# *Efficacy of amaranth grain (Amaranthus cruentus) on anaemia and iron deficiency in Kenyan pre-school children*

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This research was conducted under the auspices of the Graduate School VLAG (Advanced Studies in Food Technology, Agro-biotechnology, Nutrition and Health Sciences)

# *Efficacy of amaranth grain (Amaranthus cruentus) on anaemia and iron deficiency in Kenyan pre-school children*

*Catherine W. Macharia-Mutie*

**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 7th September, 2012
at 1.30 p.m. in the Aula.

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Efficacy of amaranth grain (*Amaranthus cruentus*) on anaemia and iron deficiency in Kenyan pre-school children
168 pages

Thesis, Wageningen University, Wageningen, the Netherlands (2012)
With references, summaries in English and Dutch
ISBN 978-94-6173-291-0

*To my beloved husband Mutie,*
*Daughters, Mwende & Muthoni ;*
*Who have been pillars of strength.*

# *Abstract*

## Background

Adding iron rich foods such as amaranth grain flour or micronutrient powders (MNP) containing low doses of highly bioavailable iron (e.g. NaFeEDTA) could be options to control iron deficiency (ID) in pre-school children. However, data evaluating the impact of such food-to-food or in-home fortification strategies is limited. The aim of this thesis was to investigate the effect of consumption of amaranth grain flour on anaemia and iron status of Kenyan pre-school children.

## Methods

First a nutrition situation was assessed through a food consumption and nutrition study. We then simulated the potential effect of fortifying maize porridge with amaranth grain flour or MNP on prevalence of inadequate iron intake and ID. Next, an acceptability study was conducted. Factors that would predict the intention of consumption of amaranth porridge among children were identified and the sensory acceptability of amaranth porridge evaluated. Finally, a controlled, randomized 16-weeks intervention trial was conducted (n=279; aged 12-59 months) to determine the efficacy of maize porridge enriched with amaranth grain flour or MNP in form of NaFeEDTA. Primary outcome was anaemia and iron status with treatment effects estimated relative to control group receiving maize porridge only.

## Results

The nutrition situation assessment showed that there were deficits in dietary energy, iron and zinc with early introduction of complementary foods. The simulation estimates resulted in a significant increase ($P$<0.005) in ferritin concentration in amaranth (1.82µg/L: CI 1.42-2.34) and MNP (1.80µg/L: CI 1.40-2.31) group compared to maize porridge group decreasing the prevalence ID to 27% in both groups.

In the acceptability study, knowledge and health value significantly predicted health behaviour identity. None of the models significantly predicted intention. A significant preference for the unfermented amaranth enriched maize porridge compared to the

fermented porridge was observed. There was no difference in preference of the 50:50 and 70:30 (amaranth to maize) unfermented porridges.

Baseline prevalence of stunting was (48.2%), anaemia (38%), ID (30%) and iron deficiency anaemia (IDA, 22%) in the clinical trial. Though consumption of amaranth grain porridge reduced anaemia, ID and IDA prevalence compared to control group, this reduction was not significant. Consumption of MNP porridge significantly reduced the prevalence of anaemia by 46% (CI: -67% to -12%), ID by 70% (CI:-89% to -16%) and IDA by 75% (CI: -92% to -20%) compared to control group. MNP porridge significantly increased haemoglobin by 2.7g/L (CI: 0.4 to 5.1) and ferritin (40% CI: 10% to 95%), and decreased TfR by 10% (CI:-16% to -4%) concentration.

## Conclusion

Addition of amaranth grain flour to maize porridge even when shown to have high iron content did not significantly reduce the prevalence of IDA in pre-school children. In contrast, consumption of maize porridge fortified with 2.5mg of NaFeEDTA can reduce the prevalence of IDA in children. Absorption studies for amaranth grain should be conducted before its public health promotion in dietary diversification as a source of iron is accelerated. Research to assess the safety of bolus doses of MNP formulated with NaFeEDTA and optimal dosing schedule is also needed. Furthermore, the effectiveness of both amaranth grain flour and MNP should be further evaluated in large scale programs.

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# *Chapter 1*

# *General Introduction*

## Introduction

In iron deficiency (ID), body iron stores are fully exhausted and some degree of tissue ID is present (1). ID develops gradually beginning with a negative iron balance (iron depletion) whereas iron deficiency anaemia is an advanced stage of iron depletion (1, 2). IDA is characterized by ID and haemoglobin concentration that is below the 95th percentile of the distribution in a population (2, 3). Iron deficiency anaemia is a significant public health problem especially in young children in developing countries as their iron needs are proportionately greater than their energy needs (1). IDA is associated with impaired cognitive development, morbidity and death (4, 5). Nutritional deficiencies (esp. vitamin A deficiency), infectious disorders (such as malaria), and haemoglobinopathies may lead to anaemia (6, 7) but iron deficiency (ID) is generally considered the main cause and assumed to account for approximately half of this problem (8).

Global estimates indicate that nearly half (48%) of pre-school children are anaemic and the highest proportions (68%) are in Africa (9). In Kenya, 70% of the pre-school children are anaemic and nearly half (43.2%) have been estimated to be iron deficient (10). Other recent smaller studies in different regions in Kenya have estimated the prevalence of iron deficiency to be 53% among children aged 2-36 months (11) and 41% among children aged 6-35 months (12).

Recent evidence indicates that the first 1000 days of life (period beginning with the woman's pregnancy and continuing into the child 2$^{nd}$ year) is the most critical as this is where nutritional deficiencies have a significant and often irreversible adverse impact on child survival and growth affecting their ability to learn in school and productivity in later life (13, 14). To achieve optimal growth, children need enough good-quality food which in turn also supports their micronutrient status. Nutritional interventions for pre-school children may therefore achieve greater benefits including improved iron intake and status.

## Dietary iron and its bioavailability

Iron is an integral part of proteins and enzymes that maintain good health and plays a major role in oxygen transport and in the immune system (3). Haemoglobin and myoglobin form the functional compartment of body iron involved in cellular metabolism while ferritin or hemosiderin is the storage form (5, 15). Iron is not actively excreted from the body and its balance is predominantly maintained by a regulation of iron absorption from the diet in the proximal small intestine (2, 6). In food, iron is present either as heme iron (mainly in animal sources) or non-heme iron mainly from plant sources. Non-heme iron is in ferric ($Fe^{3+}$) form and must be reduced to ferrous ($Fe^{2+}$) form or solubilized and chelated in the stomach to be available for absorption in the less acidic proximal small intestine (16, 17), while heme iron is more easily absorbed. Absorption is regulated by hepcidin, a hormone secreted by the liver and is dependent on iron status where iron depletion up-regulates absorption (18).

Nutritional iron deficiency arises when physiological requirements cannot be met by iron absorbed from the diet mainly due to a low level of dietary iron in combination with a low bioavailability of the iron. In developing countries particularly, where mainly plant-based diets are consumed, the main part of the dietary iron is non-heme. Contrary to heme iron with absorption of 15-35%, the absorption of non-heme iron from these diets is often less that 10% (6) due to presence of absorption inhibitors such as phytates and polyphenols (19) and the absence of enhancers such as vitamin C or meat (20).

Phytates and polyphenols precipitate iron and form insoluble hydroxides making it unavailable for absorption (16) and their effect is more pronounced with increased concentrations (21, 22). One mole of phytic acid binds 6 moles ferric iron so that even relatively small quantities of residual phytate are still strongly inhibitory (23). The molar ratio of phytic acid to iron should therefore be reduced to $<1$ and preferably to no more than 0.5 (corresponding to 20-30 mg phytic acid/100g dried product) before a meaningful increase in iron absorption is observed (24-26). Using stable isotope studies, an additive inhibitory effect of polyphenols has been found in high phytate and polyphenol plant based foods served as single meals (27). This inhibitory effect is reduced in the presence of ascorbic acid (21, 23, 27) and meat due to their ability to reduce ferric to ferrous iron and its potential to chelate iron (28). The enhancing effect of native ascorbic acid is dose and food matrix dependent (23, 28).

An ascorbic acid to iron molar ratio of at least 2:1 has been shown to enhance iron absorption from milk products and low phytate foods. However, a molar ratio of at least 4:1 is essential to reduce the inhibitory effects from foods high in phytic acid or phenolic compounds (29). The "meat factor" is thought to be within the protein fraction of muscle tissue with a possibility of involvement of other components such as cysteine-containing peptides (30). Myofibrillar proteins in meat are digested extensively by pepsin in the stomach and thus could bind iron and prevent its precipitation at the higher pH of the duodenum, supporting absorption of iron (28).

## Strategies to control iron deficiency anaemia

Nutritional strategies to combat iron deficiency anaemia focus on either iron intake and/or improving bioavailability of dietary iron. Economic costs and benefits of intervention strategies to control micronutrient deficiencies have been recognized hence positioning them on the global agenda (14, 31). The Copenhagen Consensus 2008 highly ranked iron fortification as a way of advancing global well-being particularly in the developing countries (32).

Previously, UNICEF and WHO recommended preventive iron supplementation for all children where the prevalence of ID is >30% (33) as supplementation with iron alone or iron containing multiple micronutrients have been demonstrated to significantly reduce the prevalence of iron deficiency anaemia (34-36). However, a study among pre-school children in Pemba, Tanzania, showed increased risk of adverse effects including increased risk of hospitalization and mortality especially in iron replete children (37). Following these findings, WHO currently recommends that iron supplementation for children <2 years of age in malaria endemic regions such as Kenya should only be used after screening for ID and in settings with adequate access to anti-malarial treatment (38). This recommendation however has currently been challenged as a more recent study shows that even in conditions of excellent access to health care, supplementation with iron may also be unsafe for iron deficient children in malaria endemic areas (39).

Poor consumer compliance and ineffective health distribution systems to enhance delivery and coverage are major barriers to success for many supplementation programmes in developing countries (8, 40). In addition, widespread screening for ID may not be practical due to logistics and cultural taboos (41) necessitating scaling up of food-based strategies to address ID problems in these areas.

Food-based strategies have been recommended as promising approaches to meet micronutrient needs (42) and the benefits include not only improved intakes of specific nutrients but also improved overall diets and health status (43). Food-based strategies that could be used to combat iron deficiency anaemia include dietary diversification/modification, fortification and bio-fortification. Efforts have been made to increase the level and/or bioavailability of essential nutrients in crops by traditional plant breeding or genetic engineering (bio-fortification). Food staples currently earmarked for bio-fortification include cassava, maize and sweet-potato to improve vitamin A content while rice, wheat, pearl millet and beans have been considered for iron/zinc content increment (44). Most bio-fortified crops are still in the development pipeline with the exception of orange-fleshed sweet potato that has already been successfully released (44, 45). There are still difficulties however in achieving efficacious iron bio-fortification in crops especially in those rich in phytic acid and polyphenols such as beans (27).

Fortification offers a practical alternative and has been shown to improve anaemia and iron status in pre-school children (46-49). The approach used at population level could either be mass fortification of foods regularly consumed by the general public or targeted fortification for specific population subgroups such as older infants and young children (50). In developing countries however, mass and targeted iron fortification programmes have been less effective due to the logistics of implementation, low purchasing power and use of own produce rather than centrally produced food in poor resource settings. Further, use of iron compounds with low bioavailability, low compliance, and high prevalence of infections has also reduced the impact of population level fortification (50-52). At the individual level, home fortification with micro-nutrient powders (MNP) is gradually being implemented in developing countries. MNP have been shown to be acceptable and capable of reducing iron deficiency anaemia in resource-poor settings in South Africa, Ghana, Burkina Faso and India (53-58). MNP containing high bioavailable iron in low doses have been suggested to be safer than powders with higher iron doses (38, 59) and can be easily incorporated into the recommended child feeding practices.

## Dietary diversification

Dietary diversification is a strategy that aims to enhance the availability, access, and utilization of locally available and acceptable foods with a high content and bioavailability of micronutrients throughout the year. It involves changes in food production practices, food selection patterns, and traditional household methods for preparing and processing indigenous foods (43, 60). Dietary diversification has been suggested as part of an effective strategy for reducing ID and offers key advantages in developing countries where most of the rural households largely depend on what they cultivate for their nutrition (61). In some instances a not so commonly used food may be included in a food diversification program because of its potential to be adapted to local conditions allowing it to be affordable (62). This approach can be used to enhance the awareness of micronutrient malnutrition as well as to empower the community to become more self-reliant (62). Amaranth grain is one such food which consumption in Kenya is recently increasing due to promotional activities that target its production and encourage its use as a healthy food (63).

Amaranth, a pseudo-cereal, is a subtropical gluten-free crop originally cultivated in ancient Mexico. Amaranth grain is a nutritionally promising choice to enrich maize porridge usually taken by young children (64). Amaranth grain is rich in high quality protein with lysine and sulphur-containing amino acids, which are normally limiting in maize and pulse crops respectively (65-67). The total mineral content in amaranth grain varies between and within species but is usually higher than that observed in conventional grains with iron being shown to be at least two times more than in wheat (68). Amaranth grain grown in Kenya has been found to have a phytate content of 7.9 mg per 100g, with much lower levels that could not be detected after germination (69). Levels of phytic acid have been found to be lower than that of corn and wheat but higher than that of rice and millet (70). Amaranth grain (*Amaranthus cruentus*) also contains polyphenols [for example, tannin content of 0.8% catechin equivalents (69)] which are important as dietary anti-oxidants and their content has been found to be lower than those in sorghum and millet and other pseudo-cereals such as *quinoa*, (70-72). *Amaranthus cruentus* species has been shown to give high yields in arid and semi-arid areas thereby being suggested as a crop with a potential to extend arable marginal lands in stressful environments (73).

## Determining the efficacy of dietary diversification using amaranth grain

In this thesis, we evaluated the efficacy of a dietary diversification strategy with amaranth grain on iron status among pre-school children. The approach used comprised 4 steps as depicted in **Figure 1.1**. Before successful selection of an appropriate nutrition intervention strategy, a nutrition situation analysis and knowledge of the local dietary patterns and food beliefs is required. Knowledge of the usual consumed foods can help in identifying the micronutrients that are most likely to be lacking in the diet, confirm the need and provide a rationale for an intervention program (50).

A simulation (step 2) using the result of the nutrition situation analysis is essential. The results from the simulation may inform on the potential of different strategies to improve iron intake and status thus giving direction on the best possible strategies that could be further investigated. A food belief study allows for the identification of positive and negative social cognitive factors as well as the interrelationships among these factors which may influence both production and consumption of amaranth grain (step 3). In addition, identification of sensory characteristics that may influence the uptake of amaranth grain is essential. Sensory attributes are reliable predictors of food acceptance (74) and would play an important role in the selection of amaranth grain. The next step comprises an efficacy study (step 4) to quantify the effects of dietary diversification with amaranth grain on iron status. In the efficacy study, a comparison with other strategies that have been shown to be efficacious such as home fortification should preferably be implemented.

Many studies investigate the efficacy of only one nutrition intervention strategy at a time. However, for policy makers the choice of one strategy over the other is informed by comparing different approaches for example dietary diversification with amaranth grain versus home fortification with MNP preferably in similar conditions. Together with data from other studies, these efficacy studies support informed policy decisions on interventions to be implemented on a larger scale. Large scale implementation is guided by an assessment of effectiveness (through monitoring and evaluation) to improve the impact of the approach. In the following paragraphs, knowledge gaps in the steps up to efficacy trial will be discussed. The effectiveness of a large scale implementation is not addressed in this thesis as it was beyond its scope.

**Nutrition situation: Dietary patterns and diversity**

Similar to fortification, dietary diversification program effectiveness depends on selection of appropriate foods with adequate levels of micronutrients (75). WHO and FAO recommend collection of detailed baseline information on dietary patterns and intakes of micronutrients of interest as well as biochemical data on nutritional status prior to designing and initiating food- based intervention programs (50). Consumption surveys provide information integral to the design and periodic adjustment of comprehensive nutrition interventions that meet the needs of the specific populations targeted. Lack of solid information on dietary patterns in Kenya and most of other developing countries has been a barrier to designing and implementing comprehensive interventions to predict the risk and address nutritional deficiencies due to inadequate intake (76, 77).

Previously food consumption studies have been done in Kenya including assessing micronutrient intakes but in other areas (78, 79) or in other target groups (80, 81). Regional specific information on iron intakes among pre-school children in our study area is lacking as earlier studies have described the dietary patterns limited to energy and protein intake (82-84). Moreover, dietary diversity of complementary foods which has been suggested as a simple measure for estimating adequate intake of micronutrients (85), and shown to better identify children with low micronutrient adequacy (86) has not been described in this area. There is therefore need for a systematic description of the dietary patterns and diversity in this study area with an emphasis on iron intake.

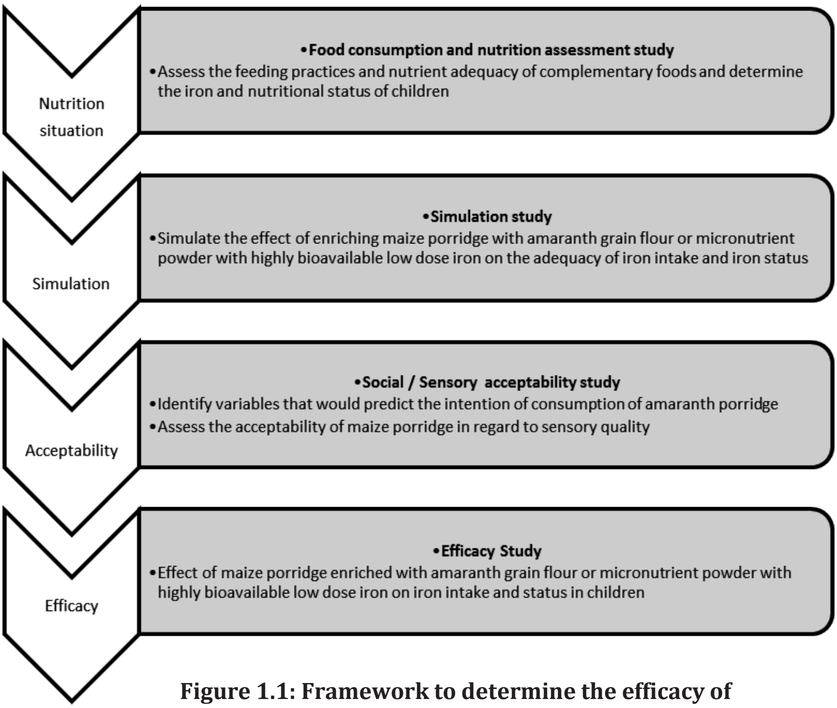



**Figure 1.1: Framework to determine the efficacy of food-based strategies**

**Simulation of the impact of fortification of maize porridge with amaranth grain**

The use of simulation models to estimate impact of strategies on the potential target population prior to actual interventions is an essential step. Previous studies have estimated intervention effects of zinc (87, 88) and iron (88) bio-fortification, as well as increased use of animal source foods (80) to reduce prevalence of inadequate zinc and iron intakes. However, none has attempted to estimate the possible effect of dietary diversification using plant-foods such as amaranth grain and in addition the possible effect on iron status. Estimation of change in prevalence of inadequate intake only may provide an overestimation of the expected effect as other factors beyond intake influence the absorption of iron and by extension affect the change in prevalence of iron deficiency in real life situation. Therefore, estimation of changes in biochemical data such as haemoglobin or serum ferritin may also provide extra information that could give indications for achievable reductions in the ID burden for a chosen intervention.

In performing simulations using individual level intake data from food consumption surveys and nutritional status, some of the assumptions are basic to predicting potential future scenarios, while others are made because of inadequate data (50, 89). General steps to simulate the effect of micronutrient additions have been highlighted by Allen *et al* (50). For purposes of this thesis, three steps would be included. These are; examination of the prevalence of inadequate iron intakes in the specific population groups (12-59 months), calculation of the reduction in the prevalence of inadequate intake (i.e. the proportion below the EAR) that would be expected to occur with the intervention and estimation using biochemical data (serum ferritin) to give indications of the intervention effect on prevalence of ID.

**Acceptability of amaranth grain in child feeding**

To achieve the intended objective of dietary diversification in the target population, a food or food product must have preferred sensory properties as well as be socially and culturally acceptable (90, 91). Sensory attributes such as taste, texture and smell can be reliable predictors of food quality and may influence an individual's intake behaviour of a particular food (91). A good prospect for improving consumer acceptance of a relatively unfamiliar food, for example amaranth enriched maize porridge, is the incorporation of a sensory evaluation study into the dietary diversification program (90). Previously sensory evaluations of amaranth enriched corn tortillas and a malted pearl millet or malted barley with amaranth weaning mixture showed that the products were considered to offer good sensory attributes (92) and were acceptable for the children (93). Though with inconsistent observations, other studies have also showed that 15-30% of the flour used for making bread and conventional pound cakes could be replaced by amaranth grain flour without negatively affecting the sensory attributes (94-96). Sensory evaluation of amaranth grain enriched maize porridge for complementary feeding however has not yet been systematically studied in Kenya as it is not yet commonly used in complementary food preparation (69). Therefore to assess whether young children would have a preference for the amaranth grain flour when incorporated into maize flour porridge, more sensory studies with young children and caregivers who make a choice on the food to give their children are necessary. The information gained from the sensory evaluation would allow for the adjustment and identification of the consumer likings and preferences and be used to prepare the best combination of maize amaranth porridge consequently improving acceptance during the intervention and beyond.

Social cultural factors have been suggested to be particularly important in influencing the development of preference for foods (97). Identification of these factors is essential when designing nutrition intervention programs. Socio-psychological theories such as Theory of Planned Behaviour (TPB) hold that a person's behaviour is determined by his/her intention to perform the behaviour, which is predicted by: behavioural intention, attitude (person's judgment about performing a behaviour) and subjective norms (perception of a person of the social pressure from 'important others' to perform or not the behaviour) and perceived behavioural control (people's perceptions of their ability to perform a given behaviour) (98). TPB proposes that the more favourable the attitude and the subjective norms, and the greater the perceived control the stronger the person's intention to perform the behaviour in question and in this case consumption of amaranth grain (98). The efficacy of the TPB as a predictor of intentions and behaviour has been evaluated and it was found that it may explain at least 20% of the variance in prospective measures of actual behaviour (99). Another psychological model, Health Belief Model (HBM), proposes that a person will take a health–related action if he/she feels that a negative health condition can be avoided, has a positive expectation that by taking a recommended action will avoid a negative health condition, and believes that he/she can successfully take a recommended action (100). A multicomponent framework which integrates salient determinants of original models has been suggested in identifying determinants of dietary behaviour (101). A combination of TPB and HBM models as applied by Sun *et al* (2006) (102) and adapted with modifications by Fanou-Fogny *et al* (103) **(Figure 4.1)** may probably therefore explain a larger variance in outcome constructs behaviour (102).

There are few studies in sub-Saharan Africa studies applying psycho-social theories such as TPB and HBM to study nutrition behaviour (103, 104), as the trend has been to use qualitative methods such as focus group discussions (105), attribute-pile-sorting (APS) and knowledge, attitudes, and practices (KAP) methodologies (106). Additionally though psycho-social theories have often been used to explain factors influencing fruit and vegetable consumption (101), studies investigating on cereal consumption are fewer (103) and none explains amaranth grain consumption. Exploring the factors influencing amaranth grain consumption provides essential information for designing nutrition education messages in the community.

**Efficacy of amaranth grain**

Evaluation of efficacy and effectiveness of dietary diversification to improve iron content from complementary foods under realistic conditions has strongly been recommended (107). Studies evaluating the efficacy of dietary diversification on iron status have primarily focused on animal source foods (108, 109) while those using plant based sources have focused on vitamin A (110-114). The potential for plant sources even when found to contain high levels of iron to make a major contribution to the control of iron deficiency in developing countries is still questioned (115) especially so due to the negative influence of phytic acid and polyphenols on iron absorption. Consequently information on efficacy of foods such as amaranth grain which have been found to contain high levels of iron (68) on iron status particularly among pre-school children is still lacking. Nevertheless, there is indication that whereas the phytate level usually increases with iron level, the phytate:iron molar ratio usually decreases possibly improving iron absorption (27). Furthermore, the inhibitory effect of phytate in cereals can be partially overcome by milling (27, 28), although this often also reduces the iron content. The role of amaranth grain therefore needs to be well quantified to demonstrate its efficacy on iron status compared to other food based approaches as its production and consumption in Kenya continues to increase.

## Aim and thesis outline

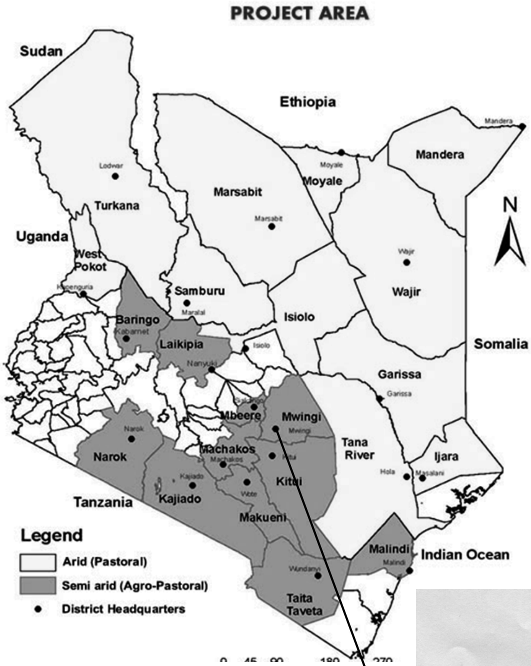
The primary aim of this thesis was to investigate the effect of consumption of maize porridge fortified with amaranth grain flour on iron intake, iron status and anaemia of Kenyan pre-school children. To achieve our primary objective, the study had the following secondary objectives;

1. To assess the dietary pattern and nutrient adequacy of complementary foods with a focus on iron intake and determine the iron and nutritional status of children.
2. To simulate the effect of enriching maize porridge with amaranth grain flour or low dose highly bioavailable iron MNP on the adequacy of iron intake and iron status among children.
3. To identify socio-cultural factors that would predict the intention of consumption of amaranth porridge.
4. To assess the sensory acceptability of fermented and non-fermented amaranth enriched maize porridge.
5. To investigate the effect of consumption of maize porridge fortified with low dose, NaFeEDTA on iron intake, iron status and anaemia of Kenyan pre-school children.

This thesis presents results of work done in four sub-studies following the framework as indicated in **Figure 1.1** and organized into six chapters. **Chapter 1** provides the background information for the study with literature review on key components of the study. To achieve the first objective, a food consumption and nutrition assessment was done through a cross-sectional survey. The dietary patterns as well as the nutrient adequacy of complementary foods of children 12-23 months are described in **Chapter 2**. In **Chapter 3,** we estimated the effect of enriching maize porridge with amaranth grain flour or micronutrient powder on the adequacy of iron intake and their potential in reducing iron deficiency prevalence using theoretical simulations. In **Chapter 4,** the sensory attributes of amaranth enriched maize porridge were assessed and the socio-cultural factors influencing amaranth grain consumption identified. The data obtained in Chapter 2-4 formed the basis for generating the hypothesis of the feeding trial in the study area. **Chapter 5** concerns the feeding trial in which the efficacy of amaranth grain on iron status was assessed. This chapter explores if enriching maize porridge with amaranth grain or MNP can improve iron status and reduce iron deficiency among pre-school children. Finally, in **Chapter 6** the main findings and conclusions as well as the implications for public health policy and future research of this thesis are discussed.

## Study area

The study was conducted from November 2007 to February 2011 in Migwani division within the larger Mwingi District in Eastern Kenya **(Figure 1.2)**. Migwani is situated 200 km North-East of Nairobi and falls within an arid and semi-arid area region. It has a population of 70,468 of whom 64% of individuals live below the poverty line defined as less than 1USD/day and are vulnerable to climate shocks and food insecurity (116). Migwani division has an average altitude of 1250m above sea level (ASL) and the highest point is 1348m ASL (116, 117). The rainfall pattern is bi-modal and sporadic with long rains falling in the months March to May and short rains from October to December with a precipitation range of 400-800mm. More than 85% of the district population is engaged in agriculture and livestock production, making them highly dependent of rainfall.




*NB: The administrative boundaries shown in this map have since been reviewed and the former Migwani division is now a fully-fledged district.*

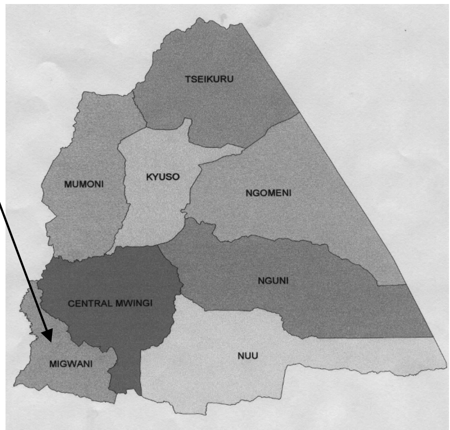



**Figure 1.2: Location of the project area in Kenya**

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# *Chapter 2*

# *Complementary feeding practices and dietary intake among children 12–23 months in Mwingi district, Kenya*

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*Int. J. Food Safety, Nutrition and Public Health (2010), Vol. 3, No. 1, pp.45–56*

## Abstract

A cross-sectional study was carried out among 280 randomly selected children aged (12-23 months), in Mwingi district, Kenya. Complementary foods were introduced at age 2.5±1.7 months and the mean duration of breastfeeding was 10.5±4.1 months. An unfortified maize porridge was the main complementary food. At least 60% of the children in all the dietary diversity terciles consumed starchy staples and oils in the preceding 24 hr. The mean dietary diversity score was 4.9±1.3 and 4.3±1.0 out of a possible score of 14 and 8 as suggested by FAO, respectively. Dietary diversity was limited in animal products. Deficits in dietary energy, iron and zinc were found due to early introduction of complementary foods and low consumption of food rich in iron. Establishing local solutions to increase dietary diversity and promote use of foods rich in iron and proteins to improve available complementary diets are needed.

## Introduction

The period of complementary feeding is a critical and vulnerable time in the growth and development of children (1). In recent years, the issue of complementary feeding in developing countries has been receiving increased attention. Rates of malnutrition usually peak when complementary foods are introduced with consequences that persist throughout life. Most of the traditional first complementary foods are prepared from cereals or starchy roots (2). In Kenya, maize meal or finger millet porridge is the principal complementary food given to children and often it does not meet the micronutrient requirements due to its bulkiness and low nutrient density (3-5). Hence, infants consume only about 80% of energy requirements and nearly half (43.1%) are stunted (6).

Poor complementary feeding practices coupled with chronic food insecurity contribute substantially to the widespread multiple micronutrient deficiencies in developing countries. This is especially a concern in arid and semi-arid lands (ASAL) where iron deficiency is not merely an outcome of overall inadequate iron intake but also of low bioavailability (5, 7). Unfortunately, the accessibility of centrally processed and enriched complementary foods in Kenya is beyond reach for many mothers in ASAL areas as many rely on home-grown cereals (4).

Dietary diversification, a key element of high quality diets that is thought to enhance the probability of adequate intake of essential nutrients, is difficult to achieve in areas of chronic food insecurity such as ASAL. Dietary diversity scores referring to the number of food groups consumed over a reference period have been positively correlated with increased mean micronutrient density adequacy of complementary foods and nutritional status of young children (8-12). The dietary diversity indicator is based on the idea that more diverse diets are more likely to provide adequate levels of a range of nutrients (13). The objective of this study, a component of a wider study with interest in iron deficiency, was to assess the feeding practices and dietary intake among children aged 12-23 months in a semiarid area in Kenya.

# Methodology

## Study area and population

This study was conducted as part of a larger study in Migwani division of Mwingi district in Kenya during the period December 2007-March 2008. This site was chosen because it is located in a semi-arid area and has been reported to have high incidence of iron deficiency, the variable of interest in the larger study (14). Frequent droughts have aggravated the poverty situation depleting any surplus food in the district (15). Two locations, Migwani and Nzauni were included in this study. All the children within the age of 12-23 months in this area were eligible for inclusion in this study. Two hundred and eighty children were included in the demographic, dietary diversity and health survey selected using a multi-stage sampling.

Verbal consent to participate in the study was obtained from the mothers or the caretaker. Ethical clearance had been obtained from the Kenyatta National Hospital Ethical Review Committee (KNH-ERC/01/4992) while research authorization was obtained from the Ministry of Higher Education, Science and Technology (MOST 13/001/37C 498).

## Demographic and health data

A household questionnaire was used to collect the demographic and health data of the study subjects. Food distribution and child feeding practices information was also collected using this questionnaire.

## Dietary diversity

Based on a simple qualitative food frequency questionnaire, the individual dietary diversity score was determined. The individual dietary diversity score (IDDS) reflects both dietary quality and adequacy (10). To calculate the dietary diversity score the food groups were summed up into 14 and 8 groups. The eight food groups were further categorized into 3 dietary diversity terciles. The lowest tercile had only three or less food groups while the high tercile had six or more food groups.

### Nutrient intake

Food consumption was measured using a repeated 24 hour recall method on a sub-sample of 43 children. Data was collected as described by Gibson and Ferguson (16). A minimum of two days and maximum of ten days separated the recall days. Three food composition databases namely; the World Food Dietary Assessment System version 2.0 (International Mini List), Kenya National Food Composition Table and the South African Food Composition Table in that order of priority were used to determine the nutrient composition of the food items (17-19). The food composition and nutrient intake data was entered in VBS-manager software. Descriptive and comparative analysis were done using SPSS version 10.0 (SPSS, Chicago, IL, USA). Data for dietary iron intake were not normally distributed and therefore presented as medians. The mean intake of other nutrients of interest was also calculated and the major food sources of the various nutrients determined. Daily energy and nutrient intakes from the complementary foods were compared with the estimated needs recommended by World Health Organization assuming average breast milk consumption and composition (20, 21).

### Focus group discussions

Focus group discussions were held with 44 mothers who had children aged between 12 and 23 months. The mothers were purposively selected from the study area and divided into six discussion groups. Standard methods in focus group discussion as a method of data collection were applied (22).

## Results

### Characteristics of the study subjects

The mean household size was 6.9±2.5. Majority (64.4%) of the mothers were between the age of 21 and 30 years. About 10% of the mothers had postsecondary education while a quarter (25.3%) had only primary education. The main source of income for the mothers was subsistence farming accounting for over 60% of their income. Only slightly more than a tenth of the study subjects had a source of income from salaried employment, casual employment or business. Nearly all the children (91.4%) had been fully immunized as per the Kenyan Ministry of Health guidelines. More than half (55.7%) of the children were reported to have had some illness within the two weeks preceding the survey and three quarters of them had sought treatment from a hospital. The major illnesses reported included upper respiratory tract infection, malaria, diarrhoea and vomiting.

**Breastfeeding and complementary feeding practices**

Amongst the mothers studied more than three quarters (86.4%) were breastfeeding at the time of data collection **(Table 2.1)**. The mean breastfeeding duration was 10.5±4.1 months. Of the children who were not breastfeeding, 70% had stopped by the age of 12 months. Nevertheless breast milk especially colostrum was considered to be very important to a child's health, growth and development. All the mothers who participated in the focus group discussions said it is strong and wholesome as seen in the following excerpt:

> *"The first milk "kithana" is very crucial to the baby's development especially the mind. So I could not afford to deny my child breast milk especially when I had just given birth to her."*

The mean age of complementary food introduction was 2.5±1.7 months. Majority (70.4%) of the children in this study were introduced to complementary foods by the third month of life. In several instances, the respondents' mothers or mother-in-laws were reported to play a great role on the age at which complementary foods were introduced.

> *"Sometimes our mothers, mothers- in-law tell us how they used to do it themselves so we follow what they tell us they practiced."*

Water was introduced to the children as early as the first day and either sugar/salt or glucose could be added to the water depending on affordability.

There were varied reasons for introduction of complementary foods including water,

> *"When I gave birth to my daughter, I didn't produce milk on the first day so I gave her some boiled water that had been added a pinch of salt and sugar to make it tasty".*

Introduction of complementary foods was also believed to reduce stomach upsets for the new-born as one mother stated:

*"All my children usually experience severe stomach upsets after birth but on introduction of diluted cow's milk at the age of one month, the problem stops."*

**Table 2.1: Distribution of children by breastfeeding duration and age at introduction of complementary foods**

| Characteristic (n) | Migwani location (%) | Nzauni location (%) | Total (%) |
|---|---|---|---|
| Breastfeeding (242) | 138 (92.6) | 104 (79.4) | 86.4 |
| Not breastfeeding (38) | 11 (7.4) | 27 (20.6) | 13.6 |
| *Breastfeeding duration* | | | |
| 0-6 months (5) | 18.2 | 12.5 | 14.3 |
| 7-12 months (21) | 63.6 | 58.3 | 60 |
| 13-18 months (9) | 18.2 | 29.2 | 25.7 |
| *Age at introduction of complementary foods* | | | |
| 1-3 months (197) | 76.9 | 70.7 | 74.1 |
| 4-6 months (67) | 22.4 | 28.5 | 25.2 |
| Over 6 months (2) | 0.7 | 0.8 | 0.8 |

Maize meal in the form of porridge or *ugali* (stiff maize porridge) was the predominant complementary food in this area.

*"My child survives only by porridge made from mixture of maize and sorghum enriched with blue band (margarine) and is very healthy."*

Tomato relish was the most common stew for children and this was attributed to the abundant availability of a locally grown variety *Nzithi* that does well even during the dry season.

**Food availability and affordability**

Overall seasonality and cost of food available in the market affected the types of foods given to the children. The type of complementary food consumed by the children was strongly influenced by what foods were locally available. This was what one mother had to say:

*"At the moment, my child has been for the past one week consuming a lot of mangoes only because they are locally available and are in season."*

Many families were from low socio-economic strata and therefore could not afford nutritionally rich foods for their children as they would want.

*"I cannot always afford to buy green bananas, liver and milk on a daily basis for my child hence I only provide what is available. Sometimes I usually get beans from githeri (mixture of maize and beans) that had been cooked for the whole family to feed my baby.*

**Table 2.2: Food groups consumed by >60% of children by dietary diversity tercile**

| *LD diversity n = 60* | *Frequency (%) 21.4%* | *MD diversity n = 194* | *Frequency (%) 69.3%* | *HD diversity n = 26* | *Frequency (%) 9.3%* |
|---|---|---|---|---|---|
| Starchy staples | 98.3 | Starchy staples | 100 | Starchy staples | 100 |
| Oils & fats | 68.3 | Oils & fats | 92.3 | Oils & fats | 100 |
| Other F&V | 63.3 | Other F&V | 92.3 | Other F&V | 100 |
| | | Dairy products | 65.5 | Dairy products | 96.2 |
| | | | | Vit A rich F&V | 92.3 |
| | | | | Legumes | 76.9 |

*LD-Low dietary diversity: ≤ 3 food groups, MD-Medium dietary diversity: 4 & 5 food groups, HD-High dietary diversity: ≥ 6 food groups. F&V: Fruit and vegetables*

The mothers were in agreement that the main complementary foods available in the area include porridge, rice, mashed potatoes and tomato soup. Ripe bananas, avocado and pawpaw are the main fruits used in complementary feeding. Fortified commercial foods including infant formulas were rarely used in the area. This was confirmed by following statement from one of the mothers.

*"I have never used NAN (infant formula) because it's too expensive for me to afford and even I have never come across anyone from this area who is using it."*

**Food choice and food avoidance**

Although no foods were prohibited to children in the area most of the mothers avoided giving tea to their children because they considered it to reduce appetite as one mother stated.

*"If I give my child tea he tends to loose appetite, so I have decided to always not give children tea."*

**Table 2.3: Two leading food sources of various nutrients in the study area**

| *Nutrient* | *Main food items* | *Absolute % contribution of food to nutrient* |
|---|---|---|
| Energy | Maize flour white | 28 |
| | Rice white | 21 |
| Protein | Maize flour white | 28 |
| | Rice white | 17 |
| Iron | Maize flour white | 56 |
| | Sorghum | 11 |
| Zinc | Maize flour white | 50 |
| | Rice white | 13 |
| Vitamin A | Mangoes | 40 |
| | Kales | 14 |
| Vitamin C | Mangoes | 30 |
| | Guava | 14 |

### Source of information on feeding practices

Maternal and child health clinics were reported to be the main source of information on child feeding practices in the study area as seen from the following excerpts:

> *"When we attend the clinics we are always advised on how to feed the children, when to introduce certain foods."*

The media was not a major source of information as the mothers claimed they had no time to read and listen to them.

> *"We don't have time to listen to the radios or read magazines since we are too busy with our household chores."*

### Anaemia and iron deficiency in children

Generally, most of the mothers were not in a position to tell the difference between anaemia and iron deficiency as the local language does not also differentiate the two. Rather, they understood it to be one and the same thing locally known as *kiala*. They agreed the causes of anaemia to be; prolonged illness, excessive bleeding and inadequate food intake.

**Dietary diversity**

The mean dietary diversity score (DDS) of 14 food groups was 4.9±1.3 while that of 8 food groups as suggested by FAO for children 1-3 years was 4.26±0.99. The difference between DDS for the children who were breastfeeding and those who had stopped was not significant. Nearly all children (over 95%) had some form of starchy staple the previous day. Slightly more than half of the children had eaten vitamin A rich fruits and vegetables. Most of the children were in the medium diversity tercile (72.1%). At least 60% of the children in all the dietary diversity terciles had consumed starchy staples, oils and other fruits and vegetables in the preceding 24 hours **(Table 2.2)**. In the medium dietary diversity tercile, dairy products formed the additional food group while vitamin A rich fruits, vegetables and legumes contributed to the increased dietary diversity within the high dietary diversity tercile group. Iron rich foods were rarely consumed in this area. Overall only 6.4% of the children had consumed a form of animal source food.

**Table 2.4: Median and contribution to estimated average requirement of two-day 24 hour intakes of energy and select micronutrients**

| *Nutrient* | *Mean ± SD* | *Median* | *25$^{th}$ and 75$^{th}$ Percentile* | *Nutrient adequacy** |
|---|---|---|---|---|
| Energy (Kcal) | 770.7±400.7 | 698.6 | 494, 944 | 64 |
| Protein (g) | 17.9±10.9 | 16.9 | 10, 23 | 153 |
| Iron (mg) | - | 5.5 | 3, 8 | 69 |
| Zinc (mg) | 3.5±2.1 | 3.0 | 1.7, 5 | 44 |
| Vitamin A (µg RE) | 376.5±400.5 | 272.2 | 59.5, 492.5 | 126 |
| Vitamin C (mg) | 39.5±44.1 | 31.6 | 14.9, 49.6 | 132 |

**Defined as intake/estimated average requirement x100*

**Dietary intakes**

Mangoes provided 40% of the vitamin A intake and this could be attributed to the fact that they were in season during the survey period **(Table 2.3)**. Maize was the major source of iron and zinc among the children contributing approximately 56% and 50% to the nutrient intake respectively. Milk contributed significantly to retinol intake. It was however noted that most of the time it was taken when combined with tea or porridge.

Estimated median intake of energy and other selected nutrients are shown in **Table 2.4**. The percentage contribution to estimated average requirement for children aged 1-3 years is also shown. Except for protein and vitamin A and vitamin C, intake of all other nutrients was below the estimated needs. Over 90% of the children (91%) had energy intake of below the estimated average requirement.

## Discussion

This study confirms that early introduction of complementary foods is a common practice in Mwingi. This is in confirmation with findings from other parts of semi-arid Kenya and other developing countries such as Malawi, Brazil and Zambia (1, 5, 23, 24). The finding that the first complementary food introduced to the children was maize porridge corresponds with the WHO review on complementary feeding in African children (21). *Ugali* (stiff maize porridge) was introduced to majority of the children by the eighth month and by one year; most children had also been introduced to the family food. This early introduction to family foods is a positive practice and concurs with the observations made in Malawi (1). The elderly mothers were influential on when complementary foods were introduced and which type food to given to children. Although water was introduced early, it was not considered as food by the local community. The socioeconomic class determined whether glucose, salt or sugar will be added to the water as reported in the focus group discussions.

Since maize contributed to most energy and micronutrient such as iron and zinc intake, it was therefore apparent that the diet was low in bio available iron. Presence of phytate found in cereals also elevated the phytate:zinc molar ratio as in other complementary foods used in developing countries (1, 25). The tomato relish and kales commonly eaten with *ugali* could probably enhance the absorption of iron due to their high levels of ascorbic acid (26, 27). However, the cooking methods of these vegetables are attributed with major losses of vitamin C before consumption thereby contributing to negligible absorption of iron (27, 28). Energy intake among the children was found to be way below the age based recommended intake contributing only 58% of the estimated needs (21). More than half of the iron requirement was met in the diet but its bioavailability was low since it was mainly non-heme iron that has been shown to be less bio available (25).

Seasonality of various foods affects the intake of various nutrients such as vitamin A and C which are mainly provided by plant based sources in developing countries (29). In this study mangoes were the main source of vitamin A and C contributing over 30% of the total intake. Probably a similar study in a different season may give a totally different source of these nutrients. This seasonality was mentioned to affect the availability and affordability of various foods used in complementary feeding. These findings are consistent with those found by Owino *et al* in Zambia (23). Use of commercially processed complementary foods in the area was almost non-existent. This was mainly due to the high cost of these foods. Considering that only about a tenth of the mothers were in salaried employment or business, it was evident that use of these foods in the short term may be minimal. It would therefore be important that when designing an intervention on child feeding in such an area, use of locally available foods or foods that could be grown in the area be promoted.

Majority of the children were classified under medium dietary diversity category as defined by FAO (10). Even the few who were classified in high dietary diversity (9.3%) had very low consumption of animal source foods. The focus group discussions showed that no belief was associated with particular animal source food withholding among children of this age. It could therefore mean that affordability of these food sources was the main reason behind low consumption. This observation compares with other studies done in the semi-arid areas in Kenya and other developing countries (1, 5). These findings imply that children may not to be getting adequate nutrients in terms of quality to sustain their growth thus a need to improve complementary foods. This underscores the need to establish local solutions to increase dietary diversity and to promote use of foods that are rich in nutrients such as iron. Dietary diversification using foods such as amaranth grain offers the prospect of substantially increasing food diversification in semi-arid areas.

Agronomic investigations indicate that amaranth grain has high iron content ranging from 7.6 to 11 mg/100g of edible portion (18, 30). Our preliminary analysis of the amaranth grain indicated an iron content of between 27.7 and 45 mg/100 g dry matter. With an assumed 5% bioavailability, feeding a child with 56g of amaranth grain flour porridge daily for 24 weeks could increase the haemoglobin by 10g/L. Amaranth grain may thus offer a good complementary food choice for combating iron deficiency anaemia in young children.

## Acknowledgements

We are grateful to the management of Migwani Sub-district hospital who provided us with field office space during this study. Our thanks extend to our research assistants. Special thanks to the mothers who provided us with the information needed and allowed their children to participate in the study. Thanks to Applied Nutrition Program, University of Nairobi, who facilitated us with transport and provided office space.

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# *Chapter 3*

# *Simulation of the impact of fortification of maize porridge with amaranth grain or with NaFeEDTA containing micronutrient powder on iron intake and status in Kenyan children*

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## Abstract

Amaranth grain high in iron and highly absorbable low iron micronutrient powders (MNP) offer the prospect of improving iron intake and status among pre-school children. We simulated the potential effect of fortifying maize porridge with amaranth grain and MNP on prevalence of inadequate iron intake and iron deficiency (ID) using data from two cross-sectional surveys among children in Mwingi, Kenya. In the first survey done in 2008, dietary intake data were collected by 2-day 24hr recalls (n= 197; aged 12-23 months). Simulating a daily consumption for 80 days of non-fortified maize porridge (60g flour), amaranth porridge (80g of flour at 70:30 amaranth to maize ratio), or maize porridge fortified with MNP (2.5mg iron as NaFeEDTA), resulted in a median iron intake of 8.6mg, 17.5mg and 11.1mg/day respectively. The prevalence of inadequate iron intake would reduce to 35% in amaranth and 45% in the MNP group. Biochemical data (n=70; aged 12-23 months) from the second survey done in 2010 showed a prevalence of anaemia, ID and IDA of 49%, 46% and 24% respectively. Using the simulated increased intake of absorbable iron from the 1$^{st}$ study, consumption of amaranth and MNP porridge was estimated to result in an increase in ferritin concentration (1.82μg/L 95%CI 1.42-2.34 and 1.80μg/L 95%CI 1.40-2.31, $p<0.005$) respectively compared to maize porridge group resulting in a decrease in prevalence of ID (27%) in the two groups. Use of dietary diversification or in-home fortification with low iron MNP has a potential to improve iron intake and status in pre-school children.

## Background

Iron deficiency and anaemia are a global public health problem especially in Sub-Saharan Africa impacting the health and economic potential of many (1, 2). Iron deficiency leads to anaemia, impaired immune functions, decreased motor activity and cognitive development among young children (1, 3). Global estimates show that the highest prevalence of anaemia (47.4%) is among children less than 5 years of age with the prevalence rising to 64.6% in Africa (1, 2). About 50% of the anaemia is assumed to be iron deficiency anaemia (1). The 1999 Kenya national survey on micronutrients estimated that seven out of every ten children under five years were likely to be anaemic and nearly half (43.2%) were iron deficient (4).

Inadequate dietary intake has been identified as an immediate determinant of micronutrient deficiencies (5). Studies on dietary patterns of infants and pre-school children in Kenya, found plant foods as the major sources of energy of which cereals contribute approximately 62-68% to the total daily energy intake (6-9). In addition, maize in the form of soft or stiff maize porridge has been shown to be the major complementary food for children 12-23 months of age contributing to over 50% of iron intake (6, 8). High prevalence of inadequate iron intake among Kenyan children of up to 77% has also been reported (9).

Supplementation has been used with various levels of success to tackle the problem of inadequate iron intake and iron deficiency. However, a recent study in Pemba supplementing children aged 1-35 months with iron and folic acid showed a 12% increase in the incidence of serious adverse events in those children classified as iron replete at baseline, presumably due to malaria (10). This prompted World Health Organisation to advise against blanket iron supplementation and use of micronutrient powders (MNP) that provide the entire iron requirement in a single dose in regions where malaria is endemic and infectious disease highly prevalent (11). Therefore, food based approaches including home fortification with low doses of highly absorbable iron or dietary diversification may be better alternatives in countries such as Kenya that are in a malaria endemic zone. Use of these micronutrient powders consumed with food has been found to improve iron status in children within short intervention periods (12-14).

Dietary diversification is thought to enhance the probability of adequate nutrient intake thereby being suggested as a suitable approach to alleviate iron deficiency (1, 15). Less studied but widely consumed whole grain cereals such as amaranth grain (*Amaranthus spp*) may have a potential to improve iron status among children. Though animal studies have indicated a bio-availability of iron from amaranth grain to be about 3%, amaranth grain is reported to have two to four times more iron than wheat, high quality proteins that are easily digested as compared to other cereals and to be a drought resistant fast maturing crop able to survive in semi-arid areas (16, 17). Amaranth grain may therefore have a potential to improve iron status among children in areas of chronic food insecurity.

Simulating the probable impact of strategies on the potential target population is an essential step in the choice and development of the appropriate actual intervention. Previous studies have estimated intervention effects of zinc and iron bio-fortification as well as increased use of animal source foods to reduce prevalence of inadequate zinc and iron intakes among women and young children (9, 18, 19) but none has attempted to estimate the possible effect on iron status. Simulation studies investigating the effect of dietary diversification using plant-foods and in-home fortification using MNP are also lacking. The objective of this study therefore was to use data on food consumption patterns of 12-23 months old Kenyan children to simulate the potential effect of enriching maize porridge with amaranth grain flour or micronutrient powder on the adequacy of iron intake and reducing the prevalence of iron deficiency.

## Methods

### Study design and selection of study participants

Two cross-sectional surveys were carried out in Migwani division of Mwingi District in Eastern Kenya purposively selected as it falls within an agro-ecological zone in a semiarid area that experiences food shortage for most part of the year. In both studies, multi stage sampling was used. Two of the six locations, Migwani and Nzauni, were randomly chosen for the purpose of these studies. The sampling frame consisted of households with children below five years. Households were selected using the random walk method until the desired sample size was achieved (20). Only one child per household within the defined age was randomly selected. Informed verbal and written consent was obtained from the principal caretaker/parent. Both studies were approved as part of a larger study by the Kenyatta National Hospital / University of Nairobi Ethical Review Committee and Ministry of Higher Education, Science and Technology in Kenya.

A quantitative food consumption survey was carried out in the first survey in March 2008 involving 197 children aged 12-23 months. Their characteristics have been described elsewhere (6). The sample size allowed us to estimate iron intake adequacy calculated from a standard deviation in iron intake among children of 6.3mg and an intended true difference estimate of the mean of 1mg/day (21). Data from this study was used to simulate the effect of fortification of maize porridge with amaranth grain or micronutrient powder on iron intake. A second survey done in the same area in January 2010 included 263 children aged 12-59 months. This sample size allowed us to measure the prevalence of iron deficiency within 6 percentage points with 95% confidence at an expected prevalence of 43% for iron deficiency in children 12-59 months. In a subsample of 70 children (12-23 months) it was estimated whether the simulated effect of the intervention on iron intake as found in the first survey would translate into a reduction of prevalence of iron deficiency in this age group assuming a similar food consumption pattern for children in the two surveys. All children were tested for malaria infection in the 2$^{nd}$ survey.

**Field data collection procedures**

***Anthropometry***

Weight measurements were taken with either a Salter spring scale distributed by UNICEF or a digital bathroom scale (Ashton Meyers® Model no 7752) with a capacity of 25 kg and 150 kg respectively. The digital bathroom scale was only used if a child refused to put on the provided weighing pants or could not fit in it. Weight was measured to the nearest 0.1 kg. The subjects were in minimum clothing which was not corrected for during analysis. Standing height (children > 2 years) or recumbent length (children < 2 years) was measured to the nearest 0.1 cm using height/length wooden boards distributed by UNICEF. All measurements were done twice. If the variation between the two measurements was more than 0.5 kg and 1.0 cm for weight and height/length measurements respectively, the measurement was done a third time (22). The two closest values were reported and afterwards used to calculate the mean value. Age was calculated from the birth date reported on clinic cards or, in rare cases, on parents/principal caretaker recall.

***Blood collection and analysis***

Non fasting venous blood samples were collected from children by venepuncture in the morning hours and divided into two portions. The sample series for the first portion were stored in EDTA tubes (Becton-Dickinson®) for determination of haemoglobin concentration while the second sample series for 2nd portion were stored in plain tubes without anticoagulant for serum separation. The samples were then stored in cool boxes with ice packs and transported to Migwani hospital. Haemoglobin concentration was determined using the Sysmex haematological analyser (KX-21, Sysmex Corporation, Japan). Separation was done within six hours and serum transferred into sterile cryo-vials, flushed with pure white spot nitrogen gas and thereafter stored in liquid nitrogen before transportation to Nairobi for further analysis. Serum ferritin was analysed using enzyme linked fluorescent assay with Mini-VIDAS® Immunoanalyser (Biomeriux SA) while CRP was analysed using a solid phase, sandwich-format, immunometric assay with NycoCard®READER II at the Centre for Public Health Research (CPHR-KEMRI). Serum transferrin receptor was analysed using fully automated particle enhanced immunoturbidimetric assay with COBAS INTEGRA® analyser (Roche Diagnostics, Mannheim, Germany) at the Pathologists Lancet Kenya Ltd, Nairobi. Certified reference kits/cell controls were used for precision determination during analysis.

***Malaria infection analysis***

Thick blood smear slides were prepared and Giemsa-stained in the field. Microscopy, the conventional gold standard for the detection and counting of malaria parasites was used to examine the presence or absence of malaria parasites (23) at the Kenya Medical Research Institute, Nairobi. The children were categorized as either parasite negative or positive.

***Dietary intake***

Food consumption was measured using repeated 24 hour recall method on 2 non-consecutive days as described previously (6, 20). The observed intakes from the 24hr recalls were adjusted for day to day variation using the National Research Council (NRC) adjustment procedure to get the estimated usual intake for the individual (24). The full probability approach was used to estimate the prevalence of iron intake inadequacy as iron requirements for children have a skewed distribution assuming an average bioavailability of 5% (25). The total amount of iron absorbed after the simulated intervention was obtained by adding the total estimated absorbable iron from the different treatments to that absorbed from the usual intake. The total amount of actual and simulated absorbable iron per child was categorized into probabilities of inadequacy ranging from 0-1 where 0 was assigned to absorbed iron that fall above the 97.5$^{th}$ percentile of requirements and 1 was assigned to absorbed iron that fall below the 2.5$^{th}$ percentile of requirements (25). The average of the prevalence of inadequacy in the different categories gave an estimate of the total prevalence of inadequacy in the population. Energy requirement was estimated at 3766kJ/day (900 kcal), the average requirement of a girl and boy aged 1-2 years at moderate physical activity (26).

Individual dietary diversity score was calculated from the 1$^{st}$ day of the 24 hr recall data. In the second survey individual dietary diversity score was based on a simple qualitative food frequency questionnaire asking information on the number of meals and types of foods/ingredients in each meal. All the ingredients mentioned in the 24hr recall (1$^{st}$ survey) or listed in the qualitative food frequency questionnaire (2$^{nd}$ survey) were assigned into 11 food groups namely: cereals roots and tubers, legumes and nuts, Vitamin A rich fruits and vegetables, dark green leafy vegetables, other fruits and vegetables, dairy, meat poultry and fish, eggs, organ meats, condiments, fats and oils. We did not include fats and oil group when awarding the scores as well as the condiments since our focus was on iron intake (27). If a food from a certain food group was consumed, this food group received a score of one and if no food in a food group was consumed, a score of zero was awarded. To calculate individual dietary diversity score, the scores for each food group were summed up and hence the minimum score was “0” and the maximum score was "9". The subjects were further classified into dietary diversity score terciles. Those consuming three or less food groups were categorized as low, those consuming 4-5 food groups were categorized as medium and those consuming six or more food groups were categorized as having high dietary diversity (27).

***Simulation***

A simulation model using deterministic approach was developed to estimate the expected change in prevalence of inadequacy of iron intake. It was based on three types of porridges namely: a) plain maize porridge (MM) with 60g of maize flour, b) amaranth maize porridge (MA) with 80g of flour at the ratio of 70% amaranth (56g) and 30% maize (24g), and c) maize porridge (60g of maize flour) with added micronutrient powder containing 2.5mg iron in the form of NaFeEDTA ($MM^{+}$) formulated for malaria endemic areas (28, 29). The amount of flour required to make the plain maize porridge was lower as the consistency was found to be thicker than for a similar amount of porridge made from amaranth and maize at 70:30 ratio (30). It was assumed that these porridges would be added to the children's diet as an extra meal as previously estimated energy and iron intake for children in the area showed that nutrient adequacy was less than 70% for both energy and iron requirements (6). We used 70% of amaranth flour in the maize porridge group as our previous sensory study showed a preference for non-fermented porridges with a lower amount of amaranth flour but no significant difference between 50:50 and 70:30 amaranth:maize ratio. We therefore used the non-fermented porridge with higher amount of amaranth in the simulation as we desired to give as much iron from the amaranth grain (30).

Simulations on the effect of the intervention on iron intake were made using data from the 1st survey. To perform the simulations, the assumptions made included; a) wet weight iron content of maize is 0.061g/kg and of amaranth grain is 0.20g/kg based on own analysis of the flours (Macharia-Mutie *et al*, unpublished results), b) bioavailability of iron from maize flour in all the porridges is 4% (31), bioavailability of the 70% amaranth flour in the amaranth maize porridge is 3% (17) and bioavailability of the 2.5mg iron in the form of NaFeEDTA is 7% (32), and c) the intervention would last for

80 days i.e. 16 weeks feeding at 5 days/ week, as treatment effects may be achieved within this timeframe and even shorter period for MNP (33). We assumed that the baseline usual iron intake for children is the median intake (4.9mg/day) obtained from the food consumption data, being 0.24mg absorbable iron per day assuming an overall iron bioavailability of 5% based on the regular diet containing enhancers such as ascorbic acid and animal source foods though in limited amounts (1). The additional absorbable iron obtained by simulating the different treatments was added to the assumed median usual absorbed iron intake of 0.24mg/day. The prevalence of inadequate iron intake was then estimated for the three groups using the full probability approach as described earlier (25).

Using the results of the 2nd survey, a simulation to estimate the expected change in prevalence of iron deficiency, ferritin concentrations and body iron stores was also done for the three earlier mentioned porridges. To estimate total iron stores to be formed from the intervention, the total median iron requirement for growth and basal losses of 0.46mg/day for a child 1-3 year old weighing 13.3kg equivalent to 34.6µg Fe/kg/day was assumed (34). It was assumed that the absorbed iron in excess of this total median requirement for growth and basal losses as simulated from the dietary intake would be used in the formation of iron stores at the end of 80 days intervention. Total iron stores after the intervention were calculated assuming that the amaranth and MNP fortified porridge will provide an extra 13.5 and 8.3 µg Fe/kg/day respectively while the maize porridge group would have a deficit of 5.3µg Fe/kg/day These values were obtained after deducting the daily median requirement of 34.6µg Fe/kg/day from the simulated iron intake assuming an average weight of 13.3kg per child. A linear relationship between log serum ferritin (SF) and iron stores per unit body weight was assumed. The equation Y=9380X-11260 where Y (µg Fe/kg) is the total body iron calculated after the intervention and X is log SF as described by Hallberg *et al* (35) was used to estimate the log SF for every child assuming that they received the different treatments. The exponential of log SF was assumed to be the extra SF formed or deficit at the end of 80 days intervention. These values were then added to the original SF values which had been corrected for acute inflammation (see next section) to give the final SF value for the three different treatments.

**Data analysis**

Anaemia was defined as haemoglobin concentration <110 g/L. Iron deficiency was defined as serum ferritin concentration <12 μg/L (36) or serum transferrin receptor (sTfR) concentration >8.3mg/L (Roche kit specific). Body iron content was calculated using the formula: body iron (mg/kg) = - (log (sTfR/SF ratio)-2.8229)/0.1207 (37). This was after recalculation of Roche kit sTfR values with the regression equation; sTfR=1.5* Roche kit values+0.35 to transform them to ELISA assay values by Flowers (38). To adjust plasma ferritin concentration for those children with elevated CRP (>5mg/L), the correction factor of 0.67 was used to yield an adjusted ferritin concentration representative of a child without acute inflammation (39).

Weight-for-height Z-score (WHZ), weight-for-age Z-score (WAZ) and height-for-age Z-score (HAZ) were calculated with WHO_ANTHRO Software version 3.2.2, January 2011 using the 2006 WHO growth standards. Stunting, underweight or wasting was defined by Z score <-2SD (40). Statistical analysis were done using SPSS version 16.0 (SPSS, Chicago, IL, USA) and Microsoft Excel (2003). Univariate analysis with planned contrasts was done to estimate treatment effects on ferritin and body iron concentration relative to maize porridge only group after simulated intervention. The level of significance was at $P < 0.05$.

## Results

Dietary intake analysis data was done using data from 197 children who participated in the 1st survey and is presented in **Table 3.1**. The mean energy intake was 2902kJ/d with 88% of the children having an intake below the daily energy requirements for children of 1-2 years. The mean energy percentage from protein, carbohydrate and fat intake were 10 en%, 74 en%, and 16 en% respectively. Maize contributed to more than half (57%) of the iron intake and about a third of the energy and protein intake in the children's diet. Nearly half (48%) of children had a low dietary diversity score (≤3 food groups per day) and only 2% had high dietary diversity score (≥6food groups/day).

The characteristics of 70 children who participated in the 2nd survey are shown in **Table 3.2**. More than half (60%) of the children were short for their age of whom 21% were severely stunted (HAZ<-3). The mean haemoglobin concentration was 108.9±10g/L with an anaemia prevalence of 49%. The prevalence of iron deficiency by serum ferritin was 44.3% while 17% of the children had tissue iron deficiency.

**Table 3.1: Adjusted usual dietary intake distributions among children 12-23 months in Migwani, 2008 (n=197)**

| | Nutrient intake | | | | | | Contribution of maize |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Median | 25th | 75th | Energy % | to nutrient (%) |
| Energy (kJ/d) | 2902 | 761 | 2908 | 2412 | 3366 | | 34 |
| Energy (kcal/d) | 694 | 182 | 695 | 576 | 804 | | 34 |
| Protein (g/d) | 16.5 | 5.2 | 16.2 | 12.8 | 20.1 | 10 | 34 |
| Fat (g/d) | 12.3 | 4.2 | 11.9 | 9.2 | 15.2 | 16 | 17 |
| CHO (g/d) | 128 | 34 | 126 | 101 | 148 | 74 | 41 |
| Zinc (mg/d) | 2.9 | 0.8 | 2.8 | 2.3 | 3.4 | | 50 |
| Iron (mg/d) | 5.1 | 1.6 | 4.9 | 3.8 | 6.1 | | 57 |

The cumulative prevalence of iron deficiency (45.7%) as indicated by serum ferritin and soluble transferrin receptor was indicative of a high prevalence of iron depletion in this age group. Prevalence of iron deficiency anaemia was 24.3% and the prevalence of malaria was 2.9%. Half of children had a low dietary diversity score (≤3 food groups per day) and only 1% had high dietary diversity score (≥6food groups/day). This observation was similar to that obtained in the 1st survey. This 2nd survey data should however be interpreted with caution as the sample size calculations were powered for children 12-59 months while we only analysed data from a subsample of children aged 12-23 months in this survey.

The simulated effects of fortification with amaranth grain and micronutrient powder are presented in **Table 3.3**. The simulations estimated that amaranth and MNP fortified porridge would provide an extra 0.013 and 0.008mg Fe/kg/day respectively while the unfortified maize porridge group would result in a deficit of 0.005mg Fe/kg/day compared to the absolute median requirement for a child aged 12-23 months.

**Table 3.2: Characteristics of children 12-23 months in Migwani, 2010**

| **Children characteristics** | | n | Frequency (%) | Mean | SD |
|---|---|---|---|---|---|
| **Demography, anthropometry & food intake** | | | | | |
| Sex | Male | 32 | 45.7 | | |
| Breastfed | | 42 | 60 | | |
| Anthropometry | WAZ | 70 | | -1.6 | 1.1 |
| | HAZ | 70 | | -2. 1 | 1.1 |
| | WHZ | 70 | | -0.8 | 1.2 |
| | Underweight † | 24 | 34.3 | | |
| | Stunted† | 42 | 60.0 | | |
| | Wasted† | 13 | 18.5 | | |
| Dietary diversity score[2] | Low | 35 | 50.0 | | |
| | Medium | 34 | 48.6 | | |
| | High | 1 | 1.4 | | |
| **Biochemical characteristics** | | | | | |
| Haemoglobin g/L | | 70 | | 108.9 | 10 |
| sTfR mg/L ‡ | | 70 | | 6.4 | (5.9, 6.9) |
| Serum ferritin µg/L ‡§ | | 70 | | 13.7 | (11.2, 16.6) |
| Body iron mg/kg‡§ | | 70 | | -0.9 | (-1.9, 0.1) |
| Anaemia | | 34 | 48.6 | | |
| Iron deficiency (ID) | SF<12µg/L[§] | 31 | 44.3 | | |
| | sTfR>8.3mg/L | 12 | 17.1 | | |
| ID anaemia ‖ | | 17 | 24.3 | | |
| CRP >5 mg/L | | 24 | 34.3 | | |
| Malaria | | 2 | 2.9 | | |

*sTfR- serum transferrin receptor, SF-serum ferritin, CRP- C reactive proteins,* WAZ-Weight for age Z score, HAZ-Height for age Z score, *WHZ*-Weight for height Z score, *†Underweight, stunting and wasting defined as <-2 Z score WAZ, HAZ and WHZ respectively, ‡Geometric mean (95% CI), § Adjusted for elevated CRP >5mg/L (39), ‖defined as concurrent anaemia and iron deficiency (SF<12µg/L or sTfR>8.3mg/L). DDS[2] classified as low (≤3food group), medium (4-5 groups), High (≥6 food groups)*

The extra iron would cause a modest increase of iron stores by 1.1 and 0.7 mg Fe/kg in the amaranth and MNP fortified porridge respectively after the intervention. Simulated serum ferritin concentrations increased by approximately 5μg/L in the amaranth and MNP groups compared to baseline. The prevalence of inadequate iron intake was found to be 93% in the 1st study.

The simulations resulted in a decrease of the prevalence of inadequate iron intake in all the simulated treatment groups. After an assumed consumption of amaranth enriched maize porridge or maize porridge with MNP, prevalence of inadequate iron intake would reduce to 35% and 45% respectively which translates to over 50% reduction. The simulated consumption of maize only porridge would result in a prevalence of 75%. In addition, based on the 2nd survey the increased estimated serum ferritin in the amaranth and micronutrient powder group reduced the prevalence of iron deficiency in the two groups to 27%. This reduction was not observed in the maize only porridge group. Planned contrasts showed that consumption of amaranth enriched maize porridge or fortified maize porridge would cause a similar and significant improvement in serum ferritin concentration (1.82μg\L CI 1.42 to 2.34, $p<0.005$ and 1.80μg\L CI 1.40 to 2.31, $p<0.005$ respectively) compared to the maize only group.

## Discussion

We simulated the estimated effect of enriching maize porridge with amaranth grain flour and highly bioavailable low iron micronutrient powder on the adequacy of iron intake and its potential to reduce iron deficiency among 12-23 months old children in Migwani, Mwingi district, Kenya. The results indicated that these treatments may comparably reduce the prevalence of inadequate iron intake and iron deficiency in our study population.

In our study, wasting, underweight and stunting levels of the participants were higher than the prevalence levels reported for both Eastern province and nationally (41). High prevalence (60%) of chronic malnutrition in our study population reflects a process of failure to reach linear growth potential as a result of suboptimal health and/ or food and feeding conditions combined with poor household socioeconomic conditions indicating the high risk of iron deficiency (5). The prevalence of iron deficiency and anaemia was high in this study and causes may range from an inadequate and monotonous diet to presence of illnesses and parasite infestations, poor infant feeding practices, inaccessibility of healthcare services, genetic determinants and environmental factors (1, 42).

**Table 3.3: Simulated impact of amaranth grain or micronutrient powder enriched maize flour on iron intake in children 12-23 months in Migwani**

| | Simulated effect | | | |
|---|---|---|---|---|
| **Dietary characteristics (1st study 2008 n=197)** | Baseline[1] | MM[2] | MA[3] | MM+[4] |
| Median daily iron intake (mg/d) [5] | 4.9 (3.8, 6.1) | 8.6 (7.5, 9.8) | 17.6 (16.4, 18.7) | 11.1 (10.0, 12.3) |
| Iron contribution from intervention (%) | 0 | 43 | 72 | 56 |
| Daily absorbed iron mg[6] | 0.24 (0.19, 0.31) | 0.39 (0.31, 0.45) | 0.64 (0.58, 0.70) | 0.57 (0.51, 0.63) |
| Bioavailable iron from intervention (mg/d) | 0 | 0.15 | 0.40 | 0.33 |
| Iron below/above the daily median requirement [7] | | | | |
| mg/d | -0.22 (-0.27, -0.15) | -0.07 (-0.12, -0.01) | 0.18 (0.12, 0.24) | 0.11 (0.05, 0.17) |
| mg $Fe.kg^{-1}.d^{-1}$ | -0.016 (-0.02, -0.01) | -0.005 (-0.01, -0.001) | 0.013 (0.01, 0.02) | 0.008 (0.004, 0.013) |
| Prevalence of inadequate iron intake (%)[8] | 93 | 75 | 35 | 45 |
| **Intervention simulation (2nd study 2010; 80 days; n=70)** | | | | |
| Deficit/extra iron stores[9] (μg $Fe.kg^{-1}$) | 0 | -421 | 1083 | 662 |
| Ferritin concentration[10] (μg/Ll†) | 13.8 (11.3, 16.8) | 9.5 (7.0, 12.6) | 18.8 (16.2, 21.9)* | 18.6 (16.0, 21.7)* |
| Prevalence of iron deficiency[11] (%) | 44.3 | 58.6 | 27.1 | 27.1 |

*Data are median (25th, 75th percentile) unless otherwise indicated. †Data are geometric mean (95% CI). *Significant difference compared to maize only group.*

*[1]This is the existing situation before simulation and the data forms the basis of the simulation assuming that these children did not receive any intervention. Bioavailability of diet considered to be 5%(1)*

*[2]MM- Plain maize porridge with 60g of maize flour (Fe content =0.061g/kg wet weight) with 4% bioavailability (31).*

*[3]MA-amaranth maize porridge with 56g of amaranth flour (Fe content = 0.20g/kg wet weight) and 24 g of maize flour. Assumed bioavailability of amaranth is 3% (17) and 4% bioavailability for iron in maize (31)*

*[4]MM+-plain maize porridge (60g maize flour) with added 2.5mg of iron in the form of NaFeEDTA (28). Assumed bioavailability of iron in the form of NaFeEDTA is 7% (32) and 4% bioavailability for iron in maize (31)*

*[5]This is the usual iron intake from other foods (4.9mg/d) plus the added iron intake from the intervention. Usual iron intake has been adjusted for day to day variation*

*[6] For the interventions we add the extra absorbable iron calculated using the intervention respective bioavailability (see footnotes 2,3and 4) to the usual bioavailable iron intake calculated using 5% bioavailability.*

*[7]Median absolute requirements for absorbed iron estimated at 0.46mg/day for a child 1-3 year old; equivalent to 34.6µg Fe/kg/day for a child 1-3 year old, weighing 13.3kg (34)*

*[8] Simulation based on median absorbable iron intake from the 1st survey food consumption study and addition of the simulated extra intake of absorbable iron assuming an 80 days intervention*

*[9]Body iron stores after the 80 days intervention assuming that the amaranth and MNP fortified porridge will provide an extra 0.013 and 0.008mg Fe.kg$^{-1}$.d$^{-1}$ respectively while the maize porridge group would have a deficit of 0.005mg Fe/kg/day obtained after deducting the daily median requirement of 0.0346mg Fe.kg$^{-1}$.d$^{-1}$.*

*[10]SF from intervention calculated using Hallberg equation Y=9380X-11260 where Y=iron stores is expressed as µg Fe/kg and X as log SF (35)*

*[11]Based on the simulated SF concentration, defined as SF<12 µg/L Baseline prevalence is the actual prevalence as measured in the 2nd survey based on biochemical parameter*

The food consumption data shows a deficit in energy and iron intake necessitating provision of extra food. Giving extra maize porridge only will improve energy intake but not iron status of the children as seen in this simulation study. Children provided with either amaranth porridge or maize porridge with added MNP will be able to not only meet their iron requirement but also have some excess to build up as buffer for use when dietary iron intake becomes inadequate (1). In case of increased iron requirement due to loss of blood from infections or other causes, the maize porridge with added MNP and the amaranth porridge will probably provide some extra iron to maintain iron status so that the children will not become iron deficient. This is however unlikely in the children receiving maize only porridge and hence they will be prone to iron deficiency in case of increased requirement because of inability to build up iron stores. With the high prevalence of iron deficiency observed in the children we suggest that providing the amaranth porridge or maize porridge with MNP would improve the iron status of children in this age.

A quantitative repeated 24 hour recall was used to collect information on food consumption. Though being time consuming and difficult to administer, the method was considered appropriate since it indicates the habitual intake and gives a valid measure of food intake in a population (43). The 24 hour recall method has been shown to give acceptable estimates of total intake in rural Kenya (44). An interactive form of 24 hour recall was used to reduce chances of under-reporting or under-estimation as the method is dependent on memory (43). Full probability approach was used to estimate the prevalence of inadequate iron intake as the iron requirements of children are not normally distributed. This method enabled us to calculate the prevalence from estimates of the children with intakes in a given intake range (25). The observed iron intake was adjusted for day to day variation. This procedure is appropriate for use in the analyses of the prevalence of inadequate or excess intakes in the group although it does not address problems of systematic bias due to under-reporting of intakes in the recall days or errors in estimation of nutrient intakes (24).

A qualitative 24 hour recall was used to collect information on the number of food groups consumed by each subject in the 2$^{nd}$ survey. This method is less time consuming and inexpensive as it simply involves listing the number of food groups consumed compared to other quantitative methods of dietary assessment. It is also preferred above list recall as it allows respondents to freely recall all the meals and snacks eaten both at home and outside by the child thus reducing over or underreporting of foods consumed (45). We did not collect quantitative breast milk intakes and so our estimates of daily iron intake for breastfeeding children may be slightly underestimated. In infants fed on breast milk however, over 90% of their iron requirements should be met by complementary feeding as early as during the second 6 months of life in order to prevent iron deficiency. Although breast milk is known to have a high bioavailability of up to 34% (46), the iron concentration has also been shown to decrease drastically by the 5$^{th}$ month of lactation up to 0.3mg/L (47). Considering that the age of our study children was even above 12 months and a reported minimal breastfeeding, we therefore suppose that the fraction of iron from breast milk is even lower for our study group and the impact on the estimated iron intakes and status minimal.

The projected values for prevalence of inadequate iron intake could be attributed to the several assumptions that were made. We used a deterministic approach in our model mainly used in nutritional sciences. This model may result in overestimations of the intake distribution as it does not take into account the uncertainties or variability in concentration or consumption data (48). Nevertheless, this model was thought to adequately estimate the expected impact of an intervention to improve dietary iron intake in this population with high prevalence of iron deficiency. The equations used to estimate ferritin concentrations have been suggested to effectively predict effects of iron fortification under different conditions with respect to dietary properties and iron requirements as it does not overestimate the amount of iron stores (35). Evidence has shown that iron stores tend to be stable or have small changes once the plateau has been reached since the body auto regulates further iron absorption (35). The simulated intervention period in this study was within a timeframe that iron stores would be expected to improve in the majority of the children as their baseline iron stores were not high.

The two surveys were conducted in the same area and the dietary diversity analysis was found to be similar in both the 1st and 2nd survey with a low intake of iron rich food for this age group. Therefore we assumed that the time interval would not significantly affect our interpretation of results. We also took a conservative estimate of 3-4% bio-availability for both the amaranth and plain maize porridge though a range of 4-12% bio-availability of non-heme iron has been reported in literature (1). In addition to the problem of inadequate iron intake, iron absorption is further reduced by high phytate intake known to inhibit absorption by forming insoluble complexes. The effect of phytate is shown to be dose dependent, starting at very low concentrations of 2-10mg/meal (49). Our preliminary results (Macharia-Mutie *et al*, unpublished results) on nutrient composition indicate that while the amaranth grain contain high amounts of iron, the phytic acid content is also high (9.6g/kg) resulting in a phytate to iron molar ratio of 3:1 which is still within the inhibitory range (49). The high phytic acid concentration may therefore negatively affect the iron absorption even when the iron intakes are increased especially in the amaranth porridge group. Absorption studies using isotopes could give further information on absorption of iron from amaranth grain porridge.

From an assumed linear relationship between iron intake and iron status, our simulated effects may suggest considerable improvement. However, the effects of absorbable iron in real life intervention maybe varied due to other factors that may either be internal/physiologic such as iron status and genetic determinants or external such as presence of infections, presence of inhibitors or enhancers and environmental factors (42). In our simulation study we have a simulated increment of serum ferritin concentration of 4.8μg/L in the MNP and 5μg/L in the amaranth group. A study in South Africa among primary school children showed an increment in serum ferritin concentration of 3.1μg/L between baseline and post intervention in the treatment group fed with MNP fortified porridge after six months of intervention (50) while an increment of >10 μg/L in ferritin concentration was observed in other studies in India (13) and Kenya (12-59 months) even within a shorter intervention period (Macharia-Mutie *et al*, unpublished results). These varied observations in relation to the simulation could be due to different age group iron requirements and presence of infections resulting to a lower increment in the South African study while the larger increment in the Kenyan study may have been influenced by regular de-worming thus reducing iron loss due to parasitic infections (1).

Our results suggest that use of amaranth grain in dietary diversification or in-home fortification strategy with multiple micronutrient powder could increase iron intake adequacy and decrease iron deficiency among children in rural Kenya. In case of policy decisions when resources are limited to do extensive intervention studies, such theoretical simulations may provide a guide to choice of appropriate intervention.

**Acknowledgements**

We thank the children, parents/caretakers who willingly participated in this study. We also thank the study field assistants who diligently collected the data.

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# Chapter 4

# Sensory acceptability and factors predicting the consumption of grain amaranth in Kenya

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*Ecology of Food &Nutrition (2011), Vol. 50, No.5, pp. 375-392*

## Abstract

This study investigated the effect of adding amaranth grain flour on sensory acceptability of maize porridge in Kenya. Factors influencing the intention of mothers to feed their children on amaranth grain were identified. A significant difference between the various porridge ratios (50:50, 70:30 and 100:0 amaranth: maize) either in unfermented or fermented form could be detected. Preference for the unfermented amaranth enriched maize porridge was observed. Intention significantly correlated and predicted amaranth grain consumption ($P < 0.001$). Knowledge and health value significantly predicted health behaviour identity. Interaction between barriers and intention negatively influenced behaviour. Findings suggest that unfermented amaranth enriched maize porridge is acceptable. Unfermented porridge with 70% amaranth can be considered for use in a program aimed at increasing dietary iron intake among children. Increasing awareness about micronutrient deficiencies and nutritional benefits of amaranth grain could enhance its consumption.

## Introduction

Maize meal in the form of porridge or *ugali* (stiff maize porridge) is a predominant complementary food in eastern and southern Africa contributing to most of the energy and micronutrient such as iron and zinc intake among children (1-3) The use of maize as the principal ingredient suggests that children may be at risk of inadequate micronutrient intake especially iron (4, 5). Inadequate intake of iron rich foods as well as low bioavailability of iron in the maize porridge contributes to occurrence of iron deficiency and iron deficiency anaemia (6). Iron deficiency anaemia is known to be a significant public health challenge especially for growing children in the developing countries (7). To improve the nutrient quality of maize porridge as a complementary food, thereby increasing iron intake and absorption, various strategies have been proposed. These strategies include fortification, enzymatic degradation of phytic acid, fermentation and dietary diversification (2, 8, 9). Dietary diversification may include increased consumption of ascorbic acid rich fruits and vegetables or other foods known to have high micronutrient content such as amaranth grain (*Amaranthus spp*). Amaranth grain is reported to be rich in proteins and iron therefore having a potential of improving the nutritional value of complementary foods (10-12).

Amaranth grain is a pseudo-cereal that was widely cultivated in pre-Columbian America and matures within a short period (11, 12). Presently, it is cultivated for both its seeds and leaves in different regions of the world including Kenya (11-14). Although amaranth grain has been promoted as a healthy food and a source of extra income for small scale farmers in the semi-arid areas in Kenya (13, 14), the effects of addition of amaranth grain flour to the ordinary maize porridge on the sensory acceptability is largely unknown.

A food product must also be socially and culturally acceptable to achieve its intended objective on the target consumers. Though consumption of amaranth grain has been promoted in Kenya, there is no information available on which factors predict the intention to consume amaranth grain. Models based on Theory of Planned Behaviour (TPB) and the Health Belief Model (HBM) have previously been used to predict factors in health and nutritional behaviour studies (15-19). The core assumption of the TPB model is that intention is the primary determinant of behaviour (in this study consumption of amaranth grain) and that this is a function of one's attitude towards that behaviour (19-21), social pressure from 'important others' to consume the amaranth grain or not (subjective norms) and perception of one's ability to perform the given action (perceived behavioural control) (16, 19, 20). The HBM is a health specific model which proposes that health behaviours are the result of a set of core beliefs (22). These beliefs include supposed vulnerability to a disease and the subjective risk of acquiring an illness if no preventive measures are taken (perceived susceptibility) and supposed physical and social consequences of getting the disease (perceived severity). A perceived benefit (health behaviour identity) which reflects whether the person thinks that performing a particular behaviour is good and barriers of the preventive behaviour to reduce risk or seriousness of impact also form part of the health beliefs in HBM model. These are categorized as internal factors in the model of Sun *et al* (18). Cue to action refers to triggers due to awareness of a threat forcing need to take action while control represents confidence of having an ability to take action (22). Perceived barriers are expected to influence the translation of intention to actual behaviour (i.e. amaranth grain consumption) (21).

The objective of this study therefore was to investigate the effect of addition of amaranth grain flour on sensory acceptability of maize porridges in a rural Kenya setting. We also aimed at identifying factors influencing the intention of mothers to feed their children on amaranth grain using the integrated model as proposed by Sun *et al* (18) with modifications. Since amaranth grain is increasingly being used in Kenya, more research about the sensory characteristics of maize porridges enriched with amaranth grain flour is required. Information obtained on sensory properties such as taste and colour will influence future promotional activities for amaranth grain production and consumption in relation to its iron content.

## Methods

### Study area

The sensory and social acceptability studies were carried out in Migwani division of Mwingi District and Makuyu division of Murang'a South District, Kenya, respectively.

Migwani and Makuyu are semi-arid areas located to the east and north of Nairobi respectively. Subjects gave verbal informed consent to participate in the study. Research authorization was given by Ministry of Higher Education, Science and Technology, Kenya.

**Sensory evaluation study**

Sixty subjects consisting of 21 adults who had a child less than two years of age and 39 teenagers participated in this study with an average age of 21.6±10.9 years. The inclusion criteria for the teenagers were that they had to be older than 12 years of age and in class seven or eight of primary school. The adults were randomly recruited from the study area through home visits and participation was voluntary. The subjects received instructions about the test and the forms to be filled before starting the evaluation.

***Preparation of porridges***

Milled white maize flour (80% extraction rate) enriched with milled whole amaranth grain flour was used in this study. Both maize and amaranth grain flours were bought separately from a local supermarket in Nairobi, Kenya. Porridges with three different proportions of amaranth grain and maize flour were prepared so as to investigate which ratio of amaranth grain and maize flour would be most acceptable in a proposed intervention study. The porridges were prepared either in fermented or unfermented form. Based on the need to use the composite flour with the highest possible iron content in the intervention study, the lowest sample ratio used in this study was 50% amaranth grain flour mixed with 50% maize flour (50:50). To assess whether addition of more than 50% amaranth is acceptable, porridges with 70% (70:30) and 100% (100:0) amaranth were included in the study.

To get the different flour ratios, manual mixing of both maize and amaranth grain flour was done for approximately 5 minutes for each ratio (i.e. 7 kg amaranth and 3 kg maize to give 70:30 ratio and 5 kg amaranth and 5 kg maize to give 50:50 ratios). The resulting mixture of 10kg for each ratio was used throughout the trial. Visual examination was done to check for even distribution of the amaranth flour in the maize flour. Cooking of both the fermented and unfermented porridges was done on the day of the test. To make the unfermented porridge, a smooth slurry prepared by adding flour to 1 litre of cold water was poured into about 5 litres of boiling water in cooking pots and the mixture was stirred for at least 15 minutes till the porridge formed a smooth paste. To make the fermented porridge natural fermentation was done by preparing smooth slurry with 2 litres of warm water and approximately 350g of each flour ratio three days before the actual cooking. The prepared slurry was poured into plastic jerry-cans and left to ferment at room temperature until use. On the day of test the fermented slurry was mixed with some more flour and 1 litre of cold water. The resulting fermented slurry was then poured into about 5 litres of boiling water in cooking pots and the mixture was stirred for at least 15 minutes till the porridge formed a smooth paste.

To ensure that the porridges had comparable consistency, the total amount of water (6300-8000 ml), flour (524-1000 g) and the cooking time (18-36 minutes) were adjusted for each of the porridge depending on the flour ratio used. The porridge with more amaranth tended to have a thinner consistency and therefore more flour and less water was used for this porridge. As much as possible we controlled for consistency through visual examination by checking the fluidity of the porridge. The cooked porridges were kept in jerry cans to maintain the serving temperature.

***Sensory tests***

Discrimination test was done by the subjects in two triangle test sessions on separate occasions one week apart. The first test session consisted of triangles of unfermented amaranth grain enriched porridge. In the second session, the triangles consisted of fermented amaranth enriched porridge. In each session, six different triangle combinations were offered. The sequence of combinations was counterbalanced across subjects. Three samples were presented simultaneously to the subjects of which two samples were identical and one was different. Each subject had to indicate which sample was the odd one based on their assessment through mouth feel and taste but they were not to report which specific attribute was different in the forms provided. The porridges were covered and the subjects were not allowed to open more than one lid of the serving bowls at the same time. Our tests were based on an alternative hypothesis that the probability that the subjects will make the correct decision when they perceive a difference between samples was to be larger than one out of three i.e. $H_a{:}P_t{>}1/3$ (23).

Paired preference test using 9 pairs of porridge was done by each participant one week after the second discrimination test. During this test, subjects received two samples with different ratios of amaranth grain flour and were requested to indicate the sample they preferred in the provided form. The subjects were requested to compare colour, smell and taste. Re-tasting was allowed during this test and the subjects were not forced to eat the whole sample. We hypothesized that the subjects will prefer the porridge with the lowest amount of added amaranth grain flour i.e. $H_a$: P (A) ≠P (B) (23).

**Social acceptability study**

One hundred and fifty women (mean age 31±7.9 years) randomly selected from Makuyu Division of Murang'a South District participated in this sub-study. The participants complied with the inclusion criteria of being a mother/caregiver of a child aged between 1 and 3 years, knew and had consumed amaranth grain before. Mothers were considered in this study as key respondents, since parental consumption has been reported as a strong determinant for children's food consumption.

***Questionnaire***

The questionnaire consisted of 131 items identified through literature search on grain amaranth and 10 questions on socio-demographic profile. The items were categorized into 12 constructs according to the model by Sun et al (2006) **(Figure 4.1)**. The constructs were further grouped into background and perception, beliefs and attitudes, external factors, intention and behaviour. Each item was translated into a statement either positively or negatively stated depending on the construct and the respondents were asked to indicate their level of agreement or disagreement on a Likert scale. Items of most constructs were scored from 1 to 5. However, for the constructs attitude towards behaviour and subjective norms, items consisted of pair statements with answer categories ranging from1 to 5 and -2 to 2. The scores of the pair statements were then multiplied to derive one score for each item ranging from -10 to 10. The questionnaire was translated into the local language (Kikuyu) and correctness checked with back translation into English. Pre-testing was done with eight women who were not part of the final study group. No change of the questionnaire was necessary after the pre-test.

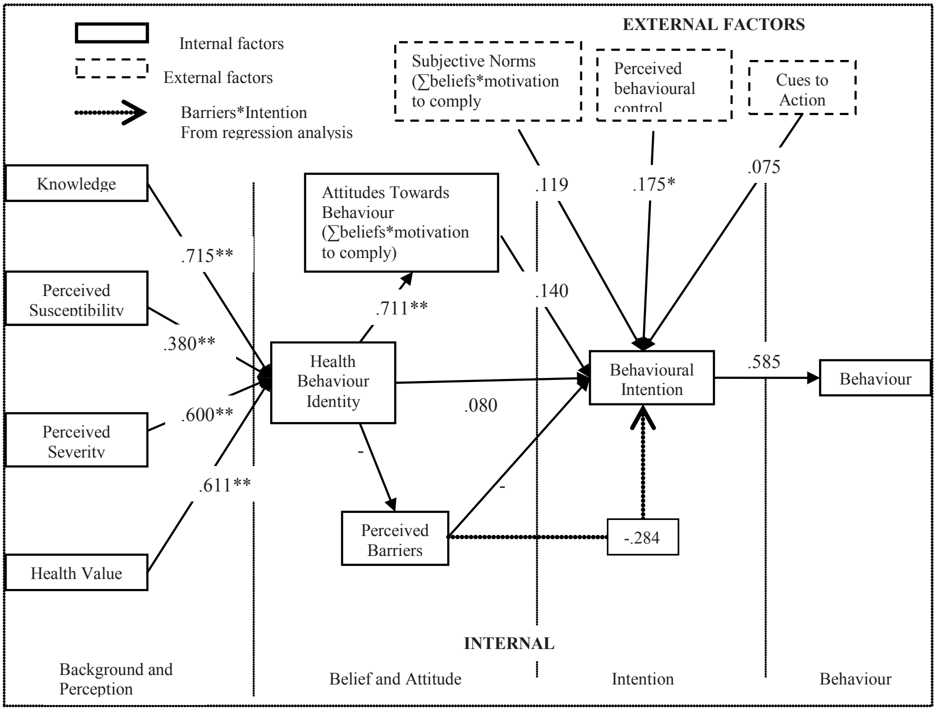


**Figure 4.1: Correlations of the various constructs using the combined health belief and theory of planned behaviour models [a].** *[a] Model adapted from Sun et al, 2006 (18), * Correlation is significant at the 0.05 level (2 tailed), **Correlation is significant at the 0.01 level (2 tailed)*

### Statistical analysis

Data analysis was performed with the Statistical Package for Social Sciences (SPSS 15.0 for Windows) and MS Excel. For sensory evaluation data the responses of our subjects were not independent from each other as they tasted the product six times. Therefore probability of variance from within and between persons existed. This multiple variance referred to as over dispersion is measured by gamma ($\gamma$), a value that ranges from 0 to 1. When $\gamma$ is significantly greater than 0, the beta-binomial model should be used to avoid an underestimation of the standard error, otherwise the binomial model would be used.

Moment estimation was used to calculate gamma ($\gamma$) and Tarone's Z statistic was used to check whether this $\gamma$ was significant. Since there was no significant subject variability, the binomial distribution model was used for analysis. To check whether the critical minimum value of correct or agreeing choices needed for significance was achieved (49 out of 120 judgments), reference was made to statistical tables on critical values (24-26). Multiple regression analysis were carried out to examine how much variance was explained by different models, and to determine which constructs significantly predicted intention. Four models used were:
Model 1: identity = knowledge + susceptibility + severity + values;
Model 2: intention = identity + barriers + attitude;
Model 3: intention = norms + control + cues to action; and
Model 4: behaviour = barriers + intention + (barriers*intention).

All models were adjusted for interviewer, place of residence, respondent's age and level of education, by including them in the models. Multi-colinearity was checked through variance inflation factor (VIF) and tolerance. No changes were necessary because VIF was <10 and tolerance was >0.1. A value of $p < 0.05$ (95% CI) was considered statistically significant (27).

For social acceptability data, descriptive statistics were carried out. Median scores were used to show the trends in answers per construct. Reliability analyses were carried out to evaluate internal consistency within the constructs. Consistency was achieved when the correlation coefficient Cronbach's alpha ($\alpha$) was 0.80 or higher for the complete set of items in a construct. Each single item had to be higher than 0.30 on the corrected item-total to remain in the set. If an exclusion of an item resulted in a considerable increase on Cronbach's $\alpha$ value for the total item set, the item was excluded for this and further analysis to enhance the consistency of construct. In total 19 items were excluded as follows: knowledge (1 item), susceptibility (1 item), barriers (12 items), attitudes (2 items) and cues (3 items). For each respondent the item scores within a construct were added to derive a total score per construct **(Table 4.1)**.

**Table 4.1: Internal consistency and median scores of the responses per construct (n=150)**

| Construct | No of items [a] | Cronbach's α (complete set) | Median | 25th | 75th | Range [b] |
|---|---|---|---|---|---|---|
| Knowledge [c] | 10 | .87 | 47 | 40 | 50 | 11;55 |
| Perceived susceptibility [c] | 3 | .81 | 12 | 11 | 14 | 4;20 |
| Perceived severity [c] | 15 | .84 | 67 | 60 | 72 | 15;75 |
| Health values [c] | 13 | .91 | 63 | 57 | 65 | 13;65 |
| Health behaviour identity [c] | 3 | .95 | 14 | 12 | 15 | 3;15 |
| Perceived barriers [d] | 10 | .90 | -6 | -14 | 2 | -50;50 |
| Attitudes towards behaviour [e] | 7 | .92 | 60 | 35 | 70 | -100;100 |
| External control belief [c] | 4 | .75 | 20 | 16 | 20 | 4;20 |
| Cues to action [c] | 8 | .80 | 30 | 26 | 34 | 11;55 |
| Subjective norms [f] | 10 | .83 | 46 | 34 | 53 | -100;100 |
| Behavioural intention [g] | 3 | .97 | 15 | 15 | 15 | 3;15 |
| Prior behaviour [g] | 2 | .98 | 10 | 8 | 10 | 2;10 |

*[a] Cronbach's α >0.3, [b] Range refers to the minimum and maximum possible scores within construct. [c] scores ranged from 1=strongly disagree to 5=strongly agree. [d] scores ranged from 2=strongly disagree to -2=strongly agree. [e] Bb (behavioural beliefs) items ranged from 1=strongly disagree to 5=strongly agree * Oe (outcome evaluation) items which ranged from -2=strongly disagree to 2=strongly agree. [f] Nb (normative beliefs) items ranged from 1=very unlikely to 5=very likely * Mc (motivation to comply) items which ranged from-2=strongly disagree to 2=strongly agree. [g] items ranged from 1= none to 5= more than 2 times a week*

Spearman's correlation was done to examine the level of agreement within the integrated model of TPB and HBM. Wilcoxon signed-rank test was used to compare the scores of the item under intention to consume amaranth grain and the item consumption of amaranth grain, hence to test whether the subjects significantly change their response in one direction (i.e. if score intention is > or < score behaviour).

## Results

### *Sensory evaluation*

In the discrimination tests, the subjects were able to detect a significant difference between all the ratios in both the unfermented and fermented amaranth enriched maize porridges ($p < 0.05$) **(Table 4.2)**. In the fermented porridges more subjects detected a difference when comparing 50:50 and 70:30 to 100:0; however when comparing 50:50 with 70:30, there was no difference in number of subjects detecting a difference.

**Table 4.2: Triangle tests results with six replications for different amaranth: maize ratios in Mwingi District[1]**

| | Porridge Serving Ratio | | | | | |
|---|---|---|---|---|---|---|
| | 50:50/70:30 | | 70:30/100:0 | | 50:50/100:0 | |
| Description | UP | FP | UP | FP | UP | FP |
| Subject variability (γ) | 0.19 | <0.0001 | 0.004 | 0.09 | 0.10 | 0.09 |
| Number of correct responses observed | 64* | 64* | 57* | 80* | 67* | 87* |
| Triangle μ Test: μ=1/3 | 0.53 | 0.53 | 0.48 | 0.66 | 0.56 | 0.73 |

**significant (α= 0.05) using Tarone's Z statistic. [1] Maximum number of responses=120; Number of responses needed for significance (α= 0.05) = 49, UP=Unfermented porridge, FP=Fermented porridge*

In the paired preference tests no subject variability was calculated because every subject tested the different comparisons only once and the binomial model was used. With the fermented porridges, the lower ratio was always preferred indicating that 50:50 was preferred above others. With the unfermented porridges both lower ratios were preferred above 100:0 but no preference was seen between 50:50 and 70:30 when comparing the unfermented with the fermented amaranth enriched maize porridges, for all the ratios a preference was found for the unfermented porridge **(Table 4.3).**

***Social acceptability of amaranth grain porridge***

The main sources of amaranth grain was buying (85.3%) and own cultivation (27.3%). More than three quarters of the respondents (80%) had the intention to feed their children amaranth grain, two or more times a week, during the next 6 months while 72% had fed their children with the grain during the past 6 months, two or more times a week Although the women scored slightly higher on intention than behaviour, Wilcoxon-signed rank test showed that there was no difference between the reported intention and behaviour (P=0.027).

**Table 4.3: Preference tests results for different ratios of amaranth enriched maize porridge in Mwingi District**

| | Porridge Ratio | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Description | F 50:50/ F 70:30 | F 70:30/ F 100:0 | F 50:50/ F 100:0 | U 50:50/ U 70:30 | U 70:30/ U 100:0 | U 50:50/ U 100:0 | F 50:50/ U 70:30 | U 50:50/ F 70:30 | U 70:30/ F 100:0 |
| Appropriate model | Binomial | Binomial | Binomial | Binomial | Binomial | Binomial | Binomial | Binomial | Binomial |
| No of responses needed for significance | 39 | 39 | 39 | 39 | 36 | 37 | 39 | 39 | 39 |
| No of responses preferring 1st stated ratio | 40* (F50:50) | 42* (F70:30) | 54* (F50:50) | 26 (U50:50) | 36* (U70:30) | 44* (U50:50) | 22 (F50:50) | 46* (U50:50) | 52* (U70:30) |
| Paired Preference Test: μ=1/2 | 0.67 | 0.70 | 0.90 | 0.43 | 0.63 | 0.76 | 0.37 | 0.76 | 0.87 |

**significant (α= 0.05)        F=Fermented porridge        U=Unfermented porridge*

The opinion scores of the constructs were high compared to the range of possible scores **(Table 4.1)**. Most respondents agreed that amaranth grain contains iron (97.3%), and that its use can prevent iron deficiency/anaemia (90.7%). They also associated iron with the health of young children (99.3%). Although most women agreed that in general adult women and young children easily suffer from iron deficiency, most (90.7%) did not find themselves at risk.

A small percentage of women did not agree that iron deficiency will lead to health problems in later life (5%), that iron deficiency can affect intelligence (18.7%) or that adults with iron deficiency will have lower work capacity (2.6%). Most of the women indicated that they were the ones who decided what is good for (99.3%) and what food to give to their child (96%). More than 30% of the women strongly perceive the availability of amaranth grain on the market (34%), the availability throughout the year (40.7%), not finding the right amaranth variety (35.3%), not finding amaranth of good quality (36%) and not having skills (32.7%) or tools (30%) to cultivate amaranth grains, as barriers. However, more than 30% of the women also feel that amaranth grain can be easily grown on the farm (39.3%), cooking amaranth grain is easy (57.3%), that colour (59.3%) and size of the grain (66.7%) were not determining whether or not to buy amaranth grains, that amaranth does taste well (45.3%), has a good texture (42%) and is easy to digest (65.3%).

The following beliefs were stated by more than half of the women: amaranth grain has nutritional qualities (59.3%); eating amaranth grain is good to prevent diseases (58.7%), amaranth grain is good to gain weight (54%) and stimulates appetite (49.3%). Amaranth grain is not seen as a food for poor (46.7%) neither for rich (32.7%). Although women find it important to feed children traditional food (65.3%) that is own cultivated (68%), about 25% of the women do not see amaranth grain as a traditional food while more than 68% do not cultivate amaranth grain on their farm. Illness of household members (43.3%) and especially children (50%) would trigger a woman to feed her child amaranth grain as well as household members (46.7%) and children (53.3%) having iron deficiency. Amaranth grain is not a food specially used during special events or celebrations (72%). According to the women, an upcoming food shortage, amaranth grain sellers or radio advertisements could make them want to eat amaranth (24.7%). Advice from either the husband (59.3%) or a nurse (48.7%) would influence nearly half of the women to give their child amaranth grain. Opinions of friends, neighbours and other women in the community were much less important according to the respondents.

**Figure 4.1** shows the correlations between the constructs of the model. All the variables within background and perception significantly correlated with identity with knowledge having the highest correlation ($r_s$=0.715 p=0.000). A high identity was significantly correlated with a positive attitude ($r_s$=0.71, $p<0.01$) and with a low level of perceived barriers ($r_s$=-0.557, $p<0.01$), but none was correlated with identity. Among the external factors, only control (perception of how easy or difficult it is to feed their children amaranth grain) was significantly correlated with behavioural intention ($r_s$=0.175, $p<0.05$).

**Table 4.4: Predictors of health behaviour identity, intention to consume amaranth grain and prior consumption among women in Murang'a South District, Kenya**

| Model [a] | Standardized β | *P Value* | $R^2$ | Adjusted $R^2$ |
|---|---|---|---|---|
| **Model 1** (Y= Health behaviour identity) | | | .593 | .576 |
| Knowledge | .603 | .000 | | |
| Perceived susceptibility | .055 | .362 | | |
| Perceived severity | -.022 | .805 | | |
| Health values | .310 | .002 | | |
| **Model 2** (Y= Behavioural intention) | | | .038 | .018 |
| Health behaviours identity | .137 | .271 | | |
| Barriers | -.065 | .510 | | |
| Attitudes towards behaviour | .017 | .886 | | |
| **Model 3** (Y= Behavioural intention) | | | .050 | .031 |
| Subjective norms | .055 | .564 | | |
| Perceived behavioural control | .162 | .063 | | |
| Cues to action | .075 | .409 | | |
| **Model 4** (Y= Prior behaviour) | | | .482 | .472 |
| Barriers | .247 | .343 | | |
| Behavioural intention | .689 | .000 | | |
| Barriers * Behavioural intention | -.238 | .362 | | |

*[a] All models adjusted for interviewer, place of residence, respondent's age and level of education. Standardized β-regression coefficient, $R^2$-correlation coefficient squared (coefficient of determination)*

The relative contribution of the variables to behaviour is shown in **Table 4.4.** The constructs within our study explained only a small variance in predicting intention.

Fifty-eight per cent of the variance of health behaviour identity could be explained by background and perception (model 1), of which knowledge about the relationship between iron deficiency/anaemia and amaranth grain ($\beta = 0.60$ $p = 0.00$) and the relative significance given by the person to the health consequences of suffering from iron deficiency anaemia (health value, $\beta = 0.31$ $p = 0.00$) were significant predictors of health behaviour identity. In models 2 and 3, none of the included constructs could significantly predict intention with only 1.8% and 3.1% of the variance explained respectively. Forty-seven per cent of the variance in amaranth grain consumption could be explained where only the construct intention could significantly predict behaviour ($\beta = 0.69$ $p = 0.00$). Although interaction between barriers and intention had a negative influence on this last model, this interaction was not significant.

## Discussion

The first objective of this paper was to investigate the effect of addition of amaranth grain flour on the sensory quality of maize porridges in a rural Kenya setting. Our results indicate that significant differences could be detected between all the porridges. The subjects used in our study were all above 12 years and therefore their cognitive skills were considerably good to provide the information required. To prevent odd sample bias in our tests, the odd sample was randomly assigned between the two similar products as follows; ABA, BAA, BBA, AAB, ABB and BAB. This reduced the probability of having only one type of porridge (ratio) as the odd one. Furthermore, there was at least one week in between the discrimination as well as the preference tests. The one week difference was expected to reduce the effect of the odd sample bias as the subjects may not have been remembering the previous odd sample.

A possible limitation during the triangle test in our study was that the lids for the serving bowls had different colours. As much as possible, we covered the bowls with lids of similar colours for each subject. There were however some instances (< 5% of the test settings) when two lids had the same colour and one lid was different in the test setting of one person. Where more than one colour of lids had to be used we ensured that similar colours did not correspond to any particular ratio. To further reduce the effect of lid colours we emphasized to the subjects prior to the test that the colours of the lids were not of importance for their choice.

Amaranth grain flour has a slightly darker colour than maize flour and therefore the porridge with more amaranth was slightly darker. To reduce the influence of porridge colour differences on the choices made by the subjects, the samples were all covered and the subjects were allowed to open one lid at a time. However, there is a probability that the respondents could sometimes remember the colour of the other porridges. It is then possible that though the discrimination test was based on mouth feel and taste, the choice was also influenced by the porridge colour. The responses of the paired preference test cannot only be based on the taste of the products but also on the visual characteristics such as consistency or smell aspects of the porridge. As such the subjects were allowed to use all their senses such as sight, smell and taste. In the statistical analysis, it was taken into account that there is 50% chance of preferring any of the two products (porridges) presented for testing when in reality there was no significant difference perceived between them. Since this chance was randomly assigned over the subjects and our preference test was not only based on taste but also on smell and sight, it was expected that the effect of choices made would not lead to incorrect conclusions.

Other studies have showed that 15-20% of the flour used for making bread and conventional pound cakes could be replaced by amaranth grain flour without negatively affecting the sensory attributes (28, 29). In this study, the minimum amount of amaranth flour added to the maize porridge was 50%. Based on the need to use a composite flour with the highest possible iron content from amaranth grain in a proposed intervention study we therefore did not compare the enriched amaranth maize products to the usually consumed plain maize porridge. The porridge with highest ratio (100:0) of amaranth was least preferred. This could be due to the intrinsic nutty taste of amaranth, which may not be familiar to the subjects (13). Although using hedonic tests, a similar low acceptability in cakes has been observed when more amaranth flour was added during their preparation in Brazil (29).

The second objective of this study was to identify factors that significantly predict the intention of mothers/caregivers to feed their children amaranth grain in Makuyu division using an integrated model of the TPB and HBM (18). In the present study the constructs explained only a small variance in predicting intention. The novelty of amaranth grain may explain the low contribution of the constructs to the prediction of intention in this study. Though the theory of planned behaviour model requires participants to describe their cognitions, this requirement is based on the assumption that the answers given will reveal pre-existing states of mind (21). Thus if the behaviour is novel and unfamiliar it is possible that the cognitions may be created simply by completing a questionnaire (30). In our study we sought to reduce the effect of unfamiliarity by setting certain requirements for participation (i.e. knowing and having eaten the grain). However, the target population was still not entirely familiar with the consumption of amaranth grain. The majority of the respondents had tried it in the past but more as a sporadic event rather than a regular behaviour.

Amaranth grain has recently been introduced to the Kenyan market and although it is currently commercialized and sold in major supermarkets in Nairobi, this has not yet reached many rural areas. In addition, amaranth grain was only being grown and sold by a small group of farmers in the study area. High opinion scores compared to the range of possible scores were observed indicating that most respondents tended to agree with the statements. Unfamiliarity and tendency of the respondents to agree rather than disagree with the statements may reflect difficulty in discriminating opinions implied by response categories provided (31). It may also reflect cultural or linguistic ambiguities in what is meant or intended by the question (31). It may be that there are also no strong believes and attitudes attached to consumption of amaranth grain yet. Lack of strong beliefs makes it difficult to set a level of agreement or disagreement towards statements, as the behaviour is not yet incorporated in the respondent's habitual context.

The observation that knowledge and health values significantly predicted intention indicates that the respondents would take a concrete health related action if they acknowledge the threat of a disease. The construct health values, has been reported as a significant predictor to take action related to health checks as in general practice (32). This indicates the extent to which the respondent values their health. This study supports these findings, as the respondents have a positive attitude towards the value of health, in particular for their children, which may be reflected in actions aiming to ensure their health. Knowledge, from a wider scope, is a determinant factor to trigger engaging actions towards healthier behaviours in this population.

Intention was used in our study as a predictor of prior behaviour which in turn is a proxy for future behaviour. This has similarly been used in other studies assessing intended food choice (33). However, to our knowledge there are no studies yet on amaranth grain consumption that confirms intention as a predictor of behaviour and therefore these findings must be taken cautiously. Two potential drawbacks are encountered when measuring intention and behaviour with self-reported questionnaires. First, answers given by the respondent on both constructs may be leading correspondingly, due to the similar way of wording resulting in an overestimation of the relationship (34). Second, when behaviour is self-reported it explains a larger part of the variance, than when behaviour is objectively measured, suggesting that individuals may provide socially desirable answers (21). On the other hand, though there could be an existent and strong linear relationship between a particular intention and behaviour in the population, when people with fairly or very strong intentions to perform the behaviour volunteer for the study, the observed correlation and percentage of variance explained in the sample will be lower than that in the population (34). Chances of this occurrence in our study were reduced by randomly selecting the participants.

The significant correlation of control with behavioural intention indicates that women in this study area are empowered to make decisions regarding amaranth grain consumption. The construct subjective norm was not a good predictor of behavioural intention and has reported as a generally weak predictor of intentions (21). In addition, African societies emphasize on communality and interdependence thereby, accentuating the importance of a referent group influence, especially in the domain of food choice (35, 36). It was therefore expected that social pressure would play an important role in intention for this population. However this trend was not observed in our study.

In conclusion unfermented amaranth enriched maize porridge is acceptable for women to give to their children. The porridge with the highest amount of amaranth, the unfermented 70:30 porridge, can be considered as a suitable food to be used in a food based approach program aimed at increasing dietary iron intake among children. The constructs explained only a small variance in predicting intention but none significantly predicted intention. Though knowledge and health value were significant predictors of health behaviour identity, this did not significantly predict intention to consume amaranth grain. To promote amaranth grain consumption therefore, increasing awareness about micronutrient deficiencies and nutritional benefits of amaranth grain (knowledge) as well as health consequences of the deficiencies (health value) could be targeted.

## Acknowledgements

We are grateful to all the schools, farmers, mothers and young people who willingly took part in this study.

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# *Chapter 5*

# *Maize porridge enriched with a micronutrient powder containing low dose iron as NaFeEDTA but not amaranth grain flour reduces anaemia and iron deficiency in Kenyan pre-school children* [1]

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*(Ahead of print J Nutr* doi: 10.3945/ jn.112.157578*)*

[1] *ClinicalTrials.gov; identifier number NCT01224535*

## Abstract

Few data have evaluated the impact of fortification with iron-rich foods such as amaranth grain and multi-micronutrients powder (MNP) containing low doses of highly bioavailable iron to control iron deficiency anaemia (IDA) in children. We assessed the efficacy of maize porridge enriched with amaranth grain or MNP to reduce IDA in rural Kenyan pre-school children. In a 16-week intervention trial, children (n=279; 12-59 months) were randomly assigned to: non-fortified unrefined maize porridge (control; 4.1 mg of iron/meal; phytate/iron molar ratio 5:1); unrefined maize (30%) fortified with amaranth grain (70%) porridge (amaranth group; 23mg of iron/meal; phytate/iron molar ratio 3:1); or unrefined maize porridge fortified with MNP (MNP group; 6.6mg iron/meal; 2.5mg iron as NaFeEDTA). Primary outcome was anaemia and iron status with treatment effects estimated relative to control. At baseline, 38% were anaemic and 30% iron deficient. Consumption of MNP reduced prevalence of anaemia (-46%; 95% CI= -67,-12), ID (-70%; 95% CI=-89,-16), IDA (-75%; 95% CI= -92,-20) and soluble transferrin receptor (-10%; 95% CI=-16,-4) concentration while significantly increasing haemoglobin (2.7g/L; 95% CI= 0.4, 5.1) and plasma ferritin (40%; 95% CI=10, 95) concentration. There was no significant change in haemoglobin or iron status in the amaranth group. Consumption of maize porridge fortified with a low dose of highly bioavailable iron MNP can reduce the prevalence of IDA in pre-school children. In contrast, fortification with amaranth grain did not improve iron status despite the large increase in iron intake, likely due to high ratio of phytic acid to iron in the meal.

## Introduction

Iron deficiency anaemia is a significant public health problem in young children in developing countries and is associated with impaired cognitive development and morbidity (1). Supplementation has successfully been used to improve iron status in Kenya (2, 3). However, following the findings that supplementation with iron and folic acid may be associated with adverse effects in iron replete children (4), WHO advised that in malaria endemic areas, iron supplementation with doses such as the entire reference nutrient intake should be administered only to those who are at risk of iron deficiency (5). This recommendation extends to untargeted use of MNP containing dosages in the range of the iron RNI so as to avoid the potential adverse effects of a large bolus of iron taken in a single dose/meal (5). Screening for iron deficiency (ID) is however not always logistically feasible in resource poor settings (6). Iron fortification of foods has been shown to be an effective strategy to alleviate iron deficiency anaemia among school-age children and infants (7-10).

Providing iron at low dosages in forms which are more bioavailable or increasing iron intake through dietary interventions are two promising strategies to overcome this problem. Home fortification with MNP can be incorporated into the existing feeding practices and ready-to-eat foods, such as porridge just before consumption. MNP with either low (11, 12) or high dose (13-15) of iron have been shown to improve iron status in children with or without malnutrition (16). Iron fortification with low dosages of iron require optimized bioavailability, however, cereal and legume based porridges used as children's foods are rich in phytate. Addition of exogenous phytase to low dose highly absorbable iron MNP has been shown to improve iron status (11). Similarly the use of sodium ferric EDTA (NaFeEDTA) has been shown to be effective in improving iron status when used to fortify high phytate foods (9). Home fortification with MNP with low dosages of bioavailable iron as NaFeEDTA without phytase may therefore be a promising strategy to improve iron status among children.

Dietary diversification can promote the use of locally available and acceptable mineral rich foods (17). Diets enriched with animal source foods have been shown to improve growth (18) and iron status (19). However, to our knowledge, limited evidence exists

(20) on the effectiveness of iron rich plant source foods in improving iron status, even if there have been promotional activities on their use. The amaranth grain *(Amaranthus cruentus)* contains high iron concentrations and is a drought resistant, fast maturing crop suitable for cultivation in semi-arid areas (21). Amaranth grain enriched maize porridge was found to be acceptable at a ratio of 70% amaranth and 30% maize (22). Food diversification with amaranth grain, which has been promoted in Kenya as a nutrient dense food, may potentially improve iron status among children. The aim of this study was therefore to investigate the efficacy of consumption of maize porridge enriched with amaranth grain or a low dose iron containing micronutrient powder on anaemia and iron status of Kenyan pre-school children. It is expected that this data may assist in the informed decision making process on the appropriate strategies to combat iron deficiency in a semi-arid rural Kenya.

## Materials and methods

### Study design, site and participants

The study was a randomized partially-blinded controlled trial conducted over a period of 16 weeks. The study was conducted from October 2010 to February 2011 in Migwani Division, Mwingi District, Kenya. Migwani, situated 200 km North-East of Nairobi was purposively selected as it falls within an agro-ecological zone in a semi-arid area that experiences food shortage for most part of the year. Children aged 12-59 months were assigned to either unrefined plain maize porridge (control group), unrefined maize porridge enriched with amaranth grain flour at the ratio of 30% maize flour to 70% amaranth (amaranth group), or unrefined maize porridge fortified at the time of consumption with a low dose iron from a MNP containing 2.5mg of iron in the form of NaFeEDTA and other micronutrients such as vitamin A, vitamin C, vitamin B12 and folic acid; MNP group) (**Table 5.1**). Informed verbal and written consent was obtained from the principal caretaker/parent. The study was approved by the ethical review committees of Kenyatta National Hospital and Wageningen University. Research permit was obtained from the Ministry of Higher Education, Science and Technology, Kenya.

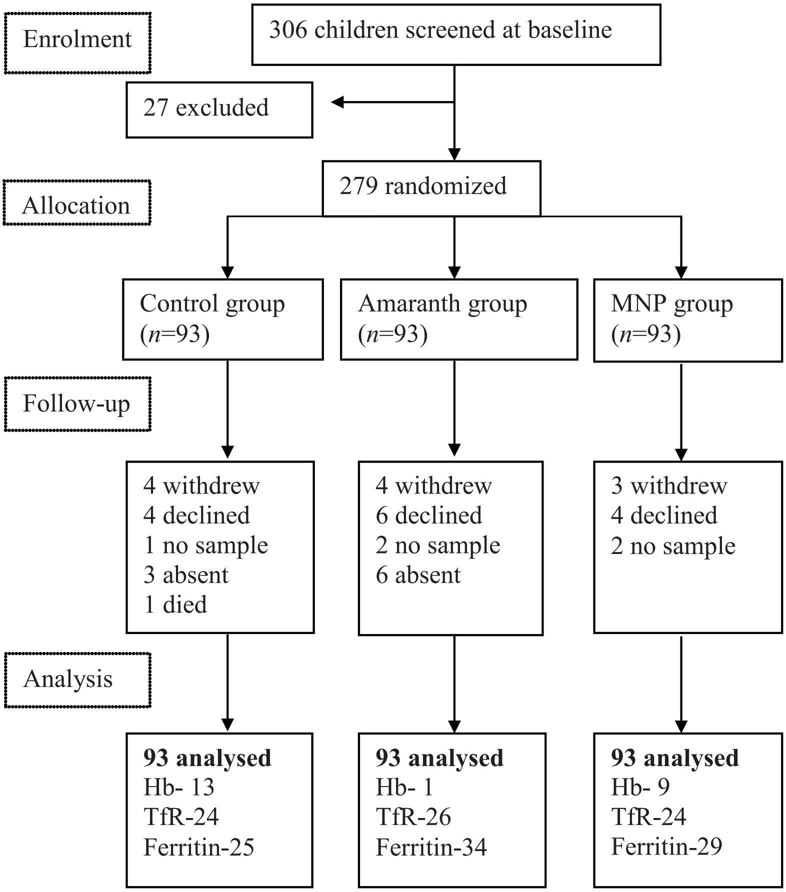


**Figure 5.1: Trial profile of the study participants**

*[1]Excluded children had fulfilled the inclusion criteria but no blood samples could be withdrawn.*
*[2]Amaranth group- maize porridge enriched with amaranth grain flour at the ratio of 30% maize flour to 70% amaranth.*
*[3]MNP group- maize porridge fortified with a low iron containing multi-micronutrient powder (2.5mg of iron in the form of NaFeEDTA)*
*[4]The number of imputed missing values either at baseline or post intervention. The imputed values include children who had sample for one or more biochemical analysis but insufficient blood samples to run all biochemical analysis.*

### Sampling procedure, sample size calculation

Children in two, out of six randomly chosen administrative locations within Migwani Division namely; Migwani and Nzauni were selected using the random walk method (23). The inclusion criteria were; age between 12 and 59 months, apparently healthy at the time of entry into the study and having lived in the village for at least 6 months prior to the intervention and continuing to live there for the next one year. A sample size of 90 per group was sufficient to detect an increase in haemoglobin concentration of 4g/L with a standard deviation of 9 (9), assuming a power of 80% and an attrition of 12% (24). A total of 306 children invited into the study were screened and 279 qualified for the study. The 279 children were randomly allocated using block randomization by age and sex generated with Excel (Microsoft, USA) by one investigator not involved in recruitment and data collection to one of the three treatment groups **(Figure 5.1)**.

### Screening procedure

A questionnaire was used to get background information of all the children. All the children underwent a clinical examination and anthropometric measurements were taken. Those who had not been de-wormed within the last three months prior to the start of the study were given a dose of albendazole (Glaxo Smithkline, Nairobi, Kenya). Children aged two years and above received 20 ml containing 400 mg albendazole whilst children under age of two years received half of that dose. Only those children who met the inclusion criteria and provided blood samples were included in the study.

### Intervention procedures

Both maize and amaranth grain flour were produced and packed into 2 kg packets by East Africa Nutraceuticals, Nairobi, Kenya. DSM Nutritional Products (Kaiseraugst, Switzerland) produced the MNP (**Table 5.1**). The target daily intake was 350 ml of porridge for all the children, considered to be an amount that they could comfortably consume in one session. Each serving of unrefined maize porridge contained approximately 60g of flour. A serving of unrefined amaranth enriched maize porridge contained 24g of maize flour and 56g of amaranth flour. Both flours were precooked, extending their shelf life and reducing the cooking time in the field to 15 minutes. The two types of porridge were cooked at three different cooking centres from where they were distributed in thermo flasks to seven additional centres for feeding. Before distributing the porridge to the feeding centres, one teaspoon of sugar was added to all the serving bowls labelled with the child's name and identification number. In addition, the MNP was also added to the bowls for those children in MNP group. Similar serving cups equivalent to 350ml of the porridge were used to serve in all the centres. The porridge was served between 8.00 and 11.00 hours. Attendance and leftovers were recorded daily.

**Table 5.1: Composition of the micronutrient powder used in the intervention study**

| Nutrients | Amount per 1g sachet |
|---|---|
| Vitamin A, *μg RE* | 100 |
| Vitamin D, *μg* | 5 |
| Vitamin E, *mg TE* | 5 |
| Phylloquinone, *μg* | 30 |
| Thiamin, *mg* | 0.5 |
| Riboflavin, *mg* | 0.5 |
| Pyridoxine, *mg* | 0.5 |
| Folic acid, *μg* | 90 |
| Niacin, *mg* | 6 |
| Vitamin B-12, *μg* | 0.9 |
| Vitamin C, *mg* | 60 |
| Iron (as NaFeEDTA), *mg* | 2.5 |
| Zinc, *mg* | 2.5 |
| Selenium, *μg* | 17 |
| Copper, *mg* | 0.34 |
| Iodine, *μg* | 30 |

**Iron and phytic acid concentrations determination**

Two samples of maize/amaranth flour and three for plain maize flour were sampled from the flour batches for iron and phytic acid concentration determination. In the laboratory, about 10g for iron and 10g for phytate analysis were taken from each packet, put in an airtight container and stored at -20°C until analysis in Wageningen University. Iron concentration was determined using microwave digestion atomic emission spectrometry (VISTA-PRO CCD Simultaneous ICP-AES, Varian; Palo Alto, USA) as described by Novozamsky *et al* (25). The phytic acid concentration was analysed as described by Makower (26), with modifications (27). The phosphate concentration was measured according to van Veldhoven *et al* (28) and results expressed as the sum of IP6 and IP5 in g/100 g dry matter (27). The difference of duplicate sample relative to the mean value was < 10%.

**Anthropometric measurements**

Weight measurements were taken to the nearest 0.1 kg with a UNICEF Salter scale or a digital bathroom scale (Ashton Meyers® Model no 7752), with a capacity of 25 kg and 150 kg respectively. The bathroom scale was only used if a child refused to put on the provided weighing pants or could not fit in it. Standing height (children > 2 years) or recumbent length (children < 2 years) was measured to the nearest 0.1 cm on a wooden height/length board (UNICEF model, item No 0114500). Weight and height measurements were done twice and the average used for further analysis. Age was calculated from the birth date reported on clinic cards or, in rare cases, by parents/principal caretaker recall. Anthropometric Z-scores were calculated using the 2006 WHO growth standards (WHO_ANTHRO version 3.2.2). Stunting, underweight or wasting was defined by Z score <-2SD for anthropometric indices (29). One child was excluded from nutrition status analysis due to biological implausible height value at baseline (30).

**Malaria Test**

All children were tested for malaria infection at baseline with a rapid diagnostic parasite lactate dehydrogenase (pLDH) test kit (OptiMAL-IT™). Due to supply problems, three different rapid diagnostic test (RDT) kits (OptiMAL-IT™, Carestart™ or Carestart™ PF/VOM Combo) were used at endpoint but only one type of the test kit was used per child which was dependent on the date of final sample collection. Thick blood smear slides were also prepared for all samples at endpoint and microscopy to examine the presence or absence of malaria parasites was performed at the Kenya Medical Research Institute, Nairobi.

### Laboratory analysis

Non fasting venous blood samples were collected by venepuncture. Haemoglobin concentration was determined using the Sysmex haematological analyser (KX-21, Sysmex Corporation, Japan). Heparinized blood samples (tubes by Becton-Dickinson®) were stored in cool boxes with ice packs in the field. Within 6 hours after collection, the samples were centrifuged at 3000 rpm for 10 minutes and separation done at Migwani hospital. Plasma was then stored in a freezer at -20$^0$C for 2-3 days before transportation to Nairobi for further analysis. Plasma concentrations of ferritin (PF), C-reactive proteins (CRP) and soluble transferrin receptor (TfR) were analysed using fully automated Cobas Integra® analysers (Roche Diagnostics, Mannheim, Germany) at the Pathologists Lancet Kenya laboratory, Nairobi. The intra and inter assay CV were <10%. Anaemia was defined as haemoglobin concentration<110g/L (31). To adjust for anaemia prevalence at an altitude of 1250M, 2.4g/L of haemoglobin was subtracted from each individual (32). ID was defined as PF concentration <12 μg/L in the absence of elevated CRP (31) or TfR concentration >8.3mg/L (test kit reference value). IDA was defined as concurrent anaemia and ID. Body iron (in mg Fe /kg body weight) was calculated using a published algorithm (33). For body iron calculation we first converted the TfR concentrations to ELISA assay equivalents using the regression equation by Pfeiffer *et al* (34). Elevated acute phase protein was defined as CRP >5 mg/L (35). A correction factor of 0.67 for those with elevated CRP was used to yield an adjusted plasma ferritin concentration representative for a child without acute inflammation (35).

### Statistical analysis

Analysis was done by intention to treat. Data was analysed using PASW Statistics Version 18.0.3 (IBM Corp, USA) and PROC MIANALYZE in SAS Version 9.2 (SAS Institute Inc., USA). Missing data values of Hb, ferritin and TfR were imputed before primary analysis using multiple imputations. The data was imputed 5 times using the fully conditional specification method with the default PASW Statistics initialization value (36). Treatment group, number of days attended, sex, age, baseline and post intervention weight, height, Hb, PF and TfR concentrations were used as predictors in the imputation model. Pooled estimates from the imputed data are reported.

ANCOVA with planned contrasts was used to estimate treatment effects relative to control group for continuous outcomes as it has more power in a model that includes covariates to detect true effects (37). Cox regression with robust variance estimates was used to estimate treatment effects relative to control group for binary outcomes with constant time at risk (38). The Cox regression was preferred to logistic regression since the prevalence ratio is easier to interpret than odds ratio while the robust variance estimate reduces the width of the CIs resulting from use of prevalence data that follows a binomial distribution (38). Exponents of the prevalence ratios obtained by Cox regression as well as effect estimates obtained from log transformed data were converted to percentage differences relative to the control group. Adjusted estimates were analysed including baseline Hb, PF and TfR concentrations as covariates. Post-hoc subgroup analysis was done to assess whether the magnitude of the treatment effect was influenced by ID at baseline. Chi-square and t- tests were used to assess the differences between and within groups. We also performed the per-protocol analysis to check whether the results of the imputation and cases with complete data were different. A value of $P < 0.05$ (95% CI) was considered statistically significant.

## Results

Two hundred and thirty nine children completed the study equivalent to 86% of the children randomized at baseline. Eleven children withdrew within the first month of the study while one child died from causes unrelated to the study as judged by the ethics committee. Endpoint measurement for biochemical indicators was not done for 19 children because either their veins could not be detected ($n$=5) or their caretakers declined ($n$=14). Nine children were absent for end measurement **(Figure 5.1)**. The baseline characteristics of these children did not differ with those that completed the study (data not shown).

The iron concentration in the amaranth fortified maize flour was 29mg/100g dry matter while that of maize flour was 7mg/100g dry matter with a phytate to iron molar ratio of 3:1. The amaranth porridge was estimated to provide an additional 18.9mg of iron/meal while the MNP provided an additional 2.5mg of iron/meal to the 4.1 mg iron/meal provided by plain maize porridge. The total number of feeding days ranged from 92 to 96 days, depending on the serving station with an average attendance of 83.7%. On average 345ml of porridge was eaten during the feeding session six days a week in a period of 16 weeks across all the groups.

**Table 5.2: Baseline characteristics of study participants by intervention group[1]**

| | Control | Amaranth group | MNP group |
|---|---|---|---|
| | *n=93* | *n=93* | *n=93* |
| Sex M/F, *%* | 49.5/50.5 | 43/57 | 51.6/48.4 |
| Age, *months* | 37.3±12.3 | 37.5±14.1 | 35.8±10.1 |
| Height for age, *Z score* | -2.0 ± 1.2 | -1.8 ± 1.0 | -1.9 ± 1.2 |
| Weight for age, *Z score* | -1.2 ± 1.0 | -1.2 ± 1.0 | -1.3 ± 1.0 |
| Weight for height, *Z score* | -0.2±1.1 | -0.3±1.0 | -0.3±1.2 |
| Stunted[2] , *%* | 51 (54.8) | 40 (43) | 43 (47.3) |
| Underweight[2] , *%* | 20 (21.5) | 21 (22.6) | 23 (24.7) |
| Wasting[2] , *%* | 5 (5.4) | 2 (2.2) | 2 (2.2) |
| Haemoglobin concentration, *g/L* | 112±11 | 112±12 | 111±11 |
| Plasma TfR concentration, *mg/L* | 5.0 (4.6, 5.4) | 5.3 (4.9, 6.7) | 5.3 (4.9, 5.7) |
| Plasma ferritin concentration, *µg/L* | 17.6 (14.9, 20.8) | 18.1 (15.3, 21.4) | 16.1 (13.6, 19) |
| Body iron[3] , *mg/kg* | 0.9 (0.02,1.9) | 0.8 (-0.1, 1.7) | 0.4 (-0.5, 1.3) |
| Inflammation (CRP>5mg/L), *%* | 15 (16.5) | 7 (7.7*) | 3 (3.4*) |
| Anaemia[4], *%* | 33 (35.5) | 36 (38.7) | 38 (40.9) |
| Iron deficiency[5], *%* | 23 (24.7) | 30 (32.3) | 30 (32.3) |
| Iron deficiency anaemia[6], *%* | 14 (15.1) | 22 (23.7) | 24 (25.8) |

*[1]Values are mean ± SD, geometric mean (95% CI) or n (%).*Different from control, P<0.05. [2]Defined as Z score < -2. [3]To convert body iron in mg/kg to mmol/kg multiply by 0.0171(11).[4]Defined as haemoglobin concentration <110g/L and adjusted for altitude. [5]Defined as plasma ferritin concentration <12 µg/L or TfR>8.3 mg/L and adjusted for presence of infection. [6]Defined as concurrent anaemia and iron deficiency*

In the baseline characteristics, only elevated CRP differed across the groups ($P<0.05$) (**Table 5.2).** This difference disappeared at endpoint and a slight increment in prevalence (13.2%) was reported. No malaria was reported at baseline while at endpoint microscopy showed that 3.8% of the children had malaria which was not different across the groups. About a quarter (24.7%) of the children had been de-wormed six months prior to the commencing of the study. There was a non-significant reduction in the prevalence of underweight in the amaranth (48%, $P$=0.10) and the MNP (26%, $P$=0.38) group relative to the control group (data not shown).

Post intervention, Hb and PF concentrations significantly increased reducing the prevalence of anaemia, ID and IDA across all the groups. Hb ($P$<0.05) and PF concentrations ($P$<0.01) significantly increased while TfR concentrations ($P$<0.01) decreased in the MNP group compared to the control group (**Table 5.3**). The increment in Hb ($P$=0.34), PF ($P$=0.82) and TfR ($P$=0.92) concentrations in the amaranth group was not significantly different from control. Consumption of MNP fortified porridge significantly reduced the prevalence of anaemia, ID and IDA compared to the control ($P$<0.05). In amaranth group, the reduction in prevalence of anaemia ($P$ =0.63), ID ($P$ =0.66) and IDA ($P$=0.51) was not different from control.

Hb concentrations increased while TfR concentration decreased significantly ($P$<0.01) among children with iron deficiency at baseline in the MNP group (**Figure 5.2**). PF concentrations significantly increased by 49% among children in the MNP group who were not iron deficient at baseline ($P$<0.05). Hb concentration had a modest non-significant increment ($P$=0.08) among children who were iron deficient at baseline in the amaranth group compared to control. Per-protocol complete-case analysis revealed a similar trend as the imputed data analysis.

## Discussion

In our study, addition of amaranth grain flour to maize based porridge did not have a significant treatment effect on anaemia and iron status among children. The group receiving maize porridge fortified with a low dose of highly bioavailable iron MNP showed a significant increase in Hb and PF concentrations while TfR concentrations significantly decreased compared to the control group. Prevalence of anaemia, ID and IDA also significantly reduced in the MNP groups compared to the control group. Although the amaranth porridge iron concentration was shown to be high compared to maize flour, it did not show significant treatment effect on anaemia or iron status compared to the control group. The phytic acid concentration and phytate iron molar ratio were high compared to the preferred <0.4:1 in meals without enhancers and could explain the low absorption of iron from the amaranth porridge (39).

**Table 5.3: Effect of amaranth and MNP fortified porridge consumption on iron status relative to control[1]**

| | Effect estimates of biomarkers concentration | | |
|---|---|---|---|
| | Control | Amaranth group | MNP group |
| *N* | 93 | 93 | 93 |
| Endpoint Hb concentration, *g/L* | 115±11 | 115±11 | 117±9 |
| Crude | Reference | 0.59 (-2.5 to 3.7) | 2.1 (-1.0 to 5.2) |
| Adjusted[2] | Reference | 1.12 (-1.2 to 3.4) | 2.7 (0.4 to 5.1) * |
| Endpoint PF concentration[3], *µg/L* | 25.5 (20.3, 32) | 26.8 (21.2, 34) | 36 (29.4, 44) |
| Crude[4] | Reference | 5 (-27 to 48) | 41(4 to 90) * |
| Adjusted[2, 4] | Reference | 4 (-28 to 49) | 40(10 to 95) * |
| Endpoint TfR concentration[3], *mg/L* | 4.8 (4.4, 5.2) | 5.0 (4.6, 5.3) | 4.4 (4.1, 4.7) |
| Crude[4] | Reference | 4 (-6 to 15) | -7 (-15 to2) |
| Adjusted[2, 4] | Reference | 0.4 (-8 to 10) | -10 (-16 to -4)* |
| Anaemia[5], *n %* | 30 (32.3) | 31 (33.3) | 18 (19.4) |
| Crude[6] | Reference | 3 (-33 to 57) | -41 (-66 to 3) |
| Adjusted[2, 6] | Reference | -8 (-36 to 32) | -46 (-67 to -12)* |
| Iron deficiency[7], *n %* | 16 (17.2%) | 14 (15.1) | 5 (5.4) |
| Crude[6] | Reference | -13 (-62 to 101) | -67 (-90 to 4) |
| Adjusted[2, 6] | Reference | -17 (-65 to 100) | -70 (-89 to -16) * |
| Iron deficiency anaemia[8], *n %* | 11 (11.8) | 9 (9.7) | 4 (4.3) |
| Crude[6] | Reference | -14 (-68 to 130) | -69 (-92 to 25) |
| Adjusted[2,6] | Reference | -29 (-75 to 101) | -75 (-92 to -20) * |

*[1]Values are mean ± SD and effect estimate (95% CI) unless otherwise indicated. *Different from control p <0.05. [2]Adjusted for baseline Hb, TfR and plasma ferritin [3]Values are geometric mean (95% CI). [4]Values indicate difference between groups expressed as percentage relative to the control group obtained after exponentiation of effect estimates from log transformed data. [5]Defined as haemoglobin concentration <110g/L and adjusted for altitude. [6]Values indicate percentage difference in prevalence estimates obtained by conversion of prevalence ratios from Cox regression with robust estimates and constant time at risk. [7]Defined as PF concentration <12 µg/L or TfR>8.3 mg/L, corrected for inflammation if CRP>5mg/L.[8]Defined as concurrent anaemia and iron deficiency*

Phytate removal, addition of an iron bioavailability enhancer such as ascorbic acid or phytic acid degradation by adding commercial exogenous phytase has been suggested as a means for improving iron absorption from high phytic acid containing foods such as amaranth grain (40-42). However, addition of a phytase as the one used in the South African study (11) for home-prepared complementary foods may still be limited due to legislation issues on use of genetically modified organisms (42).

In our study however, we could also not use a modification strategy such as fermentation since results from a previous study showed that fermented porridge was not acceptable in the population (22). Furthermore, the study duration may have been relatively short to see a significant effect using a food based approach. The prevalence of ID and IDA in the MNP group was reduced by 75% compared to the control group. This finding concurs with a meta-analysis which concluded that MNP are just as effective as supplementation on reducing anemia prevalence (43) and a recent study among South African children where iron deficiency decreased by 30% after using an MNP (11) as well as other home and centralized fortification efficacy studies in Africa (9, 13, 44).

Porridge with MNP was shown to improve the Hb and TfR concentrations particularly in those children who were iron deficient at baseline. Iron absorption has been shown to exponentially increase in individuals with ID and the higher effect of the treatment on Hb and TfR concentrations in iron deficient children may be related to their iron status, as newly available iron maybe preferentially used for erythropoiesis in iron deficient participants (45).

In contrast however, the fortified porridge significantly improved the PF concentrations in iron sufficient children at baseline, indicating that in iron sufficient participants, newly available iron will be preferentially used to increase iron stores as erythropoiesis may be down regulated. Findings from subgroup analyses should however be interpreted with caution as chance of bias is higher (46).

Although the presence of other micronutrients in the fortificant thought to have a role in anemia particularly vitamin A (47), vitamin B-12 and folic acid (48) may have contributed to the increased effect on Hb concentration, the complex interactions and the role of these micronutrients in iron absorption among children are still not sufficiently understood (48). Vitamin A may support mobilizing hepatic iron stores and increase erythropoiesis resulting in an increased Hb response and reduced ferritin concentration (49). However in our study, ferritin concentration increased in the MNP group, especially among those who were anaemic at baseline. The effects of vitamin B-12 and folic acid may have contributed to the effect seen on Hb and anaemia.

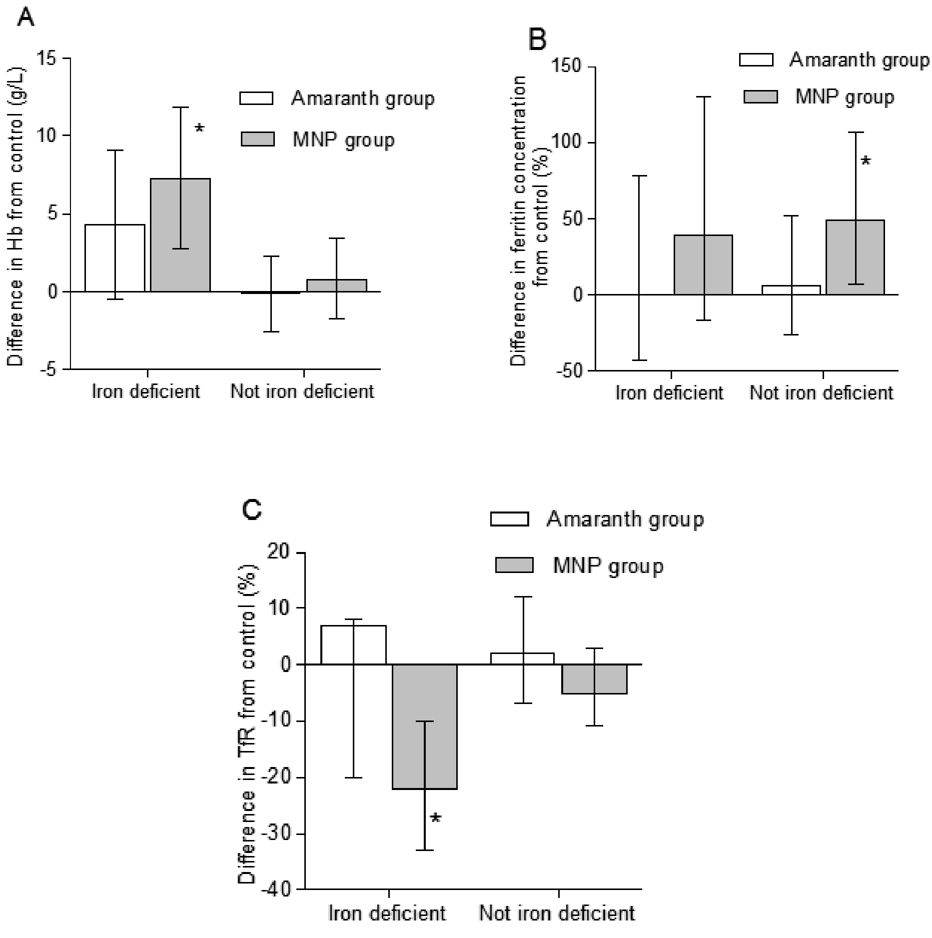


**Figure 5.2: Effect of porridge consumption on haemoglobin (A), ferritin (B) and TfR (C) in iron sufficient/deficient children at baseline relative to the control[1, 2, 3, 4]**

*[1]Values are effect estimates with line bars indicating 95% CI. [2]Amaranth group- maize porridge enriched with amaranth grain flour at the ratio of 30% maize flour to 70% amaranth. MNP group- maize porridge fortified with a low iron containing multi-micronutrient powder (2.5mg of iron in the form of NaFeEDTA). [3]ID defined as plasma ferritin <12 µg/L or TfR>8.3 mg/L and adjusted for presence of infection. [4]Effect estimates adjusted for baseline Hb, PF and TfR concentrations*

However, the evidence supporting their public health significance in general population is limited (48) and although not quantified in this study their effect is likely to be of smaller magnitude than the effect of EDTA in enhancing iron bioavailability.

Hb and PF concentrations significantly improved in the control group compared to baseline concentrations thereby reducing the prevalence of anaemia and ID. This could have been due to a combination of factors including reduction of helminthic burden due to de-worming, availability of mangoes during the study period and additional native iron, energy and protein provided by the maize porridge. Intestinal parasite infections especially hookworms increase the risk of anaemia (50). De-worming has been shown to have an effect on iron status and reduces IDA but only when the infection was high (51). Our baseline data suggest that 75% of children had not been de-wormed within the preceding six months. Nonetheless, the magnitude of the de-worming effect on haemoglobin and iron status is uncertain since we did not collect information on the parasite load in the study population. In a previous study within the area and in a similar season, it was observed that mangoes contributed to 30% of total vitamin C intake and 90% of children had energy intake below the estimated average requirement (52). Provision of extra food in form of the maize porridge could therefore have increased iron intake whereas the vitamin C from mangoes could also have enhanced iron absorption in all the groups (41).

This study has some limitations. First, though the participants were randomly allocated into three groups, the study design was only partially blinded. Whereas the maize only or maize with MNP porridge was identical in appearance and taste, for those in the amaranth group, it was evident that their porridge was different due to the beige color of amaranth and thinner consistency (22). This difference however did not affect the compliance as no significant difference was shown in attendance and amount of porridge eaten. Furthermore, we do not expect a difference between groups due to not blinding of the assistants as they were well trained and the supervisors ensured that all absent children were followed up equally. The laboratory personnel performing the analysis were also not aware of treatment allocation and therefore they may not have introduced any bias. Second, our study duration was rather short for a food diversification intervention to show significant effects and the effect on iron status outcomes may have been unnoticed. This was especially true for amaranth group and we think that with longer duration and a larger sample size it may be possible that amaranth grain may significantly improve iron status.

Pediatric blood samples could not always be collected as planned. This resulted in several missing values for the variables necessitating imputation of data. We did multiple imputation that produce outputs for each complete dataset plus a pooled output that estimates what the results would have been if there were no missing values. These pooled results are considered to be better statistical inferences than those provided by single imputation or complete case analysis methods as they preserve real observed data and reduce the standard errors of effect estimates which is due to reduced sample size (53, 54). In testing for malaria we used three different RDT tests at endpoint which have small differences in sensitivity and specificity. In addition, they did not test the same range of malaria parasites. However, we also did microscopy to diagnose malaria. All RDT results were negative while microscopy showed that 3.8% of the children were positive for malaria infection. This slide-positive/RDT-negative discordance has been reported at low parasite densities (55, 56).

In this study, only CRP was used as indicator for inflammation. Though use of two indicators of inflammation has been recommended (35, 57-59) with $\alpha_1$-acid glycoprotein (AGP) thought to better reflect the change in concentration of ferritin, CRP is currently recommended by WHO as an independent indicator of the acute phase response (58). Furthermore, CRP has recently been shown to correlate with AGP and better explains serum ferritin variance than other markers of inflammation (59). In addition we corrected the PF values for inflammation using correction factors (CF) that have been suggested as a better method than adjusting the serum ferritin cut-off value upwards or excluding those with inflammation (35, 58). Uncertainties on the impact of inflammation may however still remain as the correction procedure retains all cases and may not completely explain the variance in inflammation (57).

This study provides evidence that enriching maize porridge with MNP containing 2.5 mg iron has additional benefit of reducing anemia and iron deficiency within a short period. Such a low dose iron can probably only be effective when in the form of NaFeEDTA. WHO guidelines currently recommend use of elemental iron for home fortification for children 6-23 months while use of NaFeEDTA as a source of iron is still limited to clinical trials (60). However, recently NaFeEDTA has been added to the list of vitamin and mineral substances which may be added to food in the EU (61). In addition, for areas where prevalence of underweight is approximately 30%, 2 mg of iron as NaFeEDTA has been suggested for children 6–24 months of age (62). It is therefore evident that there is need of legislation to be put in place for countries such as Kenya to allow use of low dose iron in form of NaFeEDTA in children's food since its efficacy has been shown.

This study also shows that amaranth grain has potential to improve iron status. However, our findings also confirms that addition of plant based foods to the existing diet even when shown to have high iron concentration without reduction of phytic acid may not show significant improvement in iron status within a short study period. Addition of multi-micronutrient powder in maize porridge is more suitable than dietary diversification with amaranth grain for improving iron status among children and its effectiveness could be further evaluated in large scale programs.

## Acknowledgements

We acknowledge the support of Thomas Gichoni, Peter Muimi, Paul Musai and Joseph Mukunu from Migwani hospital for the logistical support during data collection, Joseph Wanyeki of East Africa Nutraceuticals for the flour supply logistics, Philip Ndemwa from Kenya Medical Research Institute (CPHR) for biochemical data collection and Dr. Phelgona Otieno from Kenya Medical Research Institute (CCR) as the study paediatrician.

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# *Chapter 6*

# *General Discussion*

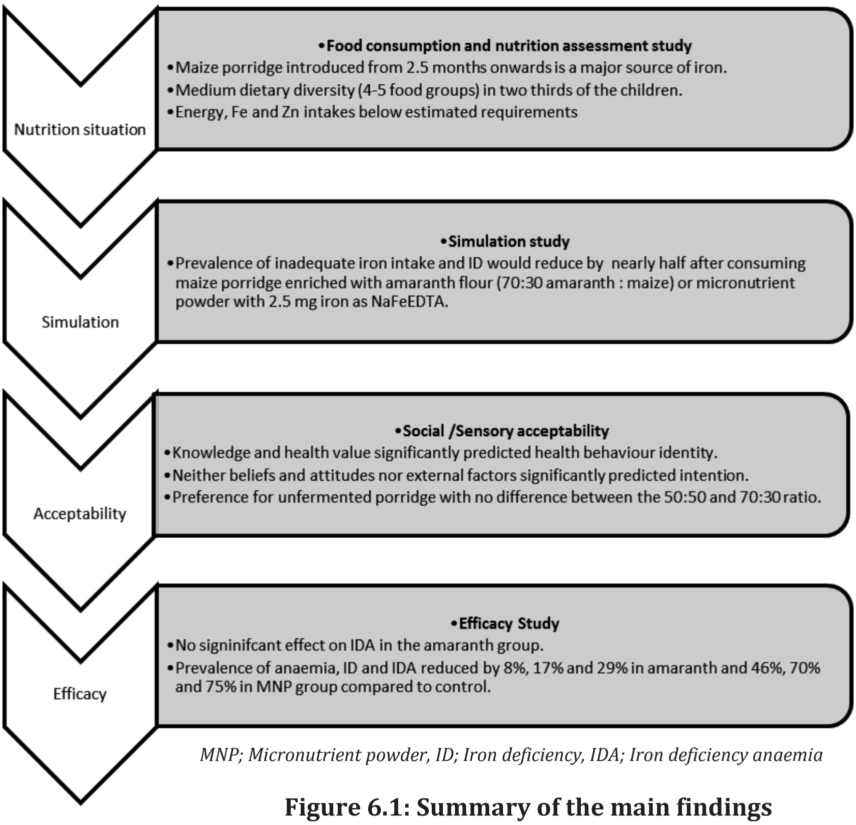
The primary aim of this thesis was to investigate the effect of consumption of maize porridge fortified with amaranth grain flour on iron intake, iron status and anaemia of Kenyan pre-school children. To realize this, the research was organized into four studies in Mwingi district, an arid and semi-arid area in Eastern Kenya following the framework as illustrated in **Figure 1.1**. In this chapter a summary of the main findings is presented. We further interpret the methodological issues (internal validity) and key findings (external validity) as well as identify policy implications and provide suggestions for future research.

## Main findings

The main findings of this thesis are summarized in **Figure 6.1** and briefly described below. In the cross-sectional study carried out among 280 randomly selected children aged (12-23 months), it was found that complementary foods were introduced at an early age, before 6 months **(Chapter 2)**. Dietary diversity was limited in animal products with unfortified maize based foods especially porridge being the main complementary food. Deficits in dietary energy, iron and zinc were found. Theoretical simulations indicated that there was likelihood to significantly improve iron intake adequacy and iron status among children in rural Kenya by enriching maize porridge with grain amaranth **(Chapter 3)**. The simulation estimates resulted in a significant increase in ferritin concentration in the amaranth and MNP group compared to maize porridge group thereby significantly decreasing the prevalence of iron deficiency in both groups.

The sensory acceptability study **(Chapter 4)** showed a preference and acceptance for the unfermented amaranth enriched maize porridge compared to the fermented porridge. There was no preference seen between the ratios of 50:50 and 70:30 (amaranth: maize) in the unfermented porridges. In the social acceptability study, 58% of the variance of health behaviour identity was explained by background and perception of which knowledge and health value were the significant predictors ($P$<0.01). Neither beliefs and attitudes nor external factors predicted intention. Prior behaviour explained nearly half of the variance in grain amaranth consumption.

In the efficacy study **(Chapter 5)**, use of low dose iron MNP significantly reduced the prevalence of anaemia, ID and IDA respectively compared to control group. Though consumption of grain amaranth porridge reduced anaemia, ID and IDA prevalence compared to control group, this reduction was not significant.




*MNP; Micronutrient powder, ID; Iron deficiency, IDA; Iron deficiency anaemia*

**Figure 6.1: Summary of the main findings**

## Methodological issues

This section evaluates the methodological decisions that may have impacted on the validity and interpretation of work presented in this thesis. Methodological issues discussed in this chapter are largely related to selection bias, information bias and confounding (Chapter 2-5). Issues discussed in the preceding chapters will not be appraised further in this section.

**Selection bias**

Selection bias encompasses any systematic difference between comparison groups and may lead to spurious associations (1). Selection bias could occur as a result of non-random selection for example due to refusals (non-response) to participate associated with a particular characteristic (2). In intervention studies, 'intention-to-treat' and blinding are examples of design features to protect against bias (1).

For this study, recruitment of children was done through random walk which may be a potential source of selection bias (2). Although the method does not produce strict probability samples, it was chosen as we did not have complete lists of elementary units (e.g., households in the project area) or address list for the area which could be used as sampling frame (3). Creation of these lists is generally prohibitively expensive (3, 4) and therefore not feasible in a budget constrained study. To minimize the risk of bias that might arise when starting points for random walk recruitment are chosen on the basis of convenience as opposed to random, the starting points in the villages were carefully determined before the field work began (4) with the assistance of the local public health officer. We also had a predetermined number of children that would be included in the study and therefore further recruitment was stopped when we reached the required number. This may have led to oversampling in areas near the centre while underrepresenting households that are on the outskirts of the cluster, which was a sub-location in this study (3, 4). In households with more than one child in the study age group we randomly selected only one of the children for the study thus minimizing selection bias. Therefore, our sample closely approaches a random sample providing valid findings representative for the study area.

When selection criteria are used to exclude participants as in the study in **chapter 5**, there is a possibility that the sample does not reflect the population introducing selection bias. However, the number of excluded children in our study was small (n=27, 9%) and except for their mean age (23 months) which was significantly lower (p=0.00) compared to those who were recruited into the study (37 months), other characteristics including stunting and underweight were similar. Though the overall attrition rate (14%) in the efficacy study was relatively higher than anticipated, the rate per treatment group was comparable and the baseline characteristics of those children who did not complete the study were similar with those who completed the study. This attrition could have resulted to non-response bias due to insufficient blood samples at endpoint and withdrawals during the intervention and at endpoint data collection. However, the reasons for this non-response were to our knowledge not related to the outcome of the study. Moreover, our analyses were done with an intention-to-treat and we therefore did multiple imputation. Multiple imputation produced outputs for each complete dataset and a pooled output to increase the power of our study (5) and reduce the potential risk of non-response (1). We found similar trends of the pooled results compared to the complete case analysis of our data and the results were similar to what has been found in other studies (5). The pooled results are considered to provide valid statistical inferences compared to those provided by simple imputation or complete case analysis (6, 7).

The intervention study for this thesis was partially blinded. The maize only and maize with MNP porridges were identical in appearance and taste as the micronutrient powder was mixed with sugar and already put in the serving dishes before serving the children. Hence, children in the MNP and control group could not differentiate whether there was MNP added or not in their porridge. For those in the amaranth group, it was evident that their porridge was different due to the beige colour of amaranth and thinner consistency (8). Furthermore, we did not separate the participants and therefore it was possible for the children to see the colour differences during the feeding sessions. This difference however did not affect the compliance as no significant differences were shown in attendance and amount of porridge eaten between the treatment groups. Though the assistants were not blinded, proper training and close supervision to ensure that all absent children were followed up equally also reduced the risk for selection bias. In addition, the laboratory personnel performing the biochemical analysis were kept unaware of treatment allocation and therefore the risk of selection bias in this study was further minimal.

**Information bias**

Information bias involves misclassification of participants with respect to disease or exposure status (9, 10) for example, iron deficient and sufficient children; or those in high or low dietary diversity category. The common types of bias in this category include recall bias and interviewer bias (10). In biomarker-based studies, information bias encompasses the issues of validity, reproducibility and stability of markers (9). The potential for information bias discussed in this section is largely related to dietary assessment (chapter 2 and 3), simulation assumptions (chapter 3) and biochemical analysis (chapter5).

***Dietary assessment***

Dietary intake cannot be estimated without error, both systematic and random, and probably never will be (11). However, measures to reduce the impact of these errors are a critical component of dietary assessment. Systematic errors reduce the accuracy of dietary intake resulting in either over or underestimation of the mean food intake of individuals while random errors lead to imprecise estimates (12). Omissions of single food item such as fruits or snacks and underestimation of amounts consumed across food categories particularly snacks and main staples has been reported with use of 24hr recall which in turn affect the estimation of nutrient intake (13, 14). To reduce this effect we used an interactive 24hr recall on 2 non-consecutive days to enhance memory recall (15). The interview protocol was standardized and included probing questions and use of food samples which were either provided by the interviewer or from leftovers provided by the respondents of similar cooked food portions (2). Although the interactive 24hr recall has been validated and found to give comparable estimates with weighed food records among women (13), the method tends to overestimate mean energy and nutrient intakes in children under 2 years (16, 17). Compared to weighed food records an overestimation of mean iron intake by up to one-third has been shown (16). There is therefore a likelihood that overestimations still occurred in our study since all children were aged below 2 years and as our results show an insufficient energy and iron intake, these may be conservative indications of inadequacy of the diet.

To estimate usual intakes from the observed dietary intake, we adjusted for day to day variation using the National Research Council (NRC) adjustment procedure (18) in chapter 3. This adjustment enabled us to get estimates which were suitable in analysis of the prevalence of inadequate or excess intake in the study population (18). Furthermore, using usual intake data gave us more precise estimates for use in the model. In chapter 2 however, the means of observed dietary intake data was reported. This method has been criticized as it is likely to result in an inaccurate estimate of the usual intake distribution because the presence of the day-to-day variability in intakes can greatly inflate the variance of the distribution of individual means (19). However, the emphasis of chapter 2 was a qualitative description of feeding practices and on dietary diversity. Finally, other methods which have been shown to provide reliable estimates of usual intakes distribution are available including the Iowa State University (ISU) method, Multiple Source Method (MSM), Age-adjusted dietary assessment (SPADE) and National Cancer Institute (NCI) method (20). These methods estimate distributions for population intakes with larges sample sizes and repeated intake data for subsamples only. The methods have been shown to have similar mean bias and a variability of usual intake that increases with smaller sample size, especially when data is less normally distributed as is the case of iron (20). NRC method was therefore most appropriate for our study as it allows for adjustment of individual intakes and we had a small sample size with equal number of observations per subject (18).

Data used to convert the intake of food into nutrients is mainly presented as raw single ingredients in many food composition tables (FCTs) including the ones used in this thesis. Use of FCTs could introduce errors in nutrient intake estimates related to the variability of values obtained from laboratory analysis or estimated based on conversion factors (21, 22). Use of FCT such as International Minilist (IML) (23) which has been adapted and suggested to simplify the task of estimating intakes in developing countries such as Kenya since it is relatively comprehensive (24) may reduce information bias. In this study, the IML provided most of the nutrient information as it has nutrient values of foods from Embu, Kenya, which are closely related to the foods in our study area.

The dietary diversity scores used in chapter 2 and 3 of this thesis may not be comparable as the aims for the two studies were different. In chapter 2, we used 8 food groups similar to another study among breastfed and non-breastfed children (25) that included fats and oils since we aimed to describe the food patterns rather than assess correlations with micronutrient density. However, in chapter 3 the focus was on describing the dietary pattern related to micronutrient intake (especially iron) and we excluded the fat and oils, because, with the exception of non-overheated red palm oil, fats and oils generally do not contribute substantially to micronutrient intake (25). In addition we also separated organ meats from animal source foods as they are considered to be rich in iron. Dark green leafy vegetables (DGLVs) were also separated from vitamin A rich fruits and vegetables [defined as foods with at least 130RE/100g (26)] resulting to 9 food groups. Use of 10 food groups have been suggested for non-breastfed children (26) and has been applied in previous studies (27). In South Africa, Steyn *et al* (28) used 9 food groups but which combined all meats, split 'other' fruits and vegetables group and included fats and oils. With this categorization it indicates that though a child may be classified in a dietary diversity category such as medium, the quality of the foods used to calculate the score in the DDS may be different. This observation is seen in our study for categories used in chapter 2 and 3. Previously, there have been efforts to provide guidance on use of DDS (26, 29). However, at present, there is still no consensus for most individual level scores on the best number, types or composition of food groups to include as proxy for micronutrient intake. This is because the decision of the number, type and composition of food groups is based on intended purpose of the study (30). Irrespective of the differences in food groupings used in studies there is consistency in indicating that increased DDS results to increased micronutrient intake (30). There remains a need however, to establish and formalize guidelines that can provide universal indicators for dietary diversity in different target groups.

The simulation model (chapter 3) was developed using a simple deterministic approach to estimate the expected change in prevalence of iron intake inadequacy. A shortcoming of this approach is that uncertainties or variability in concentration or consumption data were not taken into account as mean value or worst-case approach was applied (31). For instance, our baseline iron intake estimate was considered to be the median intake value obtained from the dietary intake data analysis. This may have reduced the inter-individual variation of iron intake simulation estimates and eventually affecting the ferritin concentration estimations. Although more complicated statistical modelling approaches have been suggested for improved estimations (31, 32), this study was limited in statistical scope as it was designed for use by policy makers who may not have extensive statistical knowhow and yet have to make a decision. It is therefore necessary that results from theoretical simulation are validated with and efficacy trial.

***Biochemical analysis***

Concentration biomarkers are a result of complex metabolic processes and are useful in evaluating the status of individuals according to their intake (33). Circulating plasma /serum ferritin levels have a strong positive correlation with tissue stores of ferritin and therefore provide a useful biomarker to indicate sufficient or inadequate iron intake (34). In addition, plasma/serum soluble transferrin receptor concentration (TfR) appears to be a sensitive and specific indicator of early iron deficiency (35) as it increases in proportion to the extent of the functional iron deficit. In our study, we used different biomarkers (ferritin, soluble transferrin receptor and haemoglobin) both before and after the intervention as the combination has been suggested to allow for reliable assessment of iron status in population (36).

As an acute phase reactant, the serum ferritin concentration is elevated by acute and chronic infections. Therefore its utility is enhanced when used in combination with acute phase protein (APP) measurements such as $\alpha_1$-acid glycoprotein (AGP) or C-reactive protein (CRP) to exclude those with inflammation or correct for inflammation (37). In this study we had CRP data only which has been shown to correlate significantly with AGP. CRP also better explains serum ferritin variance than AGP probably as a result of the distinct response times of the indicators to inflammatory stimuli and their different half-lives (38). In addition we corrected the ferritin values for inflammation using correction factors (CF). Use of CF has been suggested as a better method than adjusting the serum ferritin cut-off value upwards or excluding those with inflammation as it incorporates the effects of APP on iron status indicators (39, 40). Although the difference in ID prevalence was not significantly different when excluding those with inflammation compared to using CF in this thesis, use of CF still captured higher iron deficiency prevalence estimates that would otherwise have been underestimated. Low prevalence of inflammation in our study may explain the small difference in ID estimates using the two methods. Uncertainties on the impact of inflammation may however still remain as the correction procedure retains all cases while CRP does not indicate all inflammation and therefore the effect of inflammation may not be completely ruled out (40).

**Confounding**

In the intervention study, risk of confounding was reduced as the children were randomly assigned into three groups using block randomization by age and sex. The randomization of the subjects was successful as the baseline characteristics of the children were not significantly different across the three treatment groups except for the prevalence of inflammation (CRP>5) which was different between control and the treatment groups with the control having the highest prevalence (16.5%). The high level of inflammation would cause a higher ferritin concentration at baseline. But application of correction factors (39) led to comparable baseline ferritin concentration across the treatment groups. The groups were similar in overall average age but not in distribution over the different age groups. However, we think that this did not significantly affect the outcomes either due to different iron absorption or differences in requirements per age group. The dietary iron requirement of children 1-3yrs (11.6mg/day) is nearly the same with that of children 4-6yrs (12.6mg/day) while it is much higher for those below 12 months (18.6mg/day) (41), the last not being part of our study group.

Deworming has been associated with improved anaemia and iron status (42-44) while in other studies the positive effect was not found (45). Although there is this conflicting evidence, addressing even minor differences at baseline which may be associated with the outcome thereby confounding the treatment effects is important. We therefore dewormed all the children that participated in the intervention at baseline and at 3 months. However, the magnitude of the de-worming effect on haemoglobin and iron status is uncertain since we did not collect information on the parasite load in the study population.

## External validity

This section discusses the key findings of the thesis following the framework as illustrated in **Figure 6.1**.

***Feeding practices, dietary intake and nutritional status of children***

Chapter 2 of this thesis shows that early introduction (from 2.5 months onwards) of complementary foods with limited dietary diversity (mean 4.3±1.0) is a common practice. In chapter 3, 50% of the children are classified within the low dietary diversity category indicating that many children are not receiving the minimum dietary diversity (having at least 4 food groups) requirements as defined by WHO (46). Early introduction to complementary foods especially starchy gruels with minimal intake of dairy products, fruits and vegetables is similar to findings of a low income economies multi-country study (47). The findings showing low mean dietary diversity score among pre-school children are similar to previous studies in Kenya (48) and Philippines (27). Although not determined in our study, low dietary diversity has been associated with inadequate complementary foods (47, 48), decreased mean micronutrient density (25) and adequacy (27, 28, 49) and poor nutritional status reflected by low height-for-age and/or weight-for-age (28, 47, 50, 51) scores of young children. Also, our study shows low energy and iron intake with nutrient adequacy of 64% and 69% respectively which are probably even overestimations of adequacy as discussed previously.

In this study nearly half (48.2%), of the children were stunted while almost a quarter (23%) were underweight which could be due to insufficient dietary intake or even low dietary diversity. The stunting levels found in our study are higher compared to the stunting rate for Africa estimated at 40% (52) and the national estimates for Kenya (35%) and Eastern province (41.9%) (53). Chronic malnutrition reflects a process of failure to reach linear growth potential as a result of suboptimal health and/or food and feeding conditions (54). The high stunting levels may therefore be reflective of the adverse situation in our study area and a pointer to high risk of iron deficiency as observed in later chapters.

***Acceptability of grain amaranth***

Sensory attributes of food like taste, texture and smell are reliable predictors of its acceptance playing an important role in its selection (55). In our study **(Chapter 4)**, the porridge with amaranth only was least preferred while there was no difference in acceptability of 50:50 and 70:30 (amaranth to maize) ratio porridges. Studies have shown that at lower levels (15%-25%) addition of grain amaranth does not reduce its acceptability when compared to yam flour (56) and wheat based products (57, 58) but with increase of the ratio, its acceptability rating drops (57). The intrinsic nutty taste of amaranth, that may not be familiar to the subjects, may lower the preference for porridge with higher amounts of amaranth as observed by Kihlberg and colleagues (59). In their study, bread with amaranth scored significantly higher than did bread without amaranth for earthy aroma and astringent flavour while it scored significantly lower for intensity of colour, cereals aroma and flavour (59).

The theory of planned behaviour (TPB) and health belief model (HBM) are useful as frameworks to help predict health-related behaviour. TPB has been used to explain nutrition behaviours related to soy products (60) while HBM has been used to explain behaviour regarding fat in diets (61) and folate fortified grain products (62). In addition, an integrated TPB and HBM model has been used to explain the consumption of fortified soy sauce (63) and *fonio* (64). Our study shows that the integrated TPB and HBM partly explained the behavioural intention of grain amaranth consumption. The integrated HBM and TPB model assumes that 35% to 55% of variance in behavioural intention could be explained (63). In our study however only 1.8-3.1% of the variance could be explained by the constructs evaluated and none significantly predicted intention. The low prediction of intention by the constructs in the model could be due to the fact that amaranth grain is relatively new to the community therefore there is no belief and value system yet associated with amaranth grain consumption. Hence, according to the stages of change model the participants would be considered to be still within pre-contemplation or contemplation stage (65). During the pre-contemplation stage, people are not considering a change as they have not identified their behaviour to be a problem. In this case, although 59% of the women reported that consumption of amaranth grain is good to prevent diseases, it is still not considered a key food to improve iron intake and so no values or barriers are associated with its consumption in relation to iron deficiency. Furthermore, many women interviewed (90.7%) did not consider themselves to be at risk of iron deficiency. However if they be at the contemplation stage they would be more aware of the potential benefits of making a change, in this case starting to consume amaranth grains and possible barriers such as cost and availability (65). This may partially explain why health behaviour identity was significantly associated with barriers but could not explain intention to consume amaranth grain in this study.

The four constructs comprising background and perception were positively and significantly correlated with health behaviour identity. The present results were similar with the study of Sun *et al* who showed that health value was highly correlated with health behaviour identity in rural areas (63) and in Mali where health value was a good predictor of and correlated with health behaviour identity (64). The positive correlations may reflect a level of awareness of the women regarding disease and the role of food on health. The predictive power of intention in our study (47%) is comparable to a study among women in USA (48%) on consumption of soy products (60) and slightly lower than that reported in Mali (66%) on consumption of *fonio* (64). In conclusion, this study confirms that the integrated model could partly explain behaviour associated with grain amaranth consumption in Kenya. However, it would be essential to repeat a similar study in this area when production and consumption of amaranth grain has increased. This would probably provide a clearer picture on attitudes and barriers influencing amaranth consumption.

***Efficacy of amaranth grain and a low dose iron MNP on iron status and anaemia***

Addition of amaranth grain flour to maize based porridge did not have an effect on iron status among children in our study **(Chapter 5)**. No similar previous studies on amaranth have been reported to allow comparison on its effect on iron status. Although the iron concentration in amaranth flour was shown to be high (29mg/100g dry matter) compared to maize flour (7mg/100g dry matter), amaranth had a high phytic acid concentration. The phytate: iron molar ratio of amaranth was therefore high (3:1) compared to the preferred <0.4:1 in meals without enhancers (66) and could explain the low absorption of iron from the amaranth porridge. A previous dietary diversification/modification program using phytic reduction strategies such as fermentation in Malawi did not find a significant increment in haemoglobin among children 30-90 months after 1 year (67, 68) though the phytate/iron molar ratio was reduced but not to the level of below 0.4 (69). Consumption of bread made of *teff*, a staple cereal in Ethiopia, which is considered to be a good source of iron showed that levels of intrinsic iron (6.8-7.6 mg/100g) were not sufficient to maintain or increase average serum iron levels in women of child-bearing age. However, addition of exogenous phytase to the *teff* bread appeared to be effective in improving iron absorption (70) indicating that the levels of phytate were the main bottlenecks in iron absorption and hence reduction of iron deficiency anaemia as is probably the case in our study.

The findings in this thesis confirm that addition of plant based foods to the existing diet even when shown to have high iron content may not show significant improvement in iron status within a short period. This observation underscores the importance of incorporating strategies to enhance iron absorption from plant-based foods in dietary diversification programs either by phytate removal, addition of an iron bioavailability enhancer such as ascorbic acid or phytic acid degradation by adding commercial exogenous phytase (70, 71). Combination of dephytinization with animal-source foods and/or fortification with appropriate levels and forms of mineral fortificants has also been suggested (72) to improve the effect of plant based foods on iron status.

WHO has recently recommended the use of home fortification with micronutrient powders containing at least iron, vitamin A and zinc to improve iron status and reduce anaemia among infants and children 6–23 months of age (73). The findings of this thesis provide further evidence that low dose highly bioavailable iron (NaFeEDTA) MNP is effective in improving iron status among children 12-59 months when used to fortify high phytate foods. MNP containing iron as NaFeEDTA and provided through regular distribution together with food rations in a refugee camp was associated with minor improvements in iron status but not haemoglobin after 13 months intervention in pre-school children (74). Similar effects have been shown in an absorption study among Swiss women (75) and in a controlled clinical trial in South Africa school children (76). Conflicting observation was also made in South Africa where fortifying high extraction wheat in the form of brown bread with 2.4mg/d NaFeEDTA did not improve children's iron status (77). In our study the use of MNP reduced the prevalence of IDA by 75% which was nearly twice the reduction found among anaemic children (28%-46%) in Ghana using sprinkles containing iron either in fumarate, ferric pyrophosphate or ferrous sulphate form (78). The reduction in prevalence of iron deficiency in our study (70%) compared to the control group was higher than that found by Adu-Afarwuah *et al* (50%) among children 6-12 months (79) and almost four times that found by Zlotikin *et al* again in anaemic children (15%-30%) using sprinkles with iron as ferrous fumarate (80) which could be probably due to effect of NaFeEDTA as the fortificant in our study. Our study therefore demonstrates and confirms that highly bioavailable low dose iron in form of NaFeEDTA has a potential to improve iron status in children and especially among those who are iron deficient. A higher effect on haemoglobin and transferrin receptor concentrations particularly in those children who were iron deficient at baseline was similar to the findings of the study among Kenyan school children (81). This observation corroborates further the fact that newly available iron may be preferentially used for erythropoiesis in iron deficient subjects while in iron replete subjects, it will be preferentially used to increase iron stores (37).

***Theoretical simulation on effect of grain amaranth flour or low iron MNP on iron status***

Measurements of clinical outcomes such as haemoglobin and ferritin concentrations in case of iron should be the method of choice to assess effectiveness of a dietary intervention program. However, theoretical simulations with scientific based assumptions may provide estimates which act as a roadmap in choosing the appropriate intervention before investing in an intervention project. Simulations have been used to assess the impact of iodized salt utilization in bread on iodine intake of preschool children (82), estimate habitual iodine intake distributions in populations (83), and effects of zinc and iron bio-fortification (84, 85), as well as increased use of animal source foods (86) to reduce prevalence of inadequate zinc and iron intakes. Simulations on the impact of bio-fortified maize resulted in a reduction in the prevalence of absorption of inadequate amounts of zinc of 47% in children but had no effect on the prevalence of absorption of inadequate amounts of iron (85). No other studies have simulated the effect of consumption of amaranth grain and MNP with low dose iron in the form of NaFeEDTA on iron status to allow for comparison with our study **(Chapter 3).**

The estimations on prevalence of iron deficiency obtained after the simulations were much higher in the amaranth group while lower in the MNP group compared to what was achieved after the actual intervention (Chapter 5). This observation is related to the assumptions we made regarding the amount of absorbable iron from grain amaranth and also the available iron that is translated to stored iron in the body. For grain amaranth we used a bioavailability value of 3% that was assumed from animal studies (87) but not from human absorption studies. The simulated effect of the intervention would have been lower if we would have considered a lower bioavailability. For example using a bioavailability of 2% for amaranth grain flour resulted in an estimated effect size that is lower than that found for MNP group but higher than for the maize only group. However, we did not have scientific evidence to assume a lower iron bioavailability in amaranth porridge for our simulations. In addition, though we assumed that the bioavailability of iron from maize flour consumed together with NaFeEDTA to be still 4%, there is likelihood that more intrinsic iron from the maize was absorbed due to the presence of EDTA (88). Moreover, presence of other micronutrients in the MNP such as Vitamin A which supports mobilization of hepatic iron stores and increase erythropoiesis resulting in reduced ferritin concentration (89) may also have contributed to the higher effect seen in reduction of prevalence of iron deficiency (70%) in the MNP group after actual intervention even after correcting for infection. This may therefore partially explain the difference in intervention effects from the simulation and actual intervention. Finally though absorption may explain most of the differences observed between the intervention and simulation, there are still unexplained differences. The results from the simulation should therefore be interpreted with caution as an under/overestimation of effects may lead to a wrong choice of intervention strategy.

## General conclusions

This study provides further evidence that;

- ❖ Amaranth grain did not have an effect on iron status probably due to low iron absorption attributable to the high phytic acid content in the grain.
- ❖ Enriching maize porridge with 2.5mg highly bioavailable iron in the form of NaFeEDTA can reduce the prevalence of anaemia and iron deficiency within a short period.
- ❖ Simulations based on incomplete scientific evidence are not sufficient to replace efficacy studies. The resulting over or underestimation of effects may lead to a wrong choice of intervention strategy.

## Implications for public health policies and future research

Interventions to improve child nutrition are among the best investments in development that countries can undertake. There was strong evidence of iron deficiency in this age group as shown by the iron status indicators and the effect of home fortification with MNP on these indicators (**Chapter 5**, Table 5.3 and Figure 5.2). Iron deficiency occurred in one third of children at baseline while at endpoint iron deficiency and IDA was reduced by more than half in the MNP group. The finding that MNP can improve the iron status of children consuming high phytate foods without addition of exogenous phytase is of particular relevance for efforts to promote in-home fortification using MNP in countries where legislation on use of commercial phytase is still a concern. Though compliance in our study was high, low compliance when MNP is used in long term community based programs has been reported (74). Therefore programmatic issues to deal with availability, accessibility and compliance need to be resolved further.

Previous studies have shown that fortification with NaFeEDTA may not have adverse effects on absorption of zinc and other mineral elements (90). In our study, we did not investigate the safety of using low iron MNP since the study duration was relatively short. WHO guidelines currently recommend use of elemental iron for home fortification for children 6-23 months while use of NaFeEDTA as a source of iron is still limited to clinical trials (73). However, recently NaFeEDTA has been added to the list of vitamin and mineral substances which may be added to food in the EU (91). Further research and continuous monitoring is still needed to assess the safety of bolus doses of home fortification with MNP formulated with NaFeEDTA so as to identify any potential harm in the different age groups especially children after prolonged exposure. Additional research is needed on optimal dosing schedule including daily versus intermittent use of NaFeEDTA.

The MNP in this study also included other micronutrients whose effect was not part of the research question in this thesis. We therefore support the recommendation by WHO (73) on further research to identify the effects of other micronutrients included in the MNP on indicators of nutritional status other than ID and anaemia for instance, iodine status, vitamin A and zinc status as well as functional outcomes such as growth and motor and cognitive skills.

In the feeding trial, we found the effect of amaranth grain on iron status to be modest. The findings from the intervention study on the effect of consumption of grain amaranth on iron status underscore the need of public health policy makers to rethink on the efficacy of plant-based approaches including dietary diversification as without reduction of phytic acid the intervention may not significantly change the iron status among children. It would therefore be important that isotope absorption studies be conducted for amaranth grain and in other promising plant based foods so as to determine the amount of bioavailable iron before its public health promotion in dietary diversification as a source of iron is accelerated. Furthermore, in high physiological requirements populations, individual food based approaches may be adequate to prevent IDA but not sufficient to treat it since the iron provided by the food is in low doses. However, it may be possible that the effect of these approaches may be more effective in iron replete populations for maintenance of optimal iron levels. It is therefore proposed that studies investigating if use of iron rich plant-based foods would sustain optimal iron levels for children who are iron replete be conducted.

Since intentions are assumed to capture the motivational factors that influence behaviour, further validation on the effect of predicting actual behaviour (consumption of grain amaranth) by the integrated model is suggested. The effect of past behaviour, a variable that often strongly predicts future behaviour, may be shown to be entirely mediated by a reliable and valid proximal measure of intention (92). Further research using additional observational data such as 24 hour recalls rather than self-reports of intention and behaviour only is therefore suggested so that change can be measured and the impact on the intention-behaviour relationship assessed. Though knowledge and health value were significant predictors of health behaviour identity, identity did not significantly predict intention to consume grain amaranth. Nevertheless, to promote grain amaranth consumption, awareness and knowledge about micronutrient deficiencies and nutritional benefits of grain amaranth need to be increased. In addition, the health consequences of the deficiencies (health value) could be targeted in nutrition education programs. The integrated model could be used in areas where consumption of grain amaranth is established to predict grain amaranth consumption behaviour.

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# ***Summary***

Iron deficiency anaemia (IDA) is a significant public health problem in young children in developing countries. Global estimates indicate that 48% of pre-school children are anaemic and iron deficiency (ID) is assumed to account for approximately half of this problem. The highest proportions of preschool-age children suffering from anaemia are in Africa (68%). In Kenya, 43.2% of the preschool children have been estimated to be iron deficient while 70% are anaemic. Nutritional strategies such as dietary diversification and home fortification are promising approaches to control iron deficiency. However, there is lack of data that have evaluated the impact of these strategies on iron status in pre-school children.

The aim of this thesis was to investigate the effect of consumption of maize porridge fortified with amaranth grain flour on iron intake, iron status and anaemia of Kenyan pre-school children. To realize this objective, the study was organized into four sub-studies and organized into six chapters. **Study 1** concerned a cross-sectional survey where we assessed the dietary pattern and nutrient adequacy of complementary foods with a focus on iron intake. We also determined the iron and nutritional status of the children. From this data we simulated the effect of enriching maize porridge with amaranth grain flour or low dose highly bioavailable iron MNP on the adequacy of iron intake and iron status using theoretical estimations **(study 2)**. **In study 3**, acceptability studies were carried out where we assessed the sensory acceptability of fermented and non-fermented amaranth enriched maize porridge at the amaranth maize ratio of 50:50 or 70:30 and identified factors that would predict the intention of consumption of amaranth grain in Kenya. A randomized controlled trial **(study 4)** was then carried out to assess the efficacy of maize porridge enriched with amaranth grain or low iron MNP consumption to improve iron status and reduce iron deficiency and anaemia in rural Kenyan pre-school children. The study was conducted in Migwani division, Mwingi in Kenya between December 2007 and March 2011. An exception was the social acceptability study of which data was collected from Makuyu division in Murang'a South District. Makuyu division was chosen for the survey as a proxy of Migwani because the study subjects in Migwani did not fulfil the pre-requisites to participate in the social

acceptability study (i.e. amaranth grain to be commonly consumed or well known) and also had similar climatic characteristics with Migwani.

In **chapter 2**, data were collected using household and individual dietary diversity questionnaire among 280 children aged 12-23 months. A repeated interactive 24 hour recall was administered on a sub-sample of 43 children while 6 focus group discussions were held with 44 mothers who had children aged between 12 and 23 months. Results show that the mean duration of breastfeeding was 10.5±4.1 months with early introduction (2.5±1.7 months) of complementary foods and limited dietary diversity (mean 4.3±1.0). An unfortified maize porridge was the main complementary food. The mean dietary diversity score was 4.9±1.3 and 4.3±1.0 out of a possible score of 14 and 8 respectively. At least 60% of the children in all the dietary diversity terciles consumed starchy staples and oils in the preceding 24hr but dietary diversity was limited in animal products. Deficits in dietary energy, iron and zinc were found. This chapter concluded that establishing local solutions to increase dietary diversity and promote use of foods rich in iron to improve the local complementary foods are needed

Theoretical simulations to estimate the potential effect of enriching maize porridge with amaranth grain flour or micronutrient powder on the adequacy of iron intake and reducing the prevalence of iron deficiency were calculated in **Chapter 3**. The simulations were done with data from a quantitative food consumption survey carried out in 2008 (n=197, age 12-23 months) and iron status data from a survey carried out in January 2010 (n=263, age 12-59 months). Data was collected using repeated 24hour recall method on 2 non-consecutive days, anthropometric measurements and biochemical data from non-fasting venous blood. Simulating a daily consumption for 80 days of non-fortified maize porridge (60g flour), amaranth porridge (80g of flour at 70:30 amaranth to maize ratio), or maize porridge fortified with MNP (2.5mg iron as NaFeEDTA), resulted in a median iron intake of 8.6mg, 17.5mg and 11.1mg/day respectively. The prevalence of inadequate iron intake would reduce to 35% in amaranth and 45% in the MNP group. The prevalence of anaemia, ID and IDA was 49%, 46% and 24% respectively. Using the simulated increased intake of absorbable iron from the food consumption study, consumption of amaranth and MNP porridge was

estimated to result in an increase in ferritin concentration (1.82μg/L 95%CI 1.42-2.34 and 1.80μg/L 95%CI 1.40-2.31, $p<0.005$) respectively compared to maize porridge group resulting in a decrease in prevalence of ID (27%) in the two groups. The simulations showed that use of amaranth grain in dietary diversification or in-home fortification strategy with multiple micronutrient powder had the potential to increase iron intake adequacy and decrease iron deficiency among children in rural Kenya.

In **Chapter 4,** we investigated the effect on sensory acceptability after addition of amaranth grain flour to maize based porridge in a rural Kenya setting. We also identified factors influencing the intention of mothers to feed their children on amaranth grain using an integrated model combining Theory of Planned Behaviour and Health Belief Model. Sixty subjects consisting of 21 adults who had a child less than two years of age and 39 teenagers participated in the sensory study while 150 mothers/caregivers with a child aged between 1 and 3 years participated in the social acceptability study. A significant difference between the various porridge ratios (50:50, 70:30 and 100:0 amaranth: maize) either in unfermented or fermented form could be detected. Preference for the unfermented amaranth enriched maize porridge was observed. No significant preference between the 50:50 and 70:30 ratio unfermented porridge was observed. In the social acceptability study, 58% of the variance of health behaviour identity could be explained by background and perception of which knowledge and health value were the significant predictors ($P<0.01$). Interaction between barriers and intention negatively influenced behaviour. Neither beliefs and attitudes nor external factors predicted intention and they only explained 1.8% and 3.1% of the variance respectively. Prior behaviour explained 47% of the variance in amaranth grain consumption. The study concluded that unfermented amaranth enriched maize porridge is acceptable and the unfermented porridge with 70% amaranth could be considered in a program aimed at increasing dietary iron intake among children while increasing awareness about micronutrient deficiencies and nutritional benefits of amaranth grain could enhance its consumption.

Finally, in a controlled randomized 16-weeks intervention trial **(Chapter 5)**, children (n=279; aged 12-59 months) were randomly assigned to either: non-fortified maize porridge (control); amaranth grain fortified maize porridge at 70:30 ratio of amaranth to maize; or maize porridge fortified with a MNP containing 2.5mg Fe as NaFeEDTA. Blood samples were collected at baseline and endpoint for haemoglobin (Hb), plasma ferritin (PF), soluble transferrin receptor (TfR) and C-reactive protein (CRP) analysis. At baseline, the prevalence of stunting, anaemia, ID and IDA was 48.2%, 38%, 30% and 22% respectively. Consumption of MNP porridge reduced the prevalence of anaemia by 46% (CI: -67% to -12%), ID by 70% (CI:-89% to -16%) and IDA by 75% (CI: -92% to -20%). The MNP porridge significantly increased Hb by 2.7g/L (CI: 0.4 to 5.1) and PF (40% CI: 10% to 95%) and decreased TfR by10% (CI:-16% to -4%) concentration. In children who were iron deficient at baseline, the MNP significantly decreased TfR (-22%, CI:-33% to -10%) and increased Hb concentration (7.3g/L, CI: 2.8 to 11.8). There was no significant improvement in haemoglobin or iron status in the amaranth group. This study concluded that consumption of maize porridge fortified with amaranth grain did not improve iron status while that fortified with a low dose of highly bioavailable iron MNP can reduce the prevalence of IDA in pre-school children.

In conclusion, the work presented in this thesis provided evidence to conclude that addition of plant based foods to the existing diet even when shown to have high iron content may not show significant improvement in iron status within a short period without reduction of phytic acid. Future absorption isotope studies for amaranth and studies investigating if use of iron rich plant-based foods would sustain optimal iron levels for children who are iron replete are proposed.

# *Samenvatting*

Bloedarmoede door ijzergebrek (IDA) is een volksgezondheidsprobleem van grote omvang met name bij jonge kinderen in ontwikkelingslanden. Wereldwijde schattingen geven aan dat 48% van de kinderen onder de vijf jaar lijden aan bloedarmoede en algemeen wordt aangenomen dat 50% van deze bloedarmoede door ijzergebrek wordt veroorzaakt. De hoogste percentages kinderen met anemia worden in Afrika (68%) aangetroffen. In Kenya wordt geschat dat 70% van de kinderen onder de vijf jaar bloedarmoede heeft en 43.2% ijzerdeficiëntie. Voedingsinterventies zoals het diversificeren van het dieet en voedselverrijking zijn veelbelovende strategieen ter bestrijding van ijzerdeficiëntie. Er is echter nog te bekend over het effect van deze interventies op de ijzer status van kinderen.

Dit proefschrift heeft tot doel het meten van het effect van het eten van een maispap verrijkt met amaranth graanmeel op de ijzer inname, ijzer status en bloedarmoede van Kenyaanse kinderen onder de vijf jaar. Om dit doel te bereiken zijn vier studies uitgevoerd en beschreven in zes hoofdstukken. **Studie 1** is een cross-sectionele studie waarin het voedselpatroon werd onderzocht en werd vastgesteld of de inname van energie en nutrienten, met name ijzer, bij kinderen voldoende was. Ook werden de voedingsstatus en ijzerstatus van de kinderen gemeten. Gebaseerd op deze data en door gebruik te maken van theoretische schattingen hebben we berekend wat het effect op ijzer inname en -status zou kunnen zijn van verrijking van een maispap met amarant graan of met een micronutriénten poeder dat een hoog absorbeerbare vorm van ijzer bevat **(studie 2)**. In **studie 3** hebben we de sensorische acceptatie van gefermenteerde en niet-gefermenteerde amarant/mais pappen getest met amarant:mais ratios van 50:50 en 70:30. Ook hebben we de factoren geidentificeerd die van invloed kunnen zijn op de intentie van moeders om hun kinderen amarant/mais pap te eten te geven. Er is vervolgens een gecontroleerde en gerandomizeerde studie **(studie 4)** uitgevoerd waarin bij Keniaanse kinderen onder de vijf jaar het effect van verrijking van een maispap met amarant graan of met een micronutriénten bevattende poeder op ijzerdeficiéntie en bloedarmoede werd gemeten.

Het onderzoek is uitgevoerd in Migwani Division, Mwingi, in Kenya van december 2007 tot maart 2011. Alleen de sociale acceptatie studie is uitgevoerd in Makuyu Division in

Murang’ a District, een aangrenzende Division met vergelijkbare klimatologische omstandigheden. De vrouwen uit dit gebied, in tegenstelling to Migwani, waren al bekend met amarant graan, en dit vormde één van de voorwaardes voor deze studie.

In **hoofdstuk 2** werden bij 280 kinderen van 12-23 maanden oud gegevens verzameld over de diversiteit van het dieet op zowel het niveau van het huishouden als individueel. Een herhaalde interactieve 24-uurs navraag werd gedaan bij een kleinere groep van 43 kinderen. Er werden ook zes focus groep discussies uitgevoerd met 44 moeders van kinderen in de leeftijd van 12-23 maanden. De resultaten laten zien dat de kinderen gemiddeld 10.5±4.1 maanden borstvoeding krijgen en dat al vroeg vanaf 2.5±1.7 maanden bijgevoed wordt met voedsel van een beperkte diversiteit (gemiddeld 4.3±1.0 verschillende voedselgroepen). Met name een niet-verrijkte maispap werd gegeven. De gemiddelde diversiteit van de bijvoeding was 4.9±1.3 en 4.3±1.0 verschillende voedselgroepen uit een totaal mogelijke score van 14 en 8 respectievelijk. Ten minste 60% van de kinderen consumeerden zetmeelrijke basis voedingsmiddelen en vetten (oliën) in de voorafgaande 24 uur maar er werden nauwelijks dierlijke producten gegeten. Tekorten in inname van energie, ijzer en zink werden geconstateerd. Dit hoofdstuk concludeert dat het noodzakelijk is om lokale oplossingen te zoeken om diversiteit van het voedingspatroon te verbeteren en de consumptie van ijzerrijke voedingsmiddelen te stimuleren.

Theoretische berekeningen om het potentiele effect te schatten van het verrijken van maispap met amarant meel of met een micronutriénten bevattende poeder op ijzer inname en prevalentie van ijzerdeficiénti zijn beschreven in **hoofdstuk 3**. De schattingen werden berekend op basis van de voedselconsumptie gegevens uit de studie in 2008 bij 197 kinderen van 12-23 maanden oud en ijzerstatus data van een studie uitgevoerd in 2010 bij 263 kinderen van 12-59 maanden oud. Voedselconsumptie data zijn verzameld met een herhaalde 24-uurs navraag methode op 2 onafhankelijke dagen, met anthropometrische metingen en afname van veneus bloed bij niet-vastende kinderen. Voor de schattingen werd aangenomen dat de kinderen gedurende 80 dagen een niet-verrijkte maispap (60g meel), een amarant pap (80g amarant:mais meel met verhouding 70:30) of een maispap verrijkt met een micronutriénten poeder (met 2.5 mg ijzer in de vorm van NaFeEDTA). Deze simulaties resulteerden in een gemiddelde ijzerinname van 8.6mg, 17.5mg en 11.1mg/dag respectivelijk. De prevalentie van onvoldoende inname reduceerde tot 35% in de amarant groep en tot 45% in de micronutriënten-poeder groep. De prevalentie van bloedarmoede, ijzerdeficiëntie en bloedarmoede met ijzerdeficiëntie was 49%, 46% en 24% respectievelijk. Gebaseerd op de geschatte stijging van inname van biobeschikbaar ijzer boven op de normale ijzerinname zoals vastgesteld in het voedselconsumptie onderzoek, zou het eten van de amaranth pap en de pap verrijkt met de micronutriénten poeder in vergelijking met maismeel pap resulteren in een stijging van de serum ferritine concentratie (1.82μg/L 95%CI 1.42-2.34 en 1.80μg/L 95%CI 1.40-2.31, p<0.005) en in een daling van ijzer deficientie naar 27% in beide groepen. De simulaties laten zien dat het gebruik van amaranth ter verhoging van diversiteit van het dieet, en het thuis verrijken van voedsel met een micronutrienten poeder potentieel de inname van voldoende ijzer kunnen verhogen en de prevalentie van ijzerdeficiëntie kunnen verlagen bij Keniaanse rurale kinderen.

In **hoofdstuk 4** onderzochten we of het toevoegen van amaranth meel aan maismeelpap effect heeft op de sensorische acceptatie in ruraal Kenia. Ook identificeerden we de factoren die de intentie van moeders beinvloeden om hun kinderen amaranth te eten te geven. Dit werd gedaan met een model dat samengesteld was uit de zogenaamde Theory of Planned Behaviour en het Health Belief Model. Zestig proefpersonen waaronder 21 volwassenen met een kind jonger dan 2 jaar en 29 tieners deden mee aan de sensorische studie, terwijl 150 moeders/verzorgers met een kind tussen de 1 en 3 jaar participeerden in de sociaal-psychologische acceptatie studie. Een significant verschil tussen pappen met verschillende amarant:maize verhouding (50:50, 70:30 en 100:0 amaranth:maize) werden waargenomen, zowel in de gefermenteerde als niet-gefermenteerde pappen. Proefpersonen hadden een duidelijke voorkeur voor niet-gefermenteerde pappen, en pappen met amarant:maize verhouding 50:50 of 70:30 werden zonder onderscheid geprefereerd. Uit de sociaal-psychologische studie bleek dat 58% van de variatie in 'health behaviour identity' verklaard kon worden uit 'background and perception' en daarvan waren kennis en de waarde die aan gezondheid wordt gehecht de belangrijkste voorspellers ($P<0.01$). Interactie tussen barrieres en intentie beinvloedden het gedrag negatief. Noch overtuigingen en houding ('beliefs and attitudes'), noch externe factoren konden de intentie voorspellen en ze verklaarden slechts 1.8% en 3.1% van de variatie in intentie respectievelijk. Eerder gedrag verklaarde 47% van de variatie in consumptie van amarant. Deze studie concludeerde dat niet-gefermenteerde amarant pap acceptabel is en dat een niet-gefermenteerde pap met 70% amarant meel geschikt zou zijn voor een voedinginterventie gericht op het verhogen van ijzer inname bij kinderen. Activiteiten die de bewustwording van micronutriénten tekorten en de nutritionele bijdrage daaraan van amarant verhogen, zouden de consumptie van amarant kunnen verbeteren.

In een 16 weken durende interventie studie (**hoofdstuk 5**), werden 279 kinderen van 12-59 maanden oud geheel willekeurig in drie groepen verdeeld welke respectievelijk een niet verrijkte maispap (de controle groep), een 70:30 amarant:mais pap, of een maispap verrijkt met een micronutriénten poeder (MNP) met 2.5mg ijzer in de vorm NaFeEDTA kregen. Bloedmonsters werden zowel aan het begin als aan het eind verzameld voor het meten van hemoglobine gehalte (Hb), plasma ferritine concentratie (PF), transferrin receptor concentratie (TfR) en C-reactive protein (CRP) concentratie. Aan het begin van de studie was de prevalentie van stunting, bloedarmoede, ijzerdeficiëntie (ID) en bloedarmoede met ijzerdeficiëntie (IDA) respectievelijk 48.2%, 38%, 30% aan 22%. Consumptie van de pap verrijkt met micronutriénten poeder verlaagde de prevalentie van bloedarmoede met 46% (CI: -67% tot -12%), ID met 70% (CI:-89% tot -16%) en IDA met 75% (CI: -92% tot -20%). De MNP pap verhoogde significant de Hb concentratie met 2.7g/L (CI: 0.4 tot 5.1) en PF met 40% (CI: 10% tot 95%) en verlaagde TfR met 10% (CI:-16% to -4%). Bij kinderen die aan het begin van de studie ijzerdeficiéntie waren, verlaagde de MNP pap significant TfR (-22%, CI:-33% to -10%) en verhoogde de Hb concentratie (7.3g/L, CI: 2.8 to 11.8). We zagen geen significante verbetering in hemoglobine concentratie of ijzerstatus in de amaranth groep. Deze studie concludeerde dat maispap verrijkt met amarant de ijzerstatus van kinderen onder de vijf jaar niet verbeterd, maar dat maispap verrijkt met een micronutriénten poeder met een hoog absorbeerbare vorm van ijzer de prevalentie van bloedarmoede met ijzerdeficiëntie van kinderen onder de vijf wel kan verlagen.

Bovenstaande studies dragen bij aan het bewijs dat het verrijken van het bestaande dieet met plantaardige voedingsmiddelen, zelfs als ze een hoog ijzer gehalte hebben, waarschijnlijk geen verbetering in ijzerstatus tot gevolg hebben in een korte periode, wanneer niet tegelijkertijd ook de concentratie aan fytaten wordt gereduceerd. Het is van belang om in verder onderzoek stabiele isotopen studies met amarant uit te voeren, en studies te doen die onderzoeken of het gebruik van ijzerrijke plantaardige voedingsmiddelen een optimale ijzer status bij kinderen met ijzerdeficiëntie kunnen bewerkstelligen en behouden.

# *Acknowledgement*

It gives me great pleasure to thank those who have supported me making the completion of this thesis possible. Though, I may not mention you all by name in these few pages, I am forever grateful to you all for your support. This has been a long journey that started with an interview in Nairobi in 2002, a visit to Wageningen in 2005 and finally the start of the PhD Program in February 2007.

I owe my deepest gratitude to Dr. Inge Brouwer, my daily supervisor who walked with me all the way in this program. Inge, thank you for the deep scientific insights you have given me enabling me to develop an understanding of the subject. Not to forget those moments ☺ that you just were there and encouraged me to keep going on!! Prof. Frans Kok, it was such an honour for me to have you as my promoter. Thank you for that bird's eye view that you brought into my work. It was just great sharing with you a table as stories would be in plenty. Your support through the department when we needed finances to finish the research project was awesome and greatly appreciated. I would also like to thank my supervisor, Dr. Alice Mwangi. Alice, the acts of kindness that you showed when we needed to have funding for my proposal and support from home institution, went a long way in enabling me to achieve this academic qualification. When I moved so fast-you accelerated and still caught up with me- for this I say thank you!!

I am really grateful to Dr. Abiud Omwega who spotted a potential in me, introduced me to clinical trials research and went further to recommend me for a PhD fellowship. The initial support during proposal development and the encouragement through the program is sincerely appreciated. To Prof. Wambui Kogi-Makau, when I first met you as a 4th year undergraduate student in your office, you recognized a potential that could be nurtured, thank you for believing in me. Your support and encouragement all through my graduate studies is deeply appreciated. Dr Fré Pepping, special appreciation and thanks for your support since we met in 2002 and giving me the opportunity to be a part of VLAG. The wooden shoe-bank will always serve as a reminder of your concern to me beyond Wageningen! Special thanks to Prof. Michael Zimmermann, Dr. Diego Moretti and Dr. Anne de Wagt, for your time and academic support, especially during proposal and manuscript preparations of the clinical trial. Thank you for sharing with me a piece from your profound knowledge and experience on the subject area.

I am also sincerely grateful for the administrative support provided to me by Lidwien (RIP), Lous, Gea, Cornelia and Marion. Thank you for making my travels and stay in Wageningen comfortable. To Lucy and Alida, thank you for your friendliness. It was always fun to have those international lunches/dinners together. The institutions that provided financial support either as a study fellowship, a research grant or donation of materials in kind including; Nevin Scrimshaw International Nutrition Foundation, Nestlé Foundation, Nutricia Foundation, Clive West Micronutrient Fund, Foundation Van Dam Nutrition Plan, Sight and Life as well as Wageningen University are gratefully acknowledged.

The collection and analysis of data in this thesis would not have been possible without the support of different people. A meeting with Temi Mutia in Wageningen back in 2007 would lay a foundation on the study area. Temi, for your ideas and your support through your sister in Migwani I greatly appreciate. To Joseph Wanyeki of East Africa Nutraceuticals, thank you for ensuring that we had the flour when needed it. My research assistants Miriam Mutua, Nancy Kagwanja, Regina, Ruth, Muthoni, Patricia, Rose Joseph, Florence, Teresiah, Rose Kitavi, Esther, Jemimah, Serah, Dorcas, Caroline, Roselyn, Anne, Winrose, Grace and Nancy thank you for being so faithful and diligent during the data collection period. My drivers, Ruto, Mulei, Mwendo and Daniel who made sure that everybody was in their station in good time, I say thank you!, you made my life in Migwani bearable. Baba and mama Mungai, thank you for being there to make sure the flour was delivered promptly to Migwani. The logistical support of Thomas Gichoni, Peter Muimi, Paul Musai, Joseph Mukunu and nursing staff from Migwani hospital is highly acknowledged. I also acknowledge the support of local administration led by the Chiefs of Migwani and Nzauni location, together with the assistant chiefs in Kyamboo, Kaliluni, Nzauni, Kikiini, Kaikungu, Makengekani and Itoloni. My special appreciation goes to the head teachers and school management of the cooking/feedings centres in Kyamboo, Makengekani, Kaliluni, Kamutisya, Kangutheni, Nzauni, Kikiini, Kithalani and Kaikungu, and especially Mr Ngau and Mr Mutemi who supported this work even when it was not always convenient. The support of Christine Mwangi and Philip Ndemwa from KEMRI as well as Dr Ahmed Kalebi and Hassan Ibrahim from Pathologist Lancet Laboratory during biochemical data collection and analysis is deeply acknowledged. I am also deeply indebted to Dr Phelgona Otieno of CCR, KEMRI who supported me as the study paediatrician. For the MSc students who participated in this study; Ana-Maria, Anne, Mwai, Agnes and Natalie, I am indeed grateful for the moments we shared together during data collection and your master thesis write-up. You were just great people to work with and had such zeal to do the best in your work-bravo!

I am also indeed indebted to my colleagues and friends who supported me through life in Wageningen including Sophie, Pauline, Wanjiku, Nadia, Varsha, Maria-Elisa, Elise, Razak, Sandra, Rina, Mutuku and Por. Though some of you we only met briefly during my visits in Wageningen, your presence and the times we spent with you made a difference in my life and for this I say thank you. The social support of Kenyan student fraternity in Wageningen at different time points of my stay in Wageningen is also deeply appreciated. I am also really grateful for the family in Amazing Grace Parish, Wageningen that provided a home away from home. Pastor Farai and Busi, though more like a brother and sister, you became dad and mum. Thank you for your prayers, support and encouragement. To my paranymphs Anne and Busi thank you for your support through the last stages of the PhD.

My heartfelt gratitude is also to those who supported me and brought wholeness in my life away from books. Douglas and Alice, thank you for supporting us this far- I have enjoyed knowing and having you around us. To the Wambua's, Owours, Macharia's, Karuku's, Obel's, Mbugua's, Waweru's thank you for being there for our family-your friendship has been a refreshing fountain. My buddies, Jane Muhoro, Jane Wamondo and Anne Kamau with your families, I cherish your friendship. Karugu, Priscilla, Rosemary, Trizah, and Rhoda, thank you for the persistent prayers you made for me through this journey in the CG. I am glad that I met you and we have shared our lives in the last few years! Pastor Philip & Dinah, Pastor Fred & Pastor Leo, thank you for your support- your concern on my many travels never went unnoticed. To my "help" Margaret and Florence who graciously took care of my children when I had to be away for long periods; thank you for your support and service, I am greatly indebted.

To my parents Johnson and Jedidah Macharia who have continued to love, encourage and support me, helping me to become the person I am today, thank you for the sacrifices you made so that I could get the best. Those small acts of kindness even when it is not convenient for you do not go unnoticed and are deeply appreciated. Siblings are blossoms in the garden of life; Aunt Kui and Uncle Paul, thanks for the Sunday lunches for my family--at least there was something to look forward for and the opportunity for the girls to meet their cousins Charles and Josephine especially when I was away. Chiku, Rachael, Joan, Mumbi and Kuira, our roots say we're siblings, our hearts say we're friends, thank you for your support. To my Mom Rebecca and rest of family in Kangundo and Karatina especially cûcû na guka, thank you so much for your support, encouragement even when it was not very clear to you what, when and how it is done. To my husband Mutie and daughters Rebecca and Keren who had to endure long periods of my absence, your love, patience and support kept me going even when things were tough. I promised myself that for your sake by God's grace I will finish this thesis! I love you and hope that I will be that which God desires me to be in your lives☺.

I will extol the Lord at all times; his praise will always be on my lips (Psalms 34:1)

# ***About the author***

## Curriculum Vitae

Catherine was born on 26th December 1976 in Kiambu, Kenya. In 1982 she started her primary education and sat for the Kenya Certificate of Primary Education examination in 1989 in Gathehu Primary School, Karatina. She joined Tumutumu Girls High School in 1990 where she sat for the Kenya Certificate of Secondary Education examination in 1993. In November 1995 she joined University of Nairobi (UON) qualifying with a Bachelor of Arts (Anthropology) degree in 1999. Soon after, she joined Applied Nutrition Program (ANP) at the same University to pursue a Master of Science in Applied Human Nutrition graduating in 2002. During and after her MSc, she worked as a graduate research assistant within projects in ANP. Between 2003 and 2007 she also lectured on part-time basis in Kenyatta University. In February 2007, Catherine joined the Division of Human Nutrition, to pursue a PhD in Nutrition having received a study fellowship from the Nevin Scrimshaw International Nutrition Foundation and Wageningen University. In 2008, she joined the Directorate of Research Management and Development within the Ministry of Higher Education, Science and Technology, Kenya as a senior research officer and where she is currently an Assistant Director-Research.

# List of publications and posters

## Peer Reviewed

1. Catherine W. Macharia-Mutie, Diego Moretti, Natalie Van den Briel, Agnes M. Omusundi, Alice M. Mwangi, Frans J. Kok, Michael B. Zimmermann & Inge D. Brouwer: Maize porridge enriched with a micronutrient powder containing low dose iron as NaFeEDTA but not amaranth grain flour reduces anaemia and iron deficiency in Kenyan pre-school children. *Accepted for publication J Nutr* doi: 10.3945/ jn.112.157578.

2. Catherine W. Macharia-Mutie, Anne M. Van de Wiel, Ana M. Moreno-Londono, Alice M. Mwangi & Inge D. Brouwer (2011): Sensory acceptability and factors predicting the consumption of grain amaranth in Kenya, *Ecology of Food and Nutrition*, Vol. 5, No 5, pp. 375-392

3. Macharia-Mutie, C.W., Brouwer, I.D., Mwangi, A.M. and Kok, F.J. (2010) 'Complementary feeding practices and dietary intake among children 12–23 months in Mwingi district, Kenya', *International Journal of Food Safety, Nutrition and Public Health*, Vol. 3, No. 1, pp.45–56.

4. Macharia C.W., Kogi_Makau W. and N.M Muroki (2005). A comparative study on the nutritional status of children (6-59 months) in a World Vision project area and a non-project area in Kathonzweni division, Makueni district, Kenya. *African Journal of Food, Agriculture, Nutrition and Development.* Vol 5, No1, http://www.ajfand.net/Volume5/No1/Macharia1240.pdf.

5. C.W. Macharia., W. Kogi_Makau and N.M. Muroki (2004), Dietary intake, feeding and care practices of children in a World Vision multi-sectoral community based project in Kathonzweni division, Makueni district, Kenya. *East African Medical Journal.* Vol. 81, No 8 pp. 402-407.

## Conference abstracts and papers

1. Macharia-Mutie, C.W., Mwangi A.M. and Brouwer I.D. (2010). Dietary intake and nutrient adequacy among children in a rural Kenyan community. Poster presented at 4$^{th}$ Africa Nutritional Epidemiology Conference, Nairobi 4-8$^{th}$ October 2010.

2. Catherine W. Mutie and Florence M. Kyallo (2010). Empowering women through better healthcare and nutrition; the case of Kenya. Paper presented at the International conference on 'Empowering women in developing countries through better healthcare and nutrition, Pilani, India, 22-24$^{th}$ April 2010.

3. Macharia-Mutie, C.W., Moreno-Londono A.M., Brouwer I.D., Mwangi A.M and Kok, F.J. (2009). Factors predicting the intention to consume grain amaranth enriched porridge in Makuyu Division, Kenya. Poster presented at 19th International Congress of Nutrition, Thailand 4-9th October 2009. Annals of Nutrition Metabolism; 55 (supplement1) pp. 374.

4. Macharia-Mutie, C.W., Brouwer I.D., Mwangi A.M and Kok, F.J. (2009). Iron, zinc and phytic acid levels of grain amaranth commonly used in Kenya. Poster presented at 19th International Congress of Nutrition, Thailand 4-9th October 2009. Annals of Nutrition Metabolism; 55 (supplement1) Pp. 441.

5. Macharia-Mutie, C.W., Brouwer I.D., Mwangi A.M and Kok, F.J. (2008). Complementary feeding practices and dietary intake among children 12-23 months in Mwingi District, Kenya. Paper presented at 3rd Africa Nutritional Epidemiology Conference, Cairo 13-16th October 2008.

6. A.M. Omwega; J.M. Mutuku; D.L. Mwaniki ; C. Macharia-Mutie; L. Mwaura; R. Yusufali; A.S. Wesley; L. Ethangatta and A. Pertert (2007). Acceptability of double fortified salt (DFS) with encapsulated iron and iodine among the general and refugee populations in Kenya. Poster Presentation at 10th European Nutrition Conference, Annals of Nutrition Metabolism; 51(supplement 1): pp. 93

# Overview of completed training activities

**Discipline specific activities**

**Courses**

- Applied data analysis in nutrition and health research, Wageningen University, The Netherlands 2007
- Food policy for developing countries: governance, institutions & markets in global, national & local food systems, Wageningen University, The Netherlands 2007
- 8th International advanced course on nutritional and lifestyle epidemiology, Wageningen University, The Netherlands 2007
- Food consumption studies data collection training and use of VBS-KOMEET, Wageningen University, The Netherlands 2007
- Master Class "Linear and logistic regression, Wageningen University, The Netherlands 2010"
- Food and nutrition security impact assessment, Wageningen University, The Netherlands 2010

**Conferences and meetings**

- Food sovereignty: Promoting or undermining food security?, Seminar, Wageningen University, The Netherlands 2007
- 3rd Africa Nutritional Epidemiology Conference, ANS/ANEC, Cairo, Egypt 2008
- Fighting malnutrition efficiently: best practices and way forward, Workshop by *Gumlink* & Copenhagen Consensus Center, Nairobi, Kenya 2009
- 19th International Congress of Nutrition, IUNS, Bangkok, Thailand 2009
- International Conference on 'Empowering women in developing countries through better healthcare and nutrition, NAMST, Pilani, India 2010
- 4th Africa Nutritional Epidemiology Conference, ANS/ANEC/KEMRI, Nairobi, Kenya 2010

**General courses**

- VLAG PhD week- 17th edition, Wageningen University, The Netherlands 2007
- Information literacy for PhD including Endnote program, Wageningen University, The Netherlands 2007
- 9th and 10th Africa Nutrition Leadership Programme, North West University, South Africa 2011/2012
- Philosophy and ethics of food sciences and technology, Wageningen University, The Netherlands 2012

**Optional courses and activities**

- Research proposal development, Wageningen University, The Netherlands 2007
- Staff seminars, Wageningen University, The Netherlands 2007
- PhD Excursion to Mexico and South West USA, Wageningen University, The Netherlands 2011

The research described in this thesis was financially supported by grants/fellowships received from International Nutrition Foundation/Ellison Medical Foundation, Nutricia Research Foundation, Nestlé Foundation, Clive West Micronutrient Fund, Foundation Van Dam Nutrition Plan and Wageningen University.

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

Cover Design: Paul K. Gatere
Thesis Layout: Catherine Macharia-Mutie
Printed by GVO drukkers & vormgevers B.V/Ponsen & Looijen, Ede, The Netherlands