

## Sweetpotato-based complementary food would be less inhibitory on mineral absorption than a maize-based infant food assessed by compositional analysis

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### Abstract

The availability of micronutrients from sweetpotato-based complementary foods (CFs): oven-toasted and roller-dried ComFa, and from a maize-based infant food, enriched Weanimix, was compared using phytate/mineral molar ratios, polyphenols and  $\beta$ -carotene levels. The phytate/calcium, iron and zinc molar ratios of approximately 0.17, 1 and 15 predict better absorption of calcium, iron and zinc respectively. Generally, the sweetpotato-based CFs had at least half the phytate/mineral ratios of enriched Weanimix. The phytate/iron ratio in both the sweetpotato- and the maize-based CFs was greater than 1. Only the ComFa formulations had phytate/zinc ratio lower than 15. The level of polyphenol (iron inhibitor) was similar for the formulations. Only the sweetpotato-based CFs contained measurable levels of  $\beta$ -carotene, a possible iron enhancer. The lower phytate/mineral ratios and the  $\beta$ -carotene level of the sweetpotato-based CFs suggest that calcium, iron and zinc absorption could be better from them than from the maize-based infant food.

**Keywords:** *availability, complementary/infant food, maize, mineral, phytate, sweetpotato*

**Abbreviation:** *CFs, Complementary foods*

### Introduction

The high phytate levels of complementary foods (CFs) prepared from unrefined/non-dehulled cereals such as maize, millet, sorghum and legumes (soyabean, cowpea or groundnut), commonly used in low-income countries of sub-Saharan Africa (Greiner et al. 2006; Gibson et al. 2010), partly contribute to the high iron and zinc deficiencies among infants (Hotz et al. 2001; Hurrell et al. 2003; Hurrell and Egli 2010). Phytate forms insoluble complexes with some micronutrients (Gibson and Hotz 2001; Greiner et al. 2006; Kumar et al. 2010) and, because the intestinal phytase activity in humans is low (Sandberg and Andersson 1988; Weaver and Kannan 2002), hydrolysis of such complexes to release the bound nutrients is limited. The inhibitory effect of phytate on nutrients is dose

dependent (Hallberg and Hulthen 2000; Greiner et al. 2006). Therefore, reduction of the phytate level in plant-based infant foods is likely to improve the absorption of essential micronutrients including iron and zinc.

Strategies suggested to reduce the inhibitory effect of phytate on nutrient absorption include soaking of cereal flour in water and discarding the excess water (Hotz et al. 2001), or the addition of phytase-containing micronutrient powder to the cereal porridge before consumption (Troesch et al. 2009). Generally, thermal processing is not an effective method for reducing the phytate levels because phytate is relatively heat stable (Sathe and Venkatachalam 2002), and heating also denatures endogenous phytase

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(Sandberg et al. 1987). Importantly, lower derivatives of phytate (inositol tri- and tetra-phosphates) alone do not limit nutrient bioavailability (Sandberg et al. 1987), but do so in the presence of penta- and hexa-phosphates (Sandberg et al. 1999).

Other constituents of food such as polyphenols (Gillooly et al. 1983; Teucher et al. 2004; Petry et al. 2010) and  $\beta$ -carotene (Garcia-Casal et al. 1998, 2000; Layrisse et al. 2000; Garcia-Casal 2006) have been reported to affect iron absorption from plant-based foods. Levels of total polyphenols, determined by the Folin–Ciocalteu method, of greater than 20 mg/meal Gallic acid equivalents have been shown to inhibit iron absorption (Petry et al. 2010). Polyphenols are located in the seed coat of legumes and outer layer of cereals (Gillooly et al. 1984), so dehulling of legumes and grains could reduce the levels in foods.

A human study in which  $\beta$ -carotene was added to test diets prepared from rice, corn or wheat led to an increase in iron absorption that was 2–3 times greater compared with meals given without  $\beta$ -carotene supplementation (Garcia-Casal et al. 1998). Other findings that suggest that  $\beta$ -carotene could limit the inhibitory effect of phytate and/or polyphenols on iron absorption have been published (Garcia-Casal et al. 2000; Layrisse et al. 2000; Garcia-Casal 2006). Further, an algorithm available for predicting iron bioavailability from a vegetarian diet, which has been validated *in vitro* (coefficient of determination of 0.81) and *in vivo* (coefficient of determination of 0.79), includes  $\beta$ -carotene as a positive predictive factor (Chiplonkar and Agte 2006). It is noteworthy that there are no contradictory findings on the effect of  $\beta$ -carotene on iron absorption as have been observed for vitamin A (Walczyk et al. 2003; Hurrell and Egli 2010).

Sweetpotato is relatively low in phytate (Phillippy et al. 2003; Lukmanji et al. 2008; Lung'aho and Glahn 2009) and, depending on the variety, may contain high  $\beta$ -carotene levels (Hagenimana et al. 2001; Ssebuliba et al. 2001; Ofori et al. 2009) compared with cereals. For these reasons, it has been used to develop CFs (Amagloh et al. 2012a) as an alternative to cereal-based infant foods. Some previous researchers formulated similar sweetpotato-based infant foods (Espinola et al. 1997; Nnam 2000; Akaninwor and Okechukwu 2004; Akubor 2005; Ijarotimi and Ashipa 2006; Omwamba et al. 2007; Nandutu and Howell 2009; Adenuga 2010). However, there is little published information about the bioavailability of mineral nutrients such as calcium, iron and zinc from the sweetpotato-based food matrix (Gibson et al. 2010).

Before conducting micronutrient bioavailability trials, which are expensive and time consuming to undertake, the phytate/calcium, iron or zinc molar ratio can be a useful index to assess the potential availability of these essential micronutrients from plant-based CFs (Gibson et al. 2010). These ratios

have been used to assess the availability of calcium, iron and zinc in other CFs (Abebe et al. 2007; Chan et al. 2007; Gibbs et al. 2011; Tizazu et al. 2011). Dephytinised commercial infant cereals with lower phytate to iron and zinc ratios resulted in higher bioavailability of iron and zinc in Caco-2 cells model compared with the non-dephytinised infant cereals (Frontela et al. 2009). Furthermore, in absorption studies conducted in either humans (Adams et al. 2002; Hambidge et al. 2004) or weanling rat model (Lönnerdal et al. 2011), test meals with lower phytate/zinc molar ratios resulted in higher fractional zinc absorption compared with those with higher phytate/zinc molar ratios.

The availability of calcium, iron and zinc from sweetpotato-based CFs and a maize-based infant food was compared using phytate/calcium, iron and zinc molar ratios; and polyphenols and  $\beta$ -carotene levels.

## Materials and methods

Detailed description of the two prototype formulations of sweetpotato-based CFs – oven-toasted ComFa (household-level formulation) and roller-dried ComFa (industrial-level formulation) has been documented in an earlier publication (Amagloh et al. 2012a).

Briefly, a composite flour of 66% sweetpotato (cream-fleshed), 10% soyabean, 6.0% soyabean oil, 0.50% iodised salt, 0.50% sugar and 17% fish powder from anchovy (*Engraulis hepsetus*) was toasted for 30 min in a preheated oven at 120°C, and referred to as *oven-toasted ComFa*. Another composite flour of cream-fleshed sweetpotato (72%), soyabean (15%), soyabean oil (6.0%), iodised salt (0.50%), sugar (0.50%) and skim milk powder (6.0%) was pre-cooked in a steam-jacketed pan for 10 min (80–83°C) before roller drying with steam at 107°C under 100 kPa, and referred to as *roller-dried ComFa*.

The proportion of the ingredients was computed as described by Amagloh et al. (2012b) using Nutrition Calculator for formulating CF blends developed by Global Alliance for Improved Nutrition. The primary target for the ComFa formulations was to meet energy level of at least 1670 kJ/100 g, protein content of 15 g/100 g and fat ranging from 10 to 25 g/100 g specified in the Codex standard for CFs (Codex Alimentarius Commission 1991).

*Enriched Weanimix* is a maize-based CF and was processed as previously described (Agble 1997; Lartey et al. 1999) with some modifications (Amagloh et al. 2011). The modifications included the use of dehulled maize and soyabean flours and further addition of 17% fish powder (wt/wt) and 0.50% sugar to the basic Weanimix formulation.

Three samples of each formulation were prepared and stored at approximately –1.0°C in an airtight plastic container before analysis.

### Calcium, iron and zinc analysis

Mineral analysis was conducted by the Campbell Microanalytical Laboratory, Department of Chemistry, University of Otago, New Zealand. The samples were digested in triplicate using quartz distilled concentrated HNO<sub>3</sub> heated to 104°C. The acid strength was reduced by evaporation to near dryness at 90°C. After suitable dilution in 2.0% HNO<sub>3</sub> and the addition of reference elements, the calcium, iron and zinc levels were determined by quadrupole inductively coupled mass spectrometry (Agilent 7500ce; Agilent Technologies Inc., CA, USA). The instrument was tuned in accordance with the manufacturer's recommendations, and calibration used certified multi-element solutions. Helium collision cell gas was used to eliminate interferences. Spiked samples and blanks were run with mean recoveries 103%, 103% and 98% for calcium, iron and zinc respectively. There were no measurable minerals present in the blanks. Results were reported on a dry weight basis after correction for recovery.

### Phytate analysis

The phytate content of defatted samples of the CFs has been reported (Amagloh et al. 2012a). A *myo*-inositol-phosphate-specific phytase was used to quantify together the total di-, tri-, tetra-, penta- and hexa-phosphates as phytate content in the samples on a UV/Visible spectrophotometer (Pharmacia LKB Ultrospec II, UK) using a commercial phytate assay kit (K-PHYT 05/07) from Megazyme International Ireland Ltd., Wicklow, Ireland.

### Total polyphenols analysis

The total polyphenols in the samples, quantified as Gallic acid equivalents, were determined using the Folin–Ciocalteu method according to the procedure described by Akond et al. (2010) following extraction of total polyphenols in the samples using the method described by Zimmermann et al. (2008). Total polyphenols were extracted with acetone and Milli-Q water (acetone/water, 4:1 vol/vol). The suspension obtained was centrifuged (Heraeusmultifuge 1S-R; ThermoFisher Scientific, Osterode, Germany), and the extraction solvent was removed using a rotary evaporator (Rotavapor R-215; Büchi Labor Technik AG., Fawil, Switzerland). The absorbance of the total polyphenols in the sample was measured at 760 nm on a UV/Visible spectrophotometer (Pharmacia LKB Ultrospec II). The coefficient of determination of the standard curve was 0.994, indicating high linearity.

### $\beta$ -Carotene analysis

The level of  $\beta$ -carotene in the sweetpotato- and maize-based infant foods was determined in duplicate using

the Carr–Price method, AOAC 974.29 (4) by a commercial laboratory (Nutrition Laboratory, Massey University, New Zealand) using high-performance liquid chromatography (Shimadzu HPLC, Japan). The coefficient of determination of the standard curve was 0.999, indicating almost a perfect linearity. The percentage recovery of an internal standard spiked with  $\beta$ -carotene and run in parallel to the samples was 85%.

### Calculation of phytate/mineral molar ratio

The phytate/calcium, iron or zinc ratio was calculated by dividing the moles of phytate by moles of calcium, iron or zinc per sample. The moles of phytate in the samples were calculated by dividing the amount of phytate by the molecular mass of phytate (660.40 g/mol). The moles of calcium, iron and zinc were calculated by dividing the levels in the samples by their respective molecular masses (40.07, 55.85 and 65.38 g/mol).

### Statistical analysis

Univariate and one-way ANOVA analyses were used for the descriptive and ANOVA, respectively, using Statistical Analysis Systems software package, version 9.1 (SAS Institute, Cary, NC, USA). Tukey's Studentized range (HSD) test was used to compare differences between means when significant ( $p < 0.05$ ). Student's *t*-test was used to compare the means of  $\beta$ -carotene content of the two sweetpotato-based infant foods only.

## Results

The phytate, calcium, iron and zinc levels in the sweetpotato-based CFs (oven-toasted ComFa and roller-dried ComFa) and the maize-based infant food, enriched Weanimix, are shown in Table I. The sweetpotato-based CFs contained less phytate, approximately 26%, compared with enriched Weanimix (Amagloh et al. 2012a). The calcium content of the oven-toasted ComFa was approximately three and two times significantly higher ( $p = 0.001$ ) than the levels in the roller-dried ComFa and enriched Weanimix respectively. The level of calcium in the roller-dried ComFa and the enriched Weanimix was not significantly different ( $p > 0.05$ ). Also, the iron content in the oven-toasted ComFa and enriched Weanimix was similar and both levels were significantly higher than the content of the roller-dried ComFa ( $p < 0.0001$ ). The estimated daily intake of calcium, iron and zinc from the sweetpotato- and the maize-based CFs based on 40 g (dry weight) (Dewey 2003) for 6- to 11-month-old infants was less than half of the World Health Organization recommended levels for calcium (400 mg/day), iron (9.3 mg/day) and zinc (4.1 mg/day) from CFs processed for 6- to 8-month-old breastfeeding infants (Dewey and Brown 2003).

Table I. Levels of phytate, calcium, iron and zinc (mg/kg) in sweetpotato- and maize-based CFs\*.

Infant food	Phytate <sup>†</sup>		Calcium		Iron		Zinc	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Sweetpotato-based								
Oven-toasted ComFa	2263.70 <sup>b</sup>	320.01	6008.40 <sup>a</sup> (60%) <sup>‡</sup>	633.34	69.74 <sup>a</sup> (30%)	0.37	21.17 <sup>b</sup> (21%)	0.75
Roller-dried ComFa	1949.70 <sup>b</sup>	208.40	2017.70 <sup>b</sup> (20%)	18.28	27.88 <sup>b</sup> (12%)	1.26	15.79 <sup>c</sup> (15%)	0.25
Maize-based								
Enriched Weanimix	8032.70 <sup>a</sup>	356.81	3707.80 <sup>b</sup> (37%)	251.17	81.22 <sup>a</sup> (35%)	4.49	30.69 <sup>a</sup> (30%)	0.21
<i>P</i> -value	<0.0001		0.001		<0.0001		<0.0001	

\* Mean value ( $n = 3$ ) with respective standard error of mean (SEM) reported on dry matter basis; mean value within a column with unlike superscript letters<sup>a,b,c</sup> is significantly different ( $p < 0.05$ ); <sup>†</sup>Data extracted from Amagloh et al. (2012a); <sup>‡</sup>Value in parenthesis is percentages of the WHO desirable levels (Dewey and Brown 2003) met by the infant foods based on daily ration size of 40 g (dry weight) for 6- to 11-month old breastfeeding infants (Dewey 2003).

The only exception was the oven-toasted ComFa, which contained 60% of the recommended calcium level.

There were significant differences between the sweetpotato-based CFs and the maize-based infant food regarding the phytate/calcium ( $p = 0.001$ ) and zinc ( $p = 0.001$ ) molar ratios (Table II). All the CFs differed in the phytate/iron molar ratio ( $p < 0.0001$ ). The oven-toasted ComFa, containing 17 g/100 g fish powder, had the lowest phytate/mineral molar ratios, followed by roller-dried ComFa, which contained 6.0% milk powder. The enriched Weanimix, containing 17% fish powder as in the oven-toasted ComFa (both could be replicated at the household level), had the highest ratios. All the CFs had lower phytate/calcium ratios than the maximum recommended ratio of 0.17 (Gibson et al. 2010). The oven-toasted ComFa and the roller-dried ComFa were, respectively, about nine and three times below the maximum recommended ratio for the phytate/calcium ratio. Enriched Weanimix was slightly lower (1.3 times) than the phytate/calcium molar ratio. The phytate/iron molar ratios for the sweetpotato-based infant foods as well as the enriched Weanimix were significantly different ( $p < 0.0001$ ) and exceeded the maximum recommended ratio of 1 (Hurrell 2004) by more than 100%. However, the phytate/iron molar ratios for the oven-toasted ComFa and roller-dried ComFa were lower by approximately 67% and 30%, respectively, compared with the enriched Weanimix. The phytate/

zinc molar ratio of the oven-toasted ComFa and roller-dried ComFa was approximately two times ( $p = 0.001$ ) lower than that for enriched Weanimix. The sweetpotato-based CFs were 24% (average) lower compared with the maximum recommended phytate/zinc molar ratio of 15 (World Health Organization 1996). In contrast, the phytate/zinc ratio of enriched Weanimix was higher by 53% compared with the maximum recommended ratio.

The data in Table III indicate the amounts of total polyphenols (an inhibitor) and  $\beta$ -carotene (a possible enhancer) that could affect iron absorption from the formulations. The level of the total polyphenols, quantified as Gallic acid equivalents, in both the sweetpotato- and maize-based CFs was not significantly different ( $p = 0.43$ ). Using an estimated daily ration of 40 g (dry weight) for 6- to 11-month-old infants (Dewey 2003), the estimated total polyphenols would be 84.70 mg/meal and 76.96 mg/meal from the sweetpotato- and the maize-based CFs respectively. The oven-toasted ComFa and roller-dried ComFa contained measurable amounts of  $\beta$ -carotene content, and the levels were not significantly different ( $p = 0.19$ ). The  $\beta$ -carotene level in the enriched Weanimix was below the detection limit of 0.09  $\mu$ mol/kg.

## Discussion

The rationale and the choice of ingredients have been discussed elsewhere (Amagloh et al. 2012b).

Table II. Phytate: calcium, iron and zinc molar ratios of sweetpotato- and maize-based CFs\*.

Infant food	Phytate/calcium		Phytate/iron		Phytate/zinc	
	Mean	SEM	Mean	SEM	Mean	SEM
Sweetpotato-based						
Oven-toasted ComFa	0.02 <sup>b</sup>	0.01	2.74 <sup>c</sup>	0.38	10.72 <sup>b</sup>	1.81
Roller-dried ComFa	0.06 <sup>b</sup>	0.01	5.89 <sup>b</sup>	0.43	12.28 <sup>b</sup>	1.49
Maize-based						
Enriched Weanimix	0.13 <sup>a</sup>	0.01	8.38 <sup>a</sup>	0.24	25.94 <sup>a</sup>	1.31
<i>P</i> -value	0.001		<0.0001		0.001	
Maximum recommended ratio	0.17 <sup>†</sup>		1 <sup>‡</sup>		15 <sup>¶</sup>	

\* Mean value ( $n = 3$ ) with respective standard error of mean (SEM); mean value within a column with unlike superscript letters<sup>a,b,c</sup> is significantly different ( $p < 0.05$ ); <sup>†</sup>Source: Gibson et al. (2010); <sup>‡</sup>Source: Hurrell (2004); <sup>¶</sup>Source: World Health Organization (1996).

Table III. Levels of total polyphenols (an inhibitor of iron absorption) and  $\beta$ -carotene (a possible enhancer of iron absorption) in sweetpotato- and maize-based CFs.

Infant food	Total polyphenols (mg/kg)*		$\beta$ -Carotene ( $\mu$ mol/kg) <sup>†</sup>	
	Mean	SEM	Mean	SD
Sweetpotato-based				
Oven-toasted ComFa	2195.09	162.35	28.73	1.11
Roller-dried ComFa	2039.85	85.61	24.84	1.24
Maize-based				
Enriched Weanimix	1923.95	152.25	ND <sup>‡</sup>	
<i>P</i> -value	0.43		0.19	

\*Mean value ( $n = 3$ ) with respective standard error of mean (SEM) on dry matter basis; <sup>†</sup>Mean value ( $n = 2$ ) with respective standard deviation (SD) on dry matter basis; <sup>‡</sup>Not detected at the minimum detection limit 0.09  $\mu$ mol/kg for  $\beta$ -carotene (1  $\mu$ mol  $\beta$ -carotene = 537  $\mu$ g  $\beta$ -carotene).

In summary, the sweetpotato-based CFs (oven-toasted ComFa and roller-dried ComFa) were formulated as alternatives to maize-based CFs, which are generally low in vitamin A (Lartey et al. 1998; Dewey and Brown 2003) unless fortified, and high in phytate (Greiner et al. 2006; Gibson et al. 2010). A typical example of such maize-based CF is Weanimix, developed through collaboration between UNICEF and the Nutrition Unit of the Ministry of Health, Ghana, in 1987 (Agble 1997; Lartey et al. 1999), and formulated to be processed at either the household-level or by small-scale food manufacturers in Ghana. Lartey et al. (1999) enriched Weanimix with fish powder from anchovies to enhance the nutrient composition of infant porridge prepared at the household level. The oven-toasted ComFa was formulated for possible replication at the household level by caregivers, while the roller-dried ComFa was formulated as an industrial-level product. The roller-dried ComFa was formulated to be fortified with vitamins and minerals as done with proprietary cereal-based CFs, but the sample in this study was not fortified. The energy, macronutrients and phytate levels of both the sweetpotato- and maize-based CFs have been discussed elsewhere (Amagloh et al. 2012a). All the formulations met the stipulated energy (1670 kJ/100 g) and fat (10–25 g/100 g) contents recommended by the Codex Alimentarius Commission (1991). However, the roller-dried ComFa contained protein that was lower by 17%, but both the oven-toasted ComFa and enriched Weanimix met the requirement of 15 g/100 g.

The results of this study show that the fish powder prepared from anchovy (milled with bones) used in the oven-toasted ComFa and enriched Weanimix resulted in higher levels of calcium compared with the roller-dried ComFa. A similar observation was reported in another study where fish powder was added to cereal-based household level infant foods (Perlas and Gibson 2005). Although both the oven-toasted ComFa and

enriched Weanimix had the same levels of fish powder incorporated, which served as the main source of calcium, higher level of calcium in this sweetpotato-based infant indicates that the sweetpotato contributed about 40% of the total calcium in the formulation. The calcium content of Weanimix without the added fish powder was 155 mg/kg, which was only 4.2% of the calcium of the enriched Weanimix. Therefore, the fish powder contributed approximately 95% (3552 mg/kg) of the total calcium content of the enriched Weanimix. Hence, the sweetpotato used to formulate the oven-toasted ComFa accounted for about 2400 mg/kg of the total calcium of this product. For this reason, the incorporation of fish powder prepared from anchovies into infant foods should be encouraged especially in cultures where this practice is acceptable such as in Ghana, as suggested in an earlier report (Amagloh et al. 2011).

Without considering the inhibitory effect of phytate, the enriched Weanimix might be thought to be superior to the sweetpotato-based CFs as it was slightly higher in iron and zinc. However, when the phytate/calcium, iron and zinc molar ratios are taken into account, these minerals are likely to be more available from the sweetpotato-based CFs than from the maize-based infant food. The low phytate/calcium ratios of the CFs compared with the maximum recommended ratio suggest that the phytate in the foods would be less inhibitory on calcium absorption. On the basis of the phytate/iron ratio, the iron absorption would be limited from both the sweetpotato-based CFs and enriched Weanimix, but the inhibition is likely to be higher for the enriched Weanimix than for the ComFa formulations.

The assay method used to assess the phytate content in this study may slightly overestimate the levels of phytate as it also measured inositol di-, tri- and tetra-phosphates, in addition to penta- and hexa-phosphates, which are the usually reported phytate level in CFs (Hotz et al. 2001, Frontela et al. 2009; Gibbs et al. 2011). Tri- and tetra-phosphates, in the presence of penta- and hexa-phosphates, a likely occurrence in thermally processed foods, limit iron absorption (Sandberg et al. 1999), but this effect is ignored when only penta- and hexa-phosphates are determined. Therefore, the Megazyme assay procedure is appropriate in assessing the phytate level for processed foods.

All the CFs had a higher total polyphenols content per serving than 20 mg/meal that has been shown to reduce iron absorption (Petry et al. 2010) and is expected that the inhibitory effect of polyphenols would be similar in all the CFs.

The oven-toasted ComFa and roller-dried ComFa contained measurable amounts of  $\beta$ -carotene (26  $\mu$ mol/kg) and is likely to enhance iron absorption by off-setting the inhibitory effect of phytate and/or polyphenols as reported in other studies (Garcia-Casal

et al. 1998; Garcia-Casal et al. 2000; Layrisse et al. 2000; Chiplonkar and Agte 2006; Garcia-Casal 2006).

On the basis of the compositional analyses carried out in this study, the sweetpotato-based formulations have the potential to be a valuable CF after the period of exclusive breastfeeding, particularly for infants in low-income countries, who are especially vulnerable to micronutrient deficiencies.

## Conclusion

The phytate/mineral molar ratios for calcium, iron or zinc and possibly, the  $\beta$ -carotene level indicate that the sweetpotato-based infant foods would have less inhibitory effect on calcium, iron and zinc absorption than the maize-based product. Feeding trial among human infants is required to evaluate the impact the sweetpotato-based CFs will have on their micronutrients status.

## Disclaimer

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