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Title: The potential contribution of yellow cassava to dietary nutrient adequacy of primary school children in Eastern Kenya; the use of linear programming

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Short Title: Yellow cassava and nutrient adequacy

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Ethical Standards Disclosure: This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethical Committee in both the Netherlands and Kenya. The study was registered (clinicaltrials.gov: NCT01614483) and written consent was obtained before the start of the trial.

Title: The potential contribution of yellow cassava to dietary nutrient adequacy of primary school children in Eastern Kenya; the use of linear programming

Objective: Introduction of biofortified cassava as school lunch can increase vitamin A intake, but may increase risk of other deficiencies due to poor nutrient profile of cassava. We assessed the potential effect of introducing a yellow cassava –based school lunch combined with additional food-based recommendations (FBRs) on vitamin A and overall nutrient adequacy using Optifood (linear programming tool).

Design: Cross-sectional study to assess dietary intakes (24-hour recall) and derive model parameters (list of foods consumed, median serving sizes, food and (sub)food group frequency distributions, food cost). Three scenarios were modeled: Daily diet including; 1) no school lunch; 2) standard 5-days school lunch with maize/beans; 3) 5-days school lunch of yellow cassava. Each scenario and scenario 3 with additional FBRs were assessed on overall nutrient adequacy using recommended nutrient intake (RNI).

Setting: Eastern Kenya

Subjects: 150 primary school children (7-9 years)

Results: Best food pattern of yellow cassava-based lunch scenario achieved 100% RNI for 6 nutrients compared to no lunch (3 nutrients) or standard lunch (5 nutrients) scenarios. FBR with yellow cassava and including small dried fish improved nutrient adequacy, but could not ensure adequate intake of fat (52% of average requirement), riboflavin (50% RNI), folate (59% RNI) and vitamin A (49% RNI).

Conclusions: Introduction of yellow cassava-based school lunch complemented with FBR potentially improved vitamin A adequacy, but alternative interventions are needed to ensure dietary adequacy. Optifood is useful to assess potential contribution of a biofortified crop to nutrient adequacy and to develop additional FBR to address remaining nutrient gaps.

Key words: Linear programming, Optifood, Biofortification, Vitamin A deficiency, Yellow cassava

Introduction

Biofortification of staple foods is recognized as a sustainable food-based approach to reduce micronutrient deficiencies as it has the potential to substantially increase the micronutrient intakes of a population ⁽¹⁾. Biofortified yellow cassava, developed through conventional plant-breeding techniques, is a new staple food that is similar in use and nutritional content to white cassava but additionally contains substantially higher amounts of pro-vitamin A ⁽²⁾. Yellow cassava could potentially increase vitamin A intake and reduce vitamin A deficiency, especially in remote rural areas where other interventions have challenges to reach people in need of vitamin A ^(3, 4).

Although yellow cassava is an excellent source for pro-vitamin A and energy, it is known to be generally poor in other nutrients such as iron and zinc ⁽⁵⁾. Children whose diets consist largely of cassava may be vulnerable to micronutrient deficiencies ⁽⁶⁾. As diets in resource poor environments often lack multiple micronutrients ⁽⁷⁾, the introduction of yellow cassava, even when only the replacement of white cassava is targeted, will require additional food-based dietary adaptations to fill the existing nutrient gaps in the diet. Also, yellow cassava might displace other staple foods in the diet, so promoting its consumption may inadvertently have a negative impact on dietary adequacy.

Optifood, a software program based on linear programming, was developed to formulate affordable population-specific food-based recommendations (FBR) based on current dietary practices and costs of food ⁽⁸⁾. As these FBR are developed to resemble the local diet as close as possible, they are more likely to be followed than more general guidelines that may deviate from local habits. This mathematical modeling approach provides an objective method to predict, for example, whether fortification, supplementation or special complementary food products are needed to ensure dietary adequacy for high risk populations, and the extent to which these measures might contribute to its achievement ^(9, 10). To our knowledge, Optifood has not yet been used to inform biofortification programs planning to promote the consumption of a biofortified crop with elevated levels of a single nutrient within a set of dietary recommendations. The advantages of using this mathematical modeling approach are that it objectively determines 1) whether promoting a biofortified food, to improve dietary adequacy of one nutrient, might affect overall dietary adequacy by replacing important food sources of other nutrients; and 2) the optimal set of FBR that need to be promoted in addition to the biofortified crop to ensure dietary adequacy at an affordable cost.

School-feeding is an important and wide-spread educational intervention globally involving approximately 370 million school-age children ⁽¹¹⁾, an often neglected group providing an opportunity for micronutrient interventions in a controlled environment ⁽¹²⁾. School-feeding programs are designed to support education and food security of children living in poverty, by increasing enrolment and reducing absenteeism and drop-outs, and contributing to their learning, through avoiding short-term hunger ⁽¹³⁾. However, less attention is given to reducing or preventing micronutrient deficiencies ⁽¹⁴⁾. In Kenya, school-feeding programs provide a school lunch generally comprised of a mixture of cooked maize and beans ⁽¹⁵⁾, referred to as githeri, a common dish regularly consumed in households. In Eastern Kenya, rural school children, however, are documented to have high levels of anemia, stunting and inadequate intakes of many key nutrients in addition to vitamin A ⁽¹⁶⁾. Murphy et al (2003) showed that githeri as a school lunch did not improve the dietary quality and micronutrient intakes of schoolchildren in Eastern Kenya ⁽¹⁷⁾. The introduction of yellow cassava, currently grown experimentally in Eastern Kenya, to the school feeding program could improve the vitamin A intakes of schoolchildren but additional food-based dietary recommendations would be needed to achieve overall nutrient adequacy of the diet given the nutrient profile of cassava.

In this paper, secondary dietary intake data collected in the framework of a yellow cassava efficacy trial ⁽¹⁸⁾, were used in Optifood to 1) evaluate whether including yellow cassava in the school lunch would potentially improve vitamin A adequacy without having a negative effect on overall dietary adequacy compared to no school lunch or a standard school lunch, and 2) to identify additional FBRs needed in a yellow cassava-based school lunch program to improve adequacy of nutrients other than vitamin A at lowest cost.

Subjects and methods

Study design

This study was based on cross-sectional dietary intake data collected as part of an 18-week randomized controlled trial on the efficacy of yellow cassava in improving vitamin A status in primary school children¹⁸. In this trial, cassava was served as a mid-morning snack of boiled white or yellow cassava to children aged 5-13 years from May to November 2012. The trial also provided a school lunch made of a mix of cooked maize and beans. Cross-sectional dietary intake data were collected to quantify food intake in and outside the school and used to define inputs to model (using linear programming) three scenario's for a 7-day best diet: a daily diet including 1) no school lunch; 2) a standard 5-day school lunch with cooked maize

and beans; or 3) a 5-day school lunch of cooked yellow cassava. The yellow cassava based school lunch scenario was further optimized using nutrient dense foods. The original study was registered (clinicaltrials.gov: NCT01614483), approved by ethical committees in Kenya and The Netherlands, and written consent was obtained from parents and children.

Subjects

The original trial involved 342 children (5-13 years old) in three primary schools in Kibwezi District, Eastern Province, Kenya and were selected by screening and ranking according to serum retinol binding protein concentration and those at the lowest end of the distribution were selected for the trial. For this modelling study we selected all dietary intake data available of children between 7 and 9 years of age (n=150) as they represented the largest age group for which food based recommendation could be made.

Nutrition status assessment

Blood sample collection: All venous blood samples were obtained from fasted children at the start of the trial by venipuncture. Samples for retinol analyses were shielded from light and processed under subdued light conditions. All blood samples were stored at 2-8°C until centrifuging and in liquid nitrogen (-196°C) in Kenya, and at -80 °C during transport and storage at the laboratory of the Division of Human Nutrition in Wageningen, the Netherlands.

Blood analyses: Concentrations of retinol were measured by HPLC and described in detail elsewhere ⁽¹⁸⁾. Serum concentrations of C-reactive protein, α 1-acid glycoprotein, serum ferritin concentration, serum soluble transferrin receptor, serum vitamin B12 and serum zinc concentration were measured at the Meander Medical Hospital, Amersfoort, the Netherlands on a Beckman Coulter UniCel DxC 880i analyzer as per manufacturer's instructions. Hemoglobin was measured using a Celltac- α automated hematology analyzer (MEK-6410K) in Makindu hospital, Kenya as per manufacturer's instructions.

Nutrition deficiencies cut-off levels: Vitamin A deficiency: serum retinol concentration <0.7 μ mol/L ⁽¹⁹⁾; zinc deficiency: serum zinc concentration <9.9 μ mol/L ⁽²⁰⁾; Vitamin B12 deficiency, mild <133 pmol/L and moderate or severe <107 pmol/L ⁽²¹⁾; anemia: hemoglobin concentration <115g/L ⁽²²⁾; iron deficiency: serum ferritin concentration <15 μ g/L ⁽²²⁾.

Weight and height: Weight and height were measured at baseline according to WHO guidelines ⁽²³⁾ using a mechanical floor scale and a portable stadiometer (Seca, Hamburg,

Germany). Anthropometric indices were calculated using ANTHRO-plus (WHOV3.2.2, www.who.int/childgrowth/software/en/). Stunting was defined as z-score for height-for-age less than -2 SD.

Inflammation: Inflammation was defined as C-reactive protein >5mg/L and/or serum α_1 -glycoprotein protein concentration >1g/L ⁽²⁴⁾.

Dietary intake assessment

Dietary intakes of subjects out-of-school were assessed using a quantitative multi-pass 24-hour recall ⁽²⁵⁾ with all days evenly distributed over the week. These data were collected, during a home visit in October 2012, by well-trained interviewers in weeks 13 to 16 of the trial. Primary caretakers, in the presence of the child, were asked to recall all the foods and drinks consumed in and outside the home (except for the school lunch) by their child during the preceding day and to describe ingredients and cooking methods of any mixed dishes. Duplicate amounts of all foods or beverages consumed or of ingredients used in the preparation of mixed dishes consumed were weighed to the nearest 2g using Soehnle electronic kitchen scale. When duplicates were not available in the household, amounts were estimated in household units, in volumes, as their general sizes (small, medium or large), or as their monetary value equivalents. The total volume of each (mixed) dish cooked at the respondents' household and the volume of this dish specifically consumed by the child were measured to determine the proportion of dish consumed by the child. This proportion was multiplied by the total amount of ingredients used in the preparation of the dish to determine the amount of ingredients consumed by the child. Standard recipes were generated to estimate grams of ingredients consumed from mixed dishes eaten outside the home by averaging 3 recipes of different vendors in the local area ⁽²⁶⁾. Conversion factors were developed to convert household units, volumes, sizes and monetary values to their gram weight equivalents. Food price data were also collected from three different markets/kiosks to calculate, for each food, its mean price per 100g edible food.

Cassava intake in the schools was recorded daily as the difference in the weight of the plate of each child before and after eating during the 18 weeks intervention period and median intake was calculated. Pro-vitamin A concentration of the yellow and white cassava was analyzed ⁽¹⁸⁾. To estimate the average serving size of the maize and beans dish given at schools, the cooks (n=3) were asked to provide a standard portion per school and median portion size was calculated.

Data preparation and analysis for Optifood

Data preparation: 24-hour recall data was organized with the nutrient calculation system Compl-eat (version 1.0, Wageningen University, the Netherlands), and the model parameters were defined by using Excel 2010 (Microsoft Corporation), IBM SPSS (v21) and MS Access 2010. These parameters included: a list of non-condiment foods consumed by $\geq 5\%$ of the children; the serving size of each food defined as median serving size for all children who had consumed it; and minimum and maximum number of servings per week, for each food group and food sub-group defined as the 5th and 95th percentiles, respectively, of serve counts. Average number of servings per week for each food group was defined as the 50th percentile. Maximum number of servings per individual food within a food sub-group was estimated based on percentage of children consuming that food. For the school lunch with yellow cassava and with maize and beans, the minimum and maximum number of servings per week were set at five, assuming these foods would be provided as school lunch five days a week. Prices per 100 gram of edible food were used to estimate average and 75th percentile cost of the observed daily diet. All modeled diets had to meet (and not exceed) the energy requirement for the target group, estimated using their mean body weights and the FAO/WHO/UNU algorithm for estimating energy requirements⁽²⁷⁾. The FAO/WHO recommended nutrient intakes (RNIs) were used for all nutrients, except zinc, assuming 5% bioavailability for iron (18 mg/day)^(28, 29). For fat, average requirement of 30 energy% was used⁽³⁰⁾. For zinc, IZiNCG RNI of 7 mg/day was used⁽³¹⁾ reflecting low bioavailability and representing the average of two age groups.

Nutrient intake calculations were based on a food composition table developed specifically for this study, using the national food composition table of Kenya⁽³²⁾ as primary source complemented with data from food composition tables from South Africa⁽³³⁾, Mali⁽³⁴⁾, East Africa⁽³⁵⁾, International Minilist⁽³⁶⁾ and United States Department of Agriculture database⁽³⁷⁾. USDA retention factors⁽³⁸⁾ were applied to raw ingredients and foods to account for nutrient losses during food preparation. Pro-vitamin A and retinol were converted into retinol activity equivalent⁽³⁹⁾. Pro-vitamin A concentrations in yellow cassava are predominantly in the form of β -carotene and total β -carotene concentration of boiled yellow and white cassava was used as analyzed⁽¹⁸⁾. Conversion factors for β -carotene in cassava were conservatively assumed to be 7:1, based on a study in healthy Americans consuming yellow cassava⁽⁴⁰⁾.

Optifood analysis: All analyses were carried out with Optifood (V4.0.4.0), an approach based on linear programming to design population-specific FBR ⁽⁸⁾. Three dietary scenarios were modeled: 1) daily diet comprising foods consumed exclusively at or outside the home but not in school, 2) daily diet complemented with a school lunch of cooked maize and beans, and 3) daily diet complemented with a school lunch of cooked yellow cassava. Optifood's modules 1-3 were used in the analyses. Module 1 was run to ensure model parameters were generating realistic diets. Subsequently, for each of the three dietary scenarios, Optifood's Module 2 was run to develop 2 best diets: one creating the best diet within the average food pattern and one creating the best diet deviating from the average food pattern but constrained by the minimum and maximum number of servings per week. The total number of nutrients achieving 100% RNI in the best diet deviating from the average food pattern (best food pattern diet) were counted per scenario and compared between scenarios to determine whether including yellow cassava in the school lunch would affect overall dietary adequacy compared to no school lunch or a standard school lunch.

In Module 3, using the model parameters of the yellow cassava-based school lunch scenario, 26 different diets were modeled of which 13 were diets with the maximized content of one of 13 nutrients (best-case scenario, selecting the high nutrient dense foods within each food group to verify the highest possible nutrient intake) and 13 are diets with the minimized content of one of 13 nutrients (worst-case scenario, including the low nutrient dense foods per food group to verify the lowest possible nutrient intake). Module 3 was initially run without FBR constraints to identify problem nutrients. Problem nutrients are those that are <100% of its RNI in the best-case scenario (i.e., when nutrient content is maximized). Next, food groups with weekly servings above zero in the Module 2 best food pattern diet, and individual foods contributing at least 5% to the intake of one of the nutrients were selected. FBR were developed, by incorporating the selected food groups and individual foods, individually and in combination, and were tested to identify sets of FBR that covered >70% RNI for most nutrients. In the final phase of analyses, nutrient dense foods that were less frequently or in low quantities consumed, were incorporated in the FBRs and tested in Module 3 whether improving problem nutrient adequacy. These nutrient dense foods were identified in two ways: 1) foods consumed by less than 5% of children that had a relatively high content of at least one of the problem nutrients, and 2) foods consumed by $\geq 5\%$ of children that contributed more than 20% to the intake of problem nutrients and for which an increase in current number of servings per week was assumed to be feasible. In a last step,

FBR incorporating selected nutrient dense foods were tested in Module 3 separately. The set of recommendations (worst-case scenario) that achieved >70% RNI for most nutrients but stayed below 75th percentile of daily diet cost was selected.

Results

Baseline characteristics: The children in our study were on average 8.5 years old with 27% being stunted (**Table 1**). Thirty percent of the children suffered from vitamin A deficiency but almost none were zinc deficient (3%). Vitamin B12 status was in general low with 58% of the children being mildly deficient and 37% being moderately or severely deficient. The prevalence of anemia was only 6% but 36% of the children were iron deficient. Inflammation affected 20% of our population.

Food intake: In total 150 24-hour dietary recalls were used for data analysis and 16 out of 48 non-condiment food items were consumed by more than 5% of the children (**Table 2**). Foods most commonly consumed were maize, both as whole grain and flour, beans, onions, tomatoes and oil. Serving sizes in the diet varied from 4 g/day for onion to 165 g/day for maize flour. Most of these 16 foods had serving sizes >10 g/day (n=13, 81%). White sugar, oil and onion were consumed in small portion sizes (≤ 10 g/day). The median daily diet costs was 24 Kenyan Shilling (KES), ranging from 2 to 111 KES with the 75th percentile estimated at 38 KES per day.

Linear programming: In module 1 for each scenario 20 realistic diets were generated and, hence, no changes in parameters were needed. In module 2 only diet contents of protein, vitamin C and thiamin were above 100% RNI coverage for all three dietary scenarios in the module 2 best food pattern (**Table 3**). Furthermore, diet zinc and iron contents achieved >100% RNI in 2 scenarios: the maize and beans school lunch and cassava school lunch. Only the cassava lunch achieved >100% RNI in the module 2 best food pattern for vitamin B6. The daily cost of the 3 scenarios ranged from 34 to 38 KES. The yellow cassava module 2 best food pattern achieved >100% RNI for the highest number of nutrients (**Table 3**; 6 out of 13 nutrients). Problem nutrients, being <100% RNI in the Module 3 best-case cassava scenario, were fat, calcium, riboflavin, niacin, folate, vitamin B12 and vitamin A. A set of FBR was developed, including added fat 7 times/week, dairy products 7 times/week, grain and grain products 14 times/week, legumes 14 times/week, yellow cassava 5 times/week and vegetables 28 times/week. These recommendations ensured the worst-case scenario values

for protein, vitamin C, vitamin B6, iron and zinc achieved >70% RNI, but those of fat, calcium, riboflavin, niacin, folate, vitamin B12 and vitamin A remained less than 60% of their RNIs (**Table 4**). The nutrient dense food items that contributed $\geq 20\%$ to the intake of the problem nutrients were oil for fat, milk for riboflavin and vitamin B12 and kale/collard greens for vitamin A. Frequency of these foods were increased with seven servings per week. In addition, the hardly consumed foods chicken, beef and small dried fish were incorporated as nutrient dense foods, although only consumed by 3, 2 and 1 child, respectively, in the age group 7-9 years. Portion sizes were estimated as median grams of the foods consumed by children in the total study population (age group of 5 to 13 years). Small dried fish was consumed only 3 times in the 5-13 years old children and therefore average portion size was used instead of median. All six nutrient dense foods (oil, milk, kale/collard greens, chicken, beef and small dried fish) were added at various frequencies, individually and in combination to the yellow cassava diet with FBR and for each combination a new set of FBR was developed and tested (**Table 4**). The addition of beef and chicken increased the nutrient adequacy of only niacin to at least 70% (78% RNI) in the worst-case scenario. Small dried fish increased the nutrient adequacy for 3 nutrients to at least 70% RNI: calcium, niacin and vitamin B12. Beef and chicken were not combined with the dried fish because of negligible extra contribution of these foods to nutrient adequacy. Addition of 5 compared to 2 servings per week of fish did not increase the number of nutrients achieving >70% RNI, but would increase the daily costs of diet. Also the inclusion of more than 5 servings per week of fish in the FBR would exceed the energy requirement.

Final FBR: The final set of FBRs selected were 7 servings/w of oil, 7 servings/w of dairy products, 14 servings/w of grain products, 14 servings/w of legumes, nuts or seeds, 2 servings/w of small dried fish, 5 servings/w of yellow cassava (provided as school lunch) and 28 servings/w of vegetables (**Table 5**). This set of FBRs hypothetically ensures dietary adequacy at the population level for all nutrients except fat, riboflavin, folate and vitamin A. The increase of nutrient adequacy as % RNI coverage of the optimized diet (yellow cassava diet with FBRs with nutrient dense foods) compared to the average food pattern without school lunch is shown in **figure 1**.

Discussion

We found that, compared to no school lunch or a school lunch with maize and beans, yellow cassava provided as a school lunch can potentially improve vitamin A adequacy of the diet without lowering overall dietary quality. However, even though combining the yellow cassava school lunch with FBRs improved the nutrient adequacy of the diet, it did not ensure adequate intakes of fat (50% of the average requirement), riboflavin (55% RNI), folate (60% RNI) and vitamin A (47% RNI). The low diversity of the foods in the monotonous diet (only 16 different food items are consumed by more than 5% of the children), the low frequency and small quantity of foods rich in nutrients consumed is a major limiting factor in the modelling. This suggests it will be a challenge to cover the nutrient gaps by adapting the local diet without introducing new nutrient dense foods and/or radically modifying current food consumption patterns.

Remarkably, vitamin A remained a problem nutrient in our analyses, and even the diet with yellow cassava, rich in pro-vitamin A, covered only 49% of the required vitamin A intake. Children selected for this study represented the lowest end of the vitamin A status distribution in the area with 30% vitamin A deficiency. The diets of these children may therefore represent those that are low in vitamin A rich foods compared to diets of other school children, which may explain why their recommended nutrient intake could not be fulfilled. Collard greens/kale provided the largest contribution to vitamin A intake, but quantities consumed were small, and for carotenoids both the bioavailability and retention due to long cooking is low ⁽⁴¹⁾ leading to reduced intake of bioavailable vitamin A. In addition, we provided a conservative estimate of the potential increases in vitamin A intake as we used cassava varieties with an average β -carotene concentration of 5.4 $\mu\text{g/g}$ (fresh weight) for raw cassava and 3.9 $\mu\text{g/g}$ (fresh weight) for cooked cassava and applied a conservative conversion factor of 7:1. Another study found a higher impact of adding yellow cassava to the diet but used different modeling assumptions regarding concentration, cooking method and intake quantity ⁽⁴⁾.

In our study, iron and zinc were not identified as problem nutrients and this corresponds with the few children being zinc deficient (3%). Although 36% of the children had iron deficiency, only 6% had anemia. Major food sources of iron and zinc were maize, legumes and cassava, but animal foods were hardly consumed. For this reason iron bioavailability of 5%, reflecting a vegetarian diet, was used ⁽²⁸⁾, but the actual bioavailability of iron in the diet may have been lower ^(42, 43). This might result in the identification of iron as a problem nutrient. Also food consumption studies among children 2-5 years old in Kenya and Nigeria, showed that

children consuming cassava as staple food are at risk for inadequate intake of iron ⁽⁶⁾.

However, in our population cassava was not generally consumed as a staple food and other food sources ensured the adequacy of intakes of iron. Also our study population is slightly older and the requirements for this older age group might be easier to achieve than for younger children.

The yellow cassava school lunch complemented with small fish ensured adequate dietary intakes of calcium. Whole small fish including edible bones has proven to be a good source of calcium with a bioavailability comparable to that of milk ⁽⁴⁴⁾. However, in our population only one child in the age group 7-9 years had consumed small fish. The reason why schoolchildren do not consume small fish or fish in general is unknown. Since the research site was far from any source of fish, availability, affordability and taste could be constraints to consumption and reduce feasibility of this FBR.

Our study has limitations that should be acknowledged. First, the measurement of habitual dietary intake of groups remains a major challenge in dietary intake assessment ⁽²⁶⁾ and limitations of dietary data used as input for the Optifood analysis are related to weaknesses inherent to 24-hour recalls. Major sources of systematic bias in the use of 24-hour recall include under- or over-reporting of food intakes. To minimize bias, we used a systematic multiple-pass procedure and took precautions through training of interviewers, impromptu supervision to minimize reporting errors, proper calibration of instruments and random assignment of interviewers. Out-of-home food intake may have been omitted by mothers/caregivers and may have led to an underestimation of nutrient intake ⁽⁴⁵⁾. However, in this area, almost all meals are prepared and consumed at home and mothers/caretakers are fully involved in serving meals. Also, the presence of the children during the interviews meant they helped mothers/caregivers recall forgotten foods. Therefore we believe that underestimation of food and, therefore, nutrient intake was unlikely to have occurred.

We also assumed that the foods consumed out-of-school in our population reflected the habitual diet of schoolchildren implicitly anticipating that children in general do not take food to school and that food provided in school would not affect the amount of food consumed at home. However, the school meal can be shared with other household members or can substitute (at least partly) food normally consumed in the house ⁽⁴⁶⁾. This sharing occurs for take-home rations where children take home a given quantity of food on a regular basis ⁽⁴⁷⁾, but in our study the cassava lunch was consumed at school. Sharing may also happen

between schoolchildren, but in our study children receiving the different lunches were physically separated. Substitution may also apply when meals are consumed at school as in our study, and households opt to use the school meal as a substitute for food normally consumed at home as a cost saving strategy. A study in rural Kenya did not find evidence that schoolchildren who received supplementary snacks at school reduced food intakes at home⁽⁴⁸⁾. However, we observed during the preparation phase of the study that some children had taken food (consisting of maize and beans) from home to school for lunch, a habit they may have dropped when provided a school lunch. Our assumption that the food provided at school would not affect the food consumed outside school, may have therefore led to a slight underestimation of the Optifood model parameters related to portion size and frequency of consumption, especially concerning maize and beans. As these foods are not major contributors to the nutrients identified as being in short supply, we assume a negligible effect on the identification of problem nutrients.

The cross sectional survey captured a snapshot of dietary patterns and food cost/availability during only one agricultural season. As these vary per season, information on dietary intake in other seasons is necessary to evaluate applicability of our results through the year. Comparative analysis using dietary intake data from different seasons would be required to understand how food-based recommendations might change. It is also important to emphasize that the data used originated from a limited area in Kenya and are as such not representative for the whole of Kenya. Therefore the extent to which the developed recommendations also apply to other areas in Kenya needs to be further assessed.

Finally, our results may be sensitive to assumptions used in defining model parameters. We used iZiNCG RNI, which are below those recommended by the FAO/WHO (2004). When we did a sensitivity analysis using the FAO/WHO zinc RNI, zinc also became a problem nutrient for schoolchildren. This is not confirmed by the very low prevalence of zinc deficiency (3%) in our population, indicating that iZiNCG RNI are likely the best to use. We assumed a low bioavailability of iron (5%) based on the high consumption of unrefined cereals and legumes, and low consumption of animal sourced foods. Doing so, iron intake was not detected as below 70% RNI, even when low nutrient dense foods were used; assuming higher bioavailability would not change this finding. We included only foods consumed by $\geq 5\%$ of the children: if we would have included foods consumed by $\geq 3\%$ of the children, the number of foods in the food lists would have been increased slightly, however frequency of consumption of these foods was low and therefore it is unlikely that these foods would be

selected in the FBR. In addition, including infrequently consumed foods may decrease the feasibility of implementing recommendations.

Our study shows that providing yellow cassava in a school lunch program can ensure nutrient adequacy of the diet in our study area for 9 out of 13 micronutrients when promoted within a set of FBRs. However, proposed recommendations will require changes in the diet (such as the consumption of small fish) and also imply additional changes to the school meal program. **Optifood modelling is theoretical by nature and therefore consultations with local stakeholders are very important to determine whether these modifications to typical dietary behavior are realistic and feasible** ⁽¹⁰⁾ and recipes for the school meal program incorporating these changes should be carefully developed and tested. However, even the best modeled diet could not meet the requirements of fat, riboflavin, folate, and vitamin A. Additional interventions and solutions would be needed beyond promoting the consumption of nutrient dense foods that are commonly consumed by the children to fully eliminate the risk of inadequate nutrient intakes in this population. Since the extent of the multiple micronutrient deficits in the modeled diets would be difficult to meet with a single intervention product more biofortified crops could be introduced, or the introduction of yellow cassava could be integrated with approaches that encourage changes in the traditional diets as well as introduction of improved products such as fortified foods or improved nutrient supplements. Our study also showed that linear modeling of dietary intake data provides an excellent tool to evaluate and develop nutritional strategies for achieving better nutritional adequacy under local circumstances.

Abbreviations:

FBR: food-based recommendations

RNI: recommended nutrient intake

KES: Kenyan Shilling

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599 Figure Legend

600 **Figure 1: Nutrient intake as percentage of the RNI for the average food pattern and**
601 **optimized yellow cassava diet**

602 ¹ Values capped at 100%

603 ² Final set of FBRs selected (best optimized diet, in worst case scenario)

Table 1: Nutrition status indicators of the study population (n=150)

Characteristics		
Background		
Age, years	8.5	(0.9)
Sex, girls, n (%)	74	(49%)
Anthropometrics		
Body-mass-index-for-age ¹ z-score, SD	-1.4	(0.9)
Height-for-age ¹ z-score, SD	-1.2	(1.0)
Children being stunted ¹ , n (%)	41	(27%)
Micronutrient markers		
Serum retinol concentration, $\mu\text{mol/L}$	0.81	(0.18)
Vitamin A deficiency ² , n (%)	45	(30%)
Serum zinc concentration, $\mu\text{mol/L}$	13.7	(2.2)
Zinc deficiency ³ , n (%)	5	(3%)
Serum vitamin B12, pmol/L	123	[97, 160]
Vitamin B12 mild deficiency ^{4, 5} , n (%)	84	(58%)
Vitamin B12 moderate/severe deficiency ^{5, 6} , n (%)	54	(37%)
Hemoglobin concentration, g/L	131	(11)
Anaemia ⁷ , n (%)	9	(6%)
Iron deficiency ^{8*} , n (%)	53	(36%)
Inflammation ⁹ , n (%)	31	(20%)

Values indicate mean (SD), median [25-75th percentile] unless indicated otherwise

¹ WHO 2006 reference population (23)

² Vitamin A deficiency: Serum retinol concentration < 0.70 $\mu\text{mol/L}$ (19)

³ Zinc deficiency: Serum zinc concentration < 9.9 $\mu\text{mol/L}$ (20)

⁴ Vitamin B12 mild deficiency: Serum B12 < 133 pmol/L (21)

⁵ n=146 for Vitamin B12 and iron deficiency

⁶ Vitamin B12 moderate/severe deficiency: Serum B12 < 107 pmol/L (21)

⁷ Anemia: Hemoglobin concentration < 115 g/L for children age 5 to 11 years (22)

⁸ Iron deficiency: Serum ferritin concentration < 15 $\mu\text{g/L}$ and soluble transferrin receptor > 1.55 mg/L (22)

⁹ Inflammation: Serum concentrations of C-reactive protein > 5 mg/L and/or α_1 -acid glycoprotein > 1 g/L (24)

Table 2: Food groups and foods used for modelling as consumed by the study population (n=150)

	Percentage of children consuming	Serving size ¹ g/day	Min servings ² per week	Max servings ³ per week	Cost ⁴ KES/100g
Added fats			0	7	
Oil, vegetable	73	9	0	7	20
Added sugars			0	7	
Sugar brown	20	14	0	4	12
Sugar white	13	9	0	3	12
Dairy products			0	7	
Milk cow fresh, boiled	22	31	0	7	8
Grains & grain products			7	21	
Flour maize white	65	165	0	7	6
Flour wheat refined	14	97	0	5	7
Rice refined	8	83	0	2	12
Maize grains white, dried	62	114	0	7	5
Legumes, nuts & seeds			0	14	
Beans red, dried	36	38	0	6	10
Beans mung, dried	5	48	0	1	10
Peas pigeon, dried	24	52	0	5	8
Peas cow, dried	8	54	0	2	7
Vegetables			0	28	
Onion bulb red	68	4	0	7	10
Tomato	62	30	0	7	5
Cabbage	30	91	0	7	3
Kale/collard greens	25	39	0	7	8
School lunches					
Composites (mixed food groups)					
Maize & beans meal	72	306	5	5	0
Starchy roots & other starchy plant foods					
Cassava yellow ⁵	28	374	5	5	0
Nutrient dense foods					
Meat, fish & eggs					
Small fish, dried	1	50	0	7	33
Beef	1	55	0	7	36
Chicken	2	45	0	7	49

¹ Values are median serving sizes of the raw edible portions based on 24-h recalls. Median serving sizes for chicken and beef as well as mean portion size for fish were from 5-13 year old children

² Minimum frequencies were values in the 5th percentile of distribution

³ Maximum frequencies were values in the 95th percentile of distribution

⁴ Mean costs in Kenyan Shilling per 100 gram edible portion, estimated from 3 different kind of shops or markets during October 2012

⁵ Contains 394 µg β-carotene per 100 g boiled (fresh weight) based on own analysis (18)

Table 3: Nutrient composition (as % RNI) of the three diets of the study population for the average¹ and the best² food pattern (module 2).

	No school lunch		Maize & beans school lunch		Yellow cassava school lunch	
	Average food pattern ¹	Best food pattern ²	Average food pattern ¹	Best food pattern ²	Average food pattern ¹	Best food pattern ²
	<i>% of the RNI</i>					
Protein	240	245	217	250	194	226
Fat	45	43	49	44	55	50
Calcium	42	55	37	56	45	64
Vitamin C	201	203	199	203	428	432
Thiamin	122	117	143	137	151	145
Riboflavin	38	46	48	61	37	50
Niacin	50	55	61	71	56	66
Vitamin B-6	69	73	85	95	137	147
Folate	45	49	46	64	43	60
Vitamin B-12	3	19	4	10	4	10
Vitamin A	17	19	17	19	47	49
Iron	97	94	94	100	115	122
Zinc	95	99	98	113	89	104
Cost/Day (KES)	35	38	28	34	29	34
Number of nutrients	3	3	3	5	5	6
>100%RNI						

RNI= Recommended nutrient intake

¹ Average food pattern = best diet within average food pattern closest to median food pattern of the population

² Best food pattern = best diet deviating from average food pattern, but constrained by the minimum and maximum servings per week

636 **Table 4: Results for yellow cassava diet without and with FBR, including nutrient dense foods**

	Protein	Fat	Calcium	Vitamin C	Thiamin	Riboflavin	Niacin	Vitamin B-6	Folate	Vitamin B-12	Vitamin A	Iron	Zinc	Cost	
	% of the RNI													KES/day ¹	
Yellow cassava diet without FBR															
Best case scenario	236	58 ²	65 ²	432	158	51 ²	67 ²	148	61 ²	10 ²	49 ²	131	106	37	
Worst case scenario	161	31	22	229	92	22	38	98	28	3	30	84	63	22	
Yellow cassava diet with FBR ³															
Worst case scenario	219	49	58	432	125	43	57	130	56	10	49	121	87 ³	33	
Worst-case scenario results for nutrient dense foods added to the yellow cassava diet with FBR ⁴															
1	FBR+7 servings/wk oil	213	65	61	432	136	48	63	141	59	10	49	118	136	34
2	FBR+7 servings/wk milk	222	51	64	433	129	51	58	134	58	15	51	120	128	35
3	FBR+7 servings/wk kale/collard greens	220	49	65	382	131	39	63	133	53	10	61	123	88	33
4	FBR+2 servings/wk beef	231	53	59	432	132	48	64	137	58	21	50	123	98	38
5	FBR+5 servings/wk beef	250	59	62	432	143	54	75 ⁵	147	59	37	51	125	115	46 ⁶

6	FBR+2 servings/wk chicken	229	50	59	432	128	46	62	135	57	12	51	121	91	39 ⁶
7	FBR+5 servings/wk chicken	244	52	60	432	134	51	70 ⁵	142	58	15	54	121	98	48 ⁶
8	FBR+2 servings/wk small dried fish ⁷	243	52	116 ⁵	432	132	50	70 ⁵	141	59	81 ⁵	49	124	103	37
9	FBR+5 servings/wk small dried fish	283	58	205 ⁵	432	143	60	89 ⁵	159	62	188 ⁵	49	131	127	44 ⁶

Best-case scenario results for best optimized yellow cassava diet

8	FBR+2 servings/wk small dried fish	286	59	211	431	148	61	90	160	63	195	49	131	129	45 ⁶
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RNI= Recommended nutrient intake, FBR=Food-based recommendation

¹ Cost of the diet with or without FBR

² Problem nutrients could not reach 100% of the RNI in the best-case scenario

³ % of the RNI increased to at least 70% after FBR were applied

⁴ FBR: added fat 7 servings/wk, dairy products 7 servings/wk, grain and grain products 14 servings/wk, legumes 14 servings/wk, yellow cassava 5 servings/wk and vegetables 28 servings/wk

⁵ % of the RNI increased to at least 70% by adding the nutrient dense food to the FBR

⁶ Costs per day raised above 75th percentile

⁷ Final set of FBRs selected (best optimized diet)

Table 5: FBR developed in the yellow cassava school lunch scenario optimized with nutrient dense foods and compared with the average food pattern.

Scenario with yellow cassava as school lunch	Average food pattern ¹	With FBR ²	With FBR & optimized diet ³
<i>servings/week</i>			
Added fats	7	7	7
Dairy products	0	7	7
Grains & grain products	7	14	14
Legumes, nuts & seeds	7	14	14
Meat, fish & eggs (as small dried fish)			2
Starchy roots & other starchy plant foods (as yellow cassava)	5	5	5
Vegetables	21	28	28

FBR=Food-based recommendation

¹ Results of module 2 best diet within average food pattern of yellow cassava school lunch scenario

² Results of module 3 analysis of yellow cassava school lunch scenario with FBR including foods consumed $\geq 5\%$ of the children

³ Results of module 3 analysis of yellow cassava school lunch scenario with FBR including nutrient dense foods consumed with low frequency or low quantity

