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EFFICACY OF A PEANUT-BASED FOOD ON THE NUTRITIONAL STATUS OF WOMEN

BY

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DECLARATION

I, Isaac Agbemafle do thereby declare that with the exception of cited references, all other information in this thesis was produced by me through research under the supervision of Prof. Matilda Steiner-Aseidu and Dr. Firibu Kwesi Saalia, in the department of Nutrition and Food Science, University of Ghana, Legon.

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ABSTRACT

Background: Since the turn of the 21st century, ready-to-use foods have emerged as the preferred solution to the nutrient needs of specific target groups. This study assessed the efficacy of a peanut-based ready-to-use supplementary food (RUSF) on the nutritional status of women of reproductive age (WRA) in peri-urban settlements in the Ga East Municipality in the Greater-Accra region of Ghana.

Methods: This was a randomized controlled trial among 134 WRA aged 15-49 years. Of the 80 women who completed the study, 47 (58.8%) were in the intervention group whilst 33 (41.2%) were in the control group. The intervention group received 330ml RUSF thrice weekly for 12 weeks whilst the control group received no food. Proximate composition of the RUSF was determined using standard procedures. Baseline background characteristics, dietary intake, anthropometric, haematological and biochemical indices were measured. At 6 and 12 weeks, all measurements were repeated except for dietary intake and background characteristics. Height, weight, neck circumference (NC) and hip circumference (HC) were measured with a stadiometer, digital weighing scale and tape measure respectively. Whole blood was used to determine full blood count using the haematology automated analyzer. Sickling was determined by blood staining. Serum ferritin and iron were measured by an automated immunoassay and by the colometric agar-based method. C-reactive protein (CRP) was determined using an enhanced turbidimetric immunoassay technique. Serum total proteins, albumin and globulins were measured by Biuret, Bromocresol green and glyoxylic acid methods. Changes in baseline and endline within and between groups were compared using paired and independent sample t-tests.

Results: The mean age of the women was 29 ± 8 years. Based on dietary recall, 59.0%, 66.4%, 34.3%, 73.9% and 73.4% of the women met their recommended daily allowance (RDA) for total

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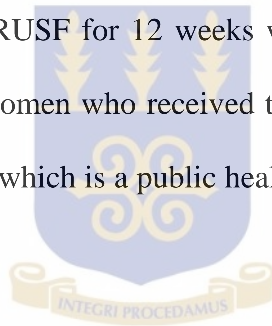
calories, protein, fats, iron and vitamin C respectively but none of the them met the RDA for folate. Nonetheless the data showed that 86.6% were food insecure. Eighty-three percent (83.0%) of the women were sickle cell negative. The energy, carbohydrate, protein, fat and ash content of the RUSF on dry matter basis were 564.52, 59.15, 22.93, 15.26 and 2.66g/100g respectively. Change in body mass index (BMI) in the intervention group was 0.96 ± 2.83 ($p<0.05$) whilst the control group was 0.42 ± 3.29 ($p>0.05$). Haemoglobin concentration in the intervention group increased from 12.26 ± 2.06g/dL to 12.43 ± 2.00g/dL and the control group decreased from 12.52 ± 1.76g/dL to 11.71 ± 1.33g/dL. Prevalence of anaemia was >30% in both groups. CRP was positive for less than 20% of the women. No differences in NC, HC, total proteins, albumin, globulins, iron and ferritin were observed between the two groups.

Conclusions: Supplementation with RUSF for 12 weeks was associated with a modest increase in BMI and haemoglobin amongst the women who received the RUSF. The RUSF has the potential to help improve iron deficiency anaemia which is a public health concern in Ghana and most developing countries.



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DEDICATION

I dedicate this work to Dr. (Mrs) Bettina K. Boohene-Andah (Bsc. Med. Sci. MB ChB)

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‘For I know the plans I have for you,’ Isaac, ‘said the Lord. Plans to prosper and not to fail you, plans to give you a future and hope.’ (Jeremiah 29: 11). Indeed, this is how far the Lord has brought me. I first thank the Almighty God for His Grace and strength to complete this thesis.

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LIST OF ABBREVIATIONS

BMI- Body Mass Index

CSB- Corn-soy blend

GDHS- Ghana Demographic and Health Survey

IDA- Iron Deficiency Anaemia

IUGR- Intra Uterine Growth Retardation

LBW- Low Birth Weight

MAM- Moderate acute malnutrition

MAM- Severe Acute Malnutrition

MDG(s)- Millennium Development Goal(s)

NTD(s)- Nural Tube Defect(s)

RCT- Randomized Controlled Trial

RDA(s)- Recommended Dietary Allowance(s)

RUF(s)- Ready-to-use food(s)

RUFS- Ready-to-Use Fortified Spread

RUSF(s)- Ready-to-use supplementary food(s)

RUTF(s)- Ready-to-use therapeutic food(s)

SAM- Severe Acute Malnutrition

SAM- Severe acute malnutrition

SFP- Supplementary Feeding Programme

WHO- World Health Organization

WRA- Women of Reproductive age

WSB- Wheat-soy blend

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CHAPTER 1

1.0 INTRODUCTION

1.1 Background information

More than half of the world's population is women, of which over 60% especially in developing countries are undernourished (UN/ECOSOC, 2007; Black *et al.,* 2008). This high prevalence of undernutrition among women in developing countries, claim one in every sixteen lives of women in sub-Saharan Africa as compared to one in 4000 in developed countries (UNICEF, 2005). Globally, undernutrition among women of reproductive age (WRA) results in 1 out of 6 infants born with Low Birth Weight (LBW) (Bryce *et al*., 2005). In Ghana, 9% of women of reproductive age (WRA) especially those in the rural areas have BMI less than 18.5, an indication of chronic energy deficiency which is a risk factor for poor birth outcome and obstetric complications (GSS, NMIMR and ORC Macro, 2004). Statistics indicate that nearly six out of every ten WRA in Ghana are anaemic (GDHS, 2008) with similarly high values for both vitamin A and Zinc deficiencies (Lartey, 2008).

Good nutrition which is a cornerstone for good health for all groups of people especially women is a necessary tool in the attainment of the millennium development goals (MDG) (MDG Report, 2010). Direct-nutrition specific interventions such as dietary diversification, supplementation and food-fortification strategies have been extensively studied in eliminating undernutrition (Black *et al.,* 2008). Micronutrient supplementation and fortification of commonly consumed products such as biscuits, cereals, wheat flour, sugar, vegetable oils and drinks have been done in a number of countries suffering from malnutrition with some improved health benefits (WFP, 2007). Iron fortification and multiple micronutrient supplementations have been found to increase haemoglobin levels among WRA during pregnancy (Thuy *et al*., 2005; Gupta *et al*., 2007). However, maternal multiple micronutrient supplementations have recently been criticized for possible association with an

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increased risk of neonatal mortality in health systems without adequate access to skilled care (Bhutta and Haider, 2009). Additionally, nutrient interactions especially in complex formulations may underlie the apparent failure of the benefits of micronutrient supplementation (Ramakrishnan *et al*., 2009). A trial in Burkina Faso concluded that multiple micronutrient supplementations should co-exist with balanced protein-energy supplements (dietary supplements) for the greatest benefits in terms of birth weight (Tofail *et al*., 2008; Huybregts *et al*., 2009). Dietary supplements are mostly made from cereals and legumes. The most commonly used dietary supplement is corn-soy blend (CSB). CSB is low in energy and micronutrient content, a limitation that can be overcome by the use of ready-to-use supplementary foods (RUSF). RUSFs are an energy-dense and a micronutrient-rich paste. In recent years there has been great success with its use for the treatment of moderate acute malnutrition in operational settings as well as among HIV/AIDS patients (Collins and Sadlar, 2002; Oosterhout *et al*., 2010). Chaparro and Dewey (2010) have proposed using similar products for other target groups.

RUSF can be considered as a macro and micronutrient intervention. Because of its food matrix (fibre), RUSF minimizes any potential hazard of single large doses of iron especially in malaria endemic areas as reported in Zanzibar (Sazawal *et al.* 2006; WHO/UNICEF 2007). Although supplementary feeding programs with RUSF have beneficial effects, the cost associated with its ingredients and production is exorbitant. There are two types of RUSF namely milk-based and non-milk based; both have comparable nutrient composition (Collins and Jeya, 2004). However, it's more expensive to produce milk-based RUSF because of the high cost of milk powder which is a component ingredient. This limits its production and accessibility for poor vulnerable individuals in developing countries where malnutrition is very high. Hence, cost effective strategies such as the use of cheap locally available ingredients are recommended in the production of non-milk based ready-to-use foods (Owino, 2010).

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Local production using alternative recipes (grains and cereals) has gained momentum because it reduces the cost of production and provides income for local manufacturers and farmers.

1.2 Rationale

Several studies have identified undernutrition among Ghanaian women (GDHS, 2008; GSS, NMIMR and ORC Macro, 2004). Nutritional status of women during and after pregnancy has extensively been studied (Huybregts *et al*., 2009; Gupta *et al*., 2007; Ceesay *et al*., 1997) but little is known of the pre-pregnancy nutritional status of WRA. Interventions aimed at assessing the nutritional status of women have mostly been through micronutrient supplementation and/or food fortification with some beneficial effects (Huybregts *et al*., 2009; Gupta *et al*., 2007). Information about the use of milk and non-milk based RUSF among moderately malnourished children, in emergency situations and among HIV/AIDS patients have been shown to offer some health benefits (Collins and Sadlar, 2002; Chaparro and Dewey, 2010; Oosterhout *et al*., 2010) but its use among WRA has not been studied.

It is therefore envisaged that, studies about the nutritional status of women before, during and after pregnancy should provide a more comprehensive knowledge and understanding of the issues relating to poor nutritional status of women as well as possible ways to improve on their nutritional status. Knowledge of the efficacy of RUSF in improving the nutritional status of WRA is necessary in order to reduce maternal mortality and morbidity. It is therefore essential to identify a locally based intervention that can be used to improve the nutritional status of WRA. A locally based intervention that takes into account the culture of the people is necessary for its acceptability and use. Its acceptability will call for large scale production and its consumption will help reduce malnutrition and its related diseases among food insecure WRA in developing countries.

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1.3 Main research question

To what extent does a peanut-based RUSF improve the nutritional status of women of reproductive age (WRA)?

1.3.1 Specific research questions

i. What is the nutritional composition of the peanut-based ready-to-use supplementary food (RUSF) and its contribution to recommended daily allowance (RDA)?
ii. What are the anthropometric indices of the women in the intervention and control group?
iii. What are the haematological indices of the women in the intervention and control group?
iv. What are the biochemical indices of the women in the intervention and control group?

1.4 Objective of the study

The study seeks to assess the efficacy of a peanut-based RUSF in improving the nutritional status of WRA in the Ga East Municipality in the Greater-Accra region of Ghana.

1.4.1 Specific objectives

i. To determine the nutritional composition of the peanut based RUSF and its contribution to recommended daily allowance (RDA)
ii. To determine the anthropometric indices of the women before and after the use of the RUSF
iii. To determine the haematological indices of the women before and after the use of the RUSF
iv. To determine the biochemical indices of the women before and after the use of the RUSF

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CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Introduction

The theory of the intergenerational cycle of growth failure linked to foetal programming through maternal undernutrition explains how growth failure is transmitted across generations through women. According to the theory, girls born with a low birth weight are more likely to become small adult women who are at a higher risk of having low-birth weight babies, partly because maternal size has a crucial influence on birth weight. Maternal size is influenced by nutritional status. Nutritional status describes the condition of a population's or individual's health as influenced by the intake and utilization of nutrients and non-nutrients. For an individual to be nutrition secured, food consumed should be nutritionally balanced, adequate and safe. If this is not achieved then malnutrition sets in.

Malnutrition has two components: undernutrition that occurs when the body does not get enough nutrients and overnutrition, when there is excess intake of nutrients. Undernutrition (underweight) remains the nutrition problem of greatest concern (Dewan, 2008), however overnutrition (overweight and obesity) in recent times has gained much attention due to its escalating trend and deleterious consequences for non-communicable diseases (NCDs). Undernutrition could be due to deficiencies in either macronutrients and/or micronutrients, the latter of which is referred to as hidden hunger. Hunger defined as undernutrition is associated with food and nutrition insecurity. According to the FAO report on "The State of Food Insecurity in the World, 2012", global prevalence of hungry people declined between 1990-92 and 2010-12, from 18.6% to 12.5% and for developing countries from 23.2% to 14.9%. These improved hunger estimates puts the Millennium Development Goals (MDGs) within reach. However, the current estimates of 870 million hungry people remain unacceptably high and this is skewed towards children and women of reproductive age (WRA) mostly in developing countries

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like Ghana. The continuing presence of hunger, undernutrition and overnutrition makes it essential to assess the nutritional status of individuals so as to improve health.

2.2 Assessment of nutritional status

Nutritional assessment is the evaluation of the nutritional status of individuals or populations through food and nutrient intake and evaluation of nutrition- related health indicators (Lee and Nieman, 1996). Screening is peculiar to nutritional assessment and identifies characteristics known to be associated with nutritional problems. The ultimate aim of nutritional assessment is to improve human health by identifying individuals who are malnourished or at risk of malnutrition. Assessment of nutritional status involves the interpretation of data obtained from anthropometry, biochemical, clinical and dietary methods to identify individuals or groups who are either or at risk of under/ over nutrition. The mnemonic "ABCD" can be helpful in remembering these four different methods which will be explored in the next few paragraphs.

2.2.1 Anthropometric indicators

Anthropometry is the measurement of physical dimensions and gross composition of the body such as weight, height, head, waist and hip circumference and skinfold thickness (Lee and Nieman, 1996). It is a widely used inexpensive and non-invasive measure of general nutritional status of an individual across various age groups. Its assessment relies heavily on age, sex, height and weight and the measurements so obtained must be compared with standard values in order to make valid deductions. For children, weight-for-height (underweight), height-for-age (stunting) and weight-for-height (wasting) are frequently reported whiles body mass index and waist-to-hip ratio (WHR) are widely used in adult populations.

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Body mass index (BMI; in Kg/m^2), a simple anthropometric measure of weight in kilograms (kg) divided by the height in metres squared (m^2) is the most widely used screening tool for underweight, overweight and obesity. Low BMI (<18.5 kg/m^2) and/or short stature (height <145 cm) are common in women in low-income countries (WHO/WHA, 2012). Prevalence of low maternal BMI ranges from 10-19% in most countries (Black *et al*., 2008). In a study conducted by Thame *et al*. (2012) in Jamaica, it was reported that lower maternal weight at the first antenatal visit was significantly associated with smaller placental volume. A meta-analysis of 25 data sets by Kelly *et al*. (1996) also demonstrated high odds ratio (OR) for both Low Birth Weight (LBW) and intrauterine Growth Restriction (IUGR) for attained weight indicators from pre-pregnancy through 9 lunar months. Conversely, about 35% of adult women worldwide are estimated to be overweight (BMI ≥25 kg/m^2), a third of whom (297 million) are obese (BMI ≥30 kg/m^2) (WHO/WHA, 2012). A study based in Manchester, United Kingdom, found that the risk of fetal macrosomia and assisted delivery increased with increasing BMI such that morbidly obese women were at greatest risk of both than women of normal weight (relative risk= 4.78 and 1.66 respectively) (Khashan and Kenny, 2009). Macrosomia leads to adverse clinical and social consequences for both the mother and the infant. Also pregnancy may place an additional metabolic burden on obese adolescents and on women who tend to retain more weight after birth. Indeed, low maternal BMI, overweight and obesity are associated with adverse effects on pregnancy outcomes and a higher risk developing non-communicable diseases in adulthood.

2.2.2 Biochemical indicators

Biochemical assessment includes measuring a nutrient or its metabolite in blood, faeces or urine or measuring a variety of other components in the blood or other tissues that have a relationship with nutritional status (Lee and Nieman, 1996). The quantity of albumin and haemoglobin in blood

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indicates the body's total protein and iron status which are influenced by diet. Although most biochemical indicators enter the human body from foods or supplements, the body itself produces some indicators in response to dietary intake or environmental exposure. Blood and urine concentrations reflect the amount of nutrients and dietary compounds actually in the body or passing through the body from all these sources. Thus they help assess if an individual is getting enough of certain nutrients or if their nutrient needs are met.

2.2.3 Clinical assessment

Clinical assessment utilizes a number of physical signs and symptoms that are known to be associated with malnutrition, deficiency of vitamins and/or micronutrients (Lee and Nieman, 1996). Symptoms are disease manifestations that the patient is usually aware of and complains about whilst signs are observations made during physical examinations by a qualified personnel. Detection of relevant signs by clinical examination of hairs, nails, skin, eyes, tongues and angles of the mouth can be helpful in ascertaining the nutritional status of an individual or group of persons. It is fast, safe, non-invasive and inexpensive to perform but cannot detect early cases.

2.2.4 Dietary assessment

Dietary assessment involves surveys measuring the quantity of food the individual consumes during the course of one and/or several days or assessing the pattern of food use during previous days and/or months (Lee and Nieman, 1996). These dietary methods can provide data on intake of nutrients or specific classes of foods. Analyzing the nutrient intake involves using food composition tables although software programs are currently available for the same purpose.

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2.3 Nutrient needs

The nutrient need of an individual is that level of energy intake from food which will balance energy expenditure and it varies with age as well as sex. An individual's daily energy needs is covered by dietary reference intakes (DRIs). On average moderately active 57kg woman needs 2000kcal/day whilst an 80kg man with similar exercise pattern needs 2800kcal/day (WHO, 1985). However an extra dietary energy is required during pregnancy to make up for the energy required for the developing foetus, the pregnant woman herself and the lactation period to follow. It is therefore recommended that pregnant women consume an additional 340kcal and 450kcal of energy during the second and third trimester respectively because total energy expenditure does not change greatly and weight gain is minimal in the first trimester. These trimester-wise energy requirements that have generated the myth 'eaten for two' may increase during multiple pregnancies based on the mother's weight and level of physical activity. This myth needs to be properly handled since excess or low weight gain during pregnancy is associated with increased risk of obstetric complications and intrauterine growth retardation respectively.

For lactating mothers however, about 350-400kcal extra energy each day is required for the first year of breastfeeding; a requirement dependent on how much milk the mother produces and how quickly she loses her pregnancy weight (Institute of Medicine (IOM), 2002). Protein intake for adult men is 56g/day and 46g/day for women (DRI reports, 2002/2005). Additional protein (25g/day) over the non-pregnant requirement (46g/day) is also needed for growth of fetal, placental and maternal tissues during pregnancy and lactation (Institute of Medicine (IOM), 2002); a requirement achievable through the consumption of quality protein. Acceptable Macronutrient Distribution Range (AMDR) of total fats for both males and females daily ranges from 20-35 (DRI reports, 2002/2005).

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The Recommended Daily Allowance (RDA) for adult males and females are 900µg/day and 700µg/day for vitamin A and 75-90mg/day and 65-75mg/day for Vitamin C (DRI report 2000/2001). Vitamin A intake increases to 770µg/day and 1300µg/day for pregnant and lactating mothers whiles vitamin C increases to 85mg/day and 120mg/day. Folate increases by 200µg/day and 100 µg/day during pregnancy and lactation over its non-pregnant non lactating intake of 400 µg/day. Iron intake for adult women is 15-18mg/day and it increases to 27mg/day during pregnancy. Iodine intake for female adults is 150µg/day whiles zinc is 8mg/day (DRI report, 2001). During pregnancy and lactation, iodine levels increase to 220µg/day and 290µg/day respectively but zinc intake is 12mg/day for both pregnancy and lactation. Dietary intakes below these recommendations lead to the physical manifestation of hunger, which is known as undernutrition. Undernutrition could be due to deficiencies in either macronutrients and /or micronutrients, the latter of which is referred to as hidden hunger.

2.3.1 Macronutrient deficiencies

Abundant epidemiological evidence indicates the relevance of maternal macronutrient deficiency on intrauterine growth restriction (IUGR), low birth weight (LBW) and foetal programming for adult chronic diseases (Barker, 1994; Rosario *et al*., 2008). Animal studies have consistently shown that restriction of maternal food intake to 30% *ad libitum* throughout pregnancy or feeding low protein diet during pre-implantation period results in the birth of growth-retarded offsprings (Woodall *et al*., 1996; Wong *et al*., 2000), which have increased risk of adult chronic diseases.

Macronutrients such as carbohydrates, proteins and fats (especially the long chain unsaturated fatty acids) have been well studied in developmental programming for adult chronic diseases (Ozanne and Hales, 1999; Storlien *et al*., 2000). A review of studies on the nutritional status of pregnant and

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lactating women showed that women in developing countries met only about two-thirds of their RDA for their energy needs and that their average weight for height was in most cases well below the 50th percentile (McGuire and Popkin, 1989). A study conducted in a Bangladeshi rural setting showed that women received disproportionate amount of household food portions especially with fish and other animal products whiles in the same household, mothers' favoured preschool boys and husbands to their own disadvantage (IFPRI, 1998). Evidence from systematic reviews of randomized controlled trials on the effectiveness of nutritional interventions has demonstrated the beneficial effects of macronutrient (protein/energy) supplementation, with an overall odds ratio of 0.77 (95% CI 0.58, 1.01) for reducing IUGR (de Onis *et al*., 1998). A community based trial in rural Gambia showed that supplementing pregnant women with a high-energy groundnut snack significantly increased birth weight by 136g but birth length was not affected (Ceesay *et al*., 1997). However affordability and access to these diets remains a public health concern.

2.3.2 Micronutrient deficiencies

It is well known that micronutrients play an important role in determining the structure and metabolism of humans. Nonetheless most individuals cannot meet their micronutrient needs from food alone. Generally children and women have difficulty getting enough of vitamin A, iron, zinc, folate and iodine from foods alone because of their increased nutritional needs. Single and multiple micronutrient deficiencies exist in most developing countries, due to insufficient availability of adequate food quality, cultural differences, poverty and infectious disease burden (Allen, 2005). Globally the burden of micronutrient deficiencies due to food insecurity account for about 4.5 billion people suffering from deficiencies in iron, vitamin A and iodine (Khan and Bhutta, 2010). Micronutrient deficiencies of zinc and vitamin A contribute to the largest disease burden among women of reproductive age (WRA); however Iron Deficiency Anaemia (IDA) and Iodine deficiency

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Disorders (IDD) are highly prevalent among women in many developing countries (Black *et al*., 2008). These micronutrient deficiencies have important public health implications; thus rectifying them will greatly benefit women and children in many ways.

2.3.2.1 Vitamin A deficiency (VAD)

Substantial amounts of placental vitamin A are transported between mother and foetus but the problem is inadequate intake from foods which results in VAD. VAD has been associated with increased risk of morbidity and mortality from diarrhoea and measles (Klemm *et al*., 2007; Holick, 2006). Low maternal vitamin A status is associated with preterm birth, intrauterine growth retardation and low birth weight mostly in developing countries (Shah and Rajalakshmi, 1984). In developed countries concern during pregnancy is about excess vitamin A intake which produces abnormalities of the central nervous system, cardiovascular abnormalities and facial abnormalities (Teratology Society Position Paper, 1987). Dietary supplementation with vitamin A or β-carotene is reported to reduce maternal mortality by 40% but has no effects on foetal loss or infant mortality rates (West *et al*., 1999; Katz *et al*., 2002). Also studies conducted in developing countries have shown that vitamin A supplementation during pregnancy improves birth weight and growth among infants born to HIV-infected pregnant women, possibly due to the enhancement of immunity (Kumwenda *et al*., 2002).

2.3.2.2 Iron deficiency

Iron deficiency (ID) and iron deficiency anaemia (IDA) are well known nutritional deficiencies in both the developed and the developing world. WHO estimates that 30.2% of non-pregnant and 41.8% of pregnant women suffer from anaemia, much of it due to ID (WHO, 2009). More importantly, ID increases the risk of mortality among anaemic women caused by haemorrhage, which remains a leading cause of death in developing countries, accounting for approximately 25% of all deaths among

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women (WHO, 2010). In the past five years, the level of anaemia among Ghanaian women aged 15-49 years has increased from 45% in 2003 to 59% in 2008 (GDHS, 2008). Parasitic infections and malaria have triggered ID among women and children in developing countries to more than 90% (Khan and Bhutta, 2010). The major clinical manifestation of iron deficiency is anaemia or low blood haemoglobin concentration. According to Rasmussen and Stoltzfus (2003), iron deficiency anaemia is significantly associated with low birth weight and preterm delivery. Due to increased nutrient requirements as a result of some physiologic and biological changes such as menstruation, pregnancy, parturition, lactation, parasitic and chronic infections; WRA are vulnerable to iron deficiency (Gulani *et al.,* 2007; Osório, 2002; UNICEF, 2002). This increased nutrient requirement is often not met through diet because of the high consumption of foods from plant sources that have low iron and nutrient bioavailability as a result of inhibitors such as phytate and fibre. Randomized trials of iron prophylaxis during pregnancy have demonstrated positive effects on reducing low haemoglobin and haematocrit, and increasing serum ferritin and iron (Gulani *et al.,* 2007). A placebo-controlled study from Cleveland also demonstrated that 30mg of ferrous iron daily in pregnancy, started before 20 weeks of gestation, gave a significantly higher birth weight compared with the placebo group (Cogswell *et al*., 2003). However, the mechanistic basis by which poor iron status may cause adverse pregnancy outcome remains poorly understood probably due to insufficient data (Rasmussen, 2001).

2.3.2.3 Iodine deficiency disorders (IDD)

Adequate dietary iodine intake is crucial for normal thyroid hormone (TH) production and its maintenance. Iodine deficiency disorders (IDD) in WRA is clinically characterized by goitre. Maternal goitre leading to foetal hypothyroidism results in cretinism, characterized by severe neuro-developmental deficits and mental retardation (Khan and Bhutta, 2010). The mental retardation of the baby resulting from iodine deficiency during pregnancy is irreversible. According to Hetzel (1994),

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about 20 million people globally have brain damage resulting from maternal iodine deficiency that could be prevented by iodine supplementation. A systematic review by the Cochrane group demonstrated that iodine supplementation during pregnancy decreased neonatal mortality [relative risk=0.71(0.56-0.96); Ibrahim *et al*., 2006]. The universal salt iodization strategy has been most effective in the fight against IDD but it needs to be scaled up.

2.3.2.4 Zinc deficiency

Women of reproductive age (WRA) are at an increased risk of zinc deficiency because of poor dietary patterns (Nguyen *et al.*, 2012). Caulfield *et al.* (1998) estimated that 82% of women in Peru are zinc deficient and this accounts for about 0.5 million maternal and child deaths annually in developing countries (Black *et al*., 2008). Zinc deficiency is associated with retarded growth (Brown *et al*., 2009), reduced fertility (Ebisch *et al*., 2007), poor pregnancy outcomes (Caulfied *et al*., 1999) and increased morbidity and mortality (Maes *et al*., 1994). Studies conducted in developing countries have shown the benefits of zinc supplementation on the child's immune function, as well as reducing diarrhoea and respiratory illnesses in infancy (Osendarp *et al*., 2000). According to Goldenberg and others (1995), the provision of 25mg zinc daily elevated plasma zinc and significantly increased infant birth weight, head circumference, arm and femur lengths and subscapular skinfold thickness. However, in a recent Cochrane review by Mori *et al*. (2012) it was concluded that, there was no convincing evidence that zinc supplementation during pregnancy results in other useful and important benefits. Thus the effect of zinc supplementation remains unclear.

2.3.2.5 Folate deficiency

Dietary folate deficiency is increasingly becoming an issue of public health concern. This is because maternal folate deficiency leads to neural tube defects (NTDs), orofacial clefts, cardiac anomalies,

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anaemia, spontaneous abortions, bleeding, pre-eclampsia, IUGR and abruptio placentae (Black, 2001). Periconceptional folic acid supplementation prevents both the occurrence and recurrence of NTDs and significantly reduces the incidence of LBW (George *et al*., 2002; Baily *et al*., 2003). An extensive meta-analysis by Goh *et al*. (2006) concluded that, maternal consumption of folic acid-containing prenatal multivitamins is associated with decreased risk of several congenital anomalies. However, a randomised controlled trial by Charles *et al*. (2005) demonstrated no difference in mean birth weight, placental weight or gestational age. Thus the effect of folic acid supplementation throughout pregnancy also remains unclear (Hovdenak and Haram, 2012).

2.4 Causes and effects of undernutrition

Maternal nutrition prior to and during gestation plays a crucial role in fetal programming (Entringer *et al*., 2012). The 'developmental origins of adult disease' hypothesis, often called the 'Barker hypothesis' after one of its leading proponents, states that adverse influences early in intrauterine life, can result in permanent changes in physiology and metabolism, which result in increased disease risk in adulthood. Controlling the intrauterine environment may be beyond man's domain but curtailing the multifaceted causes of poor intrauterine environment is an issue of concern during the first 1000 days of life.

2.4.1 Causes of undernutrition

Adequate nutrition before and during pregnancy is important for the health of the mother and her ability to meet the demands of pregnancy. Evidence from several studies indicates that, physical, environmental, social and economic aspects of life link the nutritional status of women. Women of reproductive age are particularly vulnerable to malnutrition due to inadequate dietary intake,

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inequitable distribution of food within the household, improper food storage and preparation, dietary taboos, infectious diseases, recurrent infections and limited health care (Regassa and Stoecker, 2012). Other causes include food and nutrition insecurity, heavy work burdens, high fertility rates, short birth intervals and gender inequities (Khan and Bhutta, 2010). Social and cultural norms limit women's access to food, land, labour, health care, income, education and other services in the community (Sukchan *et al.,* 2010; Dewan, 2008). Poverty contributes to poor nutrition and health among women (Teller and Yimar, 2000). Poverty has a significant effect on low birth weight (LBW), neonatal mortality, and maternal mortality rate in developing countries (Khan and Bhutta, 2010). Infectious diseases most especially HIV/AIDS has affected about 13.1 million African women of reproductive age (UNICEF, 2005) and this has worsened undernutrition among women, making pregnancy a life-threatening situation for most African women (Abdoulae, 2006). These factors undermine the woman's ability to survive childbirth and give birth to healthy children.

The high incidence of teenage pregnancy before the adolescent woman is fully grown undermines her nutritional status. Information on adolescent marriage from UNICEF (2008) shows that a third of all young women in developing countries especially in rural areas are married before they turn eighteen. Adolescent cohabitation/marriage as compared to non-adolescent cohabitation/marriage is significantly associated with stunting of offspring, higher fertility and short birth intervals; giving the mother insufficient time to replenish her nutrient stores before the next pregnancy (Raj *et al*., 2010). Also early marriages isolate adolescents from their own families, increases their workload and food insecurity which contributes to their excessive thinness (Chorghade *et al*., 2006).

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2.4.2 Effects of undernutrition

Undernutrition among women may have an impact across various age groups or life cycle and could potentially lead to intergenerational growth faltering. Years of neglect causes many women to remain undernourished at birth, stunted during childhood and become pregnant during adolescence. This increases mortality, morbidity and disability among women. Undernutrition among women accounts for approximately 50% of IUGR in developing countries (Blo¨ssner and de Onis, 2005). In adult women, low maternal pre-pregnancy BMI of less than 18.5 is a known determinant of low birth weight (LBW) and an indicator of chronic energy deficiency which ranges from 10-19% in most countries, and the persistence of low BMI from a mother's own LBW is likely to contribute to IUGR (Ronnenberg *et al*., 2003; Black *et al*., 2008; UN/SCN, 2011). Evidence from developing countries indicates that low pre-pregnancy BMI increases prenatal and neonatal mortality, stillbirths and miscarriage, which further undermines the human capital development of the family and society, and continues the vicious cycle of poverty and undernutrition (Girma and Genebo, 2002; Blo¨ssner and de Onis, 2005; Myatt *et al*., 2011).

2.5 Approaches to eliminate undernutrition

Curtailing undernutrition among women is feasible and cost-effective, but there is no magic technological bullet for it. Recent research has shown that the nutrition of women should be given the necessary attention very early in life and during the reproductive age (Linkages Project, 2001). This has necessitated emphasis on prevention or early management of the signs associated with undernutrition. Supplementation, dietary diversification and food based approaches have been extensively studied in eliminating undernutrition in emergency and non-emergency situations. Supplementary feeding is defined as the distribution of food to add-on energy and other nutrients missing from the diet of those who have special nutritional requirements. Most supplementary foods

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are micronutrient-fortified combinations of cereals and legumes. The most commonly available supplementary food for management of undernutrition is corn-soy blended flour; an inexpensive fortified cereal-legume combination that requires cooking. Often, corn-soy blend (CSB) is made from locally available, low-cost ingredients and is culturally and organoleptically acceptable in many settings. However its use has been associated with disappointing results in supplementary feeding programs among vulnerable groups due to poor infection control (Caulfield *et al*., 1999). Also, low micronutrient content and bioavailability, low energy density, high fiber and anti-nutritive content, and ration sharing (Wood and Sibanda-Mulder, 2011) may contribute to recovery rates, which are as low as 24% in operational emergency settings (Navarro-Colorado, 2007) and less than 75% in controlled research trials (Matilsky *et al*., 2009). There is increasing evidence that CSB cannot meet the nutrient needs of vulnerable groups (Owino, 2010). The nutritional requirements of malnourished individuals are usually high and therefore require diets specially made to cater for their needs. The limitations associated with CSB led to the adoption of the 'standard therapy'.

Based on accumulated scientific evidence from the early 1960s to the late 1990s, a protocol dubbed 'standard therapy' was proposed by the World Health Organization (WHO) in 1999 for the treatment of Severe Acute Malnutrition (SAM) in children. Severe acute malnutrition is defined as a weight-for-height Z-score (WHZ) < -3 plus oedema (UNICEF, 2008). In the traditional standard therapy protocol, severely malnourished children are fed for 3-4 weeks during their recovery with adapted milk feeds prepared by mixing dried skimmed milk, oil and sugar with a vitamin and mineral complex (F-100). The recovery phase is normally preceded by an initial phase that is targeted to children who are very ill and includes dietary therapy with a milk-based liquid food (F-75) containing modest amounts of energy and protein (Ciliberto *et al*., 2005). This approach, however, is difficult to implement, since these feeds are excellent growth media for bacteria, and they must be prepared and

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fed under close supervision (Briend, 2001). The formula also requires the provision of portable drinking water. These limitations prompted the formulation of an improved product known as Ready-to-Use Therapeutic Food (RUTF) (Briend, 2001).

Several studies have confirmed the superiority of RUTF over the standard therapy and the corn-soya blend (CSB) for better recovery rates and higher weight gain in severely acute malnourished children (Matilsky *et al*., 2009; Patel *et al*., 2005). Thus the acceptability and effectiveness of ready-to-use therapeutic foods (RUTFs) in the treatment of SAM are well established, but there is increasing recognition of the need to prevent and not just treat severe malnutrition. Also, the use of RUTFs for management of moderate acute malnutrition (MAM) may be unsafe given the high concentration of nutrients in the formulation. This has led to the development of ready-to-use supplementary foods (RUSFs) with formulations specifically designed to prevent malnutrition, to be used as supplements to traditional complementary foods and to treat MAM. While RUTFs provide large quantities of energy along with the quantities of nutrients needed for treatment of SAM, RUSFs provide less energy and the recommended daily allowance of most micronutrients in a small dose meant to be combined with the local diet for management and prevention of moderate acute malnutrition (MAM). More recently it has been shown that RUSF with milder nutrient concentration compared to RUTF are more effective in treating MAM (Isanaka *et al*., 2010; Grellety *et al.,* 2012; Huybergts *et al.,* 2012). Moderate acute malnutrition (MAM) is defined as a weight-for-height Z-score (WHZ) between -2 and -3 without bilateral pedal oedema (WHO, 1999).

2.6 Supplementary feeding

Supplementary feeding programs (SFP) with ready-to-use supplementary foods (RUSF) seem to be the way forward in the effective treatment of MAM. According to Chaparro and Dewey (2010), there has been great success with the use of RUSF for management of target groups such as children and HIV

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positive mothers. The interest in the use of RUSFs within nutritional programs for food insecure populations may be due to their lower cost ($0.19 per 46 g dose per day vs. $0.37 per 92 g dose per day of RUTF) (Isanaka *et al.,* 2010). Manary (2005) defined Ready-to-use foods as a generic term for different types of fortified foods, such as Lipid-based nutrient supplements (LNS), ready-to-use therapeutic food (RUTF) and ready-to-use supplementary food (RUSF) suitable for feeding severely malnourished individuals. According to Collins and Jeya (2004), ready-to-use foods should possess the following characteristics:

- Good nutritional quality (in terms of the protein, energy and micronutrient content)
- Good shelf-stability
- Should be highly palatable or delicious to taste
- Should not require any additional processing prior to feeding

RUSF is made from peanuts, powdered milk, sugar, vegetable oil, vitamins and minerals. It is an energy-dense paste with relatively low micronutrient content; manufactured in individual packages and does not require cooking before consumption; it resists bacterial growth due to the low water content of the food and can be stored without spoiling for several months (Briend, 2001). However, the cost associated with the purchase of powdered milk and vegetable oil as well as its production is exorbitant. This limits its production and accessibility for poor vulnerable individuals in developing countries where malnutrition is very high. Hence, cost effective strategies such as the use of cheap locally available ingredients are recommended in the production of non-milk based ready-to-use foods (Owino, 2010). Local production using alternative recipes (grains and cereals) has gained momentum as this will reduce the cost of production and provide income for the local manufacturer and farmers.

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The idea of formulating a local but cheap nutritious food of cereal-legume combination for treating malnutrition was started in 1955 by Brock (Briend, 2001). The composition of a ready-to-use supplementary food should contain by percentage weight: full fat milk (30%), sugar (28%), vegetable oil (15%), peanut butter (25%) and vitamin-mineral mix (1.6%) (Manary, 2005). However locally produced ready-to-use supplementary food is made from peanut (proteins, fats and vitamins source), rice (carbohydrates and vitamins source) and cowpea (proteins and vitamins source). Each of these raw materials also contributes some amount of minerals to the product. The cowpea also helps balance the amino acid profile since peanuts are deficient in methionine and lysine and cowpeas are rich in lysine. The peanut-cowpea combination is intended to make up for the protein and fat content of the locally produced non-milk based RUSF since the full fat milk/powdered milk and vegetable oil used in the production of the milk-based RUSF have been removed from the ingredients for the local production so as to minimize cost. Micronutrients are also added to the food as premixes in a powdered form so as to enrich the vitamin and mineral profile of the product (RUSF).

Through extensive research in the department of Nutrition and Food Science, University of Ghana-Legon, an alternative ready-to-use supplementary food (RUSF) has been produced from a non-milk based source using locally available ingredients (rice, peanuts and cowpea). Food analyses performed on this RUSF have shown that it is rich in energy, proteins, vitamins and minerals and compares favourably with the nutritional composition of milk based ready-to-use foods. However, clinical field trials are needed to investigate the acceptability and efficacy of the ready-to-use supplementary food produced. This efficacy trial will focus on how the RUSF can improve the nutritional status of women of reproductive age (15-49yrs) when fed the food for three months.

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2.7 Efficacy trials

Efficacy is the ability to produce an effect. It is synonymous to effectiveness, but clinicians often distinguish between the two. Efficacy trials determine the ability of a treatment to produce the expected result under ideal conditions whiles effectiveness trials measure the degree of beneficial effect under "real world" clinical settings (Godwin *et al*., 2003). Several study designs such as randomization, blinding and placebo-control are used in efficacy trials. Biases in efficacy trials are prevented by blinding: the subjects involved in the study do not know which study treatment they receive. If the study is double-blind, the researchers also do not know which treatment is being given to a subject. In a placebo-controlled design, the use of a placebo (fake treatment) allows the researchers to isolate the effect of the study treatment from the placebo effect. For randomized controlled trials (RCTs), each study subject is randomly assigned to receive either the study treatment or a placebo. RCTs are the gold standard in evaluating the efficacy of a treatment. Efficacy trials can be clinically meaningful based on several factors that determine the external validity of RCTs: patient characteristics, condition under investigation, drug regimens, costs, compliance, co-morbidities, and concomitant treatments (Finn, 1999; Pocock, 2004). Also generalization of the findings of efficacy trials is influenced by the type of study design, eligibility criteria, study duration, mode of intervention and type of statistical analysis used (Chow and Liu, 2004; Van Spall *et al*., 2007).

An essential component of initiating an efficacy trial is to recruit participants following ethical procedures using a signed document called informed consent. According to the United States National Institutes of Health, informed consent is a legally defined process of a person being told about key facts involved in a clinical trial before deciding whether or not to participate. To fully describe participation to an eligible participant, trial details such as its purpose, duration, required procedures, risks, potential benefits and key contacts are explained to the individual in a language of their choice

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and in terms the person will understand. An individual's decision to be part of a study is not an immutable contract, as the participant can withdraw at any time without penalty. Efficacy trials are closely supervised by appropriate ethics committee called Institutional Review Board (IRB). The IRB scrutinizes the study for both medical safety and protection of the participants involved before, during and after the study.

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CHAPTER 3

3.0 METHODOLOGY

3.1 Research design and study area

This study was a randomized controlled field trial. This field trial consisted of a non-food supplementation control group and a supplemented intervention group. All investigators, research assistants and participants involved in the study were not blinded to the supplementary diet given. The purpose of this study was to assess the efficacy of a peanut-based ready-to-use supplementary food (RUSF) on the nutritional status of women. Efficacy was measured by determining changes in the participants' anthropometric, biochemical and haematological indices at baseline and during the study period.

This study was conducted in Ga East municipality of the Greater Accra Region of Ghana. The communities were Ayimensa, Kweiman, Danfa, Adoteiman and Otinibi. These are peri-urban communities in the north-eastern horn of the Ga East municipality as shown in the study area map in appendix I. Abokobi is the capital and the municipality covers a land area of 166 square metres. The population density of the municipality is 1633.34 (National Population and Housing Census, 2010). There are 259,668 people living in the municipality; 127,258 are males and the remaining 132,410 are females. The main occupation of the women in these communities is farming, gari production and petty trading. A tarred road links these communities. A borehole system provides all the communities with source of drinking water; this is provided through Water and Sanitation Development Boards for piped schemes. A central health centre known at Danfa Health Centre provides all the communities with medical services.

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3.2 Study population, sampling and sample size

3.2.1 Study population

The subjects in the study were healthy women of reproductive age (WRA; 15-49 years) who reside in Ayimensa, Kweiman, Danfa, Adoteiman and Otinibi. Of the 220 WRA whose baseline information had been collected, only 134 were recruited as study participants because the remaining was not willing for their blood to be drawn and hence discontinued the study. Convenience sampling was used to recruit participants. This was based on house to house visits in all the communities to fully describe participation to an eligible woman. Trial details such as purpose, duration, required procedures, risks and potential benefits were explained to the individual in a language of their choice and in terms the person understood.

3.2.2 Sampling

Randomization into control and intervention groups consisted of 67 women each. Randomization was done by hand picking after the folded ballot papers had being placed in a box and mixed thoroughly. This simple random sampling technique was used because a computer generated code to assign participants to treatment and control groups were not available.

3.2.3 Sample size calculation

Assuming an alpha level of 0.05, power of 80% from most field trials involving humans and using an underweight prevalence of 8.6% (GDHS, 2008), the number of participants required in the intervention and control group was 60 each. The sample size was 120 and it was determined as stated by Daniels (1999).

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$$N = \frac{[Z^2 * P\,(1-P)]}{d^2}$$

where

N= Sample size, Z=Z-statistic for 95% level of confidence which is 1.96, d= Precision at a P-value of 0.05 with 95% confidence and P= Expected prevalence [8.6% of underweight women; GDHS (2008)].

$$N = \frac{[Z^2 * P\,(1-P)]}{d^2}$$

$$N = \frac{[1.96^2 * 0.086(1-0.086)]}{0.05^2}$$

$$N = \frac{[1.96^2 * 0.086(0.914)]}{0.05^2}$$

$$N = \frac{[1.96^2 * 0.086(1-0.086)]}{0.05^2}$$

N= 121

3.3 Inclusion and exclusion criteria and recruitment of participants

3.3.1 Inclusion and exclusion criteria of participants

Non-pregnant non-lactating women of reproductive age (WRA; 15-49 years) were recruited as study participants. Eligible participants should not have been on vitamin and/or mineral supplements in the last six months and should be willing to participate after the study protocol has been explained to them. Women who got pregnant or breast-feeding during the study period were automatically eliminated.

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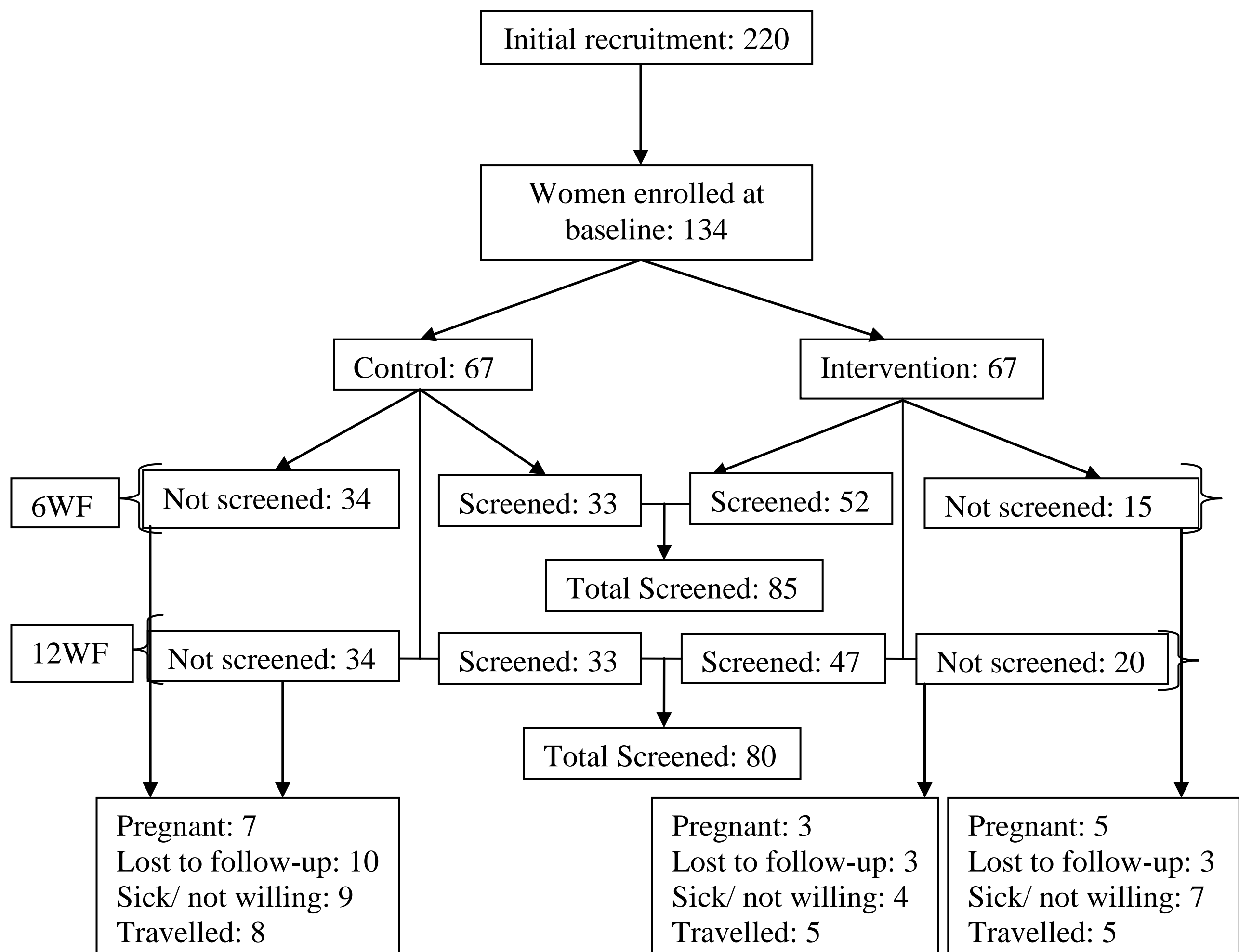
3.3.2 Recruitment of participants

All participants in the study were volunteers who willingly agreed to be part of the study. Participants in this study were recruited between January 2012 and mid March 2012. The women who were interviewed were first told about this study during community gatherings in their respective communities. Two hundred and twenty (220) women were recruited by house to house visits in the communities involved in the study after the study protocol has been explained to them. Informed consent (appendix II) was obtained from all recruited participants. Due to the invasive nature of the blood drawing component of this study, only the 134 who gave their blood were finally involved in the study (fig. 1).

This study consisted of 134 women of reproductive age (WRA) of which 85 remained after 6 weeks and 80 completed the study (fig. 1). Amongst the women who completed the study, 47 were in the intervention group whilst 33 were in the control. Attrition rate was 30% and 50% in the intervention and control group averaging 40% for the whole study. The women in the intervention group were fed three times a week with the treatment diet (RUSF product) for twelve weeks whilst the control group received no food supplement. All the women and investigators involved in the study were not blinded to the intervention diet given. The women in the intervention group who completed the study were fed 36 times whilst those who discontinued the study after the 6 weeks of feeding were fed 18 times with the treatment diet (RUSF product). The women were screened after 6 and 12 weeks of feeding. The flow diagram of the study is as shown below (fig. 1).



6WF=6 weeks of feeding; 12WF=12 weeks of feeding

Fig. 1: Flow chart of subject enrolment and completion

3.4 Proximate analysis of RUSF

The flow diagram for the production of the peanut-based ready-to-use supplementary food (RUSF) has been outlined in appendix IV. The vitamin and mineral profile of the vitamin-mineral mix added to the RUSF drink is reported in appendix V. Proximate analysis of moisture, energy, protein, fat, carbohydrate and ash of the RUSF were determined using standard procedures.

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3.4.1 Moisture content

Two previously dried and cooled moisture dishes were weighed with their covers. Approximately 2ml of RUSF was weighed into the previously dried, cooled and weighed moisture dishes. The samples were dried at 105^0C (cover off inside oven) using the air-oven overnight. After air drying, the samples were cooled in a dessicator, weighed again and the percentage moisture determined. Moisture content was determined for each batch of production.

3.4.2 Energy determination

Energy value of the RUSF was determined using a bomb calorimeter (Parr 6200 calorimeter, Parr Instrument Company, USA) as outlined in the manual for the calorimeter by Parr Instrument Company, USA (2010). Briefly RUSF was dried into a powder and made into a pellet. The weight and moisture content of the RUSF was keyed into the calorimeter and the energy value determined. Determination was done in triplicates for each batch of production and the mean ± standard deviation was reported.

3.4.3 Protein

About 5ml of RUSF was weighed into digestion flasks and digested into ammonium salts with concentrated H_2SO_4 at a high temperature. The mixture was allowed to cool and then rendered strongly alkaline with 50 % NaOH, and the ammonia was distilled into a known volume of standard boric acid with indicator. The distillate was then titrated with 0.1N HCl. The calculated amount of nitrogen found by this titration was multiplied by a factor (6.25) to give the quantity of protein present for a complete proximate analysis (AOAC, 1990). The procedure was repeated for each batch of RUSF produced.

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3.4.4 Crude fat

2ml of RUSF was weighed into an extraction thimble plug with glass wool and placed in a weighed Soxhlet flask (AOAC, 1990). The flask was filled to about two-thirds with petroleum ether and refluxing was carried out over a heater for three hours. The contents of the thimble were saved for crude fibre determination whiles the flask was dried overnight at 60^0C, cooled and weighed. Two samples were selected at random during each batch of production for crude fat determination. Crude fat was determined as shown below:

$$\text{Crude fat} = \frac{\text{Weight of flask after refluxing} - \text{initial weight of flask}}{\text{Initial weight of flask}}$$

3.4.5 Crude fibre

0.5g of celite and 200ml of boiling 1.25% sulphuric acid were added to the residue from the Soxhlet thimble in a beaker and boiled for thirty minutes (AOAC, 1990). The residue was filtered hot and washed with plenty of water through a sintered glass Buchner funnel. The residue was washed again with 200ml of boiling 1.25% sodium hydroxide solution and boiled for another thirty minutes. The residue was filtered hot and washed with hot distilled water and 1% HCl through a sintered glass Buchner funnel. The residue was dried in a crucible in an oven at 105^0C overnight. It was then cooled and weighed to obtain the crude fibre. The crude fibre content was calculated as the percentage weight loss of the washed sample per the mass of the initially weighed RUSF.

3.4.6 Ash

About 2.0g of RUSF was weighed into crucibles and heated to constant weight at a temperature of 550^0C. The ash content was calculated as a percentage of the remaining ash per the total sample used (AOAC, 1990).

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3.4.7 Total carbohydrate

Total carbohydrate content of RUSF was calculated by difference (AOAC, 1990). Under this approach, the other constituents in RUSF (protein, fat and ash) are determined individually, summed and subtracted from the total weight of RUSF (100g). This is referred to as total carbohydrate by difference and is calculated by the following formula: 100 - (weight in grams [protein + fat + ash] in 100 g of RUSF).

3.5 Mineral analyses of RUSF

Mineral profiles of the RUSF were determined using standard procedures. The minerals analyzed for were: Sodium (Na), Potassium (K), Phosphorus (P), Calcium (Ca), Chronium (Cr), Copper (Cu), Iron (Fe), Magnesium (Mg), Manganese (Mn), Zinc (Zn), Nickel (Ni) and Lead (Pb).

3.5.1 Determination of Sodium (Na) and Potassium (K)

The concentrations of Sodium (Na) and Potassium (K) were determined using Spectra AA 220FS flame photometer (Varian Co., Mulgrave, Australia) with an acetylene flame. The procedure was outlined in the manual for the fame photometer by the manufacturer (Varian Co., Mulgrave, Australia). Briefly ten parts per million (10ppm), 100ppm and 1000ppm Na and K standards were prepared from commercial stock standards of 1000µg/ml using deionised water. Deionised water was used as blank. The blank and standards were used to calibrate the machine. One (1) ml aliquot of the samples (filtered through Whatson no. 4 filter paper) was aspirated by the machine and the values obtained were converted into parts per million as shown below:

Na/K (ppm) = flame photometer reading x (vol. of extract/aliquot)

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3.5.2 Determination of Phosphorus (P)

Phosphorus (P) was determined spectrophotometrically as stated by Bray and Kurtz (1945). Briefly, to 1ml of pipetted sample that had been filtered through Whatson no. 4 filter paper was added 30ml of deionised water. A drop of P-nitrophenol and enough Ammonium solution was added until solution turned light yellow. A blue colour was developed by adding 8ml of L-ascorbic acid. The solution in the 50ml volumetric flask was made to the mark using deionised water and absorbance was read at 712nm on a Shimadzu UV-120-02 spectrophotometer. Percentage Phosphorus is calculated as depicted below:

$$\% \text{ P} = \text{Spectrophotometer reading} \times \frac{\text{vol. of extract}}{\text{Aliquot}} \times \frac{100}{10^6}$$

3.5.3 Determination of trace and heavy metals

Atomic absorption spectroscopy (AAS) was used in determining the following minerals: Calcium (Ca), Chronium (Cr), Copper (Cu), Iron (Fe), Magnesium (Mg), Manganese (Mn), Zinc (Zn), Nickel (Ni) and Lead (Pb) content of RUSF. Basically AAS makes use of absorption spectrometry to assess the concentration of a mineral in a sample. The model of the AAS machine was PERKIN ELMER Analyst 400. The AAS uses the winlab 32 software to select a wavelength, slit, current and the mineral of interest. The air-acetylene (10:2.5) flame at a temperature of about 2300^0C was used for igniting the aspirated sample. Two parts per million (2ppm), 10ppm and 100ppm standards were prepared from commercial stock standards of 1000µg/ml of each mineral using deionised water. Deionised water was used as blank. The blank and standards were aspirated by the machine and a calibration curve automatically plotted. The samples (filtered through Whatson no. 4 filter paper) were then injected and the concentration of each mineral per the amount of sample aspirated was shown on the screen as AAS

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readings. Concentration of the each mineral in the test sample was reported as parts per million (ppm) and converted to mg/100ml.

3.6 Quality assurance of RUSF drink

Initial preparation for production of the drink involves sorting out to ensure that only wholesome grains were used. Ready-to-use supplementary food (RUSF) was produced under strict hygienic conditions. The product (RUSF) has been certified by the Food and Drugs Board of Ghana (FDB/051/A11/1619) (appendix VI). Each batch of RUSF was tasted before it was sent to the field. Periodically microbiological safety checks were carried out on the product.

3.7 Data collection and instrument

A pretested questionnaire (appendix III) was administered face-to-face to solicit information on socio-demographic characteristics, food security status and usual dietary intake. De-worming of the intervention and control group was done. Feeding of the intervention group was carried out. Anthropometric indicators of weight, height, neck and hip circumference were measured. Haematological indices (full blood count) and sickling test were determined using participants' whole blood. Biochemical indicators of total protein, albumin, globulin, iron and ferritin were determined. All anthropometric, haematological and biochemical measurements were done at baseline and after 6 and 12 weeks of feeding.

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3.7.1 Socio-demographic and food security

Information on participants' socio-demographic characteristics was obtained using a pretested questionnaire (appendix III). Information on age, level of education and occupation were obtained. Food security status of participants were assessed using the standard 6-item indicator set for measuring household food security developed by the United States Department of Agriculture (Bickel *et al.,* 2000) (appendix III).

3.7.2 Dietary intake

A semi-quantitative food frequency questionnaire was used to obtain information on participants' usual dietary intake in the previous one month (four weeks) prior to the study (appendix III). For a particular food item, the frequency of intake in a week was recorded. Household food measures, food models and other eating wares were shown to the participants to improve estimation of portion sizes.

3.7.3 Anthropometric measurements

3.7.3.1 Height

Heights were measured without shoes to the nearest 0.5cm. A freestanding stadiometer (Charder height measurement manufactured by Charder Electronic Company Limited) was used. Participants were made to stand on a flat surface at right angle to the vertical board of the stadiometer. Participants were instructed to stand up straight, with heels close together, legs straight as possible, arms at the sides and shoulders relaxed. The horizontal bar on top of the stadiometer was lowered to rest on the crown of the head. The subjects were instructed to take a deep breath and then the height was measured to the nearest 0.5cm. Measurements were done in triplicates and reported as mean ± standard deviation.

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3.7.3.2 Weight

Weighing was done using a digital scale (OMRON fat loss monitor with scale). Standard measurement protocol for measuring weight was adapted from WHO expert committee report, 1995. Briefly, the participants were weighed in light clothing and without shoes. Participants were instructed to stand up straight, with feet slightly apart, legs straight as possible, arms at the sides and shoulders relaxed. Weight was recorded to the nearest 0.1kg. Measurements were taken three times and the average recorded.

3.7.3.3 Neck circumference

Neck circumference (NC) was measured to the nearest 1mm using a plastic measuring tape. Participants were asked to stand erect with their head positioned in the Frankfort horizontal plane with shoulders relaxed. The superior border of a tape measure was placed just below the laryngeal prominence (Adam's apple) and applied perpendicular to the long axis of the neck. Thus NC was measured in the midway of the neck, between mid-cervical spine and mid-anterior neck, to within 1mm with a plastic measuring tape. NC was measured thrice and reported as mean ± standard deviation for each participant.

3.7.3.4 Hip circumference (HC)

Participants were asked to stand upright with feet together and help place a measuring tape around their hips. Positioning the plastic measuring tape around the maximum circumference of the buttocks, HC was recorded to the nearest 1mm at the widest part of the hips; usually this corresponds to the groin level for women. Measurements were done in triplicates and reported as mean ± standard deviation.

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3.7.4 Biochemical and haematological determinations

A sterile syringe was used to draw 5ml of venous blood from each participant with the help of a trained phlebotomist. Blood samples were collected at baseline, after 6 weeks and 12 weeks of feeding. Two milliliters of each blood sampled was dispensed into a labelled tube containing an anticoagulant known as ethylenediaminetetraacetic acid (EDTA). The remaining blood (3ml) in the syringe was dispensed into a labelled vacutainer tube and the contents were inverted carefully about 5-6 times to mix clot activator and blood. All blood containing tubes were transported on ice to the laboratory. Blood in EDTA tubes were used for full blood count (FBC) and sickling tests whilst those in the vacutainer tubes were used for biochemical analyses (Serum total protein, albumin, globulin, iron, ferrittin and C- reactive protein). Full Blood Count and sickling tests were analyzed at the Central laboratory of University of Ghana Hospital, Legon whilst biochemical analyses were performed at the central laboratory of the Central Regional Hospital, Cape Coast-Ghana.

3.7.4.1 Full blood count (FBC)

The EDTA tubes containing 2ml of blood each were put on a Coultre mixer (UK) to ensure uniform mixing of the various blood components. The tubes were then transferred to Olympus Sysmex KX – 21N Haematology Fully Automated Analyser (France). A diluent attached to the Haematology Automated Analyser was inserted into the EDTA tubes to dilute the blood. The diluent is made up of organic and inorganic salts; anhydrous sodium sulphate, sodium chloride, dimethylurea and buffered to pH 7.4. The purpose of the dilution of the whole blood was to reduce the number of cells to a concentration, in order that the cells can pass through the detector one by one to be measured. The Haematology Automated Analyser then lyses the blood in the EDTA tube into its various components indices; red blood cells, white blood cells, platelets, haemaglobin, haematocrit, mean corpuscular

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volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) and lymphocytes. The Haematology automated analyzer runs duplicate for each sample and reports the component indices as an average that is printed out. Full blood count analyses were carried out within two days after sample collection.

3.7.4.2 Sickling test

A thin film of blood from the EDTA containing tube was put on a slide and a drop of sickling fluid (sodium metabisulphite) was added to the blood. The sodium metabisulphite served as a reducing agent; it reduces the oxygen tension in the red blood cell and subjects the red blood cells to a series of complex polymerization to induce the typical sickle-shape of red blood cells, thereby confirming that the red blood cells are sickled. The blood-metabisulphite mixture was smeared on the slide and covered with a cover slip and allowed to dry. The covered slide was observed under the electron microscope (Olympus -Germany) to identify at least a sickle shaped red blood cell in order to declare the participant as being sickling positive. All blood samples that tested positive for sickling were further examined through electrophoresis to determine those who had sickle cell disease or were carriers of sickle cell trait. The sickling test was carried out only at baseline.

3.7.4.3 Serum preparation and storage

On reaching the laboratory, the clotted blood in each vacutainer tube was centrifuged (TDL-40D centrifuge, China) at 3500rpm for 5 minutes. After centrifuging, the serum separated by the clotting factor were frozen at -30^{0}C in a Beko chest freezer until ready for analysis. The frozen centrifuged serum was allowed to thaw and the supernatant (serum) was carefully removed using a Pasteur pipette at room temperature and aliquoted into labelled eppendorf tubes for total protein, albumin, globulin,

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iron, ferrittin and C- reactive protein determinations within 12 hours. The remaining sera were returned immediately to the freezer.

3.7.4.4 Total protein determination

The colour reaction of protein molecules with cupric ions known as the Biuret method was used to determine total serum protein. The principle behind the Biuret method is that in an alkaline medium, protein molecules bind to copper ions in the Biuret reagent to form a violet colour which can be measured. The intensity of the violet colour complex is directly proportional to the total proteins in the sample.

The Kenza Max chemistry analyser was cleaned with 20ml of 10% sodium hypochlorite solution before the start of the analyses. The sera were analysed in batches, twenty samples per batch. Six test tubes were set up and labelled in duplicate as blank, standard and test. One millilitre (1ml) of Biuret reagent was pipetted into each test tube. To the blank, test and standard tubes respectively, 10µl of deionised water, serum and standard working assay was added. The content of each tube was vortexed and incubated at room temperature (25^{0}C) for ten minutes and reading taken on the Kenza max chemistry analyser at 520nm. The absorbance of the standard and test sera was measured against the blank within thirty minutes. The flow cell was cleaned after each reading done in duplicate. The procedure was repeated for each of the sera, working standards and blanks as stated. Concentration of total protein in the test sample (serum) was calculated as shown below.

$$\text{Total protein (g/dl)} = \frac{\text{Absorbance of test sample x Concentration of Protein (standard) (g/dl)}}{\text{Absorbance of Standard}}$$

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3.7.4.5 Serum albumin determination

The Bromocresol green (BCG) dye binding method was used for the serum albumin determination. At a controlled pH (4.15), BCG forms a coloured complex with albumin which is measured at 630nm. The intensity of the colour complex is directly proportional to the albumin content of the sample. The Kenza Max chemistry analyser was cleaned with 20ml of 10% sodium hypochlorite solution before the start of the analyses. The sera were analysed in batches of twenty. Three test tubes were set up and labelled as blank, standard and test. One millilitre (1ml) of BCG reagent was pipetted into each tube. To the blank, test and standard tubes respectively, 10µl of deionised water, serum and standard working assay was added. The content of each tube was vortexed and incubated at room temperature (25^0C) for ten minutes and reading taken on the Kenza max chemistry analyser at 630nm. The absorbance of the standard and test sera was measured against the blank within thirty minutes. The flow cell was cleaned after each reading done in duplicate. The procedure was repeated for each of the sera, working standards and blanks as stated. Concentration of the albumin in the test sample (serum) was calculated as shown below:

$$\text{Albumin conc. (g/dl)} = \frac{\text{Absorbance of test sample x Concentration of Albumin (standard) (g/dl)}}{\text{Absorbance of Standard}}$$

3.7.4.6 Serum globulin determination

Serum globulin was measured by reaction with glyoxylic acid and standardized with N-acetyltryptophan as described by Savory and his co-workers in 1971. The determinations depend on the violet colour formed when glyoxylic acid reacts in a strongly acidic medium with tryptophan.

10µl of serum was mixed with 1ml glyoxylic acid reagent in a reaction vessel. The content was vortexed and incubated for 4 minutes in a temperature controlled heating bath at 95^0C. The resulting

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violet colour was measured at 550nm on the Kenza max chemistry analyser. The standards for serum globulin were inserted at the beginning and completion of each batch of analysis, in order of increasing concentrations. The samples were analyzed in batches of twenty. The flow cell was cleaned after each reading done in duplicate. The procedure was repeated for each of the sera and working standards as stated. Concentration of the globulin in the test sample (serum) was calculated as shown below:

$$\text{Globulin conc. (g/dl)} = \frac{\text{Absorbance of test sample x Concentration of Globulin (standard) (g/dl)}}{\text{Absorbance of Standard}}$$

3.7.4.7 Serum iron determination

The Colorimetric Agar-Based method was used to analyse for serum iron; iron (III) reacts with chromazurol B (CAB) and cetyltrimethylammoniumbromide (CTMA) to form a coloured tenary complex with maximum absorbance at 623nm. The intensity of the colour produced is directly proportional to the concentration of iron in the sample.

Briefly a calibrated Kenza max chemistry analyser was used to measure serum iron concentrations in 50µl serum mixed with 1ml of CAB and CTMA. The test was vortexed, incubated at 37^0C for ten minutes and reading taken on the Kenza max chemistry analyser at 630nm. The standards for serum iron were inserted at the beginning and completion of each batch of analysis, in order of increasing concentrations. The samples were analyzed in batches of twenty. The flow cell was cleaned after each reading done in duplicate. The procedure was repeated for each of the sera and working standards as stated. Concentration of the serum iron in the test sample was calculated as shown below:

$$\text{Iron concentration (g/dl)} = \frac{\text{Absorbance of test sample x Concentration of iron (standard) (g/dl)}}{\text{Absorbance of Standard}}$$

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3.7.4.8 Serum ferritin determination

The AxSYM Ferritin assay offers a quantitative, automated methodology for ferritin determination as a clinically useful indicator of body iron stores. AxSYM Ferritin is based on Microparticle Enzyme Immunoassay (MEIA) technology. The AxSYM Ferritin assay was calibrated using internal and external standards. The internal standards were obtained from the manufacturer whiles the International Bureau of Standards ferritin was used as an external calibrator. Further quality control was maintained by analyzing ferritin controls with the test sample. Samples were analyzed in batches of twenty. Laboratory values of ferritin were obtained by an automated method as described by Smith and her co-workers (1993).

Briefly, sera (150µl) each and all AxSYM Ferritin reagents (Anti-Ferritin Coated Microparticles, Anti-Ferritin Alkaline Phosphatase Conjugate, Specimen Diluent and TRIS Buffer) required for ferritin determination were pipetted by the sampling probe into various wells of a reaction vessel (RV). The RV is immediately transferred into the processing center where further pipetting was done with the processing probe. An aliquot of the specimen diluent, Conjugate, Microparticles and TRIS Buffer mixture is pipetted and mixed with each serum. The ferritin, enzyme-labeled antibody and microparticles bind forming an antibody-antigen-antibody complex. An aliquot of the reaction mixture containing the antibody-antigen complex bound to the micro particles is transferred to the matrix cell. The micro particles bind irreversibly to the glass fibre matrix. The matrix cell is washed to remove unbound materials. The substrate, 4-Methylumbelliferyl Phosphate, is added to the matrix cell and the fluorescent product is measured by the MEIA optical assembly. Samples with ferritin concentrations higher than 1000µg/L were automatically diluted by the system. The AxSYM System automatically calculates the concentration of the diluted sample and reports the result. Samples with ferritin concentrations higher than 20,000µg/L were manually diluted. The concentration reported by the

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AxSYM System must be multiplied by the manual dilution factor to obtain the final sample concentration as shown below:

Final Sample ferritin Conc (µg/l) = Reported Concentration x Manual Dilution Factor

$$\text{Manual Dilution Factor} = \frac{(\text{Volume of Sample} + \text{Volume of Dilution Reagent})}{\text{Volume of Sample}}$$

3.7.4.9 C- reactive protein (CRP) determination

Serum CRP was measured as an indicator of an inflammatory state and was assessed based on a particle enhanced turbidimetric immunoassay technique (Taytec C- reactive protein latex reagent kit). In this technique the immunological reaction between CRP antisera bound to biologically inert latex particles and the CRP in the test specimen was observed for agglutination. When serum containing more than 6mg/L CRP is mixed with the latex reagent, visible agglutination occurs. The major advantage of this method is the rapid two minute reaction time. The kit was used to measure the CRP in human serum qualitatively and quantitatively.

Briefly 50µl each of CRP positive control, CRP negative control and undiluted test sample are placed in field 1, 2 and 3 of the reaction slide respectively. The remaining fields are spotted with additional unknowns. The CRP latex reagent was re-suspended and one drop added to each of the fields. The content in each field is mixed thoroughly with stirring sticks. The slides were rotated for two minutes and read immediately under an oblique indirect light. A negative reaction is indicated by a uniform milky suspension with no agglutination as observed in the CRP negative control. A positive reaction is indicated by any observable agglutination in the reaction mixture. It's always important to compare the specimen reaction to the CRP negative control.

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A positive test is followed by a semi-quantitative test. Briefly five test tubes were set up with the following dilutions: 1:2, 1:4, 1:8, 1:16 and 1:32. Positively tested sample was diluted according to the dilution factor on each test tube with saline solution. One drop of each of the positive and negative controls was spotted onto the slide ring in different fields on the reaction slide. A drop of each of the dilutions was spotted on successive fields of the reaction slides. The gently resuspended CRP latex reagent was added dropwise to each test field. The content in each field is mixed thoroughly with stirring sticks. The slides are gently rocked for two minutes and read immediately under an oblique indirect light. The approximate CRP concentration in the sample is calculated as follows: CRP Conc (mg/L) = 6×CRP titre

3.7.5 Intervention (Feeding)

3.7.5.1 De-worming

Prior to feeding, the participants were de-wormed with a dose of two tablets of ZENTEL albendazole: each tablet contains 200mg of albendazole. The purpose of the de-worming was to control for worm infestation.

3.7.5.2 Feeding

Feeding began two weeks after de-worming. The women in the control group were not given a placebo and were solely dependent on their own foods whiles those in the intervention group were fed the supplementary food for 12 weeks. Feeding was carried out from June to August 2012. The women were fed the supplementary food (RUSF) three times a week preferably in the late afternoons (2pm-6pm) on Tuesdays, Fridays and Sundays. Bottled RUSF prepared as shown in appendix III were transported to the field in a Decor ice chest. This was to ensure that the products were served chilled.

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The products were distributed within four hours on the field. Remaining products were discarded upon reaching the laboratory to prevent cross contamination. The drink was distributed to the women in their homes by home visits from one community to the other. A 330ml bottled RUSF was given to each woman on each of the days designated for feeding. Women who were at home consumed the drink on the spot. However for women who were not at home on the days of the distribution had their drink left behind with relatives whom they had already informed or introduced to the researcher. All women whose drink was left behind were identified on a separate list. During the next visit, the women were asked if they received the drink and drank it. Women who were not at home or had neither of the relatives present to receive the food on their behalf were marked absent for that day. All women who received the food were noted and marked present on the feeding list (appendix XI).

3.7.5.3 Sharing practices

Sharing practices were documented by direct observation and questioning. Sharing was understood as given RUSF to anyone other than the beneficiary. Sharing practices were documented after the 6 weeks and 12 weeks of feeding. RUSF compliance was also monitored at 6 and 12 weeks of feeding.

3.7.5.4 Morbidity data

Personal interviews were used to record information on morbidity and it was monitored monthly during the study period. Morbidity data was coded based on the type of condition (diarrhoea and respiratory infections). Diarrhoea was defined as ≥3 liquid or semi-liquid stools per day. Data on respiratory morbidity included symptoms of cough, fever, cold and running nose that lasted for ≥7 days.

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3.8 Data management and analysis

Data on background characteristics, anthropometric measurements, biochemical and haematological indices were carefully collected and kept confidential. Food security status, dietary assessments, anthropometric measurements, biochemical and haematological indices were classified based on reference values.

3.8.1 Data quality assurance

Questionnaire used to solicit information on background characteristics was pretested. All equipment used for data collection were calibrated before use. Sterile syringes, needles and blood containing tubes were used during blood drawing sessions. All information about each respondent was identified with a unique code only known to the researcher. Information was entered and checked by researcher. The data collected was validated by searching for missing variables and ensuring that they were actually missing by verification from the questionnaires. Supervisors assessed all measurements for consistency and accuracy.

3.8.2 Food security status

The level of food security was determined by rating the individual based on the kind and number of responses received (Table 3.1). The often true and sometimes true responses are considered as affirmative responses and scored zero (0) and never, don't know or refused responses are termed as negative responses and scored as one (1) as indicated by USDA Food Security Guide (Bickel *et al.,* 2000). The food security status of a household or an individual is then determined by the summation of all responses obtained from the six set of questions.

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Table 3.1: Scale for classifying food security status

Total responses	Food Security Status Level
0-1	Food secure
2-4	Food insecure without hunger
5-6	Food insecure with hunger

Source: (Bickel *et al.*, 2000)

3.8.3 Dietary intake

The responses obtained on participants' dietary intake from the food frequency questionnaire were analyzed using ESHA FPRO version 10.0.1. The frequency of food intake (for example Jollof rice) per week (Monday-Sunday) was multiplied by the portion sizes (equivalents weights) to calculate how much food was eaten in total. The average food intake for the week was calculated by dividing the total amount (weight) of food consumed in a week by 7 days. The average intake per week was taken as the usual daily food intake of the participant. The average weight of each food item consumed by each participant was then keyed into the ESHA FPRO. The ESHA FPRO was used to obtain the total of the individual nutrients in the foods that each participant consumed. The nutrients analysed for were: total calories, protein, fat, calcium, iron, vitamin A, vitamin C, iodine and folate. Each participant intake was classified as either below the Recommended Dietary Intake (RDA) or at least having met the RDA of a particular nutrient. The classification was done as follows; for a particular nutrient a participant was classified as having a low intake when the Recommended Dietary Allowance (RDA) for that nutrient was not met or at least meeting the RDA when the person's intake was within the RDA and the Tolerable Upper Intake Level (UL) as shown in Table 3.2.

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Table 3.2: RDA and Tolerable Upper Intake Level of nutrients

Nutrient	RDA*	Tolerable Upper Intake Level#
Calories (kcal)	2000-2200	-
Protein (g)[a]	50-82.5	-
Fat (g)[a]	75-165	-
Calcium (mg)[b]	1000	3000
Iron (mg) [c]	15-18	45
Vitamin A (IU) [b]	500	7500
Vitamin C (mg) [b]	40-45	1000
Folate (µg) [b]	400	1000
Iodine (µg) [b]	150	1000

*The RDA is the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97-98%) healthy individuals of a specified age range and gender. #Tolerable Upper Intake Level (UL) is the highest daily dietary intake that is likely to pose no risk of adverse health effects to almost all individuals of a specific age range. **Tolerable Upper Intake Levels were not available for calories, protein and fat; all were set within the RDA.**
Source: [a] Dietary Recommendations in the Report of a Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases (WHO Technical Report Series 916, 2003).
Source: [b] Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements (1998: Bangkok, Thailand. 2nd edition: 2004).
Source: [c] Office of dietary supplements, National institute of health: dietary supplement fact sheet: Institute of medicine, food and nutrition board. Washington, DC: National Academy of Press, 2001.

3.8.4 Anthropometric measures

Neck circumference was classified as low (<34cm), normal (34-36.5cm) and high (>36.5cm) (Ben-Noun *et al*., 2001). Currently there is no standard classification for hip circumference. BMI was calculated by dividing the participant's weight in kilograms (kg) by the height in metres squared (m^2). **BMI= kg/m^2.** The participants were then grouped into different classifications of BMI based on WHO standards as shown in table 3.3.

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Table 3.3: BMI classification

BMI classification	Cut offs (kg/m^2)
Underweight	<18.5
Normal	18.5-24.9
Overweight	25-29.9
Obese	≥ 30

Source: World Health Organisation (WHO) Global Database on Body Mass Index (2004)

3.8.5 Haematological and Biochemical indices

The women in the control and treatment groups were categorized into low, normal or high based on the reference ranges of the haematological (table 3.4) and biochemical indices (table 3.5) and these have been reported as percentages of the total study population. Their anaemia status was grouped into different classes depending on the level of haemoglobin (Table 3.6).

Table 3.4: Reference values for haematological indices

Haematological indices	Reference range
[1]**White Blood Cells (WBC)**	3.5-11.0 (K/µL)
[1]**Red Blood Cells (RBC)**	4.0-5.0 (M/µL)
[2]**Haemoglobin level (Hb)**	12.0-18.0 (g/dL)
[2]**Haematocrit (Hct)**	37.0-51.0 (%)
[2]**Mean corpuscular volume**	80.0-97.0 (fL)
[2]**Mean corpuscular Haemoglobin**	26.0-32.0 (pg)
[2]**Mean corpuscular Haemoglobin Concentration**	31.0-36.0 (g/dL)

[1]http://www.mayoclinic.com
[2]Reference values are those provided by the Ghana Health Service

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Table 3.5: Reference values for Biochemical indices

Biochemical indices	Reference range
[1]Total Serum Protein	6.6-8.7 (g/dL)
[1]Serum Albumin	3.8-5.3 (g/dL)
[1]Serum Globulin	2.0-4.8 (g/dL)
[1]Serum iron	37-145 (µg/dL)
[2]Serum ferritin	15-150 (µg/L)

[1]Reference values are those provided by the manufacturer of the reagents (Human Gesellschaft Biochemical und Diagnostica, Germany)
[2]Reference values (WHO, 2011)

Table 3.6: Classification of anaemia

Level of anaemia	Haemoglobin Cut offs (g/dL)
Normal/non anaemic	≥12.0
Any form of anaemia	<12.0

Source: Ghana demographic and health survey (2008)

3.8.6 Data analyses

Sample size, proximate composition, anthropometric measurements, biochemical and haematological indices were analysed using SPSS version 16.0 and SAS 9.1.3. SAS 9.1.3 was used for sample size calculation and SPSS version 16.0 was used for all other analysis. This was because SPSS does not give an option for calculating sample size. Descriptive statistics was used to calculate means, standard deviations and frequencies. Statistical analysis included baseline data comparisons between groups for differences in anthropometric measures, haematological and biochemical variables by using independent-sample t-tests. For women in the treatment and control groups who completed the study, Fishers' and chi-square test (for the percentages) and t-tests (for the continuous variables) were used to

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determine whether changes from baseline to endline were statistically significant. Baseline and endline comparisons within groups were conducted using paired t-tests. Change in variables were calculated for both treatment and control groups and comparisons of the difference between the change in the treatment group with that in the control group were made using independent-sample t-tests.

Difference-in-differences (DD) models were used to estimate the effects of the treatment diet. The basic DD model compares the difference in outcomes over time for a control group to the difference in outcomes during the same period for the treatment group subject to the treatment diet during the study period. The advantage for this design is that the change for the control group picks up any naturally occurring changes, whereas the treatment group change reflects both the (same) naturally occurring change and the effect of the treatment diet, thereby allowing for an accurate measurement of the effect of the treatment diet. A P value ≤ 0.05 was used to identify statistical significance.

3.9 Ethical consent and limitations of the study

3.9.1 Ethical consent

Ethical approval (Appendix VII) was obtained from the Institutional Review Board of the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon. Written informed consent (appendix II) was obtained from all recruited participants.

3.9.2 Limitations of the study

This study design had some important limitations that need to be addressed. Firstly this study was carried out in peri-urban communities amongst apparently healthy women of reproductive age (WRA). The findings therefore cannot be generalized to a population of malnourished WRA and children. Also

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this study did not include a placebo control group. Thus the effectiveness of the treatment diet (RUSF) as compared to similar products in Malawi, Kenya and Niger could not be assessed in the current study. Drop-outs may have influenced the study. However for WRA who dropped out of the study, their last measurements to the measurement points they missed out during the supplementation period were included in the analysis at 6 weeks. Furthermore the research needs to be evaluated in other studies using larger sample sizes and feeding for relatively longer periods other than the 12 weeks. Nonetheless this current research was a pilot study (to form the basis for further research) that started with a large number of WRA but attrition rate was high due to the inclusion of a non-food control group. This study lacks data on the influence of malaria episodes, seasonality of foods and the dietary intake of the participants during the supplementation period. It would have been unethical to screen for malaria parasites without treatment although both treatment and control groups were all at risk. Within group analysis may not have controlled for seasonality of foods but was likely to be similar in both groups. However, the difference in difference analysis approach was meant to control for the influence of seasonality of foods. Additionally, baseline dietary intake was similar in both groups and unlikely to change within this relatively short period.

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CHAPTER 4

4.0 RESULTS

4.1 Preamble

This study was a randomized controlled trial that focused on the background characteristics, nutrient intake, anthropometric, haematological and biochemical indices of women of reproductive age (WRA) during a 12 week supplementation period. It compared changes during the study period between the intervention and the control group. Enrolment rate was modest, group allocation was random, groups were rather similar at enrolment, and follow-ups were identical for some parameters for both groups, compliance in the intervention group appeared good and defaulting was frequent in the control group. Proximate and mineral composition of the supplementary diet has also been reported.

4.2 Background characteristics

A total of one hundred and thirty four participants were enrolled in the study. The participants' mean age was 29 years (Table 4.1). The majority of the participants were in the 20-25 age group. Majority had basic education (67.2%), most of them were employed (61.2%) and married (53.7%). Sickle cell trait was identified in 17.1% of the participants; 16.4% were sickle cell carriers and one participant had sickle cell haemoglobin C disease (Table 4.1). C-reactive protein, an indication of inflammation was positive for 17.2% of the women. Only 13.4% of the women were food secured as shown in Table 4.1 cont'd. The household asset possessed most by the participants was mobile phone (61.2%). The main source of lighting and type of toilet facility used were electricity (70.9%) and public toilet (82.1%) respectively. With the exception of age and occupation, there was no difference between intervention and control group with respect to the background characteristics of the respondents. There was no difference in baseline characteristics between the women who defaulted and those who completed the study as shown in Table 4.2.

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Table 4.1: Background characteristics of participants by study group (N=134)

Characteristics	Intervention (n=67) n (%)	Control (n=67) n (%)	Total (N=134) n (%)	p-value
Age (years) [Mean ± SD]	31 ± 8	27 ± 8	29 ± 8	0.03*
15-19	6 (9.0)	14 (20.9)	20 (14.9)	
20-29	30 (44.8)	36 (53.7)	66 (49.3)	
30-39	19 (28.4)	12 (17.9)	31 (23.1)	
40-49	12 (17.8)	5 (7.5)	17 (12.7)	
Marital status				
Single	27 (40.3)	35 (52.2)	62 (46.3)	0.23
Married	40 (59.7)	32 (47.8)	72 (53.7)	
Level of education				
Tertiary	1 (1.5)	2 (3.0)	3 (2.2)	0.85
Secondary	8 (11.9)	10 (14.9)	18 (13.4)	
Basic	47 (70.1)	43 (64.3)	90 (67.2)	
None	11 (16.5)	12 (17.8)	23 (17.2)	
Occupation				
Employed	50 (74.6)	32 (47.8)	82 (61.2)	0.002*
Unemployed	17 (25.4)	35 (52.2)	52 (38.8)	
Sickling status				
Positive	11 (16.5)	12 (17.8)	23 (17.2)	1.00
Carrier	11 (16.5)	11 (16.3)	22 (16.4)	
Sickler	0 (0.0)	1 (1.5)	1 (0.7)	
Negative	56 (83.5)	55 (82.1)	111 (82.8)	
C-Reactive Protein (CRP)				
Positive	9 (13.5)	14 (20.9)	23 (17.2)	0.25
12.0 mg/L	2 (3.0)	5 (7.5)	7 (5.2)	
24.0 mg/L	6 (9.0)	7 (10.4)	13 (9.7)	
48.0 mg/L	1 (1.5)	2 (3.0)	3 (2.3)	
Negative	58 (86.5)	53 (82.1)	111 (82.8)	

*Significant at *p<0.05*

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Table 4.1 cont'd: Background characteristics of participants by study group (N=134)

Characteristics	Intervention (n=67) n (%)	Control (n=67) n (%)	Total (N=134) n (%)	p-value
Food security				
Food secured	8 (11.9)	10 (14.9)	18 (13.4)	0.53
Food insecured	59 (88.1)	57 (85.1)	116 (86.6)	
Food insecure with hunger	48 (71.6)	49 (73.2)	97 (72.4)	
Food insecure without hunger	11 (16.5)	8 (11.9)	19 (14.2)	
Television				
Yes	37 (55.2)	38 (56.7)	75 (56.0)	1.00
No	30 (44.8)	29 (43.3)	59 (44.0)	
Refrigerator				
Yes	22 (32.8)	21 (31.3)	43 (32.1)	1.00
No	45 (67.2)	46 (68.6)	91 (67.9)	
Mobile phone				
Yes	42 (62.7)	40 (59.7)	82 (61.2)	0.86
No	25 (37.3)	27 (40.3)	52 (38.8)	
Toilet facility				
Own WC	3 (4.5)	4 (6.0)	7 (5.2)	0.91
Public WC	1 (1.5)	2 (3.0)	14 (10.4)	
Own KVIP	8 (11.9)	6 (9.0)	3 (2.2)	
Public KVIP	55 (82.1)	55 (82.1)	110 (82.1)	
Water facility				
Own pipe borne water	0 (0.0)	2 (3.0)	2 (1.5)	0.22
Public pipe borne	21 (31.3)	26 (38.8)	47 (35.1)	
Public borehole	46 (68.7)	39 (58.2)	85 (63.4)	
Type of lightening				
Electricity	47 (70.1)	48 (71.6)	95 (70.9)	1.00
Lantern	16 (23.9)	16 (23.9)	32 (23.9)	
Candle	1 (1.5)	1 (1.5)	2 (1.5)	
Touch	3 (4.5)	2 (3.0)	5 (3.7)	

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Table 4.2: Background characteristics of participants who defaulted and those who completed the study (N=134)

Characteristics	Completed (n=80) n (%)	Defaulted (n=54) n (%)	Total (N=134) n (%)	p-value
Age (years) [Mean ± SD]	30 ± 8	27 ± 7	29 ± 8	0.25
15-19	10 (12.5)	10 (18.5)	20 (14.9)	
20-29	37 (46.3)	29 (53.7)	66 (49.3)	
30-39	20 (25.0)	11 (20.4)	31 (23.1)	
40-49	13 (16.2)	4 (7.4)	17 (12.7)	
Marital status				
Single	35 (43.7)	27 (50.0)	62 (46.3)	0.48
Married	45 (56.3)	27 (50.0)	72 (53.7)	
Level of education				
Tertiary	2 (2.5)	1 (1.9)	3 (2.2)	0.14
Secondary	10 (12.5)	8 (14.8)	18 (13.4)	
Basic	59 (73.7)	31 (57.4)	90 (67.2)	
None	9 (11.3)	14 (25.9)	23 (17.2)	
Occupation				
Employed	54 (67.5)	28 (51.9)	82 (61.2)	0.07
Unemployed	26 (32.5)	26 (48.1)	52 (38.8)	
Sickling status				
Positive	14 (17.5)	9 (16.7)	23 (17.2)	1.00
Negative	66 (82.5)	45 (83.3)	111 (82.8)	
Food security				
Food secured	9 (11.3)	10 (18.5)	19 (14.2)	0.23
Food insecured	71 (88.7)	44 (81.5)	115 (85.8)	
C-Reactive Protein (CRP)				
Positive	12 (15.0)	11 (20.4)	23 (18.0)	0.36
Negative	66 (82.5)	39 (72.2)	105 (82.0)	

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4.3 Nutrient intake at baseline

Presented in Table 4.3 are the nutrient intakes at baseline of the women enrolled in the study. The caloric intake was 2352kcal and 2282kcal among the women in the intervention and control group respectively. The mean dietary intakes of more than 50% of the women were within the RDA for total calories and proteins but fats. At baseline about 70% of the women in both groups met their recommended daily protein intake. Also more than 70% of the women in both groups met their recommended daily allowance (RDA) for iron and vitamin C. The mean iron and vitamin C intakes were about 24 ± 12mg and 90 ± 71mg respectively across both groups. All other micronutrient intakes were below the RDA and interestingly none of the women in both groups met their RDA for folate and iodine as shown in Table 4.3. There was no difference in nutrient intake between the intervention and control groups at baseline.

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Table 4.3: Baseline Nutrient intake of participants in comparison to RDA (N=134)

Nutrient	Intervention (n=67)		Control (n=67)		Total (N=134)		p-value#
	Mean ± SD	Met RDA n (%)	Mean ± SD	Met RDA n (%)	Mean ± SD	Met RDA n (%)	
Calories (kcal)	2352.10 ± 927.49	39 (58.2)	2282.00 ± 909.24	40 (59.7)	2314.95 ± 915.07	79 (59.0)	0.66
Proteins (g)	68.06 ± 27.32	45 (67.2)	65.55 ± 27.22	44 (65.7)	66.72 ± 27.19	89 (66.4)	0.60
Fats (g)	68.56 ± 30.54	24 (35.8)	67.52 ± 29.27	22 (32.8)	68.00 ± 29.76	46 (34.3)	0.84
Iron (mg)	24.25 ± 11.17	52 (77.6)	23.18 ± 12.59	47 (70.2)	23.68 ± 11.91	99 (73.9)	0.61
Calcium (mg)	1005.50 ± 410.57	35 (52.2)	939.00 ± 514.42	27 (40.3)	970.27 ± 467.94	62 (46.3)	0.41
Vit. A (IU)	182.53 ± 287.37	17 (25.4)	249.09 ± 492.46	17 (25.4)	217.79 ± 408.96	34 (25.4)	0.35
Vit. C (mg)	93.14 ± 77.02	52 (77.6)	89.95 ± 66.23	49 (73.1)	91.45 ± 71.25	101 (73.4)	0.80
Folate (µg)	118.74 ± 77.09	0 (0.0)	118.18 ± 92.48	0 (0.0)	119.33 ± 85.27	0 (0.0)	0.97
Iodine (µg)	11.47 ± 10.06	0 (0.0)	10.25 ± 7.24	0 (0.0)	11.15 ± 8.66	0 (0.0)	0.42

#No statistically significant differences were observed between groups at $p < 0.05$

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4.4 Nutrient composition of Ready-to-Use Supplementary Food (RUSF)

Moisture, energy and nutrient composition of the RUSF has been shown in Table 4.4. The percent moisture content of the bottled RUSF was 83.03 ± 0.02. The protein content on dry matter basis was 22.93 ± 0.77g/100g whilst the fat content was 15.26 ± 0.04g/100g (Table 4.4). The ash content of RUSF on dry matter basis was 2.66 ± 0.20g/100g and that of iron was 2.18 ± 0.01mg/100g. Manganese and sodium respectively had the lowest and highest mineral content (0.88 ± 0.00mg/100g, 712.02 ± 0.65mg/100g; Table 4.4). The energy and protein content of the bottled RUSF drink is 316.13kcal and 12.84g respectively.

In Table 4.4, the percentage of recommended daily allowance (RDA) provided by the RUSF is reported. The carbohydrate content of the drink (RUSF) met 25.48% of the RDA for carbohydrate. The protein content of the bottled RUSF satisfied 27.91% of the RDA for protein. Consuming a bottle of RUSF met 42.75% of the RDA for fats (Table 4.4). The iron and zinc content of the bottle (330ml) RUSF drink satisfied 2.06% and 3.13% of the daily requirements for iron and zinc respectively.

Table 4.4: Nutrient composition of RUSF and percentage of daily intake met

Macronutrients	RUFS[4] (g/100g)	CSB[4] (g/100g)	g/100g DMB	g/ bottle DMB	RDA of WRA (g/day)	% RDA met for WRA
Moisture content	-	-	83.03 ± 0.02	-	-	-
Energy (Kcal)	565.32	370.33	564.52 ± 2.76	316.13	-	-
Carbohydrate	-	-	59.15 ± 0.76	33.12	130[1]	25.48
Protein	14.49	13.37	22.93 ± 0.77	12.84	46[1]	27.91
Fat	37.14	7.11	15.26 ± 0.04	8.55	20[2]	42.75
Fibre	-	-	12.70 ± 0.50	7.11	-	-
Ash	-	-	2.66 ± 0.20	1.49	-	-
Micronutrients	**mg/100g**	**mg/100g**	**mg/100g DMB**	**mg/bottle DMB**	**RDA of WRA (mg/day)**	**% RDA met for WRA**
Calcium	338.76	68.98	50.50 ± 2.61	28.28	1000[1]	2.83
Iron	3.27	4.28	2.18 ± 0.01	0.37	18[1]	2.06
Magnesium	97.96	133.69	6.84 ± 0.07	1.16	310[1]	0.35
Manganese	-	-	0.88 ± 0.00	0.15	1.8[3]	8.33
Zinc	3.27	2.14	1.47 ± 0.01	0.25	8[1]	3.13
Phosphorus	285.71	280.75	58.75 ± 1.63	9.97	700[1]	1.42
Potassium	1175.51	454.55	489.69 ± 0.36	83.10	4700[3]	1.77
Sodium	-	-	712.02 ± 0.65	120.83	1500[3]	8.06

RUFS= Ready-to-use Fortified Spread; CSB= Corn-Soy Blended flour; DMB= Dry matter basis; WRA= Women of reproductive age; [1]Recommended Dietary Allowances (RDAs) [Dietary Reference Intakes (DRI, 2002/2005)]; [2]Acceptable Macronutrient Distribution Range (AMDR) (DRI, 2002/2005), [3]Adequate Intakes (AIs) (DRI, 2002/2005); [4]Values reported on dry matter basis were obtained from Ndekha et al. (2009)

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4.5 Anthropometric indices of participants

The baseline anthropometric indices are shown in Table 4.5. The mean age for the intervention and control group was 32 and 27 years respectively. The mean body mass index (BMI) for the intervention group was 25.08 ± 6.88kg/m^2 while that of control group was 24.59 ± 5.12kg/m^2 at the beginning of the study. The neck circumference (NC) and hip circumference (HC) were 32.2 ± 6.4cm and 95.1 ± 11.1cm respectively. At baseline, all anthropometric measurements were not different between the intervention and control group. This indicates that the groupings were fairly similar.

Table 4.5: Baseline age and anthropometric indices of participants by study group (N=134)

Age and Anthropometric Indices	Intervention (n=67)	Control (n=67)	Total (N=134)	p-value
Age (years)	32 ± 8	27 ± 8	29 ± 8	<0.001*
Height (cm)	1.59 ± 0.08	1.56 ± 0.09	1.57 ± 0.09	0.13
Weight (kg)	62.66 ± 15.26	61.77 ± 13.43	61.51 ± 14.23	0.73
BMI (kg/m^2)	25.08 ± 6.88	24.59 ± 5.12	24.83 ± 6.02	0.95
NC (cm)	31.6 ± 2.4	33.1 ± 8.7	32.2 ± 6.4	0.22
HC (cm)	96.4 ± 9.9	95.10 ± 12.7	95.1 ± 11.1	0.53

BMI =Body mass index; NC =Neck circumference; HC =Hip circumference; *Significant differences were observed between groups at $p<0.05$

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Changes in anthropometric measures from baseline to 12 weeks among the women enrolled in the study are presented in Table 4.6. There was a positive change (2.74 ± 13.73kg) in weight for the intervention group. The change in weight was negative for the control group. Body mass index increased significantly from 24.76 ± 5.47kg/m^2 to 25.72 ± 5.91kg/m^2 ($p=0.02$) in the intervention group whilst the control group increased narrowly from 25.83 ± 6.23kg/m^2 to 26.25 ± 5.99 kg/m^2 ($p=0.47$). Significant changes were observed for BMI, NC and HC for the intervention group but not the control group except for HC. Within-group changes were positive for BMI, NC and HC among women in the intervention and control groups. No significant differences were observed in difference in mean change between the intervention and control groups.

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Table 4.6: Anthropometric indices of participants according to study group and with time (n=80)

Anthropometric indices	Intervention (n=47)				Control (n=33)				Differences between mean change in TG and CG #
	Baseline	12W	Change	p-value	Baseline	12W	Change	p-value	
Weight (kg)	62.80 ± 14.96	65.54 ± 18.17	2.74 ± 13.73	0.18	63.82 ± 15.14	63.68 ± 14.58	-0.14 ± 7.75	0.92	2.88
BMI (kg/m^2)	24.76 ± 5.47	25.72 ± 5.91	0.96 ± 2.83	0.02*	25.83 ± 6.23	26.25 ± 5.99	0.42 ± 3.29	0.47	0.54
NC (cm)	31.57 ± 2.29	32.64 ± 2.39	1.07 ± 1.38	<0.001*	34.05 ± 11.50	35.33 ± 11.62	1.28 ± 16.50	0.66	-0.21
HC (cm)	96.57 ± 10.09	99.76 ± 10.87	3.19 ± 4.97	<0.001*	95.25 ± 15.72	100.66 ± 11.03	5.41 ± 12.78	0.02*	-2.22

12W= 12 weeks; TG= Intervention group; CG= Control group; BMI= Body mass index; NC= Neck circumference; HC= Hip circumference; *Differences significant within-group change comparison at $p < 0.05$; #No significant differences were observed for differences in change between the intervention and control groups at $p < 0.05$

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BMI profiles with time are depicted in Figure 2. BMI at 6 weeks was highest in the control group as compared to the intervention group. This decreased slightly from 6 weeks to 12 weeks to a value ($26.25 \pm 5.99 kg/m^2$) not quite different from the baseline BMI for the control group. On the other hand there was a slow increase in BMI from baseline to 12 weeks for the intervention group.

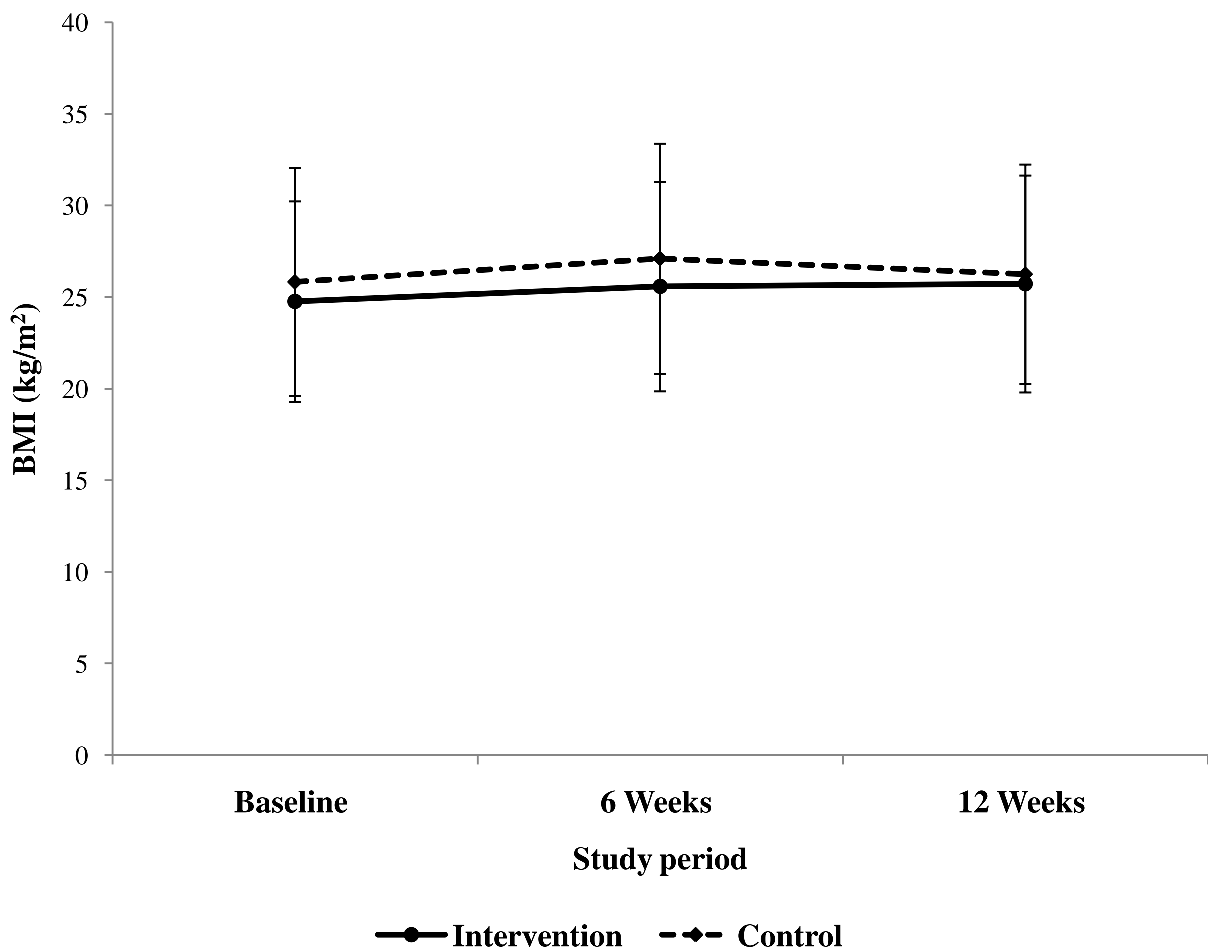

Fig. 2: Body Mass Index (BMI) profiles with time

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4.6 Haematological indices of participants

Reported in Table 4.7 are baseline haematological indices of the women enrolled in the study. Red blood cell (RBC) counts were almost the same for the intervention and control groups (4.25 ± 0.55M/μL and 4.33 ± 0.45 M/μL, respectively). White blood cells (WBC) counts were slightly higher in the intervention than control group. The mean haemoglobin concentration amongst the women was 12.09 ± 1.79g/dL. There was a difference in mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) between groups at baseline.

Table 4.7: Baseline haematological indices of participants by study group (N=134)

Haematological Indices	Intervention (n=67)	Control (n=67)	Total (N=134)	p-value
WBC (K/μL)	7.23 ± 3.57	6.75 ± 2.25	6.91 ± 2.91	0.36
RBC (M/μL)	4.25 ± 0.55	4.33 ± 0.45	4.29 ± 0.50	0.37
HB (g/dL)	12.33 ± 1.87	11.87 ± 1.70	12.09± 1.79	0.16
HCT (%)	37.15 ± 5.19	36.16 ± 4.40	36.62±4.79	0.25
MCV (fL)	87.79 ± 9.48	83.91 ± 9.43	85.69± 9.51	0.02*
MCH (pg)	29.08 ± 3.32	27.56 ± 3.65	28.25 ± 3.54	0.02*
MCHC (g/dL)	33.12 ± 1.49	32.79 ± 1.46	32.93 ±1.46	0.20
LYMP (M/μL)	24.50 ± 22.01	22.49 ± 22.88	22.54 ± 22.29	0.61

WBC = White blood cells; RBC =Red blood cells; HB= Haemoglobin; HCT= Haematocrit; MCV =Mean corpuscular volume; MCH =Mean corpuscular haemoglobin; MCHC =Mean corpuscular haemoglobin concentration; LYMP= Lymphocytes; *Difference significant at $p < 0.05$

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Table 4.8 outlines the changes in haematological profile of women enrolled in the study. A negative change for the intervention (-0.50 ± 1.80g/dL; p=0.07) and a negative change for the control group (-0.81 ± 1.17g/dL; p=0.00) were observed for the mean difference in haemoglobin at baseline and at 12 weeks. Haematocrit increased marginally in the intervention and control group from baseline to 12 weeks. Mean Corpuscular Haemoglobin (MCH) significantly decreased throughout the study period. White blood cells decreased gently (p=0.01) in the intervention group but narrowly (p=0.37) in the control group. Lymphocytes were more than twice the baseline levels at 12 weeks in both groups.

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Table 4.8: Haematological indices of participants according to study group and with time (n=71)

Haematological indices	Intervention (n=44)				Control (n=27)				Differences between mean change in TG and CG #
	Baseline	12W	Change	p-value	Baseline	12W	Change	p-value	
RBC (M/µL)	4.25 ± 0.60	4.25 ± 0.41	0.00 ± 5.51	1.00	4.40 ± 0.33	4.34 ± 0.37	-0.06 ± 0.31	0.30	0.06
HB(g/dL)	12.26 ± 2.06	11.75 ± 1.47	-0.50 ± 1.80	0.07	12.52 ± 1.76	11.71 ± 1.33	-0.81 ± 1.17	<0.001*	0.31
HCT (%)	36.91 ± 5.75	37.87 ± 3.88	0.97 ± 5.02	0.21	37.77 ± 4.64	37.93 ± 3.52	0.16 ± 3.53	0.82	0.81
MCV (fL)	87.21 ± 10.10	77.71 ± 44.86	-9.50 ± 42.59	0.15	85.84 ± 8.86	87.58 ± 6.35	1.74 ± 6.10	0.15	-11.24
MCH (pg)	28.90 ± 3.54	26.90 ± 7.48	-2.00 ± 5.96	0.03*	28.47 ± 3.50	27.09 ± 3.00	-1.37 ± 1.91	<0.001*	-0.63
MCHC (g/dL)	33.13 ± 1.60	25.73 ± 17.59	-7.41 ± 17.09	0.01*	33.10 ± 1.37	28.63 ± 11.96	-4.48 ± 11.87	0.06	-2.93
WBC (K/ µL)	7.54 ± 3.93	6.13 ± 1.50	-1.41 ± 3.32	0.01*	6.76 ± 2.43	6.39 ± 1.71	-0.37 ± 2.10	0.37	-1.04
LYMP (M/µL)	26.35 ± 21.74	62.82 ± 10.62	36.46 ± 22.40	<0.001*	19.56 ± 21.82	57.57 ± 8.42	38.01 ± 25.26	<0.001*	-6.65

12W= 12 weeks; TG= Intervention group; CG= Control group; RBC= Red blood cells; WBC= White blood cells; LYMP= Lymphocytes; MCV= Mean corpuscular volume; MCH= Mean corpuscular haemoglobin; MCHC= Mean corpuscular haemoglobin concentration; *Differences significant within-group change comparison at $p < 0.05$; #No significant differences were observed in difference in changes between the intervention and control groups at $p < 0.05$

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Figure 3 shows haemoglobin profiles with time. There was a gradual increase in haemoglobin from baseline to 6 weeks; followed by a very gentle decline from 6 weeks to 12 weeks for the intervention group that received the intervention diet (ready-to-use supplementary food, RUSF). The haemoglobin concentration in the control group was fairly constant. Generally haemoglobin concentration was higher for the intervention group as compared to the control group.

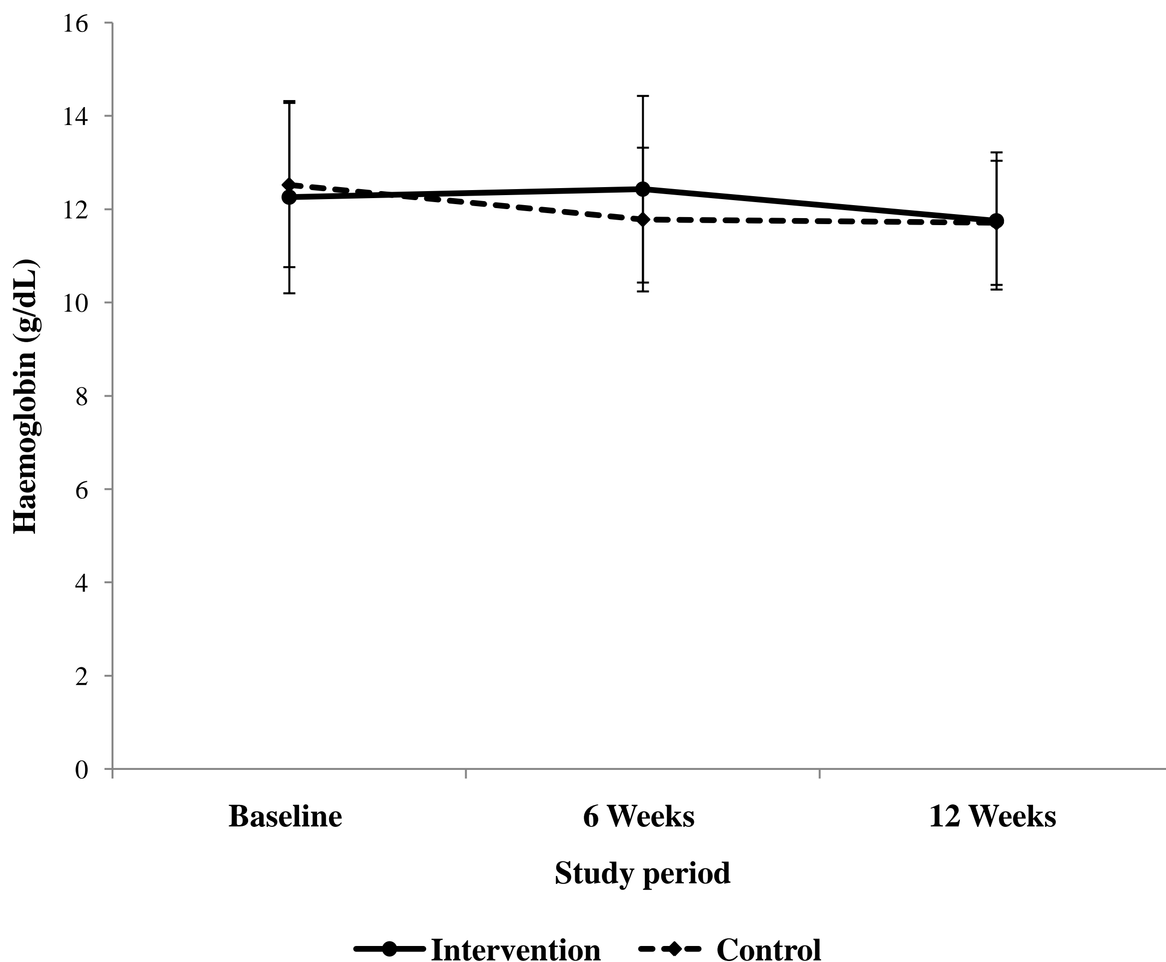

Fig. 3: Haemoglobin profiles with time

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4.7 Biochemical indices of participants

Total proteins, albumin, globulin, iron and ferritin at baseline have been outlined in Table 4.9. Serum total protein was 6.23 ± 1.57g/dL and 5.41 ± 1.83g/dL in the intervention and control groups respectively. There was no difference in serum iron between the intervention and control group at baseline but serum ferritin levels were appreciably higher for the treatment group (191.13 ± 64.64μg/L) as compared to the control group (160.77 ± 64.88μg/L). At baseline, globulin and ferritin concentrations between the groups were significant ($p<0.05$). The other biochemical indicators were not different between the groups at baseline.

Table 4.9: Baseline biochemical indices of participants by study group (N=134)

Biochemical indices	Intervention (n=67)	Control (n=67)	Total (N=134)	p-value
TPRO (g/dL)	6.23 ± 1.57	5.41 ± 1.83	5.77 ± 1.78	0.08
Albumin (g/dL)	3.56 ± 0.89	4.09 ± 8.00	3.82 ± 5.79	0.60
Globulin (g/dL)	2.67 ± 1.00	2.28 ± 0.94	2.45 ± 0.99	0.02*
Iron (μg/dL)	97.48 ± 23.32	90.49 ± 25.82	93.77 ± 24.72	0.11
Ferritin (μg/L)	191.13 ± 64.64	160.77 ± 64.88	175.20 ± 66.05	0.01*
[1]CRP (mg/L)	0.0 - 48.0	0.0 - 48.0	0.0 - 48.0	-

TPRO= Total Protein; CRP =C-reactive protein; [1]Value stated is the range; *Difference significant at $p<0.05$

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Total proteins, iron and ferritin levels of study participants from baseline to 12 weeks are presented in Table 4.10. There was a positive mean change for serum total proteins, albumins and globulins from baseline to 12 weeks during the study period in both intervention and control groups. Serum iron levels increased slightly from 98.18 ± 23.55 to 107.02 ± 27.74µg/dL in the intervention group whilst the control group was from 96.07 ± 25.99 to 107.81 ± 30.08µg/. A negative mean change (-39.34 ± 80.77 µg/L) for serum ferritin from baseline to 12 weeks was significant among the women in the intervention group but that of the control (-21.62 ± 85.06 µg/L; Table 4.10) was not significant.

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Table 4.10: Biochemical indices of participants according to study group and with time (n=71)

Biochemical indices	Intervention (n=44)				Control (n=27)				Differences between mean change in TG and CG #
	Baseline	12W	Change	p-value	Baseline	12W	Change	p-value	
TPRO (g/dL)	6.57 ± 1.54	6.80 ± 1.07	0.23 ± 1.67	0.36	6.00 ± 1.31	6.49 ± 0.99	0.49 ± 1.41	0.08	-0.26
Albumin (g/dL)	3.73 ± 0.84	5.37 ± 11.22	1.64 ± 11.19	0.34	3.53 ± 0.80	3.69 ± 0.42	0.16 ± 0.87	0.35	1.48
Globulin (g/dL)	2.84 ± 1.08	3.11 ± 0.87	0.27 ± 0.94	0.06	2.48 ± 0.66	2.81 ± 0.70	0.33 ± 0.71	0.02*	-0.06
Iron (µg/dL)	98.18 ± 23.55	107.02 ± 27.74	8.84 ± 37.88	0.13	96.07 ± 25.99	107.81 ± 30.08	11.74 ± 44.96	0.19	-2.90
Ferritin (µg/L)	193.50 ± 65.56	154.16 ± 63.39	-39.34 ± 80.77	<0.001*	186.89 ± 74.59	165.26 ± 75.85	-21.62 ± 85.06	0.20	-17.71

12W= 12 weeks; TG= Intervention group; CG= Control group; TPRO= Total Protein; *Differences significant within-group change comparison at $p < 0.05$; #No significant differences were observed in difference in change between the intervention and control groups at $p < 0.05$

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A representation of serum total protein profile with time is depicted in Figure 4. There was a slight increase from baseline to 6 weeks, followed by a gentle decline at the end of the study for the control group. A similar trend was observed in the intervention group but serum protein values were higher in the intervention group at all three points of measurements in the study.

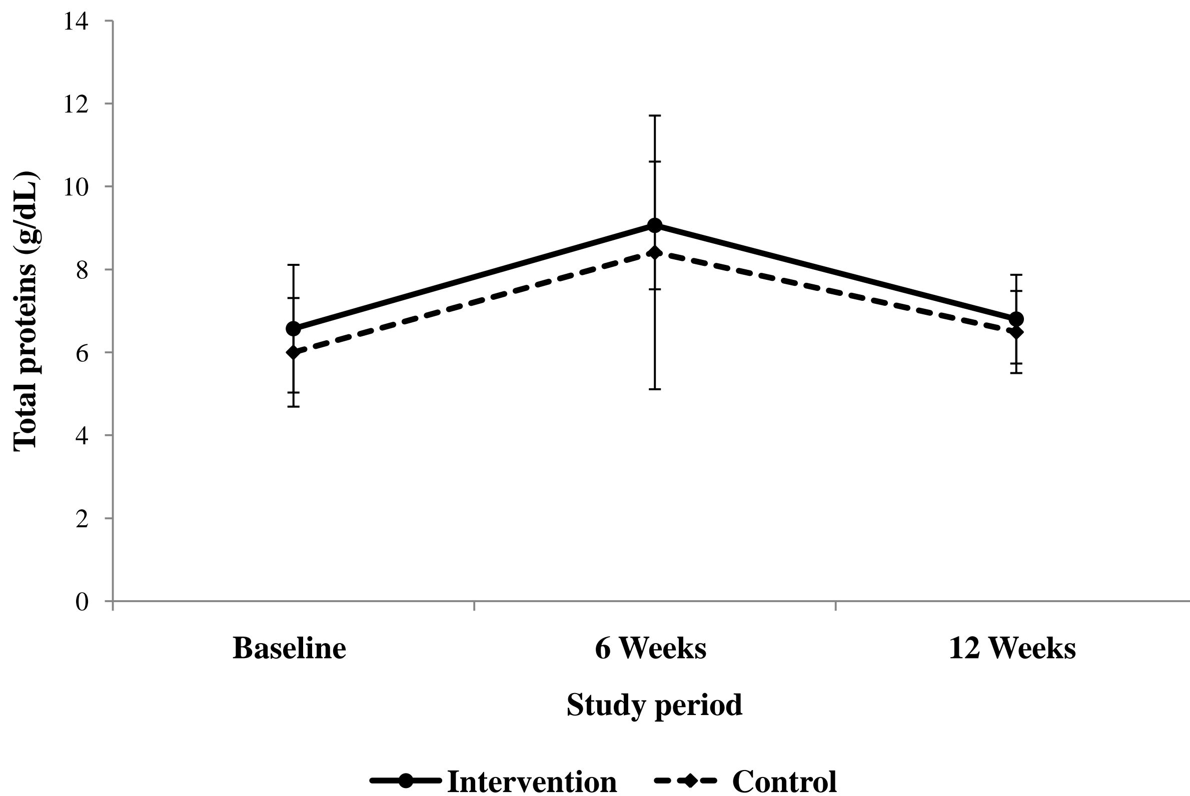

Fig. 4: Serum total protein profiles with time

Serum iron profiles with time are depicted in Figure 5. In the intervention group there was a slow rise in iron levels from baseline to 6 weeks and this remained almost constant to the end of the study. For the women in the control group, their iron levels increased marginally from baseline to 6 weeks and gradually increased to the end of the study at 12 weeks. It was interesting to note

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that differences in serum iron levels were observed among women in both groups at 6 weeks but baseline and endline (12 weeks) iron levels remained virtually the same although endline iron levels were the highest.

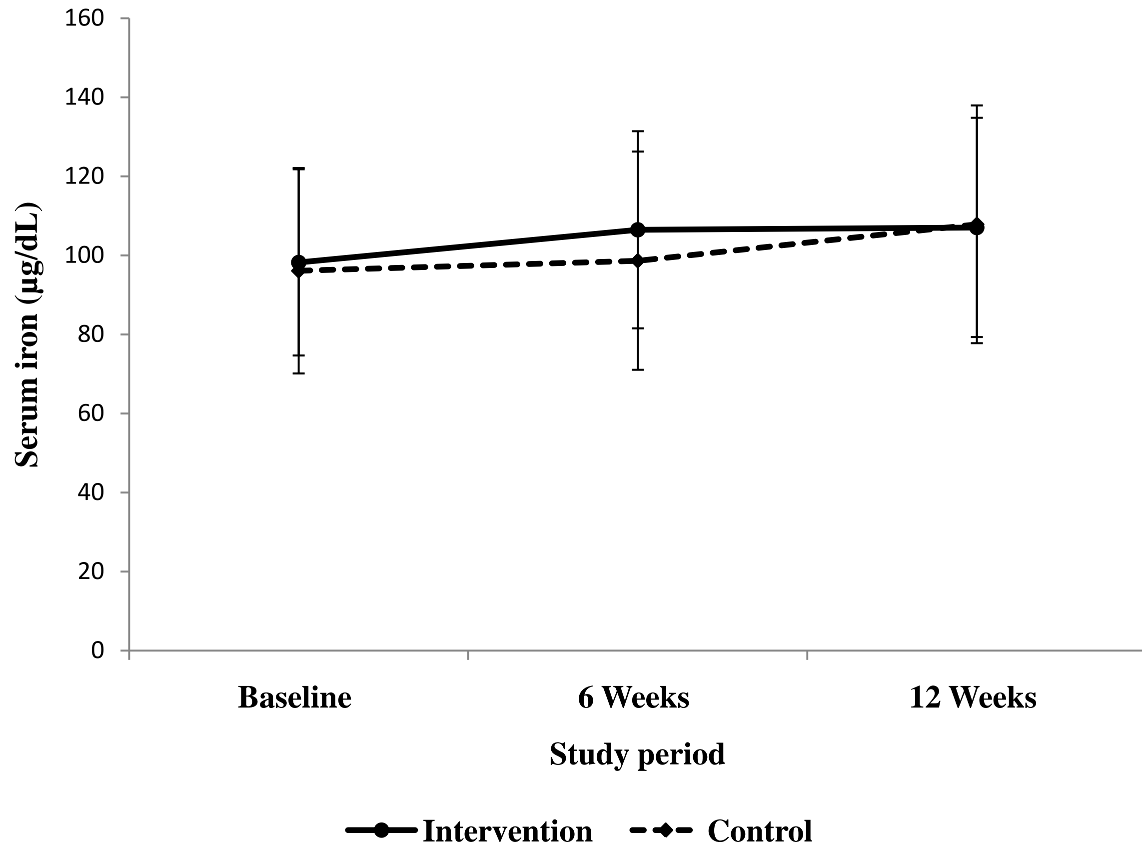

Fig. 5: Serum iron profiles with time

Ferritin levels among the women in the intervention group decreased marginally from baseline to 6 weeks, followed by a gentle decline to the end of the study (Figure 6). Concentration of ferritin among women in the control group declined very gently throughout the study. It was noticed that ferritin concentration at the end of the study (12 weeks) was higher in the control than intervention group although the reverse was observed at baseline.

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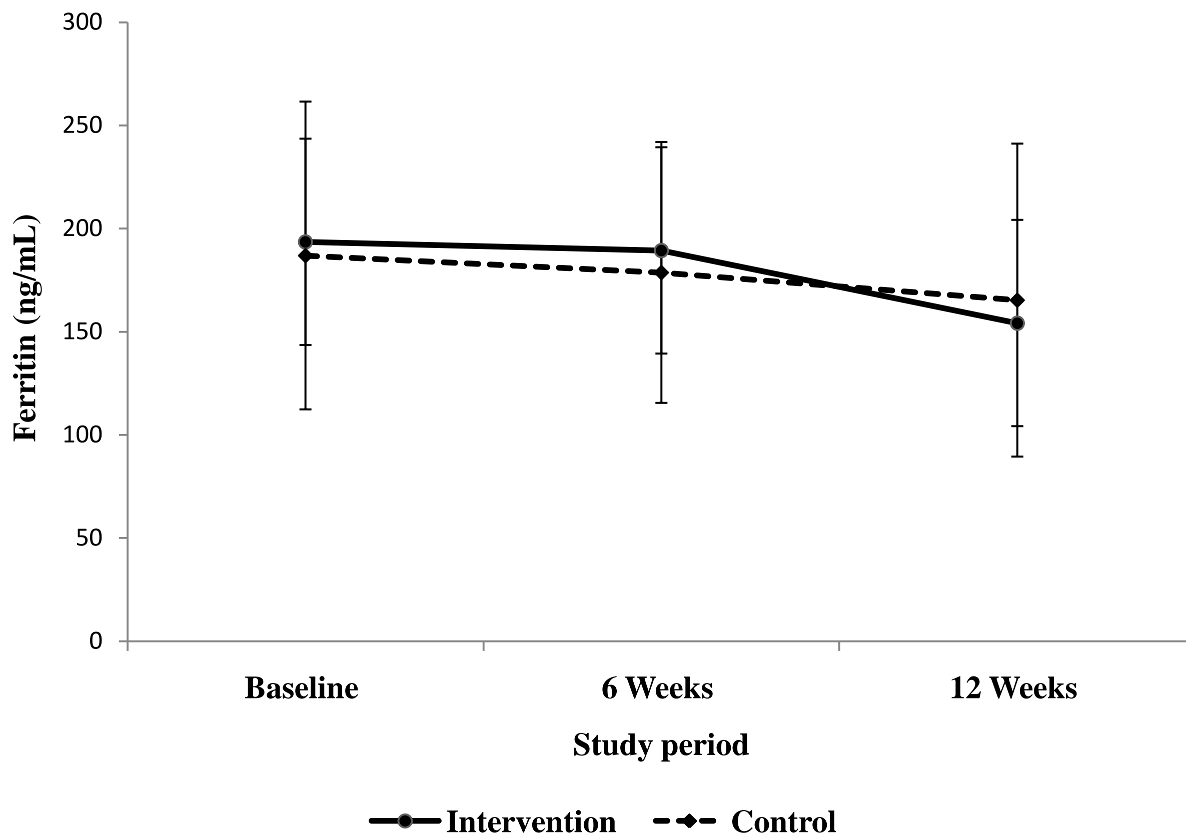

Fig. 6: Serum ferritin profiles with time

4.8 Nutritional status of participants across the study period

Anthropometric, haematological and biochemical profile of participants during the study period are outlined in Table 4.11, 4.12 and 4.13 respectively.

4.8.1 Anthropometric profile of participants

Table 4.11 shows the anthropometric profile of the women in the intervention and control group at baseline, 6 weeks (6W) and 12 weeks (12W). In this study population, a smaller proportion of the women were underweight (<18.5 kg/m^2) and obese in the intervention and control groups whilst majority had ideal BMI (18.5-24.9 kg/m^2; Table 4.11). Low neck circumference (<34cm) was predominant among the women throughout the study period.

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Table 4.11: Anthropometric profile with time of participants by study group (n=80)

Anthropometric indices	Intervention (n=47)			Control (n=33)		
	Baseline n (%)	6W n (%)	12W n (%)	Baseline n (%)	6W n (%)	12W n (%)
BMI (kg/m^2)						
Underweight	6 (12.8)	3 (6.4)	4 (8.5)	4 (12.1)	-	1 (3.0)
Normal	19 (40.4)	22 (46.8)	18 (38.3)	13 (39.4)	12 (36.4)	15 (45.5)
Overweight	15 (31.9)	14 (29.8)	14 (29.8)	10 (30.3)	13 (39.4)	9 (27.3)
Obese	7 (14.9)	8 (17.0)	11 (23.4)	6 (18.2)	8 (24.2)	8 (24.2)
NC (cm)						
Low	40 (85.1)	34 (72.3)	33 (70.2)	24 (72.7)	26 (78.8)	19 (57.6)
Normal	6 (12.8)	12 (25.5)	12 (25.5)	5 (15.2)	5 (15.2)	9 (27.3)
High	1 (2.1)	1 (2.1)	2 (4.3)	4 (12.1)	2 (6.1)	5 (15.2)

6W= 6 weeks; 12W= 12 weeks; BMI= Body mass index; NC= Neck circumference

4.8.2 Haematological profile of participants with time

Anaemia prevalence at the end of the study was about 50% in the intervention group as against 70% in the control group (Table 4.12). Low MCV levels were present among a few of the women in both groups at baseline and at the end of the study, although prevalence was much lower in the control at 12 weeks. Almost all the women had normal WBC counts throughout the study period.

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Table 4.12: Haematological indices with time of participants by study group (n=71)

Haematological indices	Intervention (n=44)			Control (n=27)		
	Baseline n (%)	6W n (%)	12W n (%)	Baseline n (%)	6W n (%)	12W n (%)
WBC (K/ µL)						
Low	-	3 (6.8)	-	1 (3.7)	3 (11.1)	-
Normal	42 (95.5)	41 (93.2)	44 (100.0)	25 (92.6)	4 (14.8)	27 (100.0)
High	2 (4.5)	-	-	1 (3.7)	23 (85.2)	-
RBC (M/µL)						
Low	10 (22.7)	14 (31.8)	13 (29.5)	3 (11.1)	10 (37.0)	6 (22.2)
Normal	32 (72.7)	26 (59.1)	30 (68.2)	23 (85.2)	16 (59.3)	20 (74.1)
High	2 (4.5)	4 (9.1)	1 (2.3)	1 (3.7)	1 (3.7)	1 (3.7)
HB(g/dL)						
Anaemia	16 (36.4)	18 (40.9)	21 (47.7)	10 (37.0)	17 (63.0)	19 (70.4)
Non anaemic	28 (63.6)	26 (59.1)	23 (52.3)	17 (63.0)	10 (37.0)	8 (29.6)
HCT (%)						
Low	44 (100.0)	21 (47.7)	18 (40.9)	27 (100.0)	19 (70.4)	10 (37.0)
High	-	23 (52.3)	26 (59.1)	-	8 (29.6)	17 (63.0)
MCV (fL)						
Low	9 (20.5)	8 (18.2)	7 (15.9)	8 (29.6)	-	2 (7.4)
Normal	27 (61.4)	34 (64.2)	32 (72.7)	15 (55.6)	12 (44.4)	23 (85.2)
High	8 (18.2)	2 (3.8)	5 (11.4)	4 (14.8)	15 (55.6)	2 (7.4)
MCH (pg)						
Low	7 (15.9)	4 (9.1)	8 (18.2)	8 (25.9)	5 (18.5)	8 (29.6)
Normal	29 (65.9)	35 (66.0)	33 (75.0)	15 (55.6)	20 (74.1)	19 (70.4)
High	8 (18.2)	5 (9.4)	3 (6.8)	4 (14.8)	2 (7.4)	-
MCHC (g/dL)						
Low	2 (4.5)	2 (4.5)	19 (43.2)	1 (3.7)	4 (14.8)	11 (40.7)
Normal	42 (95.5)	42 (95.5)	56.8 (25)	26 (96.3)	20 (74.1)	16 (59.3)

6W= 6 weeks; 12W= 12 weeks; WBC= White blood cells; RBC = Red blood cells; HB= Haemoglobin; HCT= Haematocrit; MCV= Mean corpuscular volume; MCH= Mean corpuscular haemoglobin; MCHC= Mean corpuscular haemoglobin concentration

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4.8.3 Biochemical profile of participants

In this study population, majority of the women had normal protein, albumin and globulin levels at baseline as shown in Table 4.13. However prevalence of low proteins and albumin but not globulins was quite high at the end of the study. The prevalence of low protein concentration increased from 27.3% to 52.3% (intervention group) whilst the control group was from 40.7% to 59.3% during the study period. The number of positive cases for C-reactive proteins was relatively low (about 20%; Table 4.13). Prevalence of iron deficiency anaemia in this study was below 8%. In the intervention and control groups, more than 50% (Table 4.13) of the women had high ferritin levels.

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Table 4.13: Biochemical indices with time of participants by study group (n=71)

Biochemical indices	Intervention (n=44)			Control (n=27)		
	Baseline n (%)	6W n (%)	12W n (%)	Baseline n (%)	6W n (%)	12W n (%)
TPRO (g/dL)						
Low	12 (27.3)	9 (20.5)	28 (52.3)	11 (40.7)	3 (11.1)	16 (59.3)
Normal	28 (63.6)	11 (25.0)	15 (34.1)	16 (59.3)	12 (44.4)	10 (37.0)
High	4 (9.1)	24 (54.5)	1 (2.3)	-	12 (44.4)	1 (3.7)
Albumin (g/dL)						
Low	9 (20.5)	9 (20.5)	28 (63.6)	8 (29.6)	5 (18.5)	18 (66.7)
Normal	35 (79.5)	22 (50.0)	15 (34.1)	19 (70.4)	15 (55.6)	9 (33.3)
High	-	13 (29.5)	1 (2.3)	-	7 (25.9)	-
Globulin (g/dL)						
Low	7 (15.9)	2 (4.5)	4 (9.1)	4 (14.8)	2 (7.4)	1 (3.7)
Normal	34 (77.3)	23 (52.3)	38 (86.4)	23 (85.2)	14 (51.9)	25 (92.6)
High	3 (6.8)	19 (43.2)	2 (4.5)	-	11 (40.7)	1 (3.7)
Iron (µg/dL)						
Low	1 (2.3)	-	-	1 (3.7)	2 (7.4)	-
Normal	43 (97.7)	44 (100.0)	41 (93.2)	26 (96.3)	25 (92.6)	25 (92.6)
High	-	-	3 (6.8)	-	-	2 (7.4)
Ferritin (µg/L)						
Normal	12 (27.3)	44 (100.0)	20 (45.5)	11 (40.7)	27 (100.0)	12 (44.4)
High	32 (72.7)	-	24 (54.5)	16 (59.3)	-	15 (55.6)
CRP (mg/L)						
Negative	39 (88.6)	38 (86.4)	40 (90.9)	21 (77.8)	25 (92.6)	25 (92.6)
Positive	5 (11.4)	6 (13.6)	4 (9.1)	6 (22.2)	2 (7.4)	2 (7.4)

6W= 6 weeks; 12W= 12 weeks; TPRO= Total Protein; CRP= C-reactive protein

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4.9 Associations between haematological and biochemical Indices profile

The associations between body mass index and haematological and biochemical variables at baseline (Table 4.14), 6 weeks (Table 4.15) and 12 weeks (Table 4.16) are shown below. At baseline, there was no significant association among BMI, haematological and biochemical variables as shown in Table 4.14. At 6 and 12 weeks however, some associations were observed. At 6 weeks, a significant association was observed between BMI status and RBC count amongst the women in the control group ($p<0.001$; Table 4.15). Simply put, an increase in BMI is likely to gradually increase RBC count. At 12 weeks, it was evident that a significant association exists between BMI and iron status among the women in the intervention group ($p=0.05$; Table 4.16). Thus as the BMI of the women in the intervention group increases, a corresponding gradual increase in iron levels is expected.

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Table 4.14: Haematological and biochemical profiles at baseline by study group and BMI levels (n=71)

Blood indices	Intervention (n=44)				Control (n=27)			
	BMI classification				BMI classification			
	1	2	3	p-value#	1	2	3	p-value#
Haematological								
WBC (K/ µL)								
Low	-	-	-	0.60	0 (0.0)	1 (3.7)	0 (0.0)	1.00
Normal	5 (11.4)	19 (43.2)	18 (40.9)		2 (7.4)	11 (40.7)	11 (40.7)	
High	0 (0.0)	0 (0.0)	2 (4.5)		0 (0.0)	1 (3.7)	1 (3.7)	
RBC (M/µL)								
Low	0 (0.0)	5 (11.4)	4 (9.1)	0.78	1 (3.7)	2 (7.4)	0 (0.0)	0.18
Normal	5 (11.4)	13 (29.5)	15 (34.1)		1 (3.7)	11 (40.7)	11 (40.7)	
High	0 (0.0)	1 (2.3)	1 (2.3)		0 (0.0)	0 (0.0)	1 (3.7)	
HB(g/dL)								
Anaemia	1 (2.3)	10 (22.7)	5 (11.4)	0.12	2 (7.4)	4 (14.8)	4 (14.8)	0.26
Non anaemic	4 (9.1)	9 (20.5)	15 (34.1)		0 (0.0)	9 (33.3)	8 (29.6)	
HCT (%)								
Low	1 (2.3)	10 (22.7)	5 (11.4)	0.12	2 (7.4)	4 (14.8)	4 (14.8)	0.26
High	4 (9.1)	9 (20.5)	15 (34.1)		0 (0.0)	9 (33.3)	8 (29.6)	
Biochemical								
TPRO (g/dL)								
Low	2 (4.5)	4 (9.1)	5 (11.4)	0.95	1 (3.7)	6 (22.2)	4 (14.8)	0.84
Normal	3 (6.8)	13 (29.5)	13 (29.5)		1 (3.7)	6 (22.2)	8 (29.6)	
High	0 (0.0)	2 (4.5)	2 (4.5)		-	-	-	
Iron (µg/dL)								
Low	0 (0.0)	1 (2.3)	0 (0.0)	0.54	0 (0.0)	1 (3.7)	0 (0.0)	1.00
Normal	5 (11.4)	18 (40.9)	20 (45.5)		2 (7.4)	12 (44.4)	12 (44.4)	
High	-	-	-		-	-	-	
Ferritin (µg/L)								
Normal	2 (4.5)	4 (9.1)	6 (13.6)	0.71	1 (3.7)	6 (22.2)	5 (18.5)	1.00
High	3 (6.8)	15 (34.1)	14 (31.8)		1 (3.7)	7 (25.9)	7 (25.9)	

BMI = Body mass index (1= Underweight, 2= Normal, 3= Overweight/Obese); WBC= White blood cells; RBC= Red blood cells; HB= Haemoglobin; HCT= Haematocrit; TPRO= Total Protein; #No statistically significant differences were observed at $p < 0.05$ (Fisher's exact test)

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Table 4.15: Haematological and biochemical profiles at 6 weeks by study group and BMI levels (n=71)

Blood indices	Intervention (n=44)				Control (n=27)			
	BMI classification				BMI classification			
	1	2	3	p-value	1	2	3	p-value
Haematological								
WBC (K/ µL)								
Low	0 (0.0)	1 (2.3)	1 (2.3)	1.00	-	4 (14.8)	3 (11.1)	1.00
Normal	3 (6.8)	17 (38.6)	22 (50.0)		-	6 (22.2)	14 (51.9)	
High	-	-	-		-	-	-	
RBC (M/µL)								
Low	1 (2.3)	7 (15.9)	6 (13.6)	0.40	-	6 (22.2)	3 (11.1)	<0.001*
Normal	2 (4.5)	8 (18.2)	15 (34.1)		-	5 (18.5)	12 (44.4)	
High	0 (0.0)	3 (6.8)	1 (2.3)		-	-	-	
HB(g/dL)								
Anaemia	2 (4.5)	8 (18.2)	10 (22.7)	0.90	-	6 (22.2)	10 (37.0)	0.66
Non anaemic	1 (2.3)	10 (22.7)	13 (29.5)		-	4 (14.8)	7 (25.9)	
HCT (%)								
Low	2 (4.5)	8 (18.2)	11 (25.0)	0.81	-	7 (25.9)	12 (44.4)	0.65
High	1 (2.3)	11 (25.0)	11 (25.0)		-	3 (11.1)	5 (18.5)	
Biochemical								
TPRO (g/dL)								
Low	0 (0.0)	3 (6.8)	6 (13.6)	1.00	-	0 (0.0)	1 (3.7)	1.00
Normal	1 (2.3)	8 (18.2)	5 (11.4)		-	8 (29.6)	7 (25.9)	
High	2 (4.5)	8 (18.2)	11 (25.0)		-	4 (14.8)	7 (25.9)	
Iron (µg/dL)								
Low	-	-	-	a	-	0 (0.0)	1 (3.7)	1.00
Normal	3 (6.8)	19 (43.2)	22 (50.0)		-	12 (44.4)	14 (51.9)	
High	-	-	-		-	-	-	
Ferritin (µg/L)								
Normal	1 (2.3)	8 (18.2)	4 (9.1)	0.61	-	6 (22.2)	3 (11.1)	1.00
High	2 (4.5)	11 (25.0)	18 (40.9)		-	6 (22.2)	12 (44.4)	

BMI = Body mass index (1= Underweight, 2= Normal, 3= Overweight/Obese); WBC= White blood cells; RBC= Red blood cells; HB= Haemoglobin; HCT= Haematocrit; TPRO= Total Protein; a= No p-value because grouping is a constant *Differences significant at $p < 0.05$ (Fisher's exact test)

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Table 4.16: Haematological and biochemical profiles at 12 weeks by study group and BMI levels (n=71)

Blood indices	Intervention (n=44)				Control (n=27)			
	BMI classification				BMI classification			
	1	2	3	p-value	1	2	3	p-value
Haematological								
WBC (K/ µL)								
Low	-	-	-	a	-	-	-	a
Normal	4 (9.1)	17 (38.6)	23 (52.3)		1 (3.7)	13 (48.1)	13 (48.1)	
High	-	-	-		-	-	-	
RBC (M/µL)								
Low	1 (2.3)	3 (6.8)	9 (20.5)	0.61	1 (3.7)	3 (11.1)	2 (7.4)	0.19
Normal	3 (6.8)	14 (31.8)	13 (29.5)		0 (0.0)	9 (33.3)	11 (40.7)	
High	0 (0.0)	0 (0.0)	1 (2.3)		0 (0.0)	1 (3.7)	0 (0.0)	
HB(g/dL)								
Anaemia	1 (2.3)	8 (18.2)	11 (25.0)	0.73	1 (3.7)	8 (29.6)	9 (33.3)	0.75
Non anaemic	3 (6.8)	9 (20.5)	12 (27.3)		0 (0.0)	5 (18.5)	4 (14.8)	
HCT (%)								
Low	1 (2.3)	9 (20.5)	7 (15.9)	0.15	1 (3.7)	5 (18.5)	3 (11.1)	0.15
High	3 (6.8)	8 (18.2)	16 (36.4)		0 (0.0)	8 (29.6)	10 (37.0)	
Biochemical								
TPRO (g/dL)								
Low	1 (2.3)	10 (22.7)	11 (25.0)	0.22	1 (3.7)	4 (14.8)	9 (33.3)	0.39
Normal	3 (6.8)	6 (13.6)	12 (27.3)		0 (0.0)	8 (29.6)	4 (14.8)	
High	0 (0.0)	1 (2.3)	0 (0.0)		0 (0.0)	1 (3.7)	0 (0.0)	
Iron (µg/dL)								
Low	-	-	-	0.05*	-	-	-	0.25
Normal	3 (6.8)	15 (34.1)	23 (52.3)		1 (3.7)	11 (40.7)	13 (48.1)	
High	1 (2.3)	2 (4.5)	0 (0.0)		0 (0.0)	2 (7.4)	0 (0.0)	
Ferritin (µg/L)								
Normal	1 (2.3)	12 (27.3)	8 (18.2)	0.14	0 (0.0)	6 (22.2)	8 (29.6)	0.22
High	3 (6.8)	5 (11.4)	15 (34.1)		1 (3.7)	7 (25.9)	5 (18.5)	

BMI = Body mass index (1= Underweight, 2= Normal, 3= Overweight/Obese); WBC= White blood cells; RBC= Red blood cells; HB= Haemoglobin; HCT= Haematocrit; TPRO= Total Protein; a= No p-value because grouping is a constant *Differences significant at $p < 0.05$ (Fisher's exact test)

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CHAPTER 5

5.0 DISCUSSION

5.1 Background

Malnutrition affects the well-being of millions of women as well as their children. It is therefore not surprising that on 6 March 2013, the United Nations Commission on the status of women held a panel discussion on the "Challenges and achievements in the implementation of the MDGs for women and girls". Generally, there has been accelerated progress towards the achievement of the MDGs, but less so on maternal mortality (MDG 5) (Presern, 2013). It is still the case that every year, 287,000 women die from preventable childbirth-related causes and malnutrition, 99% of them in developing countries (WHO, 2012). Inadequate access to and poor quality of diets from developing countries contributes to the wide spread of micronutrient deficiencies which sets the stage for poor pregnancy outcomes, affecting the survival and quality of life of the offspring resulting in the intergenerational cycle of growth failure. This vicious cycle of growth failure can be curtailed by channelling efforts at identifying women at risk of malnutrition and supplementing their diets with nutrient dense ready-to-use foods. This will help realise the estimated 5.5% annual rate of maternal mortality decline required to reduce by three quarters, between 1990 and 2015, the maternal mortality ratio as enshrined in MDG 5 (WHO, 2012; UNICEF, WHO, World Bank, UN, 2012). Nonetheless a ready-to-use food is not a magic bullet to maternal malnutrition and must be integrated into new and existing programs and policies.

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5.2 Study characteristics

Several studies have focused on the effectiveness and/or efficacy of ready-to-use therapeutic foods (RUTF) or ready-to-use supplementary foods (RUSF) compared to the corn-soya blend (CSB) among malnourished children, HIV-positive children and HIV-positive adults (Matilsky *et al*., 2009; Ndehka *et al*., 2009a; Nackers *et al*., 2010; Sunguya *et al*., 2012). This study adds to existing knowledge as it appears to be the first of its kind among women of reproductive age (WRA) in peri-urban settlements. This randomized controlled trial (RCT) with a non-food supplementation control group may have resulted in the high attrition rates observed for the control group (50%) as compared to the treatment group (30%). Also randomization to a non-food supplementation control group posed practical problems as participants highly appreciate food supplements when available (Ndekha *et al*., 2009a). However, the inclusion of a non-food supplementation control group in this trail design allowed for a comparison to make conclusions on the efficacy of the intervention diet on the nutritional status of the women who received the supplementary diet as compared to the women receiving no food supplements.

Supplementary feeding was done for a relatively short period (12 weeks) as was observed in some studies (Maleta *et al*., 2004; Phuka *et al*., 2009; LaGrone *et al*., 2012) but other studies have reported supplementation greater than 12 weeks (Ndekha *et al*., 2009a; van Oosterhout *et al*., 2010; Sunguya *et al*., 2012). Also the difference in the numbers between both groups have been reported in other ready-to-use supplementation trials amongst children and adult HIV patients in Sub-Saharan Africa (Ndekha *et al*., 2009a; LaGrone *et al*., 2012; Sunguya *et al*., 2012). Effectiveness studies of ready-to-use therapeutic foods (RUTF) compared with Corn/Soy blend (CSB) among acute malnourished children 6-18 months in Niger and Malawi consistently reported large differences in numbers between the two groups (Phuka *et al*., 2009; Nackers *et al*.,

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2010; LaGrone *et al.* 2012). In the clinical trial by LaGrone *et al.* (2012) 948, 964 and 978 children were allocated to receive CSB++, Soy RUSF and Soy/Whey RUSF respectively. On the contrary, a study by Ndekha *et al*. (2009b) which looked at supplementation with ready-to-use fortified spread (RUFS) and CSB in wasted adults starting antiretroviral therapy (ART) in Malawi reported almost equal numbers in the RUSF and CSB group. In order to compensate for the unequal number of women in the treatment and control group at the end of the study, many analyses focused on within-group comparisons between baseline and 12 weeks supplementation period. Additionally, because calculations were for women only, baseline differences of gender proportions between groups did not affect the interpretation of the main results. Hence the observed results were likely to be unbiased and thus representative of the population from which the sample was drawn.

5.3 Impact of RUSF on anthropometric measurements

In a succession of studies, therapeutic and supplementary versions of ready-to-use foods were shown to induce weight and body mass index (BMI) gain among malnourished children and HIV-positive adults and children (Ndehka *et al*., 2009b; Nackers *et al.*, 2010; Sunguya *et al.*, 2012). In this study, a gain in weight was observed for the intervention group whilst a lost in weight was recorded for the control group (Table 4.6). Food supplementation with peanut-based ready-to-use supplementary food (RUSF) resulted in significant increase in BMI (Table 4.6) in the intervention group after 12 weeks feeding. In the control group that received no placebo, increase in BMI was not significant. Consistent with the findings presented in this study, it was reported that Malawian adults starting ART with either CSB (245) or ready-to-use fortified spread (RUFS) (244) for 14 weeks resulted in greater increase in BMI compared to controls (104) that did not receive supplementary foods (van Oosterhout *et al*., 2010). This study is novel

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in that it provides evidence that energy dense RUSF is associated with modest weight and BMI gain in apparently healthy food insecure women. Study setting was peri-urban and thus the respondents were more likely to meet their energy intake as shown in Table 4.3. Outcomes may have been more pronounced in rural settings or if complicated respondents were studied. Unlike children who are still growing, a modest weight and BMI gain in the intervention group was expected for adult women of this age group. It therefore appears that, the effects of RUSF supplementation on adult body composition may differ from those observed in children. Also the modest increase in weight and BMI after 12 weeks of supplementation may be lost more readily as gain in weight and BMI was not rapid as reported by van Oosterhout *et al*. (2010) in an HIV-positive adult population in Malawi.

The change in BMI with time as depicted in Figure 2 indicates that during every interval in the 12 week study period, participants receiving RUSF gained modest BMI compared with control group although BMI values were much higher but inconsistent in the control group. This study does not show a difference in the mean change between the treatment and control groups as expected and this may be due to the small numbers involved as well as the duration of the study and frequency of feeding.

Neck circumference (NC) and hip circumference (HC) in this study were used as indicators of length gain as opposed to the use of mid-upper arm circumference (MUAC) and waist circumference (WC) in studies involving children and adult HIV women (Ndekha *et al*., 2009b; LaGrone *et al*., 2012). In contrast to weight and BMI, NC and HC seems to be very little affected by a 12 week supplementation with a peanut-based RUSF as evidenced by modest changes in both control and intervention groups. This was not surprising as length gain acceleration has often been shown to follow weight gain increase with a lag period of 3 months as reported in

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rural Malawi (Maleta *et al*., 2004). Hence only a longer intervention might have the potential for the primary prevention of lower NC and HC as reported in Table 4.6 and 4.11 respectively.

5.4 Clinical implications of RUSF on haemoglobin, serum iron, ferritin and CRP levels

In the human body, iron is present in all cells and acts as a carrier of oxygen to the tissues from the lungs in the form of haemoglobin (Hb). Serum ferritin, serum iron and haemoglobin are used to describe iron deficiencies; the commonest nutritional deficiency in the world. Iron deficiency is a reduced content of total body iron. Iron deficiency is believed to be the main cause of anaemia which is linked to increased maternal morbidity and mortality and impaired functional capacity in women (Haas and Brownlie, 2001). There are 3 forms of iron deficiencies namely: iron depletion, iron deficiency without anaemia and iron deficiency anaemia. Iron depletion occurs when serum iron levels falls whilst iron deficiency without anaemia is associated with low serum ferritin levels. Serum ferritin is an accurate indicator of total body iron stores especially in the absence of inflammation. Iron deficiency anaemia (IDA), occurs when the iron deficiency is sufficient to reduce erythropoiesis and therefore the haemoglobin level falls.

Prevalence of iron depletion and iron deficiency without anaemia were negligible in this study population as shown in Table 4.13. However prevalence of IDA defined as low haemoglobin concentration exceeded the threshold of 30.2% stated for non-pregnant and non-lactating women globally (WHO, 2008). Anaemia prevalence ranged from 36-70% with a higher percentage recorded for the women in the control group (Table 4.12). According to the Ghana Demographic and Health Survey (GDHS, 2008) 59% of women aged 15-49 years have some form of anaemia. Anaemia prevalence reported in this study is slightly higher than that reported by the GDHS in 2008. This is because, the women were sampled from peri-urban settlements in the Greater

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Accra region of Ghana where the standard of living may be quite high and it is possible that the prevalence of anaemia may be even higher in more remote areas where nutrition security is compromised.

The cause of anaemia is multifactorial, the commonest being iron deficiency, folate and Vitamin A and C deficiency, worm infestation and malaria (WHO, 2009). Folate deficiency is associated with macrocytic anaemia whilst iron deficiency is linked to microcytic anaemia. All the women did not meet their RDA for folate whilst only 25% met their RDA for vitamin A as presented in Table 4.3. Folate and vitamin A deficiency is likely to account for the high burden of IDA in this study population as most of the women met their RDA for iron and vitamin C. Nonetheless iron bioavailability also depends on dietary composition. Heme iron, which is found only in meat, poultry, and fish, is more absorbable than non-heme iron which is found in plant-based foods and iron-fortified foods. Vitamin C also enhances the absorption of heme and non-heme iron whilst phytate and fibre inhibits iron absorption.

In this study the effect of worm infestation may have been minimal as participants were de-wormed prior to feeding. A similar study by Manary *et al.* (2012) confirmed that de-worming during supplementary feeding is beneficial. Malaria, fever and other opportunist infections that were reported occasionally by some of the women may explain the high prevalence of anaemia in both groups during the study period. Also the presence of anaemia in the intervention group may be an indication that the peanut-based RUSF was not efficacious in improving haemoglobin levels. This can be attributed to the low iron content (0.37mg/bottle; Table 4.4) of the peanut-based RUSF. Other similar RUSF products have iron content as high as 3.27mg/100g (Ndekha *et al.*, 2009). The fibre content of the peanut-based RUSF was as high as 7.11g/bottle (Table 4.4).

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This is likely to increase the bulkiness of the food. The fibre may have a negative impact on digestion and the utilization of the food consumed. This could reduce the absorption of iron. It is important to note that the iron present in the peanut-based RUSF is in the non-heme form. This implies that it is less bio-available to the body. It is possible that the iron present in the peanut-based RUSF was poorly absorbed and hence the high prevalence of anaemia in the intervention group. Nonetheless, the presence of anaemia in the intervention group needs to be further researched using larger sample sizes and controlling for all possible factors that affect anaemia. This is because, data was incomplete as only 71 of the 134 women enrolled at baseline had complete haematological and biochemical data. Anecdotally, this was because of reluctance of women to undergo second and third venipuncture.

Haemoglobin levels cannot detect early stages of iron deficiency (Cook, 2005). The gold standard for determining iron deficiency is the staining of the bone marrow with Prussian blue for iron (Kotru *et al.,* 2004). The absence of a stain is considered as the best indicator of iron deficiency (Koulaouzidis, 2009). However this method is invasive and most laboratories may not have the facilities to conduct it; hence serum or plasma iron, transferrin, transferrin saturation, ferritin, reticulocyte haemoglobin content and soluble transferrin receptors are widely recommended (Wish, 2006; Thomas and Thomas, 2002). The use of serum iron could not identify any participant as having iron deficiency although greater than 35% were anaemic (Table 4.12). This is because the use of serum iron as an index of iron deficiency is influenced by iron absorption from meals, infection, inflammation and diurnal variations (Wu *et al*., 2002). An overnight fast is ideal but most participants would decline to give their blood very early in the morning if they had not taken a meal (personal experience). For a more accurate determination of iron deficiency, serum ferritin and C-reactive protein (CRP) were determined.

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Numerous studies of iron status conducted in developing countries have used acute phase protein biomarkers mostly C-reactive protein (CRP) to identify the influence of inflammation and infections on markers of iron status such as ferritin (Rogers *et al*., 1990; Kalender *et al*., 2002; Kalantar-Zadeh *et al*., 2004; Beard *et al*., 2006). Serum ferritin is an acute phase reactant that can become elevated during inflammation, chronic infection and other diseases (WHO, 2011). In the absence of inflammation, the concentration of plasma or serum ferritin is positively correlated with total body iron stores (Kalender *et al*., 2002). Prevalence of inflammation as measured by CRP was modest (7.4-22.2%, Table 4.13). The ferritin threshold of 150µg/L used in this study was based on the recommendation of the WHO Vitamin and Mineral Nutrition Information System that did not control for concurrent infection (WHO, 2011). Thus the high serum ferritin levels (>150µg/L; Figure 6) were more likely to be due to subclinical infections and to a lesser extent inflammation. Indeed, inflammation may not have an effect on serum ferritin unless there is enough iron stores in the body so that serum ferritin is somewhat elevated (Rogers *et al*., 1990).

5.5 Nutritional implications of RUSF on serum protein, albumin and globulin

Over the years, serum total protein, albumin and globulin concentrations have been commonly used as a laboratory test to identify protein-energy malnutrition (Rosenoer *et al*., 1977; Doweiko and Nompleggi, 1991) and hepatic function disorder (Skrede *et al*., 1975). Serum total protein is a quantitative measurement of the concentration of all proteins in the blood. The major proteins are albumin and globulin of which the former constitutes the highest percentage. Albumin helps prevent fluids from leaking through the blood vessels whiles globulins are an important part of the immune system that help the body to fight against infections. Majority of the women had low (20.5-59.3%) or normal (25.0-63.6%) total protein as shown in Table 4.13. The low total protein

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level in some participants (>30%) could hamper the transport and utilisation of nutrients in their bodies. This could affect iron metabolism and result in anaemia and iron deficiency. High total protein levels observed in four of the women at baseline also have several health implications which include chronic infections, improper functioning of the liver, excess alcohol intake and leukaemia. The albumin component indicates the quality of one's diet with respect to protein. Participants with low albumin (>20%) might have had low quality protein nutrition.

Total protein, albumin and globulin concentrations (6.00-6.80g/dL, 3.53-5.37 g/dL, 2.48-3.11 g/dL respectively; Table 4.10) were within the normal ranges for both intervention and control groups during the study period. Generally the women in this study have good protein stores. Good total protein level is an indication that the women consume enough proteins as 66% of them met the RDA for proteins (Table 4.3). Good protein stores are needed for cell growth; repair of worn-out tissues and to meet the demands of pregnancy. Meeting energy needs spares proteins and prevents wasting. It is therefore not surprising that there was no association between underweight and serum proteins as shown in Table 4.14. Also there were differences in total protein, albumin and globulin concentrations among the intervention and control group but this cannot be attributed to the peanut-based RUSF since changes in both groups were not significant.

5.6 Proximate composition of RUSF

New developments in nutrition are directed towards meeting the nutritional needs of specific target groups for survival and development. The nutrient composition of the newly developed RUSF was compared with ready-to-use fortified spread (RUFS) and the traditional Corn-Soy Blend (CSB). The energy content of the RUSF (564.52g/100g) compares well with RUFS (565.32 g/100g) but is superior to CSB (370.33 g/100g) as outlined in Table 4.4. The protein

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content of 22.93g/100g in the RUSF is quite higher than the 14.49g/100g and 13.37g/100g protein content in RUFS and CSB respectively. However the fat content of RUFS (37.14g/100g) is more than twice that in the new RUSF (15.26g/100g). This is probably because the RUFS was developed for the management of wasting in moderately malnourished children as well as severely wasted HIV-positive adults on antiretroviral therapy. On the other hand, this new product was designed to be used as a supplement to other foods and hence the low fat content. The formulation of a low fat peanut-based RUSF was to obtain a product that has little or no contribution to overweight/obesity when consumed. The iron content of the peanut-based RUSF (2.18mg/100g) is quite low as compared to RUFS (3.27mg/100g) and CSB (4.28mg/100g; table 4.4). The low iron content of the product is not desirable due to its high fibre content (12.70g/100g) which is likely to inhibit the absorption of the non-heme iron in the product. The fibre may also inhibit zinc absorption which is quite low (1.47mg/100g) in the product.

This product demonstrates that local ingredients (peanut, cowpea and rice) can be effectively mixed to give high quality supplementary food. Nonetheless selecting the right mix of foods is a herculean task since peanut and cowpea cannot easily replace milk as used in other formulations. An area of concern is that peanuts and cowpeas do not only contain proteins, vitamins and minerals, but also anti-nutrients such as phytate (phytic acid), fibre, polyphenols and enzyme inhibitors. The phytic acid content of polished rice, cowpea and peanut used in formulation of the RUSF are 0.60%, 2.38% and 1.76% of dry weight respectively (Reddy and Sathe, 2001). Several efforts were made to reduce the phytic acid content of the principal ingredients used in the formulation. The polished rice was cooked, cowpea soaked and cooked and the peanut was roasted. Soaking cowpea at room temperature for 18-24 hours resulted in an 8-20% reduction in phytate (phytic acid) levels before cooking for long hours (Mahgoub and Elhag, 1998). Roasting

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also removes 32-68% of phytic acid from peanut (Nagel, 2010). In general, the roasting, soaking and cooking are likely to drastically reduce the phytic acid, fibre and anti-nutrient content of the product. Nonetheless, the residual phytate, fibre and other anti-nutrients may have negative impacts on digestion and utilization of the food consumed; for example reducing the absorption of iron and zinc. Another area of concern is that peanuts are prone to aflatoxin contamination. A study in Kenya reported that 37% of peanut and peanut products exceeded the 10μg/kg regulatory limit for aflatoxin levels set by the Kenyan Bureau of standards (Mutegi *et al*., 2013; 2009). Aflatoxin contamination was reduced by sorting of the peanuts before and after roasting. Issues of safety are a matter of discussion since the high moisture content (83.03g/100g) of the drink as reported in Table 4.4 is likely to favour bacterial growth. For instance in 2009, *Salmonella* contamination was reported in peanut pastes by the Centre for Disease Control and Prevention.

The outcome of this human study shows that the newly developed RUSF can generally be considered safe and has been approved by the Food and Drugs Board (now Food and Drugs Authority) of Ghana (FDB/051/A11/1619; appendix VI). During this RCT, no adverse effects were reported. Diarrhoea episodes and respiratory infections were not reported by the women. The RUSF drink was palatable, well tolerated and highly accepted by the women and did not replace the consumption of other foods. A similar observation was made by other researchers amongst adults with HIV (Hebie *et al*., 2013), pregnant and lactating mothers (Mridha *et al*., 2012) and children (Adu-Afarwuah *et al*., 2008; Flax *et al*., 2009; 2010; Mridha *et al*., 2012).

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5.7 Sharing practices

The drink which was primarily consumed by the women was liked very much (personal observation). Also little or no intra-household sharing of the drink was observed. This observation agrees well with previous studies in Somalia, Malawi and Niger that reported that supplementary foods were primarily consumed by beneficiaries and that sharing was very minimal (Flax *et al*., 2009; Matilsky *et al*., 2009; Cohuet *et al*., 2012). Few women however reported being fed-up with the same food towards the end of the 12 week supplementation period.

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CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study on a ready-to-use supplementary food (RUSF) adds to existing knowledge as it appears to be the first of its kind among women of reproductive age (WRA) in peri-urban settlements in Ghana. On the whole, RUSF supplementation showed some improvements in anthropometric and haematological indices. In a nut shell, it was evident that:

- Supplementation with RUSF resulted in significant modest gains in weight and body mass index (BMI) amongst women in the intervention group from baseline to endline. There was however no difference in the mean weight and BMI of the women in the intervention and control group.
- Duration of feeding (12 weeks) with RUSF in the intervention group was too short to have any effect on neck and hip circumference.
- There was an increase in haemoglobin (Hb) levels from baseline to 6 weeks and a decline from 6 weeks to 12 weeks among the women in the intervention group. A significant decline in Hb was observed in the control group throughout the study period. Differences in mean Hb levels were not significant between both groups. Anaemia was persistent in both groups although the prevalence was higher in the control group.
- There was no difference in mean serum iron, ferritin and total protein concentration between the intervention and control group
- The energy, macronutrient and micronutrient content of the RUSF compares well with other ready-to-use foods.
- The RUSF was liked and well tolerated.

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6.2 Recommendations

Further work needs to be done to confirm that, RUSF supplementation has the potential to impact positively on maternal anthropometric and haematological indices. It is therefore recommended that the following is considered for further research:

- The energy, macronutrient and micronutrient content of the RUSF should be increased.
- Other forms of the newly developed RUSF such as biscuits and pastes need to be considered.
- Effectiveness trials of the newly developed RUSF drink in specific target groups with adequate sample, power and of sufficient duration are needed and it should include a cost effectiveness analysis.

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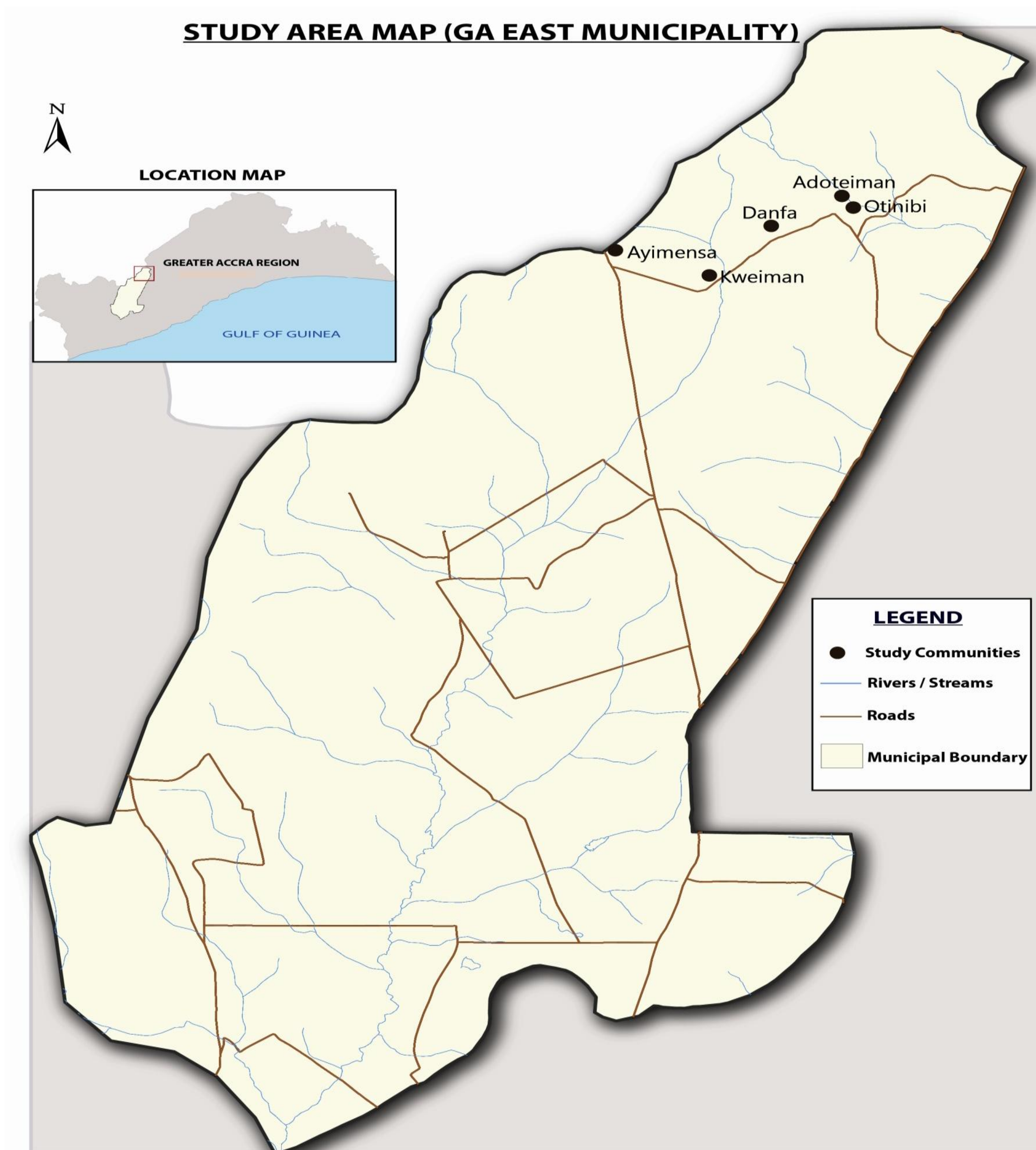
APPENDICES

Appendix I: Study Area Map (Ga East Municipality)



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Appendix II: Informed Consent Form

Study title: The efficacy of peanut based food in improving the nutritional status of women.

Principal Investigator: Prof . Matilda Steiner-Asiedu

Address: Department of Nutrition and Food Science, University of Ghana-Legon

Invitation to participate:

A research study seeks to know how a peanut based food (Ready-to-Use Therapeutic food) (RUTF) would improve on the nutritional status of women when consumed. We kindly invite your participation in the study.

Purpose:

A peanut based food product that is rich in energy, proteins, vitamins and minerals has been locally made/developed. The study would want to find out how the food would improve the nutritional status of women (18-45 years) when consumed. If found to improve on their nutritional status, it would help to correct undernutrition.

Description of procedures:

If you accept to participate in this research study, you will be asked to provide certain information such as your age, level of education, usual food intake, vitamins or mineral supplements. You will be provided three times in a week for six months an amount of the peanut based food to consume, possibly in the presence of the investigator/researcher. Body measurements, such as your weight, height, neck and hip circumferences would be taken. A sterile needle would be used to draw about 5 ml (each time) of your blood four times throughout the study; at the beginning of the study, every two months thereafter till the end of the study. Laboratory analyses would be carried out on the blood samples. Comparisons would be made with the body measurements and the blood samples taken at the beginning of the study and those within the study to find out if the food has improved on your nutritional status. The blood samples will not bear your name; therefore no one can associate you with it.

Eligibility:

Before you can participate you should be a female within the age range of 18-45 years. You will not qualify to partake in the study if you are pregnant and or lactating, on mineral or vitamin supplements or have a known allergenicity to peanuts.

Risks and inconveniences:

The inconvenience you may experience is the time you would have to dedicate to complete the interview. Possibly, some questions may make you feel uncomfortable or lead to a loss of your privacy. The drawing of the blood may be slightly painful to you. You are not compelled to answer the entire questionnaire or to complete the interview if you so wish. You are at liberty to decline to give your blood if you choose to. If you were not aware at the beginning of the study that you were allergic to peanuts and later come to know, you may decide to discontinue participation.

Benefits:

Findings from the study would help to determine if the peanut based food (RUTF) can be used to help improve the nutritional status of people. It would help to have an RUTF that is locally made.

Economic considerations:

Participation does not require any cost to you. If you choose to participate in the study the researchers would visit you and conduct the interview as well as provide you with the food (RUTF) to consume.

Confidentiality:

Any information obtained from your participation will be kept strictly confidential. Your consent form will be kept separate from the data. The data will not be available to anyone other than the researcher. The information may be used in presentations and/or research papers. However, your name will never be used in any presentations, papers, or reports.

Occasionally the institutional review board (IRB) of the Noguchi Memorial Institute for Medical Research may inspect study records as part of its auditing program, but these reviews will only focus on the researcher and not on your responses or involvement. The IRB is a group of people that reviews research studies to make sure they are safe for participants.

Compensation:

You will be given washing soap to thank you for your time.

Voluntary participation:

Participation in this study is not compulsory. You are free to opt out at any point in time if you so wish. You will not be penalized for deciding to quit.

Any questions

Kindly take enough time to make a decision. We are ever ready to answer any questions you may have about the study. If you have further questions aimed at clarifications concerning your participation in the study, you may contact the principal investigator of the study at the University of Ghana-Legon, Dr. Matilda Steiner-Asiedu on telephone (0541260704) or by email; tillysteiner@ug.edu.gh. If you have any questions concerning your right as a research subject, you may contact the chairman of the University of Ghana Institutional Review Board (IRB), Rev. Dr. Ayete-Nyampong, Chairperson, NMIMR-IRB, mobile 0208152360.

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Volunteer agreement:

The above document describing the benefits, risks and procedures for the research on the use of a peanut based ready to use therapeutic food has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

……………………………… ……..……………………….

Date signature or mark of volunteer

If volunteers cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

……………………………… ……………………………………

Date signature of witness

I certify that the nature purpose of the potential benefits and possible risks associated with participating in this study reaearch has been explained to the above individual.

…………………………………… ………………………………………………………

Date signature of person who obtained consent

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Appendix III: Study Questionnaire

Efficacy of a peanut-based food on the nutritional status of women

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Section A:

1. ID Number

2. Date of interview (DD/MM/YY)

3. Respondent's name ……………………………………………………………………

4. Respondent's address (detailed description) ………………………………………………

……………………………………………………………………………………………………
……………………………………………………………………………………………………

5. Respondent's telephone number ……………………………………………………………..

Background characteristics.

6. How old are you? …………………yrs

7. What is your marital status?

1= single [ ]

2= Married [ ]

8. What is your highest level of education?

1= tertiary [ ]

2= secondary [ ]

3= basic [ ]

4= None [ ]

9. What is your occupation?

1. Employed [ ]

2. Unemployed [ ]

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10. Do you have any of the following items in your home? (Ans 1= Yes or 2= No)

Item	Yes/No
Radio	
Television	
Refrigerator	
Telephone	
Fan	
Blender	

11. What is your mode of transport?

1= public transport [ ]

2= private car [ ]

12. What lightning facility is available to you?

1= Electricity [ ]

2= Lantern [ ]

3= Candle [ ]

13. What toilet facility is available to you?

1= Own WC [ ]

2= Own KVIP [ ]

3= Public WC [ ]

4= Public KVIP [ ]

14. What water facility is available to you?

1= Own pipe borne water [ ]

2= Public pipe borne [ ]

3= Own well [ ]

4= Public well [ ]

5= Own borehole [ ]

6= Public borehole [ ]

7= River/stream [ ]

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Household food security

These next questions are about the food eaten in your household in the last 12 months and whether you were able to afford the food you need. I'm going to read you several statements that people have made about their food situation. For these statements, please tell me whether the statement was often true, sometimes true, or never true for (you/your household) in the last 12 months.

15. The food that (I/We) bought just didn't last and (I/we) didn't have money to get more. Was that often true, sometimes true, or never true for (you/your household) in the last 12 months?
[1] Often true [ ]
[2] Sometimes true [ ]
[3] Never true [ ]
[4] Don't know or Refused [ ]

16. (I/we) couldn't afford to eat balanced meals. Was that often, sometimes, or never true for (you/your household) in the last 12 months?
[1] Often true [ ]
[2] Sometimes true [ ]
[3] Never true [ ]
[4] Don't know or Refused [ ]

17. in last 12 months, since (date 12 months ago) did (you/you or other adults in your household) ever cut the size of your meals or skip meals because there wasn't enough money for food?.
[1] Often true [ ]
[2] Sometimes true [ ]
[3] Never true [ ]
[4] Don't know or Refused [ ]

Optional screener: if any of the first 3 questions are answered affirmatively (ie., if either **Q15** or **Q16** are often true or sometimes true or **Q17** is yes) proceed to the next question. Otherwise skip to end **(Q20).**

18. **[Ask only if Q16=YES].** How often did this happen -almost every month, some months but not every month, or in only 1 or 2 months?
[1] Almost every month [ ]
[2] Some months but not every month[ ]
[3] Only 1 or 2 months [ ]
[4] Don't know or Refused [ ]

19. In the last 12 months, did you ever eat less than you felt you should because there wasn't enough money to buy food?
[1] Often true [ ]
[2] Sometimes true [ ]
[3] Never true [ ]
[4] Don't know or Refused [ ]

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20. In the last 12 months were your ever hungry but didn't eat because you couldn't afford enough food
[1] Often true [ ]
[2] Sometimes true [ ]
[3] Never true [ ]
[4] Don't know or Refused [ ]

Medical History

1. Have you been on any vitamin and or mineral supplements before?

1=Yes, if yes go to questions 2, 3 and 4

2= No

2. Who prescribed them for you? [ ]

1= A health professional [ ]

2= self medication [ ]

3= other [ ]

3. In general, how would you describe your health?

1= Excellent [ ]

2= Very Good [ ]

3= Good [ ]

4= Fair [ ]

5= Poor [ ]

Anthropometrics

a. current weight ………………………….. Kg

b. current height …………………………….m

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Food Frequency Questionnaire

For each of the following foods, mark the column to show how often respondent usually ate each food in the past month. Mark the usual amount (serving size) as small, medium or large.

`HOW OFTEN DID RESPONDENT EAT THESE FOODS IN THE PAST ONE MONTH

(Amount based on local food measures; show participant)

FOOD ITEM		FREQUENCY OF CONSUMPTION						USUAL AMOUNT*		
		1 - 2 times per day	5+ times per week	2-4 times per week	1 time per week	Never or less than once per week		S	M	L
PORRIDGES & CEREALS										
Hausa koko										
Corn koko										
Tom Brown										
Ekuegbemi										
Oblayoo										
Rice water										
Oats										
Other porridges/cereals										
BEVERAGES & OTHER DRINKS										
Cocoa drinks e.g. Milo										
Cocoa powder										
Other beverages/drinks										
Tea e.g. Lipton										
Herbal tea										
Other tea										
Coffee e.g. Nescafe										

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FOOD ITEM		FREQUENCY OF CONSUMPTION						USUAL AMOUNT*		
		1 - 2 times per day	5+ times per week	2-4 times per week	1 time per week	Never or less than once per week		S	M	L
EVAPORATED MILK										
Peak milk										
Ideal milk										
Carnation milk										
Carnation Tea Creamer										
Vega milk										
Whole milk (fresh milk)										
Skimmed milk (fresh milk)										
Yoghurt										
POWDERED MILK										
Nido										
Peak										
Cowbell										
JUICES & DRINKS										
Tampico										
Kalypo										
Vita Milk (Soya milk)										
Countre milk										
Minerals (e.g. Fanta, Coca Cola)										
Other beverages / drinks										
BREADS										
Bread (Sugar, Tea, Brown etc.)										
Bofrot										
Akara/Koose										
Pie/chips										
Biscuits e.g. Malt and Milk										
Cream Crackers										

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MAIN FOODS & STARCHES		1 - 2 times per day	5+ times per week	2-4 times per week	1 time per week	Never or less than once per week		S	M	L
Kenkey (Ga, Fante)										
Waakye										
Rice (Boiled/fried/jollof/balls)										
Fufu										
Banku										
Boiled yam										
Fried yam										
Sweet potatoes										
Boiled plantain										
Roasted plantain										
Fried plantain										
Cocoyam										
Gari										
Beans and gari										
Spaghetti										
Noodles										

FOOD ITEM		FREQUENCY OF CONSUMPTION						USUAL AMOUNT *		
		1 - 2 times per day	5+ times per week	2-4 times per week	1 time per week	Never or less than once per week		S	M	L
Millet/ Sorghum										
Maize/corn (boiled/roasted/fried)										
Tuo zaafi										
Sugar										
Other main foods/starches										
PROTEIN FOODS										
Eggs (Boiled, fried)										
Fried fish										
Smoked fish										
Fresh fish										
Seafood										

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(Crabs/shrimps/shellfish)										
Meat (farm animals)										
Bush meat										
Poultry (Chicken/duck/birds)										
Wele (cow hide)										
Sausage										
Corned beef										
Canned fish										
Beans (cowpea/soyabeans)										
Other protein foods										

FOOD ITEM		FREQUENCY OF CONSUMPTION						USUAL AMOUNT*		
		1 - 2 times per day	5+ times per week	2-4 times per week	1 time per week	Never or less than once per week		S	M	L
VEGETABLES										
Cocoyam leaves (Kontomire)										
Other leaves (Ademi, bokoboko)										
Okra (okro)										
Tomatoes (fresh)										
Onions										
Garden eggs										
Cabbage										
Lettuce										
Cucumber										
Carrots										
Green pepper										
Mixed vegetables salad										
Other vegetables										

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FOOD ITEM		FREQUENCY OF CONSUMPTION						USUAL AMOUNT*		
		1-2 times per day	5+ times per week	2-4 times per week	1 time per week	Never or less than once per week		S	M	L
FRUITS										
Orange										
Pineapple										
Water melon										
Mango										
Apple										
Guava										
Banana										
Pawpaw										
Avocado pear										
Mixed fruit salad										
Other fruits										
OILS										
Vegetable oil (cooking oil)										
Red palm oil										
Palm kernel oil										
Coconut oil										
Soyabean oil										
Groundnut oil										
Shea butter oil										

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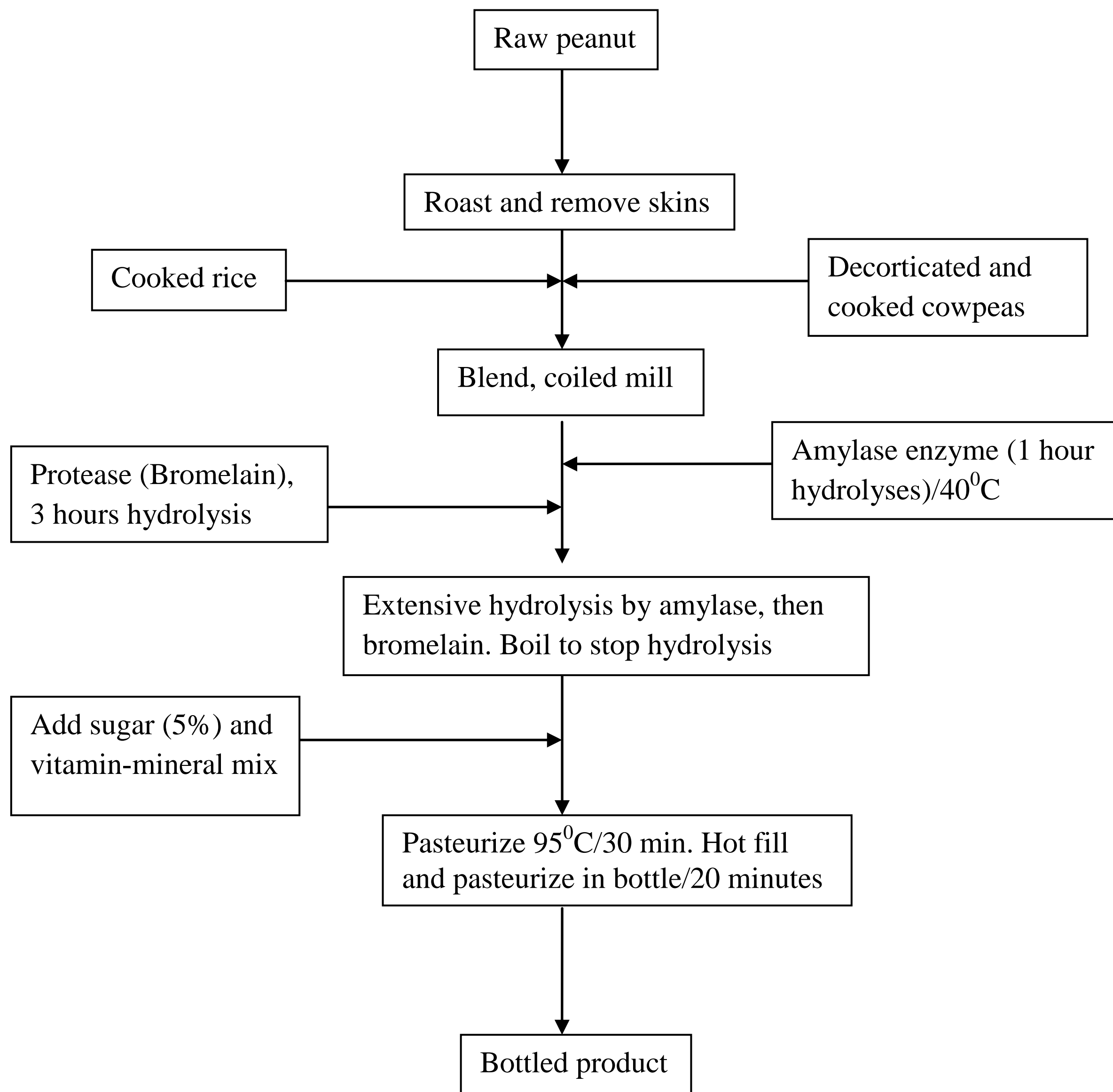
Appendix IV: Flow Diagram for Production of Ready-To-Use Supplementary Food



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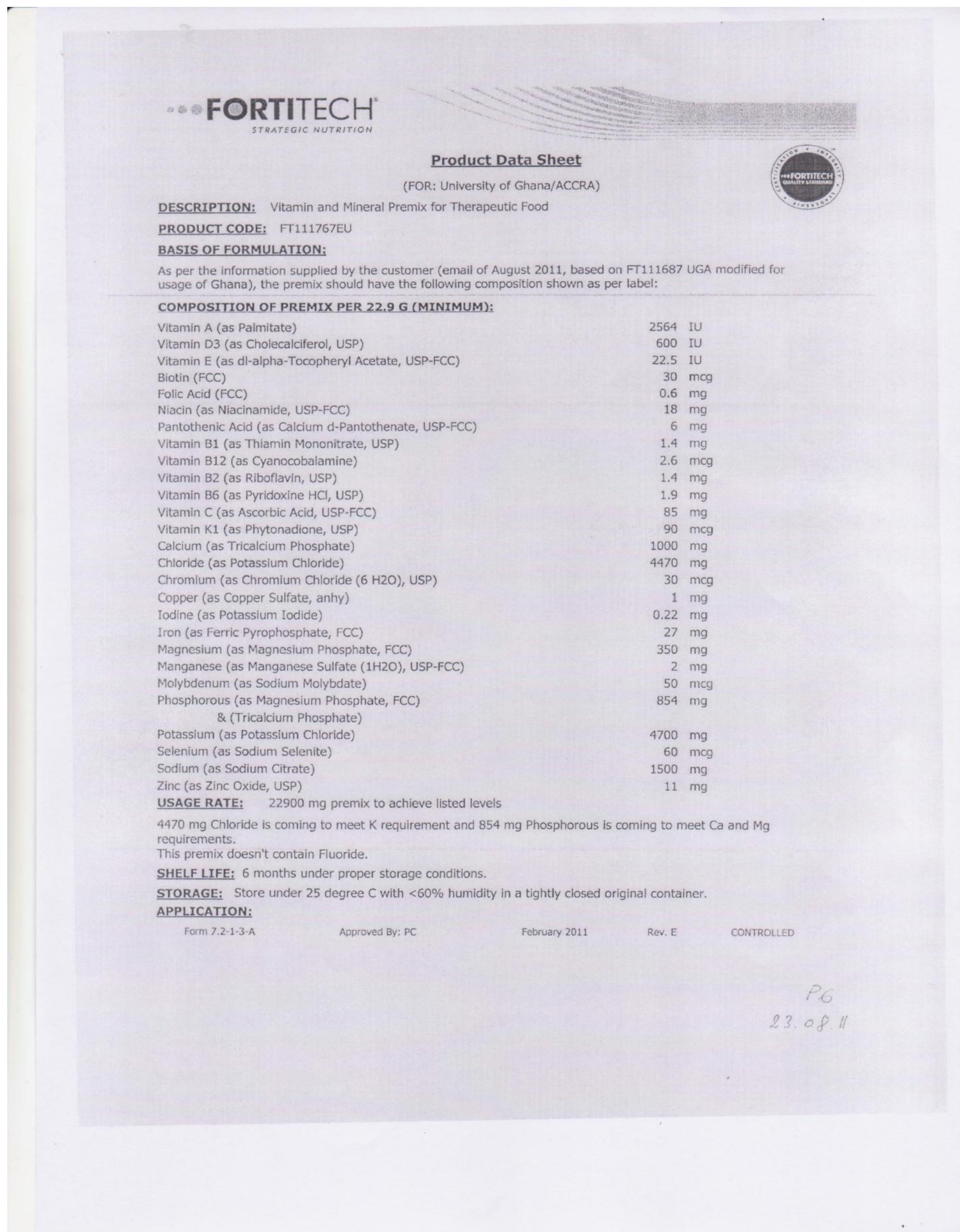
Appendix V: Vitamin-Mineral Mix Profile



FORTITECH
STRATEGIC NUTRITION

Product Data Sheet

(FOR: University of Ghana/ACCRA)

DESCRIPTION: Vitamin and Mineral Premix for Therapeutic Food

PRODUCT CODE: FT111767EU

BASIS OF FORMULATION:

As per the information supplied by the customer (email of August 2011, based on FT111687 UGA modified for usage of Ghana), the premix should have the following composition shown as per label:

COMPOSITION OF PREMIX PER 22.9 G (MINIMUM):

Nutrient	Amount	Unit
Vitamin A (as Palmitate)	2564	IU
Vitamin D3 (as Cholecalciferol, USP)	600	IU
Vitamin E (as dl-alpha-Tocopheryl Acetate, USP-FCC)	22.5	IU
Biotin (FCC)	30	mcg
Folic Acid (FCC)	0.6	mg
Niacin (as Niacinamide, USP-FCC)	18	mg
Pantothenic Acid (as Calcium d-Pantothenate, USP-FCC)	6	mg
Vitamin B1 (as Thiamin Mononitrate, USP)	1.4	mg
Vitamin B12 (as Cyanocobalamine)	2.6	mcg
Vitamin B2 (as Riboflavin, USP)	1.4	mg
Vitamin B6 (as Pyridoxine HCl, USP)	1.9	mg
Vitamin C (as Ascorbic Acid, USP-FCC)	85	mg
Vitamin K1 (as Phytonadione, USP)	90	mcg
Calcium (as Tricalcium Phosphate)	1000	mg
Chloride (as Potassium Chloride)	4470	mg
Chromium (as Chromium Chloride (6 H2O), USP)	30	mcg
Copper (as Copper Sulfate, anhy)	1	mg
Iodine (as Potassium Iodide)	0.22	mg
Iron (as Ferric Pyrophosphate, FCC)	27	mg
Magnesium (as Magnesium Phosphate, FCC)	350	mg
Manganese (as Manganese Sulfate (1H2O), USP-FCC)	2	mg
Molybdenum (as Sodium Molybdate)	50	mcg
Phosphorous (as Magnesium Phosphate, FCC) & (Tricalcium Phosphate)	854	mg
Potassium (as Potassium Chloride)	4700	mg
Selenium (as Sodium Selenite)	60	mcg
Sodium (as Sodium Citrate)	1500	mg
Zinc (as Zinc Oxide, USP)	11	mg

USAGE RATE: 22900 mg premix to achieve listed levels

4470 mg Chloride is coming to meet K requirement and 854 mg Phosphorous is coming to meet Ca and Mg requirements.
This premix doesn't contain Fluoride.

SHELF LIFE: 6 months under proper storage conditions.

STORAGE: Store under 25 degree C with <60% humidity in a tightly closed original container.

APPLICATION:

Form 7.2-1-3-A Approved By: PC February 2011 Rev. E CONTROLLED

PG
23.08.11

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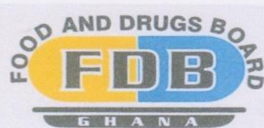
Appendix VI: Approval of Ready-To-Use Supplementary Food



Food & Drugs Board

Head Office
P. O. Box CT 2783,
Cantonments, Accra-Ghana
Tel: (+233-302) 233200, 235100
Fax:(+233-302) 229794, 225502
E-mail: fdb@fdbghana.gov.gh

CHIEF EXECUTIVE: DR. STEPHEN K. OPUNI

FDB/051/A11/1619

30th August, 2011

The Project PI,
Department of Nutrition and Food Science,
University of Ghana,
P.O. Box LG 134,
Legon-Accra.

Dear Sir,

RE: APPLICATION FOR APPROVAL OF A PEANUT BASED RECOVERY FOOD (RUTF) FOR RESEARCH STUDIES

This is to acknowledge receipt of your letter on the above subject.

Your indication to run a feeding program/study to test the efficacy of your newly formulated recovery food product on women of child bearing age has been considered.

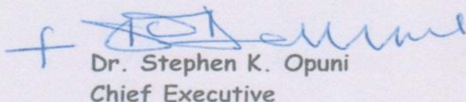
The targeted communities and age groups have also been noted.

You are hereby informed that the product has been approved for the research.

You are reminded however that the product must be submitted for registration prior to its introduction into the open market.

Thank you.

Yours faithfully,



Dr. Stephen K. Opuni
Chief Executive

University of Ghana http://ugspace.ug.edu.gh

Appendix VII: Ethical Clearance

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
Established 1979
A Constituent of the College of Health Sciences
University of Ghana

INSTITUTIONAL REVIEW BOARD

Phone: +233-302-916438 (Direct)
+233-289-522574
Fax: +233-302-502182/513202
E-mail: nirb@noguchi.mimcom.org
Telex No: 2556 UGL GH

Post Office Box LG 581
Legon, Accra
Ghana

My Ref. No: DF.22
Your Ref. No:

2nd November, 2011

ETHICAL CLEARANCE

FEDERALWIDE ASSURANCE FWA 00001824 **IRB 00001276**

NMIMR-IRB CPN 056/09-10 **IORG 0000908**

On 2nd November, 2011, the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB) at a full board meeting, reviewed and approved your revised protocol titled:

TITLE OF PROTOCOL : **The efficacy of peanut based food in improving the nutritional status of women**

PRINCIPAL INVESTIGATOR : **Dr. Matilda Steiner-Asiedu**

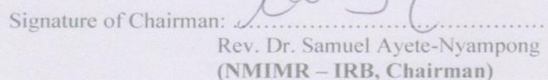
CO-PIs : **Dr. Firibu Kwesi Saalia & Matilda Anim-Fofie**

Please note that a final review report must be submitted to the Board at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to the IRB for review and approval prior to implementation.

Please report all serious adverse events related to this study to NMIMR-IRB within seven days verbally and fourteen days in writing.

This certificate is valid till 1st November, 2012. You are to submit annual reports for continuing review.



Signature of Chairman: ....
Rev. Dr. Samuel Ayete-Nyampong
(NMIMR – IRB, Chairman)

cc: Professor Alexander K. Nyarko
Director, Noguchi Memorial Institute
for Medical Research, University of Ghana, Legon

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Appendix VIII: Sample of Haematological Test Results

Parameter	No. 117	No. 118	No. 122	No. 134	No. 139	No. 141	No. 142	No. 150
Date	2012/08/29	2012/08/29	2012/08/29	2012/09/06	2012/08/29	2012/08/29	2012/08/29	2012/08/29
Time	21:41	21:44	21:43	17:30	21:21	21:20	20:57	21:32
Mode	WB	WB	WB	WB	WB	WB	WB	WB
WBC	5.0×10^3/µL	5.0×10^3/µL	7.5×10^3/µL	7.3×10^3/µL	6.1×10^3/µL	5.2×10^3/µL	5.6×10^3/µL	6.3×10^3/µL
RBC	4.99×10^6/µL	4.12×10^6/µL	4.30×10^6/µL	4.93×10^6/µL	3.87×10^6/µL	3.54×10^6/µL	4.29×10^6/µL	4.51×10^6/µL
HGB	13.2g/dL	11.1g/dL	13.5g/dL	13.2g/dL	11.1g/dL	12.7g/dL	12.4g/dL	10.6g/dL
HCT	42.1%	36.5%	41.8%	42.4%	34.4%	39.0%	38.3%	37.0%
MCV	- 84.4fL	88.6fL	97.2fL	86.0fL	88.9fL	+110.2fL	89.3fL	- 82.0fL
MCH	26.5pg	26.9pg	31.4pg	26.8pg	28.7pg	35.9pg	28.9pg	- 23.5pg
MCHC	31.4g/dL	- 30.4g/dL	32.3g/dL	31.1g/dL	32.3g/dL	32.6g/dL	32.4g/dL	- 28.6g/dL
PLT	184×10^3/µL	301×10^3/µL	176×10^3/µL	296×10^3/µL	195×10^3/µL	84×10^3/µL	297×10^3/µL	293×10^3/µL
LYM%	+ 68.0%	* 56.7%	+ 74.3%	53.6%	54.0%	+ 73.8%	+ 60.7%	* 53.7%
MXD%	T2 ---.-%	T2 ---.-%	T2 ---.-%	T2 ---.-%	T2 ---.-%	T2 ---.-%	T2 ---.-%	T2 ---.-%
NEUT%	T2 ---.-%	T2 ---.-%	T2 ---.-%	T2 ---.-%	T2 ---.-%	T2 ---.-%	T2 ---.-%	T2 ---.-%
LYM#	3.4×10^3/µL	* 2.8×10^3/µL	5.6×10^3/µL	3.9×10^3/µL	3.3×10^3/µL	3.8×10^3/µL	3.4×10^3/µL	* 3.4×10^3/µL
MXD#	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL
NEUT#	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL	T2 ---.-×10^3/µL
RDW_SD	40.6fL	43.9fL	48.1fL	46.1fL	47.0fL	+ 56.6fL	47.8fL	50.0fL
RDW_CV	11.6%	12.4%	12.5%	13.4%	13.9%	12.5%	13.4%	15.5%
PDW	11.9fL	10.8fL	14.3fL	10.6fL	10.6fL	12.2fL	9.7fL	12.5fL
MPV	9.5fL	- 8.9fL	10.4fL	8.7fL	- 8.6fL	9.2fL	- 8.2fL	9.3fL
P_LCR	22.9%	17.9%	27.5%	17.3%	15.5%	22.1%	13.3%	22.2%

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Appendix IX: Sample of Biochemical Test Results

CENTRAL REGIONAL HOSPITAL

P. O. BOX CT 1363 CAPE COAST
CAPE COAST, +233

Phone: 0332134010 - 14
Fax: 0332134016

Lab Dir: HANSEN-OWOO,EMMANUEL
CLIA ID:

Patient ID: R8	Patient Name: ., .	DOB/Age:	Sex: M	Fasting: ☐
Patient Location: LAB	Ordering Physician:	Attending Physician:		
Accession # 29394	Specimen ID: R8.	Collection Location: LAB	Collected: 9/5/2012 - 18:59 (LAB)	Released: 9/6/2012 - 07:29 (LAB)
PT Notes:				

Tests/Panels Ordered:

RESEARCH

● RESEARCH

Test Name	Normal Results	Abnormal Results	Expected Values	Units
Total Protein	7.9		6.2 - 8.5	g/dl
Albumin	4.4		3.4 - 5.0	g/dl
Total Protein (SI)	79.0		62.0 - 85.0	g/L
Globulin	3.5		2.0 - 4.8	g/dL
Albumin (SI)	44.0		34.0 - 50.0	g/L
Iron	94		65 - 175	ug/dl
Ferritin	211.0		6.9 - 323.0	ng/mL
CRP	24.			

Notified: ______ **By:** ______ **Date:** ______ **Time:** ______

Flag Legend: H = High L = Low H* = Critical High L* = Critical Low

., . **Printed on 9/6/2012 at 07:29** **Page 1 of 1**

Appendix X: Sample of Follow-up data collection form

ID	Age	Height	Waist	Wt	%BF	BMI	Sys	Dia	Pulse	Neck	Hip
154	20	5.04	73	113.4	30.4	21.8	115	80	89	33	89
179	42	5.36	81	129	33.5	22.5	147	93	74	31.2	90
159	27	5.08	85.5	153	41.9	29.1	126	89	98	35.5	105
205	43	5.38	91.4	173.2	40.9	30	124	75	74	33.2	112.5
168	26	5.33	90	157.8	41.4	27.7	114	67	93	33	107
165	49	5.05	94	145.4	41.5	27.9	127	80	81	32	101.5
160	27	5.28	92.5	169.6	44.5	30.3	104	74	76	33	109.5
180	23	5.22	81.5	128.4	32.9	22.6	109	65	79	32	98
183	36	5.04	82	119.4	32.9	22.9	115	82	81	31.5	92.7
158	23	5.07	104	172.4	44.5	32.8	112	82	58	33.5	110
204	28	5.56	84.3	159.2	39.6	26.1	109	66	75	32.8	109.8
170	30	5.29	89.5	153.6	40.4	27.2	121	76	81	33.4	101.6
169	50	5.20	72	100.8	23.5	18.4	110	65	87	29.5	85
186	39	5.18	96	189.6	47.4	35	127	85	77	36.2	114.5
184	37	5.12	81	114.4	20.6	21.4				33.5	88
206	25	5.12	83	132.6	34.4	23.9	105	58	82	33.3	94.7
163	31	5.08									
166	22	5.28	90.5	148.2	38	26.5	111	61	80	33	101
171	24	5.21	78	121.4	32	22.2	134	81	78	32	91
173	21	5.40	84	143	35.3	24.5	116	81	71	32	102
181	30	5.09	97	175.6	45.3	33.2	132	84	70	36.5	111.5
182		5.18									
157	37	5.12	72	105.0	26.2	19.7				35.8	88.5
175		5.30									
167	45	5.53	102	194.2	46.6	32.1	142	95	85	36	116
176	37	5.06	95	117.6	32.6	22.6				32.8	110.3
185	41	5.06	96	152.6	42.7	29.3	138	92	66	34	102
150	22	5.04	86	135.2	38.5	26.2	121	64	67	35.7	101
162	29	5.37	74	101.4	20.7	17.5	105	64	92	29	85.5
24	34	5.03	75	99.2	24.6	19.2	111	73	77	28.5	84
42	35	5.07	114.5	177.8	44.3	33.9	133	106	111	34	117.5
207	45	5.28	113.9	218	51.7	38.9	144	891	80	35.3	119
35	24	4.91									
14	20	5.15	91	142.4	39.2	26.5	102	74	70	31.3	105
19	19	5.40	85	167	37.6	28.7	124	82	84	34	107
43	31	5.30	79.5	112.2	27.3	19.9	151	79	104	30.4	89.7
208	22	5.17	88	139.2	37	25.7	118	80	88	31.5	98
195	49	5.32	76	103.2	23	18.1	123	85	84	31.3	84
08		5.08									
15		5.34									

Margin notes: 154 Travelled; 161 Travelled; Lost to follow up 182; 175 Travelled; Lost to follow up 176; 163.5; 8 lost to follow up 15

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Appendix XI: Sample of Field Feeding Check List

29/07 31/07 04/08 05/08 07/08 10/08 12/08 16/08 19/08 21/08

27/07 29/07 31/07 04/08 05/08 07/08 10/08 12/08 16/08 19/08

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Appendix XII: Frequency of Feeding of the Women in the Treatmet Group

ID	1st feeding	2nd feeding	Total feeding
14	18	18	36
19	18	18	36
24	18	18	36
30	11	0	11
35	18	18	36
40	11	0	11
42	18	18	36
43	18	18	36
49	18	18	36
55	18	18	36
61	18	18	36
67	18	18	36
71	18	18	36
82	7	0	7
84	18	0	18
89	18	0	18
90	18	6	24
92	18	18	36
96	18	18	36
97	18	18	36
99	18	18	36
100	18	18	36
104	18	18	36
105	18	18	36
106	18	18	36
109	18	18	36
111	18	18	36
113	18	0	18
117	18	0	18
118	18	18	36
134	18	18	36
137	18	0	18

ID	1st feeding	2nd feeding	Total feeding
139	18	18	36
141	18	18	36
142	18	18	36
146	18	18	36
154	18	18	36
158	18	18	36
159	18	18	36
160	18	18	36
162	18	0	18
165	18	18	36
168	18	18	36
169	18	18	36
170	18	18	36
179	18	18	36
180	18	18	36
183	18	18	36
184	18	18	36
186	18	18	36
195	18	18	36
199	18	0	18
203	18	18	36
204	18	18	36
205	18	18	36
206	18	18	36
207	18	18	36
208	18	18	36
210	10	0	10
213	18	14	32
216	18	18	36
218	18	18	36
219	18	18	36

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Appendix XIII: Total Protein Instruction Leaflet

TOTAL PROTEIN (BIURET) REAGENT

INTENDED USE
For the *in vitro* quantitative determination of Total Protein concentration in serum.

SUMMARY AND EXPLANATION [1]
Total Protein test system is a device intended to measure total protein (s) in serum or plasma. Measurements obtained by this device are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow as well as other metabolic or nutritional disorders.

METHODOLOGY
The color reaction of protein molecules with cupric ions, known as the Biuret color reaction, has been known since 1878. Since the Riegler[2] publications of 1914, several attempts have been made to stabilize the cupric ions in the alkaline reagent. Kingsley,[3,4] modified the procedure. In 1939 and 1942 to include the use of sodium potassium tartrate as a complexing agent. This procedure was later modified by Weichselbaum[5] and Gornall[6]. The present method is based on these modifications.

Principle

Protein + Cu^{++} —Alkali→ Colored Complex

Protein in serum forms a blue colored complex when reacted with cupric ions in an alkaline solution. The intensity of the violet color is proportional to the amount of protein present when compared to a solution with known protein concentration.

REAGENT COMPOSITION

Active Ingredients	Concentrations
Sodium Hydroxide	600mM
Cupric Sulfate	12mM
Potassium Sodium Tartrate	32mM
Potassium Iodide	30mM

pH 13.5 +/- 0.2 at 2-25°C

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Avoid Ingestion. DO NOT PIPETTE BY MOUTH. In case of ingestion drink large amounts of water and seek medical attention quickly.
3. Avoid contact with skin and eyes. The reagent contains sodium hydroxide which is corrosive. In case of contact with skin, flush with water. For eyes, seek medical attention.

REAGENT PREPARATION
Reagent is supplied ready to use.

REAGENT STORAGE
Store reagent at room temperature (15-25°C).

REAGENT DETERIORATION
The reagent should be a clear pale blue solution. Turbidity or the presence of a black precipitate indicates reagent deterioration and should not be used.

SPECIMEN COLLECTION AND STORAGE
1. Unhemolyzed serum is the specimen of choice.
2. Gross hemolysis will cause elevated results because of the released hemoglobin as well as the increase in background color.
3. Lipemic sera may cause elevated results.
4. Samples with bromosulfophthaleln (BSP) will result in falsely elevated results.[9]
5. Protein in serum is stable for one week at room temperature (18 - 25°C) and for at least one month refrigerated (2 – 8°C) when guarded against evaporation.[7]

INTERFERENCES
Studies to determine the level of interference for hemoglobin, bilirubin, and lipernia were carried out, the following results were obtained:

Hemoglobin: No significant interference (± 10%) from hemoglobin up to 100 mg/dL.
Biirubin: No significant interference (± 10%) from bilirubin up to 20.0 mg/dL.
Lipemia: No significant interference (+10%) from lipemia up to 196 mg/dL measured as triglycerides.
Young,[8] et al has reviewed a number of drugs and substances that may affect protein concentrations.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED:
1. A clinical chemistry analyzer capable maintaining constant temperature (37°C), and measuring absorbance at 540nm.
2. Iron-free deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Control and Calibrator materials, such as those provided by JAS Diagnostics.

ASSAY PROCEDURE
These instructions are to be used as a general guideline for adapting to select automated instruments. Refer to your specific JAS instrument application instructions available upon request.

System Parameters

Total Protein

TEMPERATURE:	37°C
WAVELENGTH:	540 nm
DIRECTION:	Increase
SAMPLE / RGT RATIO:	1 : 50
e.g. Sample Vol.	0.02 mL (20µL)
Reagent Vol.	1.0 mL
REACTION TIME:	5 Min

Procedure Notes
1. Final color is stable for 60 minutes at room temperature.
2. Serums with values above 15.0 g/dL should be diluted 1:1 with 0.9% saline, and the final answer multiplied by two.
3. The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements.

Calculation
Abs = Absorbance

$$\frac{\text{Abs. of unknown}}{\text{Abs. of standard}} \times \text{conc of std.} = \text{total protein (g/dL)}$$

Example:
Absorbance of unknown = 0.350
Absorbance of standard = 0.400
Concentration of standard = 8 g/dL

Then:
$$\frac{0.350}{0.400} \times 8 = 7.00 \text{ g/dL}$$

Limitations
1. Samples with values above 15.0 g/dL should be diluted 1:1 with 0.9% saline, re-run and result multiplied by two.
2. The Biuret procedure is not sensitive at low ranges (<1g/dL). Do not use for urine or spinal fluid.

CALIBRATION
Use an aqueous Total protein standard or an appropriate serum calibrator. Refer to appropriate instrument operator manual for recommended clibrator interval.

QUALITY CONTROL
The integrity of the reaction should be monitored by use of a two level control with known Total Protein values.

EXPECTED VALUES[9]
6.2 – 8.5 g/dL
1. The effect of posture, when blood is drawn, varies with the individual but recumbent values are usually lower than ambulatory. Differences may be as much as 1.2 g/dL.
2. It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE
Linearity:
When run as recommended the assay is linear from 0.1 – 15.0 g/dL.

Method Comparison:
Studies performed between this procedure and another procedure based on the same principle yielded the following results:

Number of sample pairs:	57
Range of samples:	1.6 – 13.7 (g/dL)
Correlation Coefficient:	0.9960
Slope:	1.067
Intercept:	0.01 (g/dL)

Precision:

Within Run	Level 1	Level 2	Level 3
Mean (g/dL)	4.65	7.45	13.60
S.D. (g/dL)	0.03	0.05	0.10
C.V. (%)	0.7	0.7	0.8

Total	Level 1	Level 2	Level 3
S.D. (g/dL)	0.05	0.09	0.15
C.V. (%)	1.1	1.1	1.1

Sensitivity:
A calibration factor of approximately 37.45 obtained, which is equivalent to a sensitivity of 0.027 Abs per g/dL.

Limit of Detection:
The limit of detection was found to be 0.1 g/dL.

* NOTE: Performance established on the Synchron CX.

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PI TPT2.JS04 REV:10/19/09

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Appendix XIV: Albumin Instruction Leaflet

INTENDED USE
This reagent is intended for the in vitro quantitative determination of albumin in human serum.

CLINICAL SIGNIFICANCE[1]
Observations of serum albumin level are useful as an aid in diagnosing disease states of the liver and kidneys. Moderate to large changes in the concentration of albumin have significant effects on the relative amounts of the bound and free concentrations of the ligands it carries: because free ligands are those that interact with tissue receptor sites and that can be excreted, albumin levels have important influences on the metabolism of endogenous substances such as calcium, bilirubin, and fatty acids and on the effects of drugs and hormones.
Hypoalbuminemia is very common in many illnesses and results in most instances from one or more of the following factors: 1) impaired synthesis, 2) increased catabolism, 3) reduced absorption of amino acids, 4) altered distribution which may sequester large amounts of albumin in an extravascular compartment, 5) protein loss by way of urine or feces.

METHODOLOGY[2,3]
At a controlled pH, bromcresol green forms a colored complex with albumin. The intensity of color at 630 nm is directly proportional to albumin content. The instantaneous initial absorbance is obtained as suggested by Webster

The method used by the JAS ALBUMIN reagent is based on that of Doumas

PRINCIPLE

BCG + Albumin —(Controlled pH)→ Green BCG/Albumin Complex

REAGENT COMPOSITION

Active Ingredients	Concentration
Bromcresol Green	0.25 mmol/L
Succinate Buffer	85 mmol/L
Surfactant	
PH 4.2 ± 0.1	

PRECAUTIONS
1. For *in vitro* diagnostic use only.
2. DO NOT pipette by mouth. Avoid contact with skin and eyes. If spilt, thoroughly, wash affected areas with water. For further information, consult the JAS Albumin Reagent Material Safety Data Sheet.
3. Reagent contains Sodium Azide as a preservative. This may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.
4. Do not use the reagent after the expiration date printed on the label.

REAGENT PREPARATION
Reagent is supplied ready to use.

STABILITY AND STORAGE
When stored at 2-25°C, the reagent is stable until the expiration date stated on the label.

REAGENT DETERIORATION
The reagent should not be used if:
1. The reagent is turbid.
2. The reagent fails to meet stated parameters of performance.

SPECIMEN COLLECTION AND HANDLING[3]
1. **Collection:** No special preparation of the patient is necessary and sample preservatives are not required.
2. **Sample Type:** Serum is the recommended specimen. Collect blood into the appropriate sample tube by venipuncture.
3. **Storage:** Albumin in serum is stable for one month at 2-8°C.

INTERFERENCES
1. Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:
   Hemoglobin: No significant interference from hemoglobin up to 300 mg/dL.
   Bilirubin: No significant interference from bilirubin up to 10.2mg/dL.
   Lipemia: No significant interference from lipemia up to 1020 mg/dL measured as triglycerides.
2. A number of drugs and substances may affect the accuracy of Albumin. See Young, et al.[4]

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
1. A clinical chemistry analyzer capable maintaining constant temperature (37°C) and measuring absorbance at 630 nm.
2. Deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Control and Calibrator materials such as those provided by JAS Diagnostics.

ASSAY PROCEDURE
These instructions are to be used as a general guideline for adapting to select automated instruments. Refer to your specific JAS instrument application instructions available upon request.

SYSTEM PARAMETERS

Temperature	37°C
Wavelength	630 nm
Assay Type	Endpoint
Direction	Increase
Sample/ Rgt Ratio:	1: 100
e.g. Sample Vol.	0.01 mL (10μL)
Reagent Vol.	1.0 mL
Incubation	less than 90 seconds

Procedure Notes:
1. The reagent and sample volumes may be altered proportionally to accommodate different instruments requirements.
2. The temperature of the reaction is not critical, however the temperature should be held constant.
3. Unit Conversion: g/dL x 10 = g/L

CALCULATIONS
(A = Absorbance)

$$\frac{A\ patient}{A\ standard} \times \text{Concentration of standard (g/dL)} = \text{Albumin (g/dL)}$$

Example:
A patient = 0.200
A (standard) = 0.190
Concentration of standard = 3.5 g/dL.

$$\frac{0.200}{0.190} \times 3.5 = 3.68 \text{ g/dL Albumin}$$

Limitations
Samples with values exceeding 6.0 g/dL should be diluted 1:1 with saline and re-run. The final answer should be multiplied by two.

CALIBRATION
A calibrator such as JAS Chemistry Calibrator (PN: CAL1-5), or an appropriate aqueous standard should be used to calibrate this test.

ALBUMIN (BCG) REAGENT

QUALITY CONTROL
The integrity of the reagent should be monitored by use of a two level control with known Albumin values such as JAS Chemistry Controls (PN: CON1-5 and CON2-5).

EXPECTED VALUE[1]
3.5 – 5.0 g/dL
It is highly recommended that each laboratory establish its own reference range.

PERFORMANCE *
Linearity:
When run as recommended the assay is linear from 0.0 to 6.0 g/dL

Method Comparison:
Studies performed between this procedure and a similar methodology yielded the following results:

Number of samples pairs:	57
Range of samples:	1.60 – 5.60 (g/dL)
Correlation Coefficient:	0.9768
Slope:	1.01
Intercept:	0.23 (g/dL)

Precision:

Within Run	Level 1	Level 2	Level 3
Mean (g/dL)	2.07	3.03	4.71
S.D. (g/dL)	0.04	0.05	0.05
C.V. (%)	2.2	1.8	1.2

Total	Level 1	Level 2	Level 3
S.D. (g/dL)	0.05	0.05	0.07
C.V. (%)	2.3	1.8	1.5

Sensitivity:
A calibration factor of approximately 9.74 obtained, which is equivalent to a sensitivity of 0.103 Δ Abs per g/dL.

Limit of Detection:
The limit of detection was found to be 0.0 g/dL.

* *NOTE: Performance established on the Synchron CX.*

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Appendix XV: CRP test kit Instruction Leaflet

CRP LATEX TEST KIT

Catalogue Number	Product Description
CRP/010	Test Kit 50
CRP/012	Test Kit 100

INTENDED USE
The Plasmatec CRP Latex test kit is for the qualitative and semi-quantitative estimation of C-Reactive Protein (CRP) in human serum samples.

WARNINGS AND PRECAUTIONS
For *in vitro* diagnostic use only
For professional use only
Health and Safety warnings:
All patient samples and reagents should be treated as potentially infectious and the user must wear protective gloves, eye protection and laboratory coats when performing the test.
Non disposable apparatus must be sterilised after use by an appropriate method. Disposable apparatus must be treated as biohazardous waste and autoclaved or incinerated.
Spillages of potentially infectious material should be absorbed and disposed of as above. The site of spillage must be sterilised with disinfectant or 70% alcohol.
Do not pipette by mouth.
Control reagents contain human serum. The human serum used has been tested and found to be negative for HIV, HCV and HbsAg. Nonetheless the reagent must be treated as potentially infectious and appropriate precautions should be taken when handling and on disposal. The product also contains aqueous buffer salts including sodium azide as preservative - see material safety data sheet
Analytical precautions:
Do not modify the test procedure.
Do not dilute or modify the reagents in any way.
Allow all reagents and samples to reach room temperature (18 - 30°C) before use.
Resuspend test and control cells gently but thoroughly.
Do not interchange reagents from different kit batches.

COMPOSITION
Kit contents:
Latex reagent sufficient for 50/100 slide tests (Yellow label). The latex reagent should be well shaken to ensure homogeneity.
Positive Control (Red label). This serum is human positive CRP serum. This reagent is ready for use and will give positive results when tested with the Plasmatec CRP latex test.
Negative Control (Blue label). This control is a negative CRP control serum. This reagent is ready for use and will give a negative result when tested with the Plasmatec CRP latex reagent.
10x Concentrate. Glycine Diluent Buffer (Green label). Add one part to nine parts distilled water before use. On dilution the diluent has a pH between 8.0 and 8.2.
Pipette/ Stirrers/ Reusable agglutination slide.
Pack insert.

STORAGE AND SHELF LIFE
Store reagents, upright at 2-8°C.
DO NOT FREEZE ANY OF THE REAGENTS
Do not use reagents after the stated expiry date.
Discard reagents if they become contaminated or do not demonstrate the correct activity with controls.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED.
Small glass or plastic test tubes / Serological pipettes

SPECIMEN AND SAMPLE PREPARATION
Use fresh serum obtained by centrifugation of clotted blood. The sample may be stored at 2-8? C for 48 hours before performing the test. For longer periods of time the serum must be frozen. Haematic, lipaemic or contaminated serum must be discarded.

PROCEDURE
Principle:
Latex particles coated with goat anti-human CRP antibodies are agglutinated when mixed with samples containing CRP.
CRP is a serum constituent originally defined by its ability to precipitate Pneumococcus C polysaccharide. Characteristically, CRP appears in the serum of individuals in response to various inflammatory conditions and tissue necrosis and disappears as the causative conditions subside.
It is routinely found in cases of bacterial infection, active rheumatic fever and many malignant diseases and is often seen in association with cases of rheumatoid arthritis, viral infections and tuberculosis. CRP has also been detected in patients following blood transfusions and surgical operations as well as in patients with burns, pemphigus vulgaris and other bullaous lesions.

Qualitative method
1. Allow each component to reach room temperature.
2. Gently shake the latex reagent to disperse the particles.
3. Place a drop of undiluted serum onto the circle of the test slide using the disposable pipettes provided.
4. Add one drop of the latex reagent next to the drop of serum.
5. Using the other end of the pipette (broad end) spread the reagent and serum sample over the entire area of the test circle.
6. Gently tilt the test slide backwards and forwards approximately once every two seconds for two minutes. Positive and negative controls should be included at regular intervals. Both are ready for use and do not require further dilution. At the end of the test rinse the test slide with distilled water and dry. Normal laboratory precautions should be maintained while handling patients samples.

INTERPRETATION OF RESULTS
Presence of agglutination indicates a level of CRP in the sample equal or > 6mg/l.
The lack of agglutination indicates a CRP level < 6mg/l in the sample.

Semi-quantitative determination
The semi-quantitative test can be performed in the same way as the quantitative test using dilutions of the serum in saline, phosphate buffered saline or glycine saline as follows:-

Dilutions	1/2	1/4	1/8	1/16
Sample serum	100? l	-	-	-
Saline	100? l	100? l	100? l	100? l
	?	100? l ?	100? l ?	100? l
Volume of sample	50? l	50? l	50? l	50? l
6xN°. Of dilution	6x2	6x4	6x8	6x16
Mg/I.U./ml	12	24	48	96

Normal Levels :- Adults < 6mg/l

RESULTS
The titre is expressed as the reciprocal of the highest dilution showing macroscopic agglutination: e.g. if this occurs in dilution 3, the titre is 48.

INTERPRETATION OF RESULTS
The elevation of CRP levels above normal indicates tissue damage, inflammation, or both with great reliability.
The Plasmatec CRP latex has been standardised to detect serum CRP levels at or above 6?g/ml, which is considered the lowest concentration of clinical significance.
The regular monitoring of CRP levels is often used as a means of assessing disease activity and of guiding therapy.
CRP determination is considered to be a greater practical significance than any other indicator of inflammatory disease. The erythrocyte sedimentation rate (ESR) for example, may become elevated as a result of non-inflammatory conditions. In these circumstances inflammatory disease may be excluded if CRP is absent.

PERFORMANCE CHARACTERISTICS
Analytical sensitivity: 6 (5-10) mg/L
Prozone effect: No prozone effect was detected up to 1600 mg/L
Diagnostic sensitivity: 95.6%
Diagnostic specificity: 96.2%

LIMITATIONS OF THE METHOD
High CRP concentration samples may give negative results (prozone effect). Re-test the sample again using a sample drop of 20 µL.
Haemoglobin (10 g/L), bilirubin (20 mg/dL) and lipemia (10 g/L), do not interfere. Rheumatoid factors (100 IU/mL), interfere. Other substances may interfere[7].
Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

INTERNAL QUALITY CONTROL
Control sera provided should be used to verify the test procedures.

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