

ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
CENTER FOR FOOD SCIENCE AND NUTRITION



Selenium Concentration in Cattle, Dietary Selenium Intake in Children and Zinc Bio-accessibility in agronomically biofortified teff in Amhara region, Ethiopia

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This is to certify that the dissertation prepared by Kaleab Hailu, entitled: “**Selenium Concentration in Cattle, Dietary Selenium Intake in Children and Zinc Bio-accessibility in agronomically biofortified teff in Amhara region, Ethiopia**” and submitted in partial fulfillment of the requirements for Doctor of Philosophy in Food Science and Nutrition complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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I, the undersigned, solemnly declare that this dissertation represents my own work and is not Submitted to any other institution elsewhere for the award of any degree, diploma or certificate.

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Abbreviations and Acronyms

ANOVA: Analysis of Variance

Ca: Calcium

CRM: Certified Reference Material

ENMS: Ethiopian National Micronutrient Survey

EPHI: Ethiopian Public Health Institute

Fe: Iron

GDP: Gross Domestic Product

GPS: Global Positioning System

GPXs : Glutathione peroxidase

ICPMS: Inductively Coupled Plasma Mass Spectrometry

IDI- Iodothyronine deiodinase

ILRI: International Livestock Research Institute

LMM: Linear Mixed Model

LOD: Limit of Detection

MNDs: Micro Nutrient Deficiencies

MO: Month

NIST: National Institute of Standards and Technology

SD: Standard Deviation

Se: Selenium

SOC: Soil organic content

SSA: Sub-Saharan Africa

T4: Thyroxine

T3: Tri-iodothyronine

TrxR: Thioredoxin reductase

Zn- Zinc

List of Publications

1. Kaleab Hailu, Edward J. M. Joy, Elaine L. Ferguson, Elizabeth H. Bailey, Lolita Wilson, Kenneth Davis, Martin R. Broadley, Dawd Gashu. (2023). Dietary selenium intake among Ethiopian children in areas known for selenium spatial variability. *Frontiers in Nutrition*. In press.

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Abstract

Background:

Micronutrients are vitamins and minerals need by the body in small amount and play catalytic, metabolic and structural role in the human body. However, the deficiency is prevalent in more than a quarter of the world population and those from Sub-Saharan African countries are the most affected. The deficiency has been implicated with increased risk of morbidity and mortality and slow economic growth and human capital development. Iron (Fe), iodine (I), Zinc (Zn) and folate are the most common micronutrient deficiencies. In addition, selenium (Se) for long time was known for its toxicity. However, Se through selenoproteins, plays an important role to health and metabolism of humans including thyroid metabolism, antioxidant defense system, and the immune function. But the deficiency is estimated to affect 0.5 to 1 billion people worldwide. In addition, Se deficiency in livestock has a direct and indirect impact to human nutrition by affecting cattle reproduction and amount and quality of milk and meat. The distribution of Se in nature is highly variable and Se concentration in soil is the main determinant factor. However, food crops differ in their Se absorption and accumulation capacity suggesting that dietary pattern and agro-ecology are significant contributors to the variation.

Objectives: To determine Se concentration in major food items and serum cattle and their feed from areas known for contrasting Se concentration in the Amhara region. In addition, to evaluate the Se and Zn bio-accessibility in dough and injera prepared from agronomically biofortified teff.

Methods: In a cross-sectional design, households with children in the age range 6 – 24 months old were randomly selected from west Amhara (n= 78; west Gojam) and east Amhara (n= 75; South Wollo) that are known for contrasting Se concentration in crop and soil. Dietary intake of the children was estimated by employing a non-consecutive two day 24hr weighed food record method. The sampling was repeated for 100% of children. In addition, breast milk samples (n= 121) and spot urine samples (n = 148), were collected. Blood samples were collected from jugular vein of cattle (n= 224), centrifuged and the serum was separated. Moreover, the corresponding feed samples of the cattle were collected. Se concentration in the food samples

were quantified after acid digestion, whereas in urine and cattle serum samples after dilution using Inductively Coupled Plasma Mass spectroscopy (ICP-MS). Bioaccessibility of Zinc (Zn) in dough and injera samples from Zn- biofortified teff was determined by Infogest invitro digestion method.

Results: Selenium concentration in food, urine and breast milk samples from East Amhara had significantly higher Se concentration than those from West Amhara. Similarly, there was significantly lower Se concentration in cattle serum and feed samples from West Amhara than samples from East Amhara ($p < 0.01$). 31.5 % of children and 92 % of children had deficient Se intake in east and west Amhara respectively, where as 62.5% cattle and 100 % of cattle showed Se deficiency in east and west Amhara region. Urinary Se excretion accounted for 53% and 39% of daily dietary Se intake in east Amhara and west Amhara, respectively. Se and Zn biofortification of teff uplifted grain Se and Zn concentration by ten fold and 20 %, respectively. Bio- accessibility of Zn in biofortified and control teff injera samples was not significant (11.6 vs 10.9; $p = 0.79\%$). The bio-accessibility portion of Se was not detectable.

Conclusion: There was significant Se variation in foods, cattle serum and feed samples in Amhara region affected by geographical location. This suggests the need for implementation of targeted agronomic interventions that enhance Se concentration in the edible portion of plant foods. In addition, the difference in Se concentration between foods warrants the need for nutrition education to communities in the Se deficient areas to consider selection of foods with relatively better Se concentration during food preparation.

General Introduction

1.1 Background

Micronutrients are vitamins and minerals needed by the body in smaller concentration with significant catalytic, metabolic and structural function in the human body (Tulchinsky 2010; Fairweather et al, 2011; West et al, 2012). However, deficiency of these micronutrients, sometimes termed as hidden hunger is a major global public health concern affecting an estimated of more than 2 billion people worldwide (Bailey et al, 2015). Micronutrient deficiencies (MNDs) can occur due to inadequate dietary intake or impaired absorption (Bouis, et al 2017; Katona et al, 2008). Furthermore, lack of nutritional knowledge, inappropriate dietary practices and high incidence of infectious disease are among the factors contributing to micronutrient deficiency (Diddana 2019).

MNDs of major public health importance in the world include iodine, iron, zinc and vitamin A. A review by Bailey et al shows prevalence of insufficient iodine intake in approximately 30% of the world's school-aged children. In addition, iron deficiency affects more than 30% of the world's population and, 17.3% of the global population has inadequate zinc intakes. Furthermore, quarter to half a million children are blind due to Vitamin A deficiency (Bailey et al., 2015). In addition, in Ethiopia, 18% and 35% of the population are affected by iron and Zinc deficiency, respectively according to the Ethiopian National Micronutrient Survey (ENMS) report (EPHI, 2016).

Recently, the trace mineral selenium (Se) has gained the interest of researchers in the field of nutrition and biochemistry due to its important role in many physiological processes. For example, it is an integral part of glutathione peroxidase (GPX) and thioredoxin reductase enzymes that protect the body against oxidative stress to counteracts the oxidative effects of harmful free radicals (Brown et al., 2001; Stuss et al, 2017). In addition, through iodothyronine deiodinase (IDI), Se plays significant role in normal thyroid metabolism by converting the inactive thyroxine (T4) in to the biologically active tri-iodothyronine (T3) (Mehdi et al., 2013; Taylor et al., 2009). Its deficiency is reported to affect 0.5 to 1 billion people worldwide (Haug et al., 2007). The deficiency has been associated to incidence of none communicable diseases such as cancer, cardiovascular diseases and type 2 diabetes (Kumar et al., 2014).

The biological significance of Se is not limited to humans only rather is also important to livestock in their antioxidant defenses system, strengthen immunological potential, and increase fertility rates (Mehdi et al, 2016). For example, animal feeds rich in Se can increase milk production (Krzyżewski et al, 2014; Pavel, 2015), and reduce inflammatory disorders of the mammary gland (Sordillo et al, 2013). In addition, se-rich diets relieve oxidative stress in dairy cows during their transition from late gestation to early lactation (Gong et al, 2018). On the other hand, Se deficiency in cattle has been linked to delayed conception, muscular degeneration in calf animals, myocardial necrosis and heart failure, immune dysfunction, an increased risk of mastitis, abortion and perinatal mortality, and growth retardation in young animals (Enjalbert et al, 2006). Hence, Se animal nutrition could be an important factor for Ethiopia who has the largest livestock population in Africa, with 65 million cattle, 40 million sheep, 51 million goats, 8 million camels and 49 million chickens in 2020 (CSA, 2020), contributing to 20% of the agricultural gross domestic product (GDP) (FAOSTAT 2020) . However, the livestock sector is surrounded by several challenges including poor reproductive performance and low milk production (average 1.35 liters per cow) (Mekuriaw et al, 2021) which Se deficiency could be a potential contributor.

Dietary intake, like other nutrients, is the main source of Se for humans, but it's concentration in the diet depends on the concentration in the soil where the food crops grow or animals are reared. Dietary Se concentration varies with the geographic source of foods and eating habits of the people (Thomson, 2004). Worldwide up to one in seven people are estimated to have low dietary Se intake (Jones et al., 2017). In Europe and Middle Eastern countries, Se intake is reported to be suboptimal (Stoffaneller et al., 2015). In addition, Joy et al., (2014) estimated the risk of Se deficiency in Eastern Africa to be 52%. Similarly, a recent study in Ethiopia based on national representative serum samples reported that Se deficiency is wide spread but with distinct geographical variation (Belay et al, 2020). For example, a study based on serum samples shows that majority of children from western Amhara (North-West Ethiopia) had Se deficiency (up to 92%) while few or none of the children from eastern Amhara had Se deficiency (Gashu et al. 2015a) and the deficiency has been associated with compromised thyroid metabolism even after iodine repletion (Gashu et al. 2018) suggesting the potential negative consequence of the deficiency to the ongoing salt iodization. In addition, Se deficiency among the study children was associated with lower cognitive performance (Gashu et al. 2015b).

The spatial distribution of human Se could be due to the dietary pattern difference among the two populations or difference in bioavailability of Se from the foods (Galani et al 2022) which warrant further investigation. The spatial variation of Se also suggests the need for mapping Se distribution to make appropriate decision to design targeted intervention.

In Ethiopia the effects of applying Zinc (Zn) and Selenium (Se) containing fertilizers on Zn and Se concentration of wheat (*Triticum aestivum* L.) and teff (*Eragrostis tef* (Zucc.) Trotter) were investigated under different agro ecological conditions and different fertilizer application methods. Positive outcomes in increasing grain micronutrient concentration have been reported by the authors (Manzeke-Kangara et al. 2023) suggesting that use of mineral fertilizer could be effective to enhance intake and combat the deficiency. Also, use of mineral blended fertilizers or agronomic biofortification has been recognized in the Ethiopian National Nutrition program (FDRE, 2016). In addition, the Ethiopian soil information system (EthioSIS) has collected and analyzed several thousands of soil samples to identify areas with mineral deficiency for fertilizer application. Furthermore, it can reach the largest segment of the population especially the poor, whose access to fortified and diversified food is limited (Kiran et al, 2022) However, the increase in grain micronutrient concentration doesn't guarantee adequate supply mainly because of reduced bioavailability in to the body (Galani et al 2022). Thus, evidence on the bioavailability of micronutrients from biofortified staple crops needs to be evaluated.

1.2. Statement of the problem

MND is an important cause of public health problems affecting many people worldwide, in particular pregnant women and children under 5 years of age (Bailey et.al. 2015). It is estimated to affect about 2 billion people worldwide. The deficiency is higher particularly among people living Sub-Saharan Africa (SSA) and South East Asian countries where populations rely on monotonous type of diets dominated by plant-based diets with less or no animal source foods. Addressing adequate intakes of iron, vitamin A, and Zn remains a challenge in SSA (Beal et.al. 2017; Kumssa et.al, 2015). In addition, reports based on human, crop and soil surveys also reveal the public health importance of Se nutrition in SSA (Hurst et al. 2013; Ligowe et al. 2020; Belay et al. 2020; Gashu et al. 2021). In SSA, the deficiency has been linked to endemic goiter persistence and elevated concentration of T₄ long time after iodine repletion (Kishosha et al. 2011; Gashu et al. 2016).

The distribution of Se in nature is highly variable that significantly high and low concentration can be found in small geographic areas. For example, attributed to environmental Se levels, Fordyce et al. (2020) reported the presence of Se deficiency and toxicity among population in Enshi district of China living in two areas 20 kms apart. Similarly, a study based on analysis of a nationally representative human serum by Belay et al. (2020) reported significant regional variation in serum Se concentration; none in Afar region to 81.2% in Benishangul-Gumuz region. That study also reported the spatial dependence of human Se concentration where highest concentration was found among populations living in North-East and Eastern Ethiopia and along the Rift Valley, while lowest concentration was among those living in North-West and Western Ethiopia. In addition, significant variation within in Amhara region was reported that none or few of the population from East Amhara had Se deficiency but as much as 92% of the population in west Amhara had Se deficiency (Gashu et al. 2019).

Normally, Se status of populations depends on local production for dietary sources, which is determined by Se concentration in the local agricultural soil hence the variation could be attributed to the inherent soil Se concentration. However, mineral absorption and accumulation capacity of food crops is different. For example, a survey of grain micronutrient concentrations in cereals crops in Ethiopia and Malawi shows that maize had the lowest concentration of Se, Ca, Fe and Zn indicating that people relying on maize-based diets are likely to have the lowest

mineral micronutrient intakes (Gashu et al. 2021). In addition, animal source foods tend to supply higher concentration and more absorbable minerals that are difficult to obtain from plant-based diets (Murphy and Allen, 2003). In addition to soil factors proximity to Lake Malawi that provide an opportunity for fish consumption has been reported as a significant predictor for high dietary Se intake among populations in Malawi (Chilimba et al. 2011). Hence, not only Se in the environment but also knowledge on Se concentration of local foods commonly consumed by the inhabitants is useful to identify the source for the variation.

Agriculture is the main economic stay for Ethiopia contributing to 37.7 % of GDP and 64 % of employment in 2021 (The World bank, 2021). The livestock sector in Ethiopia contributes up to 80% of farmers' incomes and 20% of the agricultural gross domestic product (GDP) (FAOSTAT 2020). Ethiopia ranks first in Africa and 10th in the world in its cattle population (Gebremedhin et al, 2007). However, in addition to the already identified factors such as feed shortage, poor productivity, poor milk production and poor resistance to diseases, Se deficiency may pose and additional challenge to limiting the contribution of the livestock sector to the Ethiopian economy. This is because Se deficiency in cattle has been associated with low milk production (Krzyżewski et al, 2014; Pavel, 2015), reduced fertility rates (Mehdi et al, 2016), and increased risk of inflammation of the mammary gland (Sordillo et al, 2013). On the other hand, in cattle, Se plays an important role to enhancing production and quality of milk, growth performance, reproduction, disease prevention and quality and fatty acid profile of meat (Mehdi and Dufresne, 2016). Cattle Se status also influence Se concentration in the food system and human nutrition.

Mineral fertilizer application to staple crops which is also termed as agronomic biofortification, increases mineral concentration in grains and human dietary supply. Compared to food-based nutrition interventions, a suitable approach to reach resource poor rural populations, providing they have access to chemical fertilizers (Tekelu et al. 2023). An agronomic biofortification trial that aimed to investigate the effect of Zn and Se mineral fertilizer application on grain Zn and Se concentration of wheat (*Triticum aestivum* L.) and teff (*Eragrostis tef* (Zucc.) Trotter) in the Amhara region over two farming seasons shows that Zn concentration was increased to 36.4 mg Kg⁻¹ from a baseline of 26.6 mg kg⁻¹ in wheat and to 31.2 mg kg⁻¹ from a baseline of 28.5 mg kg⁻¹ in teff samples. In addition, grain Se concentration ranged from 0.02-0.59 mg kg⁻¹ in wheat and between 1.01-1.55 mg kg⁻¹ in teff (Manzeke-Kangara et al. 2023). In addition, an increase in

maize Zn concentration was also obtained in Malawi in response to Zn fertilizer application (Botoman et al. 2022). However, an increase in grain mineral concentration due to agronomic biofortification cannot guarantee adequate mineral nutrition in humans as bioavailability of minerals from biofortified crops also influences the effectiveness of biofortification programs.

The present study evaluates Se dietary intake of children aged 6-24 months from two areas in the Amhara region known for contrasting human Se status. It also evaluates Se concentration in cattle and fodder samples from those same areas. Furthermore, it evaluates the bioaccessibility of Zn in dough and injera samples prepared from Se and Zn agronomically biofortified teff. The bioaccessible portion of Se was small and not detectable, hence is not included in this paper.

1.3. Literature Review

1.3.1. Epidemiology of micronutrients

Micronutrients are vitamins and minerals needed in smaller amounts that play catalytic, structural and metabolic role in the body. However, it is estimated that a quarter of the population in the world are affected by the deficiency of one or more of these micronutrients (WHO, 2000). Populations in resource poor setting of Sub-Sahara Africa (SSA) and Southeast Asian countries where the diet is less diversified and dominated by plant-based diets with less intake of animal source foods are most affected by MNDs (Consalez et al, 2022). The basic and underlying causes for the deficiency also include poverty, food insecurity, poor feeding practices, and compromised environmental sanitation with inadequate access to health services, insufficient dietary intakes compounded by impaired absorption and metabolism due to infection, disease, or inflammation (UNICEF, 2013). Failing to meet adequate intake of micronutrients is associated with poor growth and development in children, compromised immunity, poor cognitive performance, and risk of developing chronic diseases (Branca and Ferrari, 2002). It is an underlying cause of morbidity and mortality (Black, 2003) and exacerbates the severity of infections and chronic diseases (Tulchinsky, 2010). The greatest public health concerns include deficiency of iron, iodine, Vitamin A, folate zinc (Bailey et al. 2015). However, the biological importance and public health significance of the nutrient Se is also getting attention of scientists and policy makers.

1.3.2. Chemistry of Se

Se was first discovered by a Swedish Chemist Jons Jacob Berzelius in 1818, and was considered to be toxic following the poisoning of grazing animals, until Schwartz and Foltz proved that the supplementation of small amounts of Se to feed prevented muscular dystrophy and liver cirrhosis in rats in 1957 (Bodnar et al., 2012; Mehdi et al., 2013). Selenium is the 34th element in the periodic table (period = 6, group = 4 and atomic mass = 32.066). It exhibits intermediate properties between metals and non-metals. Naturally, Se occurs in soil in different oxidation states varying between plus six and minus two; selenate (Se^{+6}), selenite (Se^{+4}), elemental Se (Se^0) and selenide (Se^{-2}) (Zhang et al. 2014). The distribution of Se in the soil varies greatly

throughout the world and even within the same country due to geographic (rocks and volcanic activities), climatic variations (precipitation and aridity) as well as with changes in the soil profile (clay/loam/sand) and chemical parameters such as pH, soil organic content (SOC) and redox potential (Eh) (Ekumah e.al, 2021). It is found in a very minute amount (0.05 to 0.09 mg kg⁻¹) in the Earth crust hence, is considered a trace mineral (Fernández-Martínez Charlet, 2009).

1.3.3. Biological role of Se in humans

Se in the form of selenocysteine is a key component of the active site of diverse selenoproteins of crucial biological functions. In humans, 25 selenoproteins have been discovered including glutathione peroxidase (GPXs), thioredoxin reductase (TrxR), iodothyronine deiodinase (IDIs), selenoprotein P, selenoprotein W, and seleno-phosphate synthetase. Through these selenoproteins, it is involved in the protection of the body from damage by free radicals; catalyze activation and deactivation of thyroid hormones, strengthen the immune system, and repairing the DNA (Brown & Arthur, 2001; Ibrahim et al., 2019; Zoidis et al., 2018). In addition, Se has been found to be involved in Ca homeostasis (Bodnár et al., 2016). Furthermore, Se supplementation has been reported to improve reproductive efficiency and ovarian function in humans (Yang et al., 2019, Bodnár et al., 2016). It is also important for the synthesis and function of thyroid hormones (Ibrahim et al., 2019; Liu et al., 2019; Winkel et al., 2015). Se supplementation has shown benefits in terms of slowing the progression of HIV disease progression, morbidity, and mortality (Stone et al., 2010).

On the other hand, Se deficiency in humans has been associated with incidence of chronic diseases including cancer, cardiovascular diseases, type 2 diabetes, lung disorders, skeletal muscle disorders, and fertility (Ermakov & Jovanovi, 2010; Kumar & Priyadarsini, 2014). Muscle pain, fatigue, weakness, and elevated serum creatine kinase levels have been also linked with Se deficiency (Chariot & Bignani, 2003). In addition, the deficiency has been associated with male fertility due to impaired sperm function (Mehdi et al., 2013) and diseases such as Keshan disease, Keshin-Beck disease, and myxedematous cretinism (Ivory & Nicoletti, 2017).

1.3.4. Selenium nutrition in livestock

Se has an important role in cattle and serves as antioxidant defense and has been reported to cause positive effect on milk production (Arshad et al., 2020), increase the percentage of polyunsaturated fatty acid and reduce the incidence of metritis and ovarian cysts in cows during the postpartum period (Mehdi & Dufrasne, 2016). Se deficiency on the other hand is linked to white muscle illness in lambs, calves, and goats, sterility in ewes, and malnutrition in sheep in New Zealand (Gupta and Gupta, 2000). Moreover, the deficiency was also associated with growth retardation and milk production reduction (Enjalbert et al, 2006).

1.3.5. Distribution of Se in nature

Several reports documented that Se is distributed unevenly throughout the world. Variations in soil was observed in different regions in China, with concentrations in soils from Dashan region being the highest (reaching up to 7.65 mg kg⁻¹ on dry weight basis) (Xing et al., 2015). Fordyce et al. (2000) also reported Se deficiency and toxicity in two areas in China 20 kms from each other due to effects by geological factors. There is also soil, crop and human Se spatial variability in Ethiopia (Gashu et al.2021; Gashu et al. 2020; Belay et al. 2020). Low Se concentration in agricultural soil is very common worldwide. Available Se concentrations in soils from Scotland and Sweden were found to be low and concentration was related to organic matter than other geochemical factors (Ekumah et al., 2021). Low soil Se concentration was also reported in many countries including Malawi, Russia, UK, New Zealand (Chilimba et al. 2011; Ermakov and Jovanović, 2010; Thomson, 2004). On the other hand, very high soil Se concentration (toxic range) was found in parts of China and the USA (Tan et al, 2002; Qin et al, 2013; Banuelos et al, 2002).

1.3.6. Sources of Se in the diet

Plant foods are the primary source of Se for humans and animals. Selenium in plants is accumulated in its inorganic form (selenates +4 and +6) while the organic form of the element, selenomethionine and selenocysteine are found dominantly in animal source foods. The organic forms of Se are easily absorbable attributable to their chemical form. Dietary factors such as the presence and amount of protein determine Se bioavailability. Se is found associated with amino acids hence, protein rich foods are often good source of absorbable Se (Kieliszek, 2019). Heavy metals and sulfur are also known to reduce Se bioavailability while vitamin A, C, E, and presence of methionine enhance Se absorption from the diet (Kieliszek and Błażej, 2013). Table 1.1 contains Se concentration of selected food items. In general, cereals, meat and dairy products, fishes, seafood, milk, and nuts are considered as good sources of Se (Fraczek and Pasternak, 2013). Brazil nuts contain the highest Se concentration but the concentration varies according to its origin (Cardoso et al. 2017). Similarly, there is within and between species variation in the Se concentrations of food crops. A survey of mineral micronutrient concentration in staple crops in Ethiopia and Malawi shows that Maize compared to a variety of cereal grains (rice, teff, wheat, finger millet, sorghum, barley) shows the least Se concentration while sorghum, teff and wheat had good amount of the mineral (Gashu et al. 2021). A study reporting Se concentrations of Australian (a country known for the presence of moderate Se deficiency) foods reported that fish and animal source foods in general had the highest Se content than plant-based diets. The highest value was found among fish (12.0–63.2 µg/100 g), meats (4.75–37.9 µg/100 g) and eggs (9.00–41.4 µg/100 g), followed by cereals (1.00–20.3 µg/100 g). The least Se content was reported for fruits and vegetables (trace—3.27 µg/100 g) (McNaughton et al. 2002). However, plants such as *Astragalus bisulcatus* and *Brassicaceae* families often known as Se hyperaccumulators (Kieliszek, 2019). Moderate concentration was found in milk and milk products ranging between 2.00 and 7.89 µg per 100 gram of the food.

Table 1.1: Selenium content of food items

Food	Selenium content ($\mu\text{g/g}$)
Brazil nuts	0.2–512
Yeast	500–4000
Bread	0.09–0.20
Fish	0.06–0.63
Eggs	0.09–0.25
Chicken	0.15
Beef	0.01–0.73
Pork	0.27–0.35
Broccoli	0.012
Milk	0.01–0.06
Chocolate	0.04
Liver	0.3–0.4
Beef kidney	1.45

Source: Kieliszek, (2019).

The mammary gland has a mechanism to maintain milk mineral concentration in favor of secretion in the breast milk even at low maternal nutrient intakes. For example, in response to the increased need during pregnancy and lactation the body undergoes homeostatic adjustments in zinc utilization by increasing absorption and decreasing losses (Donangelo & King, 2012). A study reporting the associations between breast-milk concentrations of iron, zinc, and copper and maternal mineral status also shows that iron, zinc, and copper concentrations at 9 months postpartum are not associated with maternal mineral status, which suggests active transport mechanisms in the mammary gland for all 3 minerals (Domellöf et al. 2004). For elements such as Se however, there is little or hemostatic adjustment mechanism (Lönnerdal, 2000).

1.3.7. Methods to assess human Se status

Assessment of Se status of humans at individual or populations level provides an understanding of the public health significance (deficiency or toxicity) of the nutrient hence, the use of reliable methods is useful. Se status of population can be assessed by combination of a variety of methods and requirement is normally set based on intakes that maximize activities selenoproteins or prevention of certain diseases (Table 1.2)

1.3.7.1. Biochemical methods

Biochemical tests such as blood, hair and nail Se are generally useful indicators of Se status. Plasma and serum Se are short indicators of Se nutrition while RBC and toe-nail Se shows long-term status. Similarly, hair Se is a long-term indicator but the result is often confounded by the use of Se containing shampoo. In addition, urine Se concentration is regarded as a good indicator of acute Se nutrition as it is positively correlated with plasma and serum Se concentration. About 50-60% of the Se from the diet is assumed to be excreted through urine and therefore, the total intake is estimated to be twice the urinary excretion. In addition, analysis of functional biomarkers such as GPx (GPx3) and red cell GPx (GPx1) activities has been reported as an effective approach to measure Se status in areas of deficiency (Thomson, 2004).

Table 1.2 Daily selenium requirement and cut off concentration for plasma Se

Functional outcome	Requirement ($\mu\text{g}/\text{day}$)	Plasma Se ($\mu\text{mol}/\text{l}$)
Minimum requirement for the prevention of keshan disease	20	>0.25
Physiological requirement (EAR) for maximal GPx and Seleno-protein P	45-50	1.00-1.20
Requirement for optimal function of iodothyronine deiodinase	30	>0.82
Protection against some cancer	120	>1.50

Source: Thomson, (2004)

1.3.7.2. Dietary intake method

As stated previously in this document, Se content of food varies by region which limits the use of food frequency questionnaire methods and food composition tables for dietary Se intake assessment. Therefore, whenever possible, knowledge on the Se content of foods and amount of those foods consumed at local is the best approach to produce a reliable intake data (Combs, 2015). However, the high cost and time investment of this method is a challenge to apply for large scale dietary intake assessment (Gibson, 2005). The criterion for Se dietary reference intake calculations (estimated average requirements, recommended daily allowance, and nutrient reference values) was based on requirement for the maximization of plasma GPx activity (Nève, 2000). However, the idea that maximization of plasma GPX activity promotes overall human health warrants further investigation. The first physiological requirement estimation was based on data from a Chinese intervention study that reported 41µg/day was sufficient for maximum activity of plasma GPx. Taking into account differences in physical factors such as weight and height the recommended amount was later revised for US adults to be 53 µg/day. The value was later modified to be 38µg/day based on additional data from New Zealand intervention (Nève, 2000; Thomson, 2004). Even though, committees to prepare dietary reference standards use plasma GPx activity as an outcome variable, dietary standards throughout the world differ significantly (Table 3)

Table 1.3: Daily recommended intakes of selenium among adult humans from different countries.

Country/Region	AI (µg/day)	UL (µg/day)	Reference
Germany, Austria, Switzerland	70	255	Kipp et al. 2015
Nordic countries	Females 75	255	Nordic Nutritional Recommendations, 2023
	Males 90		
European Union	70	255	EFSA, 2023
Japan	*Female 25; *Male 30	350	Satoshi, 2020
Korea	60**	400	Choi and Lee, 2022

RDA: Recommended daily allowance; RNI: Recommended nutrient intake

1.3.8. Interventions to address Se deficiency

Due to the presence of low concentration of Se in the soil, human deficiency of Se is widespread globally that requires implementation of effective strategies to address the problem. One of the countries that successfully implemented reduction of Se deficiency by mineral fertilizer application is Finland. In response to the decision by the Finnish government to use Se containing fertilizers, mean Se concentration has increased by 15-fold in cereals, 6-fold in beef, 2-fold in pork, 3-fold in milk. In addition, the average human Se intake has increased from 0.04 to 0.08 mg Se/day and plasma concentration among its populations has increased from 0.89 $\mu\text{mol/L}$ to 1.40 $\mu\text{mol/L}$. The national wide supplementation program had also a positive effect on the health of animals (Alfthan et al. 2015). Reports of several agronomic biofortification trials also show the effectiveness of Se fertilizer application to increasing grain Se concentration. For example, Lyons et al. (2004) reported that soil-applied Se increased grain Se concentration by 20- to 133-fold, and foliar by 6- to 20-fold. A field trial of Se, Zn and I minerals application applied independently or together to winter wheat, maize, soybean, potato, canola, and cabbage shows that Se applied to the soil whether independently or combined with the other minerals, was effective in increasing Se (Mao et al. 2004). Se fertilizer application was also effective in increasing grain Se concentration in teff and wheat (Manzeke-Kangara et al. 2023).

Selenium supplementation has been also proven to be effective to increase blood Se concentration and bring positive changes to human health. Organic selenium than inorganic Se is more effective. Se supplementation in the form of selenite and Se-yeast among Se deficient Chinese children increased plasma Se. Compared to control groups, Se-yeast increased the Se level in red cells by six-fold and a three-fold increment was achieved among selenite supplemented children (Alfthan et al. 2000). A meta-analysis that assessed antioxidation effect of Se supplementation significantly increased GPx activity and total antioxidant activity but decreased level of plasma malonaldehyde (Hasani et al. 2019). Se supplementation was also implicated in the reduction of severity of corona virus infection (Kieliszek and Lipinski, 2020). Selenium intake in livestock can be increased either by direct supplementation or through addition of selenium to their feed (Ros et al, 2016).

1.3.9. Selenium bioavailability

Absorbable form of an element in the digestive tract can be determined by in vivo bio-accessibility techniques which mimic the human digestive system, where the results are presented as the percentage of the element that is soluble under a set of experimental parameters, including pH, enzyme addition, temperature, and contact time (Cabañero et.al. 2007). Both organic (Selenomethionine and selenocystiene) and inorganic forms (Selenide and selenate) of selenium are usually absorbed to a high extent (70% -95%), but organic forms are more bioavailable than the inorganic forms (Hadrup et.al, 2021; Lyons et al., 2007; Navarro-Alarcon & Cabrera-Vique, 2008; Huang et al, 2023). Moreover organic forms are more retained in the body than inorganic forms, which are rapidly excreted in urine (Thomson, 1998; Fairweather et. al. 2011).

The presence of dietary food components such as total protein and the presence of heavy metals have been found to have antagonistic or synergistic effects in selenium bioavailability. Protein intake from foods of animal origin has been found to enhance bioavailability because of the forms are mostly Se-Cys and Se-Met (Navarro-Alarcon & Cabrera-Vique, 2008; Thomson, 1998). Selenium absorption has reportedly been improved by vitamins A and E. Selenium absorption has been reported to decrease in the presence of heavy metals such as mercury due to formation of insoluble inorganic complexes due to binding to selenium (Cabañero et al., 2007).

1.4. Research Questions

This research was designed to address the following research questions.

- Is there a significance difference between dietary Se intake in East Amhara and West Amhara zones of Amhara region, where there is contrasting human Se status?
- Are cattle from Se deficient area in the Amhara also Se deficient? Does cattle feed predict cattle Se status?
- Is Zn in agronomically biofortified teff, dough and injera samples bio-accessible?

1.5. Objectives

1.5.1. General Objective

To determine dietary Se intake among young children, cattle serum Se and feed from areas known for contrasting human Se concentration in Amhara region and evaluate bio-accessibility of Zn in agronomically biofortified teff, dough and injera.

1.5.2. Specific Objective

- To evaluate dietary Se intakes of young children from two zones in the Amhara region, Ethiopia known for contrasting human Se status
- To evaluate Se concentration in cattle serum and corresponding feed samples from two zones in the Amhara region, Ethiopia known for contrasting human Se status
- To evaluate bio-accessibility of Zn in teff flour, dough and injera samples prepared from agronomically biofortified teff.

1.6. Significance of the Study

Selenium studies on soil, crops and human samples that have been carried out extensively in Ethiopia show Se is spatially distributed and Se deficiency exists in Ethiopia. However since dietary habits of people vary and the concentration of Se in different crops grown on same agricultural landscape vary, knowledge on the actual Se intake becomes important for designing appropriate interventions. This study would also generate data and evidence on MN intakes/status that would serve as an input for large studies. The study would provide baseline evidence for effectiveness of potential interventions such as agronomic fortification of staple crops.

1.7. Strength and limitation of the study

The present study shows presence of Se variability using analysis of different types of samples such as food items, breast milk, urine, cattle serum and cattle feed from randomly selected villages and households that give spatial balance over study areas. In addition, sufficient number of samples (643 food samples) were collected and analyzed to observe a clear difference in Se status. The dietary intake study is based on weighed records of food samples prepared in the households on two non-consecutive days where all days of the week were equally represented. This method is considered the most precise method to quantify food intake (Gibson, 2005). Biochemical markers are useful in the validation of dietary assessment methods (Gibson, 2005) therefore inclusion of urinary Se in the present study increases the reliability of the Se dietary intake estimate. Spot urine concentration is confounded by factors such as fluid intake, diet, and exercise (Cheuvront, et al. 2015.) hence, in the present study specific gravity adjustment has been used to reduce the influence of these factors to urinary Se estimate.

Availability and accessibility of foods is strongly influenced by seasons (Shahar et al. 2001). For example, diversified diet availability to household members during autumn (post-harvest season) in Ethiopia is greater than other seasons (Hirvonen et al. 2016). However, the present dietary assessment was limited to autumn and did not take into account seasonal variations or adjust for differences and therefore should be interpreted with caution. In addition, the livestock study is based on analysis of serum samples that reflect only recent Se exposure. Besides cattle serum samples were collected from 40 kebeles per zone only, which forces the results to be interpreted and utilized with caution. Furthermore, feed samples in the present analysis were both small in number and included only dominant feed types. Also, the bio-accessibility study is based on a single rate of Zn fertilizer application. Therefore, the rate of Zn application may not be sufficient to cause a noticeable difference in the absorbable fraction of Zn. In addition, neither phytate in the grain samples nor phosphorous in the study area soil samples were analyzed to explain for the absence of relationships between Zn biofortification and bio-accessibility.

1.8. Structure of the dissertation

This doctoral dissertation is organized into chapters with separate publications. The first publication focuses on determining dietary Selenium intake in children (6-24 years) from areas with contrasting serum selenium status (publication 1). The second publication is about determining selenium concentration cattle serum and feeds from east and west Amhara regions (publication 2). The third publication is about determining the bio-accessibility of Zinc in injera prepared from zinc bio-fortified teff (publication 3).

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2 . Dietary selenium intake among Ethiopian children in areas known for selenium spatial variability

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Abstract

Introduction: There is spatial variability of selenium (Se) in soil and crops in Ethiopia. We assessed the Se content of food items, breast milk and urine, among infants in Ethiopia from two areas with contrasting Se concentrations in soils.

Methods: Dietary Se intakes among children (6-24 months) were evaluated, using a weighed food record, on two non-consecutive days. Also, spot urine samples from children and breast milk samples from their mothers were collected to determine Se concentration. Selenium concentrations in the samples were analyzed using Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

Results: Injera (prepared from teff and mixtures of other cereals) with a legume-based stew were the most frequently consumed foods by the children in both areas followed by pasta. Overall, the Se concentration (mean \pm SD) of food items, breast milk (12.2 ± 3.9 $\mu\text{g/L}$ vs 3.39 ± 1.5 $\mu\text{g/L}$) and urine samples (22.5 ± 11.5 $\mu\text{g/L}$ vs 3.0 ± 1.9 $\mu\text{g/L}$) from East Amhara were significantly higher than the corresponding samples from West Amhara ($p < 0.001$). Total Se intakes by the study children from East Amhara and West Amhara were 30.2 [IQ_{25%}, 14.2 ; IQ_{75%}, 54.1] and 7.4 [IQ_{25%}, 4.2 ; IQ_{75%}, 10.6] $\mu\text{g day}^{-1}$, respectively; 31.5% of children from East Amhara and 92% of children from West Amhara were at risk of inadequate Se intakes. Urinary Se excretion accounted for 53% and 39% of daily dietary Se intake in East Amhara and West Amhara, respectively. Dietary Se intake was positively correlated with urinary Se excretion in East Amhara ($r = 0.56$; $p < 0.001$) but not among samples from West Amhara ($r = 0.16$; $p \geq 0.05$) suggesting greater physiological Se conservation in a state of deficiency.

Conclusion: There is spatial variability of Se in foods, breastmilk and urine in Ethiopia suggesting the need for implementation of targeted agronomic interventions that enhance Se concentration in the edible portion of plant foods.

Keywords: Selenium, mineral spatial variability, food mineral concentration, breastmilk selenium, dietary mineral intake

2.2. Background

Micronutrient deficiencies (MND), defined as inadequate intake of vitamins and minerals that are needed in small amount by the body, is an important global health issue affecting 2 billion people worldwide (1). The most common MND include iron (Fe), iodine (I), folate, vitamin A, and zinc (Zn). Deficiencies of these micronutrients are linked to weak physical and mental growth, compromised immunity, productivity and survival (2). Selenium is also of interest due to its importance in many physiological processes and biological functions such as antioxidant defense systems, DNA synthesis, fertility and reproduction and immune function (3). Selenium deficiency has been associated with an increased incidence of chronic non-communicable diseases such as cancer, cardiovascular diseases, type 2 diabetes, immune deficiency (4) and implicated to negatively affect normal thyroid metabolism (5,6) and impaired cognitive performance (7). A higher mortality risk from COVID 19 has also been reported among Se deficient individuals (8).

Soil physico-chemical characteristics (e.g. pH, organic matter content) and its Se content control grain Se concentration and in humans and animals that consuming these foods (9,10). Populations dependent on subsistent agriculture are particularly vulnerable to Se deficiency, when local soils are low in Se. Previously, we reported spatial variability of Se in agricultural soils, cereal grains (11,12) and human serum (13), in Ethiopia. There is also strong evidence that the Se status of livestock differs from two areas in the Amhara region that are a short distance apart (East Amhara and West Amhara, Ethiopia) (14). A similar pattern was observed among children in Amhara with a high prevalence of biochemical Se deficiency in West Amhara and little or no deficiency in East Amhara (7). This contrast could arise due to differences in food choices and feeding practices as affected by agro-ecology (15). In the present study, we evaluated the feeding practices and dietary Se intakes of young children (aged 6-23 months) from the Amhara region known for contrasting soil, crops, livestock and human serum Se concentrations.

2.3. Materials and Methods

2.3.1. Study area and study population

The study was conducted in South Wollo (East Amhara) and West Gojjam (West Amhara) zones of the Amhara region where contrasting Se concentrations in soils, food crops and human serum samples have been observed (12,13). Three woredas (Dessie Zuriya, Kalu and Tehuledere) from South Wollo zone and two woredas (Bure Zuriya and Jabi Tenna) from West Gojjam zones were selected based on proximity to facilities and road access for proper sample collection, handling and storage. In the selected districts, eight villages from South Wollo and seven villages from West Gojjam were randomly selected. Lists of households were obtained from health posts. With the help of health extension workers and available records, households with at least one child between the age of 6 mo and 23 mo who had resided in the area for at least 6 mo before study's recruitment period were listed and 75 households were randomly selected from each zone. In cases where more than one eligible child were available in a household, a lottery method was used to pick a child. An additional three households (not originally selected) from selected villages in West Amhara were included because a household member made a request to participate in the study.

2.3.2. Study design

A cross-sectional survey was conducted from March to May, 2020. In this survey, dietary, anthropometric, biochemical and questionnaire data were collected from the children or their parents, and food samples were collected. Data collectors with a first degree in health, agriculture or social sciences who were familiar with the local area, were recruited and trained in the standard procedures for administering the questionnaire and dietary intake assessment. They were initially trained, in the classroom, including standardized methods for questionnaire and weighed food record data collection, and the recording procedure, followed by pilot testing in the field. Nurses from local health facilities took the anthropometric measurements after refresher training and they were required to pass an evaluation of their accuracy and precision in the anthropometric measurements.

2.3.3. Socio-demographic and anthropometric characteristics

Information on socio-demographic characteristics, occupation, and education level of mothers/guardians were collected using a structured and pre-tested questionnaire. Measurements of both child weight and length were taken in triplicate. The weight of each child was measured with light clothes on, in the arms of an adult participant to the precision of 0.1 kg on an electronic battery-powered digital balance (UNICEF SECA 874 U, UNICEF Supply Division, Copenhagen, Denmark). The weight of adults was measured using the same balance and subtracted from total weight (adult + child). The balance was calibrated at least twice a day. Child recumbent length was measured to the nearest 0.1 cm using a UNICEF measuring board (UNICEF Supply Division, Copenhagen, Denmark). The Z-scores for length-for-age (LAZ), weight-for-age (WAZ), and weight-for-length (WLZ) were calculated using WHO multicenter growth reference data (16).

2.3.4. Dietary intake assessment and food sample collection

A weighed food record was collected on two non-consecutive days, to estimate the food and beverage consumed by each participating child from the time the child woke up in the morning until they went to bed for the night (17). Each data collector was assigned to visit two nearby households in a day. All days of the week were proportionately represented to account for any day-of-the-week effect on food intake (17). All foods and beverages consumed by a child were weighed using a digital balance (SF-400A). Accuracy of the balance was checked using a known weight (500 g) prior to weighing the child's food intakes. Serving plates/cups were dried with a dry cloth and their weight was recorded prior to serving food to the child (W1). Mothers/guardians were asked to place foods on the weighed plates/cups in amounts exceeding the usual serving size and the serving plates/cups were weighed with foods (W2). Any leftover food was weighed (W3) and collected in closed polyethylene zipper bags and wrapped with aluminum foil prior to storage in a refrigerator. The weight of food eaten by the study child was calculated by difference as $W = (W3 - W1) - (W2 - W1)$. The weight of any spillage was estimated using spoons and was deducted from W2. Data collectors stayed outside when child was fed to minimize distractions.

2.3.5. Breast milk collection

Breast milk was collected when mothers felt comfortable using a manual breast milk pump. Before milk collection, the nipples and areolas of the breasts were cleaned with deionized water (18). Samples were transported and temporarily stored at -20 °C in the nearest health facility, (approximately 1-3hrs after collection) before being transported to Addis Ababa University, Center for Food Science and Nutrition Laboratory in cold storage.

2.3.6. Urine Collection

Spot urine samples of children were collected on the first day using adhesive pediatric urine bags after cleaning the genital regions. The urine samples were transferred to urine tubes and tightly sealed, stored in ice boxes, and transferred to a freezer in nearby health facilities for storage at -20°C. The samples were then transported to Addis Ababa University in cold storage and stored at -80°C. Aliquots of frozen urine samples were shipped on dry ice to the University of Nottingham, UK under material transfer agreement (MTA) for elemental analysis.

2.3.7. Sample preparation

The food samples (with the exception of cow milk and breast milk) were freeze dried using a Lyophilizer (Mini Lyodel, India) at the Center for Food Science and Nutrition Laboratory, Addis Ababa University. Dried samples were ground in an acid cleaned mortar and pestle and 20 g of each sample was packed in to airtight bags. Aliquots of 15-20 mL of cow's milk and breast milk samples were transferred to labeled containers and frozen. Samples were transported on dry ice to the University of Nottingham, under MTA for multi-element analysis.

Dried and ground food samples (ca. 0.4 g) and breast milk and cow milk samples (1 mL) were digested in concentrated nitric acid (68% HNO₃ Primar Plus™ for Trace Metal Analysis, Fisher Scientific) using a hotplate digestion system (Anton Parr-PFA coated graphite hot block). Milli-Q® (MQ) water (18.2 MΩ cm) was used for final dilution of the digests (1 in 10) prior to analysis. Operational blanks (n=14) were digested alongside samples in each batch. Samples of certified reference material, National Institute of Standards and Technology (NIST) wheat flour SRM 1567b and ERM-BD150 dried milk powder were included in digestion batches of food and milk samples.

Urine samples were diluted in 2% Trace Element Grade HNO₃ prior to analysis. Seronorm Trace Elements Urine L-1 and L-2 (SERO, Billingstad, Norway) were prepared and analyzed alongside the urine samples.

2.3.8. Se analysis in food, urine and breast milk samples

Multielement analysis was conducted using ICP-MS (Thermo-Fisher Scientific iCAP-Q, Thermo Fisher Scientific, Bremen, Germany). Samples were introduced (flow rate 1.2 mL min⁻¹) from an autosampler (Cetac ASX-520) incorporating an ASXpress™ rapid uptake module through a perfluoroalkoxy (PFA) Microflow PFA-ST nebuliser (Thermo Fisher Scientific, Bremen, Germany). Sample processing was undertaken using Qtegra™ software (Thermo-Fisher Scientific) utilizing external cross-calibration between pulse-counting and analogue detector modes when required. Selenium (m/z 78) was measured in hydrogen (H₂) reaction mode to reduce polyatomic interferences. Calibration standards (Claritas-PPT grade CLMS-2, SPEX Certiprep Inc., Metuchen, NJ, USA) for Se were prepared in the range 0 – 100 µg L⁻¹ (0, 20, 40, 100 µg L⁻¹). Internal standards, used to correct for instrumental drift, were introduced to the sample stream on a separate line (equal flow rate) via the ASXpress unit. Internal standards typically include combinations of Sc (10 µg L⁻¹), Ge (10 µg L⁻¹), Rh (5 µg L⁻¹), and Ir (5 µg L⁻¹). The matrices used for internal standards, calibration standards and sample diluents were 2% v/v trace analysis grade HNO₃ (Fisher Scientific, UK) with 4% methanol to enhance ionization of Se.

The limit of detection (LOD, 3 x SD blank digest) for Se in food samples was 0.0039 mg/kg (n = 14), in milk samples 0.0068 mg/kg (n = 10), and in urine was 0.013 µg/L (n = 10). The limits of quantification (LOQ, 10 x SD blank digest) were 0.013 mg/kg, 0.023 mg/kg and 0.043 µg/L for food, milk and urine samples, respectively. Recovery of Se was 87% (n = 4) for NIST wheat flour SRM 1567b and 91% (n = 3) for ERM-BD150 Milk Standard. Seronorm Trace Elements Urine L-1 (n = 2) and L-2 (n = 2) gave average values of 19.0 µg/L (acceptable range 8.3-19.5 µg/L) and 78.7 µg/L (acceptable range 41.9-98.3 µg/L).

2.3.9. Specific gravity analysis for urine samples

Specific gravity was measured using a temperature corrected refractometer (PAL-10S, Atago, Japan) on 300 µL samples to enable correction of Se concentrations for hydration status as described in Phiri et al. (19).

2.4. Statistical analysis

The WHO Anthro software was used for analysis of anthropometric data. Height-for-age, weight-for-age, and weight-for-height were used to classify the study children into categories of nutritional status using the WHO Multicenter Growth Reference (16). Children below -2 height-for-age z-score (HAZ), -2 weight-for-age z-score (WAZ), and -2 weight-for-height z-score (WHZ) were classified as stunted, underweight, and wasted, respectively. The Se intake from breast milk was calculated using the assumption of average daily breast milk intake (mL/day) of 641 for infants between 6–8 mo, 598 for infants between 9–11 mo and 533 for infants between 12–23 mo (20). The amount of food, and the Se content of the foods and breast milk consumed by the study participants in the 24 hrs preceding data collection was used to calculate daily Se intake. Age aggregated reference values (15 µg/day, 4 months to under 4 years old children) for adequate Se intake by Kipp et al. (21) were used to determine adequacy. Statistical analysis was performed using SPSS for Windows (v18). Normality of data was checked using the Kolmogorov-Smirnov test before conducting the correlation and comparison analysis. Pearson correlation and student t-test were conducted on log-transformed data to conform normality to study bivariate correlations and comparison of variables, respectively.

2.5. Ethical Review

This study was conducted according to the guidelines laid down in the Helsinki Declaration for all procedures involving human subjects. The study was approved by the National Research Ethics Review Committee at the Ministry of Science and Technology, Ethiopia (Reference 310/177/2018) and Addis Ababa University Institutional Review Board (Reference CNSDO/450/10/2018). Written informed consent was obtained from all mothers or guardians.

2.6. Results

2.6.1. Socio-demographic and anthropometric characteristics

The socio-demographic characteristics of the study households are presented in Table 2.1. The mean age of mothers/caretakers was 27.6 years in East Amhara and 29.4 in West Amhara. The majority of the mothers/caretakers had primary education. The percentages of mothers who were breast feeding at the time of data collection were 76% in East Amhara and 95% in West Amhara. Some, 81.3% of mothers in East Amhara and 62% in West Amhara, reported starting complimentary feeding of their child at 6 mo. The study children had a male to female ratio of 1.04: 0.95. children from East Amhara had comparable mean age with those from West Amhara (15.3 mo vs 14.5 mo).

Table 2.1: Socio-demographic characteristics of study households and infants

Variable	East Amhara, n (%)	West Amhara, n (%)
Mothers' educational status		
Can read and write	27 (37.1)	47 (60.3)
≤8 th grade	29 (39.7)	22 (28.2)
9 th -12 th grade	15 (20.5)	6 (7.7)
≥12 grade	2 (2.7)	3 (3.8)
Marital Status		
Married	67 (90.5)	71 (91)
Unmarried	2 (2.7)	1 (1.3)
Divorced	5 (6.8)	5 (6.4)
Widowed	0 (0.0)	1 (1.3)
Occupation		
Farmer	22 (30.1)	48 (61.5)
Daily laborer	17(23.3)	14 (17.9)
House wife	24 (32.9)	8 (10.3)
Other	10 (13.7)	8 (10.3)
Source of drinking water for the household		
Tap water	67(90.5)	66(86.8)
Protected borehole	7 (9.5)	10 (13.2)
Complementary food preparation training		
Yes	0 (0.0)	74 (97.4)
Complementary food feeding frequency		
≤5 times	54 (73.0)	66 (85.7)
> 5 times	20 (27)	11 (14.3)

Stunting was prevalent both in East Amhara and West Amhara. In addition, both underweight and wasting are prevalent among children from West Amhara (Table 2.2).

Table 2.2: Anthropometric characteristics of study subjects

Measured Characteristics	East Amhara					West Amhara				
	Minimum	Maximum	Mean (SD)	<-2SD, (%)	n	Minimum	Maximum	Mean (SD)	<-2SD, (%)	n
WAZ	-2.8	4.2	-0.3 (1.4)	8 (11.1)		-4.2	2.2	-1.3 (1.17)	15 (19.5)	
HAZ	-5.1	2.8	-1.6 (1.8)	28 (38.9)		-4.3	1.9	-1.1 (1.26)	15 (19.5)	
WHZ	-3.0	5.2	0.6 (1.5)	1 (1.4%)		-5.2	1.9	-1.1 (1.23)	19 (24.7)	

2.6.2. Dietary and breast milk Se concentration

Injera (prepared from teff and mixture of other cereals) with legume stew was the most frequently consumed food item followed by pasta by the infants/young children from both areas (Table 2.3). Too early (4.1%) and late (21.8%) introduction of complementary feeding was common in both areas. About two-third of children from East Amhara and majority from West Amhara were breastfeeding at the time of data collection.

Table 2.3: Most frequent types of complementary food consumed by infants in Northern Ethiopia, 2020

Food items	East Amhara, n (%)		West Amhara, n (%)	
	Day 1	Day 2	Day 1	Day 2
Injera with legume-based stew	49(67.1)	51(69.9)	54(69.2)	59(75.6)
Pasta‡	41(56.2)	37(50.7)	27(34.6)	14(17.9)
Bread	17(23.3)	19(26.0)	21(26.9)	8(10.3)
Cow's milk	16(21.9)	16(21.9)	5(6.4)	4(5.1)
Cereal based porridge	13(17.8)	11(15.0)	40(51.3)	27(34.6)

‡pasta, macaroni, instant noodles

Breast milk Se concentration was 12.2 ± 3.9 $\mu\text{g/L}$ in East Amhara and 3.4 ± 1.5 $\mu\text{g/L}$ in West Amhara with a significant difference at $p < 0.05$. The mean Se concentration of the predominant food types are listed in Table 2.4. There was significant difference in Se concentration in injera, gruel/porridge, bread and breast milk samples between the two areas while no difference was seen in pasta/macaroni, a food item that is centrally processed and distributed in the market.

Table 2.4: Comparison of mean Se concentration ($\mu\text{g}/100\text{g}$) of predominantly consumed food items by study area in Ethiopia, 2020

Food items	Study areas, (n)	Mean	Min	Max	SD	p value
Injera with legume-based stew	East Amhara, (n=110)	17.78 ^a	1.6	46.9	9.5	<0.001
	West Amhara, (n=86)	3.34 ^b	0.6	20.1	2.5	
Pasta/maccoroni	East Amhara, (n=77)	9.4 ^a	1.0	33.58	6.8	0.007
	West Amhara, (n=28)	8.75 ^a	2.0	17.3	3.56	
	East Amhara, (n=19)	‡8.2 [2.1, 16.7] ^a	0.27	89.8		<0.001
Gruel/Porridge	West Amhara, (n=70)	1.6 [0.5, 3.5] ^b	ND	116.3		
	East Amhara, (n=43)	7.4 ^a	1.7	20.81	4.05	0.144
Bread	West Amhara, (n=24)	1.4 ^b	ND	3.2	0.84	
	East Amhara, (n=17)	0.34 ^a	0.16	0.70	0.15	<0.001
Cow's milk ($\mu\text{g}/\text{L}$)	West Amhara, (n=5)	0.21 ^b	0.10	0.35	0.09	
	East Amhara, (n=51)	12.2 ^a	ND	20.2	3.9	<0.001
Breast milk ($\mu\text{g}/\text{L}$)	West Amhara, (n=73)	3.4 ^b	1.3	7.4	1.56	

Means with different superscripts are significantly different at 0.05

‡Such values are median [Q_{25%}, Q_{75%}]

ND: Not detected

2.6.3. Se intake

Mean Se intake of children from East Amhara was significantly higher than those from West Amhara on both days when samples were collected (Table 2.4). Total Se intake among children from East Amhara and West Amhara was 30.2 [IQ_{25%},14.2; IQ_{75%},54.1] and 7.4 [IQ_{25%},4.2; IQ_{75%}, 10.6] $\mu\text{g day}^{-1}$, respectively (Table 2.5); with 31.5% of the study subjects from East Amhara and 92% from West Amhara had inadequate Se intake. Daily Se intake variation was not significant between the two measurement days. There was no significant within day variation in Se intake or meal frequency (3.5 vs 3.6 meals per day). The mean Se intake from breast milk in East Amhara (6.8 $\mu\text{g/day}$) was significantly higher than those in West Amhara (1.9 $\mu\text{g/day}$), $p < 0.001$.

Table 2.5: Comparison of Se intake ($\mu\text{g/day}$) among infants between two measurement days from East Amhara and West Amhara

	East Amhara (n=74)	West Amhara (n=75)
Day 1	25.4 [11.9, 46.1] ^a	7.1 [3.6,10.3] ^b
Day 2	29.8 [12.5, 52.2] ^a	6.5 [3.2,11.7] ^b

Numbers with different superscripts in the same row are significantly different at 0.01

2.6.4. Urine Se concentration

Urinary Se excretion comprised 53% and 39% of daily Se intake, respectively. Urine Se concentration was significantly higher among study children from East Amhara compared to West Amhara ($22.5 \pm 11.5 \mu\text{g/L}$ vs $3.0 \pm 1.9 \mu\text{g/L}$, $p < 0.001$). Overall, Se intake was positively correlated to urinary Se excretion ($r = 0.56$; $p < 0.001$), however the correlation between dietary Se intake and urinary Se excretion among study subjects from West Amhara was not significant ($r = 0.16$, $p \geq 0.05$).

2.7. Discussion

The present study assessed Se concentration in complementary foods, breast milk and urine of children (aged 6-23 months) from two areas in Ethiopia. Consistent with previous reports of Se spatial variability in soils, crops, humans and livestock in Ethiopia (11-14), most food items, breast milk and urine samples analyzed from East Amhara had significantly higher Se concentrations compared to the samples from West Amhara. These results also agree with previous studies showing none or lower Se deficiency prevalence (none to 41%) among populations in East Amhara but higher (91.1%) in West Amhara, respectively (22).

There is large variation in the Se content of foods as affected by geographical location, environmental and soil factors. A review of published literature on the Se concentration in 148 food items reported the greatest variation that food items in general from the United States had the greatest Se content while foods from the United Kingdom and New Zealand had lower Se concentration (23). That same study also reported presence of Se variability in foods from the same country and regions. Similarly, in the present study, food items from East Amhara had high Se concentration compared to food items from West Amhara. Also, human breast milk, and cow's milk samples showed a similar pattern of Se concentration. In addition, compared to the age reference dietary Se intake by Kipp et al. (21) children from West Amhara had low median Se intake, 7.4 [IQ_{25%}, 4.2; IQ_{75%}, 10.6] $\mu\text{g day}^{-1}$ and 92% of the participants had inadequate Se intake but those from East Amhara had median Se intake of 30.2 [IQ_{25%}, 14.2; IQ_{75%}, 54.1] $\mu\text{g day}^{-1}$ with 31.5% inadequate prevalence. This is consistent with findings of previous studies on cereal grain and soil (11,12), human (20,22) and livestock (14) blood from the two areas. Significant variation in Se content across food groups is known with animal source foods such as fish, meat and eggs having greater Se concentration than cereals, with fruits and vegetables having the lowest Se concentrations (23,24). The food items in the present study were dominated by cereal based foods and consumption of animal sources was very limited.

The release of essential minerals such as Cu, Fe and Zn into breast milk from the body is typically independent on maternal mineral status and is regulated by the mammary gland (25). However, in the present study, maternal Se status has been positively correlated with breast milk Se concentration indicative of an absence of homeostatic regulation (26). In the present study, lower breastmilk Se was found among mothers from West Amhara, an area known for Se

deficiency (11,12). WHO recommends exclusive breast feeding of infants until the age of six months and timely introduction of complementary feeding afterwards (27). However, in the present study, 26% of study subjects had early (before 6 mo) or late (several days or months after 6 mo) introduction of complementary feeding. Early or late introduction of complementary feeding is associated to lower breast milk production by the mother, reduced absorption of essential nutrients by children, nutritional inadequacy and increased in infection rates (28).

Results of the present study have shown that children from West Amhara obtain very low concentrations of Se both from breastmilk and complementary foods suggesting a need to design and implement interventions to address the deficiency. Biofortification of staple cereals has been reported to be cost effective in Ethiopia (29). Animal sources foods are known to contain good amount of Se but are only infrequently consumed by the children in the present study hence nutrition education for the inclusion of animal source foods in complementary food preparation is important.

Urine is a major excretory route for Se hence, urinary Se concentration is considered as viable biomarker for assessing Se status at population level (19). There is direct correlation between dietary Se intake and urinary excretion. A study on the relationship between dietary intake and urinary Se excretion among Japanese adults reported urinary Se excretion of 73% and 77% of dietary intake in men and women, respectively as assessed by 24 hr recall (30). In the present study, urinary Se excretion was positively correlated with 24 hr dietary Se intake ($r=0.56$; $p<0.001$). In the present study, the predictive rate of urinary Se was lower than in other studies often conducted in high Se areas. It suggests that there may be homeostatic regulation to conserve Se in a state of deficiency (31).

This study is based on weighed records of food samples prepared in the households on two days, a method considered the most precise to quantify food intake (32). Biochemical markers are useful in the validation of dietary assessment methods (32) therefore, inclusion of urinary Se in the present study increases the reliability of the Se dietary intake estimate. Spot urine concentration is confounded by factors such as fluid intake, diet, and exercise (33.) hence, in the present study specific gravity adjustment has been used to reduce the influence of these factors to our urinary Se estimate. Availability and accessibility of foods is strongly influenced by seasons (34). For example, diversified diet availability to household members during autumn (post-

harvest season) in Ethiopia is greater than other seasons (35). However, the present dietary assessment was limited to autumn and did not take into account seasonal variations or adjust for differences and therefore should be interpreted with caution.

In general, there was lower Se concentration in food items, breast milk and urine samples from West Amhara than East Amhara. Breast milk Se seems not to be subjected to homeostatic regulation, unlike other micronutrients such as Zn where their release into the breastmilk is favored. There was a positive correlation between dietary Se intake and urinary Se excretion among children from East Amhara but the relationship was not significant among participants from West Amhara suggesting that the body has an ability to conserve Se when intake is inadequate. The children in the present study were from subsistent farming households that depend upon own/ local food production thus implementation of targeted interventions, such as agronomic biofortification of food crops widely consumed by the local population could be important. In addition, because the analyzed foods had different Se concentration, community nutrition education to select food items with better Se content (without compromising the intake of other nutrient) during food preparation is essential.

2.8. References

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3. Selenium concentration in cattle serum and fodder from two areas in Ethiopia with contrasting human selenium concentration

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Abstract

Introduction: Selenium (Se) is an essential mineral for livestock health and productivity. In cattle, Se deficiency is associated with delayed conception, growth retardation, and increased morbidity and mortality.

Methods: We conducted a survey of cattle serum (n=224) and feed (n=81) samples from two areas with contrasting human and cereal grain Se concentration in Ethiopia. The fodder samples include stover, straw, hay and pasture grass. Se concentration of the samples were measured using inductively coupled plasma-mass spectrometry.

Results: Serum Se concentration ranged from 14.9 to 167.8 $\mu\text{g L}^{-1}$ (median, 41.4 $\mu\text{g L}^{-1}$). Cattle from East Amhara had significantly greater serum Se concentration compared to cattle from West Amhara (median: 68.4 $\mu\text{g L}^{-1}$ vs 25.7 $\mu\text{g L}^{-1}$; $p < 0.001$). Overall, 79.8% of cattle had Se deficiency ($< 81 \mu\text{g L}^{-1}$). All of the cattle from West Amhara were Se deficient compared with 62.5% of those from East Amhara. State of lactation of cows or age of cattle was not associated with serum Se concentration. The Se concentrations of feed samples ranged from 0.05 to 269.3 $\mu\text{g kg}^{-1}$. Feed samples from East Amhara had greater Se concentration than samples from West Amhara. Cow serum and cattle feed Se concentrations showed strong spatially correlated variation, with a strong trend from East to West Amhara.

Conclusion: This study shows that cattle Se deficiency is likely to be highly prevalent in Ethiopia, which will negatively affect the health and productivity of livestock. The deficiency appears to be geographical dependent. More extensive surveys to map Se concentration in soil-feed-livestock-human cycle are required in Ethiopia and elsewhere.

Keywords: Amhara region, Cattle feed; cattle selenium deficiency; Ethiopia

3.2. Introduction

Selenium (Se) is an essential mineral for humans and livestock. In livestock, Se is important for fertility and disease prevention [1]. Sufficient Se increases fertility rate, improves antioxidant defense systems and immunological potential [2]. Selenium-enriched diets improve milk production [3], reduce inflammatory diseases of the mammary gland [4-6], and improve growth performance [7]. Animal feeds high in Se help dairy cows to relieve oxidative stress during their transition from late gestation to early lactation due to increased metabolic activity [8]. Selenium deficiency in cattle is associated with several problems, including delayed conception, muscular degenerative disease in calves, myocardial necrosis and heart failure, immune dysfunction, increased risk of mastitis, abortion and perinatal mortality, and growth retardation in young animals [9]. It is also associated with white muscle disease which causes mortality to newborn calves and reduced productivity in growing and adult cattle [1].

We previously reported the presence of widespread human Se deficiency among the population of Ethiopia, with a strong spatial component [10]. For example, in the Amhara region, there was strong evidence of greater prevalence of Se deficiency among all demographic groups in western Amhara, but little or no deficiency was observed in eastern Amhara. This was consistent with studies of children reported previously [11]. Studies have since shown that the Se status of the Ethiopian populations is correlated with grain Se concentration of the most consumed cereal crops in Ethiopia (including maize, teff, and wheat), which in turn is linked to variation in soil and landscape properties [12,13].

To our knowledge, there is no published information on Se status of cattle in Ethiopia. However, based on studies of soils, grains, and blood Se in people, we can expect Se deficiency to be widespread. Crop residues and stover are commonly utilized as cattle feed in Ethiopia, whose grains have already been shown to be highly variable in Se status [12,13]. The constraining effect of Se deficiency to the livestock production is likely to be of great importance to countries like Ethiopia where the livestock sector contributes up to 80% of farmers' incomes and 20% of the agricultural gross domestic product (GDP). In addition, Ethiopia is among the countries with the largest cattle population in the world [14]. Selenium improves the chemical composition of milk through increased Se concentration, and can reduce the concentration of saturated fatty acids, increase polyunsaturated fatty acids, and improve the organoleptic property of associated

dairy products [15]. The present study analyzed Se concentration in serum of free grazing cattle, and in feed samples, using a design informed by previous studies [10-13]. We hypothesized that cattle serum Se concentration in Ethiopia have similar spatial variation pattern with Se concentration in cereal grain and humans.

3.3. Materials and methods

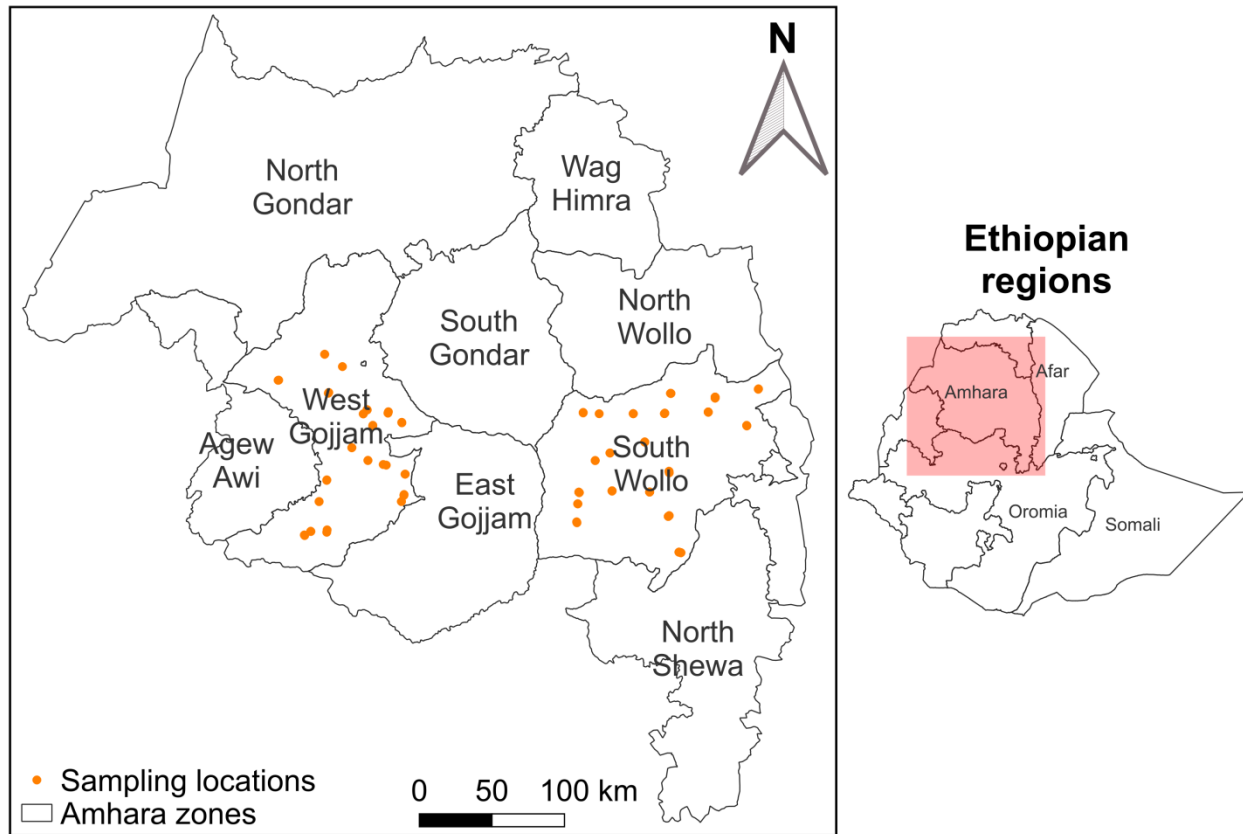
3.3.1. Study Area description, sampling design and field sampling

The study was conducted in East Amhara (South Wollo) and West Amhara (West Gojjam), Ethiopia (Figure 3.1). These areas were selected because we previously reported the presence of spatial variability among humans and staple crop Se status such that few or none of the populations from East Amhara had Se inadequacy while the majority (91.1%) of the populations in West Amhara were Se deficient [11,12].

Using satellite imagery, 40 villages (20 in each zone) were selected to give reasonable spatial coverage over the target sampling areas of the two selected zones of the Amhara region. In each village, two farming households possessing cattle were selected from a pre-defined list and invited to participate in the study. The lists of farming households were obtained from the district agriculture bureau.

Sampling was conducted by personnel working at the respective zonal agriculture bureaus of the Amhara region. Vets and extension workers were part of the sampling teams. Training was provided on sample collection and preparation and assessment of potential risks on the field using standard procedures. Cattle blood sampling was conducted by qualified personnel after refresher training. The locations of sampling villages were loaded on computer tablets for mapping. In addition, the teams were provided with GPS devices to navigate to the villages from where cattle feed and blood samples were to be collected. Blood samples from cattle and feed samples were collected, subject to farmers' consent.

Figure 3.1. Sampling village locations (n=20 villages per zone).



3.3.2. Blood collection and processing

Cows (lactating or non-lactating), confirmed as healthy by vets, were targeted for blood sampling (up to 3 per farm). Oxen or heifers were also included in the sampling in cases where the number of healthy cows in a farm were inadequate. The study included 224 cattle, including Zebu local breeds (n=200) and Holstein Zebu cross breeds (n=24). The cattle were in the age range of 1 to 13 years. The animals were squeeze chuted (allowed to pass through parallel fences that allows them to move forward only) and their heads elevated to locate the jugular vein. The neck area was wiped with antiseptic gauze to remove dirt and any debris. Approximately 10 mL of whole blood was drawn from the neck jugular vein using vacutainer needles by venipuncture. The samples were kept in an icebox until coagulation. Serum was separated by centrifugation in the field and transferred into vials. Duplicate subsamples were stored at -20°C in the nearest health facility and transported to the laboratories of the International Livestock Research Institute (ILRI) in Addis Ababa, Ethiopia. One aliquot of each sample was transported on dry ice to the University of Nottingham, UK, for analysis.

4.3.3. Cattle feed collection and preparation

Only the dominant feed types in the study areas, such as stover, straw, hay, or pasture grass, were included in the sampling. Commonly, cattle in Ethiopia graze freely on pasture and farmers were asked to locate the pastureland for their herds, where grass samples were collected from five spots 200–300 m apart and combined to represent one sample. In addition, samples of straw, stover and husks were collected (five sub-samples to represent one sample) from the center of stalks to avoid contamination with soil particles. Photographs of the sampling area and sample bags were taken for quality assurance purposes. The feed samples were kept in paper bags that allowed moisture escape, and transported to the laboratory at the Center for Food Science and Nutrition, Addis Ababa University. The samples were oven dried (60°C, 12 h) and milled in a stainless-steel coffee grinder. The grinder was wiped clean before use and between samples with a non-abrasive cloth. About 20 g of each sample were shipped to the University of Nottingham for multi-element analysis. Only Se concentration result is included in the present study.

Approximately 0.4 g of dried and milled feed samples were digested in acid (68% HNO₃ Primar Plus™, for Trace Metal Analysis, Fisher Scientific) in multicube 48 digestion system (Anton Parr-PFA coated graphite hot block). Milli-Q water (18.2 MΩ cm) was used for final dilution of the digest. Two operational blanks were digested alongside samples in each batch to control for other sources of contamination. Duplicate samples of certified reference material (National Institute of Standards and Technology (NIST) wheat flour SRM 1567b and CRM tomato 1573 A, and European Commission Joint Research Centre Hay powder BCR-129) were included in every digestion batch.

3.3.4. Selenium analysis

The concentration of Se in serum and feed samples was determined using inductively coupled plasma mass spectrometry (ICP-MS) at the University of Nottingham (Thermo Fisher Scientific iCAPQ, Thermo Fisher Scientific, Bremen, Germany). The detailed analytical procedure and conditions of the instrument are reported elsewhere [10,12]. Accuracy was verified by the use of two Seronorm™ reference materials: L-1 (Lot 1801802) and L-2 (Lot 1801803) (Nycomed Pharma AS, Billingstad, Norway). These were reconstituted and then diluted in an identical way to the samples. Average Se recovery (%; n = 2) when compared to accredited values determined was 102% and 104% for L-1 and L-2, respectively.

3.4. Statistical analysis

Statistical analysis of data was performed using R software [16]. Descriptive statistics were computed for the residuals from an exploratory linear model for cattle serum Se concentration, with fixed effects set as sample origin (sub-region), livestock group (lactating or non-lactating) and livestock age. The analysis only considered cows (lactating and non-lactating) because, unlike oxen and heifers, these were reasonably balanced in terms of sample size between east and west Amhara. Similar exploratory analysis was done with a model for feed Se concentration with fixed effects set as sample origin and feed type.

The residuals from the exploratory models for both serum and feed samples were positively skewed. In addition, there was a pronounced increase in the dispersion of the residuals as the corresponding fitted value increased for both variables. These were both corrected by transformation of the Se data to natural logarithms. For the feed samples, one datum appears to be an outlier on the log scale falling out with Tukey's outer fences [17]. This was one maize straw sample from west Amhara. This outlier was removed to avoid undue influence on variance parameter estimates.

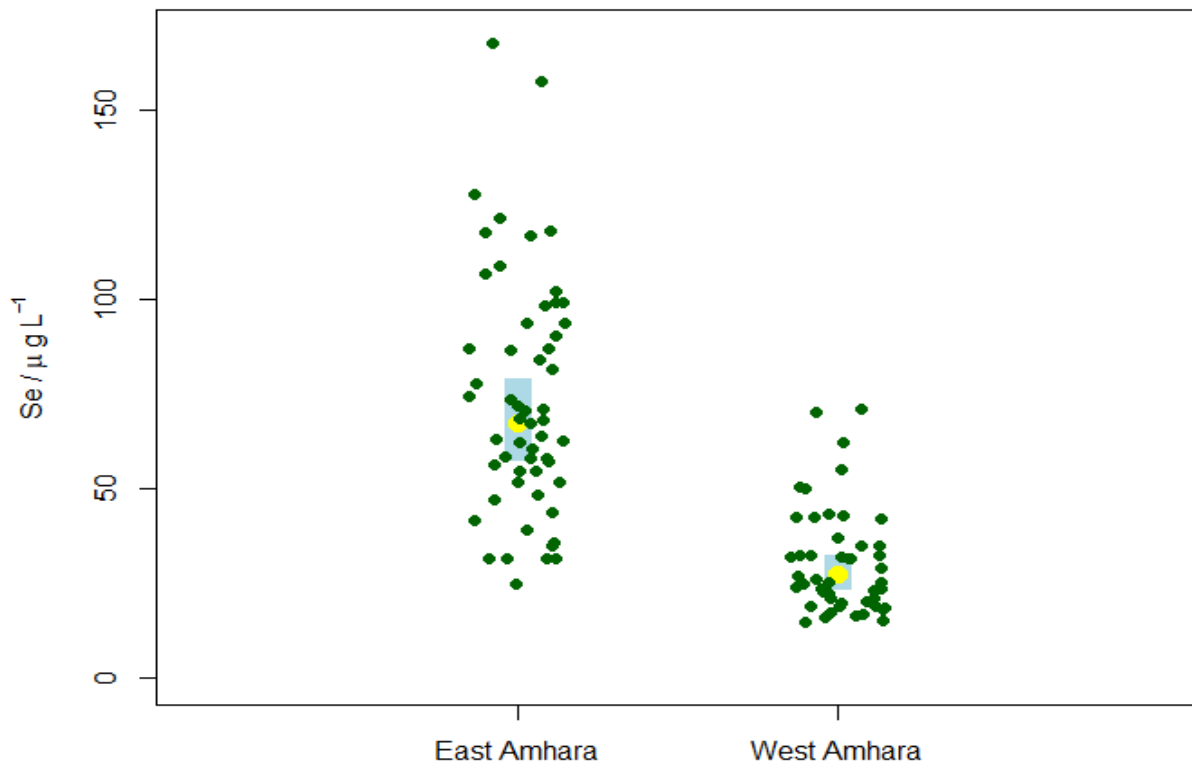
Because the villages selected for sampling were chosen to give good spatial coverage, rather than randomly and independently, a linear mixed model was used for the final analysis of the data. The model had a spatial auto-correlated random effect with a Matérn correlation function. Distances between locations were computed from latitude and longitude by a spherical approximation, and so the smoothness parameter of the correlation function has an upper bound of 0.5. The profile likelihood function was used to select a value, which was at this upper bound for both variables. The model was fitted with fixed effects, in sequence as follows (i) a constant mean the only fixed effect, (ii) separate means for east- and west-Amhara as the fixed effects and (iii) sub-regional mean plus livestock type (lactating or non-lactating) as fixed effects (livestock type not retained). (iv) sub-regional mean plus livestock age as fixed effects (age not retained). On the addition of each fixed effect from (ii) onward, the null hypothesis of no relation between serum Se and the new effect was tested by the log-likelihood ratio [18], and the effect was retained or dropped accordingly. Similarly, for the feed samples, the linear mixed model (LMM) was fitted with (i) a constant mean the only fixed effect, (ii) separate means for east- and west-Amhara as the fixed effects and (iii) sub-regional mean plus feed type as fixed effects.

To compare Se concentration among feed types and feed sample origin (east vs west Amhara), four prior contrasts were selected before data analysis and presented standardized by their standard errors so that values outside $[-2, 2]$ are of interest. These comparisons were included because showed a strong east-west trend in Se in staple grains [12, 13]. In addition, sorghum grain seemed to have a larger concentration of Se than did grain of other crops, and barley grain had a smaller concentration than others [13]. This defines two of the prior contrasts. In addition, we compared grazed grass with the other (dry) feeds, and compared grass hay with crop straw. After this analysis of prior contrasts, all pair-wise comparisons were computed among the main crop residues barley, maize, teff and wheat, and the p-values for these contrasts were adjusted by the criterion of [19] to control the family-wise error rate over the whole set of post-hoc comparisons at 0.05.

3.5. Results

Statistics are reported for cows in the study set. Their serum Se concentration ranged from 14.9–167.8 $\mu\text{g L}^{-1}$ (median, 41.4 $\mu\text{g L}^{-1}$). Cows from East Amhara, range 24.8–167.8; median 68.4 $\mu\text{g L}^{-1}$ had significantly greater serum Se concentration compared to cows from the East Amhara region, range 14.9–71.2; median 25.7 $\mu\text{g L}^{-1}$, $p < 0.001$. Overall, 79.8% of cows had serum Se concentration below the optimal threshold, 81 $\mu\text{g L}^{-1}$ [20]. All of the cows from West Amhara had Se deficiency but there was relatively lower Se deficiency prevalence (62.5%) in cattle from East Amhara (Figure 3.2).

Figure 3.2: Comparison of serum Se concentration among cattle from East and West Amhara region, Ethiopia. The green discs represent the original serum Se data. The yellow discs are the back-transformed mean estimates for each group (median-unbiased) with the 95% confidence interval (blue band). Note that, within each region, a small lateral random "jitter" is added so that the individual data points can be visualized.



There was marked spatially-correlated variation in cow serum Se, but the spatial dependence is very short-range after the sample origin (East or West Amhara) is included in the model. Table 3.1 shows the log-likelihood ratio statistic which measures the effect of sub-regions, livestock type and livestock age on cattle serum Se concentration. There is no evidence for a difference between lactating and non-lactating cows, nor for any dependence on animal age. Only the area where the sample originated is associated with Se concentration in serum.

Table 3.1. Log-likelihood ratio statistic testing the relationship between (a) zones, type and age of livestock with cattle serum Se concentration and (b) zones and feed type for concentration of Se in feed.

Fixed effects	L	df	p-value
a). Cattle serum Se concentration			
Zones	12.43	1	0.00042
Lactating or non-lactating cows	0.45	1	0.461
Age of livestock in years	0.44	1	0.506
b). Feed Se concentration			
Zones			
Feed type	12.10	1	0.0005
	27.97	8	0.0002

The Se concentration of feed samples (n = 81) was in the range of 0.05–269.3 $\mu\text{g kg}^{-1}$ (Table 3.2).

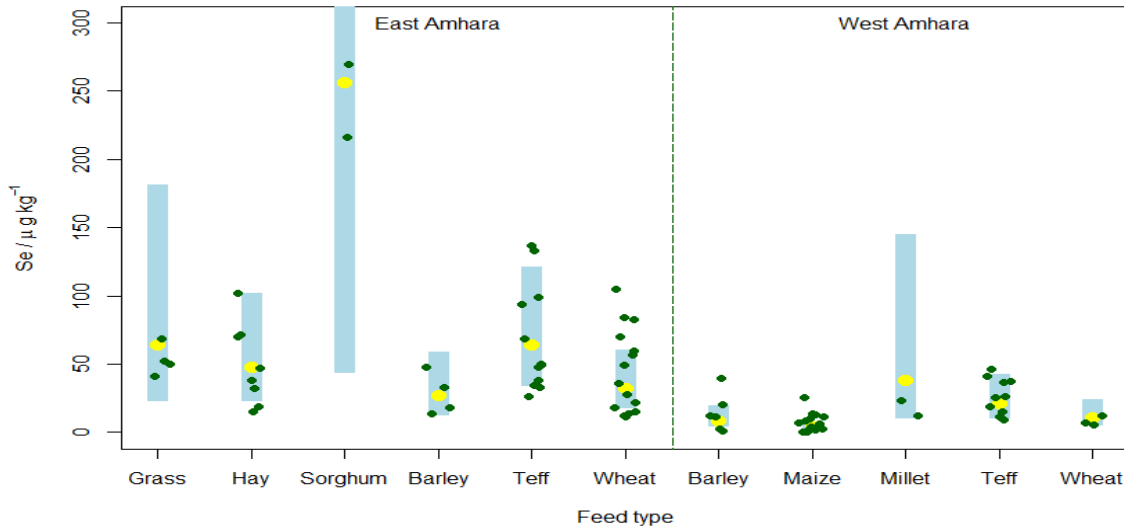
Table 3.2. Summary of selenium concentration ($\mu\text{g kg}^{-1}$, dry matter) of feed samples collected from the Amhara region, Ethiopia

Feed type	n	minimum	maximum	median [Q1, Q3]
Barley straw	11	0.9	47.7	13.4 [2.2,32.6]
Wheat straw	18	5.3	105.0	24.8 [11.9,62.5]
Teff straw	22	9.5	136.5	37.7 [25.9,54.4]
Maize stover	14	0.05	25.2	6.4 [2.2,11.6]
[†] Hay	8	15.3	101.5	42.6 [22.2,71.2]
Sorghum stover	2	215.9	269.3	*242.6 \pm 37.7
Finger millet straw	2	12.1	23.4	*17.8 \pm 8.0
[‡] Pasture grass	4	41.1	68.4	*53.0 \pm 11.4

*All such values are mean \pm standard deviation, [†]indigenous grass, [‡]grass-weed mixture

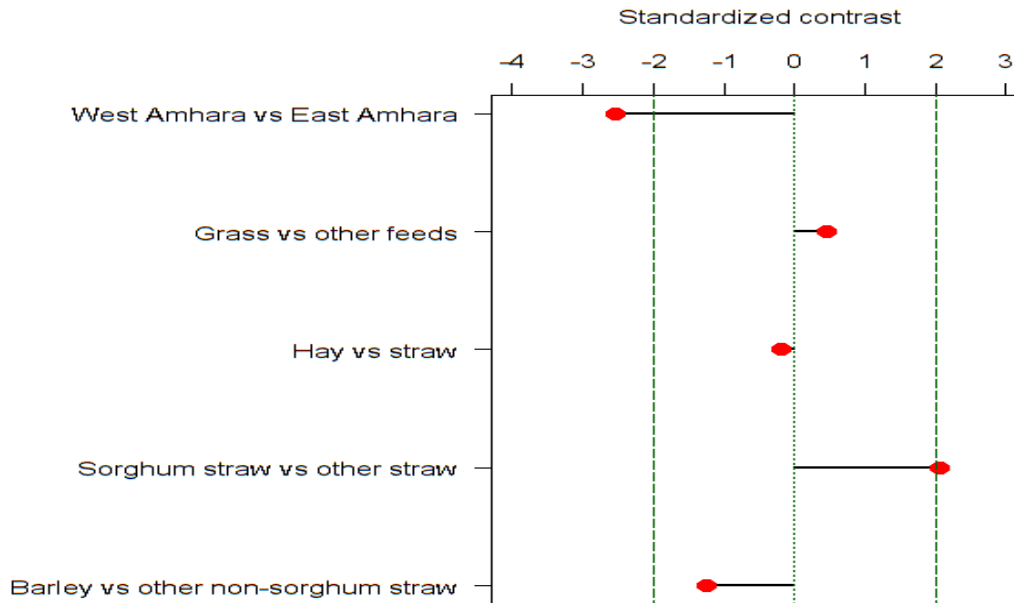
In general, feed samples from East Amhara had greater Se concentration compared to the samples from West Amhara (Figure 3), although the sample size is small there is evidence for such a difference ($p = 0.0005$, Table 1b). There is also evidence for differences among the feed types ($p = 0.0002$, Table 1b) even when the East-West difference is accounted for. Figure 3.3 shows the dominant effect of the regional trend. It is also notable that the sorghum straw appears to have a larger Se concentration than other straws. Although there were only two samples these were both much larger than for other feeds.

Figure 3.3: Selenium concentration of feed samples from East Amhara and West Amhara region, Ethiopia. The green discs are the original Se content of the feeds. The yellow discs are the back-transformed mean estimates for each group (median-unbiased) with the 95% confidence interval (blue band). Note that, within each category, a small lateral random "jitter" is added so that the individual data points can be visualized.



The contrast in Figure 3.4 shows only weak evidence that barley straw has a smaller Se concentration than other straws (sorghum excluded).

Figure 3.4: Comparison of Se concentration in feed samples from east Amhara and west Amhara region (the red dots are the standardized contrasts, i.e. the contrast divided by its standard error).



There is marked spatial variation in Se content of feeds, even after the regional (East-West) trend and feed differences were considered. The contrast between East and West Amhara feed Se concentration was consistent with the difference in serum Se concentration observed. This warrants regional surveys to map Se content of feeds and forage.

Results of log-likelihood ratio tests for the effects of sub-region and feed type on feed Se concentration shows that both geographical location ($p=0.0005$) and type of feed ($p=0.0026$) significantly influence feed Se concentration. Table 3 contains the log-likelihood ratio statistic for each comparison. The post-hoc comparisons suggest that teff straw is a richer Se source than barley straw and maize straw and (marginally) wheat straw. In addition, there is at least initial evidence that sorghum straw is the richest source of Se among feeds, although this is based on a very small sample.

Table 3.3: Comparison of Se concentration in stover samples

Comparison	L	df	P	<i>P</i> _{adj}
Barley vs Maize	1.35	1	0.2446	0.4893
Barley vs Teff	7.11	1	0.0077	0.0383
Barley vs Wheat	0.59	1	0.4425	0.4893
Maize vs Teff	12.98	1	0.0003	0.0019
Maize vs Wheat	3.41	1	0.0647	0.1940
Teff vs Wheat	5.75	1	0.0165	0.0658

3.6. Discussion

The present study assessed Se concentrations in cattle serum and feed samples from West and East Amhara region where contrasting human Se status, and crop grain concentrations, had previously been established. The median serum Se concentration was 41.4 $\mu\text{g L}^{-1}$. However, there was greater serum Se concentration among cattle from East Amhara compared to cattle from West Amhara. Similarly, there was greater Se concentration in feed samples from East Amhara than in samples from West Amhara. This is consistent with previously established contrasts in human Se status in these two areas [10-13].

Ethiopia is one of the countries in the world with the largest cattle population [14]. However, the contribution of the livestock sector to the national economy is below its potential. Research has identified several factors hindering the development of the livestock sector in the country, including low rates of reproduction, poor feeding and healthcare practices and facilities, poor breeds, and low capital investment related to the sector [21]. The present study shows high prevalence (79.8%) of Se deficiency in cattle from the Amhara region but with spatial variation such that significantly greater deficiency prevalence was observed in cattle from West Amhara than from East Amhara. This result may suggest that the deficiency could be affecting the performance of the livestock sector in the country. Selenium deficient cattle are at higher risk of reduced growth or weight gain affecting animal fattening and meat production. The deficiency is also associated with weakened immunity in cattle which further exacerbates morbidity and low

productive efficiency [22]. Selenium deficiency is also a problem of animals in other countries. For example, in Poland, Se deficiency was found in 50% of cattle. The study also reported the presence of Se deficiency variation among cattle type that 40% of cows, 80% of calves, 100% of heifers and 90% of bulls were affected [23]. However, such analysis was not possible in our study due to the unbalanced nature of the samples. Similar to results of the present study, Se deficiency was prevalent in cattle from all regions, however, there was apparent regional variation. Unlike the present study where age of cattle was not a significant factor for serum Se concentration variation, in Poland, Se deficiency was observed more frequently in young animals [23]. An earlier study in the United States also reported that about 18% of cows had severe or marginal Se deficiency and the Se status varied by geographical region and those from Southeastern states had higher rates of Se deficiency compared to cattle from other regions [20]. Se deficiency is also an important challenge to the pastoral sector in New Zealand [24].

Consistent with the distribution trend of cattle serum Se concentration, feed samples from West Amhara had lower Se concentration than East Amhara samples. Previous studies in the region [10] and the country [11] reported the presence of spatial dependence of human Se status. In addition, similar trends of spatial variability of Se concentration were observed in grain samples attributable to environmental and soil factors including soil pH, soil organic matter, temperature, rainfall and topography [12, 13].

Previously between-species variation in grain Se concentration has been reported in Ethiopia, where sorghum, finger millet and teff (*Eragrostis tef* (Zucc.) Trotter) had typically higher Se concentration, while barley and maize grains had the lowest Se concentration [13]. Consistent with the grain data, in the present study there was between-species variation in crop straw Se concentration, with sorghum being the richest source of Se, and teff straw containing higher Se than barley, maize, and wheat straw. This suggests that environmental controls, via the grain crops which can be cultivated at a given location, and the management effects where these are then grown by the farmer, will influence their livestock dietary supply of this essential micronutrient. Yet, this is an invisible trait to the farmer, with no role confirmed for Se in plant health. It is also likely that inter-connected geographic and species controls influence other mineral nutrients in livestock diets in this region, and these also warrant further investigation for their effects on well-being and productivity of the animals.

Results of the present study and previous studies reveal the presence of a high rate of Se deficiency in humans, plant foods and cattle from wider areas of Ethiopia. This suggests the need to communicate this aspect of deficiency to identify solutions that will increase Se nutrition for improving animal wellbeing and productivity, and human health. Crop Se fertilization helps to alleviate Se deficiency in humans [25]. This strategy may also help to improve Se status of cattle feeding on crop stovers, and the Se concentration in livestock products including milk and meat. Large-scale future studies to investigate the association between location and cattle Se deficiency in Ethiopia are important to design targeted interventions. Studies estimating the economic cost of Se deficiency to the agricultural sector in general and the livestock sector in particular is key to seek the attention of policy planners and justify the importance of the subject among other competing priorities.

The strength of this study includes generation of data on feed and serum from collocated areas, random selection of farms from villages that give spatial balance over study areas, and sufficient number of samples to observe a clear difference in Se status. Consequently, the design and sampling approach for larger surveys can now proceed on an informed basis. However, this study is based on serum analysis which reflects only recent Se exposure. In addition, this study had small area coverage. Furthermore, feed samples in the present analysis were both small in number and included only dominant feed types.

3.7. Conclusion

This study reveals that Se deficiency in cattle is highly prevalent though spatially variable in the Amhara region. Feed and cattle serum samples from east Amhara had significantly higher Se concentrations compared to respective samples from west Amhara. In addition, feed types were significantly associated with feed Se concentration. Selenium deficiency may affect productive efficiency of the livestock sector in Ethiopia, which should be explored together with other micronutrients, allowing interventions to enhance nutritional status in cattle to be identified. The potential for geographic variation in the nutritional quality of animal products (meat and dairy) entering the human food chain should also be studied. Further studies with larger sample size covering a larger area are needed. In addition, studies to better understand the consequences of Se deficiency to cattle productivity and the livestock sector are required.

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4. Evaluation of zinc bioaccessibility in zinc biofortified teff

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Abstract

Background: Zinc deficiency is highly prevalent among all demographic groups of the Ethiopian population. *Teff* () is a staple crop consumed by large segment of the population in the country hence, mineral fertilizer application to this crop could increase grain mineral concentration and Zn dietary supply. We evaluated the bio-accessability of Zn in dough and injera samples, at different time, prepared from Zn biofortified teff grain.

Methodology: Zn biofortified teff was produced in participatory approach with local farmers in west Gojjam, Amhara region Ethiopia for a feeding study that aimed to determine whether consumption of teff flour biofortified with Zn fertilizer application would be effective at improving human Zn status. 21% ZnSO₄.7H₂O was applied twice, at seedling stage of teff. Dried and matured feff samples were harvested in standing position and hand threshed to avoid soil contamination. The teff grain samples were milled in stainless steel coffee grinder and injera was prepared from dough samples with different fermentation time (24hr, 48hr, 72hr, and 96hr). Zinc concentration of teff grains, flour, dough and injera samples was conducted by Inductively Coupled Mass Spectrometer (ICP-MS). Bio-accessability of Zn in dough and injera samples was carried out following the modified INFOGEST invitro digestion method.

Result: Mean Zn concentration in biofortified and control teff grain samples were 31.8 (SD 1.71, n=24) mg/kg and 26.4 (SD 1.67, n=12) mg/kg, respectively, representing a 20% uplift in grain Zn concentration with agronomic biofortification. In addition, the experimental and control flour had 30.2mg/kg and 25.3 mg/kg Zn, respectively. However, percent bio-accessability of Zn in biofortified samples dough (14.9 vs 14.7; p=0.91) and injera (11.6 vs 10.9; p=0.79) was not significantly different from the control samples. Zn concentration in dough and injera samples had a decreasing trend with increasing fermentation time for both biofortified and control samples. On the other hand, there was an increasing trend in pH of both dough samples with increasing fermentation time.

Conclusion: Agronomic biofortification can increase teff grain Zn concentration but has no significant impact on bio-accessability.

Key words: Agronomic biofortification, Bio-accessability, Dough, Injera, Teff, Zinc,

4.2. Introduction

Zinc, due to its physico-chemical properties, has structural and regulatory roles in the human body. It also plays a catalytic role as it is required for normal function of more than 300 enzymes (Stefanidou et al. 2006). In addition, Se through seleno-proteins, plays significant roles in normal thyroid metabolism, defense against free radicals, the immune system, and brain function (Brown and Arthur, 2001). However, Se and Zn deficiency are found widespread among all demographic groups of the Ethiopian population. A report based on analysis of a national representative human serum samples reported that 72% of the Ethiopian population are Zn deficient with a median serum Zn concentration of $57.5 \mu\text{g dL}^{-1}$. However, the distribution of Zn status was not uniform across regions, demographic groups and residency. In general, populations from Tigray region had the lowest serum Zn concentration while those from Addis Ababa had the highest Zn concentration. In addition, the least Zn concentration was observed among young children and the highest among men participants and those from urban areas had higher Zn concentration than those resided in rural areas (Belay et al. 2021).

Risk of Zn deficiency has been associated with high rates of stunting, and prevalence of stunting is used as a functional indicator for population Zn nutrition (Wessells & Brown, 2012). Zn deficiency can lead to substantial infant and child morbidity and mortality mainly due to diarrhea (Krebs et al, 2014). Zinc deficiency in pregnant women has been associated with incidence of giving low birth weight child (Khadem et al. 2012; Alemu and Gashu, 2020). On the other hand, improved Zn nutrition help in a reduction of diarrhea and pneumonia associated child mortality (Yakoob et al. 2011).

Deficiency is mostly common among populations predominantly consuming less diversified and unrefined plant-based diets that supply low amount of Zn. Additional factors also include increasing demand due to physiological condition, impaired absorption caused by pathological conditions, excessive loss, poor metabolism, and low consumption of diets high in bioavailable such as animal source foods (Gibson, 2012). On the other hand, cereal and legume diets may not necessarily be low in Zn but its absorption can be affected by phytic acid, a main inhibitor of Zn absorption in to the body system (Sandstead and Freeland-Graves, 2014). Phytic acid (PA), [*myo*-inositol 1,2,3,4,5,6-hexakisphosphate] is the main phosphorous storage compound. It's negative charges and chelate divalent metal ions such as Zn^{+} and form an insoluble salt. Because,

humans lack phytase enzymes in their digestive track, phytate holds and prevents absorption Zn into the body (Kumar et al. 2021). However, traditional processing methods such as fermentation, soaking and germination have been used to reduce phytate from cereals and grains through activation of endogenous phytase enzymes in the grain, by providing optimum amount of water and pH condition. Phytic acid mainly is located in the bran of grains and can be removed during milling (Gupta et al. 2015).

Implementation of multilevel nutrition interventions employing dietary diversification, food fortification and supplementation must be put in place for mitigation of Zn deficiency. Agronomic biofortification, a term used to describe, application of optimized mineral containing fertilizers to the soil or on the leaves (De Valença et al. 2017) has proven to be very effective for increasing Zn concentration in cereal grains. A review of studies on the relevance Zn agronomic fortification to prevent human Zn deficiency in African countries reported that soil Zn application can increase Zn concentration in maize, rice and wheat grains by 23, 7 and 19 %. It also shows that Zn foliar application can increase grain Zn concentration by 30, 25 and 63 % to maize, rice and wheat grains (Joy et al. 2015). In addition, to increasing grain Zn concentration, Zn fertilization can increase human Zn bioavailability by reducing soil phosphorous uptake there by reducing phytate content of cereal or legume grains (Wang et al. 2015). A field experiment of Fe and Zn agronomic biofortification of finger millet conducted over two seasons at two different location in Ethiopia reported an increase in grain Zn concentration by 20% in response to joint application of Fe and Zn and by 18% when only Zn was applied to the soil (Teklu et al. 2023). A trial in Ethiopia that aimed to test the effects of Zn and Se mineral fertilizer applications to wheat (*Triticum aestivum* L.) and teff (*Eragrostis tef* (Zucc.) Trotter) grown under different landscape positions shows both mineral application and landscape position had significant effect on grain Zn and Se concentration (Manzeke-Kangara et al. 2023). A positive teff yield response to Zn fertilization was also reported (Haileselassie et al. 2011). The present study evaluates bioaccessability of Zn in teff grains harvested with the application ZnSO₄ in Ethiopia. Teff is a dominant crop in Ethiopia. It accounted for a quarter of grain-cultivated area coverage and involve the participation of about 6.5 million smallholder farmers in its production, suggesting its importance for food and nutrition security and economy of the nation (Tadele and Hibistu, 2021).

4.3. Materials and methods

4.3.1. Teff production

Teff was produced in participatory approach by engaging local farmers in Bure woreda, west Gojjam zone, Amhara region, Ethiopia. In consultation with the Zonal, woreda and local agricultural extension officers, an area previously known for production of teff was selected and a farmer cooperative was identified and farmers were invited to participate in production of the crops. Following several rounds of engagement and consultation meetings, the farmer cooperative agreed to produce the crop under contracts that guaranteed 20% premium above market value. The study team had no role in land preparation, seeding, deciding or advising on rate or method of inorganic fertilizer, or any of the farming practices. However, the study team worked with farmers and the agriculture extension officers to apply selenium and zinc fertilizers, and oversaw key stages of crop production, harvesting and processing.

4.3.2. Zinc concentration

Matured and dried teff (both biofortified and control) in standing position was harvested and hand threshed to avoid soil contamination. The teff grains were milled in stainless steel coffee grinder. The grinder was cleaned with clean cloth in between milled samples to avoid cross contamination. The flour samples were shipped to Nottingham University, UK.

4.3.3. Sample preparation

Flour samples were made into dough by mixing flour and deionized water (18.2 M Ω cm) in the ratio of 1:2 (w/v) and left to ferment for 24hr, 48hr, 72hrs, and 96hrs. A small portion of dough was taken and added to boiling water and was thoroughly mixed by stirring, a process called *absit* preparation to speed up the fermentation process (reference). pH of the dough samples was measured using ...pH meter (country, brand) by taken a small portion of the slurry at each fermentation time. The *absit* was added to the dough and stirred to make a thin consistency and left for about 30 minutes and was baked to make injera (a thin pancake).

Dough samples (ca. 500g) of different fermentation time were taken and freeze dried until the samples appear dry using a Lyophilizer (Mini Lyodel, India) at the Center for Food Science and Nutrition Laboratory, Addis Ababa University. The samples were packed in to airtight Ziplock bags and shipped to the University of Nottingham. In addition, injera samples were oven dried

(40°C), ground using clean mortar and pestle and packed in to airtight Ziplock bags and shipped to the University of Nottingham. The samples were shipped to the University of Nottingham under Material Transfer Agreement.

4.3.4. Sample preparation for Zn analysis

Zn concentrations were measured in the study grain, with samples taken from sacks immediately prior to storage. Level of Zn in biofortified and control teff grains samples was determined using ICP-MS. Samples were acid digested in a hot plate as described in Gashu et al., (2021). Briefly, about 0.2 g of sample was weighed into digestion tubes and placed into a heating block (Multicube 48, Anton Paar Ltd, UK). Concentrated HNO₃ (8 mL, trace metal grade, Fisher Chemical, USA) was added to each tube and left for 30 minutes at room temperature. The samples were then heated for 2 hours at 115°C and left to cool before dilution to 50 mL using MilliQ water (18.2 MΩ cm; Fisher Scientific). A further 1 in 10 dilution was undertaken immediately prior to analysis by inductively coupled plasma-mass spectrometry (ICP-MS) (Thermo Fisher Scientific, Bremen, Germany). A certified reference material (CRM, Wheat 1567b, National Institute of Standards and Technology, Gaithersburg, MD, USA) was used to determine % recovery. Operational blanks (n=20) were analyzed at the same time to determine the limit of detection (LOD) for each element.

4.3.5. Zn bio-accessibility test

Zinc bio-accessibility in fortified teff samples and products were carried out following the *in vitro* digestion followed by dialysis INFOGEST method (Minekus et al., 2014) as latter modified by Muleya et al., (2021). Milli-Q water was added to freeze dried dough samples to make a 30% dry flour slurry.

1. Oral phase digestion: 2.5 g of sample flour slurry was mixed with 2.488 mL SSF that contains 0.012 mL CaCl₂ and 75 U mL⁻¹ amylase. Then the mixture was incubated at 37 °C, in a shaking water bath for 2 minutes after the pH was adjusted to 7.0.
2. Gastric phase digestion: 5 mL of SGF containing 2000 U mL⁻¹ of pepsin was added. Then, the mixture was incubated for 90 minutes after adjusting pH at 3.0. The dialysis bag containing 17.5 mL of 0.05 M pipes buffer (pH 6.7) was added to the sample digestion tubes incubated for a further 30 minutes.

- Intestinal phase digestion: incubation continued for 2 hours after 5 mL of SPF complete and 5 mL of SBF complete were added and pH was adjusted to 7.0. Then tubes were cooled using ice to stop enzyme activity. The dialysate was carefully transferred to clean storage tubes after removing the dialysis bag. Then, 4 mL of the dialysate was mixed with 2 mL of 50% HNO₃ and heated using microwave. Finally, bio-accessible fraction Zn was analyzed using ICP-MS.

4.4. Results

Mean Zn concentration in intervention and control grains were 31.8 (SD 1.71, n=24) mg/kg and 26.4 (SD 1.67, n=12) mg/kg, respectively, representing a 20% uplift in grain Zn concentration with agronomic biofortification. In addition, the experimental and control flour had 30.2mg/kg and 25.26 mg/kg Zn, respectively. Zn concentration in dough and injera samples had a decreasing trend with increasing fermentation time for both biofortified and control samples (Table 4.1). On the other hand, there was a decreasing trend in acidification (increase pH) of both dough samples with increasing fermentation time (Table 4.2).

Table 4.1: Zn concentration (mg/kg) in dough and injera samples prepared at different fermentation time from Zn biofortified teff

Fermentation time	Dough		Injera	
	Control	Zn biofortified	Control	Zn biofortified
24	25.6	27.04	25.04	25.26
48	24.59	23.27	22.83	24.05
72	22.04	20.71	22.63	23.92
96	22.04	17.35	22.17	21.45

Note: Both samples are in dry weight basis

Table 4.2: pH of dough samples prepared from Zn biofortified and control teff at different fermentation time.

fermentation time	control	experimental
24	3.64	3.60
48	3.82	3.78
72	4.11	4.06
96	4.11	4.06

With an increase in fermentation time, there was an increasing trend in the percentage bio-accessibility of Zn in both control and biofortified injera samples. However, dough samples showed no clear pattern in Zn bio-accessibility with fermentation time (Table 4.3).

Table 4.3: Zn bio-accessibility (%) in dough and injera samples prepared at different fermentation time from Zn biofortified teff

Fermentation time	Dough		Injera	
	Control	Zn biofortified	Control	Zn biofortified
24	19.32	13.42	9.11	6.34
48	17.29	18.19	10.70	10.3
72	10.3	12.31	10.56	14.06
96	12.85	14.73	13.41	15.55

4.5. Discussion

Multiple micronutrient deficiency is highly prevalent in Ethiopia. Zinc deficiency is one of the most notorious micronutrients among the Ethiopian population. Agronomic biofortification of teff increase Zn dietary supply. However, studies on the bio-accessibility of Zn from Zn biofortified Zn is lacking. The present study reports Zn bio-accessibility in dough and injera samples of teff harvested through the application of Zn mineral fertilizer.

In the present study, Zn fertilizer application at a rate of increases grain Zn concentration by 20%, control grains had mean Zn concentration 26.4 ± 1.8 mg/kg and increases to 31.8 ± 1.7 mg/kg in response to Zn fertilization. Findings of similar studies also conform that Zn application can effectively increase Zn grain concentration. A report of a study on micronutrient fertilizer application effect on wheat and teff grain Se and Zn concentration at different slope positions and soil types shows that wheat grain Zn concentration increased from a baseline of 26.6 to 36.4 mg kg⁻¹ with co-application of basal and foliar Zn fertilizer yielded the highest Zn concentration compared to in the basal only (32.7mg kg⁻¹) and basal + side dressing application (32.7 mg kg⁻¹). An increase in teff grain Zn concentration was also attained from 28.5 to 31.2 mg kg⁻¹ in response to Zn fertilizer application. Similar to the trend observed for wheat, Zn application form had a significant effect such that co-application of soil and foliar Zn fertilizer had the greatest effect in grain Zn concentration (Manzeke-Kangara et al. 2023). Furthermore, an agronomic biofortification study based on a randomized complete block design with 15 treatment combinations comprising crop variety, two landscape positions, five basal fertilizer treatment combinations including 20 kg ha⁻¹ Fe SO₄ + 25KG ha⁻¹ ZnSO₄ + NPKS; 25KG ha⁻¹ ZnSO₄ + NPKS; NPKS (control) shows an increase in grain Zn concentration by 20% in response to the application of Fe and Zn combined while a 19% increase was recorded due to 25KG ha⁻¹ ZnSO₄. The authors also reported that an interaction between Fe and Zn was a significant effect in Zn concentration suggesting the presence of synergy in combined application of Fe and Zn fertilizers on grain Zn concentration (Teklu et al. 2023).

Inadequate dietary Zn supply is considered as the main factor for the high prevalence of Zn deficiency among populations in resource poor settings. In addition, regular consumption of high phytate plant-based diets such as cereal grains with little or no inclusion of animal source foods

in the daily diet contributes to Zn deficiency. However, studies reported agronomic biofortification can increase Zn bioavailability by decreasing phosphorous plant absorption hence, decreased phytate concentration. In the present study however, Zn bio-accessibility in biofortified dough (14.9 % vs 14.7%; $p=0.91$) and injera (11.6 % vs 10.9%; $p=0.79$) was not significantly different from the control samples. This may be because, although biofortified samples had higher Zn than the control samples, the total absorbable Zn fraction in biofortified samples was not great enough to cause a noticeable difference. In addition, phytate concentration, unfortunately was not analyzed in the present study, was not reduced significantly in response to Zn biofortification. Results of the present study warrants the need for further bio-accessibility studies (including phytate content analysis) on teff harvested with higher Zn fertilizer rate.

Fermentation provides an optimum pH for endogenous phytase enzymes to hydrolyze phytate and increase mineral bio-accessibility/bioavailability (Leenhardt et al. 2005). Similarly, in the present study, there was an increasing trend in bio-accessibility of Zn with increasing fermentation time. However, this was observed in injera samples (not dough samples) which may suggest the importance of the synergy between fermentation and thermal treatment to cause a significant Zn bio-accessibility from cereals. Heat treatment during food preparation can cause loss of phytate (Kumar and Anand, 2021). In the present study, Zn concentration had an inverse relationship with fermentation time in both biofortified and control samples. The reduction could be attributed to that portion of the Zn in the dough that migrate to the slurry was lost every time the slurry was decanted and dilution effect the water added to make the volume of fluid from the dough due to the removal of the slurry.

In general, Zn fertilization increases teff grain Zn concentration. However, there was no significant difference in bio-accessibility of Zn between biofortified and control teff flours. Still biofortification can increase dietary Zn intake. The present study is not without limitation including lack of phytate data and availability of only a single point moderate Zn fertilizer application that may result a small absorbable Zn fraction, to explain the absence percent bio-accessibility difference between the two samples.

4.6. References

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5. General discussion

Mineral micronutrients are very essential for proper physiological activity and prevent morbidity and mortality in the human population. The deficiency of micronutrients including Ca, Fe, Se, and Zn is a public health importance affecting over two billion people worldwide. The deficiency is very common among populations in resource poor settings with narrow food choices (WHO and UNICEF, 2006). In addition, for populations dependent on subsistent agriculture for local food production, soil mineral concentration determines the supply of these nutrients to humans through consumption of staple cereals and animal source foods (Joy et al, 2015). However, the distribution of these minerals in the soil is not uniform even within a country emphasizing the need for mapping and identifying areas with low soil mineral concentration to design proper interventions. In addition to soil mineral level, mineral absorption and accumulation capacity of food crops are different (Yan et al, 2021), and thus understanding the dietary pattern of population is an important factor. For example, a survey in Ethiopia and Malawi shows that compared to other cereal grains, maize had the least mineral concentration including Se, indicating that populations dependent on maize for energy source are highly likely to be Se deficient (Gashu et al. 2021). On the other hand, populations even from Se deficient areas with access to Se hyperaccumulating plants (0.1–0.5% of plant DW) such as the families of *Asteraceae*, *Brassicaceae*, *Chenopodiaceae*, *Lecythidaceae*, *Fabaceae*, *Rubiaceae* and *Scrophulariaceae* (Galeas et al, 2007) and with a fair intake of animal source foods could be less likely to be affected by the deficiency. This suggests that soil mineral concentration may not necessary predict human Se status.

Dietary intake of children in the present study was dominated by plant-based diets while only small percentage of children consumed animal source foods. Compared to plant-based diets, animal source foods are efficient source of readily available micronutrients (Neumann et al. 2002). However, in the present study, only 9% of children (n=7) from west Amhara but 37.3% (n=28) children from East Amhara consumed animal source foods.

Injera (flat bread prepared from mixture of teff and other cereals) with stew that was prepared from legume flour (bean, chickpea, pea) was the most frequently consumed food item followed by pasta. Injera meals from west Amhara were significantly lower in Se than from east Amhara. Plant based diets in general are known to contain low mineral concentration. In addition, they

contain anti-nutritional factors that hinder absorption of minerals by the human body (Sandberg, 2002; Platel and Srinivasan, 2016). However, it is important to remember that some plants such as some *Brassica juncea* cultivars are considered as a nature source of concentrated minerals, such as Se, and has been suggested as mineral dietary supplement (Elless et al, 2000). In this study, 61% of the mother's in west Amhara were farmers who reported to be dependent on their own farm produce for their diets, as compared to 30% in east Amhara. This would mean that families from west Amhara would be consuming Injera with less Se as compared to families from east Amhara, who reported purchasing majority of their foods.

As stated above, pasta was among the dominant food items consumed by the study children. Since wheat grain losses a great deal of micronutrients during milling stage (Rao and Deosthale, 1981), pasta from unfortified flour supplies low amount of minerals to consumers. As a solution, Ethiopia legislated mandatory wheat flour fortification in 2022 after the data collection period of the present study. Also, Se is not among the he minerals included in mandatory wheat flour fortification standard.

There was an apparent difference in Se concentration of the food and breast milk samples from east Amhara and west Amhara samples. Such spatial variability of Se was also reported in the previous studies based on analysis of soil, crop and human serum samples in the Amhara region (Gashu et al. 2019; Gashu et al. 2020). Other studies also reported presence of Se distribution variability (Fordyce et al. 2000; Tan et al 2002; Hao et al. 2021). Se concentration in breast milk samples had also similar pattern with other food items suggesting that unlike other nutrients such as Zn that their secretion in the breast milk is homeostatically regulated in a state of deficiency in favor of the breastfeeding child (Lönnerdal 2000). Regarding the non-significant difference in Se concentration of the pasta samples it's expected that populations in both areas have similar source for pasta and macaroni.

Three-fourth of the study children were breastfeeding at the time of data collection. Breast milk samples from East Amhara were significantly higher than those from West Amhara area, which might reflect Se concentrations in foods consumed by mothers (Dorea 2002; Lönnerdal 2000). Injera with legume stew (which was found to be low in selenium) in west Amhara region is the staple food eaten by mothers most frequently in a day while pasta/macaroni and rice constitute the diets of mothers from East Amhara, which might contribute to higher selenium intakes since

these items are industrially prepared from imported wheat or are imported themselves. The findings indicate neither breast milk nor complementary foods deliver adequate Se to children in west Amhara.

Urinary Se excretion is a useful biomarker of Se intake which can be used to assess Se status (Phiri et al, 2020). Hays et al (2014) cited Institute of Medicine (IOM) and reported urinary Se concentrations of 10 μ g/L if EAR values are met. In the current study only one child (1.3%) from west Amhara had urinary Se above 10 μ g/L, which indicates Se deficiency in the West Amhara area. On the other hand two children (2.8%) from East Amhara had urinary Selenium concentrations less than 10 μ g/L which might indicate relatively higher Se intake in the area. However 57% of children from East Amhara had urinary Se within the accepted range 20-90 μ g/L (Godebo et al, 2023).

Adequate micronutrient status positively influences livestock health and performance (Upadhaya et al, 2020). It is also an important consideration for human micronutrient nutrition particularly because animal source foods provide high amount and readily available micronutrients. Adequate Se nutrition to cows has been linked to an increase in the production and quality and fatty acid profile of milk, growth performance to new born, reproduction, and improvement in overall health (Mehdi and Dufrasne, 2016). This suggests that proper Se nutrition would highly benefit the livestock sector. This is particularly important to nations like Ethiopia possessing large cattle population and has a significant share to the agricultural national GDP (20%) (FAOSTAT, 2020). In the present study, similar to the Se distribution pattern of the food samples, Se concentration in cattle samples from west Amhara was low (prevalence=100%, cut off =81 μ g/l). In addition, Se concentration in all feed samples from East Amhara was greater than West Amhara samples. This may pose a challenge to the dairy and animal fattening sectors due to low milk production, poor growth performance and reproduction. This may indicate the need for cattle Se supplementation or Se biofortification of cattle feed. Besides the geographical effect in the variation of Se concentration, the selenium content of forages varies with the type of feed. Attributable to their chemical similarity, cattle feed with higher sulfur amino acids can accumulate more Se (Mehdi and Dufrasne, 2016). For example, in the present study sorghum stover samples had 2-10-fold greater Se concentration than other samples (242.6 \pm 37.7 μ g/kg, dry matter).

Coupled with other dietary based interventions (dietary diversification, supplementation and food fortification) agronomic biofortification, the deliberate addition mineral micronutrient fertilizer to increase specific nutrients in the edible portion of plant foods, plays an important role to improve human nutrition by increasing grain mineral micronutrient concentration. It has the potential to alleviate the deficiency of coexisting micronutrient deficiency by simultaneous addition of multiple mineral (White and Broadley, 2009). A multicounty field experiments conducted to study the effect individual application of I and Zn, and a mixture solution containing I, Zn, Se and Fe on wheat mineral concentration shows that wheat grain Zn concentration increased from 28.6 mg kg⁻¹ to 46.0 mg kg⁻¹ and 47.1 mg kg⁻¹ with Zn spray alone and application multiple micronutrients, respectively. Iodine increased from 24 µg kg⁻¹ to 361 µg kg⁻¹ and 249 µg kg⁻¹, respectively. In addition, grain-Se increased 90 µg kg⁻¹ to 338 µg kg⁻¹. Furthermore, 12% grain-Fe concentration increment was achieved (Zou et al. 2019). Agronomic biofortification of various foods such as winter wheat, maize, soybean, potato, canola, and cabbage with Zn, Se, and I was also reported to be effective in increasing mineral micronutrient concentration in the edible portion of the crops. However, the degree of effectiveness was dependent on crop species, fertilizer forms and application methods (Mao et al. 2014). Field trials in Ethiopia also show that soil or foliar application of mineral fertilizers was effective in increasing grain mineral concentration (Teklu et al. 2023; Manzeke-Kangara et al. 2023). However, an increase in grain mineral concentration doesn't necessarily guarantee optimal nutrition status due to reductive effect of antinutritional factors to the bio-availability of minerals. On the other hand, mineral fertilizer application such as Zn substantially reduces phytate concentration by competitive inhibition of soil phosphorous (Chattha et al. 2017). Measuring bio-accessibility of target micronutrients in crop is thus important to optimize nutrient availability for body absorption and to improve programs targeting micronutrient deficiency (Huey et al. 2022).

In the present study, compared to control samples, there was a 20% increase in Zn concentration of biofortified teff samples. However, there was no significant difference in the bio-accessibility of Zn in control and biofortified teff dough (14.9 vs 14.7; p=0.91) and injera (11.6 vs 10.9; p=0.79) samples. This could be because, the added Zn was not adequate enough to cause significant inhibition effect on phosphorous absorption hence, phytate synthesis or the total absorbable Zn fraction in biofortified samples was not high enough to cause a noticeable

difference. Unfortunately, neither phosphorous content nor phytate level was measured in this study.

Traditional processing including fermentation are known to improve the bioavailability and bio-accessibility of minerals from foods by creating optimal condition for endogenous enzymes to break dietary fibers and phytate (Nkhata et al. 2018). Similarly, in the present study, Zn bio-accessibility percentage has increased with increasing fermentation time both in control and biofortified samples. However, this trend was observed in injera samples but not in dough samples implying that fermentation and heat treatment than fermentation alone causes significant effect to increase bio-accessibility. Thermal processing can cause denaturation to endogenous phytates enzymes (Urbano et al. 2000). On the other hand, it can reduce phytic acid concentration and increases over all bio-accessibility (Mahesh et al. 2015). A study reporting the influence of thermal treatment on antinutritional factors and invitro bioavailability of minerals of different chick pea cultivars in India found that heat processing had a significant reduction effect on the level antinutritional factors in all cultivars and bio accessibility of Zn and Fe (Sharma et al. 2018). Similar result was also reported by others (Kumar et al. 2023)

6. Conclusion and recommendation

6.1. Conclusion

Micronutrients play catalytic, metabolic and structural role in the human body. The deficiency of these micronutrients is a major global concern affecting more than 2 billion people worldwide. Populations in low income countries are the most affected due to poor dietary-quality and frequent infection. The condition is sometimes referred as ‘Hidden hunger’ because of absence of the feeling of hunger but is manifested with clinical symptoms. This is associated to intrauterine growth restriction, low birth weight, chronic growth faltering, mental retardation, frequent morbidity, and mortality (Black, 2014). It has deleterious impact on economic growth and human capital development at population and global level. The most common micronutrient deficiencies in the world include Fe, I, Zn and folate (Bailey et al. 2015). In addition, it is estimated that half to one billion people worldwide are affected by Se deficiency (Zhang, 2023).

Se through seleno-proteins is important in several metabolic pathways in humans including thyroid hormone metabolism, antioxidant defense systems, and immune function (Brown and Arthur, 2001). It is generally assumed that Se status of populations dependent on subsistent agriculture for food consumption is a mirror reflection of Se concentration in the soil (Hurst et al. 2013). The presence of spatial variability of Se in humans, soil and crop samples in Ethiopia was reported (Gashu et al. 2019; Gashu et al. 2020). Similarly, in the present study, Se dietary intake among children from east Amhara was significantly higher than children from west Amhara regions. More children from west Amhara (92%) than those from east Amhara (31.5%) had higher prevalence of Se intake inadequacy. Urinary Se excretion ($< 10\mu\text{g/L}$) also showed similar trend, suggesting children from West Amhara (98.7) were Se deficient than those from east Amhara children (2.8%). In addition, major food items and breast milk samples from east Amhara had significantly higher Se concentration than the samples from west Amhara. Furthermore, there was similar distribution trend of Se in cattle serum and their feed samples from the Amhara region such that all of the cattle from west Amhara had low Se concentration ($< 81 \mu\text{g L}^{-1}$). This is particularly an important finding firstly, because cattle are major players to maintain the food system by supplying nutrition through milk and meat. Secondly, Se plays an important role in reproduction and health of cattle which covers significant portion of the Ethiopian economy.

Food based interventions such as dietary diversification, food fortification and supplementation are effective approaches to increase human micronutrient intake and alleviation of deficiency. However, compared to these strategies, agronomic biofortification in poor rural areas has better effectiveness provided they have access to micronutrient fertilizers (De Valença et al, 2017). Agronomic trials show that mineral fertilizer application can significantly increase grain mineral concentration (Teklu et al, 2023, Manzeke-Kangara et al, 2023). In addition, agronomic biofortification of crops with Zn is thought to increase Zn bioavailability by competitive inhibition of phosphorous which is the main component of the antinutritional factor, phytate (Gibson et al, 2010). In the present study, mean Zn concentration in biofortified and control teff grain samples were 31.8 (SD 1.71, n=24) mg/kg and 26.4 (SD 1.67, n=12) mg/kg, respectively, representing a 20% uplift in grain Zn concentration with agronomic biofortification. In addition, the experimental and control flour had 30.2mg/kg and 25.3 mg/kg Zn, respectively. However, the bio-accessibility of biofortified and control dough and injera samples was not different.

6.2. Recommendations

1. Nutrition interventions to address Se deficiency among populations in west Amhara are important. Furthermore, because of difference in Se concentrations in food items, nutrition education to Se deficient areas to select the food items with better Se content or accumulation capacity (without compromising other nutrients) during meal preparation is important.
2. Ethiopia has an agrarian economy with significant contribution from the livestock sector. Hence, addressing Se deficiency in cattle may significantly improve cattle reproduction, health and quality and production of milk. Response to Se supplied feeds by local cattle breed needs to be investigated. In addition, the potential of Se deficiency from animal source foods (meat, milk and eggs) entering into the human food chain needs to be explored at larger scale.
3. Biofortification of different cereal crops has improved mineral concentration of the crops in Ethiopia. However further studies regarding bio-accessibility of minerals from biofortified crops needs to be studied to provide evidence for policy makers.

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Annexes

Annex 1: Ethical Approval Document

COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES
Addis Ababa University



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OFFICE OF THE DEAN
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Ref. No.
ቁጥር CNSDO/450/10/2018
Date
ቀን April 19, 2018

To Whom It may Concern

The College of Natural & Computational Science Institutional Review Board (CNS-IRB) Committee in its meeting held on 30/03/2018 Minute No. IRB/032/2018 has examined the project proposal entitled "Dietary Selenium Intake and Bioavailability in Areas of Distinct Selenium Nutrition in Amhara Region, Ethiopia", by Kaleab Hailu from the Addis Ababa University.

The proposal is approved for implementation.

With regards,

Shiferaw Amessega
Dean, College of Natural & Computational Science

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Please Quote our reference number in you correspondence
"የተሰጠውን መርጫ ማሳካት ይቻላል"

"Examine all things, hold fast that which is good"

Annex 2: Information and Consent Form (English Version)

Title: Dietary Selenium Intake and Bioavailability in Areas of Distinct Selenium Nutrition in Amhara Region, Ethiopia

Principal Investigator: Kaleab Hailu

Institution: Addis Ababa University

Introduction

Selenium is a nutrient in our food that helps our bodies fight infections and contributes to making our children learn and perform well. Selenium also has a role in helping the body use iodine and have a healthy thyroid gland. If the diet is low in selenium, children may have more illnesses, not perform well in school, and have problems of goiter and other related health issues.

Previous studies on children in Amhara region indicated deficiencies in the western part of Amhara region unlike the eastern region where very high amounts of Se were analyzed in children. This study aims to investigate the intake of selenium from foods in children living from these two regions, in order to understand the cause of this Se variation. This study will also give indications on the kind of food that are rich in selenium, and if the body is able to utilize the Se from the foods. This information can be used as a basis for studies in the future where supplementary interventions may be suggested.

In order to undertake the study we are asking for you and your child who is under two years of age to participate in the study. Information on the food intake will be collected by collecting a two day non-consecutive food the child eats in 24 hours. Breast milk samples will also be collected for the study. Moreover the child's urine will also be collected and the mother will be interviewed to tell us how frequently she eats the foods from different food groups.

Due to lack of laboratory facility in the country, the breast milk and urine samples will be transported to and analyzed in UK, Nottingham University. And we are asking your consent for you and your child to participate in the study.

Procedures

If you agree to participate, we will collect spot urine from your child and breast milk from you and it will be transported to UK for analysis.

Risks

Nothing harmful will come from you and your child participation as the method only involves analysis of the urine and breast milk samples.

Benefits

There are no other direct benefits to you or your child in participating. However, the findings will possibly help others. If we find that selenium intake from the foods your child eats is not adequate for normal biological functions, we will inform policy makers to work on agronomic strategies to ensure adequate supply of Se.

Cost

There is no cost to you for participating.

Compensation

There will be no compensation for participating.

Participant Rights

If I have said things that are not clear to you, you may ask without hesitation and I will answer. You may feel free and ask questions. Your child's participation in the study is entirely voluntary and up to you to decide. There is no penalty if you do not agree to participate. If you don't agree to participate, you can say 'no' without worry. Your health center and extension health worker will continue to provide health services to you as usual.

Confidentiality

Test results and any information about your child will be kept private. Only the research team will have access to your information. When we write a report, everyone's information will be put

together so that information about you or any other individual cannot be seen. Your child will be identified with random numbers on serum vials.

Persons to contact:

If you have any question, you can ask at any time. If you have additional questions about the study, you may contact: Kaleab Hailu, Addis Ababa University, Phone no: 0911 34 27 32 Email: kaleab2000@gmail.com

If you have questions about your rights as a participant of a research study, you may contact: Addis Ababa University, College of Natural Science Institutional Review Board (CNS-IRB), Tel: 251-111-23 94 72 Fax: 251-111-23 94 69 Email: dean_cns@aau.edu.et

If you agree to participate in the study, please sign or give your left thumb impression at the space indicated below.

Thank you for your cooperation.

Signature:

Child's name _____

The study has been explained to me and my questions have been answered to my satisfaction. And I agree to participate in this study and I agree to have my child participate in this study.

_____	_____	_____
Signature or left thumb impression	Printed Name	Date
_____	_____	_____
Signature of the data collector	Printed Name	Date

Annex 3: Household Information and Child feeding questionnaire (English Version)U

Identification

No		
1	Date information collected	___/___/_____ Date/ Month/ Year/
2	Field worker's Name	_____
3	Field site	_____
4	Supervisor's Name	_____
5	Date Reviewed by Supervisor	___/___/_____ Date/ Month/ Year/

1. Background Information about the Area



	Questions	Choices or Answers	Identification Code	Skip
1.1	Zone	South Wollo.....1 North Wollo2 West Gojam.....3 East Gojami.....4	<input type="text"/>	
1.2	Woreda	_____		
1.3	Kebele	_____		
1.4	Agro-ecological character	Dega1 Woynadega.....2 Kolla.....3	<input type="text"/>	

DEMOGRAPHIC INFORMATION

Please fill out all sections of this form completely, recording information for all children 0-59months and their mothers/caregivers.

2. Socio-Demographic Information

	Questions	Choices or Answers	Identification Code	Skip
2.1	Name of the mother/care taker	_____		
2.2	Age of the mother/care taker (In years)	_____ Years		

2.3	To which religious group do you belong?	Orthodox.....1 Protestant.....2 Muslim.....3 Catholic4 If other specify _____5	<input type="text"/>	
2.4	What is your occupation?	Unemployed.....1 Daily laborer2 Farmer.....3 Selling Enjera, Kolo, Areke ,Tella.....4 Privately owned Business.....5 Government/NGO employee6 If other specify _____7	<input type="text"/>	
2.5	What is your marital status?	Married.....1 Divorced.....2 Widowed.....3 Never Married.....4	<input type="text"/>	Skip to 2.7
				
2.6	What is the occupation of your husband/partner?	Unemployed.....1 Daily laborer2 Farmer.....3 Selling Enjera, Kolo, Areke ,Tella.....4 Privately owned Business.....5 Government/NGO employee6 If other specify _____7	<input type="text"/>	
2.7	What is the highest educational attainment of your husband/partner?	Illiterate1 Can Read and/or Write.....2 Grade 1-4.....3 Grade 5-8.....4 Grade 9-12.....5 Technical school certificate.....6 University/College Certificate..... 7	<input type="text"/>	
2.8	Does your household own or rented any land for agriculture?	Yes, owned land1 Yes, rented land2 No.....3	<input type="text"/>	Skip to 3.1
				
2.9	How many (local units) of agricultural land does the	_____ Units. Write the local unit here (_____)	<input type="text"/>	

	household owned/rented?	I don't know/Not sure.....999		
--	-------------------------	-------------------------------	--	--

3. Initiation about Child Feeding Practice

3.1	How many children (who are living with you) do you have?	One Two If other specify _____	
-----	--	--------------------------------------	--

No	Name of Children	Sex	Age
1			
2			
3			
4			
5			
6			
7			

	Data Collector: <ul style="list-style-type: none"> Identify child/children who are below 2 years of age If more than one child is there, select the child using simple random sampling technique 	
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3.2	Name of the selected child	_____	
-----	----------------------------	-------	--



3.3	When did you give birth to (NAME)? (Data Collector: Ask for any evidence that shows date of birth.)	___/___/_____ Date/ Month/ Year/ Use Ethiopian calendar, if date of birth is not available use 15, which is middle of month)	Source of date of birth Birth certificate.....1 Baptism Certificate.....2 Immunization card.....3 Event calendar.....4 Recall5 other (specify).....6	
-----	--	---	---	--

3.4	Age in completed months of (NAME)	_____ Months	
-----	-----------------------------------	--------------	--




3.5	Gender of (NAME)	Female1 Male.....2	<input type="checkbox"/>	
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4. Breastfeeding Practice and Initiation of Feeding

4.1	Did you ever breastfed (NAME)? <i>Sometimes babies are fed breast milk in different ways, for</i>	Yes1 No2	<input type="checkbox"/>		Skip to 4.6
-----	--	-------------------------	--------------------------	--	--------------------

	<i>example by spoon, cup or bottle.</i> Did (<u>NAME</u>) consume breast milk in any of these ways?		
4.2	How many months did you give breast milk alone to (NAME) without any food, liquid or water?	____ months Still being exclusively breastfed	
4.3	Are you still breastfeeding (NAME)?	Yes1 <input type="checkbox"/>  <input type="checkbox"/> No2	Skip to 4.5
4.4	In total, for how many months did you breastfeed (NAME)?	____ months I don't remember.....99	
4.5	How many times do you breastfeed (NAME) during the daylight hours?	____ times I don't remember.....99	
4.5	How many times do you breastfeed (NAME) between sunset and sunrise?	____ times I don't remember.....99	
4.6	At what months of age did you start to give additional food to (NAME)?	____ months	
4.7	Did (NAME) fully complete the transition to family food?	Yes1 <input type="checkbox"/>  <input type="checkbox"/> No2	Skip to 5.1
4.8	If yes, at what age?	____ months	
5. Existing Local Foods/Recipes, Preparation and Consumption			

5.1	<p>What type of solid, semisolid, or soft foods do you most commonly feed to (NAME)?</p> <p>(Data Collector: Multiple answers are possible)</p>	<p>Teff gruel Teff porridge Maize gruel Maize porridge Wheat gruel Wheat porridge Barely gruel Barely porridge Sorghum gruel Sorghum porridge Bulla gruel Bulla porridge Soybean gruel Soybean porridge Mashed potato porridge Gruel from composite flour of cereal and legumes Porridge from composite flour of cereal and legumes Gruel from mixture of grain and enset/tuber Porridge from mixture of grain and enset/tuber Enjera with wot If other specify _____</p>	
5.2	<p>In the foods that you most commonly feed to (NAME), (which is mentioned in 5.1) what major additional ingredients do you commonly add?</p>	<p>_____ _____ _____ _____</p>	
5.3	<p>If you want to cook/prepare a meal (which is mentioned in 5.1) adequate for (NAME) only for a day and night, what amount of the main ingredient (SPECIFY), do you use?</p>	<p>_____ number of coffee cups</p> <p>(Data Collector: Convert the volume to the number of standard 70ml coffee cups)</p>	
5.4	<p>How do you obtain the ingredients of the foods that you feed to (NAME)?</p>	<p>Produced by the household1 Purchased at the market.....2 Some are purchased while others are produced.....3 <input type="checkbox"/> Food aid4 Safety net5 If other specify _____6</p>	
5.5	<p>Specify the purchased and produced ingredients.</p>	<p>Produced by the household: _____</p>	

	(Data Collector: Ask for all ingredients identified under section 5.6)	_____ Purchased at the market: _____ _____ Food aid/safety net: _____ _____	
5.6	Are these ingredients available throughout the year?	Yes1 <input type="checkbox"/>  No2 <input type="checkbox"/> Some are available while others are not available.....3	Skip to 5.9
5.7	List all the ingredients with limited availability.	_____ _____	
5.8	When such ingredients become unavailable, what coping mechanisms do you employ? (Data Collector: Probe on exhaustive list. For example: food aid, changing the ingredients, reducing the volume and frequency of feeding etc)	_____ _____ _____ _____ _____	
5.9	Are these ingredients affordable throughout the year?	Yes1 <input type="checkbox"/>  No2 <input type="checkbox"/> Some are available while others are not available.....3	Skip to 5.11
5.10	When such ingredients become unaffordable, what coping mechanism do you employ? (Data Collector: Probe on exhaustive list. For example: food aid, changing the ingredients, reducing the volume and frequency of feeding etc)	_____ _____ _____ _____	
5.11	At what age did you start giving meat to (NAME)?	_____ months I did not start giving meat to him/her88 <input type="checkbox"/> I don't remember/Not sure.....99	
5.12	What is the usual water source for the preparation of the food and drinking?	Tap water1 <input type="checkbox"/>  Protected well or spring2 Unprotected well or spring3 <input type="checkbox"/> Surface water4 If other specify5	Skip to 5.15

5.13	Do you treat the water in any way to improve its quality?	Yes.....1 No.....2	<input type="checkbox"/>	Skip to 5.15
5.14	If yes, what water treatment technique do you employ? (Data Collector: Multiple answers are possible)	Use of chemical disinfectants like “Wuha Agar”1 Boiling2 Filtering by cloth or other techniques3 Storing and sedimentation4 If other specify5	<input type="checkbox"/>	
5.15	Was (NAME) included in supplementary feeding program (SFP) in the last 6 months?	Yes.....1 No.....2	<input type="checkbox"/>	Skip to 5.18
5.16	If yes, specify what types of foods were given to (NAME).	_____ _____		
5.17	Did you fully feed (NAME) the food aid that you receive to him/her?	He was fed the whole1 He was fed about half of it2 He was fed about quarter of it3 He was not fed at all.....4	<input type="checkbox"/>	
5.18	In the last 3 months did you get any kind of education on how to prepare food for babies?	Yes.....1 No.....2	<input type="checkbox"/>	Skip to section 6
5.19	If yes who provided you the education?	Health workers/Health extension worker1 Community health workers/volunteers.....2 Food distributing agents.....3 If other specify4	<input type="checkbox"/>	

6. CHILD FEEDING

Please describe everything that (**NAME**) ate yesterday during the day or night, whether at home or outside the home.

a) Think about when (**NAME**) first woke up yesterday. Did (**NAME**) eat anything at that time? If yes: Please tell me everything (**NAME**) ate at that time. Probe: Anything else? Until respondent says nothing else. If no, continue to Question b).

b) What did (**NAME**) do after that? Did (**NAME**) eat anything at that time?

If yes: Please tell me everything (**NAME**) ate at that time. Probe: Anything else? Until respondent says nothing else.

Repeat question b) above until respondent says the child went to sleep until the next day.

If respondent mentions mixed dishes like a PORRIDGE, sauce or stew, probe:

c) What ingredients were in that (**MIXED DISH**)? Probe: Anything else? Until respondent says nothing else.

As the respondent recalls foods, underline the corresponding food and circle ‘1’ in the column next to the food group. If the food is not listed in any of the food groups below, write the food in the box labeled ‘other foods’. If foods are used in small amounts for seasoning or as a condiment, include them under the condiments food group.

Once the respondent finishes recalling foods eaten, read each food group where ‘1’ was not circled, ask the following question and Circle ‘1’ if respondent says **Yes**, ‘2’ if **No** and ‘8’ if **Don’t Know (DK)**:

	Question	Coding	Skip
6.1	Yesterday during the day or night, did (NAME) drink/eat any (FOOD GROUP ITEMS)?		
No	Questions	Coding Categories Yes[1] NO [2] DK [8]	
A	Porridge, bread, rice, noodles, or other foods made from grains		
B	Pumpkin, carrots, sweet potatoes that are yellow or orange inside		
C	White potatoes, cassava, or any other foods made from roots		
D	Any dark green leafy vegetables		
E	Ripe mangoes, ripe papayas		
F	Any other fruits or vegetables		
G	Liver, kidney, heart, or other organ meats		
H	Any meat, such as beef, pork, lamb, goat, chicken, or duck		
I	Eggs		
J	Fish, seafood		
K	Any foods made from beans, peas, lentils, nuts, or seeds		
L	Cheese, yogurt, or other milk products		
M	Any oil, fats, or butter, or foods made with any of these		
N	Any sugary foods such as chocolates, sweets, candies, pastries, cakes, or biscuits		
O	Condiments for flavor, such as chilies, spices, herbs		
		IF all ‘No’ →	Go to
		If at least one	6.2
		‘yes’ →	Go to
		or all ‘DK’	6.3

6.2	Did (<u>NAME</u>) eat any solid, semi-solid, or soft foods yesterday during the day or at night? <i>IF 'YES' PROBE:</i> What kind of solid, semi-solid, or soft foods did (<u>NAME</u>) eat?	Yes.....1 Go back to 6.1 and record foods eaten and continue to 6.3 No.....2 Don't Know.....8	Skip to section 6.4
6.3	How many times did (<u>NAME</u>) eat solid, semi-solid, or soft foods other than liquids yesterday during the day or at night?	Number Of Times..... __ __ Don't Know..... 98	
6.4	Did (<u>NAME</u>) drink anything from a bottle with a nipple yesterday during the day or night?	YES..... 1 NO..... 2 DON'T KNOW 8	

Thank you for your participation

End time: _____

Duration: _____

Comments of the Interviewer:

Comments of the data entry operator: