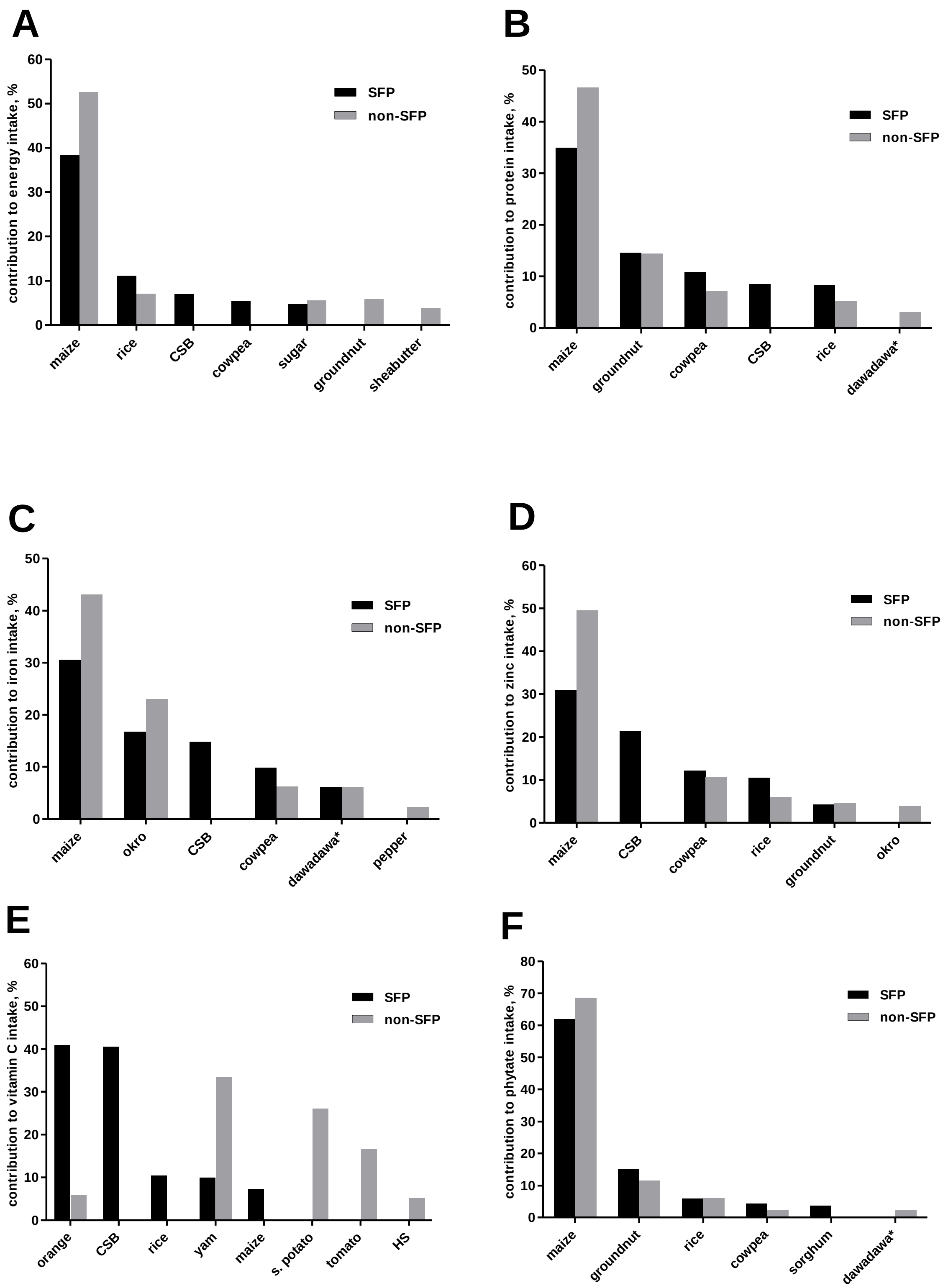

Figure 1. The top five foods contributing to energy (A), protein (B), iron (C), zinc (D), vitamin C (E) and phytate (F) intakes among school feeding and non-school feeding participants in northern Ghana. CSB, corn-soy blend; *dawadawa*, local condiment made from fermented African locust-bean (*parkia biglobosa* seeds); HS, *hibiscus sabdariffa*; s. potato, sweet potato; SFP, school feeding programme; non-SFP, non-school feeding programme

Home-consumption and the contribution of school lunch to energy and nutrient intake

Based on home-consumption, energy, fat, carbohydrate, calcium, vitamin C and phytate intakes were not different between the two groups of children ($P>0.05$). Home intake was significantly higher among non-SFP for protein ($P<0.05$), iron ($P<0.05$), zinc ($P<0.01$) and vitamin A ($P<0.01$). For energy, macronutrients and selected minerals, 20 – 37% of daily intakes were supplied by the school lunch served to SFP participants. For vitamins A and C however, > 90% of the daily intakes were supplied by the school lunch (**Table 4**). School lunch provided approximately 418 kJ more than home lunch (2,351 (SD 209) v. 1929 (SD 280) kJ; $P<0.001$) and about 2 g of protein more (18 (SD 1) g v. 16 (SD 3) g; $P<0.001$). The contribution of school lunch to EER for energy among SFP participants was significantly larger than the contribution of home lunch to EER for energy among the non-SFP participants (37 (SD 7) v. 31 (SD 8) %; $P<0.001$). However, the contribution of school lunch to daily protein requirement among SFP participants did not differ from the contribution of home lunch among non-SFP participants (88 (SD 17) v. 84 (SD 26) %; $P=0.096$).

Relative contribution of individual foods and food groups to energy and nutrient intakes

In **Figure 1**, the five topmost individual foods contributing to ≥ 70% of energy, selected nutrients and anti-nutrient related to iron absorption are shown. Except for vitamin C, maize was the main source of total energy and selected nutrient intakes. The relative contribution of maize to energy and selected nutrient intakes among the non-SFP group ranged from 43 – 70% but ranged from 30 – 60% for SFP group. Among the SFP participants, cowpeas and corn-soy blend (CSB+, from the World Food Programme) were additional sources of energy and nutrient intakes.

For both groups of children, the main food groups that contributed to dietary intake were cereals (maize, rice and sorghum), vegetables (dried okra and green leaves), nuts (groundnuts) and fish (*amani*). Food groups such as meat, eggs and fruits were rarely consumed by children in the non-SFP (**Figure 2**). The SFP children received meat at school twice per week but the average quantity per serving was <10 g/day. The overall dietary variety (number of different food groups consumed) among the SFP participants was greater than among the non-SFP participants (8.5 (SD 0.9) v. 6.2 (SD 1.1); *P*< 0.001).

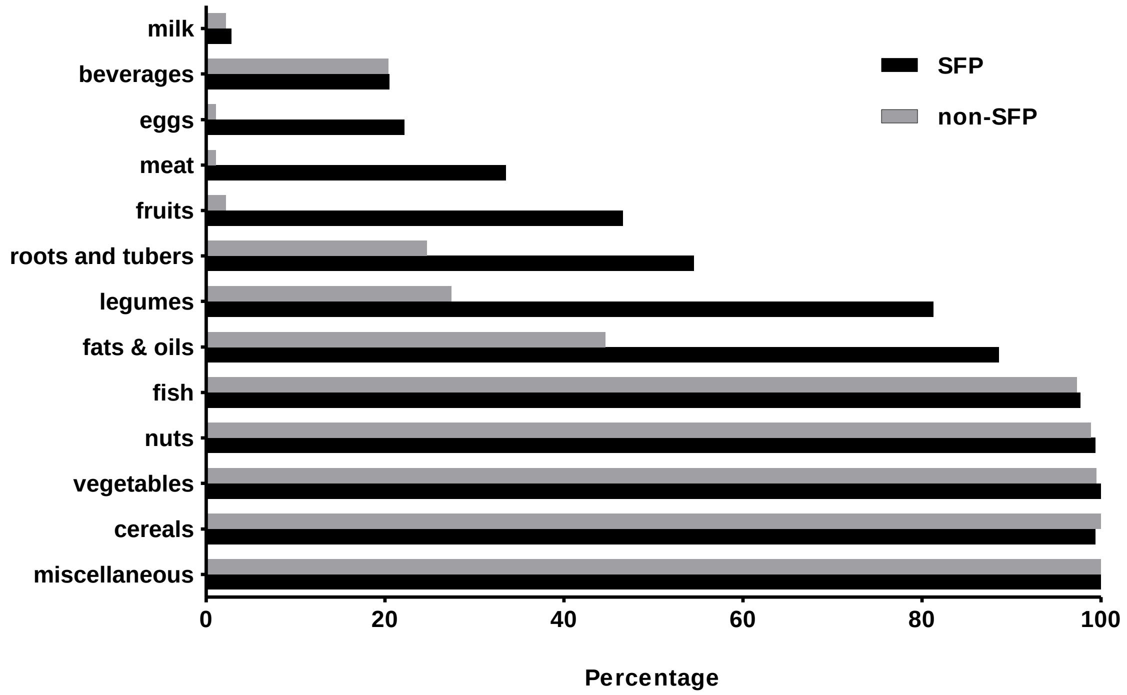

Figure 2. Proportion of school feeding and non-school feeding children in northern Ghana consuming foods from 13 food groups. SFP, school feeding programme; non-SFP, non-school feeding programme

Eating moments among schoolchildren

Almost all children in both groups ate at each of the three main eating moments per day; breakfast, lunch and dinner. Whereas larger proportion of SFP children consumed a meal before the main breakfast meal (36% v. 25%; *P*=0.018), the reverse was true for children who ate a meal before lunch (13% v. 40%; *P*<0.001). Before the main dinner meal, almost every SFP child consumed a meal compared to only 20% of non-SFP children. For the SFP participants, the meal before the main dinner meal could best be described as a second lunch

(**Figure 3**). On the average, SFP participants had more eating moments (meals) compared to non-SFP (4.5 v. 3.8; *P*<0.001).

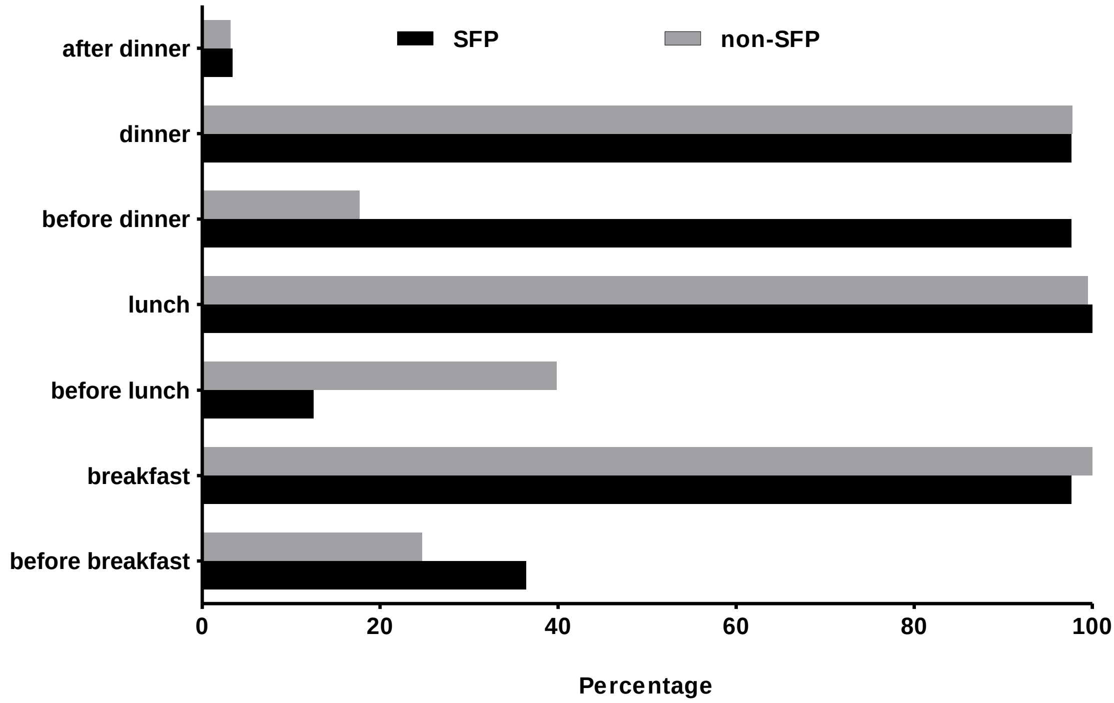

Figure 3. Proportion of school feeding participants and non-school feeding participants in northern Ghana who ate meals across the seven daily eating moments. SFP, school feeding programme; non-SFP, non-school feeding programme

Iron and nutritional status of schoolchildren

Mean haemoglobin concentration of the children was 100 (SD 16) g/l. SFP had 6 g/l higher haemoglobin concentration than non-SFP (*P*<0.001) even after controlling for household and child characteristics. Serum ferritin concentration did not differ between the two groups. Soluble transferrin receptor concentration was significantly lower among SFP compared to non-SFP (*P*=0.04). Calculated body iron store was not different between groups (*P*=0.08). There was no difference in the mean concentrations of C-reactive protein and the proportion of children with inflammation between the two groups.

Table 4. Difference between home-consumption between school feeding and non-school feeding participants and the contribution of school lunch to nutrient intake among school feeding participants

	SFP	non-SFP	*P*
n	174	178	
Energy (kJ)			
Home-consumption[3]	7439 (5234, 9996)	7858 (5933, 10682)	0.064
School lunch	2397 (2033, 2858)	-	
% of total intake	24	-	
Protein (g)			
Home-consumption	50 (36, 67)	53 (40, 75)	0.041
School lunch	19 (13, 24)	-	
% of total intake	28	-	
Fat (g)			
Home-consumption	29 (19, 48)	33 (20, 52)	0.135
School lunch	18 (14, 22)	-	
% of total intake	38	-	
Carbohydrate (g)			
Home-consumption	292 (205, 386)	309 (232, 409)	0.147
School lunch	81 (68, 93)	-	
% of total intake	22	-	
Iron (mg)			
Home-consumption	20.4 (15.4, 29.3)	22.8 (17.4, 31.8)	0.011
School lunch	7.0 (3.2, 12.5)	-	
% of total intake	26	-	
Zinc (mg)			
Home-consumption	8.2 (5.9, 11.2)	8.8 (6.6, 11.4)	0.005
School lunch	4.2 (2.4, 8.2)	-	
% of total intake	34	-	
Calcium (mg)			
Home-consumption	230 (169, 336)	257 (190, 420)	0.110
School lunch	134 (74, 240)	-	
% of total intake	37	-	
Vitamin A (μg RAE)			
Home-consumption	50.8 (24.6, 116.2)	64.0 (31.1, 122.3)	0.005
School lunch	556.6 (76.5, 1090.3)	-	
% of total intake	92	-	
Vitamin C (mg)			
Home-consumption	1.8 (0.44, 10.7)	1.3 (0.8, 19.8)	0.143
School lunch	63.4 (2.6, 81.6)	-	
% of total intake	97	-	
Phytate (mg)			
Home-consumption	2940 (1984, 4167)	3091 (2449, 4327)	0.064
School lunch	242 (201, 333)	-	
% of total intake	8	-	

RAE, retinol activity equivalent. [3] Including foods bought outside home and consumed by children

The prevalence of anaemia was marginally lower (*P*=0.06) in SFP whilst the prevalence of ID and IDA were not significantly different between the two groups.The SFP children were about 3 cm taller however the difference was not significant after controlling for age differences. Z-scores of weight-for-age and height-for-age were similar between the groups. BMI-for-age Z-score was significantly larger for the non-SFP group (*P*<0.05). After controlling for age difference between groups, the prevalence of stunting was significantly lower among SFP participants but prevalence did not differ for underweight and thinness (**Table 5**).

DISCUSSION

We compared the energy and nutrient intakes, iron and nutritional status of schoolchildren in school feeding and non-school feeding schools. Energy and nutrient intakes, and their adequacies as well as iron status were significantly higher among school feeding participants than non-participants. Also, the prevalence of stunting was significantly lower among SFP participants.

The significantly higher intake of energy and nutrients among the school feeding participants is attributable to the complementary effect of school meals [41-43] and superior energy density of the school lunch. School lunch meals were served before 12.00 hours so children were probably hungry again by the time school closed at 14.00 hours and therefore were still able to eat a late lunch at home. The school lunch also improved diversity in the meals of participating children which has been shown to be related to increased quality and quantity of nutrient intakes [44-48]. For both groups, all children met their safe level of intake for protein. However the biological value of the protein may be low given that only an average of 4% is animal source protein and cereal protein is limiting in growth supporting lysine. Even though we did not adjust for protein quality [49, 50], the digestibility of the protein may also be compromised given the high concentration of dietary fibre in the meals of both groups [49].

Table 5. Iron and nutritional status of children in school feeding and non-school feeding schools in northern Ghana

	SFP		non-SFP		P^2
	Mean	SD	Mean	SD	
n	194		180		
Iron Status markers					
Hb (g/l)	103	15	97	18	<0.001
SF (µg/l)	41.3[1] (22.3, 75.4)		35.9 (19.3, 70.6)		NS
SF, excluding elevated CRP[3] (µg/l)	37.7 (21.3, 69.8)		34.4 (18.5, 67.3)		NS
sTfR (mg/l)	11.4 (8.7, 14.4)		12.6 (9.3, 16.5)		0.04
Body Iron[4] (mg/kg body weight)	3.2	4.1	2.3	4.3	0.08
Inflammation marker and classification					
CRP (mg/l)	1.7 (0.8, 2.8)		1.9 (0.8, 3.6)		NS
CRP >10 mg/l (%)	9.8		10.5		NS
Iron Status Classification					
Anaemia (%)	75.8		84.9		0.06
ID, SF <15 µg/l (%)	14.4		17.8		NS
ID, SF <15 µg/l excluding elevated CRP[3] (%)	14.9		18.6		NS
ID, SF <15 µg/l and/or sTfR >8.5 mg/l (%)	77.8		80.7		NS
IDA[5] (%)	62.7		69.4		NS
Nutritional Status Indices					
Weight (k)g	23.6	5.0	22.8	5.2	NS
Height (cm)	125.3	10.4	122.1	11.9	NS
Weight-for-age[6] (Z-score)	-0.95	1.1	-0.92	1.1	NS
Height-for-age (Z-score)	-1.1	1.6	-1.1	1.5	NS
BMI-for-age (Z-score)	-0.93	0.84	-0.62	0.82	0.008
Nutritional Status Indicators (%)					
Underweight[6]	16.3		14.4		NS
Stunting	23.3		28.9		0.04
Thinness	11.9		5.6		NS

[1] Values are geometric mean (IQR). [2] Adjusted for age difference between groups. [3] *n* = 175 (SFP) and 161 (non-SFP). [4] To convert body iron from mg/kg to mmol/kg multiply by 0.0171 [51]. [5] Defined as anaemia and SF <15 µg/l and/or sTfR >8.5 mg/l. [6] Anthroplus software allows weight-for-height calculation only for children 5 – 10 years old, *n* = 136 (SFP) and 139 (non-SFP)

Few food items contributed to the improved micronutrient intake among the school feeding participants; orange for vitamin C, fortified corn-soy blend for iron, vitamins A and C, and palm oil for vitamin A. The multiple-micronutrient fortified corn-soy blend in particular appears to play a key role in increasing micronutrient intake and adequacy among the school feeding participants. This may thus suggest that adequate micronutrient intake may not be achieved by the mere provision of an extra meal through school lunch, but by deliberate supply of micronutrient dense foods [(3, 52)]. However, the bioavailability of the relatively higher iron and zinc intake among SFP may be reduced given the high phytate content of the diet in general [(53)] and the meagre contribution of animal protein to total dietary intake [(54)]. More so the oranges that were served (two times a week) with lunch, which could improve iron bioavailability when consumed together with the school lunch [(55, 56)], were rather sent home and often shared with younger siblings not in school.

Out-of-home food intake may have been omitted by mothers/caregivers and may lead to an underestimation of nutrient intake [(19)]. However in this area almost all meals were prepared and consumed at home and mothers/caregivers were fully involved in serving meals. Also, the presence of children during the interviews helped mother/caregivers to recall likely forgotten foods. We therefore believe that underestimation of nutrient intake was unlikely to have happened.

The high prevalence of anaemia among these children is not unexpected. The survey area is malaria endemic and malaria is among the leading causes of anaemia [(14)] in this area. Since this survey was conducted during the peak of malaria transmission (November-December), it is most likely that malaria has contributed to the high prevalence of anaemia among these children [(57)]. Notwithstanding the apparent contribution of malaria to anaemia, the high prevalence of iron-deficiency anaemia among these children may indicate that a large proportion of the anaemia is due to iron-deficiency. The low ID observed based on SF alone rather than when combined with sTfR highlights the difficulty of reliably measuring ID in settings where infections and infestations may be high. In the same area, Abizari et al. [57] in an intervention trial found that SF decreased, from baseline values similar to those observed in this study, in response to deworming and malaria treatment. However the low prevalence of elevated CRP (an acute-phase protein) among these children does not seem to suggest that SF values in this survey are possibly influenced by inflammation. Unlike CRP, AGP (α_1-acid glycoprotein) rises and returns to baseline values slowly [58] and therefore it may be better to measure both CRP and AGP as composite marker of cross-sectional inflammation [57, 59, 60] but AGP was not measured in this survey.

The higher haemoglobin concentration, better iron status (based on sTfR) and the relatively lower prevalence of anaemia among SFP participants may be associated with the overall better iron content of school lunch. Health related interventions associated with SFPs such as deworming could have also contributed to the relatively better haemoglobin and iron status [4], but neither group of schools reported receiving deworming treatments within six months preceding our study. In the same area it was shown in a randomized trial that school feeding coupled with deworming and malaria treatment significantly improved haemoglobin concentration, iron status and reduced anaemia prevalence [57].

Contrary to our expectation the improved energy and nutrient intake among the SFP participants did not result into significant improvement in weight related indices. It is however possible that the improved energy and nutrient intake improved activity level of the children at the expense of weight gains [61]. It is unclear whether the lower prevalence of stunting among SFP participants could be attributed to school lunch given that interventions beyond 2 years of age are deemed unlikely to improve stunting [62].

Based on the differences between the estimated energy requirements (EER) for the children and the adjusted energy intakes, majority in both groups of schoolchildren were in positive energy balance. However there was no evidence of the positive energy balance in the nutritional status of the schoolchildren. On the other hand, it is also possible that the schoolchildren were more physically active and thus required more energy than our estimates based on moderate physical activity level.

The evidence of the impact of SFPs (e.g. Government-run SFPs) have been described as lacking rigor because of their non-experimental design [(4)]. The absence of measures of nutrient intake, iron status and nutritional status for both groups at start of the SFP makes us unable to isolate the impact of school lunch albeit we controlled for differences in child and household characteristics in the analysis. We matched SFP communities with non-SFP communities that were otherwise also qualified to receive school feeding but were not enrolled at the time of our research. We examined our assumptions that intervention communities had similar starting status as their controls by comparing outcomes of interest between all four SFP-non-SFP pairs (2SFPs by 2non-SFPs) to see if differences were consistently in favour of school feeding. We observed consistent differences in favour of school feeding with respect to energy and nutrient intake but not with iron status and nutritional status suggesting that our assumption for similarity may not be strongly supported. It is possible however that the paired comparisons lacked power to detect consistent direction of effect because sample sizes were half of what our study was powered for. More so, unobservable differences between communities may have blunted the effects due to SFP [(63)].

Therefore we conclude that school feeding contributed to energy and nutrient intake and their adequacies which probably improved iron status and stunting prevalence. The results also suggest an important role of targeted flour fortification in achieving micronutrient adequacies within school feeding programmes.

ACKNOWLEDGEMENTS

We are grateful to the following teachers for their cooperation during the survey: Y. Abdul-Majeed (Wayamba primary school), A. Alaru (Wayamba primary school), A. A. Suhuyini (Jagbo primary school), I. Norgah (Tibung primary school) and S. Inusah (Kpalgung primary school). We also thank L. van der Heijden for her technical support during the training of research assistants and R. K. Adatsi of the Tamale Teaching Hospital for his technical support during blood samples collection and measurement of haemoglobin concentration. This research was supported by the Interdisciplinary Research and Education Fund (INREF) of Wageningen University through the TELFUN (Tailoring food sciences to Endogenous patterns of Local food supply for Future Nutrition) Project.

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Chapter 3

Cowpeas in northern Ghana and the factors that predict caregivers' intention to give them to schoolchildren

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Submitted for publication

ABSTRACT

Background: Cowpeas are important staple legumes among the rural poor in northern Ghana and have the potential to contribute to food sovereignty. Our objectives were to assess the iron and zinc content of cowpea landraces and identify factors that predict the intention of mothers to give cowpeas to their schoolchildren.

Methods and Findings: We performed biochemical analysis on 14 landraces of cowpeas and assessed the opinion of 120 caregiver-child pairs on constructs based on the combined model of the Theory of Planned Behaviour and Health Belief Model. We used correlations and multiple regressions to measure simple associations between constructs and identify predictive constructs. Cowpea landraces contained iron and zinc in the range of 4.9 – 8.2 mg/ 100 g d.w and 2.7 – 4.1 mg/100 g d.w respectively. The landraces also contained high amounts of phytate (477 – 1110 mg/100 g d.w) and polyphenol (327 – 1055 mg/100 g d.w). Intention of mothers was strongly associated (r_s=0.72, P<0.001) with and predicted (β= 0.63, P<0.001) behaviour. The constructs barriers (β= –0.42, P=0.001) and attitudes towards behaviour (β= 0.25, P<0.028) significantly predicted intention albeit the predictive ability of the model was weak.

Conclusions: We conclude that some cowpea landraces from northern Ghana have appreciable amounts of iron and zinc but probably with poor bioavailability. Attitudes towards giving cowpeas and perception of barriers are important predictors of mothers' intention to give cowpeas to their schoolchildren. Finally our results suggest that increasing knowledge on nutritional benefits of cowpeas may increase health values mothers hold for their children in support of giving cowpeas to schoolchildren.

INTRODUCTION

Iron-deficiency is of public health significance in developing countries [1] and likely to co-exist with zinc deficiencies [2, 3]. In northern Ghana, more than two-thirds of schoolchildren are likely to suffer from iron-deficiency [4]. Long term consequences include decreased physical work capacity and future productivity [5, 6]. An integration of approaches has been proposed as key to the reduction in prevalence of iron-deficiency [7, 8]. Strategies to fight iron-deficiency need to be culturally sensitive, acceptable and carefully linked with the food culture of communities. One of such strategies is the promotion of the consumption of local staple foods that are rich in iron. However local staples in settings like northern Ghana are largely cereals, roots and tubers, and legumes, which often do not contain high native iron. Therefore targeted breeding for higher concentration of native iron (biofortification) has been proposed as a sustainable intervention to increase dietary iron intake [9]. Early in the steps towards such biofortification is the identification of varieties with high native iron and/or zinc to serve as breeding parents [10].

Being one of the widely consumed staple foods in Ghana, cowpeas (*Vigna unguiculata* (L.) Walp) have received attention as a candidate crop for biofortification to improve its native iron as well as zinc concentration. Cowpeas are native to northern Ghana [11]. They have high nutritional significance due to their good quality protein content and significant amounts of vitamins and minerals like iron and zinc [12, 13]. Over the years cowpeas have grown from being regarded as “poor man’s meat” [14] to one that is consumed across socio-economic strata. Therefore cowpeas may have the potential to contribute to food sovereignty in a long term and if targeted at schoolchildren, in a school feeding programme, improved cowpeas may also contribute to better iron status.

Even though cowpeas are already an intricate part of the food culture of northern Ghana [13], it is not known what factors influence mothers to give cowpeas to their schoolchildren.

Understanding the significant factors that predict cowpea consumption can provide important insight for the development of effective interventions leading to increased cowpea consumption not only within a school feeding programme but also at the household.

To date, two popular psychosocial theories (Theory of Planned Behaviour (TPB) and Health Belief Model (HBM)) have found wide use in explaining influential variables in food-related behaviours. According to the TPB, behaviour is a conscious act proximally mediated by intention [(15)]. The HBM on the other hand posits that a health behaviour results from a set of core beliefs [(16)]. It has been proposed that a combination of these two complementary theories will help broaden our understanding of factors that influence dietary behaviour [(17, 18)].

In this paper, as part of identifying cowpeas with potential for biofortification, we assessed the iron and zinc content of available cowpea landraces in northern Ghana. Secondly, using a combined model as proposed by Sun et al. [(17)], we aimed at identifying factors that influence the intention of mothers/caregivers to give cowpeas to their schoolchildren.

MATERIALS AND METHODS

Study Area

This study was conducted in the Tolon-Kumbungu district of the Northern Region of Ghana. The region is well-suited for cowpea production and Tolon-Kumbungu district is among the three top production-processing-consuming sites for cowpea in the region [(19)]. The research area is located within the Guinea Savannah agro-ecological zone and has two distinct seasons; rainy season (April – September) and a dry season (December – March) characterized by relatively high day temperatures (35 – 40 ^{0}C). The people of this area are largely subsistence farmers [(20)]. Two communities, Kpaligung and Tibung, were purposively selected because they were participating in a larger nutrition study that sought to investigate the potential role of cowpeas in improving iron status through school feeding. At the time, these two communities were the only ones piloting the Government-supported school feeding programme in the district.

Study Design and Subjects

Cowpea landrace study

Key informant interviews with 3 farmers and 3 market women were conducted in March 2008 to identify locally available landraces of cowpeas in the selected communities and the largest market in the main city, Tamale. Landraces were identified based on the local knowledge and experience of local farmers and market women. For each landrace, a sample of 200g was collected after cleaning and separating mixed landraces. Seeds with holes or weevil attack were removed by hand. All collected samples were kept in transparent polythene bags and labelled with their corresponding local names as given by the key informants. The samples were sent to Wageningen University, The Netherlands, and stored at -20 ^{0}C pending analysis.

Cowpea acceptability study

This was a cross-sectional study conducted in November 2008. In each school 60 schoolchildren (6 – 12 years) in lower primary (classes 1 – 3) were randomly selected to participate, a sample size assumed to be adequate in research based on the TPB [(21)]. The corresponding 120 caregivers of the selected children were interviewed. The study was approved by the Institutional Review Board of Noguchi Memorial Institute for Medical Research, University of Ghana (NMIMR-IRB 022/08-09). Each volunteer gave verbal informed consent prior to participation.

Table 1. Operational definition of constructs used to examine factors that predict intention of caregivers to give cowpeas to their schoolchildren

Construct	Operational definition
Knowledge	The caregiver's knowledge about the relationship between cowpeas and health, and specifically to malnutrition and iron-deficiency anaemia
Perceived susceptibility	The caregiver's subjective perception of her schoolchild being malnourished and anaemic
Perceived severity	The caregiver's feelings concerning the seriousness of her schoolchild being malnourished and anaemic
Health value	The importance caregiver places on the consequences of her schoolchild being malnourished and anaemic
Health behaviour identity	The caregiver's opinion of the expected consequence of giving cowpeas to the schoolchild
Attitudes towards behaviour	Favourable or unfavourable disposition of the caregiver towards giving cowpeas to the schoolchild
Perceived barriers	The caregiver's beliefs about costs or negative aspects of cowpea consumption by the schoolchild
Cues to action	Triggers that stimulate the caregiver to give cowpeas to her school child.
Subjective norms	The caregiver's perceived social pressure to give or not to give the schoolchild cowpeas (who is important for the behaviour and is the opinion of that person important?)
External control belief	The caregiver's perceived presence of factors that may facilitate or impede giving cowpeas to the schoolchild
Behavioural intention	The caregiver's readiness to give cowpeas to the schoolchild
Behaviour	Giving cowpeas to the schoolchild

Questionnaire Development

An 89-item (grouped into 12 constructs, see **Table 1**) research questionnaire was developed along the recommendations of Francis et al [21] and based on the Theory of Planned Behaviour

[15] and the Health Belief Model [16]. These two models were combined into the study model as described by Sun et al [17], **Figure 1**.

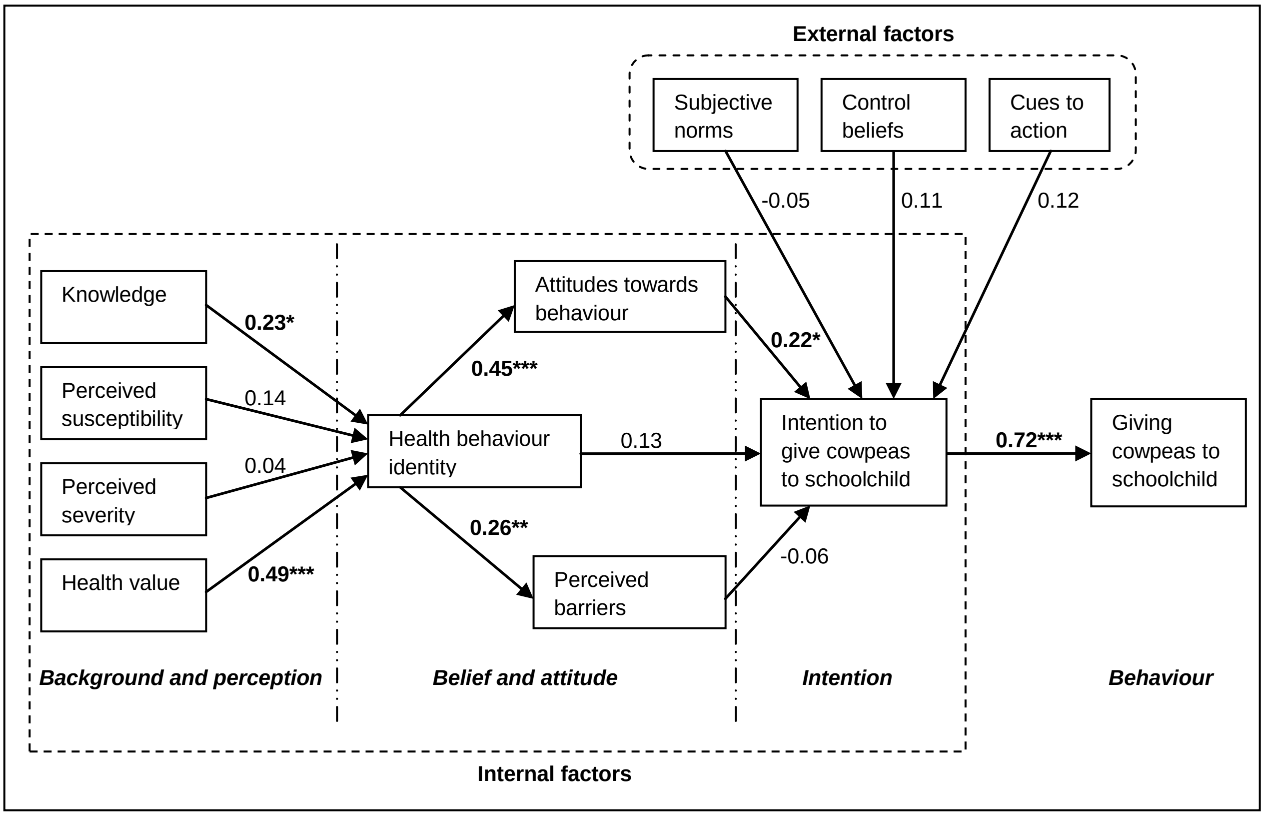

Figure 1. A combined model of the Theory of Planned Behaviour and Health Belief Model with correlation coefficients between related constructs. Adapted from Sun et al. [17]. *P<0.05; **P<0.01; *** P<0.001 (2-tailed).

Relative to behaviour, constructs were grouped into internal and external factors. The internal factors were further grouped into: background and perception, belief and attitude, and intention. The construct subjective norm, left out of Sun et al.'s model, was added to the study model because in an African setting the values of extended family and community significantly influence behaviour of an individual [22]. The items included in each construct were drawn from previous studies [17, 23] and literature review on cowpea attributes from West African countries (Nigeria, Ghana and Senegal) [24-29]. The questionnaire also included questions concerning background information of the respondents and their schoolchildren.

The items in the 12 constructs were verified in a focus group discussion and pre-tested in a site similar to the study site. Where applicable, constructs were modified to suite local knowledge and practice. The questionnaire was translated into the local language (Dagbani) and administered by well-trained research assistants who were familiar with the research area and spoke the local language.

Scale measurements and analysis

Individual items, phrased as statements, of each construct (except Intention and Behaviour) were rated on 5-point Likert [30] response options: strongly disagree, disagree, neutral (neither disagree nor agree), agree and strongly agree; recoded as unipolar (+1 to +5) or bipolar (–2 to +2) depending on the nature of the question. The score for each construct was computed as the sum of individual item scores. The scores for the constructs *"Attitudes towards behaviour"* and *"Subjective norms"* were sums of products of paired items; *attitudes* × *evaluation of attitudes*, and *normative beliefs* × *motivation to comply*, respectively. To show negative, neutral or positive influences, item scores of *attitudes* and *normative beliefs* ranged from –2 to 2 and the scores of the *evaluation of attitudes* and *motivation to comply* ranged from +1 to +5. This resulted in a paired-item score range of –10 to 10. For intention and behaviour, scores were based on the number of times caregivers intended to or had given cowpeas to their schoolchildren in the succeeding or preceding month respectively. Intention was considered high if it was greater than the median intention score of the group (10 times per month) and low if it was equal to or lower than the median score.

Cowpea chemical analysis

Iron and zinc concentration of cowpeas were measured using inductively coupled plasma atomic emission spectrophotometer (ICP-AES, Varian Vista-Pro, Palo Alto, CA, USA) after microwave digestion with a mixture of hydrofluoric and nitric acids (HNO_3-HF-H_2O_2). Analytical variation was ~6% for both iron and zinc. Phytic acid determination was done using a modified Makower method [31] in which the inorganic phosphate liberated from the phytic acid degradation is measured according to the van Veldhoven's method [32] and expressed as inositol hexaphosphate (IP6). A modified Folin-Ciocalteau method [33] was used to measure total polyphenol concentration of the cowpea seeds.

Calculation of phytate-to-mineral molar ratios

The respective phytate-to-iron and phytate-to-zinc molar ratios for each landrace were calculated as: phytate content of cowpea (mg) $\cdot$ 660^{-1}/ iron content of cowpea (mg) $\cdot$ 56^{-1}; phytate content of cowpea (mg) $\cdot$ 660^{-1}/ zinc content of cowpea (mg) $\cdot$ 65.4^{-1} respectively, where 660, 56 and 65.4 are the molecular weights of phytate, iron and zinc respectively [34, 35].

Statistical analysis

Data processing and analysis was done in SPSS software (version 18.0, Armonk, NY, USA). Descriptive statistics were used to examine background characteristics of study participants, constructs and cowpea landraces. Student's t-test for independent samples was used to compare the difference in chemical composition between white and coloured landraces of cowpeas. Cronbach's alpha was computed as measure of reliability for each construct. A construct was reliable if Cronbach's alpha was >0.7 [36]. The corrected item-total correlation of all items in a construct was set at 0.30 [36]. When the item-total correlation was lower than 0.30 the item was deleted from the construct. As such, a total of 7 items were deleted from two constructs; one item from *cues to action* and 6 items from *attitudes towards behaviour*.

Spearman correlations were computed to determine association between related constructs. For constructs that influenced intention, the Mann-Whitney-U test was used to compare whether subjects with a high intention to consume cowpea scored significantly different on any of the constructs from subjects with a low intention. The Wilcoxon signed rank test was used to test the differences in the scores of behaviour and intention.

Four multiple linear regression models were used to determine the relative importance of the predicting constructs for the following outcomes: health behaviour identity, intention and behaviour. All models were controlled for background characteristics of caregivers. Significance value for all tests was set at 0.05 (2-tailed).

Model 1: Health behaviour identity = *f* (Knowledge, Susceptibility, Severity and Value)

Model 2: Intention = *f* (Barrier, Health behaviour identity, and Attitudes towards behaviour)

Model 3: Intention = *f* (Subjective norms, Control beliefs, and Cues to action)

Model 4: Behaviour = *f* (Barrier, Intention)

RESULTS

Iron, Zinc, Phytate and Polyphenol concentrations of cowpea landraces

A total of 14 landraces were identified as common landraces from the two communities and the central market. Iron and zinc concentrations ranged from 4.9–8.2 mg/100 g d.w and 2.7–4.1 mg/100 g d.w respectively. Phytate and polyphenol concentrations ranged from 477–1110 mg/100 g d.w and 327–1055 mg/100 g d.w respectively (**Table 2**). With respect to colour of the cowpeas, there was no significant difference ($P > 0.05$) in iron, zinc and phytate concentrations between the white and coloured landraces. Molar ratios of phytate-to-iron also did not differ between white and coloured landraces ($P > 0.05$). Coloured landraces however had significantly higher concentrations of polyphenols and significantly larger ($P < 0.05$) phytate-to-zinc molar ratios (**Table 3**).

Background characteristics

More than 50% of the children in school were male. Ages of the school children ranged from 6 – 12 years with about one-third in the age groups 8 – 9 years and 10 – 11 years. Majority (61%) of households indicated that they had more than 10 people in their households. More than 50% of the caregivers of the school children were older than 35 years, 61% of them were mothers and 62% were in polygamous marriage. Only 4% of the caregivers were literate and more than 70% of them were either engaged in farming or trading as their main economic activity (**Table 4**).

Table 2. Iron, zinc, phytate and polyphenol composition of landraces of cowpeas locally available in northern Ghana

Local name	**Colour**	**Iron**	**Zinc**	**Phytate**[1]	**Polyphenols**[2]
		mg/100g dry weight			
Dagban tuya	white	6.8	3.4	477	445
Apagaba ala1	white	8.2	3.9	519	481
Komtuya	white	5.8	4.1	679	335
Black eye	white	6.2	3.5	745	385
Tuchicherigu	black & white	5.5	3.0	888	327
Apagaba ala2	white	5.7	2.7	487	368
Gampabgi 1	brown	6.3	3.7	1110	744
Sanzi sabinli	brown	7.0	3.3	664	621
Gampabgi 2	black	6.2	3.5	561	662
Milo	brown	6.5	3.1	610	NA
Yaminu	red	5.4	2.7	537	1055
Sanzi zie	red	7.7	4.1	895	942
Brown eye	white	5.8	3.7	605	NA
Marfu tuya	white	4.9	3.6	NA[3]	NA

[1] Inositol hexaphosphate (IP6). [2] Gallic acid equivalent (GAE). [3] Not analysed due to insufficient sample

Table 3. Comparisons of iron, zinc, phytate, polyphenol and phytate-to-mineral composition of white and coloured landraces of cowpeas locally available in northern Ghana

Parameter	**Colour of cowpea**		***P***
	White	**Coloured**	
Iron	6.2 ± 1.1 (7)[1]	6.4 ± 0.8 (7)	0.738
Zinc	3.6 ± 0.4 (7)	3.3 ± 0.5 (7)	0.398
Phytate (PA)	570 ± 108 (7)	752 ± 215 (7)	0.077
Polyphenols	403 ± 59 (5)	725 ± 257 (6)	0.023
PA : Iron[2]	8.0 ± 2.1 (6)	10.1 ± 3.0 (7)	0.171
PA : Zinc[2]	16.5 ± 2.9 (6)	22.3 ± 5.4 (7)	0.037

[1] Values are mean ± SD (number of landraces). [2] Molar ratio

Knowledge, attitude and perceptions of caregivers about cowpeas and giving cowpeas to schoolchildren

Ninety-two percent (92%) of caregivers had the intention to give cowpeas to their schoolchildren at least once per week within the referent month while 82% indicated that they had given cowpeas to their schoolchildren at least once per week within the referent month. Almost all the caregivers agreed that cowpeas contain iron (94%), can prevent iron-deficiency (97%) and support growth (97%) of their schoolchildren. They think of cowpeas as a food that is nutritious (98%), traditional (97%) and tasty (99%), and adds variety (97%) to the diet of their schoolchildren. More than half (57%) of the caregivers however think that cowpeas are not easily digested by their schoolchildren and leaves them feeling uneasy. Nevertheless 97% of them said their schoolchildren like to eat cowpeas. Generally the caregivers agreed that availability on the market (73%), prices (85%), time required to cook cowpeas (71%), weevils (70%), high prices (80%) and preservation (81%) were barriers to giving cowpeas to their school children. In line with their health-related opinions about cowpeas, 70% of the caregivers indicated that "illness" serves as a cue for them to give cowpeas to their schoolchildren.

Reliability of constructs and their correlations

Reliability (Cronbach's α) of the multiple item constructs ranged from (0.67 – 0.88). Except for the construct *susceptibility*, the reliability of all other constructs was ≥ 0.80. Two of the four constructs classified as "background and perception" were significantly correlated with *health behaviour identity*; *knowledge* (r_s = 0.23, P = 0.013) and *health value* (r_s = 0.49, P < 0.001). Within the "belief and attitude" group of constructs, *attitude towards behaviour* (r_s = 0.45, P < 0.001) and *perceived barriers* (r_s = 0.26, P = 0.004) showed significant correlations with *health behaviour identity*. *Attitude towards behaviour correlated* (r_s = 0.22, P = 0.019) significantly with *intention*.

Table 4. Background characteristics of schoolchildren and their caregivers in northern Ghana

Characteristic	***n* (Percentage)**
n	120
Sex of child, male	69 (57.5)
Age of child, years	
6 – 7	34 (28.3)
8 – 9	42 (35.0)
10 – 11	41 (34.2)
≥ 12	3 (2.5)
Household size	
3 – 6	11 (9.2)
7 – 10	36 (30.0)
>10	73 (60.8)
Age of caregiver, years	
19 – 34	54 (45.0)
35 – 49	36 (30.0)
>49	30 (25.0)
Relationship of caregiver to child	
Mother	73 (60.8)
Stepmother	10 (8.3)
Grandmother	22 (18.3)
Other relation	15 (11.7)
Marital status of caregiver	
Married (monogamous)	32 (26.7)
Married (polygamous)	74 (61.7)
Widowed/divorced	14 (11.6)
Education of caregiver	
% illiterate	115 (95.8)
Occupation of caregiver	
Farming	38 (31.7)
Trading	53 (44.2)
Housewife	25 (20.8)
Other	4 (3.4)

None of the three constructs classified as "external factors" significantly correlated with caregivers' intention to give cowpeas to schoolchildren. Intention to give cowpeas to schoolchildren was positively and strongly correlated (r_s = 0.72, P < 0.001) with the behaviour of giving cowpeas to schoolchildren (**Table 5**).

Since *attitudes towards behaviour* correlated significantly with *intention*, we checked and found that scores were higher for the high intenders but did not significantly differ the scores of low intenders ($z = -0.64$, $P = 0.52$). All median scores of the items within *attitudes towards behaviour* were positive for both the low and high intenders. Paired comparisons between *intention* and *behaviour* showed that intention to give cowpeas was significantly higher than the behaviour of giving cowpeas ($z = -3.177$, $P = 0.001$). Of the 120 caregivers interviewed the intention-behaviour paired observations were: intention > behaviour, $n = 62$; intention < behaviour, $n = 24$; intention = behaviour, $n = 34$.

Predicting health behaviour Identity, intention and behaviour

The relative contribution of the predictor variables to the outcome variables for models 1 – 4 are shown in **Table 6**. Model 1 explained 36% of the variance in *health behaviour identity* and the constructs *knowledge* ($\beta = 0.20$, $P = 0.030$) and *health values* ($\beta = 0.49$, $P < 0.001$) significantly predicted health behaviour identity. Model 2 explained only 8% of the variance in *intention* and the constructs *barriers* ($\beta = -0.42$, $P = 0.001$) and *attitudes towards behaviour* ($\beta = 0.25$, $P < 0.028$) significantly predicted *intention*. In model 3, none of the external factors significantly explained intention. Model 4 accounted for 40% of the variance in behaviour and intention significantly predicted behaviour ($\beta = 0.63$, $P < 0.001$).

Table 5. Sample item statements, number of items, reliability and summary values of the constructs from the combined model of the Theory of Planned Behaviour and the Health Belief Model

Construct	**Example of item statement**	**Number of items**	**Cronbach's α**	**Median score (IQR)**	**Range of values**
Knowledge	Cowpeas are a blood giving food	11	0.84	45 (44, 49)	11 – 55
Susceptibility	My schoolchild easily becomes sick	4	0.67	12 (10, 16)	4 – 20
Severity	Shortage of blood causes poor growth of my schoolchild	7	0.87	28 (24.3, 29)	7 – 35
Health value	The health of my schoolchild is very important to me	7	0.80	31 (29, 33)	7 – 35
Health behaviour identity	Giving cowpeas is one of the best things I can do for my schoolchild	2	0.80	8 (8, 9)	2 – 10
Barriers	I worry about the availability of cowpeas on the market	16	0.85	38.5 (32.3, 45.8)	16 – 80
Control beliefs	I am the one who decides to give my school child cowpeas	1	–	4 (4, 4.8)	1 – 5
Cues to action	Important ceremonies like weddings or funerals make my schoolchild want to eat	5	0.81	16 (14, 22)	5 – 25
Attitudes towards behaviour	(Cowpeas have a good taste) × (my schoolchild prefers foods that taste good)	16	0.88	38 (32, 47)	-160 – 160
Subjective norms	(My mother-in-law advices me to give cowpeas to my schoolchild) × (the opinion of my mother-in-law is important to me)	14	0.83	-4.5 (-20, 15.5)	-140 – 140
Behavioural intention	How many times do you intend to give cowpeas to your schoolchild in the coming month	1	–	10 (5, 15)	0 – 30
Behaviour	How many times have you given cowpeas to your schoolchild last month	1	–	8 (4, 12)	0 – 30

Table 6. Constructs predicting health behaviour identity associated with cowpeas, intention to give cowpeas and giving cowpeas to schoolchildren in northern Ghana[1]

Model description	Standardized β	P	R^2	Adjusted R^2
Model 1				
Y = Identity			0.43	0.36
Predictors				
Knowledge	0.20	0.030		
Susceptibility	0.02	0.847		
Severity	-0.04	0.652		
Values	0.49	<0.001		
Model 2				
Y = Intention to consume cowpeas			0.17	0.08
Predictors				
Identity	0.06	0.611		
Barriers	-0.42	0.001		
Attitudes	0.25	0.028		
Model 3				
Y = Intention to consume cowpeas			0.07	-0.03
Predictors				
Control	-0.09	0.334		
Cues	-0.001	0.994		
Subjective norms	-0.05	0.637		
Model 4				
Y = Consumption of cowpeas			0.46	0.40
Predictors				
Intention	0.63	<0.001		
Barriers	0.07	0.469		

[1] All models were controlled for community, interviewer, caregiver and child characteristics

DISCUSSION

Cowpea landraces

Our first objective was to identify the cowpea landrace (s) that would be most suitable to promote as source of bioavailable iron and zinc. We found that the locally available landraces contained appreciable amount of iron and zinc but also contained high concentrations of phytate and polyphenols.

The range of values we observed for iron (4.9 – 8.2 mg/100 g d.w) and zinc (2.7 – 4.1 mg/100 g d.w) were somewhat lower than the 5.6 – 10.4 mg/100 g d.w and 3.7 – 5.4 mg/100 g d.w respectively observed among cowpea landraces in Benin [37]. Based on iron and zinc concentrations the results suggest that *zanzi zee* could be promoted as the most suitable landrace with the potential to improve iron intake. However, the phytate and polyphenol concentrations of the landraces were high but within range of values reported by Madode et al. [37]. Iron and zinc absorption are partly influenced by phytate and polyphenol concentration [35, 38].

A proxy measure of iron and zinc bioavailabilty is the molar ratio of iron-to-phytate and zinc-to-phytate respectively [34, 35, 39, 40]. Iron-to-phytate and zinc-to-phytate molar ratios of <1 and ≤15 respectively are considered predictive of iron [40, 41] and zinc [34, 35] bioavailabilty. As such, all the landraces have low bioavailable iron. For zinc however, *apagaba ala-1* and *dagban tuya* are likely to contain zinc with higher bioavalability. Abizari et al [42] found that iron bioavailability in cowpeas was <2% and their results suggested that rather than polyphenols, phytate-to-iron molar ratio may predict the low bioavailability.

Cowpea acceptability

The second objective was to identify factors that influenced mothers to give cowpeas to their schoolchildren. We found that intention of mothers was strongly associated with and predicted behaviour. *Knowledge* of mothers about cowpeas and the *health values* they hold for their children were together associated with intention through *health behaviour identity* and *attitudes towards behaviour*. Knowledge and health values also predicted health behaviour identity. *Attitudes towards behaviour* and *perceived barriers* were the two internal constructs that predicted intention significantly albeit the predictive ability of the intention models was weak.

Other studies using the combined models of TPB and HBM [17, 23, 43] or the TPB alone [44, 45] have also shown strong association between intention and behaviour. The studies that measured behaviour and intention (cross-sectional or prospective) have also shown that intention is predictive of behaviour and may sometimes do so through an interaction with perceived barriers [23]. This seems to suggest that giving cowpeas to schoolchildren in northern Ghana may be largely driven by conscious efforts of mothers. It has however been shown that the proximity between the measurement of intention and behaviour can influence their association [46]; measured together (as in our case) can strengthen the association or, when behaviour is assessed after 5 weeks of measuring intention, weaken the association [47, 48]. However, in a related study in the same communities, frequency of consumption of cowpea-based meals at the household level was on average 2 – 3 times per week (Abizari, unpublished results), similar to values recorded here as intention and behaviour. As such, proximity may have had minimal influence on our measurements of intention and behaviour.

We did not observe a significant role of external factors (subjective norms, control beliefs and cues to action) on caregivers' intention to give cowpeas to their schoolchild [23, 49]. This partly demonstrates that cowpeas are well accepted in the research area [13] and their consumption is not influenced by the opinion of health workers, husbands, mothers in-law and significant others. In line with the observations of Sheeran et al. [50], the absence of external influential factors would suggest that intention to give cowpeas is more likely to be stable if mothers have favourable attitudes. We doubt however whether if we had much younger reference children (<2 years) the outcome would have been the same. From our experience in the area, health workers encourage mothers of such children to give cowpeas to their children (especially when they are undernourished) ostensibly to improve iron status and growth. This may partly explain why we observed in this study that "child's illness" is an important cue to give schoolchildren cowpeas. In our predictive model for behaviour we also observed age of child was a significant explanatory factor. It means that if iron-deficiency is presented in the context of illness, mothers would be more likely to accept a cowpea-based food promoted to contribute to reduce iron-deficiency.

Similar to the role of intention in the TPB model [15], the *health behaviour identity* was expected to mediate between “background and perception constructs” and *intention* in the combined model used in our study [17]. Our results confirm this mediating role of health behaviour identity [17, 23, 43]. In our work it means that the *knowledge* of mothers about cowpeas in combination with the *health values* they hold for their children made them have positive *health behaviour identity*. The positive *health behaviour identity* in turn yielded positive attitudes towards giving cowpeas to schoolchildren which subsequently predicted the intention of mothers to give cowpeas. It implies that if we reinforce mothers’ knowledge that “cowpeas give blood” and “support the growth of schoolchildren” coupled with the positive health values mothers hold for their children, it should be possible to promote cowpeas as likely vehicles to contribute to reduce iron-deficiency. Such a promotion may not be completely successful without addressing the barriers to behavioural intention. For instance, price of cowpea on the market was one of the barriers mentioned by caregivers. In their work Mishili et al. [51] reported that cowpea prices on the market start rising a few months after harvest. The implication is that rural households who have run out of cowpea stock may find it expensive to buy. Our results support the findings of Ndubuaku et al. [27] that abdominal discomforts, presence of weevils and long cooking time are barriers to cowpea consumption.

Internal reliability measures of our constructs were generally good and were within range of values observed by others [23, 43]. However, there is no prior indication of the reliability of the predictive models with regards to giving or intention to give cowpeas. Two other studies [23, 43] in Africa that utilized these predictive models have shown similar trend in low predictive abilities of the two models on intention. In our case the low predictive ability could be attributed to the generic reference to cowpeas rather than a specific cowpea-based food. In a study on iron-fortified soy sauce the predictive ability of the intention model was higher (15).

In summary what we have shown is that cowpea landraces from northern Ghana contain appreciable amounts of iron and zinc, but probably with a poor bioavailability. Attitudes towards giving cowpeas and perception of barriers are important predictors of mothers' intention to give cowpeas to their schoolchildren. We have also shown that health behaviour identity may mediate but not predict intention of mothers. Finally our results suggest that knowledge about cowpeas and health values mothers hold for their children are key areas to focus attention in order to promote giving cowpeas to school children.

ACKNOWLEDGEMENTS

We are grateful to the following teachers for their cooperation during the survey: I. Norgah (Tibung primary school) and S. Inusah (Kpalgung primary school). We thank all mothers and their schoolchildren who participated in the survey. We also thank all the research assistants for their contribution during data collection. This research was supported by the Interdisciplinary Research and Education Fund (INREF) of Wageningen University through the TELFUN (Tailoring food sciences to Endogenous patterns of Local food supply for Future Nutrition) Project.

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Chapter 4

Phytic acid-to-iron molar ratio rather than polyphenol concentration determines iron bioavailability in whole cowpea meal among young women

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Journal of Nutrition 2012; 142, 1950-1955

ABSTRACT

Limited data exists on iron absorption from NaFeEDTA and $FeSO_4$ in legume-based flours. The current study compared iron absorption from NaFeEDTA and $FeSO_4$ as fortificants within and between red and white varieties of cowpea with different concentrations of polyphenols (PP), but similar phytic acid (PA) to iron molar ratios. We performed a paired crossover study in young women (n = 16). Red (high PP) and white (low PP) cowpea test meals (*Tubani*) were each fortified with (^{57}Fe) labelled NaFeEDTA or (^{58}Fe) labelled $FeSO_4$ and were randomly administered. Iron absorption was measured as erythrocyte incorporation of stable iron isotopes. Per serving, the mean (± SD) PP concentration of the white and red cowpea-based meals was 74 ± 3.6 and 158 ± 1.8 mg, respectively, and the molar ratio of PA to iron was 3.0 and 3.3. Iron bioavailabilities from red and white cowpeas were 1.4% and 1.7%, respectively, in NaFeEDTA fortified meals and 0.89% and 1.2%, respectively, in $FeSO_4$ fortified meals. Compared with $FeSO_4$, fortification with NaFeEDTA increased the amount of iron absorbed from either of the cowpea meals by 0.05 mg to 0.08 mg ($P < 0.05$). Irrespective of the fortificant used, there was no significant difference in the amount of iron absorbed from the two varieties of cowpea. The results suggest that NaFeEDTA is more bioavailable in legume-based flours compared to $FeSO_4$. In cowpea-based flours, the major determinant of low iron absorption may be the high molar ratio of PA to iron, and not variations in PP concentration.

INTRODUCTION

Iron deficiency is one of the most prevalent single nutrient deficiencies in the world [1] and is most prevalent among populations who consume monotonous diets of cereals and legumes [2, 3]. Next to cereals, legumes contribute substantially to nutrient intake among poor populations [4] and thus may have broad potential as vehicles for improving iron status.

Cowpeas (*Vigna unguiculata* (L.) Walp), like many food legumes, are an important source of good quality plant protein [5], particularly in West and Central Africa, and contribute to household food security [6]. Cowpeas also have a higher non-haem iron concentration compared to commonly consumed cereals such as rice and maize. However, human absorption studies in other legumes, especially the common beans (*Phaseolus vulgaris*), have shown low fractional iron absorption [7, 8], suggesting that varieties of common beans with higher concentration of iron may not lead to a higher absolute amount of iron absorbed. Thus, the usefulness of biofortification (targeted breeding for higher concentration of intrinsic iron) as a strategy to improve iron status has been questioned for some of the currently available breeds of common beans [8, 9].

The potential usefulness of fortification of legume flours, such as cowpea flour, has been questioned because in many cultures cowpeas are eaten whole without milling [9]. However, there are many recipes based on legume flours in Africa, including cowpea-cereal complementary blends. Fortification has been widely used to improve the iron concentration of cereal flours but the main difficulty is the inhibition of iron absorption by high phytic acid (PA) concentration in cereals [10]. At the weakly acidic to neutral pH in the duodenum, PA has a strong tendency to chelate iron to form insoluble complexes [11]. Sodium iron ethylene diamine tetraacetate (NaFeEDTA), as a fortification compound, has shown higher iron bioavailability in presence of PA than electrolytic iron, ferrous fumarate and ferrous sulphate [12-14] and is thus recommended as suitable fortificant for PA rich flours. However, cowpeas also contain polyphenols (PP), and some PP inhibit iron absorption [15]. Unlike PA rich flours, little is known about the efficacy of fortification of legume flours like cowpea (which is rich in both PA and PP) with NaFeEDTA. The aim of the current study was therefore to compare iron absorption from NaFeEDTA and $FeSO_4$ as iron fortification compounds within and between two varieties of cowpea with different concentrations of PP but similar PA to iron molar ratios.

METHODS

Participants

The study was carried out at the Division of Human Nutrition, Wageningen University, The Netherlands, among sixteen apparently healthy young women. Participants were recruited from the student population of Wageningen University through advertisements. The inclusion criteria were: age 18-40 y; body weight < 65 kg; non-pregnant, non-lactating and not planning to become pregnant; free of chronic illnesses and not taking chronic medication except oral contraceptives; not taking supplemental iron; no intake of vitamin and mineral supplements in the last two 2 wk prior to the study, or willingness to discontinue; no blood donation in the last 6 mo preceding the study, and no participation in studies that administered enriched stable iron isotope labels. Eligible participants were invited for information sessions where the study, requirements for participation, and risks were explained after which an informed consent was obtained. A sample size of sixteen participants was estimated to be adequate to detect an intra-individual variation in log (iron absorption) of 0.3 [16] and a 35% increase in iron bioavailability with a power of 0.8 and a significance level of 0.05. Ethical approval was obtained from the Medical Research Ethics Committee of Wageningen University, the Netherlands, and the Internal Review Board of Noguchi Memorial Institute for Medical Research, Ghana.

Study design

A paired crossover study design was used to allow within-participants comparisons. Cowpea based test meals (*Tubani*) labelled with ^{57}Fe or ^{58}Fe were administered over two pairs of consecutive days. Therefore each participant consumed a total of four test meals (**Table 1**) in a randomized fashion. On d 0, participants were invited to the study location, a baseline blood sample (7 mL) was collected, and weight and height were measured following standard procedures [(17)]. On d 1 and d 2, the test meals were administered. Fourteen days later, on d 15, a second blood sample was collected after which a third (d 16) and fourth (d 17) test meals were administered. Fourteen days later, on d 30 of the investigation, a final blood sample was collected.

Table 1. Description of cowpea test meals consumed by participants[1]

Test meal	Description
WC-EDTA	White cowpea meal (low polyphenol) + 4 mg ^{57}Fe as NaFeEDTA
WC-FS	White cowpea meal (low polyphenol) + 4 mg ^{58}Fe as $FeSO_4$
RC-EDTA	Red cowpea meal (high polyphenol) + 4 mg ^{57}Fe as NaFeEDTA
RC-FS	Red cowpea meal (high polyphenol) + 4 mg ^{58}Fe as $FeSO_4$

[1] RC-EDTA, red cowpea plus NaFeEDTA; RC-FS, red cowpea plus ferrous sulphate; WCEDTA, white cowpea plus NaFeEDTA; WC-FS, white cowpea plus ferrous sulphate

All meal ingredients were purchased and cooked in bulk and used for the entire study. The test meal portions were kept frozen until use. Participants consumed the four test meals as breakfast after an overnight fast each time and were not allowed to eat or drink until 3 h post feeding. All test meals were served between 0700 and 0900 h under close supervision of investigators. Each portion was defrosted for 1.5 min in a microwave oven on the day of feeding. The isotopically labelled iron compounds were added quantitatively to the test meals ~ 5 min prior to consumption. Isotope labels were carefully spread on the surface of test meals. After consuming the entire portion of the test meal, the glass bowl used to serve the test meal was rinsed with Milli-Q water (Millipore SAS, Molsheim, France) for the participants to drink. Test meals were served with a maximum of 300 mL of Milli-Q water (Millipore SAS, Molsheim, France).

Test meals

The test meal served to all participants was *Tubani*: a local Ghanaian dish made from cowpea (*Vigna unguiculata* (L.) Walp) flour. There were two different *Tubani* test meals: one *Tubani* test meal was made from cowpea known to have high concentration of polyphenols (red variety, “Sanzi Zei”) and the other made from cowpea known to have low concentration of polyphenols (white variety, “Kom tuya”). The white variety was chosen because it is widely cultivated and most preferred for consumption, and the red variety was chosen because of its higher concentration of PP. Each portion (150 g) of test meal contained 66 g of whole cowpea flour, 13 mL of 5% (w/v) food grade sodium bicarbonate solution (serves as softener and rising agent) mixed into a paste in 71 mL of Milli-Q water (Millipore SAS, Molsheim, France). The resulting paste was wrapped in aluminium foil and steamed at 100^{o}C for 45 min. Each *Tubani* was served with a standard amount (31 ± 1 g) of sauce made up of groundnut oil, salt, fried onions, chili and “false sesame seeds” (*Ceratotheca sesamoides*). In each type of test meal 4 mg of ^{57}Fe or ^{58}Fe was added as either NaFeEDTA or $FeSO_4$, respectively.

Test meal analysis

Iron concentration of cowpea seeds, cowpea flour and cowpea meal were measured using inductively coupled plasma atomic emission spectrophotometer (ICP-AES, Varian Vista-Pro, Palo Alto, CA, USA) after digestion with HNO_3-HF-H_2O_2. Phytic acid determination was done using a modified Makower method [18] in which the released inorganic phosphate is measured according to the van Veldhoven’s method [19] and expressed as inositol hexaphosphate (IP6). A modified Folin-Ciocalteau method [20] was used to measure total polyphenol concentration of the cowpea meal and expressed as gallic acid equivalent (GAE).

Preparation of stable isotope labels

Isotopically labelled $^{58}FeSO_4$ was prepared from isotopically enriched elemental iron (^{58}Fe, enrichment 99.6%, Chemgas Boulogne, France) by dissolution in diluted sulphuric acid. The solutions were stored in Teflon® containers and flushed with nitrogen to keep the Fe in the +II oxidation state. Isotopically labelled $Na^{57}FeEDTA$ (^{57}Fe enrichment 97.6%, Chemgas Boulogne, France) was prepared according to the method described by Loots et al. [(21)].

Blood analysis and iron isotope measurements

Haemoglobin concentration in whole blood was measured on the day of blood collection using a Beckman-Coulter LH750 HmX haematology analyzer (Beckman Coulter, Miami, FL, USA). Serum ferritin (SF), soluble transferrin receptors (sTfR), C-reactive protein (CRP) and α_1-acid glycoprotein (AGP) were measured simultaneously using an in-house sandwich ELISA technique [(22)]. All measurements were done in duplicates and if CVs were $\geq$10% measurements were repeated. The CVs (inter-assay) for the various indicators were: SF, 2.6%; sTfR, 2.4%; CRP, 7.2% and AGP, 2.9%. Certified quality control samples from the CDC/Atlanta and Bio-Rad Liquicheck (Bio-Rad, Munich, Germany) were used.

Whole blood samples were mineralized and separated as described by Schoenberg and von Blanckenburg [(23)]. Iron isotopic analyses were performed employing a high-resolution, multicollector-inductively coupled plasma-mass spectrometer (Thermo-Finnigan Neptune, University of Bonn, Germany; see [(24)] for details). Copper was added (1 µg/g) to the solution immediately prior to analysis to correct for mass bias [(23, 25)]. Each isotopically enriched solution was measured in triplicate using standard sample bracketing [(23, 25, 26)]. One third of the samples were re-measured as external duplicates for quality control. Analysis was done under chemical blank monitoring using ^{57}Fe or ^{58}Fe indicator solutions as an external quality control.

Calculation of iron absorption

Iron absorption measurement was based on erythrocyte incorporation of iron stable isotope labels 14 d after intake of the labelled test meals [(27)]. Circulating iron was calculated on the basis of blood volume, which was estimated from the participant's height and weight [(28)]. Calculation of iron absorption was based on the shift in the isotopic ratios after a 14-d incorporation period as previously described [(27)] taking in to account that isotopic labels are not monoisotopic [(29)]. An incorporation rate of 80% of the absorbed iron into red blood cells was assumed.

Statistical analysis

Data were analyzed in Excel (Microsoft Office 2007, Microsoft, Seattle, USA) and GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego, CA, USA) and SPSS (version 18.0, Armonk, NY, USA). Variables were checked for normality and if not normally distributed were log transformed before use in analysis. Student's *t* test was used to compare iron, PA and PP concentration of the red and white cowpea meals. A general linear model (Two-Way ANOVA) for repeated measures was used to evaluate the main effects of variety and fortificant as well as their interaction (V x F). Correlations between ln(SF) and ln(% iron absorption) were checked using Pearson correlation. The level of statistical significance was set at $P < 0.05$ for all analysis. Summary values of iron status and iron absorption are reported as geometric means. Fractional iron absorption data for participants was standardized to an SF value of 15 µg/L with the method proposed by Cook et al.[(30)].

RESULTS

Participants

None of the participants dropped out of the study or missed any of the test meals. The mean body mass index of participants was within the normal range, respectively. One participant was iron deficient based on serum ferritin concentration <15 µg/L. None of the participants was anaemic or had evidence of inflammation as indicated by elevated CRP or AGP (**Table 2**). The ^{56}Fe /^{54}Fe isotope ratio in the samples obtained during the study was compared to the isotopic reference material (IRM) and was in the range of the values previously reported for healthy young women [(31, 32)].

Table 2. Anthropometric, iron and inflammation status of participants at baseline[1]

Variable	Summary value
Age, *y*	23.3 ± 3.6
BMI, *kg/m²*	21.0 ± 2.6
Haemoglobin, *g/L*	134 ± 7
Serum Ferritin, *µg/L*	30.0 (22.7, 42.5)
Serum Transferrin Receptor, *mg/L*	4.5 (4.0, 5.1)
Body Iron[2], *mg/kg body weight*	5.3 ± 1.8
Serum C-reactive Protein, *mg/L*	0.41 (0.21, 1.15)
Serum α_1-acid glycoprotein, *g/L*	0.62 (0.48, 0.72)

[1] Values are mean ± SD, geometric mean (IQR), *n*=16. [2] To convert body iron from mg/kg to mmol/kg multiply by 0.0171 [(33)]

Test meals

Test meals made from red cowpea had significantly higher ($P < 0.05$) concentrations of native iron, PA and PP, than test meals made from white variety. However both varieties of cowpea had similar molar ratios of phytic acid to iron (**Table 3**). Each participant consumed the entire test meal portion on each of the 4 d.

Iron absorption

Geometric mean iron absorption from the four test meals ranged from 0.89% - 1.7% but when absorption was adjusted to SF of 15 µg/L, the geometric mean iron absorption ranged from 1.8% - 3.4%. Irrespective of cowpea variety used for the test meals, fortification with NaFeEDTA resulted in significantly higher amount of iron absorbed than from $FeSO_4$ (*P* <0.05); in the white cowpea meal fortification with NaFeEDTA resulted in 0.05 mg more iron absorbed compared to fortification with $FeSO_4$ whereas in the red cowpea meal NaFeEDTA fortified meals resulted in 0.08 mg more iron absorbed compared to $FeSO_4$ fortified meals. Conversely, irrespective of the fortificant used, there was no significant difference in the amount of iron absorbed between the two varieties of cowpea. The interaction between variety and iron fortificant was not significant (**Table 4**). Iron status (serum ferritin concentration) was inversely and significantly correlated with fractional iron absorption from all test meals: WC-EDTA (r= -0.61, P<0.05); WC-FS (r= -0.79, P<0.01); RC-EDTA (r= -0.74, P<0.01); RC-FS (r= -0.67, P<0.01).

DISCUSSION

In a paired crossover design iron absorption from NaFeEDTA and $FeSO_4$ as iron fortification compounds within and between two varieties of cowpea with different concentrations of polyphenols were compared. The results show that iron absorption from whole cowpea meal fortified with NaFeEDTA was significantly higher than when fortified with $FeSO_4$. In addition, variety of cowpea used did not significantly influence iron absorption.

Low fractional iron absorption

Fractional iron absorption from whole cowpea meal was generally low (< 2%) regardless of type of fortificant or variety of cowpea. Previous iron absorption studies in humans have been mainly conducted with common beans (*Phaseolus vulgaris*) but are difficult to compare because of differences in iron status of participants in different studies. Donangelo et al. [8] observed low iron bioavailability of ~2% from two varieties of common beans despite low iron status of participants.

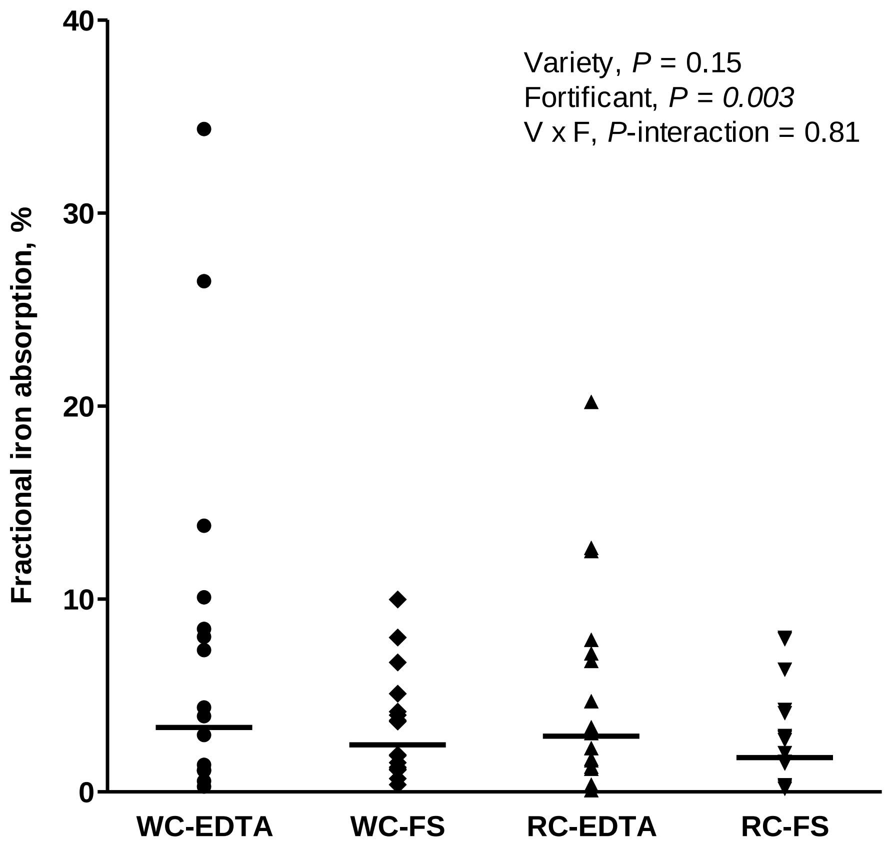

Figure 1. Fractional iron absorption of young women from four cowpea test meals – adjusted to SF of 15 µg/L. Data points for each symbol represent participants. Horizontal bars indicate geometric means for each column, $n = 16$. *P*-values are from Two-Way ANOVA for repeated measures. V x F, interaction between cowpea variety and type of iron fortificant used. Test meals: RC-EDTA, red cowpea plus NaFeEDTA; RC-FS, red cowpea plus ferrous sulphate; WC-EDTA, white cowpea plus NaFeEDTA; WC-FS, white cowpea plus ferrous sulphate.

Table 3. Iron, phytic acid and polyphenols concentration of cowpea test meals served to young women[1]

Test meal	Iron *mg/serving*		Phytic Acid *mg/serving*	Polyphenols *mg GAE/ serving*	Phytic Acid : Iron per serving	Extrinsic EDTA : Iron
	Native	Fortification				
WC- EDTA	5.3 ± 1.1*	4	330 ±15.9*	74 ± 3.6*	3.0 : 1	1 : 1
WC-FS	5.3 ± 1.1	4	330 ± 15.9	74 ± 3.6	3.0 : 1	-
RC- EDTA	8.8 ± 0.8	4	494 ± 21.5	158 ± 1.8	3.3 : 1	1 : 1
RC-FS	8.8 ± 0.8	4	494 ± 21.5	158 ± 1.8	3.3 : 1	-

[1] Values are means ± SD, absolute amount or molar ratios. *Different from red cowpea, $P < 0.05$. GAE, gallic acid equivalents; RC-EDTA, red cowpea plus NaFeEDTA; RC-FS, red cowpea plus ferrous sulphate; WC-EDTA, white cowpea plus NaFeEDTA; WC-FS, white cowpea plus ferrous sulphate.

Table 4. Fractional iron absorption and total iron absorbed per cowpea test meal served to young women[1]

	Test meals				Effect[2] (*P* value)		
	WC-EDTA (A)	WC-FS (B)	RC-EDTA (C)	RC-FS (D)	Variety	Fortificant	V x F
Fractional Iron absorption, *%*							
Observed	1.7 (0.41, 6.4)	1.2 (0.67, 3.1)	1.4 (0.59, 3.7)	0.89 (0.27, 1.9)	0.11	0.006	0.90
Adjusted[3]	3.4 (1.1, 9.7)	2.4 (1.2, 4.9)	2.9 (1.4, 7.7)	1.8 (0.64, 4.2)	0.15	0.003	0.81
Iron absorbed, *mg*	0.16 (0.05, 0.53)	0.11 (0.06, 0.19)	0.19 (0.08, 0.47)	0.11 (0.08, 0.22)	0.69	0.016	0.99
Absorption ratios							
EDTA : FS	A:B = 1.4	-	C:D = 1.6	-	-	-	-
WC : RC	-	-	C:A = 0.82	D:B = 0.74	-	-	-

[1] Values are geometric mean (IQR) unless otherwise stated, *n*=16. EDTA, NaFeEDTA; FS, ferrous sulphate; RC, red cowpea; RC-EDTA, red cowpea plus NaFeEDTA; RC-FS, red cowpea plus ferrous sulphate; V x F, interaction between cowpea variety and type of iron fortificant used; WC, white cowpea; WC-EDTA, white cowpea plus NaFeEDTA; WC-FS, white cowpea plus ferrous sulphate. [2] Two-Way ANOVA for repeated measures. [3] Adjusted to serum ferritin of 15 µg/L [(30)].

Among iron replete participants, Beiseigel et al. [7] and Petry et al. [34] observed < 2% and between 2.4% – 2.6% fractional iron absorption, respectively from common beans. Iron absorption is inversely related to iron status [35], an observation also made in this study. After correcting absorption for low iron stores, iron bioavailability was significantly higher and ranged from 1.8 – 3.4% ($P < 0.05$) (**Figure 1**), but lower than the data reported among Rwandan women with low iron status, where Petry et al. [9] observed fractional iron absorption ranging between 3.4 – 4.7% from two varieties of common beans.

In our specific case however, the fractional iron absorption could have been affected by the large amount of isotopic labels added to the test meals to reflect a hypothetical iron fortification level of 60 mg/kg cowpea flour. Studies have shown that increasing amounts of iron label decreases fractional absorption albeit larger absolute amounts of the label get absorbed [36]. Another possible explanation for the low iron absorption in our study could be the use of sodium bicarbonate in the test meal (*Tubani*) recipe. Sodium bicarbonate could buffer the stomach acidity and promote chelation of ferric iron [37] to PA or PP. When administered 1 – h after a meal, 6.17 g of sodium bicarbonate increased gastric pH of adult males from basal 1.8 to a range of 6.7 – 7.0 after 15 min of ingestion [38]. In our study 0.65 g (13 mL of 5% (w/v)) of sodium bicarbonate was ingested per meal. Therefore, it is likely that the concentration of bicarbonate per meal was insufficient to offset stomach acidity and significantly decrease iron absorption.

Difference in absorption between NaFeEDTA and $FeSO_4$

As expected, fractional iron absorption from *Tubani* fortified with NaFeEDTA was significantly higher than when fortified with $FeSO_4$. Despite both being water soluble fortificants [39], unlike $FeSO_4$, the EDTA moiety in NaFeEDTA chelates iron at low pH and to some extent protects it from binding with ligands such as PA in the stomach and subsequently releases iron for uptake in the duodenum and jejunum [(40)]. Absorption of native iron is also improved by NaFeEDTA [(40)] and this makes it a more suitable fortificant for inhibitory cereals and legumes than $FeSO_4$.

Absorption ratios: NaFeEDTA vs. $FeSO_4$

In our study the ratio of fractional iron absorption between NaFeEDTA and $FeSO_4$ (1.4 – 1.6) was somewhat lower than expected. A number of studies have demonstrated that in inhibitory meals, fractional iron absorption from NaFeEDTA could be 2 – 4 times higher than $FeSO_4$ [(40, 41)] but these comparisons have largely been done in PA-only rich meals, not in meals like cowpeas that are rich in both PA and PP. However, the effect of the extra PP in cowpeas on the absorption ratio between NaFeEDTA and $FeSO_4$ has not been previously reported. For $FeSO_4$, the absorption ratio we observed between high and low PP cowpeas was similar to that observed by Petry et al. [(9)] in common beans with high and low PP concentration.

No significant difference between low and high PP cowpea varieties

Fractional iron absorption from red (high PP) cowpea was not significantly different from white (low PP) cowpea ($P > 0.10$). Another investigation involving two varieties of common beans reported no significant difference in iron absorption from $FeSO_4$ between white and brown beans [(7)]. Petry et al. in a double meal design observed a significant 27% lower iron absorption in red (high PP) compared to white (low PP) common bean variety and attributed the difference to the additional inhibition of PP [(9)]. In our study, the difference in PP between the two cowpea varieties was 2-fold compared to the 7-fold in the study by Petry et al. Comparing PP concentration in foods is difficult due to the wide range of polyphenol measurement methods currently employed even when PP concentrations are expressed as Gallic Acid Equivalents. Nevertheless, PP (as measured by the Folin-Ciocalteu method) has been shown to be predictive of iron bioavailability in legumes [(34)] and phenolic containing beverages [(42)].

The lack of significant difference between varieties, despite significant difference in PP, could be due to the similar phytic acid to iron molar ratio (PA:Fe) in both cowpea varieties used in this study. In inhibitory foods, an important determinant of iron absorption is the PA:Fe of the food [(43)]. For non-composite meals, PA:Fe greater than 1:1 is described as inhibitory [(44)]. In our study both fortified white and red cowpea meals had similar PA:Fe of ~3:1, in contrast to ~5:1 without fortification iron. However it remains to be shown whether in the absence of PP white and red varieties of cowpea with similar PA:Fe will show similar iron absorption.

Our study involved a single non-composite meal and as such the design may have overemphasized the combined inhibitory effects of PA and PP on iron absorption [(45)]. However, in cowpea consuming areas of West Africa (e.g. Ghana), traditional cowpea recipes for both children and adults hardly include iron absorption enhancers, and the test meals consumed in this study are representative of typical cowpea-based meals [(46)]. As shown by Petry et al [(9)], the effect of PP was statistically significant when beans with high and low PP concentration were given alone, but when the same beans were consumed as part of a multiple meal design fed over several days with rice and potatoes the effect of PP was no longer significant.

In conclusion, the results of our study indicate that NaFeEDTA has better bioavailability than $FeSO_4$ in legume-based flours, such as cowpea, and is thus likely to be a better fortificant for cowpea flour irrespective of the colour of the cowpeas. At similarly high molar ratios of PA to iron, the higher concentration of polyphenols in red cowpeas did not significantly reduce iron absorption from either fortificant. However, the low bioavailability of iron fortificants in legume-based flours may limit their ability to improve iron status in deficient populations, and this should be tested in an efficacy trial in a target population. This data also suggests that phytic acid concentration may be the main inhibitor of iron absorption in cowpea based meals.

ACKNOWLEDGEMENTS

We thank L. Lemmens for her support during the preparation of test meals and feeding. We also thank C. Zeder, and C. Münker for their technical support. Supported by a research grant from Nestle Foundation for Nutrition Research, and the TELFUN project, Wageningen University.

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Chapter 5

Whole cowpea meal fortified with NaFeEDTA reduces iron-deficiency among Ghanaian school children in a malaria endemic area

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Journal of Nutrition 2012; 142, 1836-1842

ABSTRACT

Cowpeas, like other legumes, contain high amounts of native iron but are rich in phytic acid and polyphenols inhibiting iron absorption. NaFeEDTA may overcome the combined inhibitory effect of phytic acid and polyphenols. Our objective was to test the efficacy of NaFeEDTA fortified cowpea meal in improving iron status of school children in a malaria endemic area. We conducted a double-blind controlled trial with 5 – 12-y-old school children from two rural communities in northern Ghana (n = 241). Eligible children were randomly assigned to two treatment groups to receive either cowpea meal fortified with 10 mg Fe/meal as NaFeEDTA, or an identical but non-fortified cowpea meal. Meals were provided 3 d/wk for a period of about 7 mo under strict supervision. Mass deworming and malaria antigenemia screening and treatment were carried out at baseline and 3.5 mo into the trial. Consumption of cowpea flour fortified with NaFeEDTA resulted in significant improvement of Hb ($P < 0.05$), SF ($P < 0.001$) and body iron stores ($P < 0.001$) and reduction of TfR ($P < 0.001$) compared to non-fortified flour. Fortification resulted in 30% and 47% reduction in the prevalence of iron-deficiency (ID) and iron-deficiency anaemia (IDA) ($P < 0.05$), respectively. The results indicate that fortification of cowpea flour with NaFeEDTA overcomes the combined inhibitory effect of phytic acid and polyphenols and, when used for targeted school-based fortification of cowpea flour, is effective in reducing the prevalence of ID and IDA among school children in malaria endemic rural northern Ghana.

INTRODUCTION

Recent estimates indicate that about 40% of school-aged children from countries with a low human development index suffer from anaemia [(1)], half of which is likely to be due to iron-deficiency (ID) [(2)]. Consequences of iron-deficiency anaemia (IDA) among school-age children include delayed psychomotor development and impaired cognitive performance [(3)]. Low iron bioavailability rather than low dietary iron intake is considered an important contributing cause of IDA in sub-Saharan Africa and other developing regions [(4)] in addition to habitual consumption of inhibitory cereal and legume staples [(5)].

Iron fortification of foods is one of the strategies that may contribute to reducing ID and IDA prevalence. It is practical, sustainable and cost effective in the long term at the population level [(6, 7)], and considered safer than supplementation but it has shown little success in Africa [(8)]. Cereal flours (wheat and maize) are often the target vehicles for fortification [(7)], however fortified flours hardly reach rural African households. Therefore targeted interventions such as school feeding programs may be more successful in reaching vulnerable groups [(7)]. School feeding programs may not only improve nutritional status but also improve the educability of pupils [(9, 10)].

Legume-cereal blends are often promoted for use in school feeding programs e.g. corn-soy blend. In cowpea consuming West Africa, cowpea flour or blend may provide an alternative to corn-soy blend but this potential has not been explored. Cowpea (*Vigna ungiuculata* (L.) Walp) is a good source of energy and plant protein [(11)]. Relative to other cereals and grain legumes, cowpeas are a good source of non-haem iron which if made bioavailable can contribute significantly to dietary iron intake in a targeted intervention. Non-haem iron in cowpea, similar to that in common beans, may have low bioavailability because of the high native phytic acid (PA) and polyphenols (PP) concentration, both inhibitors of iron absorption

[12, 13]. Therefore an ideal fortification compound for cowpea would be one that can overcome the inhibition of PA and PP.

Sodium iron ethylene diamine tetraacetate (NaFeEDTA) does overcome the inhibitory effect of phytic acid and also improves the bioavailability of native iron in food [14]. When used to fortify foods rich in phytic acid, iron bioavailability from NaFeEDTA can be 2-3 times higher than ferrous sulphate [15]. The aim of this study therefore is to investigate whether NaFeEDTA could overcome the combined inhibitory effect of PA and PP and improve iron status of school children.

METHODS

Study design and randomization

This study was a randomized double-blind controlled trial. The primary outcome of the study was iron status of the participants as determined by the serum concentration of soluble transferrin receptor (sTfR). This choice was made because the study site is malaria endemic, and sTfR is a sensitive marker of iron status less affected by infection and inflammation compared serum ferritin (SF). Secondary endpoints include haemoglobin concentration, serum ferritin, and prevalence of iron-deficiency, iron-deficiency anaemia and anaemia. Eligible children were randomly assigned to two treatment groups using block randomization with block size of four. Randomization was performed by a member of the investigating team who was not present at screening and enrolment in Ghana.

Study area

This trial was carried out in two primary schools from two rural communities in Tolon-Kumbungu district of northern Ghana between October 2010 and May 2011. The two communities have similar socio-demographic characteristics and school infrastructure, and are ~50 km away from the main city in the region, Tamale, and ~10 km apart. The area has a typical tropical climate with two main seasons – a dry season (December – March) characterized by high temperatures and a rainy season (April – September). Malaria is hyperendemic in this area [(16)] and is the main cause of morbidity among children [(17)]. Malaria transmission peaks towards the end of the rainy season (October and November) [(18)]. People in this area are mostly subsistence farmers [(19)]. A Government-supported school feeding program, the Ghana School Feeding Program (GSFP), has been introduced in selected schools in the district [(20, 21)] but the two schools in this study were not beneficiaries of the program.

Study Population and sample size

Children in the two rural primary schools participated. Inclusion criteria were: 5 – 12-y-old, regularly attending school, apparently healthy and not taking medication or supplemental iron at time of enrolment and having haemoglobin concentration >70 g/L. Children who met the inclusion criteria were enrolled by the study doctor who reviewed their medical questionnaire. Two children with severe anaemia (haemoglobin concentration <70 g/L) were referred to the clinic for treatment. A sample size of 240 (120 per group) was estimated to be sufficient to detect a difference in serum transferrin receptor of 2 mg/L [(22)], assuming an SD of 5 mg/L (A.R. Abizari 2010, unpublished) at a 5% significance level with 80% power assuming an attrition rate of 20%.

Ethical approval for this trial was obtained from the Medical Research Ethics Committee of Wageningen University, the Netherlands and the Internal Review Board of Noguchi Memorial Institute for Medical Research, University of Ghana. Permission was obtained from the district administration, and chiefs and opinion leaders of respective communities. Thumb printed informed consent was obtained from a parent or caregiver. Our trial is registered at ClinicalTrials.gov, identifier number NCT01208363.

Treatment of participants

Meal served to children

The main meal served to children was *Tubani:* a local Ghanaian dish made from cowpea (*Vigna unguiculata* (L.) Walp) flour. The recipe of a portion was: 66 g whole cowpea flour, 10 mL of 5% (w/v) bicarbonate of soda solution (serves as softener and rising agent) mixed into a paste in ~ 75 mL of water. The resulting paste was wrapped in broad leaves (similar to *Maranta leuconeura*) and steamed for 35 min. The cooked weight of a single portion was ~150 g which was served with ~30 g of sauce made of 16 g groundnut oil, salt, fried onions, chili and 12 g of *Bungu* or "false sesame seeds" (*Ceratotheca sesamoides*). The total caloric content of the meal was ~ 430 kcal. A short run-in period of 3 d showed that the portion size was suitable and well accepted by the school children.

Preparation of fortified cowpea flour

Based on the data of a food consumption survey we conducted earlier in this area (results not shown here), we aimed at supplying between 40 – 70% of the RNI for children 5 – 12 y. Guided by an earlier acceptable daily intake (ADI) of 2.5[1] mg EDTA/kg body weight set by the Joint FAO/WHO Expert Committee on Food Additives [23] and 24 kg as the average body weight of targeted children (A.R. Abizari, unpublished data), we defined a fortification level of 10 mg Fe/66 g of cowpea flour in the form of NaFeEDTA. Cowpeas were purchased in bulk (2,300 kg) at the Tamale central market. A group of local women winnowed and sieved out sand, stones and debris, and picked out spoilt cowpea seeds. YEDENT AgroFood Processing Company, Ghana, milled and fortified the cowpea flour. To prevent spoilage, milling and fortification were done in two batches. Each batch of flour was used for ~ 3.5 mo.

[1] This value represents the ADI for calcium disodium EDTA set in 1974 by JECFA. Based on the 2007 JECFA recommendations the ADI used in calculating the fortification level in this thesis should have been 1.9 mg EDTA/kg body weight rather than 2.5 mg EDTA/kg body weight. Using the ADI of 1.9 mg EDTA/kg body weight, the allowable fortification level would have been 8.9 mg/day (0.37 *24). We used a fortification level of 10 mg/serving for three non-consecutive days in a week.

Half (500 kg) of each batch of cowpea flour was fortified with a water soluble food-grade ferric sodium EDTA from AkzoNobel (Ferrazone) (AkzoNobel Chemicals Pte Ltd, Arnhem). The Ferrazone was sent to DSM (DSM Nutritional Products South Africa (Pty) Ltd, South Africa) for premix preparation to suit the requirements of the food processing company in Ghana. The iron-fortified and non-fortified cowpea flours were sealed in double-layered 40 kg polyethylene storage bags and were received as color-coded flour from the factory. The key to the code was withheld from both researchers and participants until data analysis was completed. The fortified and unfortified flours were not visibly different. We evaluated the acceptability of the fortified versus the unfortified *Tubani* among 16 children of similar age to the trial children using a simple pairwise preference ranking. The results showed that 10/16 of the children preferred the unfortified *Tubani*, while 6/16 preferred the fortified *Tubani*. We concluded that even though the fortified *Tubani* was less preferred it was acceptable in this population group.

Preparation of Tubani and Feeding

Tubani was prepared in the two schools by trained women experienced in *Tubani* preparation. In each school two parallel cooking units were set up to match colour-codes. Each cooking unit was under the supervision of a dedicated research assistant (graduate nutritionist) who ensured strict adherence to the recipe and protocol. *Tubani* was served to pupils during the 1000 h break 3 d/wk (Mondays, Wednesdays and Fridays) for seven consecutive months (including school vacation time). It was possible to feed during vacation because all the children in each school were from same community, and the school was within walking distance for all participants. Feeding sessions were under the supervision of teachers and research assistants in parallel classrooms and lasted for about 45 min. Each child was served 150 g of *Tubani* and ate *ad libitum* but could not share or take food out of the classroom. Leftovers for each child, if any, were weighed with an electronic kitchen scale (precise to 2 g)

(Soehnle, Nassau, Germany) and recorded in a daily feeding register. Each child had an identification (name and ID number) card. Total *Tubani* consumed over the trial period was calculated as total weight of *Tubani* served minus total weight of leftover.

Deworming

Prevalence of hookworm infection among children in Northern Ghana is estimated to range between 45 – 50% [24, 25]. All children in the schools received treatment against intestinal parasites with a single dose of mebendazole 500 mg chewable tablets (Remedica Ltd. Cyprus). The first dose was given 4 d before screening and 2 wk before the start of feeding and a second dose given at 3.5 mo into the intervention [26].

Malaria screening and treatment

We used malaria rapid diagnostic cassettes (First Response; Premier Medical Corporation Limited, India) to screen for current or recent malaria at baseline, midway and at the end of intervention based on the presence of the Histidine-Rich Protein-2 (HRP2) in whole blood. HRP2 is specific to *Plasmodium falciparum*, a known cause of more than 80% of malaria cases in Ghana [18, 27]. The cassette used has a sensitivity of 95% and a specificity of 99.5% (First Response; Premier Medical Corporation Limited, India). A 10% subsample of the blood samples was also checked for malaria by microscopy. We treated children who were positive to the malaria antigen with Arthesunate Amodiaquine 100 mg/270 mg (Winthrop; Maphar Laboratories, Morocco), in line with the guidelines of the Ghana Health Services adopted from WHO [28]. Each child received one tablet a day for three consecutive days under supervision of research assistants and teachers.

Measurements

Anthropometry

Weight and height of children were measured at baseline and after the intervention according to standard procedures [29]. Height was measured to the nearest 0.1 cm with a microtoise (Bodymeter 208; Seca GmbH, Germany). Weight was measured with an electronic scale (UNIscale; Seca GmbH, Germany) to the nearest 0.1 kg. At baseline and endpoint, both weight and height were measured twice for each child and the average of the two measurements was taken. The scales were calibrated with known weight each day measurements were taken. Age was calculated using verifiable records (birth certificate, health record, community birth register) or, estimated based on another child's record or event on a traditional calendar (12 children).

Chemical and biochemical measurements

Iron concentration of *Tubani*, cowpea flour and cowpea seeds were measured using inductively coupled plasma atomic emission spectrophotometer (ICP-AES, Varian Vista-Pro, Palo Alto, CA, USA) after digestion with HNO_3-HF-H_2O_2. Phytic acid determination was done using a modified Makower method [30] in combination with the van Veldhoven's method [31] and expressed as inositol hexaphosphate (IP6). A modified Folin-Ciocalteau method [32] was used to measure total polyphenol concentration of the *Tubani*.

At baseline and after the intervention, 2 mL and 6 mL of whole blood was withdrawn into K_2EDTA-coated and silica-coated serum separator vacutainers (Becton-Dickinson diagnostics, Belgium), respectively. Whole blood in K_2EDTA vacutainers was stored in a cool box while in field and during transportation. Haematological analysis was done on the same day with a Pentra 60C+ automated analyzer (HORIBA ABX, Montpellier, France). ABX Minotrol 16 (HORIBA Medical, Benelux, Belgium) quality control samples were used.

Whole blood in silica-coated vacutainers was kept at ambient temperature while in field and during transportation. Serum was separated using a centrifuge (Hettich GmbH, Germany) at 500 x *g* for 5 min at room temperature. Separated serum was aliquoted, kept frozen at -80°C (Thermo Fisher Scientific, Asheville, USA) and subsequently transported on dry ice to Germany via the Netherlands for measurements of serum indicators. Both baseline and endpoint serum samples were analyzed together.

Serum ferritin (SF), soluble transferrin receptors (sTfR), C-reactive protein (CRP) and α_1-acid glycoprotein (AGP) were measured simultaneously using an in-house sandwich ELISA technique [(33)]. All measurements were done in duplicates and if CVs were ≥ 10% measurements were repeated. The CVs (inter-assay) for the various indicators were: SF, 2.6%; sTfR, 2.4%; CRP, 7.2% and AGP, 2.9%. Certified quality control samples from the CDC/Atlanta and Bio-Rad Liquicheck controls (Bio-Rad, Munich, Germany) were used. Anaemia was defined as haemoglobin concentration <115 g/L for children <12 y and <120 g/L for children ≥12 y old; iron-deficiency as ferritin concentration <15 µg/L [(8, 34)]; inflammation as CRP >10 mg/L and/or AGP >1.0 g/L [(35)]; tissue iron-deficiency as serum transferrin receptor concentration >8.5 mg/L (Ramco equivalents) [(36)]; iron-deficiency anaemia as concurrent anaemia and SF <15 µg/L and/or sTfR >8.5 mg/L. Body iron was calculated using Cook's formula [(37)].

Statistical analysis

Data entry and processing was done in SPSS software (version 18.0, Armonk, NY, USA). Anthropometric Z-scores (height-for-age and BMI-for-age) were calculated using WHO AnthroPlus (version 1.0.3). Data distribution were checked by visual examination of Q-Q plots and histograms and tested for normality with Kolmogorov-Smirnov test. Variables not normally distributed were log-transformed and used in subsequent analysis. Analysis was by intention to treat. Treatment effect was measured as group differences in outcome variables at the end of intervention [38]. Effect sizes were evaluated for all continuous outcome variables (Haemoglobin, SF, sTfR and BI) using ANCOVA while controlling for baseline covariates [39]. We present the results as crude and adjusted effect sizes. For log-transformed continuous variables (SF and sTfR), the β-estimates obtained from ANCOVA were exponentiated to obtain effect sizes in percentages which were in turn converted to their corresponding absolute values. Cox regression with adjusted variance (covsandwich) and constant time-to-event [40, 41] was used to obtain prevalence ratios for binary outcomes like Anaemia, ID, IDA and malaria antigenemia in SAS (SAS Institute Inc., Cary, NC, USA).

Table 1. Nutrient composition of cowpea-based meal served to children during intervention

	Tubani[1]	Sauce[2]	Total
Energy, *kcal*	222	209	431
Fat, *g*	1.3	16.0	17.3
Protein, *g*	12.3	1.9	14.2
Carbohydrate, *g*	40.2	4.6	44.8
Fe, *mg*	11.4 ± 2.4[3]	2.2 ± 1.2	13.6 ± 2.6
Phytate (Inositol hexaphosphate), *mg*	256 ± 13.0	119 ± 10.0	374.3 ± 16.4
Polyphenols, *mg GAE*	24.8 ± 4.1	20.3 ± 1.0	45.1 ± 4.2

[1] Unfortified Tubani. GAE, Gallic acid equivalent. [2] Contained 12 g false sesame seeds (*Ceratotheca sesamoides*) and 16.5 g groundnut oil. [3] Arithmetic mean ± SD, all such values.

RESULTS

Composition of Tubani and compliance

Proximate, iron, phytic acid and polyphenol concentration of *Tubani* served during the intervention are presented in **Table 1**. The native iron in the cowpea seed used in this trial was ~6 mg/100 g dry weight (~4.3 mg/serving) however the iron concentration of the unfortified cowpea flour was 13.1 ± 3.9 mg/serving due to added contaminant iron during milling. Based on the total *Tubani* consumed over the intervention period, the NaFeEDTA group consumed on average 17.2 ± 2.5 mg Fe/day whilst the control group consumed 10.3 ± 1.3 mg Fe/day. Apart from iron concentration due to fortification, the composition of *Tubani* per serving for both groups was the same. Fortification iron remained stable over the period of intervention and did not cause clear discoloration (data not shown).

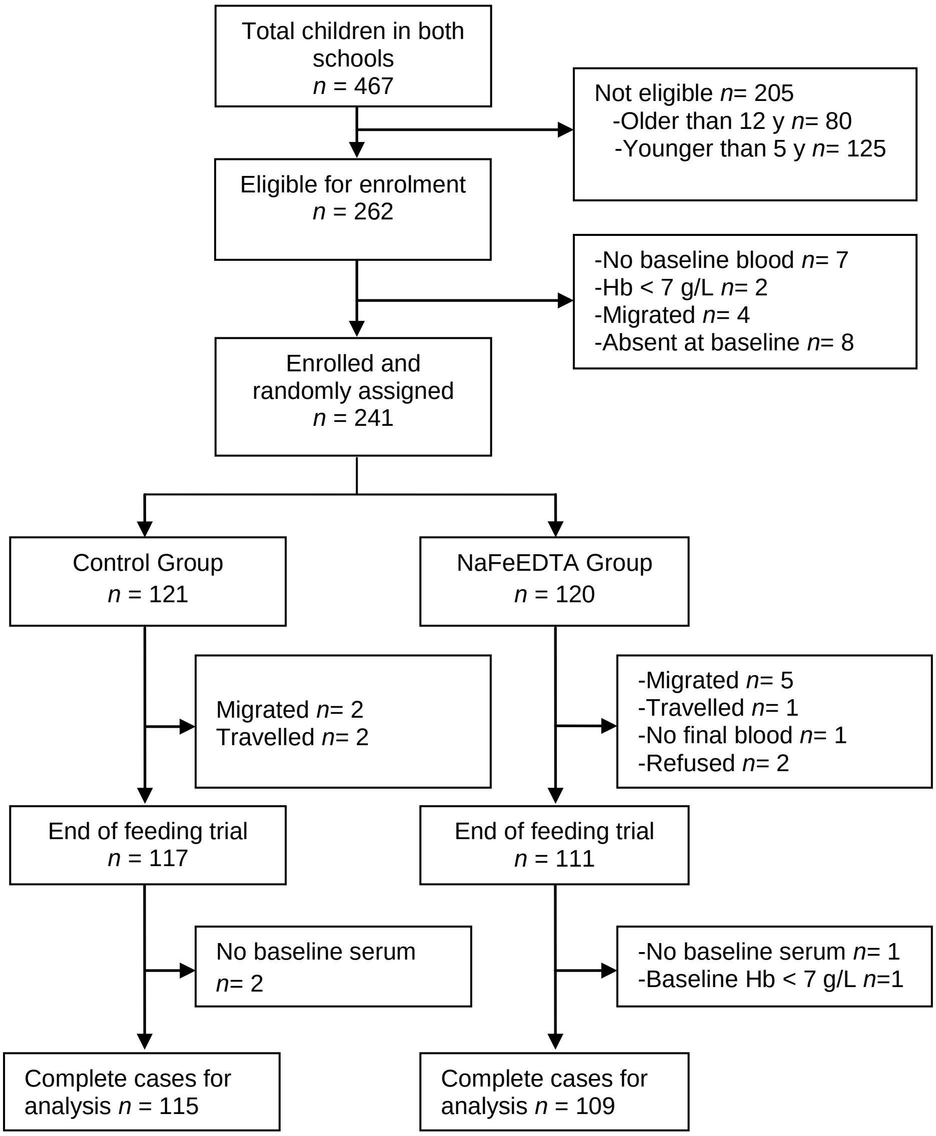

Figure 1. Cowpea intervention trial profile: enrolment followed by 7 mo intervention. Hb, Haemoglobin concentration; NaFeEDTA, Sodium iron ethylene diamine tetraacetate.

Compliance was not different between groups. There were 81 feeding days in total and on average children were present for 94% of the feeding days. The control group consumed an average of 86% of the total quantity of *Tubani* served over the 7 mo compared to 88% in the fortification group. Of the 241 children enrolled, 228 completed the trial (**Figure 1**). The main reason for non-completion of trial in both groups was migration from the study area. Two children (both from iron fortified group) discontinued the study due to non-migratory reasons. One of them was not regular at school and subsequently stopped attending; the other did not like *Tubani*, information we did not have at enrolment. Background characteristics of children who dropped out were not different from those who completed the study (data not shown).

Baseline characteristics

At baseline only 19% of the children were negative for malaria antigenemia, and 47% had inflammation as marked by elevated CRP or AGP. The proportion of children with anaemia, iron-deficiency and iron-deficiency anaemia at baseline was 63%, 68% and 46% respectively. Summary values of iron status markers (Haemoglobin, SF, sTfR and BI), inflammation markers (CRP and AGP) and nutritional status, and prevalence of inflammation, iron-deficiency and iron-deficiency anaemia were not significantly different between the groups at baseline. Typical for this area, 63% of school children in school were boys as seen in both groups **(Table 2)**.

Table 2. Baseline characteristics of Ghanaian school children by intervention group[1]

	Group	
	Control	NaFeEDTA
n	115	109
Sex (male), *%*	63.5	62.4
Age, *y*	8.17 ± 2.2[1]	7.83 ± 2.1
Weight, *kg*	23.4 ± 5.5	22.1 ± 4.8
Height, *cm*	122.2 ± 12.1	120.2 ± 11.4
Height-for-age Z-score	-1.36 ± 1.20	-1.56 ± 1.14
BMI-for-age Z-score	-0.46 ± 0.75	-0.49 ± 0.83
Malaria antigenemia, *%*	81.7	80.7
Iron Status markers		
Haemoglobin concentration, *g/L*	109 ± 13	109 ± 14
Serum Ferritin (SF) concentration, *µg/L*	48.5 (27.2, 91.6)	52.1 (33.5, 92.4)
SF, *µg/L* excluding elevated CRP and/or AGP[2]	36.4 (22.3, 65.9)	39.1 (23.5, 84.5)
Transferrin receptor concentration, *mg/L*	11.0 (8.0, 12.9)	10.8 (8.2, 13.4)
Body Iron[3], *mg/kg bw*	3.8 ± 3.8	4.3 ± 3.3
Inflammation markers and classification		
C-reactive protein concentration (CRP), *mg/L*	2.4 (0.3, 6.9)	2.1 (0.3, 3.7)
α_1-acid glycoprotein (AGP), *g/L*	1.0 (0.8, 1.1)	1.0 (0.8, 1.2)
CRP >10 mg/L, *%*	22.6	20.2
AGP >1.0 g/L, *%*	38.3	49.5
CRP >10 mg/L and/or AGP >1.0 g/L, *%*	41.7	53.2
Iron Status Classification		
Anaemia[4], *%*	63.5	65.1
ID based on SF <15 µg/L, *%*	7.8	6.4
ID based on SF <15 µg/L excluding elevated CRP or AGP[2], *%*	11.9	9.8
ID based on SF <15 µg/L with Thurnham correction [(42)], *%*	9.6	7.3
ID based on SF <30 µg/L, *%*	29.6	22.0
ID as SF <15 µg/L and/or sTfR >8.5 mg/L, *%*	66.1	70.6
IDA[5], *%*	47.0	45.0

[1] Values are mean ± SD, geometric mean (IQR) unless otherwise stated. BI, body iron; Hb, Haemoglobin; ID, iron-deficiency; IDA, iron-deficiency anaemia; SF, serum ferritin; sTfR, soluble transferrin receptor. [2] *n* = 67 for Control and 51 for NaFeEDTA group. [3] To convert BI from mg/kg to mmol/kg multiply by 0.0171[(43)]. [4] Defined as Hb <115 g/L for children <12 y or Hb <120 g/L for children >12 y. [5] Defined as anaemia and SF <15 µg/L and/or sTfR >8.5 mg/L.

Effect of the intervention on haemoglobin, ferritin, transferrin receptors and body iron stores

Relative to baseline we observed a significant increase in haemoglobin concentration in both the NaFeEDTA group and the control group. Serum ferritin and transferrin receptor concentration, and body iron stores decreased significantly in both treatment groups after 7 mo of intervention relative to baseline. Compared to non-fortified flour, consumption of cowpea flour fortified with NaFeEDTA resulted in significant increase in haemoglobin (P < 0.05), serum ferritin concentration (P < 0.001), transferrin receptor concentration (P < 0.001) and body iron stores (P < 0.001) (**Table 3**). Prevalence of inflammation in both groups decreased from baseline by 80% (data not shown).

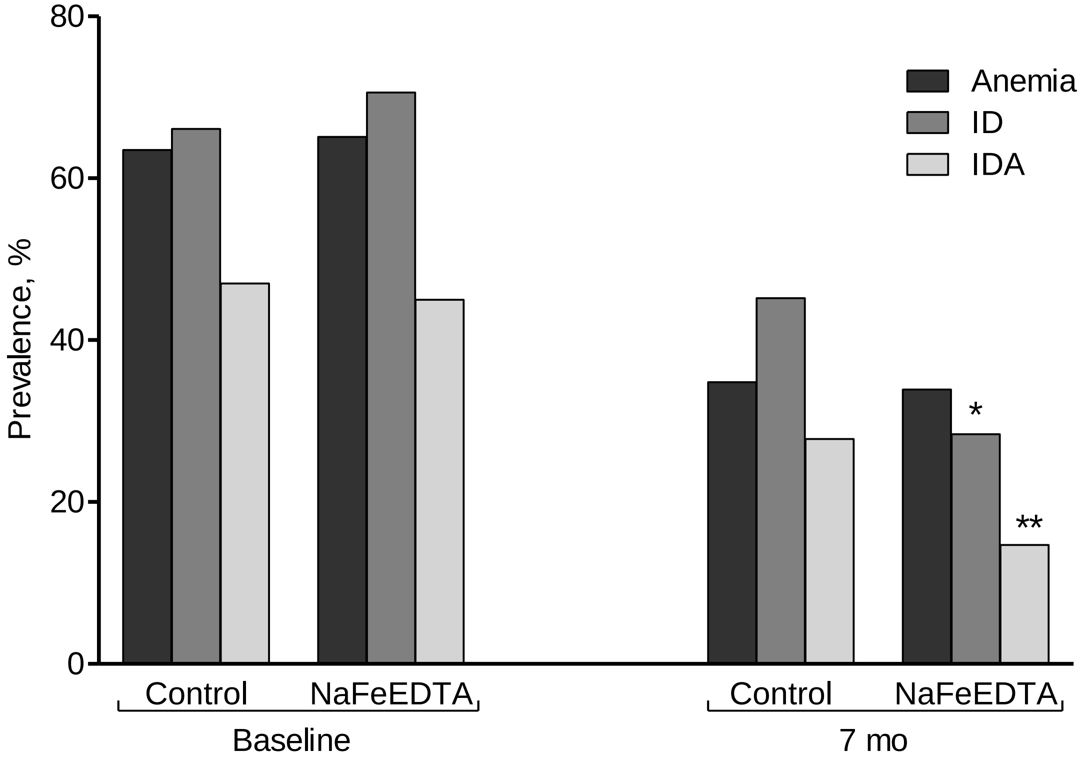

Figure 2. Prevalence of anaemia, iron-deficiency and iron-deficiency anaemia in control group and NaFeEDTA group at baseline and after 7 mo of intervention. n = 115 for Control and 109 for NaFeEDTA group. NaFeEDTA, Sodium iron ethylene diamine tetraacetate. *,** Different from corresponding bars in control group at 7 mo (Cox regression with adjusted variance (covsandwich)). $^{*}P = 0.04$, $^{**}P = 0.002$.

Effect of intervention on prevalence of anaemia, ID and IDA

Post-intervention prevalence of anaemia, iron-deficiency and iron-deficiency anaemia was significantly lower compared to baseline in both groups (**Figure 2**). However, consumption of NaFeEDTA fortified *Tubani* did not result in a significant decrease in the prevalence of anaemia relative to control, but did result in 30% and 47% reduction ($P < 0.05$ in both) in the prevalence of iron-deficiency and iron-deficiency anaemia relative to control, respectively (**Table 4**). No adverse health outcomes related to the trial were observed.

Table 3. Effect of NaFeEDTA fortification on iron status of school children in Ghana[1]

Variable	Group		P^2
	Control	NaFeEDTA	
n	115	109	
Hb endpoint, *g/L*	117 ± 13	120 ± 11	
Crude effect	-	3.4 (0.3–6.6)	0.030
Adjusted effect[3]	-	3.5 (1.2–5.7)	0.002
SF endpoint, *µg/L*	26.2 (15.6, 42.3)	35.1 (27.2, 48.1)	
Crude effect	-	8.6 (2.8–17.8)	0.001
Adjusted effect[3]	-	7.8 (2.8–14.7)	0.000
sTfR endpoint, *mg/L*	8.8 (6.8, 10.6)	7.4 (6.1, 8.6)	
Crude effect	-	-1.4 [-2.1– (-0.63)]	0.010
Adjusted effect[3]	-	-1.2 [(-1.7– (-0.76)]	0.000
BI endpoint[4], *mg/kg body weight*	2.4 ± 3.5	4.1 ± 2.9	
Crude effect	-	1.7 (0.79–2.5)	0.000
Adjusted effect[3]	-	1.3 (0.7–2.0)	0.000

[1] Values are mean ± SD, effect size (95% CI) or geometric mean (IQR). BI, body iron; Hb, Haemoglobin; SF, serum ferritin; sTfR, soluble transferrin receptor. [2] ANCOVA. [3] Adjusted for baseline factors (Hb, SF, sTfR and BI respectively). [4] To convert BI from mg/kg to mmol/kg multiply by 0.0171 [(43)].

Table 4. Effect of NaFeEDTA fortification on iron status prevalence among school children in Ghana[1]

Iron Status	Group		P^2
	Control	NaFeEDTA	
n	115	109	
Anaemia endpoint[3], *%*	34.8	33.9	
Crude effect	-	0.98 (0.68 – 1.40)	>0.05
Adjusted effect[4]	-	0.99 (0.72 – 1.37)	>0.05
ID endpoint[5], *%*	45.2	28.4	
Crude effect	-	0.63 (0.44 – 0.90)	0.010
Adjusted effect[4]	-	0.70 (0.50 – 0.98)	0.038
IDA endpoint[6], *%*	27.8	14.7	
Crude effect	-	0.53 (0.31 – 0.91)	0.020
Adjusted effect[4]	-	0.53 (0.36 – 0.80)	0.002

[1] Values are prevalence ratios (95% CI) unless otherwise stated. BI, body iron; Hb, Haemoglobin; ID, iron-deficiency; IDA, iron-deficiency anaemia; SF, serum ferritin; sTfR, soluble transferrin receptor. [2] Cox regression with constant time to event and adjusted variance (covsandwich). [3] Defined as Hb <115 g/L for children <12 y or Hb <120 g/L for children >12 y). [4] Adjusted for baseline factors (HB, SF, sTfR and BI respectively).
[5] Defined as SF <15 µg/L and/or sTfR >8.5 mg/L. [6] Defined as anaemia and SF <15 µg/L and/or sTfR >8.5 mg/L.

Prevalence of malaria antigenemia during the trial

Malaria antigenemia was found in 81% of the participating children in both groups at the start of the trial. At midpoint during the trial the prevalence decreased to 35% and 45% in control and NaFeEDTA groups, respectively [PR: 1.29 (95% CI = 0.93, 1.79)] and to 5% in both groups at the end of the trial (data not shown). A subgroup analysis among iron deficient children did not show significant difference in prevalence of malaria antigenemia between groups [PR: 1.35 (95% CI = 0.92, 1.98)].

DISCUSSION

We investigated the efficacy of cowpea meal (*Tubani*), fortified with NaFeEDTA to supply extra 10 mg Fe/d for 3 d/wk, in improving iron status of school children in northern Ghana. Our results indicate that the consumption of fortified cowpea meal significantly improved both functional and storage iron status and consequently reduced the prevalence of iron-deficiency and iron-deficiency anaemia in school aged children. Reduction in anaemia prevalence however was not attributable to consumption of fortified cowpea meal.

Our study was carried out in a malarious area and baseline measurements were taken at the peak of malaria transmission (October – November). Therefore there was high prevalence of malaria antigenemia at the start of the study. In the presence of infection and chronic inflammation (e.g. asymptomatic malaria) iron distribution shifts towards storage and sequestration [(44)]. This may explain why at baseline we observed a significant positive correlation between SF concentrations and inflammation markers (CRP and AGP). As such, SF at baseline may not be a sensitive measure of ID. However, there was a decrease in concentrations of CRP and AGP corresponding to a 40% and 25% decrease in SF in control and NaFeEDTA group respectively at end of trial. This may be partly due to the lower malaria transmission in May and partly due to the malaria treatment and mass deworming given twice in the trial at 0 and 3.5 mo. Since both malaria antigenemia and inflammation decreased by more than 80% at end of trial, SF is most likely a sensitive marker of post intervention ID status of the children in our study.

In malarious and high infection areas, the iron sequestration processes induced by hepcidin may be prominent, creating a condition similar to anaemia of inflammation [(8, 45)]. This may explain why a number of efficacy studies from malarious areas with high infection and inflammation only show marginal effects on haemoglobin [(46, 47)]. In our study however, we found a significant fortification effect on haemoglobin concentration. Contrary to our expectation we found no fortification effect on anaemia despite significant reduction in ID due to fortification. The lack of fortification effect on anaemia suggests, strongly, that in these children ID may not be the main cause of anaemia, but other factors such as malaria [(48)] and helminth infestation [49, 50] may play a role, for which both groups in our trial received the same treatment. Since we did not have a placebo group we are unable to determine the effect of malaria and helminth treatment on anaemia. Another reason for the lack of fortification effect on anaemia may also be that the magnitude of the improvement in haemoglobin concentration due to fortification was not sufficient to result in relative reduction in anaemia and perhaps a longer duration of feeding could lead to reduction in anaemia due to fortification.

Our findings add to the growing body of evidence from field trials that NaFeEDTA is effective in improving iron status of vulnerable groups in developing countries. Earlier evidence was shown in condiments; curry powder (masala) in South Africa [51] and fish sauce in Vietnam [52]. Recent evidence of efficacy in Africa came from a trial involving school children who were fed fortified whole maize porridge in Kenya [22]. Unlike earlier studies, our trial used a highly inhibitory staple (cowpea) as the fortification vehicle. Cowpea is rich in both inhibitory phytic acid (PA) and polyphenols (PP) [53], unlike maize that is mainly rich in PA . In an iron absorption study, Petry and colleagues found that PA and PP as found in common beans inhibited iron absorption from $FeSO_4$ independently and when concomitantly present but their combined inhibition did not seem to be additive [54]. However, the ability of NaFeEDTA to overcome the combined inhibitory effect of PA and PP has not been investigated in a field trial. The current trial shows that NaFeEDTA overcomes the combined inhibitory effect of PA and PP in white cowpea varieties.

Based on a difference in body iron stores of 1.7 mg/kg body weight between iron-fortified and control group at end of intervention relative to difference in fortification iron ingested over the same period, fractional absorption of fortification iron was ~7%. A previous fortification trial with similar level of fortification (10 mg Fe/d) despite higher frequency of consumption, reported ~12% fractional iron absorption from NaFeEDTA in fish source eaten with rice [52]. The difference in fractional absorption may be due to the fact that study participants were all anaemic women in whom iron absorption may be more efficiently up-regulated [55, 56]. Another reason for the difference in fractional absorption could be the difference in fortification vehicle; cowpea is more inhibitory than fish sauce and rice due to its higher concentration of PA and PP.

The findings of this trial may be an important proof of concept for the fortification of legumes and pea flours in general. These foods may be recommendable for use in school feeding programs due to their protein content. However, cowpeas contain high concentrations of absorption inhibitors (PP and PA). In addition, consumption of *Tubani* and other legume source foods in schools may be limited to 2–3 times/wk to decrease boredom, therefore a hypothetical fortification program should utilize a highly bioavailable iron compound. A limitation of the use of NaFeEDTA may however be that level of fortification in a future cowpea fortification will be restricted by ADI of EDTA since current JECFA recommendation allows only 0.2[2] mg Fe/kg body weight as FeEDTA [57].

Although our study was not designed and powered with malaria as endpoint, no significant differences in malaria antigenemia were found between the two groups at any point in the trial. In a subgroup analysis at 3.5 months, children with ID or who were iron replete at baseline did not show differential malaria antigenemia risk between groups, contrary to the findings of earlier supplementation trials with iron and other nutrients among pre-school children in malarious areas [58, 59]. There is, therefore, need for further investigation.

The challenge with iron fortification is the alteration of organoleptic properties of the fortified food. In the current trial the fortified flour was not distinguishable from the unfortified flour.

[2] Based on the 2007 JECFA report, this value should be 0.37 mg Fe/kg body weight.

After cooking, *Tubani* from the fortified flour looked slightly darker but the colour change was not immediately noticeable unless the two samples were placed side-by-side. The fortified *Tubani* also had a slight but bearable iron after-taste. These together could have offset the blinding. However since the feeding was done in parallel rooms and the children never got to see or taste the fortified and unfortified *Tubani* together, the integrity of the blinding was maintained. Moreover compliance did not differ between NaFeEDTA group and the control group.

The results of this trial may be particularly relevant for northern Ghana; cowpea is widely used in the Government-sponsored school feeding program but it is only in northern Ghana that there is an opportunity to use cowpea flour-based recipes (e.g. *Tubani*). Therefore fortification of cowpea flour will be more useful as a school-based targeted intervention, combined with adequate treatment against helminths and malaria. In the absence of central milling and commercial cowpea flour on the market, it is unlikely that fortified cowpea will find use outside schools despite the popularity of cowpea flour-based recipes.

In conclusion, our results have shown that NaFeEDTA overcomes the combined inhibitory effect of PA and PP. When used for targeted school-based intervention, fortification of cowpea flour is effective in improving iron status and consequently reducing the prevalence of IDA among school children in rural northern Ghana.

ACKNOWLEDGEMENTS

We are grateful to the teachers of the two schools: Y. Abdul-Majeed, A. Alaru, A. A. Suhuyini and A. Wumbei. We thank M. Nagasu, L. van den Broeck, N. Creemers, J. Hulst and J. B. Ziem for their contribution during field work. We also thank S.K Ntim, K.A.O Atiemo and A.S Amponsah all of YEDENT Agro-food processing company, Ghana and M.

van Doorn of Akzo Nobel Functional Chemicals B.V. Deventer, the Netherlands for their respective technical assistance during the trial. Supported by a research grant from the Nestle Foundation for Nutrition Research and Wageningen University (TELFUN project). Akzo Nobel chemicals provided the fortificant and DSM South Africa formulated the NaFeEDTA premix.

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Chapter 6

General Discussion

Iron-deficiency (ID) among schoolchildren in Ghana is a public health problem, especially among rural communities. A sustainable strategy would be one that utilizes local foods to contribute to solving ID while having the added potential to improve food sovereignty of local communities. The main aim of the research in this thesis is to improve iron status of schoolchildren through the consumption of locally produced cowpeas within a school feeding programme. To achieve this aim, five related studies were conducted, mainly in Tolon-Kumbungu district of Ghana. The studies comprised cross-sectional studies, chemical analysis of cowpea landraces, an *in vivo* bioavailability study and a randomized intervention trial. In this chapter a summary of the main findings from all the studies in this thesis is given (temporal linkages shown in **figure 6.1)**. A synthesis of the internal and external validity of the studies, the public health and policy implications of the findings as well as suggested future research directions are also presented.

MAIN FINDINGS

The status quo investigation indicated that iron-deficiency and iron-deficiency anaemia are severe public health problems among schoolchildren in northern Ghana with 8 and 7 out of every 10 schoolchildren affected respectively. It also showed that the probability of adequate dietary iron intake is close to 0.30 but much higher (~0.90) if schoolchildren benefitted from a school feeding programme (**Chapter 2**). The chemical analysis of cowpea landraces available in northern Ghana indicated that cowpeas contain appreciable amounts of iron (4.9–8.2 mg/ 100 g d.w) and zinc (2.7–4.1 mg/100 g d.w) but also contain high amounts of inhibitory phytate (477–1110 mg/100 g d.w) and polyphenol (327–1055 mg/100 g d.w). Mothers/caregivers intended to give cowpeas to their schoolchildren 2–3 times per week. The positive attitudes of mothers towards cowpea predicted their intention to give them to their schoolchildren but they were worried about the cost, long cooking time and the discomfort their children may feel after consuming cowpeas (**Chapter 3**). The main findings in **chapter 4** showed that the iron bioavailability from red and white cowpeas were 1.4 and 1.7%, respectively, in NaFeEDTA-fortified meals and 0.9 and 1.2%, respectively, in FeSO4-fortified meals. Compared with FeSO4, fortification with NaFeEDTA increased the amount of iron absorbed from red and white cowpea meals by 0.05 and 0.08 mg (P < 0.05) respectively. Irrespective of the fortificant used, there was no significant difference in the amount of iron absorbed from the 2 varieties of cowpea despite difference in polyphenol concentration. In **chapter 5**, the efficacy trial among schoolchildren showed that fortification of whole cowpea flour with NaFeEDTA resulted in almost one-third and one-half reduction in the prevalence of iron-deficiency (ID) and iron-deficiency anaemia (IDA) (P<0.05), respectively.

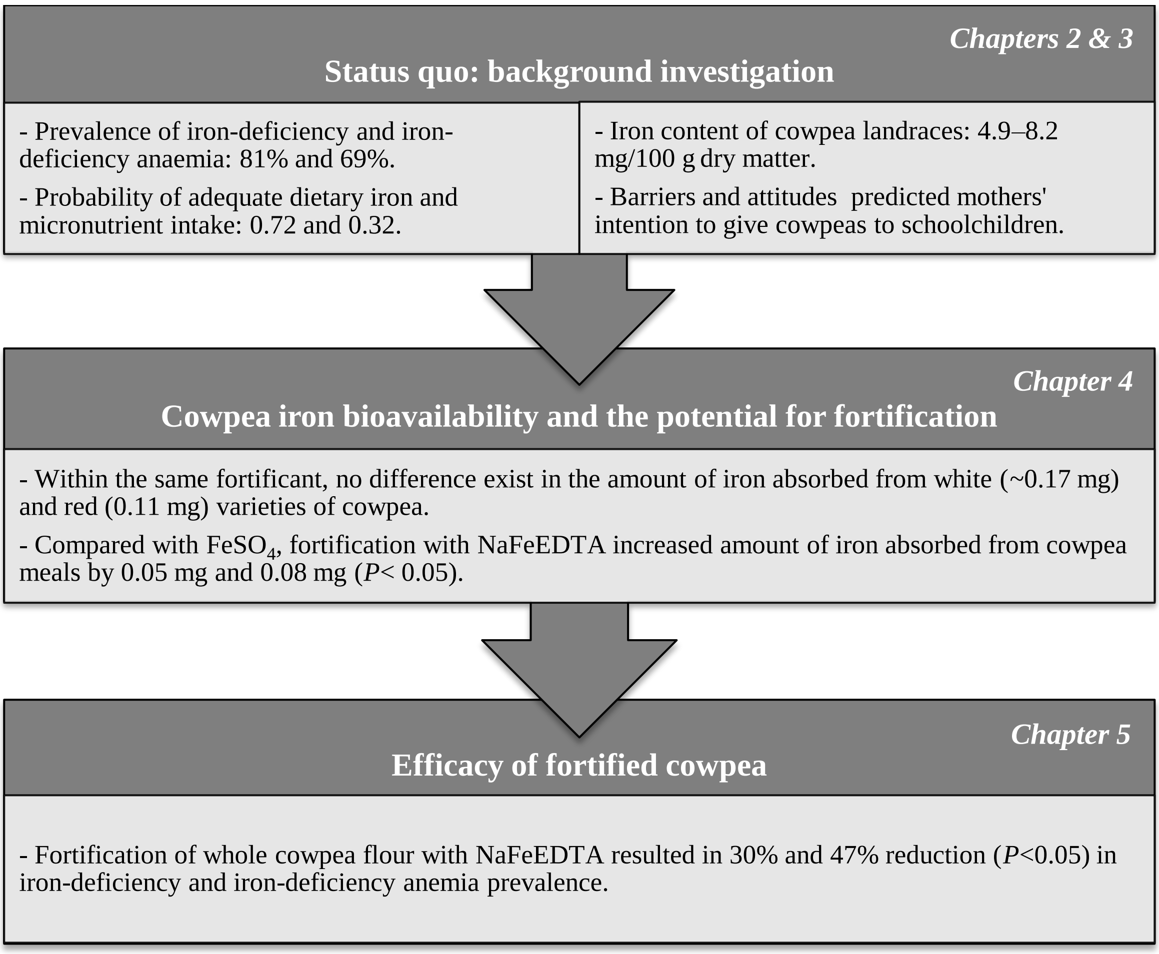

Figure 6.1. Summary of main findings and their temporal linkages

INTERNAL VALIDITY

The subsequent section in this chapter discusses the methodological considerations for the studies in chapters 2–5 which may potentially influence the conclusions in this thesis, namely selection bias, information bias and confounding.

Selection bias

Systematic differences in the recruitment of study participants or comparison groups lead to selection bias which may cause the relationship between exposure and outcome among those selected to participate to be different from that of those in the population who did not participate [(1)]. Self-selection by volunteers and refusal to participate in a study are examples of selection bias that can lead to spurious conclusions [(2)]. None of the studies in chapters 2, 3 and 5 were based on self-selection thus eliminating the potential for self-selection bias. A sampling frame was constructed by pooling class registers in the respective schools from which schoolchildren were randomly selected to participate in the studies. One child per household was included in the studies and in cases where more than one child in a household was qualified one of them was randomly selected to participate.

Another source of selection bias arises from refusal to participate but this did not occur as all children and parents who were selected consented and did participate. In chapter 5, systematic difference between treatment groups in dropouts and in compliance to the treatments is another potential source of bias. But checks showed that there was no difference in the characteristics of the children who dropped out from both treatment groups. Similarly compliance was high and similar between intervention and control group (88% vs. 86% respectively) indicating that a systematic difference between groups is unlikely. Our use of the intention-to-treat analysis maintained the integrity of the initial randomization [(3)].

Information bias

Information bias occurs when there are either random or systematic differences in the measurement of exposures, outcomes and possible confounders or when some respondents provide different information from the rest of the sample [1]. Common sources of information bias discussed here include misclassification/measurement bias, non-blinding of intervention allocation and outcome measurements, and recall and interviewer bias.

Misclassification of iron status

In both chapters 2 and 5, iron status is a key outcome measure. Classifying iron status of children in malaria endemic areas such as in this thesis is difficult and the risk of misclassifying children is high [4, 5]. Serum ferritin (SF) is a sensitive biomarker of iron stores so it is recommended as a measure of iron status [6]. However SF is also an acute phase protein and its level rises in response to inflammation independent of iron status [7] masking an otherwise deficient iron store. Prolonged exposure to malaria parasites may confer varying levels of immunity among schoolchildren and increase asymptomatic malaria [8]. The presence of these malaria parasites can produce a chronic or mild acute phase response among asymptomatic children resulting in elevated SF [8]. The baseline assessment of malaria antigenemia in chapter 5, showed high prevalence (81%) among the schoolchildren studied. Proportion of children with inflammation was also high (>40%). It is therefore most likely that SF values were elevated and could have underestimated iron-deficiency. To reduce misclassification iron-deficiency was defined as low SF and/or elevated sTfR. It is known that sTfR is a sensitive marker of tissue iron need and is not affected by inflammation [9, 10] so such a combination improved the classification of iron-deficient schoolchildren [4].

Misclassification of anaemia status could also have occurred. Given the range of ages, schoolchildren fell into two categories for the classification of age specific haemoglobin status. Wrong age classification could lead to wrong assignment of anaemia status and either underestimate or overestimate anaemia prevalence. WHO reference cut-offs for haemoglobin were used and care was taken to obtain accurate ages of schoolchildren based on verifiable documents such as birth certificates, community birth register and health cards. In the absence of these verifiable documents, age estimation is difficult because over 90% are not literate; however using events on a local calendar and verifiable record of a sibling good estimate was made for a small proportion (10%) of children.

Blinding of intervention treatments

The aim of blinding is to prevent information bias which may occur through knowledge of the nature of the allocated intervention and also through the preference of the researcher for a particular outcome [(11)]. To minimize this form of information bias in chapter 5, both subjects and researchers were blinded to the intervention allocation and the code was not broken until data analysis was completed. Production, fortification and coding of cowpea flour were done by an independent food company. Integrity of blinding was maintained and the likelihood of cross-over treatment was eliminated by assigning different feeding rooms to colour codes throughout the intervention. Biochemical analysis was done by people unaware of the intervention treatment allocations.

Dietary assessment

Accurate and precise assessment of habitual dietary intake of individuals and groups remains a major challenge in dietary assessment [(2)]. Both systematic and random errors may occur, and measures to reduce the impact of these are critical components of a dietary assessment [(2)]. In chapter 2, we aimed to estimate group level habitual nutrient intake and our use of 24-hour recall with a non-consecutive duplicate recall among a subsample has been recommended and shown adequate for such measurement [(2, 12)]. With a duplicate recall from a 20% subsample of schoolchildren we were able to estimate usual intake from the observed dietary intake by adjusting for day-to-day variation using the National Research Council's (NRC) method [13]. The use of the NRC adjustment method allowed precise estimation of the prevalence of inadequate nutrient intake among the schoolchildren based on probabilities. The alternative to the NRC method would have been to compare nutrient intakes to the RDA (recommended dietary allowance but this method seriously overestimates the proportion of a group at risk of inadequate nutrient intake [12].

Major sources of systematic bias include under or over-reporting of intake and the processing of dietary information [2, 14]. Misclassification of nutrient intake adequacy could have occurred as a result of under or over-estimation of food intake by mothers. To minimize misreporting of food intake, caregivers were taken through a systematic multiple-pass procedure which aided recollection of foods and ingredients used in preparation of meals at home [2]. Also, because mothers were likely to omit foods consumed out-of-home such as fruits and snacks [15], children were present to assist with foods that were consumed out of home. The use of household measures from the respondents' own home aided them to remember and to estimate quantities and portion sizes, and when food ingredients were available actual weights were taken to avoid mistakes with estimation. In addition to these measures, we examined likely misreporting by comparing age and sex specific estimated energy requirement (EER) [16] to energy intake based on 24-recall. We found that most of the children were in positive energy balance but the anthropometric data did not confirm this as none of the children was overweight or obese. Therefore it is likely that caregivers overestimated the dietary intake of their schoolchildren. However this misreporting is less likely to affect the differences observed in nutrient intakes between the two groups since total energy intake from home-consumption were not significantly different.

The use of food consumption tables to convert food intake into nutrients could introduce bias into nutrient intake estimates [2, 14, 17]. The newly released West African food composition table (WAFCT) [18] was the primary source of nutrient composition because apart from capturing local foods in Ghana it also covered a wider range of nutrients compared to the Ghana food composition table. The food composition values in WAFCT are averages from 9 countries and updated with data from scientific literature, theses, university reports and food composition databases. Nutrient values of cooked foods were calculated by using appropriate yield factors and nutrient retention factors [19]. In addition the table was compiled following international standards for food composition and compilation [20, 21] thereby improving the quality and the validity of the dietary assessment.

Mixing of stable isotope tag with test meals and sequence of test meals consumption

A possible source of bias in chapter 4 is inadequate mixing of extrinsic stable iron isotope labels with test meals prior to consumption [22, 23]. The consequence is that extrinsic iron may not equilibrate with intrinsic iron in the test meal and may result in underestimation of bioavailability of extrinsic iron. It is not known how long it takes for extrinsic iron label to equilibrate with intrinsic iron but we believe that mixing prior to consumption (for 5 minutes in our case) was adequate [24]. There is evidence that even when the tag is not homogenously mixed with the test meal but put in a component of the meal, there is subsequent equilibration in the stomach [25]. Also our use of a test meal made from flour rather than whole grain improved equilibration and exchange between intrinsic and extrinsic iron [24]. Cook et al. [26] have demonstrated that in single meal studies the effect of iron absorption inhibitors is likely to be exaggerated. This observation implies the reported bioavailability in chapter 4 could be higher in composite meals. However the test meal as used in this thesis is representative of how it is traditionally consumed in Ghana.

Confounding

A measure of an association between exposure (e.g. fortified cowpea meal) and outcome (e.g. iron status of schoolchildren) is confounded if an explanatory/third factor is associated with both exposure and outcome at the same time [3]. In chapter 2, unobserved/unmeasured community-level characteristics could be prognostic for the observed differences in dietary intake and/or iron status between school feeding participants and non-participants. We had no control over the selection of the communities for the pilot school feeding programme but tried to match community characteristics in the selection of control communities. Where there were differences in measured community-level characteristics, they were accounted for in analysis but residual confounding of unobserved or unmeasured/inaccurately measured characteristics could still be present [3]. Even though we selected communities that were otherwise qualified but not selected for the pilot school feeding programme, there is the likelihood of selection bias and confounding by indication [27] which we could not eliminate.

In chapter 5, the possibility for confounding was reduced by the randomization of schoolchildren into respective treatment groups [3]. Possible confounders of the association between intervention and iron status were identified during design stage to include age, sex and nutritional status (weight and height) of schoolchildren. Block randomization was used to assign children into the treatment groups by a researcher who was not present in the field and had no personal contact with the children enrolled into the trial. The similarity between intervention groups with respect to baseline characteristics showed that randomization was successful albeit unknown/unmeasured confounders may be present. Malaria and helminthic infestations are two factors that could influence the outcome of intervention in the research area [4, 28]. Therefore mass deworming as well as screening for malaria antigenemia among schoolchildren was carried out twice during the intervention however the possibility of re-infestation during the intervention cannot be ruled out.

EXTERNAL VALIDITY

Under this section, the main findings in this thesis as outlined in **Figure 6.1** will be discussed in relation to other findings in literature and the extent to which our findings may be applied in different settings.

Status quo: nutrient intake, micronutrient adequacy, and prevalence of iron-deficiency and anaemia among schoolchildren.

The findings in chapter 2 indicate that in the absence of school feeding the probability of adequate micronutrient intake (especially iron) among schoolchildren is low (~0.30). This to a large extent reflects the poor quality of diets at the household level since almost all meals consumed by the non-school feeding children were from home. Micronutrient quality of cereal and legume-based diets of rural African households has been reported as poor and contributes to inadequate intake of bioavailable iron [(29, 30)]. Although the school feeding programme is associated with an increased iron intake of about 5 mg (P<0.05), we did not find a difference in prevalence of iron-deficiency anaemia (~65%) between beneficiaries and non-beneficiaries of the school feeding programme. This supports the suggestion that iron bioavailability plays a major role in causing ID among children in this area and sub-Saharan Africa in general [(31)]. The increment in iron intake in school feeding programme was largely due to the consumption of micronutrient fortified Corn-Soy Blend (CSB+) from World Food Programme, confirming that fortification is probably needed to achieve adequate iron intake [(32, 33)]. Combined with the observation that school meals did not replace home meals, long term intake of fortified school meals may augment iron intake and contribute to iron status.

The prevalence of ID (~80%) reported in this chapter is higher than values reported among schoolchildren in other West African countries irrespective of whether ID was defined by only SF as in Benin (49%) [(34)] or in combination with sTfR and/or ZnPP as in Cote d'Ivoire (45%) [(4)]. However it is consistent with the high prevalence reported in chapter 5 (~70%)

although measured two years apart. It is unclear why ID prevalence is higher among the children in this thesis but the findings together point to the severity of ID as a public health problem in West Africa.

The prevalence of anaemia (~80%) is also a severe public health problem in our study population and is similar to the 70% observed among schoolchildren in Cote d'Ivoire [28]. Findings from northern Benin, in the same ecological zone as the research area in this thesis, suggest seasonal influence on anaemia; 70% in the post-harvest but 33% in the pre-harvest season [34]. Even though malaria was not measured, the authors speculated that the difference was due to high malaria prevalence in the post-harvest season. Anaemia prevalence in chapter 2 was measured in the post-harvest season; a period marking the end of peak malaria transmission in northern Ghana. Therefore, similar to Benin, malaria likely contributed to the high anaemia prevalence in accord with other studies [4, 28, 35, 36]. Moreover the high anaemia prevalence is again consistent with the baseline findings in chapter 5, also measured in early post-harvest season but two years apart, where malaria antigenemia was found among 80% of the children.

Status quo: cowpea landraces and factors predicting consumption

Human dietary behaviour is complex and getting a model that accurately predicts it is challenging. Two cognitive models have found wide use in predicting health and dietary behaviour: the theory of planned behaviour [37] and the health belief model [38]. The overlapping constructs in these models have led to recommendation for their application as combined model in anticipation of improved predictive ability [39]. The combined model was useful in predicting intention to consume fortified soy sauce in China [39]. It has also been used to identify important factors influencing fonio consumption in Mali [40] and amaranth consumption in Kenya [41]. In this thesis, the combined model identified two constructs as significant predictors of mothers' intention to give cowpeas to their schoolchildren; barriers and attitudes towards behaviour (chapter 3). Whereas barriers negatively predicted intention, attitudes of mothers towards cowpeas positively predicted intention. Consistent with previous applications of the intention model in Mali [40] and Kenya [41], the variance explained by the model was low despite the extensive literature search that was done to identify factors for inclusion into the behavioural models used in this thesis. The low predictive ability of the intention model suggests that other factors still need to be explored. It does not however invalidate the significant predictors (barriers and attitudes towards cowpeas) of intention identified in this thesis [42].

Chapter 3 confirmed cost [43], long cooking time [44] and uneasiness after consumption [44, 45] as important barriers to cowpea consumption. Cost is driven by market forces but investigation into processing techniques show that the use of cooking aids such as bicarbonate (rock or pure powder) have been reported to decrease cooking time without affecting the nutrient quality of cowpeas [46]. The feeling of uneasiness has been reported to be related to flatulence producing properties [47] and can be reduced by soaking or fermentation [46].

As shown in chapter 2, the prevalence of iron-deficiency is high and the probability of iron intake is low. For cowpeas to contribute significantly to dietary iron intake they must have a high concentration of potentially bioavailable iron [48]. However the analysis of cowpea landraces in chapter 3 showed that even though cowpeas may have an appreciable amount of iron its bioavailability is likely low due to the high phytate-to-iron molar ratio: a ratio associated with inhibition of iron absorption [49]. An assessment of commonly consumed cowpea dishes in Benin also suggest that phytic acid-to-iron molar ratios are still high enough to inhibit iron absorption [44]. This is likely because phytate is heat stable up to a temperature of ~100 ^{0}C thus making conventional heat processing at the household unable to degrade it [50].

Cowpea iron bioavailability and potential for fortification

The 2×2 cross-over stable isotope study in chapter 4 showed that the absolute amounts of iron absorbed from both white and red cowpeas were similar. Investigations of iron absorption from common beans support these findings. Among iron replete women, Beiseigel et al. [51] found that the amounts of iron absorbed from white and brown varieties of common beans were similar. The same observation was made among Rwandan women with marginal iron stores when iron absorptions from white and red common beans were compared [52]. Donangelo et al. [53] observed that even within the same variety of common beans, the amount of iron absorbed from a high iron genotype was similar to that from a low iron genotype. These findings point to a common observation; varietal or genotypic differences may not influence the amount of iron absorbed from cowpeas or common beans. Thus conventional biofortification of cowpeas for improved iron concentration alone may not be adequate to improve intake of bioavailable iron unless native phytate and polyphenols are both reduced drastically. Independent of each other, phytate and polyphenols decrease iron bioavailability [54]. Breeding a cowpea variety with very low phytate and polyphenol is a challenge for breeders. Due to the difference in the location of phytate and polyphenols in cowpeas [50], pre-consumption treatment/processing of cowpeas such as soaking, milling, dehulling, germination, fermentation and roasting may have different effects on their concentrations. It may therefore require multiple processing techniques to drastically reduce both inhibitors which may lead to loses of other important minerals such as zinc, magnesium and calcium [50, 54].

The findings in chapter 4 also showed that iron absorption from cowpea fortified with NaFeEDTA is better than when fortified with $FeSO_4$ although the fractional absorption was generally low (<2%). In other absorption studies, the potential of NaFeEDTA as a fortificant has been shown in vehicles other than cowpeas [(55-58)]. A simulated calculation using the low bioavailability observed in chapter 4 showed that even the consumption of cowpeas with iron content similar to biofortification target of ~10 mg/100 g [(48, 59)] may not improve iron status unless it is fortified with a highly bioavailable iron compound like NaFeEDTA (Abizari, unpublished data). Madode et al.'s work on traditional processing to improve iron availability of cowpea foods in West Africa, also supports the need to explore the fortification of cowpea flour [(44)].

Efficacy of fortified whole cowpea flour

Whole cowpea flour fortified with NaFeEDTA improved body iron stores and reduced the prevalence of iron-deficiency by 30% and iron-deficiency anaemia by 47% among schoolchildren (Chapter 5). To our knowledge, no previous study has reported on the efficacy of fortified whole cowpea flour. However in Kenya, whole maize flour fortified with NaFeEDTA decreased iron-deficiency and iron-deficiency anaemia by 91% and 89% among 3–8-year-old children [(60)]. NaFeEDTA fortified whole wheat flour was also efficacious in reducing iron-deficiency and iron-deficiency anaemia by 67% and 51% among iron depleted 6–15-year-old Indian schoolchildren [(61)]. These two interventions have shown markedly different effect sizes albeit similar fortification levels. Relative to Kenya, it was expected that the intervention effect in India would be larger because of the absence of potential blunting by both malaria [(62)] and helminths [(28)] coupled with inclusion of children with low iron status; a known determinant of iron absorption [(63)]. It is not immediately clear what accounts for the difference but low detection (precision) of iron status in Kenya could have been masked by inflammation and the few detected were truly iron-deficient and responded well to the intervention. The smaller effect size in our study relative to Kenya and India could be attributed partly to our use of cowpea as vehicle which may have dual inhibition by both phytic acid and polyphenols [64] compared to maize and wheat that have main iron inhibition from phytic acid.

There are concerns that a highly bioavailable iron compound such as NaFeEDTA may not be desirable for flour fortification in malarious areas because it is suspected to create a milieu of non-transferrin bound iron (NTBI) which may be used by malaria parasites to proliferate and thereby worsen infestation [65]. Troesch et al. showed that 6 mg fortification iron as NaFeEDTA used in a stable isotope study did not increase NTBI among healthy women [66] suggesting that this may mitigate the safety concerns associated with using NaFeEDTA for fortification in malaria endemic areas [67]. However this proof of principle investigation is yet to be demonstrated on the field. There is therefore need to monitor malaria morbidity in the implementation of fortification programmes in malaria endemic areas so that potential harm may be identified [65]. The school setting provides a favourable environment for such monitoring. Another safety concern regarding the use of NaFeEDTA as fortificant has been related to adverse effect on zinc and other important minerals [68]. In developing countries, iron-deficiency often coexists with zinc deficiency [69, 70] and it is feared that using NaFeEDTA as a fortificant may further worsen zinc deficiency. In this thesis zinc status was not measured but other field studies in Kenya [60] and India [71] showed that fortification with NaFeEDTA within recommended levels [72] for schoolchildren is unlikely to worsen zinc status or urinary zinc excretion respectively.

In 2009, the WHO-led expert committee issued a consensus directive to use NaFeEDTA as the only recommended fortificant for whole maize and high extraction wheat flour fortification [73]. The findings in chapter 5 indicate that NaFeEDTA is not only effective in phytic acid-rich cereals but can be extended to include whole legume flours that are phytic acid as well as polyphenol-rich. The relatively high cost of NaFeEDTA still make people favour the use of elemental iron in flour fortification [74] albeit poorly absorbed and has previously not shown efficacy in Africa [28, 60]. It is however envisaged that increasing utilization of NaFeEDTA will drive its cost towards levels that would allow wider use as whole flour or high extraction flour fortificant.

GENERAL CONCLUSIONS

Overall, the research in this thesis has shown that in a malarious region with high iron-deficiency like (northern) Ghana, iron status of schoolchildren can be improved through the consumption of cowpeas within a school feeding programme. The improvement in iron status is however unlikely to result from the usual/conventional consumption of cowpeas but through fortification of whole cowpea flour with a highly bioavailable iron compound. This thesis has also shown that NaFeEDTA is a suitable iron compound for whole cowpea flour fortification irrespective of whether the cowpea has low or high concentration of polyphenols.

IMPLICATIONS FOR PUBLIC HEALTH PRACTICE AND FUTURE RESEARCH

The consistency in the findings of intervention studies involving NaFeEDTA in inhibitory cereals [60, 61] and legume vehicles collectively establish it as the compound of choice for fortification in Africa and similar settings. However its application at the population level in a place like Ghana may still be hampered by lack of central milling of staple cereal flours [75]. Currently there is no legislation for mandatory fortification in Ghana [75] but even if enacted it will only affect industrially processed flours. Cowpea flour is not in this category confining it to small scale processing. Supplying cowpea flour to schools would require centralized processing and perhaps this could trigger the development of a business model for the fortification of legume flour.

A school feeding programme is a social protection intervention with potential to improve educational participation and nutritional status of school-going children [76]. Even though this thesis shows that participation in school feeding programme seems to benefit micronutrient intake in general and iron intake in particular (chapter 2), iron bioavailability may remain poor due to high native phytate of staples [31]. Using NaFeEDTA fortified food within a school feeding programme will not only improve absorption of fortification iron but also that of native iron in cowpeas [74] thus contributing substantially to dietary iron intake among schoolchildren. There is need however for complementary regular deworming of schoolchildren because apart from preventing blunting of fortification [77] it can also improve anaemia status independent of other interventions [28]. Currently deworming of schoolchildren is not an integral part of the school feeding programme so the Government and partner organizations should consider making deworming an integral and regular component of the school feeding programme.

An important observation in chapter 2 is that home meals were not replaced by school lunch of school feeding beneficiaries. This is contrary to what was reported in Southern Ghana [78]. Given that only two beneficiary schools in one district were studied in this thesis, the observation (no replacement) may not be generalizable to northern Ghana and thus calls for similar studies in other districts.

The assessment of malaria antigenemia halfway through the intervention in this thesis suggests that the fortification group had higher prevalence (45% compared to 35%) though it did not reach statistical significance. A subgroup analysis to further examine whether there was differential prevalence between iron replete and iron-deficient children was not possible because iron status was not measured at mid-intervention therefore baseline iron status was used. This could have affected the relationship between antigenemia and fortification since both were not measured at the same time. The very low prevalence of antigenemia at the end of intervention did not also allow for comparison. There is the need to further examine the influence of NaFeEDTA fortification on malaria morbidity.

The acceptable daily intake (ADI) expressed on body weight basis, is the estimated amount of food additive that can be ingested daily over a lifetime without appreciable health risk [(79)]. For EDTA, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has set it at 1.9 mg/kg body weight [(72)]. Given the inhibitory nature of cowpea flour coupled with the quantity of cowpea flour consumed *per capita* in this thesis, fortification guidelines suggest fortification level of 40 ppm [(73)]. Future research should evaluate the efficacy of cowpea flour on iron status at lower enrichment.

Over the seven months duration of the intervention in this thesis, cowpea flour was milled in two batches and none of the batches was used for more than four months. Monthly samples of the cowpea meal showed stability of fortification but we could not measure effect of extended period of storage on sensory qualities of both the flour and the cowpea meal. Such investigation is desirable in future.

THE TELFUN PROGRAMME – A REFLECTION

The TELFUN programme was designed to demonstrate how focusing on a local and culturally accepted index crop could contribute to improve food sovereignty in developing regions of the world. The hypothesis is that by improving food sovereignty, nutritional status (iron) of the people would also improve. The causes of such a problem as iron deficiency are varied and cut across disciplines. An interdisciplinary team is thus appropriate to find a solution. In the Ghana/Benin arm of the programme the index crop that was investigated by the team (breeder, food technologist, nutritionist and social scientist) is cowpea. The nutrition component has contributed to the TELFUN programme aim by demonstrating that a local, well-embedded index crop like cowpea could be adapted to improve iron status of schoolchildren within a school feeding programme. Specifically the nutrition research has shown that whole cowpea flour is a good vehicle for NaFeEDTA fortification.

The interdisciplinary approach of the programme created a platform to first disagree across disciplines, understand why there was disagreement and to find reasons to agree. Such constructive process eventually created consensus and focus, and the need to coordinate disciplinary studies. The coordinated network study (CNS) at the start of the programme allowed a comprehensive understanding of the problems relevant to actors in the cowpea network and offered opportunity to identify discipline-specific as well as cross-disciplinary questions for further investigations. Interdisciplinarity also created interdependence which did not always work as planned but forced innovation. For instance, the nutrition component was expected to receive and test a cowpea variety with “improved iron and zinc content” from the breeder and “improved processing technique” from the food technologist. Whereas it was unrealistic to receive an improved variety of cowpea from breeder within a short period, it became also clear with time that an improved processing technique of existing cowpeas was unlikely. Thus the links of nutrition with breeding and food technology became weak. But the continuous interactions among these disciplines still helped to move the nutrition research forward.

Even though the original plan to test an improved variety and processing technique was no longer feasible, the food sovereignty concept continued to inform the design of the nutrition intervention trial. Preliminary investigations prior to selection of intervention (chapters 3 and 4) suggested that fortification rather than mere consumption of cowpeas with high native iron could improve iron status of school children. Staying within the concept of food sovereignty, the local food system was explored further for a flour-based local dish that was acceptable and could serve as a fortification vehicle. *Tubani* (a cowpea flour-based steam cooked paste) was eventually identified as a suitable vehicle in consultation with communities and was tested in the intervention. Food sovereignty advocates for the use of local resources to solve local problems, but in the context of cowpea flour fortification as tested in this thesis an external input (fortificant) was introduced. Since the introduction of this external input was in consultation with the stakeholders in the communities and was found acceptable, it supported food sovereignty.

The annual workshops organised by the TELFUN programme improved interaction within and between disciplines. It also sustained interest in the programme and prevented stagnation of individual projects. The rotation of the annual workshops helped the researchers to appreciate the common challenges in the different locations around which the TELFUN programme was formulated. Through this, it was easier to understand and appreciate the socio-cultural context within which each researcher was working; each researcher had a global picture of their local research.

In a semi-controlled setting, the TELFUN programme has served as proof-of-principle that it is possible to work on a common theme despite disciplinary differences and contribute to food sovereignty.

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Summary

Summary

In sub-Saharan Africa children are more likely to have survived the critical first 1000 days of life carrying along unresolved micronutrient deficiencies into the school-age. In addition, the growth spurts during the school-age period also impose high nutrient requirements which are seldom met by dietary intakes and often cause micronutrient deficiencies. Iron-deficiency is the most prevalent micronutrient problem affecting school-age children in sub-Saharan Africa and yet the most difficult to resolve. It is necessary to ensure an adequate iron intake through the diet. However, if in school, the dietary intake of school-age children is less closely supervised by parents and this may affect micronutrient intake. School-based feeding interventions may therefore be a way to improve iron intake of school-age children. Such a feeding intervention would be more sustainable if it embeds locally produced food(s) with the potential to support food sovereignty. In this context, this thesis investigates whether foods based on cowpeas, an indigenous legume crop originating from Africa, can be used in a school feeding setting to improve iron status of school-age children in Ghana. This research was done as part of an interdisciplinary programme known as TELFUN (Tailoring food sciences to Endogenous patterns of Local food supply for Future Nutrition) involving breeders, food technologists, nutritionists and social scientists from Ghana/Benin, India and Ecuador.

The investigations in this thesis comprised cross-sectional studies, chemical analysis of cowpea landraces, an *in vivo* bioavailability study and a randomized intervention trial conducted mainly in Tolon-Kumbungu district of Ghana. In a cross-sectional study, we first investigated the status quo of schoolchildren (5-13 years old) comparing iron status, iron intake and its adequacy between beneficiaries and non-beneficiaries of the Ghana school feeding programme (**Chapter 2**). The results indicated that iron-deficiency and iron-deficiency anaemia are severe public health problems among schoolchildren in northern

Ghana with 8 and 7 out of every 10 schoolchildren affected respectively. It also showed that the probability of adequate dietary iron intake is 0.32 but much larger (~0.90) if schoolchildren benefitted from a school feeding programme. The high but similar prevalence of iron-deficiency among school feeding beneficiaries and non-beneficiaries coupled with the difference in iron intake and adequacy between these two groups suggested that: (i) school feeding did not close the iron gap and (ii) iron bioavailability from the diet may be low. This indicated towards the need to promote consumption of iron dense foods with good bioavailability. Compared to commonly consumed cereals like maize and rice, cowpeas have a higher content of iron and perhaps landraces with high iron content could be promoted to improve iron intake within a school setting.

To understand what factors would be important to consider in promoting consumption of a cowpea variety with high iron content, we conducted a cross-sectional study among 120 mothers-schoolchild pairs using a combined model of the Theory of Planned Behaviour and Health Belief Model in **chapter 3**. Mothers/caregivers intended to give cowpeas to their schoolchildren 2–3 times per week. The positive attitudes of mothers towards cowpea predicted their intention to give them to their schoolchildren but they were worried about the cost, long cooking time and the discomfort their children may suffer after consuming cowpeas. We also analysed the iron, zinc, phytic acid and polyphenols content of 14 locally available cowpea landraces in northern Ghana and found that they contain appreciable amounts of iron (4.9–8.2 mg/ 100 g d.w) and zinc (2.7–4.1 mg/100 g d.w) but also high amounts of inhibitory phytate (477–1110 mg/100 g d.w) and polyphenol (327–1055 mg/100 g d.w). Polyphenol concentration in particular differed (P<0.05) between white and coloured landraces. This led to the conclusion that the conventional consumption of cowpeas may not improve bioavailable iron intake.

This led to the studies in **chapter 4** where *in vivo* iron absorption from cowpea (stable iron isotope labelled) was measured and potential fortificants for cowpea flour were evaluated in a 2×2 cross-over design involving 16 apparently healthy young women. The setup compared white and red varieties of cowpea with different concentrations of polyphenols but similar phytic acid-to-iron molar ratios. The findings showed that iron bioavailability from red and white cowpeas were 1.4 and 1.7%, respectively, in NaFeEDTA-fortified meals and 0.89 and 1.2%, respectively, in FeSO4-fortified meals. Compared with FeSO4, fortification with NaFeEDTA increased the amount of iron absorbed from white and red cowpea meals by 0.05 and 0.08 mg (P < 0.05) respectively. Irrespective of the fortificant used, there was no significant difference in the amount of iron absorbed from the 2 varieties of cowpea. The results suggested that rather than polyphenols, phytic acid-to-iron molar ratios may determine iron absorption from cowpeas. The results further suggested that NaFeEDTA is more bioavailable fortificant in cowpea flour than $FeSO_4$.

In **chapter 5**, the efficacy of NaFeEDTA-fortified cowpea flour in improving iron status was tested in a double-blind randomized controlled trial among 241 schoolchildren (5–12-year-old). Eligible children received either cowpea meal fortified with 10 mg Fe/meal as NaFeEDTA or an identical but unfortified cowpea meal on 3 non-consecutive days for 7 months. The results showed that fortification of whole cowpea flour with NaFeEDTA resulted in improvement of haemoglobin (P<0.05), serum ferritin (P<0.001) and body iron stores (P<0.001), and reduction in transferrin receptor concentration (P<0.001). Fortification also resulted in 30% and 47% reduction in the prevalence of iron-deficiency (ID) and iron-deficiency anaemia (IDA) (P<0.05), respectively.

Overall, the research in this thesis has shown that in a malarious region with high iron-deficiency like (northern) Ghana, iron status of schoolchildren can be improved through the

consumption of cowpeas within a school feeding programme. The improvement in iron status is however unlikely to result from the usual/conventional consumption of cowpeas but through fortification of whole cowpea flour with a highly bioavailable iron compound. This thesis has also shown that the most suitable iron compound for such whole cowpea flour fortification is NaFeEDTA irrespective of whether the cowpea has high or low concentration of polyphenols.

This thesis has provided evidence that cowpea flour fortification can be explored within a school setting to improve iron status and consequently educability of schoolchildren an African setting. Future research should evaluate the efficacy of cowpea flour on iron status at lower enrichment than used in this thesis and assess the effect of extended period of storage on sensory qualities of both the flour and the cowpea meal.

Samenvatting

Samenvatting

Kinderen in sub-Sahara Afrika die hun eerste kritische 1000 levensdagen hebben overleefd, gaan vaak met onopgeloste micronutriëntendeficiënties hun schooljaren tegemoet. Daarnaast vraagt de groeispurt in de schoolleeftijd ook extra nutriënten en deze worden zelden via het voedsel aangeleverd. In sub-Sahara Afrika is ijzertekort het meest voorkomende micronutriëntenprobleem bij schoolgaande kinderen. Helaas is deze ook het moeilijkst te bestrijden. Het is daarom noodzakelijk dat er voldoende ijzer in de voeding zit. Wanneer kinderen naar school gaan, kunnen ouders de voedselinname van hun kinderen echter minder in de gaten houden en dit kan dan ook bijdragen aan een ijzertekort. Interventies op school zouden daarom een goede manier kunnen zijn om de ijzerinname van schoolkinderen te verbeteren. Zo'n schoolvoedingsinterventie zou duurzamer zijn wanneer het gebruik maakt van lokaal geproduceerd voedsel ter ondersteuning van voedselsoevereiniteit. Binnen deze context onderzoekt deze studie of voedsel gebaseerd op *cowpeas*, een inheemse leguminose van Afrikaanse oorsprong, gebruikt kan worden in een schoolvoedingsprogramma ter verbetering van de ijzerstatus van schoolkinderen. Deze studie vormt een onderdeel van een groter interdisciplinair onderzoek met de naam TELFUN ('Tailoring food sciences to Endogenous patterns of Local food supply for FUture Nutrition') waarbij plantenveredelaars, voedseltechnologen, voedingskundigen en sociaal-wetenschappers uit Ghana, Benin, India en Ecuador betrokken zijn.

De studies in dit proefschrift omvatten cross-sectionele studies, chemische analyse van cowpeas rassen, een *in vivo* biobeschikbaarheidsstudie en een gerandomiseerde voedingsinterventie, allen uitgevoerd met name in Tolon-Kumbungu District in Ghana. In een cross-sectionele studie bepaalden we eerst de ijzerstatus, de ijzerinname en of deze inname voldoende was bij schoolkinderen (5-13 jaar oud), en vergeleken inname en status tussen kinderen van scholen die wel en die niet betrokken waren bij het Ghanese schoolvoedingsprogramma (Hoofdstuk 2). De resultaten lieten zien dat 8 van de 10 kinderen ijzerdeficiënt waren en dat 7 van de 10 kinderen bloedarmoede door ijzerdeficiëntie hadden en dit wijst er op dat zowel ijzerdeficiëntie als bloedarmoede door ijzerdeficiëntie ernstige gezondheidsproblemen vormen in noord Ghana. De studie gaf ook aan dat de kans op voldoende ijzerinname via voedsel 0.32 was en dat deze kans veel hoger is (0.90) wanneer het kind deelneemt aan het schoolvoedingsprogramma. Dat de ijzerinname hoger is bij schoolkinderen die deelnemen aan het schoolvoedingsprogramma maar dat de prevalentie van het ijzertekort niet verschilt tussen beide groepen wijst erop dat: (1) de schoolvoeding het tekort aan ijzerinname niet volledig opheft en dat (2) de biobeschikbaarheid van het ijzer in het voedsel mogelijk laag is. Dit geeft aan dat het noodzakelijk is om de consumptie van voedsel met een hoog gehalte aan biobeschikbaarijzer te stimuleren. Vergeleken met gangbare granen zoals mais en rijst, hebben cowpeas een hoger ijzergehalte en misschien kunnen rassen met een nog hoger gehalte gevonden worden die gebruikt kunnen worden om ijzerinname van kinderen in een schoolvoedingsprogramma te verbeteren.

Om te begrijpen welke factoren belangrijk zijn bij de promotie van de consumptie van cowpeas rassen met een hoog ijzergehalte, hebben we een cross-sectionele studie gedaan bij 120 moeder-kindparen. In deze studie maakten we gebruikt van een gecombineerd gedragsmodel gebaseerd op de ‘Theory of Planned Behaviour’ en het ‘Health Belief Model’ (Hoofdstuk 3). Moeders gaven aan dat ze van plan waren hun schoolkinderen 2 tot 3 keer week cowpeas te laten eten. De positieve houding van moeders ten opzichte van cowpeas bepaalde in belangrijke mate hun voornemen om hun kinderen cowpeas te geven. Ze maakten zich echter zorgen over de kosten, de hoeveelheid tijd die nodig is om de cowpeas te koken en het ongemak dat de kinderen ondervonden na het eten van cowpeas. We analyseerden ook het gehalte aan ijzer, zink, phytaten en polyfenolen van 14 lokaal beschikbare cowpeas rassen in noord Ghana. We vonden dat zij aanzienlijke hoeveelheden ijzer (4.9-8.2 mg/100 g drooggewicht) en zink (2.7 – 4.1 mg/100 g drooggewicht) bevatten maar ook hoge gehaltes aan fytaten (477-1110 mg/100 g drooggewicht) en polyfenolen (327-1055 mg/100 g drooggewicht). De polyfenolen concentraties verschilden met name tussen witte en gekleurde rassen. Aangezien fytaten en polypfenolen de absorptie van ijzer remmen, concludeerden we dat de consumptie van conventionele cowpeas rassen de inname van opneembaar ijzer waarschijnlijk niet verbetert.

Dit leidde tot de studies beschreven in hoofdstuk 4. Daarin werd in een *in vivo* stabiele isotopenstudie de ijzerabsorptie uit cowpeas gemeten en werden potentiele ijzerfortificanten geëvalueerd in 16 gezonde jonge vrouwen in een 2 X 2 cross-over studie-opzet. De studie vergeleek witte en rode cowpeas rassen met verschillende polyfenolenconcentraties maar met vergelijkbare *fytaat:ijzer molar ratio's*. Resultaten lieten zien dat de ijzerbiobeschikbaarheid in rode en witte cowpeas 1.4% en 1.7% was in het geval van fortificatie met NaFeEDTA en 0.89% en 1.2% wanneer gefortificeerd werd met $FeSO_4$. Vergeleken met $FeSO_4$, verhoogde de fortificatie met NaFeEDTA de geabsorbeerde hoeveelheid ijzer uit witte en rode cowpeas-maaltijden met 0.05 en 0.08 mg ($P<0.05$). Er was geen significant verschil in de geabsorbeerde hoeveelheid ijzer tussen de twee cowpeas rassen, onafhankelijk van welke fortificant was gebruikt. De resultaten suggereren dat de fytaat:ijzer molar ratio's belangrijker zijn voor de absorptie van ijzer in cowpeas vergeleken met polyfenolen. Ook wijzen ze er op, dat NaFeEDTA een betere beschikbare fortificant is in cowpeas dan $FeSO_4$.

In hoofdstuk 5 is het effect van NaFeEDTA-gefortificeerd-cowpeas meel op een verbetering van de ijzerstatus van 241 schoolkinderen (5-12 jaar oud) in een dubbel-blind gerandomiseerde trial getest. Kinderen ontvingen óf een cowpeas maaltijd die gefortificeerd was met 10 mg Fe per maaltijd in de vorm van NaFeEDTA, óf een identieke niet-gefortificeerde cowpeas maaltijd op 3 niet-opeenvolgende dagen per week gedurende een periode van 6 maanden. De resultaten laten zien dat fortificatie van een cowpeas maaltijd met

NaFeEDTA leidde tot een verhoging van hemoglobine (P<0.05), serumferritine (P<0.001) en ijzeropslag in het lichaam (P<0.001) en tot een verlaging van transferrin-receptor concentratie (P<0.001). Fortificatie leidde ook tot een significant verlaging van de prevalentie van ijzerdeficiëntie (30%), en van de prevalentie van bloedarmoede door ijzerdeficiëntie (40%).

Samenvattend laten de studies in dit proefschrift zien dat in gebieden waar malaria heerst en waar veel ijzerdeficiëntie voorkomt, zoals in noord Ghana, de ijzerstatus van schoolkinderen verbeterd kan worden door consumptie van cowpeas in een schoolvoedingsprogramma. De verbetering van de ijzerstatus vindt waarschijnlijk niet plaats wanneer de conventionele cowpeas worden gegeten maar alleen wanneer de cowpeas maaltijden verrijkt worden met een ijzerfortificant met een hoge biobeschikbaarheid. Dit proefschrift laat ook zien dat NaFeEDTA de meest geschikte ijzerfortificant voor cowpeas meel is ongeacht een hoge of lage concentratie aan polyfenolen.

De studies in dit proefschrift leveren bewijs dat fortificatie van cowpeas meel onderzocht kan worden in een schoolsituatie ter verbetering van de ijzerstatus en het daaropvolgend leervermogen van schoolkinderen in Afrika. Vervolgonderzoek zou zich moeten richten op de evaluatie van het effect van cowpeas meel op de ijzerstatus met fortificant concentraties die lager zijn dan die wij gebruikt hebben in ons onderzoek en op het effect van langdurige cowpeas-opslag op de sensorische kwaliteit van zowel het cowpeas meel als de cowpeas maaltijd.

Acknowledgement

Acknowledgement

Like a symphony orchestra, the final outcome of a PhD thesis is a collaborative effort and support of individuals around the candidate. At the risk of inadvertently leaving out some people, I would like to say THANK YOU to all who have contributed in diverse ways to the composition of my PhD thesis but have not been singled out for mention here.

I first want to thank my family for their love and support throughout my academic journey. I am indebted to my wife (Jennifer Anankani) for her unflinching support and understanding, and for keeping home stable anytime I was away. To my daughter, Noyo Kamilah Abizari, in whom I found renewed strength to strive to the finish line.

Inge D. Brouwer, words are simply not enough to express my gratitude. I got the opportunity for this PhD because you believed in my ability to deliver and throughout the period you never doubted my ability. I am grateful! Your contribution to my research competency, academic and professional development is invaluable. Like a guitarist, you tightened the strings and gave me sleepless nights and when necessary you loosened the strings for me to catch a respite. Like a curator, you nurtured my scientific outlook. You did not give the answers readily even if you had them but your persistent “why?”, “how?” “are you sure?” and “so what?” have always brought out more out of me than I felt capable. Accept my gratitude for the mentorship and friendship you have provided me over the years. You have turned supervision into a super-relationship! To my local supervisor, Margaret Armar-Klemesu (a.k.a Mum), I am grateful for the confidence you had in me, for the unflinching support and for the guidance. I appreciate your crucial role in obtaining ethical approval for my research. Diego Moretti, thank you for being part of my scientific journey. I am grateful for all the critical comments and insight you gave during the formulation and execution of the iron absorption study and the feeding trial. You are such a nice person to work with. Michael B.

Zimmermann, thank you for being my promoter and the global direction you gave to my research. I want to thank you, Joseph Mensah-Homiah, for being part of my supervision team.

I thank members of my thesis examination committee (Prof. Dr. Ir. C de Graaf, Dr. Ir. JCM Verhoef, Dr. Ir. MJR Nout and Dr. S de Pee) for accepting to be on my examination committee and the critical comments during the review of the thesis.

At a point I thought my research was not going to take off because there was no money in my project kitty. After trying unsuccessfully from a number of funding organisations I finally got support from the Nestle Foundation. I am grateful for your financial support and for believing that my research was relevant. To this end I also want to express my deepest gratitude to Anne Mburu de Wagt for her support and for helping identify funding organizations.

At the Division of Human Nutrition in Wageningen, a number of people have contributed in diverse ways to arrange my travels, accommodation and permit, and others have helped me immensely in the laboratory during my numerous analyses. To you, Lous Duym, Eric van Munster, Riekie Janssen, Lucy Elburg, Paul Hulshof, Nhien Ly, Robert, Tineke van Roekel and Marlies Diepeveen-de Bruin, I say *Dank u wel!*

The head teachers of Wayamba (Y. Abdul-Majeed and A. Alaru), Kpalgun (S. Inusah), Tibung (I. Norgah) and Jagbo (A. A. Suhiyini) primary schools have been instrumental in the smooth running of the surveys and the feeding trial. As is always said to teachers "your reward is in heaven" but accept my sincere gratitude here on earth.

During the feeding trial, it was such a pleasant sight to see the kitchen staff (Awuraba Yussif, Latifa Dawuda, Sadia Mutaru and Zeenatu Sulemana) do their work with so much dedication and joy, and also handle the children like their own. You earned the trust of the children with your motherly instincts. *Ni yi tuma pam! Naawun deemi yi suhugu.*

I am grateful to the following research assistants who worked with me during different stages of the research project: Kamal-Deen Djabaku, Sadia Mamani, Dawuda Issahaku, Eliasu Yakubu, Alhassan Osman, Nasira Abizari, Abdul-Razak Wumbei, Yussif Ayine, Jane Pedavoah, Abdul-Wadudu Mohammed, Sharifa, Zulfawu and Mohammed Lawal.

During the different stages of the field work in Ghana, I also worked with eight uniquely different MSc students from Wageningen who have contributed in diverse ways to the success of my research project. I am grateful to you; Christiana Buxton, Vera Lugutuah, Nerisa Pilime, Liselot Lemmens, Lien van den Broeck, Miwako Nagasu, Noortje Creemers and Judith Hulst.

To you my colleagues in the international PhD student community; Catherine Macharia-Mutie, Nadia Fanou, Yara Koreissi, Sophie Ngala, Varsha Rani, Maria-Elisa, Elise Talsma, Rina Agustina, Sueppong Gowachirapant, Akwilina Mwanri and Wanjiku Gichohi, I say thank you for your support and company through the years.

The interdisciplinary nature of the TELFUN project has been an eye-opener for me and provided the platform for cross fertilization and constructive conflict of ideas. I am thankful for the opportunity to work on the project. I say to all the supervisors and project leaders *bedankt, shukria, merci, gracias!* Thank you (Joost Jongerden) for the diligent coordination of the project activities.

Kwadwo Adofo (the consultant), Yann Madode (team leader) and Wilhemina Quaye (sovereignty specialist) you made my integration into the TELFUN family much easier and shared with me insights into the programme that gave me leap into my research. It has been nice working with you. To the rest of my colleagues on the TELFUN project from India and Ecuador, I am grateful for your friendship and scientific intercourse.

A family away from home is what you need to help you break away from the "room-office-room" pattern that characterizes the sandwich PhD student's life in Wageningen. The Hoek family (Steven, Monica and children) was there for me. Thank you for allowing me into your home and often sharing your meals with me. Thank you, Samson Nibi and the Ghanaian student community in Wageningen for all the "Azonto" parties and friendship.

I am thankful to Akzo Nobel functional chemicals in The Netherlands, DSM South Africa and YEDENT Agro-food processing company in Ghana for their technical support during the formulation of the fortified flour for the intervention trial.

Elise Talsma and Martin Ndegwa Mwangi, I thank you for accepting to be my paranymphs and for all the effort you put into organizing the graduation ceremony.

I am grateful to the Dr. Judith Zwartz Foundation for the financial support in printing this thesis.

Finally I thank all parents/caregivers and their children, and opinion leaders of respective communities for their participation and cooperation throughout the different phases of the field world.

Thank you ALL

About the author

About the Author

Abdul-Razak ABIZARI was born on 25th August 1974 in Tamale, Ghana. He completed his secondary school education in 1994 and enrolled to be a professional teacher at the Bagabaga Training College, Tamale, Ghana. In 1996, halfway through the teacher training, he left to start his bachelor degree in the University for Development Studies (UDS). He graduated in 2000 with BSc in Community Nutrition.

He did his National Service in 2001 with the Food and Nutrition Security Unit (FNSU) of UDS as a research assistant on a UNICEF/IFPRI/UDS collaborative research. In 2002 he was employed as a senior research assistant in the FNSU of UDS where he continued to work on the UNICEF/IFPRI/UDS collaborative research. Later in 2002 he obtained post-BSc diploma in Food and Nutrition Security for Developing Countries at the International Agricultural Centre (now known as Centre for Development Innovation), Wageningen, Netherlands.

Abdul-Razak was back in the Netherlands in 2003 for MSc degree in Nutrition and Health at the Wageningen University. When he completed in 2005 he went back to the University for Development Studies and took up a teaching position in the School of Medicine and Health Sciences of UDS, a position he holds till date. He also coordinates the Third Trimester Field Practical Training (TTFPT) and the Community-Based Education and Service (COBES) for the School of Medicine and Health Sciences.

In 2008 he got a sandwich PhD position on the interdisciplinary TELFUN research programme where he worked as the nutrition researcher on the West African arm of the project. He won a research grant from the Nestle Foundation to support his PhD research in Ghana and the Netherlands.

List of publications

List of Publications

Peer reviewed articles

Leroy JL, **Abizari AR**, and Habicht JP. Only Children of the Head of Household Benefit from Increased Household Food Diversity in Northern Ghana. J. Nutr. 138: 2258–2263, 2008

Quaye W, Adofo K, Madode Y and **Abizari AR**. Exploratory and Multidisciplinary Survey of the Cowpea Network in the Tolon-Kumbungu District of Ghana: A Food Sovereignty Perspective. African Journal of Agricultural Research Vol. 4 (4), pp. 311-320 April, 2009

Abizari AR, Moretti D, Zimmermann MB, Armar-Klemesu M, Brouwer ID. Whole Cowpea Meal Fortified with NaFeEDTA Reduces Iron Deficiency among Ghanaian School Children in a Malaria Endemic Area. J Nutr. 2012 Oct;142:1836-42.

Abizari AR, Moretti D, Schuth S, Zimmermann MB, Armar-Klemesu M, Brouwer ID. Phytic Acid-to-Iron Molar Ratio Rather than Polyphenol Concentration Determines Iron Bioavailability in Whole-Cowpea Meal among Young Women. J Nutr. 2012 Nov;142:1950-5.

Conference Papers and Posters

Dittoh, S, **Abizari, AR** and Akuriba, M. Agriculture for Food and Nutrition Security: A must for Achieving the Millennium Development Goals in Africa. African Association of Agricultural Economists (AAAE), 2007 Second International Conference, August 20-22, 2007, Accra, Ghana.

Abizari, AR. Contribution of Food-Based Interventions to the Consumption of Vitamin-A-Rich Foods Among Children 6 - 36 Months in Savelugu-Nanton District of Northern Ghana. Poster presented at the Second Africa Nutritional Epidemiology Conference, October 2008, Cairo, Egypt.

Abizari, AR, Pilimi, N, Armar-Klemesu, M and Brouwer, ID. Predictors of Cowpea Consumption Among School Pupils in Northern Region of Ghana. Poster presented at the International Congress of Nutrition (ICN), October 2009, Bangkok, Thailand.

Abizari, AR, Buxton, C, Armar-Klemesu, M and Brouwer, ID. Contribution Of School Lunch to Improvement In Micronutrient (Iron) Status Of School Pupils In Tolon-Kumbungu District Of Northern Ghana presented at the 19th International Congress of Nutrition (ICN), October 2009, Bangkok, Thailand.

Abizari, AR, Hulst, J. Moretti, D. Armar-Klemesu M and Brouwer ID. The association between malaria morbidity, anaemia and the consumption of iron fortified cowpeas among school children in rural northern Ghana. Poster presented during PhD study tour, Monterrey, Mexico, October 2011.

Abizari AR, Moretti D, Zimmermann MB, Armar-Klemesu M, Brouwer ID. The effect of NaFeEDTA fortified cowpea meal on iron status of school children in northern Ghana. World Nutrition Congress, April 2012, Rio de Janeiro, Brazil.

Abizari AR, Moretti D, Schuth S, Zimmermann MB, Armar-Klemesu M, Brouwer ID. A comparison of iron bioavailability from NaFeEDTA and $FeSO_4$ added to cowpea-based meals. Nutrition Congress Africa, October 2012,Bloemfontein, South Africa.

Training activities - overview

DISCIPLINE SPECIFIC ACTIVITIES (DSA)

Courses	***Place***	***Date***
Training in Food Consumption studies	Wageningen, Netherlands	2008
Principles of Research in Medicine and Epidemiology	Erasmus Univ. Rotterdam	2009
Clinical Trials	Erasmus Univ. Rotterdam	2009
Production, Management and Use of Food Composition Data, VLAG	WICC, Wageningen	2009
Lecture/seminar		
Sovereign wheat production: integrated and environmentally friendly production, processing and consumption of wheat (Zeeuwse Vlegel)	Zeeland, Netherlands	2008
Food sovereignty: origins, meaning and relation with other discourses	Wageningen, Netherlands	2008
Technologies for sustainable food networks: does locality matter?	Wageningen, Netherlands	2008
Seminar on food Sovereignty	WICC, Wageningen	2008
TELFUN workshops with oral presentation		
Second TELFUN Workshop	Quito, Ecuador	2008
Third TELFUN Workshop	Haryana University, India	2009
TELFUN mini-workshop	Wageningen, Netherlands	2009
Fourth TELFUN Workshop	Univ. for Devt. Studs, Ghana	2010
Conferences		
3rd African Nutritional Epidemiology Conference	Egypt, Cairo	2008
19th International Congress of Nutrition	Bangkok, Thailand	2009
Wageningen Nutrition Forum	Arnhem, Netherlands	2009
World Nutrition Conference	Rio de Janeiro, Brazil	2012
Nutrition Congress Africa (NCA 2012)	Bloemfontein, South Africa	2012
Africa Study Day	Wageningen, Netherlands	2012

GENERAL COURSES (GC)		
Presentation Skills	Wageningen, Netherlands	2008
Information literacy and Endnote	Wageningen, Netherlands	2008
Introduction to Data Analysis	Erasmus University, Rotterdam	2009
Regression Analysis	Erasmus University, Rotterdam	2009
Scientific Writing	Wageningen University	2010
Epigenesis and Epigenetics, 2nd Edition	Wageningen University	2011
10th Africa Nutrition Leadership Programme	South Africa	2012
Master Class - Management of SAM	Bloemfontein, South Africa	2012

OPTIONAL COURSES AND ACTIVITIES (OCA)		
Preparation PhD research proposal	Wageningen, Netherlands	2008
Journal Club (International Nutrition)	Wageningen, Netherlands	2009 – 2010
Staff seminar	Wageningen, Netherlands	2008 – 2012
International PhD Study Tour, VLAG	Mexico, South-West USA	2011
Supervision of 8 MSc students	Tamale, Ghana	2008 – 2011

The research described in this thesis was financially supported by: the Interdisciplinary Research and Education Fund (INREF) of Wageningen University through the TELFUN Project and Nestle Foundation for Nutrition Research.

Financial support from Dr Judith Zwartz Foundation for printing this thesis is gratefully acknowledged.

Cover
Inspired by Abdul-Razak ABIZARI and designed by Mubarak Abdul-Rahaman

Layout
Abdul-Razak ABIZARI

Printing
GVO drukkers & vormgevers B.V., Ponsen & Looijen