

**Feasibility of field implementation of fortified lentils to improve iron status status  
of adolescent girls in Bangladesh**

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By

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## ABSTRACT

The purpose of this crossover study was to establish methodology and logistics to conduct future large-scale community-based efficacy studies. A crossover trial was carried out in which adolescent girls consumed fortified lentil consisting of 2 different cooking preparation styles (thick vs thin) and 3 different lentil 'portion size' of raw weight 25 g, 37.5 g and 50 g. The cooked lentils were served 5 days a week over 12 weeks mixed with a standard cup (250 mL) of cooked rice among 100 adolescent girls. Small cotyledon lentil (iron 75-90 ppm) were fortified using NaFeEDTA iron fortificant solution with 1600 ppm of Fe at the Crop Development Centre (CDC), University of Saskatchewan. Visual Analog Scales (VAS) were used to measure the rating of hunger, satiety, and palatability before the serving of the meal, after one bite of the meal and after finishing the meal. In-depth interviews and focus group discussions were carried out with the participants' mother/guardian to evaluate acceptability and lentil preparation styles at the end of the trial. Mean (SD) age and age of menarche of the adolescent girls were 12.85 (2.00) and 12.06 (0.94) years, respectively. Fifty-four percent heard about iron deficiency anemia, and 48% were of the opinion that eating more iron-rich foods could prevent anemia. The results showed that thick preparation of cooked lentil at 37.5 g portion size had higher positive scores in all VAS variables compared to the thin preparation. Considering the raw amount served, the thick preparation of lentil at 37.5 g would provide 6.9 mg Fe/d. This would cover approximately 86% (and 66% who have started to menstruate) and 46% of the RDA for adolescent girls aged 9-13 years and 14-18 years, respectively. A thick preparation of cooked lentil at 200 g (37.5 g raw lentil amount) would be preferable for a fortified lentil intervention in future human efficacy trials.

**Keywords:** Fortification, Iron, Adolescents, Iron Deficiency Anemia (IDA), Community-based trial, Lentils, Dietary Intake, Study Design.

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## LIST OF ABBREVIATIONS

WHO	World Health Organization
FAO	Food and Agriculture Organization of the United Nations
EDTA	Ethylene Diamine Tetra-acetic Acid
NaFeEDTA	Sodium Ferrous Ethylene Diamine Tetra-acetic Acid
PMTDI	Provisional Maximum Tolerable Daily Intake
JECFA	The Joint FAO/WHO Expert Committee on Food Additives (JECFA)
IDA	Iron Deficiency Anemia
SK	Saskatchewan
AC	Adolescent Club
VAS	Visual Analog Scale
FGD	Focus Group Discussion
IDI	In-Depth Interviews
MUAC	Mid-Upper Arm Circumference
CDC	Centers for Disease Control and Prevention
RDA	Recommended Dietary Allowance
ICDDRDB	The International Centre for Diarrhoeal Disease Research, Bangladesh
UNICEF	The United Nations Children's Fund
GAIN	Global Alliance for Improved Nutrition
GIFS	Global Institute for Food Security
USAID	The United States Agency for International Development
HNPP	Health, Nutrition and Population Program
UofS	University of Saskatchewan
GoB	Government of Bangladesh
JPGSPH	James P Grant School of Public Health
SD	Standard Deviation
SEM	Standard Error of the Mean

## **CHAPTER 1: INTRODUCTION**

### **1.1 Background**

Fortification is commonly understood as the process of adding nutrient or non-nutrient bioactive components to food products in order to minimize or prevent wide-spread nutrient inadequacies (Dwyer et al., 2015). It has been adopted by many countries including high-, middle- and-low income countries and played an important role in improving nutrient deficiencies at a large scale (Darnton-Hill and Nalubola, 2002; Dwyer et al., 2015). As a part of a food-based approach, the World Health Organization (WHO) recognized fortification as a valid technology for reducing micronutrient malnutrition and it is likely to have benefits for combating micronutrient malnutrition (Tontisirin, Nantel and Bhattacharjee, 2002; WHO & FAO, 2006). It is considered as one of the most attractive and widespread solutions for introducing missing micronutrients to vulnerable groups (Briend, 2001). Micronutrient fortification with iron (Fe), vitamin A and zinc is cost-effective due to its low production cost and significant economic profit. It significantly reduces infant and child deaths (Horton, 2006). The most common food fortificants are iodine, iron (Fe), vitamin A, vitamin D, vitamin E, vitamin C, and B-complex vitamins (FAO, 1995).

Micronutrient deficiency is a form of malnutrition, a recognized food security and public health problem in many developing countries. World Health Organization statistics reported around 800 million people are chronically undernourished (WHO, 2016b), and around 165 million children under 5 years of age are stunted (Black et al., 2013). Among other nutritional deficiencies, Fe deficiency anemia (serum ferritin <12.0 µg/L) is the most common nutritional disorder in the world. It affects more than 2 billion people around the globe, however in recent years the prevalence of iron deficiency has declined but still it remains one of the main cause of anemia (McLean, Cogswell, Egli, & Wojdyla, 2009). Africa and Asia continent jointly contributed more than 40% of iron deficiency among children under 5 and pregnant women in the world (Black et al., 2013). This is more prevalent in food-insecure households in the developing countries (Tontisirin et al., 2002). Iron deficiency anemia was considered one the top ten risk factors for disease by the World Health Organization (WHO, 2002). Chronic blood loss within the body such as peptic ulcer disease, colorectal cancer etc, lack of iron in the daily diet, intestinal disorder that inhibits iron absorption in the circulation, and pregnancy are the major causes of the iron deficiency anemia (Mayo Clinic, n.d.-b). It is reported that iron deficiency is the major cause of anemia; however, deficiencies of vitamin B12 and folate, and chronic infection or inflammation is also responsible for developing different forms of anemia or could contribute to the severity of anemia (Siu, 2015). Other forms anemia includes aplastic anemia (due to bone marrow damage), pernicious anemia (due to vitamin B12 and folate deficiencies), hemolytic anemia (unable to make and replace new red blood cells due to problems with the genes that control red blood cells), sickle cell anemia (due

to the genetically defective form hemoglobin that makes irregular red blood cells to die prematurely), anemia of chronic disease (inhibit the production of red blood cell due cancer, HIV/AIDS, kidney diseases etc.), thalassemia (inherited blood disorder due to formation of abnormal forms of hemoglobin) and malarial anemia (due to the ejection of red blood cells from the blood circulation followed by reduced production of red blood cell in the bone marrow) (Mayo Clinic, n.d.-a; National Heart Lung and Blood Institute, n.d.). Iron deficiency anemia (IDA) adversely affects adult work capacity, impedes children and adolescents cognitive development, and impairs visual and auditory functioning (Algarin, Peirano, Garrido, & Pizarro, 2003; Halterman, Kaczorowski, Aligne, & Auinger, 2001; Hass, 2001; Verdon, Burnand, Stubi, Bonard, Graff, & Michaud, 2003).

Earlier estimated statistics in Bangladesh showed that 40% of adolescents are anemic, and 50% of that is due to iron deficiency (serum ferritin <12 µg/L plus hemoglobin <120 g/L) (Ahmed et al., 2010; Hyder et al., 2007). However, Bangladesh National Nutritional Survey (NNS) recently published results in 2013 using the data from 2011-12 and reported national prevalence of iron deficiency (serum ferritin level <15.0 µg/L) was 3.9% and 9.5% among children of 6-11 years and 12-14 years, respectively. This reported further showed national prevalence of iron deficiency anemia (hemoglobin <11.5 g/dl plus ferritin level <15.0 µg/L in children 6-11 years and hemoglobin <12.0 g/dL plus ferritin level <15.0 µg/L in children 12-14 years) was 1.3% and 1.8%, respectively (ICDDRDB, UNICEF Bangladesh, GAIN, & Institute of Public Health and Nutrition, 2013). Adolescence is generally a nutritionally vulnerable time because of their significant growth, lifestyle and food habit (McNulty et al., 1996). Especially, females are more susceptible to iron deficiency without anemia due to monthly menstrual losses. Despite the inability to identify the exact mechanism in humans, endurance and cognitive performance has been shown to be impaired in laboratory studies of non-anemic women with low Fe stores. However, it is not because of oxygen transport (hemoglobin), but due to tissue oxidative capacity of the muscle, and dopamine biology in the brain (Dellavalle & Haas, 2012b; Hinton, Giordano, Brownlie, & Haas, 2000; Murray-Kolb & Beard, 2007b; Zhu & Haas, 1998a). Daily Fe supplementation is often a problem due to its low compliances, and using food-based methods to increase Fe intake is preferable to improve Fe status if efficacy with alternative methods such as fortification can be proven. One potential solution to combating IDA is fortifying food with iron and it could be the long-term sustainable solution for combating Fe deficiency (Huma, Salim-Ur-Rehman, Anjum, Murtaza, & Sheikh, 2007; Hurrell, 1997). However, identifying the Fe compound is the key to successful implementation of Fe fortification (Hurrell, 2002) and phytic acid is the main inhibitory compound which is widely present in cereal grains and legume seeds such as lentils (Hallberg, Brune, & Rossander, 1989; Hurrell, 2002).

Lentils are generally considered to be nutrient-dense and a good source of Fe. The lentil research and breeding group at the University of Saskatchewan (Uof S) have been engaging in lentil fortification research in the past decade. Research conducted at the University of Saskatchewan has shown that lentils grown in Saskatchewan (SK) province in Canada are rich in Fe, and there is a potential to further increase the Fe level through plant breeding (DellaValle, Thavarajah, Thavarajah, Vandenberg, & Glahn, 2013a; Thavarajah et al., 2011; D. Thavarajah, Thavarajah, Sarker, & Vandenberg, 2009a). Initial investigations profiled the micronutrient levels in existing lentil cultivars being produced in Saskatchewan (SK). SK lentils are rich in micronutrients Fe (73 – 90 mg/kg), Zn (44 – 54 mg/kg), and Se (425 – 673 µg/kg) (Thavarajah et al., 2011; Thavarajah et al., 2009a). Further research showed that phytic acid was naturally low in SK grown lentils compared to the reported low phytic acid mutant wheat (1.24-2.51 mg g<sup>-1</sup> of total phytic acid P) and common bean (0.52-1.38 mg g<sup>-1</sup> of total phytic acid P) which indicates that Fe and Zn from SK lentils would likely have high bioavailability (Thavarajah et al., 2011; D. Thavarajah et al., 2009a; P. Thavarajah, Thavarajah, & Vandenberg, 2009). DellaValle et al., (2013) analyzed the Fe concentration, in vitro Fe bioavailability, and phytic acid concentration of 23 lentil genotypes grown in 5 different locations in SK (DellaValle, Vandenberg, & Glahn, 2013). Significant location effects were observed for Fe concentration, Fe bioavailability, and phytic acid concentration. Negative associations were observed between Fe concentration and phytic acid concentration, Fe concentration and relative Fe bioavailability, and Fe bioavailability and phytic acid content. Lentils are a dietary staple in the Indo-Gangetic plain of South Asia. It is a highly nutritious food, containing high concentrations of Fe and zinc (Zn) relative to many staple grains (Erskine, Sarker, & Kumar, 2011). Therefore, a tremendous opportunity exists for lentil to be part of a whole-food based, sustainable solution to the global micronutrient deficiency problem.

It is therefore, a community-based human efficacy trial is warranted to examine the effect of the iron fortified lentils on the Fe status of adolescent girls in Bangladesh. It could follow a double-blind, community-based, cluster-randomized control trial which will propose to establish novel evidence on the efficacy of iron fortified lentil in improving body Fe status of non-pregnant adolescents of rural Bangladesh. Multiple hematological test such as serum ferritin and soluble transferrin receptor (sTfR), and details dietary intake information along with socio-economic status will assess the attribution of the iron fortified lentils on the adolescents' body iron status. This efficacy trial expects that the supplemental Fe from the iron-fortified lentils will improve body Fe status after controlling for baseline Fe status and dietary Fe intake of adolescent girls. To the best of our knowledge, no study investigated the effect of iron-fortified lentils on the body iron status. The evidence generated by this study will have three potential implications. First, the results of the study will be used to garner support and substantiate large-scale production and market expansion of fortified-Fe lentils by Saskatchewan.

Second, BRAC's- the extensive country-wide programmatic network of BRAC, the largest NGO in the world, has high potential for efficient marketing of high-Fe lentils. Third, the results from this study will contribute to knowledge about how to enhance the Fe status of adolescents and women worldwide in resource-poor settings. However, before carrying out such a trial, an implementation feasibility study was needed.

### **1.2 Research question:**

What is the most feasible intervention and field implementation strategy for future human efficacy trials using fortified lentils to improve the Fe status in adolescent girls in Bangladesh?

### **1.3 Statement of purpose:**

The study aims to establish methodology and logistics to conduct a large-scale community-based efficacy study in adolescent girls in Bangladesh.

### **1.4 Study aims:**

The study is designed to determine the following components that are critical for the efficacy study to be conducted in adolescent girls in Bangladesh.

1. To determine the maximal acceptable daily intake of lentils over a 12-week period.
2. To determine a standardized preparation of lentil (to maximize acceptability and compliance).
3. To reveal family- and community-level implications of the proposed long-term diet intervention.
4. To determine the feasibility of lentil distribution through the local BRAC program network.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 Current global practice and challenges of fortification

It is a well-established fact that iron fortification plays a vital role in reducing the iron deficiency burden. Fortification is generally meant to add nutrient or non-nutrient bioactive components to food products with an aim to reduce and/or prevent large-scale nutritional deficiencies (Dwyer et al., 2015). The Codex General Principles for the Addition of Essential Nutrients to Foods amended in 1989, 1991 defined fortification as *“the addition of one or more essential nutrients to a food whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups”* (Codex Alimentarius Commission. General principles for the addition of essential nutrients to foods CAC/GL 09-1987, 1987 (amended 1989, 1991). It would be a beneficial public health intervention if an adequate amount of iron-fortified food is consumed by groups that have a high risk for iron deficiency (Allen, Benoist, Dary and Hurrell, 2006). Although several studies have been conducted on specific iron fortification of different foods in various populations around the world, review studies on iron fortification using common fortificants on various foods are currently limited. Therefore, a thorough literature review including journal articles, reports, and policy briefs on iron fortification was warranted to understand the efficacy of specific iron fortificants on food vehicles in different parts of the world. This literature review examined effective iron fortification, challenges and future directions of iron fortification globally.

Common food fortificants are iodine, iron (Fe), vitamin A, vitamin D, vitamin E, vitamin C, and B-complex vitamins (FAO, 1995). Iron fortification is considered the key towards treatment and prevention of iron deficiency anemia (Miller, 2013). It is suggested that common foods should be the prime focus for iron fortification (Allen et al., 2006). These foods include wheat flour, pasta, rice, milk (both dry and fluid), cocoa products, salt, sugar, sauce (soy and fish), juice, soft drinks, and cereal-based complementary foods. Although each of the food vehicles has different recommended fortificants, ferrous sulfate and ferrous fumarate are most commonly used as they have the highest bioavailability (Allen et al., 2006). The color of the iron fortificant plays an important role in selecting the potential food vehicle particularly for the lightly colored foods because they can alter the appearance of the food (FAO, 1995). There is increasing evidence that Fe-EDTA (Ethylene diamine tetra-acetic acid) complex is a preferable iron fortificant as it remains bioavailable even after interaction with the iron absorbents (FAO, 1995; Martínez-Torres, Romano, Renzi, & Layrisse, 1979). However, NaFeEDTA has some limitations. For instance, while it has been shown to be the most effective compound for iron fortification with cereal and legume-based foods, it is not as effective for other fortificants even though they have high bioavailability. Several clinical trials confirmed its efficiency

as a potential fortificant and no evidence of a toxic effect has been noted to date using suggested amounts of NaFeEDTA (5-10 mg) as a food fortificant (Bothwell & MacPhail, 2004; Hurrell et al., 2004). However, it is suggested to take into account that the total contents of the iron including the host food vehicle iron content as this amount should not exceed the provisional maximum tolerable daily intake (PMTDI) of 0.8 mg/kg body weight/day (as iron) (FAO, JECFA, & WHO, 2010; WHO & JECFA, 2007).

## **2.2 Significance in global and regional context**

Iron deficiency occurs when serum ferritin concentration levels become  $<12 \mu\text{g/L}$  (Cook & Reusser, 1983). It is the most conclusive test to detect iron deficiency (Guyatt et al., n.d.). Iron deficiency anemia occurs when the body's existing amount of iron is insufficient to upkeep production of erythrocytes, and depends on the balance of body's iron intake, stores, and excretion (Miller, 2013). Hemoglobin, one of the most abundant iron-containing proteins in humans, contains more than half of the body's total iron. Anemia as a characteristic traits of iron deficiency, based on the location of hemoglobin in erythrocytes (Andrews, 2008; Miller, 2013). Iron deficiency anemia (IDA) is the most well-known cause of nutritional deficiency around the world (Schneider et al., 2005). Indications of IDA may vary, be non-specific, and may appear only when the body is suffering severe iron deficiency (Akman et al., 2004; Beard & Connor, 2003; Grantham-McGregor & Ani, 2001; Lozoff, 2000; Oski, Honig, Helu, & Howanitz, 1983). The World Health Organization (WHO) estimated, using the data from 1993-2005, among the total global burden (1.6 billion), one fourth (42%) of pregnant women, one third (30%) of non-pregnant women (aged 15 to 50 years), around half (47%) of preschool children (aged 0 to 5 years), and more than one-tenth (12.7%) of men aged  $> 15$  years are suffering from anemia (Benoist, McLean, Egll, & Cogswell, 2008). Study published in 2008 based on data from 1993-2005 estimated that 24.8% of the world's population has been suffering from anemia (Benoist et al., 2008). Among these, iron deficiency anemia contributed more than half (60%) of the global anemia burden and of which 89% of the total burden lies in developing countries (Kassebaum, 2016). This study added that hemoglobinopathies, infections, chronic kidney diseases, gastrointestinal and gynaecologic conditions are the most common causes of anemia, and that preschool children and women of reproductive age are particularly affected by anemia.

In Bangladesh, using data from 2011-2012, 26% of non-pregnant non-lactating women and 17.1% children aged 12-14 years are anemic ( $<12.0 \text{ g/dL}$ ) and women and children (12-14 years) residing in the rural areas had higher prevalence of 27.4% and 18.1%, respectively. National prevalence of iron deficiency (serum ferritin level  $<15.0 \mu\text{g/L}$ ) reported 7.1% and 9.5% among non-pregnant non-lactating women and children aged 12-14 years. Rural non-pregnant non-lactating women and children



(12-14 years) contributed 6.7% and 10%, respectively. This report estimated that non-pregnant non-lactating women (4.8%) and children aged 12-14 years (1.8%) are suffering from iron deficiency anemia (Rahman et al., 2016). Endurance and cognitive performance are impaired in laboratory studies of non-anemic women with low Fe stores likely due to tissue oxidative capacity of the muscle, and dopamine biology in the brain and not because of decreased oxygen transport (hemoglobin), (Dellavalle & Haas, 2012a; Murray-Kolb & Beard, 2007a; Zhu & Haas, 1998b). Food-based methods that is fortification to increase micronutrient intake is preferable to improve nutrition status as it requires the less active involvement of the targeted group whereas compliance with daily Fe supplementation needs extra attention in resource poor-communities, especially in low-income households. (Davidsson & Nestel, 2004; Thavarajah et al., 2011; Thavarajah, Thavarajah, Sarker, & Vandenberg, 2009b). Menstruating and pregnant women including the intra-uterine fetus are the highest risk group for IDA and in poor resource settings, it is recommended that iron should be provided with other micronutrients in order to reduce the burden of anemia (Lemaire et al., 2011). To mitigate iron deficiency, four strategies - dietary diversification, breeding and genetic engineering to increase iron concentration, iron fortification of industrially manufactured foods, and iron supplementation with pharmaceutical doses, have been advocated (Hurrell et al., 2010). Food-based approaches such as dietary diversification and food fortification along with the nutritional education should be included in public health programs to increase micronutrient intake. Micronutrient supplementation provides a much faster improvement in the nutritional status, however, food fortification may be slower while having a much wider and more sustainable effect, and should be regarded as an integrated approach to tackle micronutrient deficiencies (Allen et al., 2006). Furthermore, in a systemic review, iron fortification significantly increased the haemoglobin level of women of reproductive age including pregnant women (Das, Salam, Kumar, & Bhutta, 2013a). Other than the numerous health benefits of iron fortification, it is also a cost-effective intervention. Based on the data from Venezuela, a unit fortification cost of iron fortification per person is US\$ 0.12, whereas the economic benefit of that patient in the population is US\$ 4.04; the study concludes that this cost-effective intervention may reduce the iron deficiency anemia prevalence by 24% (Allen et al., 2006).

### **2.3 Successful iron fortification projects and key challenges**

Past research has established the fact that iron fortification can improve body iron level and therefore could reduce iron deficiency anemia. Table 1 compiles efficacy trials conducted in different parts of the world using varying iron fortificants, food vehicles, and populations. The selection criteria of this review matrix were limited to human efficacy trials using various iron fortificants that have been published in scientific journals. An efficacy trial conducted in Vietnam showed that consumption of fish sauce fortified with NaFeEDTA (10 mg Fe/d) significantly enhanced body iron levels at a dose of

10 mL/day for a period of 6 months in anemic Vietnamese women when compared to those who did not consume fortified fish sauce (Ho, 2005; van Thuy et al., 2003, 2005). In a randomized trial conducted in South Africa, male and female participants ( $\geq 10$  years) consumed curry powder fortified with NaFe(111)-EDTA for 2 years. This method was found to be effective in reducing iron deficiency anemia (Ballot, Macphail, Bothwell, Gillooly, & Mayet, 1989). Another study based in South Africa used brown bread fortified with NaFeEDTA, electrolytic iron, and ferrous fumarate, but neither of the fortificants affected iron status (van Stuijvenberg, Smuts, Lombard, & Dhansay, 2008). In China, several iron fortification trials have been conducted using electrolytic iron, FeSO<sub>4</sub>, and NaFeEDTA in soy and fish sauce and found increased haemoglobin levels, and reported NaFeEDTA as more effective than FeSO<sub>4</sub> and elemental iron (Chen, Zhang, Yin, & Piao, 2005; Sun et al., 2007). In Guatemala and Morocco, iron fortification was done in sugar and salt and both studies concluded significant reduction of iron deficiency anemia (Viteri et al., 1995; Zimmermann et al., 2003). Studies in Vietnam, Kenya, Thailand, and Kuwait using different iron fortificants, found a significant increase of iron store due to their intervention (Andang'o et al., 2007; Biebinger et al., 2009; van Thuy et al., 2005; Zimmermann et al., 2005). A series of successful iron fortification studies with different food vehicles in different countries were listed in the Mannar & Gallego's (2002a) published article. They included that school children who consumed iron-fortified rice for 6 months had increased haemoglobin in the Philippines. In Caracas, Venezuela, precooked wheat flour fortified with iron (a mix of ferrous fumarate and electrolytic iron) was served to school children of aged 7, 11, and 15 years for 1 year. The study reported a substantial reduction of the prevalence of iron deficiency (37% in 1992 to 16% in 1994) which was measured by the serum ferritin concentration level in the study population (Layrisse et al., 2002). In China, soy sauce was iron-fortified as it has been consumed commonly by the Chinese population; consumption of iron-fortified soy sauce ranging from 5 mg to 20 mg for 3 months was an effective in treatment of iron-deficiency anemia among the Chinese children. Furthermore, a double-blind, placebo controlled trial found significant reduction in the prevalence of anemia among the high risk all age groups (children and women) after 6 months consumption of iron-fortified soy sauce [as NaFeEDTA, 4 mg/(adult/d)] compared to their control group (Mannar & Gallego, 2002b). In the United States, iron fortification in infant formula was effective in reducing anemia in children under 5 years of age ( Yip, Walsh, Goldfarb, & Binkin, 1987; Fomon, 2001). Wheat and maize flours were iron-fortified in Venezuela, and pre-and-post intervention demonstrated significant reduction of iron deficiency anemia (Layrisse et al., 1996). In Chile, prompt reduction of iron deficiency was observed among infants and young children due to the consumption of iron-fortified (+ vitamin C combined) milk (Hertrampf, 2002; Stekel et al., 1988).

Table 1: Review matrix of iron fortification trials

Authors & year	Study title	Length of the study	Used fortification doses (mg/day)	Food vehicles	Food Fortificants	Target groups	Benefited groups	Country	Key findings (results in Tx vs Ctl sequence)
(Ballot et al., 1989)	Fortification of curry powder with NaFe(III)EDTA in an iron-deficient population: report of a controlled iron-fortification trial.	24 months	7.1mg/day	Curry powder	NaFe (111)-EDTA	Male and Female $\geq 10$ years	Premenopausal women, multipara women, and women with prolonged menstruation	South African of Indian descent	Hemoglobin (g/L): Female: $124.7 \pm 13.9$ vs $132.9 \pm 11.3$ , $P < 0.0001$ Male: $146.9 \pm 16.4$ vs $151.5 \pm 16.7$ , $P < 0.05$ Serum Ferritin ( $\mu\text{g/L}$ ): Female: $12.0 \pm 4.42$ vs $27.0 \pm 10.73$ , $P = 0.0002$ Male: $31.0 \pm 9.106$ vs $56 \pm 20.159$ , $P < 0.05$
(Viteri et al., 1995)	Fortification of sugar with iron sodium ethylenediaminetetraacetate (FeNaEDTA) improves iron status in semirural Guatemalan populations	32 months	1 g FeNaEDTA/kg and 15 mg retinol palmitate/kg	Sugar	FeNaEDTA	Male and Female $\geq 1$ year	All semirural population	Guatemala	Hemoglobin (g/L), and Serum Ferritin ( $\mu\text{g/L}$ ) increased in all age groups, $P < 0.05$ .
(van Thuy et al., 2003)	Regular consumption of NaFeEDTA-fortified fish sauce improves iron status and reduces the prevalence of Anemia in anemic Vietnamese women	6 months	10 mg Fe/d	Fish sauce	NaFeEDTA	Women 17–49 years	Non-pregnant factory workers	Vietnam	Hemoglobin (g/L): $116.3 \pm 8.7$ vs $107.6 \pm 11.0$ , $P < 0.0001$ Serum Ferritin ( $\mu\text{g/L}$ ): 30.9 (95% CI: 23.4, 40.6) vs 14.6 (CI: 11.3, 19.0) g/L, $P < 0.001$ Soluble transferrin receptor (sTfR) (mg/L): 7.2 (CI: 6.4, 7.9) vs 9.0 (CI: 8.1, 9.9), $P < 0.001$
(Zimmermann et al., 2003)	Dual fortification of salt with iodine and microencapsulated iron: a randomized, double-blind, controlled trial in Moroccan schoolchildren.	9 months	1 mg iron/g salt	Salt (bread, fava beans)	Ferrous sulfate hydrate encapsulated with partially hydrogenated vegetable oil	Male and female 6-15 years	Children	Morocco	Hemoglobin (g/L): $127 \pm 12$ vs $116 \pm 12$ , $P < 0.05$ Serum Ferritin ( $\mu\text{g/L}$ ): $40 \pm 25$ vs $17 \pm 12$ , $P < 0.05$ Soluble transferrin receptor (sTfR) (mg/L): 6.5 (3.0–15.3) vs 8.9 (3.8–118.0), $P < 0.05$

(Chen, Zhang, Yin, & Piao, 2005)	Studies on the effectiveness of NaFeEDTA-fortified soy sauce in controlling iron deficiency: a population-based intervention trial	18 months	29.6 mg Fe/100 mL	Soy sauce	NaFeEDTA	Male and female $\geq 3$ years	All age and sex subgroups	China	Hemoglobin (g/L): Fortified group- Baseline vs Endline ( $P < 0.05$ ) of all age groups and by sex. Serum Ferritin ( $\mu\text{g/L}$ ): Fortified group- Baseline vs Endline ( $P < 0.05$ ) of all age groups and by sex.
(van Thuy et al., 2005)	The Use of NaFeEDTA-Fortified Fish Sauce Is an Effective Tool for Controlling Iron Deficiency in Women of Childbearing Age in Rural Vietnam	6, 12, and 18 months	9 mmol (500 mg) Fe/L	Fish sauce	NaFeEDTA	Non-pregnant woman between 16 and 49 years	Non-pregnant woman	Vietnam	Hemoglobin (g/L): 125.6 (CI 124.4-126.8) vs 131.1 (CI 129.5-132.7), $P < 0.05$ Serum Ferritin ( $\mu\text{g/L}$ ): 29.8 (CI 24.8-35.7) vs 66.1 (CI 52.0-84.1), $P < 0.001$
(Zimmermann et al., 2005)	Comparison of the efficacy of wheat-based snacks fortified with ferrous sulfate, electrolytic iron, or hydrogen-reduced elemental iron: randomized, double-blind, controlled trial in Thai women	8 months	12 mg Fe/d for 6 d/wk for 35 wk as ferrous sulfate, electrolytic iron, or hydrogen-reduced iron	Wheat flour baked snacks	Ferrous sulfate	Female 18-40 years	Women	Thailand	Hemoglobin (g/L): 121 $\pm$ 12 vs 125 $\pm$ 9 Serum Ferritin ( $\mu\text{g/L}$ ): 13 (CI 3-69) vs 29 (CI 4-109) Soluble transferrin receptor (sTfR) (mg/L): 5.8 (2.1-17.8) vs 4.5 (CI 1.7-10.3)
(Sun et al., 2007)	Effects of wheat flour fortified with different iron fortificants on iron status and Anemia prevalence in iron deficient anemic students in Northern China.	6 months	Electrolytic iron 60 mg Fe/kg, FeSO <sub>4</sub> 30 mg Fe/kg and NaFeEDTA 20 mg Fe/kg	Wheat flour	Electrolytic Iron, FeSO <sub>4</sub> and NaFeEDTA	Male and Female 11-18 years	Anemic students	China	Hemoglobin (g/L): 14.9 $\pm$ 8.0 vs 132.4 $\pm$ 10.2, $P < 0.01$ Serum Ferritin ( $\mu\text{g/L}$ ): 46.0 $\pm$ 20.5 vs 60.0 $\pm$ 24.5, $P < 0.01$ Soluble transferrin receptor (sTfR) (mg/L): 35.7 $\pm$ 1.2 vs 23.0 $\pm$ 1.2, $P < 0.01$
(Andang'o et al., 2007)	Efficacy of iron-fortified whole maize flour on iron status of schoolchildren in Kenya: a randomised controlled trial	5 months	High dose NaFeEDTA (56 mg/kg), low-dose NaFeEDTA (28 mg/kg),	Maize flour	NaFeEDTA and electrolytic iron	Male and Female 3-8 years	School going children	Kenya	Hemoglobin (g/L): 117.2 $\pm$ 8.5 vs 115.7 $\pm$ 9.7 Serum Ferritin ( $\mu\text{g/L}$ ): 23.0 (CI 16.0-36.0) vs 35.0 (CI 24.5-47.0)

			or electrolytic iron (56 mg/kg).						Soluble transferrin receptor (sTfR) (mg/L): 2.5 (CI 2.1-3.1) vs 2.3 (CI 1.9-2.7)
(Le et al., 2007)	The effect of NaFeEDTA on sensory perception and long-term acceptance of instant noodles by Vietnamese school children	2.5 months	10.7 mg iron per 52 g of noodles	Noodles	NaFeEDTA	children (6–8 years) and adults (17–30 years)	Children and adults	Vietnam	NaFeEDTA fortified noodles can be suitable food vehicle for food fortification programme
(van Stuijvenberg et al., 2008)	Fortifying brown bread with sodium iron EDTA, ferrous fumarate, or electrolytic iron does not affect iron status in South African schoolchildren	8 months	2.35 mg iron as NaFeEDTA; 4.70 mg iron as ferrous fumarate; and 8.30 mg iron as electrolytic iron	Brown bread	NaFeEDTA, electrolytic iron and ferrous fumarate	Male and Female 6-11 years	School children	South African	Hemoglobin (g/L): Effect (estimated marginal means difference) -0.9 (CI -2.8, 1.0) Serum Ferritin ( $\mu\text{g/L}$ ): Effect (estimated marginal means difference) 0.49 (CI -3.9, 4.9) Soluble transferrin receptor (sTfR) (mg/L): Effect (estimated marginal means difference) -0.16 (CI -0.6, 0.2)
(Biebinger et al., 2009)	Efficacy of wheat-based biscuits fortified with microcapsules containing ferrous sulfate and potassium iodate or a new hydrogen-reduced elemental iron: a randomized, double-blind, controlled trial in Kuwaiti women	6 months	10.2 (SD 0.9) mg per snack of 25 g for the encapsulated FeSO <sub>4</sub> biscuits	Wheat flour biscuits	Encapsulated ferrous sulfate	Female 18-35 years	Female post graduate college students	Kuwait	Hemoglobin (g/L): 131 $\pm$ 10 vs 131 $\pm$ 9 Serum Ferritin ( $\mu\text{g/L}$ ): 10.5 $\pm$ 6.5 vs 19.8 $\pm$ 10.5, P < 0.001 Soluble transferrin receptor (sTfR) (mg/L): 7.5 $\pm$ 4.9 vs 6.0 $\pm$ 1.5, P < 0.01
(Ziegler, Nelson, & Jeter, 2009)	Iron status of breastfed infants is improved equally by medicinal iron and iron-fortified cereal	23 months	7.0–7.5 mg	Medicinal iron (FeMed), Iron fortified infant	Ferrous sulfate	Infants 1-24 months, significant results found at 9 months.	Breastfed infants	United States	Hemoglobin (g/L): 116 $\pm$ 7 vs 115 $\pm$ 8 Serum Ferritin ( $\mu\text{g/L}$ ): 28 $\pm$ 17 vs 48 $\pm$ 32, P < 0.005 Soluble transferrin receptor (sTfR) (mg/L): 6.14 $\pm$ 1.17 vs 5.91 $\pm$ 1.05, P < 0.01

				cereal (FeCer)					
(Lozoff et al., 2012)	Iron-Fortified vs Low-Iron Infant Formula: developmental outcome at 10 years	6 months (age from 6-12 months)	Iron-fortified (mean, 12.7 mg/L) or lowiron (mean, 2.3 mg/L)	Infant formula as the supplementation vehicle	infant formula	Infants	Full-term infants	Chile	Iron-fortified (effect size: rangng from -0.096 to -1.25, P<0.05) could adversely affect infants' long-term development

Key challenges in fortification efforts exist for both interventionists and consumers. Challenges for interventionists include implementation challenges, the capacity of the country and/or institute to follow the standard protocol of iron fortification. Consumers, lack of knowledge, awareness, and perception either positive or negative contributed the most. The World Health Organization (WHO) recommended that each member country should follow certain steps before implementing any iron fortification nation/region-wide public health intervention (Allen et al., 2006). Firstly, each country should quantify the daily iron intake of the risk group for iron deficiency. Secondly, estimation of the bioavailability of iron from the diet that is commonly consumed should be determined. Thirdly, estimated iron intake should be compared with bioavailability with iron requirements (based on dietary iron bioavailability). Fourthly, deficit amount of iron in the diet should be calculated. Finally, the calculated amount of iron should be added to the daily mean potential food fortificant consumption of the predetermined risk groups. The key challenge of iron fortification, as well as fortification in general, is that very few countries in the world have the capability to follow this procedure (Allen et al., 2006).

Another study portrayed a successful and sustainable fortification program from a different point of view. In their pyramid-shaped model, Mehansho (2002) highlighted that identification of the deficiencies should be in the bottom of the pyramid followed by the development of the fortification technology, efficacy evaluation of the fortified foods (Mehansho, 2002). In the top of the pyramid, two tasks should be undertaken simultaneously, i.e production, distribution and marketing in one arm, and education, social engagement, and marketing in another arm (Mehansho, 2002). Another study mentioned that finding a suitable iron fortificant for a particular food vehicle remains a challenge (van Thuy et al., 2003), since the nature of changing color raising questions on NaFeEDTA and ferrous fumarate as iron fortificants, although they are recommended and commonly used as iron fortificants (van Stuijvenberg et al., 2008). Effectiveness of iron-fortified food vehicle oriented randomized trial should be rigorously evaluated under realistic conditions before the scale-up iron-fortification program at the national level (Van Thuy et al., 2003).

Other challenges include the perception of the consumers for whom iron fortification initiatives were taken. Nutritional knowledge along with focused nutritional education is positively associated with the perception of iron-fortified food (Pounis et al., 2011). A similar study in China suggested that the improvement of nutritional knowledge on iron fortification is crucial for a successful iron fortification program (Sun, Guo, Wang, & Sun, 2006). A study based in India found that pregnant women and children residing in rural areas have less awareness on consuming targeted fortified foods than the urban residents (Nagaraj, Yousuf, & Ganta, 2013).

Multiple studies showed that iron fortification has an incremental effect on the body's iron status. These studies have been conducted in different study context, using altered food fortificants in

various food vehicles among different age groups regardless gender. Each country has its own mandatory fortification strategies and they implement accordingly. Regarding iron fortification, there are two contrary things that could make iron fortification a complex issue. On one hand, there is a demand of iron fortification particularly for the high-risk group, i.e., children, adolescents and women in order to reduce the iron deficiency burden in a country (Das, Salam, Kumar, & Bhutta, 2013b). On the other hand, spontaneous withdrawal of existing iron fortification could increase the prevalence of iron deficiency which eventually will exacerbate the burden of iron deficiency. Some evidence suggests that withdrawal of iron fortification (mandatory) could adversely affect iron status. In Denmark, a mandatory iron fortification in wheat flour was withdrawn in 1987 which resulted in decreased serum ferritin status among blood donors (Milman, Byg, Ovesen, Kirchhoff, & Jurgensen, 2002). Sweden experienced that same, i.e., prevalence of iron deficiency anemia increased 20% in the high risk group within 6 years of closing its mandatory iron fortification program (Leif Hallberg & Hulthén, 2002).

A small number of studies did not find any positive response of iron fortification. The reason for this rather contradictory result is not entirely clear even though each study was conducted using iron deficiency risk groups with recommended doses. Inconsistent research findings in a same topic threaten its validity, therefore influence in decision making (Jones, Keil, Holland, Caughey, & Platts-Mills, 2015). One of the potential reasons could be the limitation of controlling the confounding factors. For example, a study in South Africa did not find any effect of iron fortification using 3 different fortifications in brown bread (van Stuijvenberg et al., 2008). They highlighted their limitations as not having the option of controlling the food intake at home as well as respondents' usual school feeding practices (van Stuijvenberg et al., 2008). Another possibility may be that all studies found a positive effect of iron fortification on iron status and were published. On the contrary, it is also possible that there might be an existence of bias towards positive results. It is a well-known perception that positive results, effects or significant attribution to the outcome variable are more likely to be published in a journal (Dwan et al., 2008). Another dimension could be that the public health program interventions are not evaluated properly. Guidelines for iron fortification of cereal food staples reported that not all iron fortification programs are evaluated which limit our knowledge on this issue (SUSTAIN, 2011). The guideline furthermore suggested that efficacy and effectiveness of iron fortification are urgently needed to reduce the knowledge gap in this issue (SUSTAIN, 2011). In recent years, all randomized trials are suggested to register the trial, i.e., authors must report their results whether positive or negative (DeAngelis et al., 2004).

The Food and Agriculture Organization (FAO) of the United Nation recommended that a comprehensive surveillance program should be designed to track the impact of iron fortification, since not all age groups have an equal demand of body iron regardless of gender. For instance, menstruating



women and children would not be the concern if the iron overload occurs as they require much higher iron than the adult male (FAO, 1995). There is a need for investigating the long-term effect of unnecessary extra iron intake for the adult male population as the consumption of iron-fortified staple foods are usually not restricted to a particular and/or high-risk groups. Therefore, determining the optimal amount for iron fortification of food is warranted (FAO, 1995). Related to this, a decade-long trial on iron-fortified or low-iron formula in infants in Chile reported the long-term adverse effect on the infants who received 12.7 mg/L of iron-fortified formula compare to those who did not get iron fortified formula and suggested that determining the iron amounts in infant formula require further study (Lozoff et al., 2012). A nutrition-related educational campaign was recommended in order to clarify the misconception on iron fortification within the community (Truzyan, Crape, Petrosyan, & Grigoryan, 2011), and there is need for nutritional counselling to the targeted group, particularly for cooking process and frequency along with quantity in order to fulfil the purpose of the fortification (Nakkeeran N, Chaturvedi A, Bhagwat S, Sankar R, & Patel R., 2015).

Iron fortification in various food is a food-based approach that could potentially increase iron levels and consequently could reduce iron deficiency anemia. Challenges of implementing iron fortification programs exist due to issues such as the country's and/or other stakeholders' capacity and community engagement. These areas need to be given high priority by countries wherein iron deficiency is prevalent in order to reduce the iron deficiency burden and to sustain an iron sufficient population.

#### **2.4 Iron fortification strategies- rationale, challenges and alternatives for Bangladesh**

Recent statistics showed that Bangladeshi women and children aged 12-14 years have high prevalence of anemia (26% and 17.1% respectively (Rahman et al., 2016). The national prevalence of anemia was reported as 7.1% among non-pregnant non-lactating women and 9.5% in children aged 12-14 years. However, members of the population who live in rural areas experience much greater incidences of iron deficiency (27.4% non-pregnant non-lactating women and 18.1% children aged 12-14 years) (Rahman et al., 2016). This rural subset makes up a large portion of the total Bangladeshi population, therefore it would be reasonable to assume that Bangladesh is in need of urgent attention to reduce this burden.

In order mitigate the iron deficiency burden, food fortification has been implemented within a few food vehicles in Bangladesh although fortification is not a new concept globally. In 1999, a trial fortification program was initiated by The United States Agency for International Development (USAID) Micronutrient Program, to understand the usefulness of known technologies that are appropriate to the local food industry and market. Based on the evidence, white flour was suggested as a potential food vehicle to increase vitamin A and iron among Bangladeshi women and children (The

USAID Micronutrient Program, 2003). In Bangladesh, white flour was fortified with multiple micronutrient agents such as vitamin A, iron, zinc, B1, B2, niacin and folic acid (The USAID Micronutrient Program, 2003). Given the limited evidence on iron fortification, it was warranted to summarize the rationale of iron fortifications, implementation challenges and alternative food-based approaches for Bangladesh.

#### **2.4.1 Iron fortificants suitable for Bangladesh**

Iron supplementation may not be a feasible option for countries like Bangladesh as previous evidence suggested that it requires extra attention in resource-poor settings because its effect depends on the local factors such as the prevalence of diarrhoea, infections and other dietary factors (WHO, 2001). However, iron fortification may be a good option for Bangladesh as it requires the less active involvement of the targeted group than iron supplementation (Davidsson & Nestel, 2004; Thavarajah et al., 2011; D. Thavarajah et al., 2009b). Evidence suggests that iron fortification with NaFeEDTA could increase body iron stores (Ballot et al., 1989; Biebinger et al., 2009; Lozoff, 2000; van Thuy et al., 2003, 2005; Viteri et al., 1995; Zimmermann et al., 2003). Bangladesh may benefit greatly from iron fortification due to the large portion of the population that is iron deficient, particularly children and women (Rahman et al., 2016).

Choosing an appropriate food vehicle is a difficult task. It is preferable that the food vehicle is already iron-rich and commonly consumed by the target population. Dietary iron sources may be designated as non-heme iron from plant sources and heme iron from animal sources. Due to an inability to fortify animal sources, plant sources such as grain, legumes such as dried beans, chickpea, cowpea, and lentils can be the preferred choice for food vehicles because these plant sources are reportedly rich in iron (Dietitians of Canada, 2016; Government of Canada, Health Canada, Health Products and Food Branch, Food Directorate, Bureau of Nutritional Sciences, 2016; Iqbal, Khalil, Ateeq, & Sayyar Khan, 2006; Quinteros, Farré, & Lagarda, 2001). However, non-heme iron is not as bioavailable as its heme counterpart. Only approximately (1-5%) of plant-based iron is digested within our body due to compounds which inhibit iron absorption such as phytates (inositol-hexaphosphate), polyphenols, and calcium ions (Hunt, 2003). Therefore, any of the grain legumes may be ideal for iron fortification using EDTA as it remains bioavailable even after interaction with these inhibiting compounds (Allen et al., 2006; FAO, 1995; Martínez-Torres et al., 1979). Multiple studies confirmed NaFeEDTA's efficiency as an iron fortificants and no direct toxic effect (within the limit of NaFeEDTA (5-10 mg)) has been reported so far (Bothwell & MacPhail, 2004; Hurrell et al., 2004). Considering the fact of the global evidence, Bangladesh may choose to fortify grain legumes using NaFeEDTA as an iron fortificants to reduce the iron deficiency burden.

Iron fortification presents several possible limitations. Allen, Benoist, Dary, & Hurrell (2006)

highlighted limitations of food fortification which are also applicable for iron fortification and relevant to the current situation in Bangladesh. They stated that the poorest economic segment often remains out of reach to fortified food because of their low purchasing power and faulty distribution settings, although they need the intervention. Choosing the right food vehicle also requires special attention as this economically disadvantaged group may not be able to afford and/or consume foods other than the staple food within recommended amounts (Allen et al., 2006).

#### **2.4.2 Alternatives to iron fortification**

Due to the limitations of interventions such as supplementation, fortification, and diversification, biofortification has been proposed as an a possible long-term, sustainable alternative (Combs, Duxbury, & Welch, 1997; Pfeiffer & McClafferty, 2007; Welch, 2002; White & Broadley, 2005; Zhu et al., 2007). It may be because that traditional dietary interventions such as supplementation, fortification, and diversification efforts have been unsuccessful (White & Broadley, 2005). Given all the available evidence, it is, therefore, reasonable to assume that biofortification may be the better option for Bangladesh. It is defined as “.....*process by which the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or modern biotechnology*” (WHO, 2016a). As of 2010-2011, more than half (57.41%) of the total land of Bangladesh is used for crop production and that creates a great potential for Bangladesh to adopt biofortification to reduce malnutrition (Hasan, Hossain, Bari, & Islam, 2013). Furthermore, biofortification is a food-based approach that is primarily targeted for rural areas. This will not only reduce the burden of malnutrition of Bangladesh but also help to continue the nutritional well-being (Meenakshi et al., 2010; Singh, Praharaaj, Chaturvedi, & Bohra, 2016). In addition, given the resource-poor setting of the target population, it would be a practically adoptable initiative for Bangladesh as the investment of time and money for biofortification is only required during the research and development stages (Frossard, Bucher, Machler, Mozafar, & Hurrell, 2000; Nestel, Bouis, Meenakshi, & Pfeiffer, 2006). Studies suggest that various cereals, such as rice, and legumes, such as lentils, have high potential for biofortification because of their high iron content and low phytate content which increase iron and zinc absorption in the body (Beyer et al., 2002; Lucca, Hurrell, & Potrykus, 2002; Ye et al., 2000). Furthermore, Hossain & Mohiuddin, (2013a) reported that biofortification of Bangladeshi staple foods, including rice in particular, with Fe, Zn, and Vit-A had great potential in reducing micronutrient deficiency among the rural residents. Biofortification of lentils could be the potential solution for combating high rates of iron deficiency anemia in Bangladesh. Lentils are a dietary staple food for Bangladesh and contain a high amount of iron and zinc compared to the other staple foods which make them an ideal food vehicle (Erskine et al., 2011). A study was conducted in 5 different locations in Saskatchewan, Canada to evaluate the iron concentration, *in vitro* Fe bioavailability, and phytic acid

concentration of 23 lentil genotypes (DellaValle, Thavarajah, Thavarajah, Vandenberg, & Glahn, 2013b). The study concluded that due to the variability of iron concentration, relative iron bioavailability, and phytic acid content, lentils have high biofortification potential (DellaValle et al., 2013b).

Challenges includes on iron biofortification in Bangladesh is the limited evidence-based data, although there are a few recent biofortification experiments on rice with Zn and found successful (“Bangladesh Releases New Improved Zinc Rice Variety,” 2015; Bashir & Miah, 2016). For instance, Bangladesh Rice Research Institute first developed Zn enriched rice variety (BRRI dhan 62) in 2013 which contains around 20-22 mg Zn kg<sup>-1</sup> (S. M. Hossain & Mohiuddin, 2013b). To the best of our knowledge, no study has been found that investigated iron biofortification in Bangladesh.

## **2.5 Iron fortification strategies- rationale, challenges and alternatives for Canada**

The need and demand for food fortification in Canada vary from province to province and is based on many factors such as sunlight exposure, climate, soil biogeochemistry and population size (Institute of Medicine; Food and Nutrition Board; Committee on Use of Dietary Reference Intakes in Nutrition Labeling, 2003). The purpose of this short report is to summarize successful food fortification attempts, challenges in fortification efforts, and alternatives to combat the iron deficiency burden in Canada. Several research and leading health institutes advocated that iron fortification could play a key role in reducing iron deficiency anemia (Allen et al., 2006; Briend, 2001; Tontisirin et al., 2002; WHO & FAO, 2006). Unlike other countries, Canada has its own fortification legislation to reduce the iron deficiency burden among the Canadian population. The Canadian Food Inspection Agency created legislation in 2009 of mandatory fortification, where vitamin B, iron and folic acid must be added to white flour. It is directed that in each 100 g white flour, 0.64 milligrams of thiamine, 0.40 milligrams of riboflavin, 5.30 milligrams of niacin or niacinamide, 0.15 milligrams of folic acid, and 4.4 milligrams of iron should be added to enrich the white flour (Canadian Food Inspection Agency, 2016).

### **2.5.1 Iron fortificants suitable for Canada**

Research suggests that iron fortification using Sodium Ferrous Ethylene Diamine Tetraacetic Acid (NaFeEDTA) fortificants may lead to success in reducing iron deficiency anemia, particularly for high risk populations (Ballot et al., 1989; Biebinger et al., 2009; B Lozoff, 2000; van Thuy et al., 2003, 2005; Viteri et al., 1995; Zimmermann et al., 2003). NaFeEDTA has higher efficacy in increasing body iron status compared to other iron fortificants such as ferrous sulphate and electrolytic iron (Sun et al., 2007). Research undertaken in different parts of the world using rigorous research methods, i.e., randomized control trial among the high-risk groups, i.e., children and women to evaluate the effectiveness of the iron fortification on the body iron status. Mostly white flour and foods made by it (bread, biscuits), maize, noodles, and sauce were used as food vehicles (Andang'o et al., 2007;

Biebinger et al., 2009; Chen, Zhang, Yin and Piao, 2005; J. Sun et al., 2007; van Stuijvenberg et al., 2008; van Thuy et al., 2005; Zimmermann et al., 2005). Given the global evidence, Canada may consider adopting iron fortification using NaFeEDTA on new food vehicles. Although the Canadian Food Inspection Agency (2016) has been implementing mandatory tri-fortification in all products made with white flour, recent statistics report that 86.9% females aged 12-19 years and 90.9% females aged 20-49 years sufficient serum ferritin (Iron sufficiency of Canadians, 2015). This is significantly lower than their counterpart male (Iron sufficiency of Canadians, 2015). Therefore, it may be useful for Canada to devise a new iron fortification intervention using popular foods to reduce iron deficiency in its at-risk population. It would be an important attempt for Canada as this particular female age group suffers most from iron deficiency anemia (Kassebaum, 2016).

Mass fortification may not be feasible for Canada as its male population has already sufficient body iron level (Iron sufficiency of Canadians, 2015). Moreover, there is a chance of overload of iron storage among the male population as they would not require extra iron like the high-risk group (FAO, 1995). This chronic iron overload may pose risk to male Canadians of developing cancer, vomiting and diarrhoea, articular pain, hormonal disturbance, heart disorder, and osteoporosis (Edwards et al., 1988; Schümann, 2001; Sempos, Looker, Gillum, & Makuc, 1994). Targeted iron fortification may be the potential solution in this scenario.

A study in South Africa reported that NaFeEDTA iron fortificants used in bread changed its color even after the low level of fortification (van Stuijvenberg et al., 2008). Other studies supported that color change is an issue for sodium iron ethylenediaminetetraacetate (NaFeEDTA) iron fortificants as it may result in undesirable color and flavour changes in the food vehicle (Alam, Shah, Saleemullah, & Riaz, 2007; Bovell-Benjamin, Allen, Franke, & Guinard, 1999; Hurrell, 2002). Furthermore, it becomes a great concern particularly for the light colored food vehicles that are to be fortified (FAO, 1995). If Canada chose to fortify grain legumes with NaFeEDTA there may not be a noticeable issue with the appearance changing due to the grain legumes naturally darker color. Nonetheless, the combination of fortificants and food vehicles should be acceptable to the target population in order to run a successful iron fortification program (Adelia C. Bovell-Benjamin, & Guinard, 2003). Another challenge is the issue of packaging as it should be designed to maintain the balance of moisture content (below 5%), otherwise, textural deterioration could create a mould (FAO, 1995).

### **2.5.2 Alternatives to iron fortification**

Supplementation and biofortification are two alternatives to increasing iron status in the body. Iron supplementation requires extra caution during implementation and fortification requires less active involvement to the targeted group (Davidsson & Nestel, 2004; Thavarajah, et al., 2011; Thavarajah et al., 2009b). Moreover, the unit cost of iron supplementation is higher than that of iron fortification

(Allen et al., 2006).

## **CHAPTER 3: MATERIALS AND METHODS**

### **3.1 Field set-up logistics**

#### **3.1.1 Study setting:**

The study was implemented in collaboration with BRAC – previously known as Bangladesh Rural Advancement Committee is one of the top ranked most effective multinational non-government organization (NGO) in the world- headquarter based in Dhaka, Bangladesh (BRAC, 2016). It works with the Government of Bangladesh (GoB) to improve the life of the socially disadvantaged people. BRAC approximately reaches 138 million Bangladeshi population out of its total population close to 160 million (BRAC, 2016; Central Intelligence Agency, 2017). It is currently implementing its operation in 14 countries covering South-East Asia, and Africa with offices in the USA and UK. It employs around 125,000 staffs mostly women employed in Asia and African region with the mission to empower people and communities in poverty, illiteracy, disease, and social injustice (BRAC, 2016). Among many development programmes, BRAC has a specific programme for adolescents- named BRAC Adolescent Development Programme which formed adolescent clubs as a part of their project. These clubs targeted all adolescent girls in the community regardless of their school attendance, marital status, pregnancy, or socio-economic status. The clubs provide a unique place and opportunity for adolescents to socialize both in rural and urban settings. Each club comprises of 25-40 adolescents, mainly girls and a few boys member between ages 10 to 19 years. These clubs operate in afternoons in BRAC schools, while in the absence of such facilities, a room is rented locally by BRAC.

#### **3.1.2 Sourcing of lentils:**

CDC Maxim- a widely grown and popular cultivated lentil variety developed at the Crop Development Centre of the University of Saskatchewan, Canada was selected for fortification. Lentils were grown in the farmers field by the farmers in the Saskatchewan province of Canada in 2015. Prairie Pulse Limited- a processing company based in Saskatoon collected the lentils from farmers field in one bulk and dehulled (removing the seed coat) in their processing plant. Lentil fortification was carried out at Saskatchewan Food Industry Development Centre Inc., Saskatoon at a time. Fortification was conducted five months before it shipped to Dhaka. No significant changes on organoleptic attributes of the fortified lentils after one year of fortification was found in laboratory room temperature (Podder et al., 2017). BRAC inspected the lentils every two months in detecting the quality of physical appearance of the lentils. Individual samples, drawn from the clubs contributed to each composite samples at the Area Office (AO) level. AO is the lowest level (below upazila) from where lentils were handed over to the adolescent clubs.

### **3.1.3 Required amount of lentils and shipment**

A total of 225 kilograms of fortified lentils were required for this study. Considering the expected waste during the shipment, local handling errors/wastages, and cooking process, we air-shipped 300 kilograms of fortified lentils to Dhaka in 20 kg bag each. The air-shipped lentils arrived in Dhaka (sent from Canada) on December 12, 2016 and sent to BRAC storage facility in Gazipur after Bangladesh customs clearance at the airport.

### **3.1.4 Lentil fortification with iron**

Lentils were fortified with iron in the lab setting at the Crop Development Centre of the University of Saskatchewan, Canada. We selected small cotyledon lentil because its higher iron (75-90 ppm) content compare to other available lentils. Podder et al., (2017) assessed iron level of all available lentils in the market (polished football, polished splits, unpolished football and unpolished splits) for fortification to identify the best type regards to their uniform iron absorption, drying time and iron concentration. As per the guidelines on food fortification with micronutrients of WHO and FAO, lentils were fortified by spraying a solution of the approved iron fortificant NaFeEDTA (Podder et al., 2017). NaFeEDTA iron fortificant solution with 1600 ppm of Fe were applied by a small sprayer placed at the beginning of the lentils (locally named in Bangladesh as ‘daal’) polishing machine at a commercial lentil mill located near Saskatoon, Canada. The iron solution was applied as a fine mist which is absorbed into the daal as it travels through the polishing drum. As the fortified daal leaves the drum it was bagged in 20 kg food grade water sealed bags. They used colorimetric method to determine the appearance of the lentils after fortification and measured the relative bioavailability by using *invitro* cell culture bioassay. The Fe concentration in the final product was approximately 10 mg of Fe (of which 6.5–7 mg of Fe from the iron fortificant and 3.5 mg from the Saskatchewan grown lentils) of 50 g of fortified lentil (Podder et al., 2017). This will supply the dietary Fe equivalent of a major part of the RDAs depending on gender, age, pregnancy [Recommended Dietary Allowances (RDAs)] for iron - 8 mg (9-50 year old males), 8 mg (females 9-13 years) + additional 2.5 mg/d who have started to menstruate, 15 mg (females 14-18 years) and 27 mg females during pregnancy (Institute of Medicine; Food and Nutrition Board; Committee on Use of Dietary Reference Intakes in Nutrition Labeling, 2003)

### **3.1.5 Pre-trial informal discussion**

We conducted an informal discussion session with the potential adolescent girls to make the intervention culturally appropriate before we served cooked lentil (daal). The topic of the discussion was whether we should include cooked rice (locally known as ‘Baat’) with the daal? If yes, then when should the meal be served? And what portion size of rice should be added with daal? It was because daal is normally consumed with cooked rice. The group discussion included 10-15 adolescent boys and girls in a typical ‘Adolescent Club day’. It was found that adolescent club members come to the club



on the club days regularly. Normally their school ends around 2 pm, and some of them have coaching class 2-4 pm (mostly those who are in class 8-10). Adolescents return home from school around 2:30 pm. Their daily meal pattern described below:

- 6-7 am - Breakfast
- 12:00 pm school break- snacks
- 2:30- 3:00 pm - Lunch.
- 9-10 pm - Dinner
- 5-6 pm – Window of opportunity for intervention

Adolescents studying in class 8-10 arrive at the club in club days around 5 pm. And they return home around 6 pm. In their daily usual intake, adolescents eat daal 2-3 days a week (twice in each day). For example, if daal is cooked at home, they eat the same daal for both lunch and dinner, and they usually eat thick daal. It was noted that all of them are willing to eat daal if we provide them for 12 weeks. During the discussion of adding rice, 3 possible options came out: daal with 2-3 piece of Roti (Bread), or daal with rice or only daal. However, the majority agreed, giving daal with rice would be more appropriate than the other 2 options, regardless thin and thick preparation and the different amount. They reckon, daal tastes good with rice. They reasoned, they eat lunch around 3:00 pm (+/-) and at around 5-6 pm, they feel slightly hungry. Others added that if only daal is provided then first few days they will eat, but it may not be possible to eat daal for 3 months without adding anything. Including rice with daal was considered as a small lunch. With regards to the amount of rice, three different sized bowls were demonstrated. Measurement of the rice was undertaken to the point where the majority supported. To standardize the intervention, a tea cup volume of the cooked rice was added in the cooked daal throughout the intervention regardless the amount and cooking preparation.

### **3.1.6 Standardized daal recipe:**

BRAC Program Organizer (field level manager) asked each mother of adolescent girls residing in the study areas volunteering from the study about a set of instructions for preparing a daal (for thin preparation), including a list of the ingredients required and we set a standardized daal recipe to assess the variation of main ingredients, and especially concentration of lentil, and thus the Fe content. Based on the recipes collected, a home experiment was conducted on 100 g raw lentils at researchers' home to determine the approximate amount of ingredients required for lentil cooking, i.e., daal and to make sure that the taste is good. After a series of experiments, the following recipes were finalized (Table 2). Both thick and thin preparations were served in three different sizes each with 25 g, 37.5 g, and 50 g to each participant over 12 weeks after adjusting the proportional increment of the ingredients for cooking daal.

Table 2: Cooked lentil recipe for thick and thin preparation

<b>Thick lentil cooking preparation &amp; amount (Based on 100g uncooked daal)</b>		<b>Thin lentil cooking preparation &amp; amount (Based on 100g uncooked daal)</b>	
<b>Ingredients</b>	<b>Amount</b>	<b>Ingredients</b>	<b>Amount</b>
Daal	100 g	Daal	100 g
Chopped onion	40 g	Chopped onion	40 g
Garlic	8 g	Garlic	8 g
Green chili	3 small pieces	Green chili	3 small pieces
Salt	1.5 teaspoon	Salt	1.5 teaspoon
Bay Leaf (Tejpata)	1 small piece	Bay Leaf (Tejpata)	1 small piece
Turmeric	5 g	Turmeric	3 g
Water	700 mL	Water	1.5 L
Soybean oil	10 teaspoon	Soybean oil	10 teaspoon
Time	18 minutes	Time	53 minutes
Weight after cooking: 585 g		Weight after cooking: 1166 g	

### 3.1.7 Data collection workforce and responsibilities

- **Cook** (*1 per adolescent club*)
  - i. Prepare, deliver, and portion standardized daal & rice for participants
  - ii. Coordinate with BRAC for weekly lentil delivery
- **Field Research Assistant** (*1 per adolescent club*)
  - i. Supervise cook
  - ii. Ensures girls receiving correct amount (g) and preparation (thick/thin)
  - iii. Direct observation
  - iv. Monitors compliance of visual analog scale
  - v. Record consumption of daal (all, 2/3, 1/4, etc)
- **Survey Data Enumerator:**
  - i. Externally daily-wise paid staff.
  - ii. Collect cross-sectional data by face to face interview from the study participants.
- **BRAC Field Monitor:**
  - i. Supervise Field Research Assistant
  - ii. Monitors all 4 adolescent clubs

### 3.1.8 Workforce plan

The implementation partner of this study was BRAC and it contributed program organizers, supervisory personnel, and lentil distribution logistics. Community Volunteers were in-charge of cooking/preparation of lentil meals. Research Assistants (RAs) were recruited by BRAC who were involved in the monitoring of compliance, collection of data (VAS, other assessments as described), and was supervised by the University of Saskatchewan (U of S) graduate student.

### 3.1.9 Lentil consumption schedule

- *Adolescent clubs* run 2 days per week (Thursday + other day)
  - 2 days/week: girls consume daal once daily at adolescent club
  - 3 days/week: girls come to adolescent club (*no club activities running*) to consume daal. BRAC field level staff encouraged adolescent girls to come to the club for consuming daal. These adolescent girls reside near the clubs. We, therefore, assumed that there will be full participation in non-club days. Furthermore, we screened the potential participants based on our pre-selected criteria where we asked each respondent whether they were interested to join the club in non-club days. Those who were willing to participate were included in the study.
- 5 days/week: Visual Analog Scale (VAS) completed independently, under supervision of field research assistant.

## 3.2 Feasibility study

### 3.2.1 Study design

We adopted a mix-methods study design (quantitative followed by qualitative) to achieve the specific aims of this study. For the quantitative method, we used cross-over design to examine the preparation style and optimal dosage of lentil. For the qualitative method, we conducted a focus group discussion (FGD) and an in-depth interviews (IDI) using WHO guideline in which all activities and logistics related to the implementation of the study were recorded through process documentation (Dawson & Manderson, 1993; WHO, 2000).

### 3.2.2 Study site and population:

The study included girls attending BRAC's adolescent clubs in rural Bangladesh (Figure 1). This target population for the study was chosen from a similar setting in terms of their socioeconomic and demographic characteristics. It is similar as those who have the most potential to benefit from the consumption of Fe fortified lentils, given the prevalence of iron deficiency or IDA in this region stated in the Bangladesh National Nutritional Survey (ICDDR, 2013). The study was conducted in 4 BRAC adolescent clubs located in 2 *upazilas* (sub-districts) of Gazipur district – Sreepur and Tongi puroshova (municipality). To test the implementation (delivery, transportation, and storage) logistics

for 4 ACs, two from each *upazila* were purposively selected from two different levels of storage and transportation accessibility as characterized by the BRAC Health, Nutrition and Population Program (HNPP). Healthy study participants who attend the ACs were included in the study if between the ages of 10-17years; non-smoking, not pregnant, and not breastfeeding.

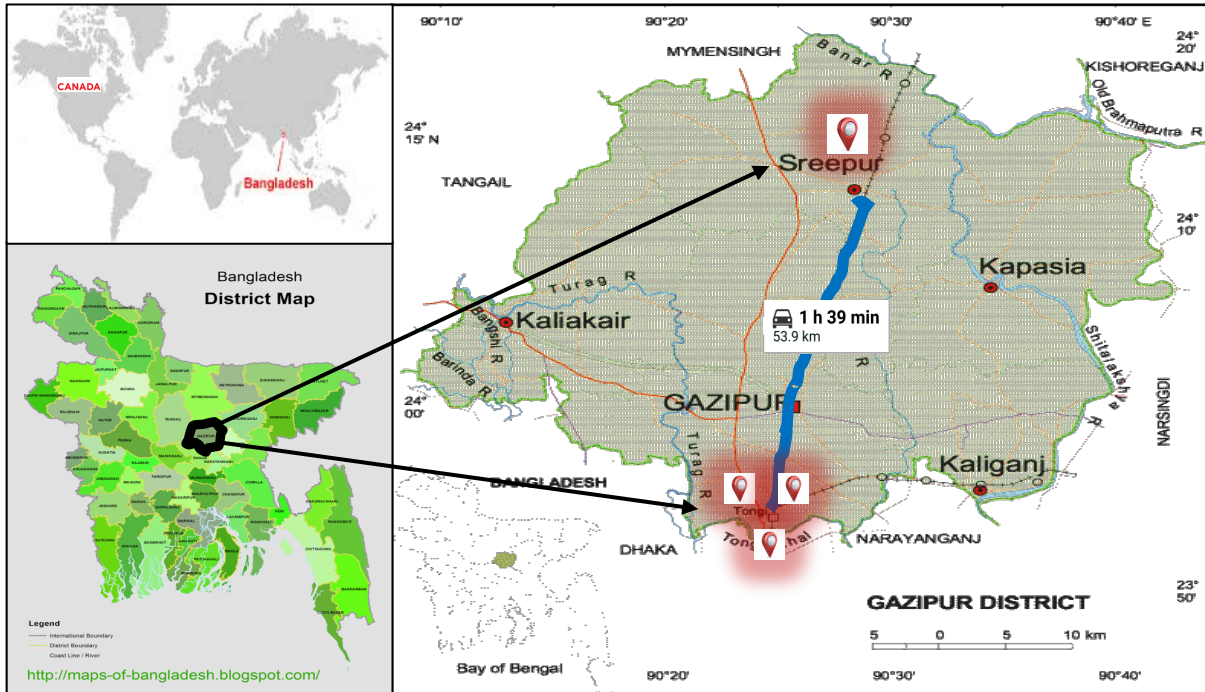


Figure 1. Map of Bangladesh

### 3.2.3 Crossover trial

A crossover trial of lentil consumption ( $n = 100$ ), each consisting of 2 different preparation styles (thick vs thin) and 3 different lentil portion size (raw weight = 25 g, 37.5 g and 50 g) were conducted in our target population (Figure 2). Each portion size was consumed 1 portion/d, 5 d/week, for 2 weeks. The trial was conducted in 2 different locations to assess transportation distance / logistics of delivery, as well as to assess differences in acceptability based on demographic and other characteristics. During the trial, volunteer adolescents came to the BRAC adolescent clubs to consume their portion of lentils along with a standard, controlled portion of rice across study conditions- both of which were prepared and served by a locally hired cook and research assistants at the BRAC ACs. This was a controlled setting, enabling direct assessment of compliance, and other measures described below. Each portion consumed by participants in this trial was needed to be completed in this setting for the 5d/week, which has been shown to be a successful ‘exposure’ of biofortified food items in previous trials in school children (Finkelstein et al., 2015; Haas et al., 2011).

Both thick and thin preparation were cooked and served in three different sizes each with 25 g, 37.5 g, and 50 g to each participant over 12 weeks after adjusting the proportional increment of the ingredients for cooking daal. We served 5 days a week while rest 2 days of the week was not served

because of the wash-out period. We first provided thin lentils for a total of 6 weeks, each of the amount (uncooked 25 g, 37.5 g and 50 g) contributes 2 weeks. Then we served thick preparation of cooked lentil with the same amount and for the same duration. To be culturally sensitive as daal is not normally eaten alone but with rice in most of the cases, we provided a standard cup of cooked rice mixed with each of the portion of cooked lentil in both thick and thin preparation between 3-6 pm Sunday through Thursday.

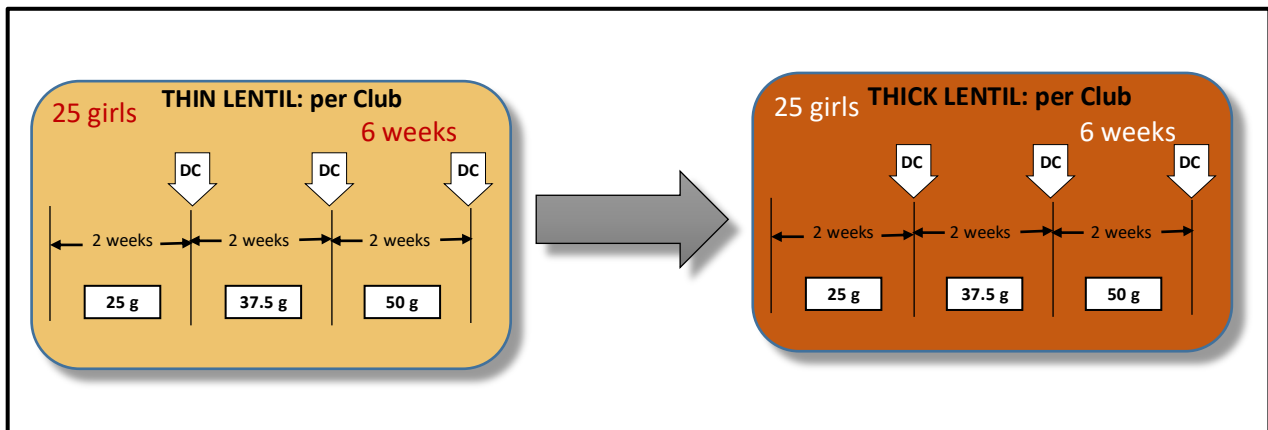


Figure 2: Cross-over trial

### 3.2.4 Measurement of raw lentils and rice

The amount of fortified lentil served in each club each day in two different cooking preparations is presented in Table 3. Measurement was assessed based on the adolescents who attended regularly in the club. Cooked rice was not weighed as it was served as volume. Digital weight balance (with +/- 1 g precision) was used to measure the exact amount of daal before cooking.

Table 3: Amount of fortified lentils served in different cooking preparations per day

Thin preparation daal (raw amount of lentils)					Rice
Trial amount (uncooked)	Club no	No of Adolescents/club	Duration	Final amount per day	Rice amount (50 g/person/day)
25 g	AC 1	33	2 weeks	825 g	1650 g
	AC 2	35		875 g	
	AC 3	30		750 g	
	AC 4	33		825 g	
37.5 g	AC 1	33	2 weeks	1238 g	
	AC 2	35		1313 g	
	AC 3	30		1125 g	
	AC 4	33		1238 g	
50 g	AC 1	33	2 weeks	1650 g	
	AC 2	35		1750 g	
	AC 3	30		1500 g	

	AC 4	33		1650 g	
<b>Thick preparation daal (raw amount of lentils)</b>					<b>Rice</b>
<b>Trial amount (uncooked)</b>	<b>Club no</b>	<b>No of Adolescents/club</b>	<b>Duration</b>	<b>Final amount per day</b>	<b>Rice amount (50 g/person/day)</b>
25 g	AC 1	33	2 weeks	825 g	1650 g
	AC 2	35		875 g	
	AC 3	30		750 g	
	AC 4	33		825 g	
37.5 g	AC 1	33	2 weeks	1238 g	
	AC 2	35		1313 g	
	AC 3	30		1125 g	
	AC 4	33		1238 g	
50 g	AC 1	33	2 weeks	1650 g	
	AC 2	35		1750 g	
	AC 3	30		1500 g	
	AC 4	33		1650 g	

### 3.2.5 Post-trial qualitative interviews

At the end of the feeding trial, in-depth interviews and focus group discussion was conducted with the mother/guardian of the participants to evaluate acceptability, lentil preparation styles, and lentil delivery logistics that will inform the efficacy trial from an individual participant level. Interviews were conducted throughout the study with BRAC management at different levels (e.g. supervisory, delivery) to assess quality control/quality assurance procedures, and methods for process documentation.

### 3.2.6 Outcome measurement

#### Quantitative:

We collected data on socio-demographics and economic characteristics such as age, sex, family history, and family member's characteristics, religion, ethnicity, education, occupation, marital status and housing condition of the adolescents by using structured questionnaire. We collected their food habits, practice, and knowledge on nutrition, iron deficiency and anemia. Detail anthropometric measurement such as height, weight, waist, hip and mid-upper arm circumference (MUAC) were taken by trained female interviewers under the direct supervision of researchers. Anthropometrics were measured in bare-footed and within minimal clothing avoided carpet, sloping surface, and rough, uneven surface based on the guideline of WHO and CDC (Bacopoulou, Efthymiou, Landis, Rentoumis, & Chrousos, 2015; National Health and Nutrition Examination Survey, 2007; WHO, 1995). For weight, adolescents were first asked to step into the digital body weight bathroom scale after removing their

shoes, slippers, sandals and socks. They were requested to stand still by face forwarding and placing palm on their respective side. Frankfurt horizontal plane was ensured before height was measured. Adolescents were asked to put their heels placed together, and other backward curved body parts such as buttocks, and shoulders blades along with heels and head placed against the plane. MUAC was measured at the left hand placing the arm at 90° angles towards the body using the constant tension tape. Upper and lower points of the left arm measured and mid-point was marked. Then, wrapped the tape around the mid-point and adjusted the tape neither too tight or too loose and record the measurement accordingly. Waist circumference was measured using constant tension tape in a private setting by a female enumerator with the presence of a witness. After the end of normal expiration, each waist measurement was taken under the point of the midline of the armpit, at the midpoint between the lower part of the last rib and the top of the hip. Same constant tension tape was used to measure the hip circumference. It was measured at the maximum diameter of the buttocks. These measurements were collecting in centimeter (cm) and kilogram (kg) where appropriate as the unit. Mean teen Body Mass Index (BMI) was calculated using BMI Percentile Calculator for Child and Teen (Centers for Disease Control and Prevention, 2017). Using the visual analog scale (VAS), we measured after one bite of daal meal (palatability / taste, texture, appearance, portion size), and before and after daal meal (hunger, fullness, thirst, nausea, prospective consumption)

### **Qualitative:**

In-depth interview and focus group discussion were conducted with the participants and her mother/guardian to evaluate acceptability, lentil preparation styles, and lentil delivery logistics that will inform the efficacy trial from an individual participant level.

### **3.2.7 Sample size**

The study was conducted on girls between 10 to 17 years in 4 adolescent clubs. This age group comprises most of the adolescent population in the clubs. However, due to the ethical and cultural context, we served each of the adolescents who attended the clubs during the meal serving time. In this crossover trial, each participant was served as her own control. Total sample size was 100, each trial was  $n = 50$ , based on previous food intake studies in similar populations and to account for a 20% drop-out rate (Dibari et al., 2013). In this trial, each group (thick daal and thin daal) had 25 girls and each of the two groups received three different amounts of raw lentil 25 g, 37.5 g, and 50 g over 12 weeks per club meaning 4 adolescent clubs comprised of total of 100 adolescents. For the qualitative study, purposive sampling techniques were chosen meaning that choosing participants purposively that are of interest of the topic. It was because only a few BRAC staff worked in this project and practically carried out the study at the field level. Therefore, it was warranted to interview them to acquire more insight information.

### **3.2.8 Data collection tools, technique, and procedure**

We recruited external data enumerators through the BRAC Research and Evaluation Division (RED)- Field Management unit who maintain a database of almost 350 professional enumerators (minimum completed bachelor degree). We used the database to identify potential enumerators and through the interview committee, we recruited them as daily-wise paid staff. The interview committee consisted of the researcher, BRAC RED admin department, and a member of field management unit. Before interview, we set a standard qualification for selection of the enumerators. We developed a standard training module for the enumerators and conducted training among the interviewers all together in order to adhere to the question, and answer the format strictly, with the same degree of questioning on objective measures (hard data) for all the participants. Before the interview, each interviewer read and comprehensively explained the consent form in front of each potential participant (adolescents) and their guardians/caregivers, since they voluntarily accepted to participate in the study, they were then asked to sign or put a thumbprint on the written informed consent paper.

#### **Quantitative components:**

##### ***Baseline questionnaire:***

To collect data, we conducted a baseline cross-sectional survey using close-ended structured questionnaires consisting of easy-to-understand questions with appropriate response options. Data included their socio-demographics and economic characteristics such as age, sex, detailed family history and family member's characteristics, religion, ethnicity, education, occupation, marital status. We also collected their food habits, practice, and knowledge on nutrition, iron deficiency and iron deficiency anemia as well as standard body measurements (height, weight, mid-upper arm circumference, waist and hip circumferences). The researchers developed the questionnaire and were pretested in the neighboring clubs similar to the study area. Questionnaires had a separate section of confirming inclusion criteria, household structure information, detail demographic data, knowledge of nutrition, iron deficiency and anemia, and anthropometric measurement scales.

##### ***Visual analog scale (VAS):***

VAS measurement tool was used to measure before, after one bite of the meal and after finishing the whole meal. It is a well-established measuring instrument that attempts to measure such attitude or characteristics for which the probable response lies within a range of values that cannot be captured directly and easily (Wewers & Lowe, 1990). Other studies validated and used this tool for measuring the hunger, satiety, and appetite (Flint, Raben, Blundell, & Astrup, 2000; Lindeman, Huang, & Dawkins, 2016; Parker et al., 2004; Sadoul et al., 2012). This pre- and post-meal measured the rating of hunger & satiety and palatability after taking one bite of the daal. Assessments of hunger, fullness, gastrointestinal discomfort were assessed before and after consuming each portion in both trials using



Visual Analog Scales (VAS) (Figure3). Sensory characteristics of both preparations were also assessed using VAS in both trials. Compliance with lentil consumption was assessed using portion size guides completed by participants in both RC trials.

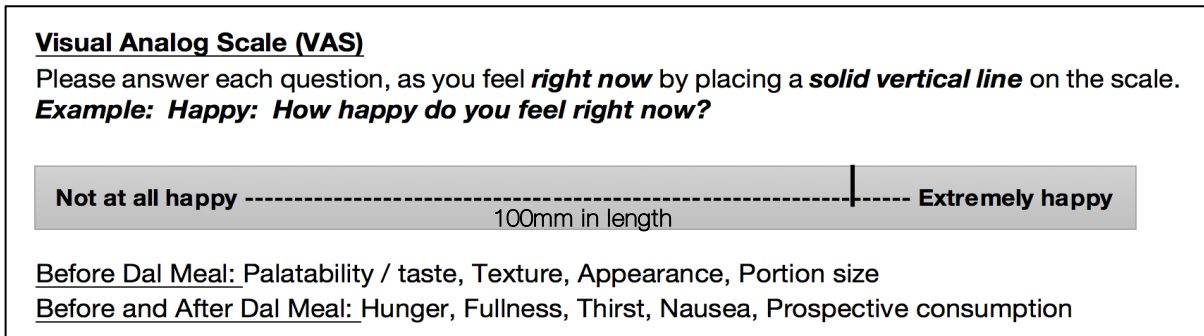


Figure 3 Visual Analog Scale

### Qualitative component:

We interviewed the mother/guardian, BRAC field level managers, and BRAC head office managers. Mothers of the adolescents had a wide range of education ranging from no education to class 10 completion. Some of them work in the garment factories and some are housewives. Majority of them were young and migrated from their hometown to near capital city for better living. Two BRAC's field level managers were interviewed- one area manager and the other is program organizer (PO). Area manager worked as supervisor of Program organizer/Field organizer. She was responsible for local budgeting, local purchase, maintain liaison with the local people, report to the head office, solve any unexpected issues, make sure everything goes well. She was responsible for 3 of our club out of 4 clubs. Program organizer (PO) maintain warehouse register, distribute lentil to the research assistants, assign club leader, cooks, and make sure the adolescent attendants. She was involved in the local purchase, organize parents meeting, community awareness of the clubs for the study. She reports to area manager. She was also responsible for 3 of our club out of 4 clubs. Majority tasks had carried out by her. BRAC Head office manager worked as primary tag person from BRAC's side to the University of Saskatchewan. She did the entire budget, making sure everything is well, logistics, liaison with BRAC adolescent program and solving the problems. However, she did not participate in data collection either survey and/or VAS tools, storage, distribution, club activities etc. She worked more of a research manager and overview the entire study.

Qualitative data were retrieved from mother/guardian of the adolescents and BRAC staff (head office and field office level) using a different checklist. One FGD was conducted among the mother/guardian of the adolescents and 2 IDI were conducted among BRAC staff (head office and field office level). The checklist included in the FGD were usual food practice of the adolescents, their preference of food menus, and decision maker of the family for the selecting food items/menus. IDI

focuses on the issues raised during implementing the study such as transportation, local budget, packing of the lentils, storage etc. Data collection tools summarized in table 4 below:

Table 4: Summary of qualitative tools and checklist topics

<b>Participants</b>	<b>FGD</b>	<b>IDI</b>	<b>Topic discussed</b>
Mother/guardian of the adolescents	1		Meal time- frequency, daily usual menus, decision maker for selecting food items/menus, cooked lentil (daal) eating habits, health benefits of daal, preferred preparation of daal, amount of daal consumed.
BRAC Field level staff	-	2	Local transportation of the lentils, local budget/purchase issue, lentils packaging at the local level and rice purchase, field level budgeting, transparency of the inventory, community engagement.

### 3.2.9 Statistical analyses

#### Crossover trial:

For the quantitative data analysis, both descriptive and inferential statistics were used. We presented summary statistics using percentage and total number, and Pearson’s chi-square, and Students independent ‘t’ test to investigate the association and mean difference of different categorical and continuous variables. One-way Analysis of Variance (ANOVA) was conducted to determine whether any of the continuous variables (subject characteristics, lentil ratings, etc) affected the relationship between lentil portion size, preparation condition and lentil intake by weight and to see if there were a significant difference between each six conditions - thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g and thick 50 g. A mixed linear model with repeated measures was used to analyze the main outcomes of lentil intake by weight (g), ratings of hunger, satiety and lentil meal characteristics. A modified Bonferroni adjusted post hoc differences test was used for pairwise comparisons of means (RMANOVA). It was conducted to compare visual analog scale scores of adolescent girls within each intervention group of different clubs with a static test at condition 1 (thin 25 g raw lentils), condition 2 (thin 37.5 g raw lentils), condition 3 (thin 50 g raw lentils), condition 4 (thick 25 g raw lentils), condition 5 (thick 37.5 g raw lentils), and condition 6 (thick 50 g raw lentils). This test was chosen because of the assumption that each of the observations must not be influenced by any other observations (independence of observations). Post-hoc comparison among clubs was

undertaken because intervention mean hunger scores were significantly different from each other. Results were considered significant at  $p < 0.05$  at 95% confidence interval. All analyses were done using SPSS for WINDOWS PAWS version 22.

### **Qualitative information**

Data were analyzed using the three-phase coding system (Neuman, 2000). For the first phase, taping and transcribing data were scanned by each researcher which leads to a certain degree of familiarization with the factors considered and locating the themes. Each researcher looked at the range, content, and diversity of the raw data. Secondly, priori codes, inductive codes and sub codes were generated, and identified, and defined in broader group. Definition includes: code abbreviations, color coding, full description, when to use, when not to use, and examples from transcripts. At the end, researcher re-read the sorted data and finalized the final theme. The transcripts were checked by other researchers to increase the validity of the data.

#### **3.2.10 Data collection management and quality assurance**

It was essential to thoroughly and directly train field staff in applying the study materials and implementing quality control procedures for assessments conducted in the field. UofS and BRAC proposed a training model that BRAC has used several times before, in which a training-of-trainers approach was used for the piloting phases of instrument and field procedure development, followed by the direct training of enumerators within each district in the weeks just prior to the commencement of data collection in order to achieve reliability and fidelity of implementation. We trained the trainers on both quality control visits in the field and follow-up training to uncover any misunderstandings about the data collection procedures, answer questions from trained assessors that have emerged as they practice what they have learned, ensure that data were being handled correctly to protect confidentiality and establish assessor reliability.

### **3.3. Ethical review**

The study protocol was submitted to the Institutional Review Board (IRB) of University of Saskatchewan, Marywood University, USA and BRAC University James P Grant School of Public Health (JPGSPH), Bangladesh for ethical review and approval. The subjects and their families were informed separately about the purpose of the study, the procedures used, and the potential scopes derived from this feasibility study. Two separate written informed consent from parents/guardians and participants (adolescents) was considered as a prerequisite to recruitment of the subjects. The severely anemic adolescents were symptomatically treated for Fe deficiency. The study conformed to all regulations of the Government of Bangladesh (GoB).

## CHAPTER 4: RESULTS

### 4.1 QUANTITATIVE FINDINGS

#### 4.1.1 Socio-demographics of the participants

A total of 100 adolescent girls aged 10-17 years were included for data analysis in this study. Table 5 summarizes the demographics of the study population. Mean age (SD) of the study population was 12.85 (2.00) and mean menarche age (SD) was 12.06 (0.94). Among those who experienced menarche, 54% experienced normal menstrual cycle (once a month) and 97% were unmarried. The majority (67%) had either completed and/or continuing education at the secondary level and 28% were in the primary level education. Regards to the participants' household condition, 82% house floor was made of brick/cement, 95% house roof made of tin, and 52% house wall was from brick/cement. Regards to the household assets, 98% of the participants' house had electricity connection, and almost all of them had mobile phones (94%) in their house. 84% of mothers were primarily responsible for preparing food for their household. Majority of the adolescents had taken their last meal >3 hours before having the served cooked lentil.

Table 5: Socio-economic status of all participants (N 100)

Variables	Adolescent girls (age 10-17years) %
Age [mean (SD)]	12.85 (2.00)
Age of menarche [mean (SD)]	12.06 (0.94)
Menstruation (Yes)	64
Menstrual regularity (Once in every month)	54
Marital status (No)	97
Education (Secondary)	67
Socioeconomic status	
House wall Brick/Cement)	52
House roof (Tin)	95
House floor Brick/Cement)	82
Electricity	98

#### 4.1.2 Anthropometric measurements of the adolescents:

Table 6 presented anthropometric measurements of the study participants. Mean (SD) height was 146.8 (10.0) cm, and weight 37.3 (9.2) kg. Mean BMI corresponded to adult 18.5, placing the BMI-for-age at the 50<sup>th</sup> percentile for girls mean aged 12 years 8 months. Participants' waist, hip and Mid-upper arm Circumference (MUAC) were 64.9 (7.0) cm, 78.1 (7.9) cm, and 20.8 (3.2) cm

respectively.

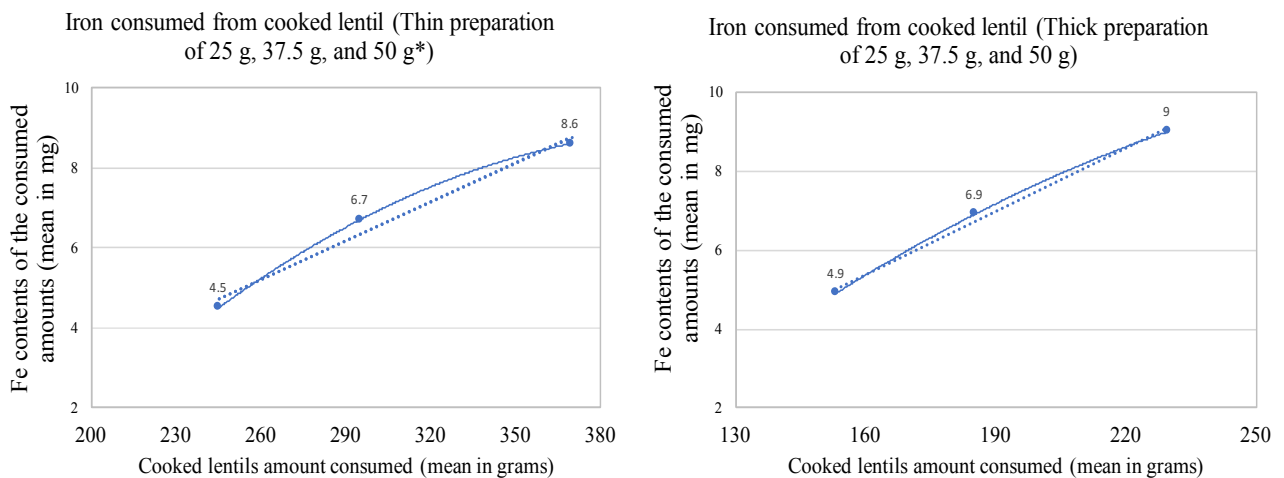
Table 6: Anthropometric measurements among all participants (N 100)

Measurements	Adolescent girls (age 10-17) mean (SD)
Height (cm)	146.8 (10.0)
Weight (kg)	37.3 (9.2)
Waist circumference (cm)	64.9 (7.0)
Hip circumference (cm)	78.1 (7.9)
Mid-upper Arm Circumference (MUAC) in cm	20.8 (3.2)
*BMI	17.3 (Adult corresponded to 18.5)

\*Mean teen BMI was calculated using BMI Percentile Calculator for Child and Teen (CDC, 2017).

#### 4.1.3 Measurement of cooked lentils (daal) and iron content of consumed Dal

Amount of cooked lentil was measured each time before served to the participants and another measure was undertaken after they returned the bowl once they were not willing to eat anymore. A linear relationship was noted with the increased amount of cooked lentil served with greater iron contents regardless the cooking preparation of lentils (Figure 4).



\* 50 g of fortified raw lentil = 10mg of Fe after cooking (Podder et al., 2017)

Figure 4: Iron (Fe) content of consumed cooked lentils.

#### 4.1.4 Normal distribution of adolescents age by clubs:

A frequency distribution histogram was generated of the adolescents' age by four different clubs. This illustration would help to understand the VAS score response on their hunger, satiety, and palatability.

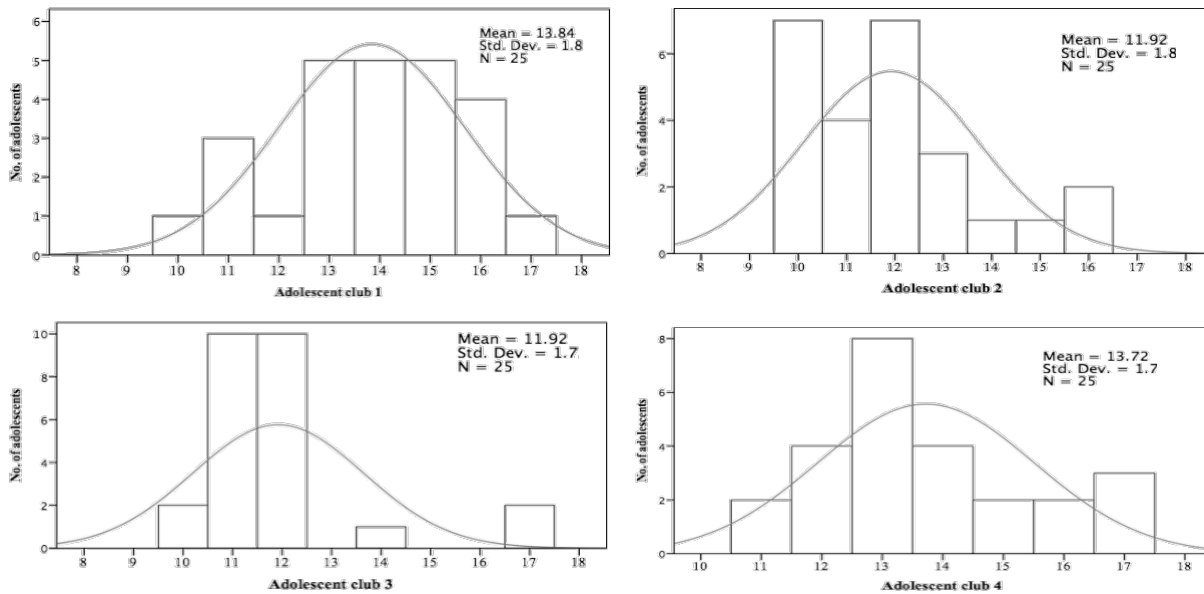


Figure 5: Normal distribution of adolescent age by four different clubs.

#### 4.1.5 Variation of Visual Analog Scale variables score (before, after one bite and after finishing daal meal) by cooking preparation and amount served

Analysis of VAS variables were carried out in three sequential steps. At first, mean difference of VAS variables by cooking preparation (thin vs thick) was undertaken using Independent student ‘t’ test. We checked the test of normality and Levene’s test for equality of variance assumption for Independent student ‘t’ test. We noticed both the test of normality and Levene’s test for equality of variance assumption were violated. Therefore, we performed the nonparametric Mann-Whitney U test as data were not normally distributed. Secondly, mean difference of VAS variables by 3 different raw amount of lentil (25 g, 37.5 g, and 50 g) that were cooked and served were undertaken using nonparametric Kruskal-Wallis test, since the data were not normally distributed. However, these tests were unable to explain the assumption that each of the observations must not be influenced by any other observations (independence of observations). Therefore, thirdly, we carried out a modified Bonferroni adjusted post-hoc differences of repeated measure ANOVA (RMANOVA) after creating six combined continuous variables (treated as adolescent response on six conditions) of the cooking preparation and raw amount of lentils that were cooked and served such as thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g and thick 50 g.

Table 7 shows higher mean score in thick preparation of cooked lentils over thin preparation among all VAS variables. This would mean that adolescents preferred thick preparation of cooked lentil than thin preparation. All visual analog scale measurement mean scores were found significantly different except nauseated (before meal) and thirst (after meal) between thin and thick preparation of cooked lentils which would mean their preference on thick preparation over thin preparation were

noteworthy different.

Table 7: Mean difference of VAS variables\*\* by cooked lentil preparation (N 100)

Time of VAS response	Visual Analog Scale measurements	Cooked lentil preparation	Mean (SD)	p value*
Before meal	Hunger	Thin	70.6 (25.1)	<0.001
		Thick	81.9 (21.5)	
	Thirst	Thin	62.6 (27.8)	<0.001
		Thick	56.2 (27.9)	
	Prospective consumption	Thin	75.9 (20.9)	<0.001
		Thick	89.3 (15.9)	
	Feeling full now	Thin	15.6 (26.3)	<0.001
		Thick	11.8 (23.1)	
	Nauseated	Thin	0.15 (2.8)	0.07
		Thick	0.04 (1.3)	
	Odour	Thin	16.3 (16.6)	<0.001
		Thick	7.9 (9.8)	
After one bite of meal	Taste pleasant	Thin	87.9 (12.9)	<0.001
		Thick	94.5 (5.5)	
	Pleasant texture/mouthfeel	Thin	87.3 (12.5)	<0.001
		Thick	93.9 (5.3)	
	Pleasant visual appearance	Thin	88.0 (12.6)	<0.001
		Thick	94.4 (5.5)	
	Prospective consumption	Thin	82.1 (12.6)	<0.001
		Thick	91.1 (11.6)	
	Portion size about to consume	Thin	79.2 (18.3)	<0.001
		Thick	88.9 (13.7)	
After finishing meal	Hunger	Thin	5.0 (16.3)	<0.001
		Thick	7.5 (14.1)	
	Thirst	Thin	10.7 (26.1)	0.09
		Thick	13.1 (29.7)	
	Prospective consumption	Thin	27.6 (29.8)	<0.001
		Thick	14.1 (16.7)	
	Feeling full now	Thin	79.3 (24.5)	<0.001
		Thick	85.1 (14.9)	
	Nauseated	Thin	0.2 (3.9)	<0.01
		Thick	0.0 (0.6)	

\*Nonparametric Mann-Whitney U test significant at  $p < 0.001$ , and  $p < 0.01$

\*\* Ratings of VAS score: 0 -100mm, higher score = intense, lower score = Not at all intense

Table 8 shows 50 g raw lentil category had significantly higher VAS variable mean score (except before meal- thirst, feeling full now and nauseated) compared to the other two raw amounts 25 g and 37.5 g that were cooked and served. However, the highest mean score was observed in prospective

consumption (before meal and after one bite of meal) and portion size about to consume in the 37.5 g raw lentil category. Thus, cooked amount of raw lentils of 37.5 g could be reasonable amount to serve for future efficacy trial.

Table 8: Mean difference of VAS variables\*\* by raw lentil portion size (N 100)

<b>Time of VAS response</b>	<b>Visual Analog Scale measurements</b>	<b>Lentil portion size (raw amount served in cooked meal)</b>	<b>Mean (SD)</b>	<b>p value*</b>
Before meal	Hunger	25 g	72.4 (26.5)	<0.001
		37.5 g	76.9 (22.1)	
		50 g	79.0 (23.0)	
	Thirst	25 g	58.7 (29.2)	0.12
		37.5 g	60.9 (26.8)	
		50 g	59.0 (28.2)	
	Prospective consumption	25 g	80.3 (21.3)	<0.001
		37.5 g	83.8 (17.8)	
		50 g	83.2 (20.0)	
	Feeling full now	25 g	13.3 (25.3)	0.10
		37.5 g	13.4 (23.9)	
		50 g	14.5 (25.4)	
	Nauseated	25 g	0.1 (2.9)	0.60
		37.5 g	0.1 (2.0)	
		50 g	0.1 (1.6)	
Odour	25 g	16.3 (18.2)	<0.001	
	37.5 g	11.8 (12.5)		
	50 g	8.9 (10.1)		
After one bite of meal	Taste pleasant	25 g	88.5 (13.9)	<0.001
		37.5 g	91.4 (9.2)	
		50 g	93.5 (6.5)	
	Pleasant texture/mouthfeel	25 g	87.9 (13.6)	<0.001
		37.5 g	90.7 (8.8)	
		50 g	92.8 (6.4)	
	Pleasant visual appearance	25 g	88.5 (13.5)	<0.001
		37.5 g	91.3 (8.9)	
		50 g	93.5 (6.8)	
	Prospective consumption	25 g	84.4 (17.4)	<0.001
		37.5 g	87.7 (13.5)	
		50 g	87.3 (15.2)	
	Portion size about to consume	25 g	82.8 (18.5)	<0.001
		37.5 g	85.0 (14.9)	
		50 g	84.3 (17.2)	
	Hunger	25 g	9.9 (18.7)	<0.001



After finishing meal		37.5 g	5.1 (12.7)	<0.001
		50 g	3.6 (13.2)	
	Thirst	25 g	15.2 (29.2)	
		37.5 g	9.9 (26.4)	
		50 g	10.6 (27.7)	
	Prospective consumption	25 g	34.0 (27.9)	
		37.5 g	18.8 (22.2)	
		50 g	10.6 (19.2)	
	Feeling full now	25 g	74.0 (23.9)	
		37.5 g	82.9 (16.5)	
		50 g	89.9 (17.7)	
	Nauseated	25 g	0.3 (4.8)	
		37.5 g	0.1 (1.1)	
		50 g	0.0 (0.0)	

\* Nonparametric Kruskal-Wallis test significant at  $p < 0.001$ .

\*\* Ratings of VAS score: 0 -100mm, higher score = intense, lower score = Not at all intense

#### 4.1.6 Repeated measure anova (RMANOVA) test of the VAS variables

VAS was used to measure the rating of taste, texture, appearance, prospective consumption and portion size about to consume after one bite of the served cooked daal. Once adolescents finished their meal, rating of hunger, prospective consumption, feeling full right now and nauseated were measured using the VAS scale.

##### *Taste pleasant (after one bite of cooked meal)*

Significant main effect for pleasant taste (after one bite of cooked meal) was observed across all 6 conditions- thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g and thick 50 g (Wilks Lambda,  $F 60.7$ ,  $p < 0.001$ , partial  $\eta^2 0.77$ ). It means that there was a change in pleasantness across the 6 different conditions. Adolescents taste was significantly different among all thin preparation portion size (thin 25 g, thin 37.5 g, and thin 50 g) but not significantly different among the thick preparations portion size (thick 25 g, thick 37.5 g and thick 50 g). This would mean that adolescents rated pleasant taste differently among all thin preparation portion sizes but the pleasant taste were constant (not different) among thick preparation portion sizes. It indicates that changes in pleasantness induced by increasing concentration significantly differed between taste qualities.

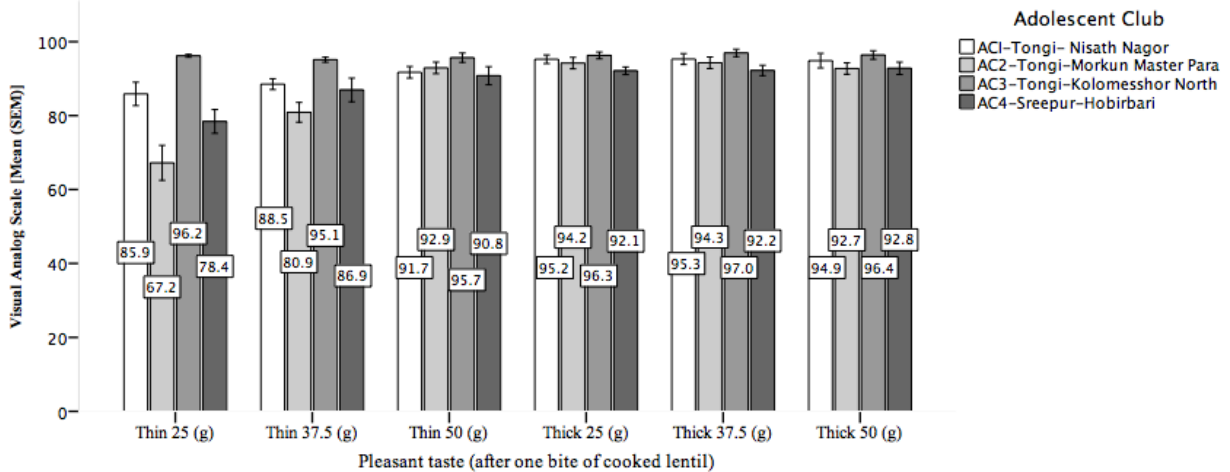


Figure 6: Mean (SEM) pleasant taste (after one bite of cooked meal) of all adolescent clubs for all intervention conditions. Repeated measures ANOVA and simple contrasts with Bonferroni correction were used to test for significant differences of pleasant taste among 6 conditions. Pleasant taste was significantly different ( $p < 0.001$ ) among all thin preparation portion size thin 25 g, thin 37.5 g, thin 50 g but all thick preparation portion size thick 25 g, thick 37.5 g and thick 50 g were not significantly different.

### *Pleasant texture/mouthfeel (after one bite of cooked meal)*

Significant main effect for pleasant texture/mouthfeel (after one bite of cooked meal) was observed across all 6 conditions- thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g and thick 50 g (Wilks Lambda,  $F 73.3$ ,  $p < 0.001$ , partial  $\eta^2 0.80$ ). It suggests that there was a change in pleasant texture/mouthfeel across the 6 different conditions. Adolescents pleasant texture/mouthfeel were significantly different among all thin preparation portion size (thin 25 g, thin 37.5 g, and thin 50 g) but not significantly different among thick preparations portion size (thick 25 g, thick 37.5 g and thick 50 g). This would mean that adolescents rated pleasant texture/mouthfeel differently among all thin preparation portion sizes but the pleasant taste was constant (not different) among thick preparation portion size. It implies that changes in texture/mouthfeel induced by increasing concentration in cooking preparation significantly differed between taste qualities.

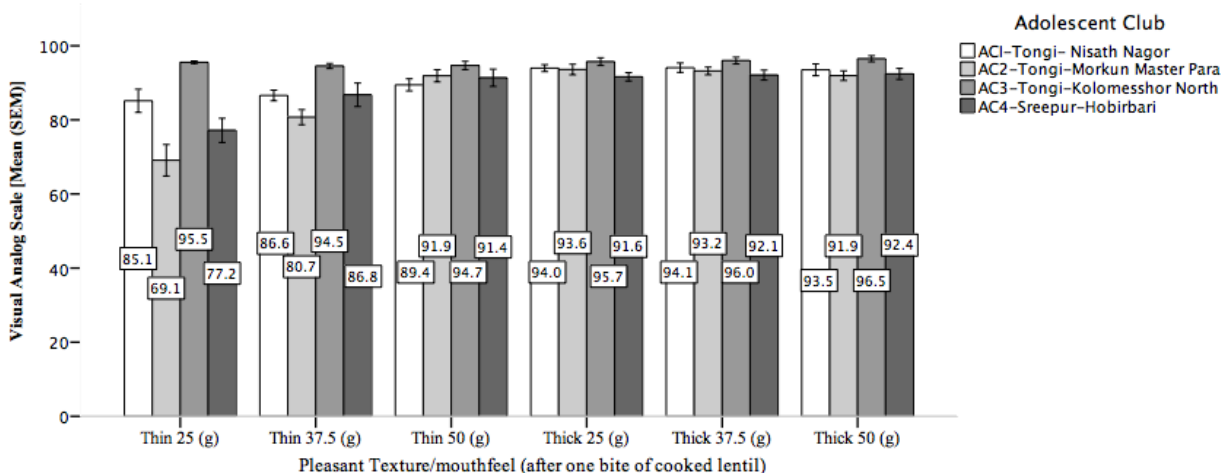


Figure 7: Mean (SEM) pleasant texture/mouthfeel (after one bite of cooked meal) of all adolescent clubs for all intervention conditions. Repeated measures ANOVA and simple contrasts with Bonferroni correction were used to test for significant differences of pleasant texture/mouthfeel among 6 conditions. Test score of pleasant texture/mouthfeel were significantly different ( $p < 0.001$ ) among all thin preparation portion size thin 25 g, thin 37.5 g, thin 50 g but were not significantly different among all thick preparation portion size thick

25 g, thick 37.5 g and thick 50 g.

*Appearance (after one bite of cooked meal)*

Significant main effect for visual appearance (after one bite of cooked meal) was observed across all 6 conditions-thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g and thick 50 g (Wilks Lambda, F 85.1, p <0.001, partial eta<sup>2</sup> 0.82). It specifies that there was a change in visual appearance across the 6 different conditions. Adolescents visual appearance were significantly different among all thin preparation portion size (thin 25 g, thin 37.5 g, and thin 50 g) but not significantly different among thick preparations portion size (thick 25 g, thick 37.5 g and thick 50 g). This would mean that adolescents considered visual appearance differently among all thin preparation portion sizes but were not different among thick preparation portion size. It indicates that changes in visual appearance induced by increasing concentration in cooking preparation significantly differed between sense of taste qualities.

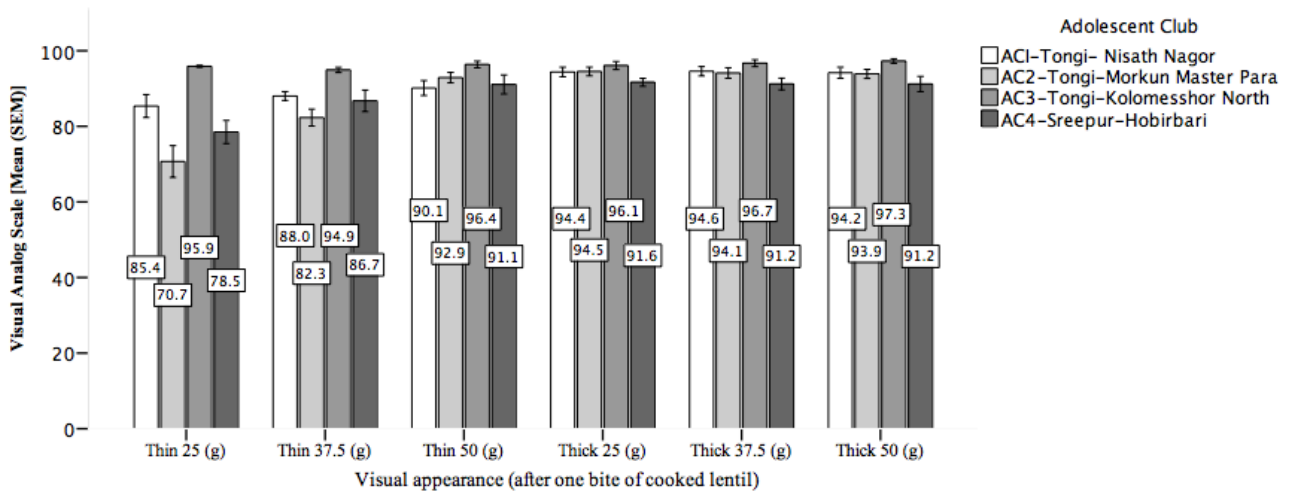


Figure 8: Mean (SEM) visual appearance (after one bite of cooked meal) of all adolescent clubs for all intervention conditions. Repeated measures ANOVA and simple contrasts with Bonferroni correction were used to test for significant differences of visual appearance of the cooked daal among 6 conditions. Visual appearance test score was significantly different (p <0.001) among all thin preparation portion size thin 25 g, thin 37.5 g, thin 50 g but were not significantly different among all thick preparation portion size thick 25 g, thick 37.5 g and thick 50 g.

*Prospective consumption (after one bite of cooked meal)*

Significant main effect for prospective consumption (after one bite of cooked meal) was observed across all 6 conditions- thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g and thick 50 g (Wilks Lambda, F 103.1, p <0.001, partial eta<sup>2</sup> 0.85). It suggests that there was a change in prospective consumption across the 6 different conditions. Adolescents’ prospective consumption was significantly different among all thin preparation (thin 25 g, thin 37.5 g, and thin 50 g) and thick 50 g portion sizes but not significantly different between thick 25 g, and thick 37.5 g portion sizes. It indicates that adolescents’ prospective consumption after one bite of the cooked meal were the same when they were served the thick 25 g, and thick 37.5 g portion sizes.

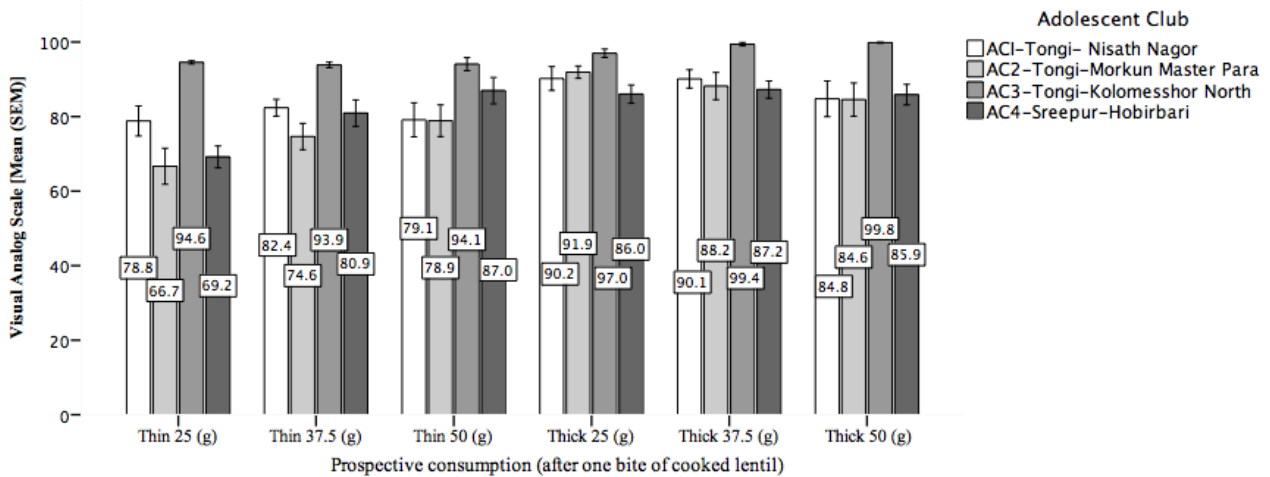


Figure 9: Mean (SEM) prospective consumption (after one bite of cooked meal) of all adolescent clubs for all intervention conditions. Repeated measures ANOVA and simple contrasts with Bonferroni correction were used to test for significant differences of prospective consumption of the cooked daal among 6 conditions. Prospective consumption test score was significantly different ( $p < 0.001$ ) among all thin preparation portion size thin 25 g, thin 37.5 g, thin 50 g and thick 50 g portion sizes but were not significantly different between thick 25 g, and thick 37.5 g portion size.

*Portion size about to consume (after one bite of cooked meal)*

Significant main effect for portion size about to consume (after one bite of cooked meal) was observed across all 6 conditions-thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g and thick 50 g (Wilks Lambda,  $F 70.5$ ,  $p < 0.001$ , partial  $\eta^2 0.80$ ). It suggests that there was a change in portion size about to consume across the 6 different conditions. Portion size about to consume were significantly different among all thin preparation portion size except between thin 37.5 g, and thin 50 g. However, thick preparation portion sizes (thick 25 g, thick 37.5 g and thick 50 g) were not significantly different. It indicates that changes in perception of portion size about to consume induced by increasing concentration in cooking preparation were not different among the thick portions sizes.

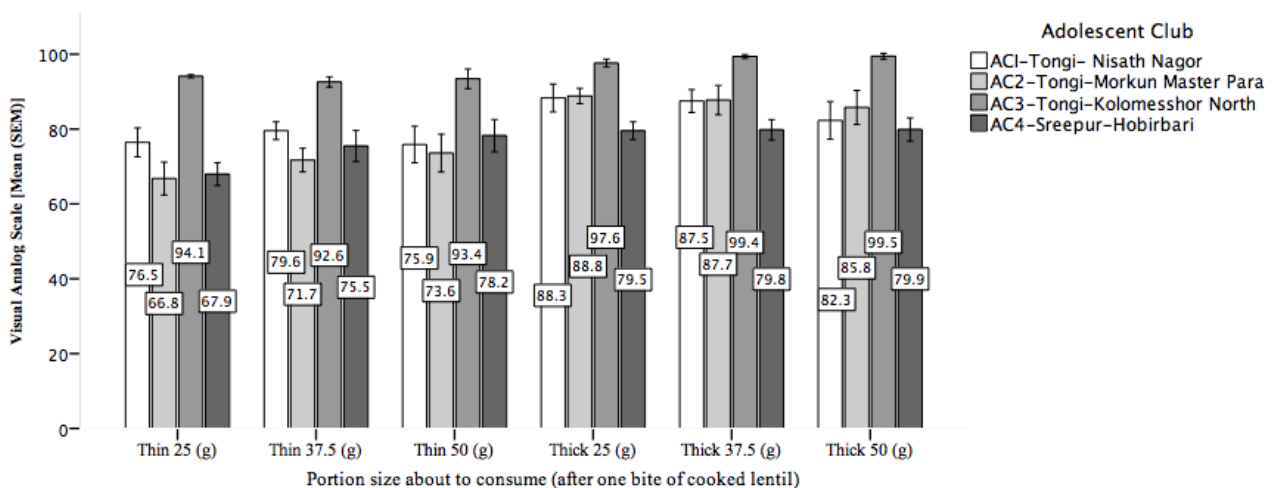


Figure 10: Mean (SEM) portion size about to consume (after one bite of cooked meal) of all adolescent clubs for all intervention conditions. Data are expressed as mean (SEM). Repeated measures ANOVA and simple contrasts with Bonferroni correction were used to test for significant differences of portion size about to consume of the cooked lentils among 6 conditions. Portion size about to consume test score was significantly different ( $p < 0.001$ ) among all thin preparation portion sizes except between thin 37.5 g and thin 50 g but were not significantly different among all thick preparation portion sizes.

### Hunger (after meal)

Significant main effect for hunger (after meal) was observed across all 6 conditions- thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g and thick 50 g (Wilks Lambda,  $F_{22.3}$ ,  $p < 0.001$ , partial  $\eta^2$  0.55). It suggests that there was a change in after meal hunger across the 6 different conditions. Hunger after meal were significantly different between thin 25 g, and thin 37.5 g, and thin 25 g and thin 50 g portion sizes. Furthermore, significantly different hunger was observed among all thick preparation portion sizes. It indicates that changes in after meal hunger by increasing concentration in cooking preparation among the adolescents were different across the thick preparation portions sizes.

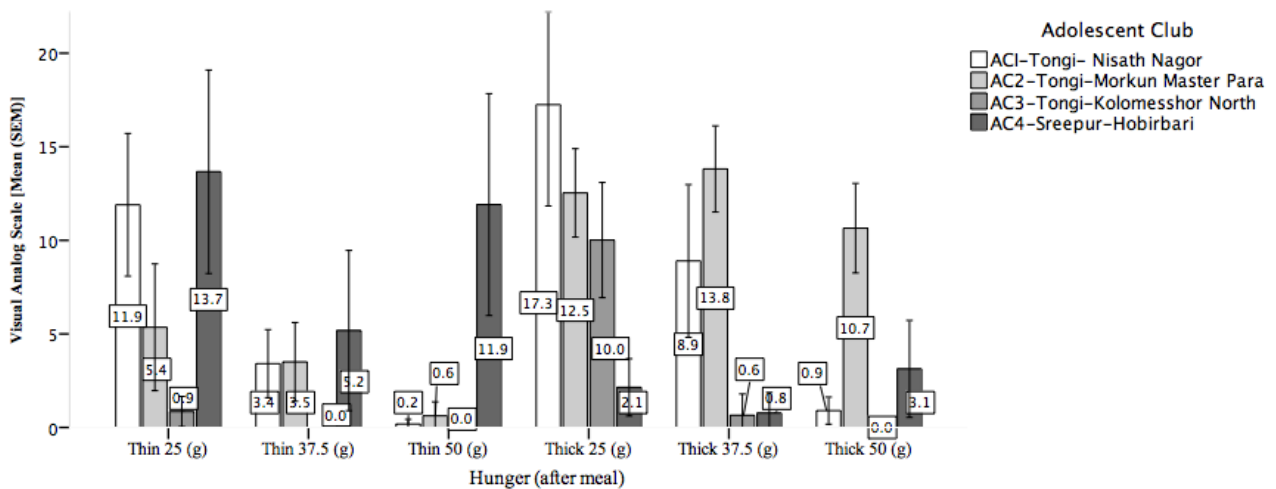


Figure 11: Mean (SEM) Hunger (after meal) of all adolescent clubs for all intervention conditions. Repeated measures ANOVA and simple contrasts with Bonferroni correction were used to test for significant differences of hunger after meal of the cooked lentils among 6 conditions. Hunger were significantly different ( $p < 0.001$ ) between thin 25 g, and thin 37.5 g, and thin 25 g and thin 50 g portion sizes. All thick preparation portion sizes were significantly different.

### Prospective consumption (after meal)

Significant main effect for prospective consumption (after meal) was observed across all 6 conditions- thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g and thick 50 g (Wilks' Lambda,  $F_{136.9}$ ,  $p < 0.001$ , partial  $\eta^2$  0.88). It suggests that there was a change in after meal prospective consumption across the 6 different conditions. After meal prospective consumption were significantly different across all 6 conditions except thick 25 g and thick 37.5 g portion sizes. It indicates that changes in after meal prospective consumption induced by increasing concentration in cooking preparation were not significantly changes when they were served thick 25 g and thick 37.5 g portion sizes.

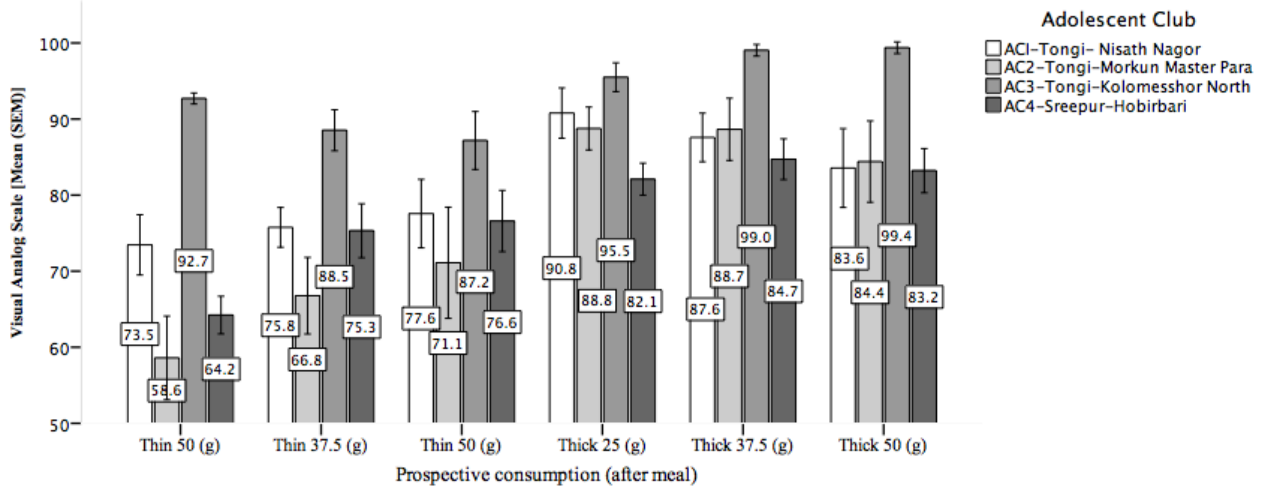


Figure 12: Mean (SEM) prospective consumption (after meal) of all adolescent clubs for all intervention conditions. Repeated measures ANOVA and simple contrasts with Bonferroni correction were used to test for significant differences of prospective consumption after meal of the cooked lentils across 6 conditions. After meal prospective consumption were significantly different ( $p < 0.001$ ) among all 6 conditions except between thin 25 g, and thin 37.5 g, and thin 25 g and thin 50 g portion sizes.

### Feeling full now (after meal)

Significant main effect for feeling full now (after meal) was observed across all 6 conditions—thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g and thick 50 g (Wilks' Lambda,  $F 89.8$ ,  $p < 0.001$ , partial  $\eta^2 0.83$ ). It suggests that there was a change in after meal feeling full now across the 6 different conditions. After meal feeling full right now were significantly different among all 6 conditions except between thin 37.5 g and thick 37.5 g, and thin 50 g and thick 50 g portion sizes. It signifies that changes in after meal feeling full now induced by increasing concentration in cooking preparation differed across all 6 conditions but no changes were observed when they were served both thin and thick preparation of 37.5 g and 50 g portion sizes.

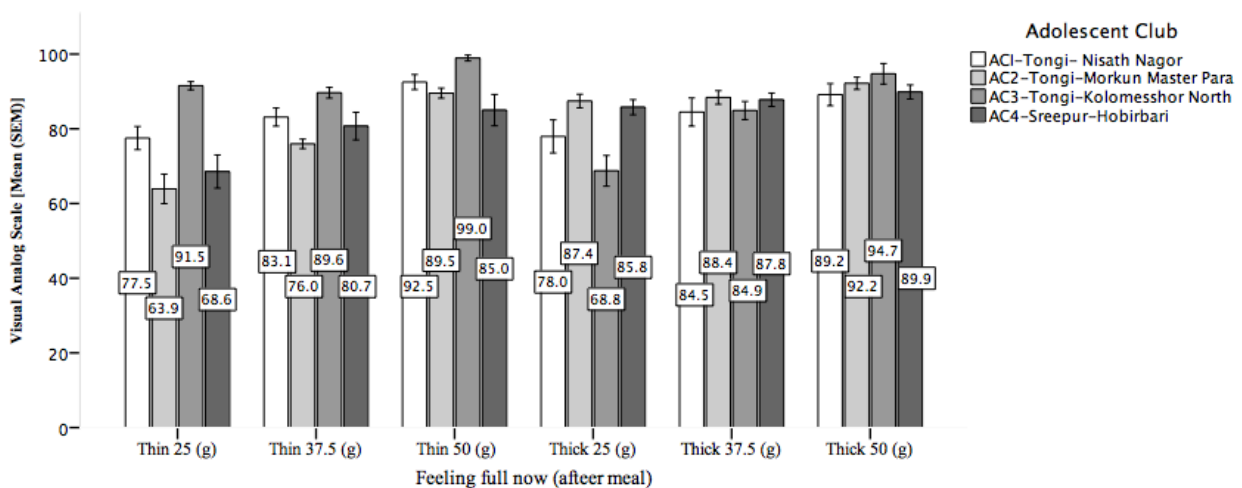


Figure 13: Mean (SEM) feeling full now (after meal) of all adolescent clubs for all intervention conditions. Repeated measures ANOVA and simple contrasts with Bonferroni correction were used to test for significant differences of feeling full now after meal of the cooked lentils across 6 conditions. After meal prospective consumption were significantly different ( $p < 0.001$ ) among all 6 conditions except between thin 37.5 g and thick 37.5 g, and thin 50 g and thick 50 g portion sizes.

### *Nauseated (after meal)*

Adolescent feeling nauseated before meal were not significantly different across all conditions (thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g, thick 50 g). It indicates that adolescents sense of nausea did not change after consuming each 6 portion sizes.

## **4.2 QUALITATIVE FINDINGS**

We carried out the qualitative interviews to explore the acceptability of the served cooked lentils, perception on the lentil preparation style among the mother/guardian of the study participants. It was also intended to understand the lentil delivery logistics within Bangladesh (from central store to local store to study site) and from Canada to Bangladesh such as customs clearance documents, transport system etc. Before moving to human efficacy trial, it was warranted to understand the different aspect of the study from an individual point of view from those who closely worked in this feasibility study. Our analysis of the interviews with mother/guardian, BRAC field level managers, and BRAC head office managers identified eight broader themes such as understanding the usual food practice, transportation, local Budget/purchase issue, packaging of the lentils, rice purchase, budgeting, transparency of the inventory, and community engagement.

### **4.2.1 Usual food practice:**

During the focus group discussion with the mothers/guardians of the adolescents' usual food practice was discussed. They eat meal three times a day- breakfast, lunch and dinner. However, they cook twice a day- majority cook their lunch early in the morning and then cooked again at night for dinner and next day breakfast. The usual daily recipe includes daal (lentils), vegetable, rice, curry- either with fish or meat or egg. Their breakfast ends at around 7 am in the morning because of the school and office start (some of the mother works in the garments factory). Breakfast menu include rice mixed with daal and leftover curry from yesterday's dinner. Usually, there is no exact time for the adolescents to have lunch. Some of their school time starts from 10 am to 4 pm, and some begin early morning. Usually, they have their dinner around 10-11 pm. Mostly women i.e adolescents' mother decides the daily menus; however, they often discuss with their husband if he has a certain preference. One of the mothers stated

“We decide what to cook.....” (FGD)

Regards to the lentil consumption (daal) practice, they make daal usually 3-4 times in a week.

“Daal is common in our menu.....I sometimes cook daal in almost everyday, sometimes at least in every 1-2 days” (FGD)

Once they cook, they eat twice a day- evening and next day morning. Majority of the mother were aware of the health benefits of the consuming daal. They perceived that daal is good for health. They consider that the water of the thin daal is rich in vitamin.

“We believe daal (thin preparation) has more vitamins.....particularly in the daal water” (FGD)

“I don’t like daal at all.....but have to cook for my children and husband” (FGD)

Their best recipe is the thin daal and smashed potato with rice. They, however, cook thick lentil often. When they cooked thin daal, they consider this as an extra item to the menu, and when thick daal, they consider as the main item on the menu. It is their understanding that daal would make their children healthy, and look nourished. One of the mothers stated:

“Even if someone goes to doctor, they will be asked to eat daal.....” (FGD)

“We eat thin daal because of vitamin” (FGD)

All of them considered daal as the main food item in their menus and they expressed that everyone likes it. Although there are a few varieties of lentil available in the market, they prefer Lentils (*Lens culinaris L.*) locally known as ‘Moshur daal’. They prefer to eat thin preparation of daal over thick preparation.

“We prefer to eat thin daal.....” (FGD)

“for some member, it became a habit to eat daal after finishing the main food.....” (FGD)

However, they could not reason why they particularly like one over other. Whatever the daal preparation, they usually eat a small full a bowl or sometimes 4-5 spoon of the daal.

#### **4.2.2 Transportation:**

BRAC field level managers opinioned that it would be good to transport an entire lot of lentils to the respective local office and store there rather than store in the central warehouse. They believe that it would save the time of the manager to get those from the warehouse small amount again and again, and reduce transportation cost, and hassle. It is also difficult for a woman to arrange a small truck (pick-up van) and travel in it with those people who are not used to travel with women in their truck. It’s uncomfortable for both of field manager. Regards to the local storage, it’d be good to put all lentils in the big drum, so that it won’t get bugs and remain safe from rats.



“It’s not difficult to transport lentils from central storage to local storage.....but its bit of uncomfortable for women to seat beside the truck driver.....” (IDI)

#### **4.2.3 Local Budget/purchase issue:**

It is important to include branch account manager in the field level research team and she/he should attend the research orientation seminar. It is because that they would aware the requirement of the study and cooperates with the field team, and understand the priority of the materials rather asking much more question to make it complicated. It kills time and hampers relationship within the office. It is particularly important for the perishable items such as onions, thai chills as its price varies in every day.

“branch accountant does not realize the importance of buying same standard of product required for the study.....because they were not briefed” (IDI)

“buying the perishable items is a challenge.....price varies everyday” (IDI)

#### **4.2.4 Packaging and rice purchase:**

Both BRAC field staff thinks that the recent packaging of the lentil was good- it was in a sealed box. However, BRAC Head office manager opinioned that it’d be good to have special instruction from the research team regards to the making sure the good quality of the lentils. Nevertheless, BRAC itself usually follow standard procedure for keeping the quality such as good ventilation, temperature. Even if BRAC don’t have extra facilities at the field level, it could arrange special storage facilities for ensuring the lentil quality i.e freezer, cooling system. They believe it’d be good to buy a full rice bag rather than buying it in a small portion. They reasoned that price of the rice also varies each day and it could also affect the response of the adolescents because of the rice variety and quality. Adolescents don’t want/would not like to eat poor quality rice which has a bad smell.

“There are many rice qualities available in the market...its difficult to make them (purchasing committee) understand and ensure the same quality of rice.....but if we buy the whole a lot at once, then this will be no issue anymore.” (IDI)

#### **4.2.5 Budgeting:**

Not estimating the budget properly could be disastrous for the project. Therefore, to be in safe side, it’d good to estimate the budget to a bit high. They argued that if the price of the perishable items goes down, there should be any problem, but if it goes high, then the budget needs to revise and require approval. It usually takes 15-20 days to get the budget approval. This is time-consuming and becomes

difficult to deliver the product in-time. An approved budget remains valid for 3 months as per the existing financial guideline.

“It is our mistake this time that we made budget tight based on the current market price.....but if we could make the budget mark at a bit higher rate then we would not face any challenges due to the price variation of the market.” (IDI)

“Our budget remains valid for 3 months.....it applies for all BRAC programmes” (IDI)

#### **4.2.6 Transparency of the inventory:**

BRAC maintains the security of the warehouse at a very high level at all times. During delivering the lentil to the research assistants, lentils were measured by a digital weight scale and wrote it down in a stock register - maintained by the field level program manager. Each log/account of the product maintained at high level. From the head office, two different programs person were assigned – a monitor person and an audit person. However, as in the current storeroom, all the product of BRAC education program are placed in the same room which includes paper, books, pen, pencil, leaflets, rice, lentils, and much more. It'd be very good if lentils can be stored in a separate store room which will be only for this project – this will make sure the better security.

“Everything in or out is maintained in stock register.” (IDI)

#### **4.2.7 Community engagement:**

Community engagement i.e relation with parents, influential person is crucial for this study. It'd be easier for us to convince people if we able to get a prior approval from the Government Official – may be District Commissioner or Upazilla Nirbahi Officer (Sub-district superior official).

“We can apply and get written clearance for running the study from the local government office before we start the trial..... this would ease to convince community people. (IDI)

## CHAPTER 5: DISCUSSION AND CONCLUSIONS

We carried out this feasibility study to find out the appropriate intervention i.e portion size and cooking preparation for the future human efficacy trial. The qualitative part was to understand the usual food practice of adolescents' family and better understand the implementation strategies at the field level. Combining two results would help to better design and implement plan for the future human efficacy trial. It is also noted that around 50% of our study participants lied about age 10-12 years. Our further analysis revealed that adolescents' taste, texture, visual appearance, prospective consumption and portion size had a significant effect on their preference of type and amount of meal we served. We found that thick preparation of cooked lentil was preferable than thin preparation and 37.5 g raw amount (served amount 200 g) could be reasonable amount to serve for future efficacy trial. This result was based on the findings of the analysis. For instance, firstly, mean comparison between VAS variables and cooking preparation of lentils was undertaken to see if adolescents' preference on certain cooking preparation (thin vs thick) were meaningfully different. It was found that thick cooking preparation of lentil was preferable than its counterpart. This result may be partly explained by the fact that in each measurement of palatability/taste, texture, visual appearance, and portion size score were higher in thick preparation of cooked lentils regardless the three amounts 25 g, 37.5 g, and 50 g raw lentil categories compare to thin cooked preparation. Therefore, it would be reasonable to be in opinion that Bangladeshi adolescents would likely to prefer thick cooking preparation of lentils than thin preparation if future efficacy carry out using the same recipe among similar study context. Secondly, on the issue of lentil amount to be served for future efficacy trial, highest positive mean score in 'prospective consumption' (before meal and after one bite of meal) and 'portion size about to consume' were noted in the 37.5 g raw lentil category compare to other amounts 25 g and 50 g.

Determining the suitable amount of lentils for future human efficacy trial is complex. It depends on four main determinants i.e residual amount of served amount, Fe content consumed by the adolescents, adolescents iron RDAs level and bioavailability of the fortified lentils. The residual amount of cooked lentil was calculated from the cooked amount served and left over after finishing the meal. We found that lowest mean residual amount for 25 g raw lentils (157.9 g thick served amount) was 3% and highest (14.4%) in thin 50 g raw amount (431 g thick served amount). It was noted that higher the amount, higher the left-over. However, the potential challenge with this amount is the Fe content consumed by the adolescents which are 4.9 mg Fe. Iron content of the served cooked lentil daal was calculated based on the recent findings of cooked fortified lentils which reported that 50 g of fortified raw lentil provides approximately 10 mg of Fe after cooking, of which 6.5–7 mg of Fe from the Fe fortificants and 3.5 mg from the lentil (Podder et al., 2017). In this study, we have two age groups that fall under iron RDAs for adolescent i.e age between 10-13 years and 14-18 years. RDAs of iron

for girls- 8 mg for 9-13 years + additional 2.5 mg/d who have started to menstruate, and 15 mg for 14-18 years (Institute of Medicine; Food and Nutrition Board; Committee on Use of Dietary Reference Intakes in Nutrition Labeling, 2003). The amount consumed (4.9 mg/d) may be appropriate for the adolescents aged between 10-13 years as they have lower Fe RDAs (8mg Fe/d) and covers 61.2% of their Fe RDAs. Contrary, this amount may not be sufficient for 14-18 years girls to increase their daily dietary iron intake as they have higher -almost double the RDAs (15mg Fe/d) than younger girls and covers only 32.6% of their Fe RDAs. Therefore, this 25 g raw amount (157.9mg thick served amount) can be ruled out due to this difference of adolescents' Fe RDAs. The next lower raw lentil 37.5 g (served amount 202 g) had 17.7 g residual amount (8.7%). This amount seems to be reasonable as adolescent girls consumed higher amount than the previous amount. Fe content of this amount consumed by the adolescents was 6.9 mg Fe/d which covered approximately 86% (and 65.7% who have started to menstruate) of their respective RDAs for 9-13 years [8mg Fe/d (+ 2.5 mg/day of iron who have started to menstruate) and 46% of the RDA for 14-18 years (15mg Fe/d) respectively for adolescent girls aged between 10 to 17 years. This amount could covers approximately 50% of the adolescents' daily iron requirements. The other raw amount 50 g (served amount 256.5 g) can be eliminated because of its Fe content (9 mg Fe) which exceeded the RDAs (8 mg) of the younger adolescents (10-13 years). However, a potential argument can be made in favour of the 50 g raw amount of lentils oppose to 37.5 g raw lentil even though it has been exceeded the iron RDA. It is because Tolerable Upper Intake Levels (ULs) for iron is 40 mg and 45 mg for females aged 9-13 years and 14-18 years respectively (Institute of Medicine; Food and Nutrition Board; Committee on Use of Dietary Reference Intakes in Nutrition Labeling, 2003). It would be therefore reasonable to argue that exceeding iron RDA from its upper boarder line would not affect adversely to the adolescents. But the drawback of this exceeding iron RDA amount could be several because Bangladesh National Nutritional Survey (NNS) reported low prevalence of the iron deficiency, even lower proportion of the adolescent were suffering from iron deficiency anemia. This would mean that majority of the children were neither iron deficit nor anemic, and serving them above their RDA could increase their body iron status high. In addition, it would mean that they were consuming sufficient iron from their usual daily dietary intake. For example, natural existence of iron in water. Bangladesh national drinking water quality survey conducted in 2009 reported that iron content of Gazipur district (our study area) was average 1.66 mg/L with a maximum of 9.1 mg/L (Bangladesh Bureau of Statistics & UNICEF, 2011). It futher reported that high levels of iron in drinking water were widespread in Bangladesh- affecting approximately 40% of its population as they were drinking more than Bangladeshi iron in drinking water limit of 1.0 mg/L (Bangladesh Bureau of Statistics & UNICEF, 2011). Other example related to the common foods in Bangladesh could include- 1 egg (hard boil) could serve 1 mg/L Fe, potato 2 mg/L, rice 1 mg/L etc (Institute of

Medicine; Food and Nutrition Board; Committee on Use of Dietary Reference Intakes in Nutrition Labeling, 2003). Serving more than the RDA of iron to certain population who are not iron deficit may be not recommended and could pose continuous threat to that population if in case their daily iron intake from dietary source increase at high level. However, these arguments lose its weight as this study did not collect detail dietary information from the adolescent. It would be a conclusive argument related to the adolescents' dietary intake information if this study could collect these information and we consider it as one of the major limitation of this study. Another drawback with the 50 g raw amount serving was the residual amount. Although we found that higher the amount served, higher the amount consumed, but we also noted that higher served amount had the higher residual amount. We found that average 10.6% of the cooked lentil were spoiled in the 50 g raw amount arm whereas 8.7% was spoiled in the 37.5 g raw amount arm. Given the limitation, 37.5 g raw amount could be better choice compare to the 25 g or 50 g raw amount because of the assumption that it will covers approximately 86% and 46% of the iron RDA of the children aged 9-13 years and 14-18 years and rest of iron could be fulfilled by their usual daily foods which commonly included green leafy vegetables, carrots, and natural existence of iron in drinking water.

Relative bioavailability of iron of cooked lentils is another concern that needs to be considered before suggesting lentil amount for the future human efficacy trial. As iron-fortified lentil is a plant source, therefore non-heme iron in nature. It is significantly less absorbed than heme iron which comes from animal source foods (Hurrell & Egli, 2010). Although increasing non-heme relative bioavailability of legumes (eg. lentils and pulses) depends on natural presence of anti-nutrients such as polyphenolic compounds and phytic acid, previous studies reported that lentils cooking with water could reduce these anti-nutrient substances, thus increases bioavailability (Quinteros, Farré, & Lagarda, 2003; Viadel, Barberá, & Farré, 2006a, 2006b; Wang, Hatcher, Toews, & Gawalko, 2009). Previous study reported that removal of the seed coat (dehulled lentil) increases relative iron bioavailability (DellaValle & Glahn, 2014). To increase its bioavailability based on the previous evidence, we chose dehulled lentils for iron fortification. Further study revealed that cooked iron-fortified lentils have significantly lower level of phytic acid compared to uncooked fortified lentils and an increment of 32-36% of higher absorption was observed among the fortified cooked lentils (Podder et al., 2017). Earlier studies reported that neither iron contents of the iron-fortified food vehicles reduce after cooking nor adversely affect consumers' consumption (Losso et al., 2017; Naozuka & Oliveira, 2012; Prom-u-thai, Rerkasem, Fukai, & Huang, 2009). We, however, to be cautious, did not wash or rinsed iron-fortified lentils before cooking for both thin or thick preparation rather those were washed before iron fortification process. This would mean higher relative iron bioavailability of iron-fortified lentils and made it a potential candidate for reducing global iron deficiency burden through the non-heme source.

The population we studied belonged to semi-urban and rural Bangladesh. As a proxy for socioeconomic status, almost all their household had electricity connection. However, having electricity connection does not ensure uninterrupted electricity supply particularly in the semi-urban and rural areas in Bangladesh. Majority of the adolescents lives in Tin-shed houses which indicates that they belong to low socioeconomic status. Around 85% of the country's population lives under the tin-shed roofing and it is considered as large identifiable difference between urban and rural housing in Bangladesh (National Institute of Population Research and Training (NIPORT), Mitra and Associates, & IFC International, 2014). Bangladesh Demographic and Health Survey reported that 24.8% and 25.3% of rural population fall under lowest and second lowest quintile in the national wealth index (National Institute of Population Research and Training (NIPORT) et al., 2014). As this study area cover semiurban and rural areas, it would be reasonable to assume that they belong to same socioeconomic status. It is however, to be noted that this type of housing may not be considered as low socio-economic group in other countries. For instance, bamboo is used to make houses among the low-income household in Equador (Housing and planning in urbanizing countries, n.d.). In kenya, houses made of mud wall with grass-thatched roofs would consider low economic factors (Ayaya, Esamai, Rotich, & Olwambula, 2004). We converted their BMI corresponding to the adult scale using BMI Percentile Calculator for Child and Teen (CDC, 2017) and found a mean of BMI score 18.5. Furthermore, their mean of waist-hip ratio was 0.8 which would indicate that they are healthy. This ratio considered as an indicator for measuring body fat deposition and indicates that higher the ratio-higher the risk of complication of obesity (WHO, 2008). It is observed that the leftover (after finishing the meal) was considerably less ranging from 3% thick preparation to the lowest amount served 157.9g (25 g raw amount) and 14.4% for the highest amount thin preparation served 431.1g (50 g raw lentil). We found similar scenario during the pre-trial qualitative adolescent discussion. Another study on iron absorption using typical Bangladeshi meal plan showed that adding rice with cooked lentils significantly increases the relative iron bioavailability of the meal (DellaValle & Glahn, 2014). Considering their palatibility score and residual amount, it may be reasonable to conclude that adolescents are keen to eat both preparation of lentils that we served with the standard amount of rice.

One potential implication of this study was to pave the way for the future human efficacy trial. It will follow a double-blind, community-based, cluster-randomized control trial which will test the efficacy of consumed iron fortified lentils in improving Fe stores of rural adolescent girls in Bangladesh. This cluster- randomized controlled trial will eliminate selection bias and bias from confounding variables, allows to reliably assess the causal effect of the iron fortified lentils. Ruling out a few questions related to the intervention at the first instance were crucial such as at what portion size of raw lentils, which cooking preparation, for how long, when to serve, mixed with other foods or not

and would adolescent comply, does community accept such meal before initiative human efficacy trial. However, there is no certainty that answering these questions will ensure ideal efficacy trial. One potential argument could be made against iron intervention in Bangladesh because of the recent low prevalence of national iron deficiency among Bangladeshi adolescents. Recent statistics (data from 2011-12 published in 2013) showed the national prevalence of iron deficiency (serum ferritin level  $<15.0 \mu\text{g/L}$ ) among children of 6-11 years and 12-14 years were 3.9% and 9.5% respectively. This national nutritional survey further reported that national IDA (haemoglobin  $<11.5 \text{ g/dL}$  plus ferritin level  $<15.0 \mu\text{g/L}$  in children 6-11 years and haemoglobin  $<12.0 \text{ g/dL}$  plus ferritin level  $<15.0 \mu\text{g/L}$  in children 12-14 years) prevalence among children of 6-11 years and 12-14 years were 1.3% and 1.8% respectively (ICDDRDB et al., 2013). Contrary to the expectation, Bangladesh National Nutritional Survey (NNS) did not shown specific iron status for adolescents aged 15-18 years rather it shows iron status as non-pregnant non-lactating women (15-49 years). It was essential information for this specific age 14-18 years because of their iron RDA (15mg Fe/d) (Institute of Medicine; Food and Nutrition Board; Committee on Use of Dietary Reference Intakes in Nutrition Labeling, 2003). Given the recent national statistics, we, therefore, set out iron fortification as a food-based approach rather than adopting iron supplementation to reducing iron deficiency of the adolescents. Furthermore, given the facts from the Bangladesh National Nutritional Survey (NNS), we propose for future fortified lentil efficacy trial to enrol adolescents above the cut-off point (Serum Ferritin  $<15.0 \mu\text{g/L}$ ) of iron deficiency meaning that iron deficiency anemic adolescents should be excluded from the efficacy trial. However, excluding only anemic adolescents from the future efficacy trial would not have positive implications to its outcome because there are other forms of anemia that are prevalent in Bangladesh. For instance, pernicious anemia are caused by vitamin B12 and folate deficiencies, and it is reported that 8.6% and 6.1% of the rural population in Bangladesh were folate and vitamin B12 deficit (National Institute of Population Research and Training (NIPORT) et al., 2014). Therefore, future efficacy trial should consider serum ferritin level as its outcome because adolescents who will be participated in the efficacy trial may still found to be anemic which may not be because of iron. Study reported that iron deficiency was not found to be associated with high prevalence of anemia in Bangladesh (Rahman et al., 2016). Although, data on the prevalence of hemoglobinopathies in Bangladesh is scarce (Hossain et al., 2017), a study found 28% rural women have beta thalassemia (Merrill et al., 2012). The consumption of iron-fortified lentil would act as a lasting sustainable solution which will ensure much of daily iron demand through staple food approach. For instance, if future human efficacy trial aim to provide 37.5 g raw lentils in thick preparation, that would essentially ensure 86% RDA (8 mg Fe/d) for 9-13 years and 46% of the RDA (15 mg Fe/d) for 14-18 years. To support this, WHO recognized fortification as

broader and sustainable solution for reducing malnutrition (Tontisirin et al., 2002; WHO & FAO, 2006). Other study acknowledged fortification as the most effective way to deal with the micronutrient deficiency of the vulnerable population and it is also less expensive and economically profitable (Briend, 2001; Horton, 2006). Given the positive results from the future human efficacy trial, it would create industry at large scale that could potentially cover daily iron demand of the vulnerable population.

One major strength of the study is the cross-over design itself as it balances all interventions and times periods among all subjects equally. Furthermore, the design reduces the influence of confounding factors because the design allows each intervention receiving participants to serve as her own control on the given intervention for equal time-period. Therefore, it creates an ideal situation to compare for a certain intervention to an individual. However, it is possible that there might be the existence of ‘carry over’ effect, i.e., one intervention’s affect the next intervention. In order to mitigate the ‘carry over’ effect, sufficient wash-out period (2 days) were given all interventions. Despite challenges of the cross-over trial design, the study was prone to certain biases due to the survey that we carried out before the trial. Selection bias was less likely to occur in this study because it did not have issue of exposure of certain variables among the study population. Selection criteria were based only on the age limit and adolescents who already been recruited in the existing BRAC adolescent clubs. However, the difference of motivation participating in the study between BRAC adolescents and non-BRAC adolescents residing in the community were less likely to affect negatively the study outcome. It is because this study was about consuming daal which is a common food that all Bangladeshi eat in their typical meal day and any fluctuation of the consuming the daal should likely to affect equally among Bangladeshi adolescents. Based on the recipe we used, it is possible that iron could be present in the water that we used to cook the lentils. However, any fluctuation of natural presence of iron in the water could likely to affect equally to the study participants. Questions may arise on the recipes which were slightly varied particularly in the amount of turmeric and volumn of water between thin and thick preparation of lentils that may affect taste. The study was flexible on the recipe because it was aimed to provide acceptable cultural taste using their own variation of recipes and identify their prefencce between thick and thin preparation of lentils. Although, we provided cooked rice mixed with each of the portion of cooked lentil in both thick and thin preparation, it is unlikely to affect adolescents’ responses because volumn of rice was standardized by serving in a standard small cup (250 mL) across all 6 conditions-thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g and thick 50 g. To reduce interviewer bias, we trained enumerators all together for the equal duration by the same trainer in order to adhere them to the questionnaire and capturing answers precisely, with the same degree of questioning on the close-ended structured questions. We recruited trained female enumerators to



conduct the survey interviews which includes question related to adolescents' menstrual cycle and to carry-out the anthropometric measurements in order to be culturally appropriate and being gender sensitive. Furthermore, we recruited four female research assistants to serve the cooked meal to the adolescents and collecting visual analog scale data. Recall bias is less likely to occur when enumerator asked adolescents on their menstrual age because it is likely that adolescents could recall their menstrual age as it is a memorable life event for women. One of the major weakness of the study was not assigning 6 conditions-thin 25 g, thin 37.5 g, thin 50 g, thick 25 g, thick 37.5 g and thick 50 g randomly among the randomly selected clubs. Another major limitation was not collecting data on other risk factors responsible for iron deficiency such as history of chronic blood loss, vitamin B12 and folate deficiencies, and intestinal disorder that inhibits iron absorption in the circulation. It would be useful for future efficacy trial to know the prevalence of other risk factors of iron deficiency anaemia among the Bangladeshi adolescents. The study has several limitations of the qualitative design. For the interviews, it is possible that participants viewpoint might not be effective for a future human efficacy trial because of their views which were based on their own set of meaning. However, their stances helped to describe the palpable context of the local situation during the study. It is important to be cautious before making quantitative predictions.

### **5.1 Recommendation for future efficacy trial implementation:**

- *Low profile strategy during the intervention:* As researchers do not belong to the community, frequent visit to the study area with numerous people together may raise many questions and confusion among the local residents. In such cases, recruiting a supervisor, whose task would be to visit each of the clubs on a random basis to ensure research assistants are doing what they are supposed to do.
- *Quality of rice:* Consistency of the rice quality is important. Rice was normally purchased in installments. Therefore, a chance of different quality of rice is possible. The problem of the different quality rice is the different smell; some rice quality is not pleasant. Serving the same quality (variety) of rice is important as it could adversely affect the response of the taste of the cooked daal intervention among the study participants, therefore, could contaminate the intervention outcome. This could lead to drop-out of the study participants.
- *Daily update of the intervention:* It is important to update daily wise lentils served i.e amount, recipe (+/-) and preparation, so that researcher based in Canada could track those and make sure things are going well. If require it would help to make an immediate decision.
- *Lentil shipment to study site:* Implementation partner of the study – BRAC, has multiple storage facilities – usually in all district level. Therefore, it is recommended that required amount of

lentil for future efficacy trial could be shipped from the Canada to Dhaka in one single installments. It would be useful to distribute required amount of lentil directly to the study site field office. Using a central storage facility which usually located far from the field office would be not be useful as it would require an extra logistic arrangement for transferring the lentil in multiple installments.

- *Package of lentils:* These must be waterproof. It is because Bangladesh is a tropical country and often has been experiencing unusual rain which could spoil lentils due to the prolong contact with water. *Community engagement:* Several community engagement tools would increase the participation. For instance, organizing picnic, teaching cycling to the participants.

## **5.2 Recommendation for future efficacy trial design:**

- *Challenges with age and self-administered questionnaire:* particularly, aged 10-11 years are crucial for the self-administered questionnaire. Adolescents who will be studying in class five, it would be difficult for them to read and understand. They would require special attention to make sure that they understand the questions.
- *Sample size:* Although, this feasibility study was carried out among 100 adolescent girls, future efficacy trial sample size should be calculated with at least 80% power given the high response rate (100%) of the adolescents. It is understood from the study that future efficacy trial is scalable in similar settings. However, it would be safer to have 20% dropout rate as it is expected that sample would be higher in the efficacy trial.
- *Study participants:* Future efficacy trial may face challenges if it aims to capture adolescents aged from 10-17 years because of the different iron recommended dietary allowance (RDAs) – 8mg/dL and 15 mg/dL for 9-13 years and 14-18 years respectively. Balancing iron RDAs through iron fortified lentil between these two groups would be challenging because iron RDA of these two groups are almost double to each other.
- *Dietary intake:* This feasibility study did not collect dietary intake information from the adolescents, therefore failed to generate evidence for future efficacy trial. However, it is recommended to collect dietary intake data for explaining the dietary pattern of the study participants before claiming the effectiveness of the iron fortified lentils on their body iron status.
- *Socio-economic data:* Future efficacy trial should be collecting detail socio-economic data as it would be important to explain the study population. It is learned from this study that limited socio-economic data (household condition and status of electricity connection) would not be sufficient to explain the study participants.

- *Blood sample:* This feasibility study did not collection any sort of biological sample. Therefore, could not direct the future efficacy trial in this regard.
- *Qualitative component:* Haiving a qualitative component of the study helps enormously to understand the study context and adoelscents perception on the fortified lentil intervention. This fesibility find it helpful in explaining such scenario. Therefore, future human efficacy trial could consider to having an component of process evaluation.

In conclusion, it can be inferred from this crossover study that an uncooked amount of raw lentils (37.5 g) cooked in thick preparation using Bangladeshi recipe for the period of 12 weeks is feasible to carry out a future human efficacy trial.

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# APPENDICES

## A: Conceptual framework

