THE EFFICACY OF IRON-FORTIFIED WEANING FOOD IN IMPROVING IRON STATUS OF INFANTS AND YOUNG CHILDREN

BY

THEODOSIA ADOM

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT FOR THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN NUTRITION.

DEPARTMENT OF NUTRITION AND FOOD SCIENCE
UNIVERSITY OF GHANA, LEGON

SEPTEMBER 2000

DECLARATION

I declare that, with the exception of references cited, which have being duly acknowledged the work described in this thesis was carried out by me under the supervision of Dr. Matilda Steiner-Asiedu of the Department of Nutrition and Food Science, University of Ghana, Legon.

THEODOSIA ADOM
(Student).

DR. MATILDA STEINER-ASIEDU
(Supervisor)

DEDICATION

Dedicated to all the children who particapated in the research and to

David

Miriam

Miranda.

ABSTRACT

Iron deficiency is the most widespread nutritional problem in the world. It is common throughout childhood and prevalent among 6 – 12 month olds or 1 – 2 year olds when 70% and 50% of the respective requirements arise from the rapid rate of tissue growth.

Despite large-scale iron supplementation programmes, the prevalence of iron deficiency anaemia remains high especially in developing countries. Low iron intake and poor absorption or bioavailability of iron in the diet can cause negative iron balance in children.

This study involved the use of cowpea-fortified fermented maize flour as a vehicle for iron fortification. The efficacy of iron-fortified maize-cowpea flour in improving iron status was investigated in fifty-six (56) infants and young children aged between 6 and 18 months in two peri-urban communities. In addition the effect of treatment on physical growth was measured. Subjects were randomly assigned to one of the two project foods: (i) iron-fortified food (18.4mg Fe/100g; n=29) and (ii) non-iron fortified food (7.5mg Fe/100g; n=27). The foods were fed daily for six months. Haemoglobin concentrations, serum iron, total iron-binding-capacity, weight, length and mid upper arm circumference (MUAC) were measured at baseline and at the end of the study.

The study revealed that about 70% of the subjects were anaemic (defined as haemoglobin concentration < 11g/dl) at baseline. Prevalence of malnutrition (defined as Z-scores < - 2 SD for weight-for-length/height, length/height-for-age, weight-for-age, and MUAC < 13.5 cm) was low; stunting was about 11% and wasting 20%. At the end of the intervention period, there was a decrease in the prevalence of anaemia in the iron-fortified group. Significant differences were observed in gain in haemoglobin concentration (1.08 ± 1.43 compared with –0.40 ± 1.72g/dL, p= 0.0009), length gain (5.11 ± 2.13 compared with 3.73 ± 2.18cm, p= 0.020) and MUAC gain (0.37 ± 0.93 compared with –0.004 ± 0.71, p=0.048) between the iron-fortified and non-iron-fortified groups respectively. There however were no significant differences in serum iron, total iron-binding-capacity, plasma transferrin saturation and weight gain.

It could be concluded that the iron fortified maize-cowpea flour is efficacious in controlling anaemia, and in improving linear growth.

ACKNOWLEDGEMENT

I am deeply indebted to my supervisor, Dr. Matilda Steiner-Asiedu of the Department of Nutrition and Food Science, University of Ghana, Legon for her guidance, helpful suggestions and constructive scrutiny of this work. My deepest appreciation to all my lecturers especially Dr. Esther Sakyi-Dawson, Dr. Anna Lartey, and Mr. Tayie for their constructive criticism and suggestions.

I am grateful to Mr. Addo of Noguchi Memorial Institute for Medical Research, Legon, who helped with the laboratory work, also to the technicians and drivers especially Mr Korankye and Eddie Ashitey of Department of Nutrition and Food Science. I am indebted to Dr. Johnson and Mr Armah, both of Food Research Institute (Pilot Plant), the technicians of the Soil Science Department who helped in diverse ways to make this research possible.

The study was facilitated by financial support from the United States Agency through the Bean/Cowpea Collaborative Research Support Programme (CRSP) for International Development. I wish to express my gratitude to CRSP for the assistance and to Shell Foundation for sponsoring my MPhil Programme. The cooperation of the chiefs and people of Otinibi and Danfa are also appreciated.

Finally, I appreciate the assistance and encouragement of my family that made my study possible. I am thankful to Cynthia Gadegbeku, Richard Banson and Gloria Adu-Aboagye for their immense contributions and to all my friends who contributed either directly or indirectly towards my study.

TABLE OF CONTENT

DECLARATION i

DEDICATION ii

ABSTRACT iii

ACKNOWLEDGMENT v

TABLE OF CONTENT vi

LIST OF TABLES xii

LIST OF FIGURES xiii

LIST OF APPENDICES xiv

1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION 1

1.2 STATEMENT OF THE PROBLEM 3

1.3. OBJECTIVES 4

1.3.1 Main Objectives 4

1.3.2 Specific Objectives 4

2.0 LITERATURE REVIEW 5

2.1. `DIETARY FACTORS AFFECTING IRON ABSORPTION AND BIOAVAILABILITY 5

2.1.1 Form of Iron 5

2.1.2 Other Dietary Constituents 6

2.1.3 Dietary Iron Content 6

2.1.4 Nutrient Interactions 7

2.2 ASSESSMENT OF IRON STATUS 8
2.2.1 Assessment Tools 9
2.2.1.1 Biochemical Assessment 9
2.2.1.2 Dietary Assessment 11
2.3 PREVALENCE OF IRON DEFICIENCY ANAEMIA 12
2.4 IRON REQUIREMENTS 13
2.5 CAUSES OF IRON DEFICIENCY ANAEMIA 14
2.5.1 Diet 14
2.5.2 Malaria 15
2.5.3 Sickle-cell disease 17
2.5.4 Low-birth-weight babies 17
2.5.5 Parasitic worm infestation 18
2.6 CONSEQUENCES OF IRON DEFICIENCY ANAEMIA 18
2.6.1 Compromised development in young children 19
2.6.2 Child growth failure and poor physical development 19
2.6.3 Pregnancy outcome 19
2.6.4 Increased morbidity 20
2.6.5 Lowered physical activity, mental concentration, and productivity 20
2.6.6 Other consequences 21
2.7 IRON IN INFANT AND YOUNG CHILD NUTRITION 22
2.7.1 The role of iron in complementary feeding 22
2.7.2 Infant feeding practices in Ghana 23
2.7.3 Efficacy trials of iron – fortified foods 24
2.7.4 Iron in human growth 26

3.0 METHODOLOGY 28

3.1 RESEARCH DESIGN AND SETTINGS 28

3.2 STUDY POPULATION 28

3.3 SAMPLE SIZE CALCULATION 28

3.4 PREPARATION OF TEST FOOD 29

3.5 STUDY GROUPS 30

3.6 MODE OF DISTRIBUTION OF TEST FOOD 30

3.7 BIOCHEMICAL ASSESSMENT 30

3.7.1 Haemoglobin Concentration 30

3.7.2 Serum iron and Unsaturated iron – binding capacity (UIBC) 31

3.7.2.1 Principle 31

3.7.2.2 Reagents and conditions 31

3.7.2.3 Procedure for serum iron 32

3.7.2.4 Procedure for UIBC 32

3.7.2.5 Calculation 32

3.7.3 Total iron – binding capacity (TIBC) 33

3.7.4 Transferrin saturation (TS) 33

3.8 DIETARY ASSESSMENT 34

3.9 ANTHROPOMETRIC MEASUREMENTS 34

3.10 CHEMICAL ANALYSIS 35

3.10.1 Wet ashing of the test foods 35

3.11 DATA ANALYSIS 36

4.0 RESULTS........ 37

4.1 BACKGROUND INFORMATION 37

4.1.1 Maternal and household characteristics 37

4.1.2 Maternal education 38

4.13 Maternal employment 38

4.2 BASELINE CHARACTERISTICS OF STUDY CHILDREN 47

4.2.1 Infant feeding practices 43

4.2.2 Effect of maternal education and employment on infant feeding practices 48

4.2.3 Child care practices 49

4.2.4 Immunisation and health 49

4.3 DIETARY DATA 51

4.3.1 Dietary intakes of study children at baseline 51

4.3.2 Dietary patterns of children 52

4.3.3 Energy and nutrient composition of diets 54

4.3.4 Energy and nutrient intake of test foods 54

4.3.4 Relationship between dietary intake and iron status 66

4.4 BIOCHEMICAL DATA 55

4.4.1 Anaemia prevalence and iron status 55

4.5 ANTHROPOMETRIC DATA 57

4.5.1 Anthropometric indices and nutritional status of children 57

4.6 EFFECT OF INTERVENTION ON GROWTH 58

4.7 EFFECT ON INTERVENTION ON ANAEMIA AND IRON STATUS 60

5.0. DISCUSSION 65

5.1. ANTHROPOMETRY 68

5.2. IRON DEFICIENCY AND ANAEMIA AT BASELINE 67

5.3. EFFECT OF INTERVENTION ON BIOCHEMICAL MEASURES 69

5.4. EFFECT OF INTERVENTION ON GROWTH 72

6.0 CONCLUSIONS AND RECOMMENDATIONS 78

6.1. CONCLUSIONS 75

6.2. RECOMMENDATIONS 76

REFERENCES 77

APPENDICES 95

LIST OF TABLES

Table 2.1 Examples of diets with estimated overall bioavailability ... 7

Table 2.2 Haemoglobin and Haemotocrit levels below which anaemia is judged present ... 10

Table 2.3 Median requirements for absorbed iron (mg/day) at different stages of the life cycle ... 13

Table 3.1 Interpretative guide of characterising anaemia ... 34

Table 3.2 Interpretative guide of classifying malnutrition ... 35

Table 4.1 Educational level of female guardian (n=56) ... 38

Table 4.2 Baseline characteristics and infant feeding practices of study children (n=56) ... 42

Table 4.3. Effect of maternal education and employment on infant feeding practices ... 66

Table 4.4 Mean daily dietary intakes of study children at baseline (n=56) ... 51

Table 4.5 Nutrient composition of diets per kilogramme dry weight ... 54

Table 4.6 Ranges of energy, protein and iron intake from test food ... 55

Table 4.7 Baseline biochemical measures of iron status ... 56

Table 4.8 Classification of anaemia at baseline using blood haemoglobin levels ... 56

Table 4.9 Baseline anthropometric indices of study children in Intervention groups ... 57

Table 4.10 Nutritional status of children after 6 – months in intervention period ... 58

Table 4.11. Nutritional status of children after 6 – months using MUAC (n=56) ... 58

Table 4.12. Effect of intervention on growth of pre-school children after 6-months intervention period (n=56) ... 60

Table 4.13. Effect of intervention on biochemical measures (n=56) ... 61

Table 4.14. Classification of anaemia at the end of intervention (n=56) ... 64

LIST OF FIGURES

Fig. 4.1. Occupation of female guardians (n=56) ... 40

Fig. 4.2. Age at which breast milk was supplemented ... 44

Fig. 4.3. Reasons for supplementing breast milk ... 45

Fig. 4.4. Reasons given for cessation of breast feeding ... 47

Fig. 4.5. Health symptoms of study children one month prior to start of intervention ... 50

Fig. 4.6. Frequency of feeding some common foods ... 53

Fig. 4.7. Mean haemoglobin over intervention period ... 62

Fig. 4.8. Anaemia prevalence over intervention period ... 63

APPENDICES

APPENDIX I Questionnaire and consent form 97

APPENDIX II Results of biochemical analysis 104

APPENDIX III Results of anthropometric assessment 106

APPENDIX IV Analytical settings for dietary iron 108

1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

Iron deficiency is the most widespread nutritional problem in the world. Two thousand million people are at risk (ACC/SCN, 1991). Two thirds of children and women of childbearing age in most developing countries are estimated to suffer from iron deficiency; one third of them have the more severe form of the disorder, anaemia (ACC/SCN, 1991). The prevalence of iron deficiency anaemia remains high especially in developing countries despite large-scale iron supplementation programmes.

Unlike classical nutritional diseases, iron deficiency is found in all physiological groups and in all societies, developing and industrial alike. In the United States, Japan and Europe, between ten and twenty percent of women of childbearing age are anaemic (Scrimshaw, 1991).

Malaria, dietary factors, parasitic worm infestation and other infections are the causes of iron deficiency anaemia in the world. In developing countries however, dietary factors are the major causes of iron deficiency anaemia (Scrimshaw, 1991; WHO/ICC/IDD, 1998). Iron in these diets comes predominantly or totally from cereals and vegetables. For the poor, meat is expensive and consumed in small quantities or not at all. Iron deficiency and anaemia affect the majority of individuals in such populations.

Iron deficiency is not life threatening, but the more severe form of anaemia is, and can have detrimental effects on work performance, learning ability and resistance to infection. These consequently result in compromised development in young children and child growth failure (ACC/SCN, 1991).

The International Community has suggested a combination of strategies that will have the required impact on a target population (ACC/SCN, 1991). These are:

(i) Short-term intervention such as iron supplementation. Iron supplementation has been shown to be a successful intervention, but problems of limited outreach and sustainability as well as poor compliance constrain its implementation on a large-scale.

(ii) Medium- to long-term intervention such as iron fortification. This is geared towards improving the nutritional quality of the food supply. It has been found effective and feasible when the vehicle chosen is widely consumed by the population at risk.

(iii) Long-term intervention such as dietary modification. This offers a more permanent alternative, but will take a long time before that objective is realised.

Iron fortification of foods has been recommended as the optimal approach to reducing the high prevalence of iron deficiency anaemia (IDA) in developing countries. Technology for fortifying wheat flour and bread is well established and the use of these vehicles has probably had a significant impact on iron status in Western countries (Cook and Reusser 1983).

Trials conducted during the past two decades have shown that fortifying a broad range of foods and condiments with iron could lead to significant improvement in iron status, particularly in iron deficient groups. For example, refined sugar in India and Guatemala (Nadiger *et al.*, 1980; Viteri *et al.*, 1981); fish sauce and paste in the Philippines and fish-based condiments in Thailand (Garby and Areekul, 1974) and curry powder in South Africa (Ballot *et al.*, 1989).

1.2 STATEMENT OF THE PROBLEM

Iron deficiency is a common problem throughout childhood (Dallman *et al.*, 1980). In the first two months of life, there is minimal dietary iron absorption and stores are mobilised to meet iron requirements (FAO/WHO, 1988). Thereafter dietary iron absorption becomes increasingly significant, and by about four- to- six months of age, iron stores have been significantly depleted. Since breast milk alone will not suffice to meet the infant's requirement after this age, the diet becomes critically important. Iron deficiency among infants is prevalent among 6- 12 months and 1- 2 year olds when 70 percent and 50 percent of the respective requirements arise from the rapid rate of tissue growth (FAO/WHO, 1988). Low iron intake and poor absorption or bioavailability of iron in the diet can cause negative iron balance in children.

In practice, the first foods that infants consume in most developing countries tend not to be favourable for iron absorption. These include cereal and pulse based gruels because they are readily available and culturally acceptable staple foods. However these are rich in inhibiting phytate. Other foods given include cow's milk which contains low concentration of iron and high levels of calcium, phosphorus and proteins that form insoluble complexes with iron in the intestines thereby reducing iron absorption (Hurrell *et al.*, 1989). In many cultures, meat is introduced relatively late or is not affordable. Moreover, the intake recommended from 6-12 months can only be obtained through the use of iron-fortified formulas or iron-enriched infant cereals (Guthrie, 1986).

In Ghana, West Africa, the traditional weaning food fed to infants is a fermented maize porridge called *koko,* which has been shown to have low energy and nutrient density (Eyeson *et al.*, 1975). The development of cowpea-fortified food and its use in intervention studies has been well-documented (Sefa-Dedeh *et al.*, 1997). Sosi (1999) investigated the effects of iron fortification and fermentation on product characteristics of cowpea-fortified fermented maize-flour. Iron fortification did not affect colour and taste and the product was acceptable.

1.2 OBJECTIVES

1.2.1. Main Objective

To investigate the efficacy of iron-fortified maize-cowpea flour in reducing the prevalence of iron deficiency and anaemia among infants.

1.2.2. Specific Objectives

1. To compare iron nutrition status of infants and young children on iron-fortified maize-cowpea flour to those on non iron-fortified maize-cowpea flour using the following indices:

- Serum iron
- Haemoglobin concentration
- Total iron-binding capacity
- Plasma transferrin saturation

2. To compare the growth of the two groups of children using selected anthropometric indices namely length, weight and mid-upper arm circumference.

2.0 LITERATURE REVIEW

2.1 DIETARY FACTORS AFFECTING IRON ABSORPTION AND BIOAVAILABILITY

2.1.1 Form of Iron

Dietary iron exists as either haem iron (in meat, poultry and fish) or non-haem iron (milk, eggs, cereals, vegetables and fruits). The source of dietary iron strongly influences the efficiency of its absorption. The amount of iron absorption varies from less than 1% to more than 20% depending on the food (Monsen *et al.*, 1978).

Haem iron is twenty to thirty percent absorbed in normal individuals and this process is relatively unaffected by dietary and physiological variables (FAO/WHO, 1988). However, only ten to fifteen percent of dietary iron is in the haem form, even in diets where meat consumption is high.

Absorption of non-haem iron is governed by its solubility in the upper part of the small intestine (Charlton and Bothwell, 1983). The absorption may be as low as five percent in many cereal-based diets commonly consumed in developing countries. Some non-haem iron derives from contamination during food preparation; for example, by iron cooking pots in fermentation or low pH environments.

2.1.2 Other Dietary Constituents

Enhancers of iron absorption, such as ascorbic acid and citric acid form soluble complexes with iron thereby preventing precipitation and polymerisation. Ascorbic acid reduces ferric iron to ferrous iron, which is better absorbed at pH values greater than 3 found in the duodenum and small intestine. Citrate is present in fruits and vegetables (Hazell and Johnson, 1987). Haem iron, usually within meat, and meat protein also enhance absorption of non-haem iron.

Inhibitors such as phytate and polyphenols, act through forming large insoluble polymers, thus decreasing the overall solubility of iron in the meal. Tea is the most potent inhibitor of dietary iron. Regular consumption has been proposed as a means of decreasing iron absorption in patients with iron overload. Tannins extracted from tealeaves produce similar inhibition. Phytate inhibits trace mineral absorption in general, and is the main cause of the inhibitory action of bran on iron absorption (Hallberg *et al.*, 1987). Coffee inhibits iron absorption in a dose-dependent manner by rendering a large proportion of dietary iron unavailable. Table 2.1 shows examples of diets with estimated overall bioavailability.

2.1.3 Dietary Iron Content

This is also important in iron absorption and bioavailability. The iron dose is generally inversely related to the percentage that is absorbed, both with dietary iron and iron supplements (Hahn *et al.*, 1951). The higher the level of iron to which the intestinal mucosal cells have been exposed, the lower the relative efficiency of iron absorption.

However, provided that the iron is in an assimilable form, the actual amount absorbed will rise progressively with increasing dietary intake (Fairweather-Tait, 1995).

Table 2.1: Examples of diets with estimated overall bioavailability

Bioavailability of Iron	Typical diet
Low (5% absorption)	Cereal-based, roots or tubers, and legumes with negligible meat, fish, or ascorbic acid-rich foods.
Intermediate (10% absorption)	Cereal-based, roots or tubers with negligible quantities of food of animal origin or containing ascorbic acid (higher than for the low diet); or a diet with still higher levels of animal source foods or ascorbic acid but also large amounts of tea or coffee consumed with meals.
High (15% absorption)	Diversified diet containing generous quantities of meat, poultry, and fish; or foods containing high amounts of ascorbic acid.

Source: FAO/WHO (1988).

2.1.4 Nutrient Interactions

These are important considerations with respect to iron absorption and utilisation. For example, dietary calcium inhibits absorption of both haem and non-haem iron possibly through inhibition of iron transport (Hallberg *et al.*, 1992; Gleerup *et al.*, 1995). Folate and vitamin B_{12} modify iron utilisation through their role in nucleic acid synthesis and red blood cell production (Velez *et al.*, 1966). Vitamin A reduces the inhibition of iron absorption by phytate and polyphenols (Layrisse *et al.*, 1998), and it is involved in iron store mobilisation (Mejia and Arroyave, 1982). Riboflavin acts synergistically with iron in the catalysis of several steps in iron metabolism. Iron absorption, mobilisation of intracellular iron and retention of absorbed iron are all sensitive to changes in riboflavin status (Powers *et al.*, 1983).

2.2 ASSESSMENT OF IRON STATUS

At least two thirds of the body iron is functional iron, mostly haemoglobin within circulating red blood cells, with some as myoglobin in muscle cells and part of iron-containing enzymes. Most of the remaining body iron is storage iron (existing as ferritin and haemosiderin) that serves, as a deposit to be mobilised when needed. There are three main stages in the reduction of body iron (WHO/UNICEF/UNU, 1996).

(i) **Iron depletion** is the first stage of the decrease of body iron stores. It is measured by a reduction in serum ferritin concentration and represents a borderline state of iron nutrition. Any further reduction is associated with a decrease in the level of functional compounds such as haemoglobin.

(ii) **Iron deficiency erythropoiesis** develops only in the second stage of iron depletion. It is characterised by biochemical changes that reflect a lack of sufficient iron for normal production of haemoglobin and other essential iron compounds. There is decrease in transferrin saturation, or increases in serum transferrin receptor and erythrocyte protoporphyrin. At this time haemoglobin synthesis starts to become impaired and haemoglobin concentrations fall yet there is no frank anaemia. This stage is often described as iron deficiency without anaemia.

(iii) **Iron deficiency anaemia** is the severe degree of iron deficiency and ensues if the haemoglobin concentration falls below a statistically defined threshold lying at two standard deviations below the median of a healthy population of the same age, sex and state of pregnancy (WHO/UNICEF/UNU, 1996). By this stage the restriction in haemoglobin production is severe enough to lead to the distortion of red cells, with microcytosis and hypochromia. Iron deficiency anaemia (IDA) represents one extreme state of the iron deficiency spectrum. For every case of IDA found in a population, there are at least two cases of iron deficiency (Yip 1994; WHO/UNICEF/UNU, 1996)

2.2.1 Assessment Tools

2.2.1.1 Biochemical Assessment

The most common method for assessing iron status is the measurement of haemoglobin or haematocrit levels as a measure of anaemia. Although anaemia is not a specific indication of iron deficiency, given that other causes are possible, a population with a high prevalence of anaemia is likely to have a high prevalence of iron deficiency (Yip *et al.*, 1996). The determination of haemoglobin concentration can be done in the field by using a Hemocue based on laboratory cyanmethemoglobin method of assessment. The Haematocrit or packed cell volume method is also simple, although more variable than haemoglobin assessment, and can be done using a hand-cranked microcentrifuge. Critical levels of haemoglobin and haematocrit with regard to age, sex, and physiologic status are shown in Table 2.2.

Serum ferritin is the most specific biochemical test indicator of total body iron stores and is a useful indicator of iron status where prevalence of IDA is low (WHO/UNICEF/UNU, 1996). However, serum apoferritin is an acute-phase reactant protein that is elevated in response to infection- thus constraining interpretation in environments where the incidence of infection is high (WHO/UNICEF/UNU, 1996). Serum ferritin is present in small quantities. It is measured by radio immuno-assay or by enzyme-linked immuno-assay. An advantage of measuring the serum ferritin level is that it permits an evaluation of Fe status not only in persons with deficiency, but also in those with excess. At all ages, a serum ferritin value of less than 10-12μg/L indicates a depletion of Fe stores (DeMaeyer *et al.*, 1989).

Table 2.2: Haemoglobin and Haemotocrit levels below which anaemia is judged present.

Group	Critical Level	
	Haemoglobin (g/dl)	Haematocrit (%)
Children		
6 months -5years	11.0	33.0
5 -11 years	11.5	34.0
12 –13 years	12.0	36.0
Men	13.0	39.0
Women		
Non pregnant	12.0	36.0
Pregnant	11.0	33.0
Severe anaemia	7.0	
Very severe (Life threatening)	4.0	

Source: WHO/UNICEF/UNU (1996), adapted from WHO (1968).

Serum transferrin saturation is an equally good indicator of body iron stores. It measures the iron supply to the erythroid bone marrow. Transferrin saturation is calculated by expressing total serum iron as a percentage of total iron binding capacity. Normal range is 30 – 40% (Yip *et al.*, 1984.). Decreasing transferrin saturation values occur with iron deficiency.

Serum iron content reflects the number of iron atoms bound to the iron transport protein transferrin. Each molecule of transferrin can be bound to one or two atoms of iron, although rarely are both binding sites occupied. The colometric assay of serum iron employs ferrozine as the chromogen. Iron deficiency results in a fall in serum iron levels. Low serum iron levels also occur in conditions such as infections, inflammation, and malignancy arising from defects in the release of iron from the reticuloendothelial cells. High serum iron levels occur in conditions such as haemochromatosis, haemolytic anaemia, liver damage and excessive absorption of iron transferrin among others (Yip *et al.*, 1984.). Both diurnal and day-to-day variations occur in serum iron concentration.

Total iron binding capacity (TIBC) is related to the total number of free-iron binding sites on transferrin. In iron deficiency, there is an increase in the number of free iron-binding sites. TIBC is therefore elevated. TIBC also tends to be above normal in conditions associated with erythropoiesis (example haemolysis and polycythemia). TIBC is less subject to diurnal effects.

2.2.1.2 Dietary Assessment

The measurement of iron intake through dietary assessment is only useful as a complement to the direct measurement of haemoglobin concentrations because iron intake and iron nutritional status are usually poorly correlated when bioavailability and host factors are not accounted for. Dietary assessment may, however be useful for infants who consume few other foods in a predominantly milk-based diet.

2.3 PREVALENCE OF IRON DEFICIENCY ANAEMIA

Over two thousand million people worldwide are iron deficient, with a total prevalence estimated at about 40% of the world's population (WHO, 1991). Globally, prevalence among various sub-groups are estimated at 51% for pregnant women, 48% for infants and 1-2 year old children, 35% for non pregnant women, and 25% for pre-school children. According to WHO (1996), overall rates for IDA in developing countries are 26% for men and nearly 50% for women and children. Ten percent of infants in industrialised countries and 30 - 80% of those in developing countries are anaemic at one year of age (WHO, 1996).

The IDA situation in Ghana is no different. A national survey conducted between 1979-1983 listed IDA to be a problem among pre school children, pregnant and lactating women. IDA caused 3.4% of all infant deaths and anaemia alone contributed 6.8% of under-five mortality (The Child Cannot Wait, 1992) during the period. The situation does not seem to have changed much. Surveys by the Department of Nutrition and Food Science in several communities in the Greater Accra Region since 1985 have consistently found high prevalence of anaemia among all age groups and in both sexes.

A recent survey revealed a high prevalence of iron deficiency anaemia among pre-school children (Ministry of Health, 1998). In terms of public health importance, prevalence was

83% in pre-school children (aged 6 – 59 months), 71% in school-aged children, 65% in pregnant women and 58% in lactating women. Anaemia prevalence of 70% was recorded among children in the Sekyere West District of the Ashanti Region (Asibey-Berko *et al.*, 1999).

2.4 IRON REQUIREMENTS

Iron requirements vary tremendously during different stages of the life cycle and between the sexes. Table 2.3 shows median requirements for absorbed iron at these stages.

Table 2.3 Median requirements for absorbed iron (mg/day) at different stages of the life cycle

Group	Age (years)	For growth	To replace losses		To replace losses	
			Basal	Menstrual	Total (mg/day)	Ratio (mg/1000cal)
Infants	0.25 – 1	0.56	0.21		0.77	0.98
Children						
Preschool	1 – 2	0.24	0.25		0.49	0.42
Preschool	2 – 3	0.22	0.34		0.56	0.36
School-aged	6 – 12	0.36	0.56	-	0.94	0.40
Adolescent						
Girls	12 – 16	0.36	0.79	0.47	1.62	0.76
Boys	12 – 16	0.66	0.80	-	1.46	0.58
Adult men	17 – 45	-	0.91	-	0.91	0.31
Women						
Non Preg.	17 – 45		0.77	0.48	1.25	0.59
Preg						
1st trimester	-	0	0.77	0	0.77	0.33
2nd trimester		0.83	0.77	2.75	4.35	1.89
3rd trimester	-	2.75	0.77	2.75	6.25	2.72
Lactating	-	-	1.05		1.05	0.40
Post menopausal	45+	-	0.77	-	0.77	0.41

Source: Modelled after FAO/WHO (1970) and updated to iron requirements from FAO/WHO (1988) and energy requirements from FAO/WHO/UNU (1985)

2.5 CAUSES OF IRON DEFICIENCY ANAEMIA

Anaemia is usually related, at least in part to iron deficiency. This is particularly true in Asia and the Americas, where prevalence of ID generally significantly exceeds that of anaemia. In Sub-Saharan Africa, particularly tropical Africa, this may not be the case and other causes of anaemia such as malaria, folate deficiency, parasitic worm infestation and other infections may be prevalent.

2.5.1 Diet

Iron has diverse biological functions, and it is this diversity that accounts for the wide-ranging impact of its deficiency. All the iron needed to execute these diverse tasks comes from the diet. Iron deficiency occurs when there is an imbalance among iron uptake, iron utilisation and iron loss. It relates both to the actual quantity of intake and to the bioavailability of a given intake.

Although vegetables, particularly spinach, are regarded as impressive sources of iron, plant (non-heme) iron is relatively poorly absorbed. For instance, the body can take in only 1.4% of the iron from spinach. Other vegetables yield slightly more; 1.6% from black beans, 4.4% from lettuce and 7% from soybeans. In contrast, 20 percent of iron from red meat, in the form of heme iron can be absorbed. Iron from breast milk is equally well assimilated, but the concentrations are lower (Scrimshaw, 1991).

The composition of a meal can influence the amount of iron that is retained. For example, if a meal contains heme and nonheme iron the former will improve the absorption of the latter.

Absorption also changes in accordance with the amount of iron in the body: it decreases if individuals are iron replete and increases if they are iron deficient (Scrimshaw, 1991). By far the major proportion of dietary iron in developing countries is in the inorganic non-heme form derived from cereal-based diets. In China, for example, of the mean daily per caput iron intake of 11.7mg, 10.3mg derives from plant sources, mainly rice, wheat, and vegetables (ACC/SCN, 1992). Poor absorption from the predominantly vegetarian diets of most people in developing countries is thus a primary cause of iron deficiency (Scrimshaw, 1991). Food from animal sources has more bioavailable iron; however because meat is expensive and consumed in small quantities or not at all, iron deficiency and anaemia affect the majority of individuals in such populations.

2.5.2 Malaria

Malaria increases the prevalence of anaemia and worsens its severity (Brabin, 1992), through two major routes. First, and most importantly, it causes a haemolytic anaemia that leads to less iron in the haemoglobin mass and more sequestered in stores, and second, as with chronic infections, it is associated with some impairment in the release of iron from reticulo-endothelial stores.

There is some evidence that persistent or recurrent parasitaemia induces iron deficiency, although the mechanisms are uncertain. These include the following:

(i) There is reduced absorption of iron during the acute period of the illness (Molyneux *et al.*, 1989).

(ii) Low haptoglobin levels, which results from intra-vascular haemolysis, will reduce the formation of haptoglobin/haemoglobin complexes, which are removed from the circulation by the liver, reducing iron availability (Boreham *et al.*, 1981). Once this happens, the iron in any additional free haemoglobin will be lost to the body either acutely (as haemoglobinurea) or, more commonly as haemosiderinurea over a longer period (Kariks, 1969).

(iii) There is immobilisation of iron in haemozoin complexes (malaria pigment) (Abdalla, 1990; Fulton and Maegraith, 1949).

Differences attributable to diet or other confounding factors affects comparisons of the prevalence of iron deficiency (ID) in malarious and non-malarious areas. Where iron status is already marginal, or latent ID is already present, the additional losses produced by malaria may be sufficient to tip the scales towards overt ID.

Malaria has been implicated in the aetiology of IDA among infants. In infancy, marked changes in haemoglobin values occur in healthy children in the first months of life. However, this pattern of change alters in malarious areas. The lower haemoglobin values in malaria-exposed infants are related to the high incidence of malaria infection from the first months of life (Brabin, 1992). Moreover there have been studies, which estimate the improvement in haemoglobin in children when malaria is controlled. The increase is most evident in younger children (who are less immune) and is likely to be greatest when acute malaria infections are more frequent (McGregor, 1988). In young children living in endemic areas, malaria associated anaemia may present with features compatible with Fe deficiency and folate deficiency states (Draper, 1960).

Megaloblastic anaemia in infants and children other than the severely malnourished has been reported infrequently from tropical countries (Walt *et al.*, 1956). Other studies in Nigeria indicate that ID is of greater importance in children than folate deficiency, which was not considered a major cause of anaemia (Akenzua *et al.*, 1985).

2.5.3 Sickle cell disease

Sickle cell disease is another consideration. In Africa, between one and two percent of infants are born with sickle cell disease (Nagel and Fleming, 1992). In countries such as India, Mediterranean, Americas, and United Kingdom, 30,000 infants are born with the condition each year (Fleming, 1998). Individuals with sickle cell disease tend to have haemoglobin values of 6-10g/dL at birth.

2.5.4 Low-birth-weight babies

Low-birth-weight (LBW) babies are at greater risk of IDA. At birth, the full-term baby has haemoglobin level of approximately 17g/dL. After birth, haemolysis occurs and by the sixth to eighth week, the haemoglobin falls to 11g/dL. The iron set free is stored, principally in the liver, and utilised during the period of milk feeding. At the end of nine months, the haemoglobin should have risen to 13g/dL (Passmore and Eastwood, 1986).

Premature and full-term infants of low birth weight, for example twins, have a small blood volume and hence smaller stores of iron to tide them through the milk-feeding period. Moreover, their rate of growth is greater and hence the requirements for iron are increased (Passmore and Eastwood, 1986).

2.5.5. Parasitic worm

Hookworm infestation increases with age and prevalence rates are higher among adults than children. Hookworms infect about one billion of the world's population. They cause intestinal blood loss by feeding on the intestinal mucosa, the amount of blood loss being directly proportional to the number of worms infecting the host (Stoltzfus and Dreyfuss, 1997). A hookworm infection of moderate intensity in a woman amounts to a faecal iron loss of 3.4mg/day (Stephenson, 1987). In a study in Nepal, 29 percent of IDA was found attributable to hookworm infection (Dreyfuss *et al,* 1996).

Schistosoma, and to a lesser degree *trichuriasis* and *ascaris* infestation can adversely affect iron status through provoking gastric or intestinal ulceration and blood loss. A strong association has been found between urinary schistosoma and iron status in Sub-Saharan Africa (Greenham, 1978; Stephenson *et al.*, 1985; Stephenson *et al.*, 1989). A severe *schistosoma haematobium* infestation can lead to a daily iron loss of 2.1mg in a woman.

A recent study on anaemia prevalence in Ghana revealed that hookworm infestation and infection with *schistosoma haematobium* contribute significantly to ID and anaemia in pregnant and lactating women but not in preschool children (MOH, 1998).

2.6 CONSEQUENCES OF IRON DEFICIENCY ANAEMIA

2.6.1 Compromised Development in Young Children

The peak prevalence of iron deficiency among young children coincides with the latter part of the spurt in brain growth (6 - 24 months) when motor and cognitive abilities take shape

(Martorell, 1997). An infant who becomes anaemic through iron deficiency is at high risk of long-term, even permanent impairment in mental and motor development. This has been demonstrated in field studies in countries like Chile, Costa Rica, Guatemala, Indonesia, Egypt, India, Indonesia, Thailand and the US (Lozoff, 1990; Lozoff *et al.*, 1991)

2.6.2. Child Growth Failure and Poor Physical Development

The child has a particularly high risk of iron deficiency from 6-24 months of age when growth velocity is particularly high, the consequences of which could impair energy utilisation and physical growth (Judisch *et al.*, 1986). The high risk period for developing iron deficiency is actually the 6-12 month age group. Children aged between 6 and 8 years with low iron stores were significantly more stunted than their more iron-replete counterparts in a study in South Africa (Kruger *et al.*, 1996).

2.6.3 Pregnancy Outcome

IDA increases the risks of maternal mortality, foetal growth retardation, prenatal and peri-natal mortality and infant mortality. Anaemia is a major contributory cause of postpartum maternal mortality and may "shift a pregnant woman's balance towards death" during delivery (Koblinsky, 1995). In fact anaemic women are five to ten times more likely to die in childbirth than non-anaemic women. The risk of maternal mortality attributable to anaemia is 20% in Africa and 22.6% in Asia (Ross and Thomas, 1996).

Anaemia is directly related to risk of pre-term delivery and inadequate gestational weight gain (McGregor, 1963; Garn *et al.*, 1981; Murphy *et al.*, 1986). The more severe the anaemia, the greater the risk of LBW baby because of poor intrauterine growth. It is well known that LBW babies have a higher risk of dying in infancy and early childhood (Villar *et al.*, 1990). Anaemia may increase the risk of infant death by 600 percent.

2.6.4 Increased Morbidity

Iron deficiency may adversely affect specific cell-mediated immunity, even before frank anaemia (Bhaskaran and Reddy, 1975; Stinnert, 1983) as well as non-specific immunity related to oxygen-dependent defence mechanisms. Lowered resistance manifests itself in increased morbidity from diarrhoea, respiratory, and other infections (Hussein, *et al.*, 1988). The fear that extra iron may predispose an infant to infection has been disapproved by the fact that iron-binding serum transferrin is only one-third saturated in infants and not influenced by recommended iron doses (Enwonwu, 1990). Subsequent raising of iron status of iron-deficient children through supplementation or fortification can reduce morbidity (Enwonwu, 1990).

2.6.5 Lowered Physical Activity, Mental Concentration, and Productivity.

A positive linear relationship between iron deficiency and work capacity has been demonstrated from agricultural workers in Colombia, Guatemala, Indonesia, Kenya and Sri Lanka (Scrimshaw, 1996), and hospital patients in Sri Lanka (Ohira, 1979). Lowered attention span will adversely affect mental concentration, which can further reduce productivity.

The effects are reversible and iron supplementation has been shown in many studies to lead to significant improvement in physical work performance. There have been increases in work output and gains of 10% to 30% percent in productivity and take-home pay (Basta *et al.*, 1979; Edgerton, 1981; Hussaini *et al.*, 1981; Ohira *et al.*, 1981; Levin, 1986; Tumbi and Dodd, 1990; Li, *et al.*, 1994; Scholz *et al.*, 1997; Zhu and Haas, 1998).

2.6.6 Other Consequences

Iron deficiency is associated not only with increased absorption of iron (Watson *et al.*, 1980; Yip, 1990), but also of other toxic heavy metals including lead and cadmium. Children living in polluted urban environments will be particularly at risk. The microcytic anaemia thought in the past to be due to lead poisoning is, in fact, iron deficiency anaemia, which is particularly common among lead-poisoned children (Clark *et al.*, 1988). Iron treatment may also reduce lead poisoning (Ruff *et al.*, 1993).

An impaired capacity to maintain body temperature in a cold environment is characteristic of iron deficiency anaemia. When exposed to cold, those suffering from iron deficiency anaemia may be at higher risk of hypothermia because of reduced thyroid function and altered epinephrine and prostaglandin production and metabolism (Dillman *et al.*, 1982; Martinez-Torres *et al.*, 1984; Beard and Borel, 1988; Beard *et al.*, 1990).

Iron deficiency has also been associated with altered gastrointestinal function relating to mal-absorption of vitamin D (Heldenberg *et al.*, 1992) and fat (Naiman *et al.*, 1964).

2.7 IRON IN INFANT AND YOUNG CHILD NUTRITION

Infancy is a time of rapid physical growth as well as physiological, immunological, and mental development. During the first year of life, nutritional requirements are at the highest in the entire life cycle. Deficiency in energy or any of the essential nutrients can have adverse consequences, some of which are irreversible.

2.7.1 The Role of Iron in Complementary Feeding

In the first four-six months, the infant's nutritional requirements can be totally met by breast milk. Afterwards, complementary foods need to be introduced to augment energy and nutrient intake. Complementary foods are therefore, transitional foods consumed between the time when the diet is composed exclusively of breast milk and the time when it is mostly made up of family foods. Complementary foods make up a large proportion of the baby's diet and contribute significant amount of the nutrients that are needed for growth and development. The foods must therefore contain nutrients to complement breast milk.

Iron deficiency is a problem of public health concern among infants globally. While overt anaemia may not be common, ID often occurs during the second half-year of life. At this stage, weaning foods are introduced and fed as a complement to breast milk, bovine milk, or formula (Lonnerdal, 1991). As many weaning foods, such as cereals and legumes, contain factors that inhibit iron absorption, it is difficult to meet the iron requirements of growing infants by diet alone. This is accentuated by the fact that intake of meat, a good source of available iron, is very limited in this age group.

Breast-feeding of short duration and lack of exclusive breast-feeding are other factors. In developed countries, the availability of dietary iron can be restricted by the early introduction of cow's milk in infancy and by an excessive cow's milk intake in young children (Hurrell *et al.*, 1989). Milk not only displaces iron rich foods from the diet, but the high levels of protein, calcium and phosphorus form insoluble complexes with iron in the intestine ((Hurrell *et al.*, 1989). Social and cultural practices may exacerbate the problem.

In industrialised countries, approximately 15 percent of infants consume insufficient amounts of dietary iron (Yip, 1989). The figure is markedly higher in developing countries and therefore the possibility of fortifying complementary foods with iron for children from this age is the most common form of targeted fortification (INACG, 1986; Rush *et al.*, 1988). The importance of iron-fortified infant foods is borne out by the fact that anaemia among infants remained quite high in countries that do not fortify infant foods or before such foods were fortified (Chan-Yip and Gary-Donald, 1978; Calvo and Ginazzo, 1990). In survey carried out in Peru and Mexico (Brown *et al.*, 1995), it was observed that only liver could cover the iron requirements of children particularly those aged between 6 – 11 months (provided that two-third of children's energy intake comes from a single food).

2.7.2 Infant Feeding Practices in Ghana

Traditional weaning foods in West Africa are known to be of low nutritive value and are characterised by low protein, low energy, and high bulk. In Ghana, most mothers give the traditional weaning food, which is a thin gruel, or porridge prepared from fermented maize dough up to the six-month of age. After six months, the infants are given the family diet with complementary breast-feeding. Some examples of the family diet are dishes made from cereal, starchy tubers, legumes and vegetables (Armar-Klemesu and Wheeler, 1991). Low-cost, nutritious, well-balanced weaning foods rich in protein and energy have been developed. Weanimix, a blend of legume (groundnut and/ cowpea) and cereal (maize) is an example. It was introduced in 1987 by the Ghanaian Ministry of Health, Nutrition Division and the United Nations Children's Fund UNICEF/Ghana to improve the quality of weaning foods. Cereals generally have low protein content and are deficient in the amino acid lysine, but adequate amounts of sulphur-containing amino acids. Legumes on the other hand have high protein content and are rich sources of lysine but containing low levels of sulphur-containing amino acids.

2.7.3 Efficacy Trials of Iron-Fortified Foods.

In recent years, addition of more bioavailable iron to infant cereal has been shown to protect against ID when significant amounts are consumed daily (Pizzaro *et al.,* 1991). In the U.S., iron fortification of infant formula has led to significant reduction of prevalence of IDA. This was contained in a report of the Women, Infants and Children (WIC) Programme (Yip, 1989). Regular consumption of the iron-fortified infant and cereals by infants from disadvantaged families resulted in improved haematological indices of iron nutritional status.

Large-scale clinical studies were conducted in Canada to investigate the efficacy of iron-fortified infant cereals in reducing the risk of iron deficiency (Green-Finestone *et al.,* 1989). A six-month, double blind trial involving healthy infants showed that significantly fewer infants given iron-fortified cereal were at risk of reaching low haemoglobin values at the end of the trial than controls. Furthermore, the infants who receive iron-fortified cereal had lower haemoglobin and serum ferritin responses to ferrous sulphate supplement given at the end of the initial trial than controls. Thus, infants who received iron-fortified cereals had better iron status and greater storage than those who did not. The study confirms the efficacy of iron-fortified cereals in reducing the risk of iron deficiency.

Yeung et al (1981) confirmed the evidence that wide usage of fortified cereals is effective in preventing iron deficiency in a population. For example close to 96 percent of infants aged 4-10 months were customarily given the cereals, and that the cereals were clearly the main source of iron in the infant's diets. It was apparent that the recommended dietary allowance for iron could not be met without the presence of iron-fortified cereals in the diet.

Two trials in Chile and one in South Africa clearly demonstrated the efficacy of fortification in reducing anaemia prevalence. In one, feeding children aged 3 – 15 months with iron-fortified milk reduced the incidence of anaemia significantly from 36% in the control group to 13% in the fortified group. The inclusion of vitamin C in another group of children was associated with a more significant decline from 28% to 2% (Stekel *et al.*, 1986).

The iron status of Chilean school children also improved when cookies fortified with heme iron were used in school feeding programme (Stekel *et al.*, 1986). A recent study in South Africa (Kruger *et al.*, 1996) showed a significant effect of iron-fortified soup on the iron status of six-to eight- years old children after five months.

In the Philippines, a clinical trial conducted with school children for six months showed a greater increase in haemoglobin in subjects on iron-fortified rice than in those on non-fortified rice (Florentino and Pedro, 1996).

2.7.4 Iron in Human Growth

The risk of iron deficiency increases with growth velocity. During infancy, rapid tissue growth requires high iron needs. When this is not readily available, growth is impaired. The growth of iron deficient pre-school and school-aged children has been found to improve after iron supplementation. This is an indirect evidence of the effect of iron on growth (Bhatia and Seshadri, 1993; Allen, 1994). It is however, not clear whether this is an independent effect of iron because appetite, and possibly food intake, also increases as iron deficiency is corrected (Lawless *et al.*, 1994).

In one study, there were decreased rates of stunting among anaemic Indonesian pre-school children through iron supplementation (Angeles *et al.*, 1993). In another study, there was a significant improvement in physical growth velocity of rural Indonesian children upon iron supplementation (Chwang *et al* 1988). Other researchers (Briend *et al.*, 1990; Latham *et al* 1990; Lawless *et al.*, 1994; Aukett *et al.*, 1986) observed that growth of preschool and school-aged children improved upon iron supplementation. Tulchinsky *et al.*, (1994) found that iron had a highly positive effect on length-for age. Children who were not fed iron had low scores for length-for age, especially those aged nine- to fifteen- months.

The efficacy of iron supplementation for iron-deficient subjects is in no doubt. However, the assumption that iron supplementation of iron-replete subjects is harmless may not be valid. Idjradinata *et al.*, (1994) found adverse effect of iron supplementation on weight gain of iron-replete young children. These results suggest that iron supplementation of iron-replete children may retard their growth, although no reduction in linear growth occurred-suggesting that any such negative effect was not significant. Also this finding had not been apparent in three other small studies (Stephenson, 1995).

3.0 METHODOLOGY

3.1 RESEARCH DESIGN AND SETTINGS

This was a community intervention trial conducted between April and September 1999. The study sites were Otinibi and Danfa, which are peri-urban communities in the Greater Accra District about 14km from Accra, with Amasaman being the district capital.

The population is mostly of Ga extraction. Both communities have primary school facilities. A health post at Danfa serves both communities for primary health care.

3.2 STUDY POPULATION

All children in the community aged between six and eighteen months, whose mothers consented (Appendix I), participated in the study.

3.3 SAMPLE SIZE CALCULATION:

The variance of haemoglobin of normal adults is 13 and those with iron deficiency anaemia 19 (Punnonen *et al.*, 1994). Assuming between group difference of haemoglobin change as equal to or greater than 1.0g/dl over the six-month intervention period at 95% confidence level, the expected range of variance of haemoglobin is $13^2 - 19^2$ and using the following formula by Punnonen *et al.*, (1994) the sample size was 26 – 55 per group.

$$N= \frac{(2)^2 x\ (1.96)^2\ x\ (SD)^2}{(\text{Desired change in outcome})^2}$$

Where N= number of subjects

SD= Standard Deviation

Sixty preschool children were recruited based on their initial haemoglobin levels. The sample was made up of 26 boys and 34 girls. Inclusion criteria were children whose haemoglobin levels ≥ 9g/dl and those who were likely to stay in the community throughout the study period. Exclusion criteria included a child who was on iron supplements, a child who had had current blood transfusion and a child who was not available for baseline haematological screening.

3.4 PREPARATION OF TEST FOOD

The maize-cowpea blend used in this study consisted of 80% maize and 20% cowpea. Maize was cleaned and soaked for 24 hours then rinsed thoroughly. Dehulled cowpea was added to the maize and milled using a disc attrition mill. Dough was prepared and then fermented using traditional methods. The fermented dough was dehydrated in an oven at 45°C for 9 –12 hours and ground into fine flour. Iron-fortified sample was prepared by adding ferrous fumarate powder to the fermented maize-cowpea flour. This was done at Food Research Institute- Pilot Plant near Legon. The prepared blends were then packaged into polythene bags (500g) and stored at room temperature prior to distribution.

3.5 STUDY GROUPS

Two groups were used in the study. Subjects were randomly assigned to one of the two project foods: (i) iron-fortified food and (ii) non-iron fortified food. Thirty-one (31) children received iron-fortified food and 29 received non-iron-fortified food.

3.6 MODE OF DISTRIBUTION OF TEST FOOD

This was done on take-home basis. Mothers were given pre-packaged rations (500g) weekly. They were encouraged to feed at least three times a day to the study children for an intervention period of six months.

3.7 BIOCHEMICAL ASSESSMENT

About 5ml of venous blood was taken from each subject at baseline and at end of the intervention into centrifuge tube, allowed to stand for about 30 minutes and centrifuged for 10 minutes at 1000g. The serum was then refrigerated at –20°C until ready for analysis.

3.7.1 Haemoglobin Concentration

This was done using a Hemocue Haemoglobinometer (Hemocue AB, Angelholm,Sweden) (Van Schenck *et al.*, 1986). The system consists of a battery-operated photometer and a disposable cuvette that is coated with the dried reagent (sodium azide) and serves as the blood collection device. A drop of fresh venous blood was drawn into a Hemocue cuvette by capillary action and read directly within 30 seconds.

3.7.2 Serum Iron and Unsaturated Iron-Binding-Capacity

Colorimetric methods were employed (Goodwin *et al.*, 1966; Artiss *et al.*, 1981).

3.7.2.1 Principle

In the measurement of serum iron, ferric iron is dissociated from its carrier protein, transferrin, in an acid medium and simultaneously reduced to the ferrous form. The ferrous iron is then complexed with the chromogen, a sensitive iron indicator, to produce a blue chromophore, which absorbs at 595 nm. The unsaturated iron binding capacity (UIBC) is measured by adding a known amount of ferrous iron in excess, to the serum at alkaline pH. This saturates the unoccupied iron binding sites on the transferrin. The amount of free iron thus measured is subtracted from the total amount added to calculate the UIBC. Chemicals were obtained from Randox Laboratories Ltd.

3.7.2.2 Reagents and Conditions

1. Chromogen (Ferene)
2. Reductant (Ascorbic acid)
3. Iron buffer (Acetate buffer Dimethyl sulphoxide Sulfactant)
4. UIBC buffer (Tris buffer)
5. Ferrous reagent (Ferrous ammonium sulphate)
6. Low standard (8.95 µmol/L)
7. High standard (89.5 µmol/L)

Wavelength: 595 nm (590 –610)

Cuvette: 1 cm light path

Temperature: 15 -25° C/37° C (Iron) 15 - 25°C/37° C (UIBC)

3.7.2.3 Procedure for Serum Iron

Two millilitres buffer was pipetted into iron- free test tube. A little (0.1ml) reductant was added and 0.5 ml sample also added. Reagent blank was prepared by adding 0.5 ml iron-free water instead of sample. Standard was also prepared by adding 0.5 ml standard to buffer and reductant in test tube. Reagent blank, standard and sample were mixed and the initial absorbances of the sample and of the standard read against the reagent blank. A little (0.1ml) chromogen was then added to each test tube, mixed and incubated for 5 minutes at 15 – 25° C/37° C. The final absorbance was read against reagent blank. Initial absorbance was subtracted from final absorbance to give change in absorbance (▵A) for sample and standard.

3.7.2.4 Procedure for Unsaturated iron binding capacity (UIBC)

Two millilitres buffered ferrous reagent was pipetted into iron-free test tube. A little (0.1ml) reductant was added and 0.5 ml sample also added. Reference was prepared by adding 0.5ml iron-free water instead of sample. The reference and sample were mixed and incubated for 7 minutes at 15 – 25° C/37° Initial absorbances of sample and reference were read against deionized water. A little (0.1ml) chromogen was then added to each test tube, mixed and incubated for 5 minutes at 15 – 25° C/37° C. The final absorbance of sample and reference were read against deionized water. Initial absorbance was subtracted from final absorbance to give ▵A for sample and reference. Pooled sera from Korle-Bu Teaching Hospital served as quality control for both serum iron and UIBC.

3.7.2.5 Calculation

A calibration curve was plotted by assaying the iron standards using the iron procedure described above. This was used to calculate the iron concentrations in the UIBC procedure.

The reference tube provided a measure of the total amount of iron added to each sample. The absorbance of the sample tube was subtracted from that of reference tube. The difference represents the UIBC, which is determined from the calibration curve.

Thus $\triangle Ac = (\triangle Af - \triangle As)$

Where $\triangle Ac$ = UIBC absorbance

$\triangle Af$ = abs. of reference

$\triangle As$ = abs. of sample

The iron concentration corresponding to $\triangle Ac$ was read from the calibration curve. This was the UIBC of the sample.

3.7.3 Total iron-binding-capacity (TIBC)

Total iron-binding-capacity (TIBC) was expressed as the sum of serum iron concentration and unsaturated iron-binding-capacity.

3.7.4 Transferrin saturation (TS)

This was expressed as a percentage of serum iron and total iron binding capacity. TS below 16% is considered iron deficient and values above this cut-off point is taken as adequate iron stores.

Table 3.1: Interpretative guide for characterising anaemia

Category	Haemoglobin concentration (g/dl)
Normal	>11
Mild anaemia	10 – 11
Moderate anaemia	7 – 10
Severe anaemia	< 7

Source: (WHO/UNICEF/UNU 1996).

3.8 DIETARY ASSESSMENT

Mothers were interviewed on child feeding practices using structured questionnaires (Appendix I). Dietary iron intake was assessed at the start of the study by using 24-hour recall. Daily intake of test food was estimated by weighing sachet and its contents after every four weeks. Energy and nutrient intakes were evaluated using Food composition tables (Eyeson *et al.*, 1975).

3.9 ANTHROPOMETRIC MEASUREMENTS

Subjects were weighed naked to the nearest 10g with a digital beam balance (Perspective Enterprises, Portage, MI). Recumbent length was measured to the nearest 0.1cm with a portable measuring board (an infantometer). Mid-upper arm circumference (MUAC) was taken to nearest 0.1cm at the midpoint of the upper left arm between the acromion and olecranon using an insertion tape. Weight and length measurements were transformed into Z-scores of the National Center for Health Statistics reference standards (NCHS, 1975)

Table 3.2 Interpretative guide of classifying malnutrition

Category	Malnutrition indicators
Normal	WLZ*, LAZ** and WAZ*** >-2 SD
Stunting	WAZ and LAZ < -2SD, WLZ > -2 SD
Wasting	WAZ and WLZ <-2SD, LAZ >-2 SD
Underweight	WAZ, WLZ and LAZ < -2 SD

Source: (ACC/SCN, 1992).

*WLZ= Weight-for-length; **LAZ= Length-for-age; ***WAZ= Weight-for-age.

For MUAC, the cut-off points for malnutrition were: MUAC > 13.5 cm normal, MUAC 12.5 – 13.5 cm moderate malnutrition: MUAC < 12 cm more severe malnutrition. One was classified malnourished when MUAC < 13.5 cm (ACC/SCN, 1992).

3.10 CHEMICAL ANALYSIS

In the determination of minerals in biological materials, it is usually necessary to eliminate the organic materials as a first step. Wet-ashing (Osborne and Voogt, 1978) was used to extract the iron. The iron content of both fortified and non-fortified maize-cowpea flours were then determined by AAS (Appendix IV)

3.10.1 Wet Ashing of the Test Foods

About 1 g of the experimental diet was weighed into a 250ml Erlenmeyer flask and left to stand overnight after 12ml of reagent grade nitric acid had been added. This was heated on a hot plate at low heat (100°C) for an hour, after which the heat was increased to 200°C for 3 hours. The sample was then cooled to room temperature. Two millilitres of perchloric acid was added, and the heat increased gradually to 400°C. Digestion continued until the volume reduced to approximately 2ml. After cooling, 40ml double distilled water was added, brought to boil and allowed to cool. The digest was then transferred quantitatively into a 100ml volumetric flask and diluted to volume with double distilled water. Digestion was done in duplicate.

3.11 DATA ANALYSIS

Data were entered using Epi Info Version 6.0 and Stata Version 5 Software Programme used to analyse data. Baseline characteristics were compared using Student t-test and chi-square test. Differences in outcome variables at the end of the intervention were compared using student t test and chi-square. Regression and correlation were used to establish relational associations.

4.0 RESULTS

4.1 BACKGROUND INFORMATION

4.1.1 Maternal and Household Characteristics

The socio economic backgrounds of the women were comparable. All the families lived in peri-urban communities with similar environmental and sanitary conditions. There is no market in the communities so people travel to nearby towns for marketing and other business activities. The population is ethnically heterogeneous; the households in this study were mostly Gas (48.2%) and Ewes (35.7%). The rest were Akans (10.7%) and other ethnic groups, which formed only a minor percentage.

The housing units are mostly compound in nature. Most of the houses (87%) are built from clay. The average family size was between 3 and 6 people (59%); a few (about 10%) had more than seven members. There was a maximum of two rooms to each family, which in most cases either rented or owned by the extended family. A few of the houses were however owned by the respondents themselves. A high number of the respondents (about 62%) have a wireless radio set as source of information. The source of drinking water for majority (about 57%) of the people in the study area is a pond at outskirts of town. A few (about 14%) have access to treated water which they purchase from water tankers, owned by private individuals.

4.1.2 Maternal Education

Generally, educational level of the respondents was low (Table 4.1). About thirty-six percent (35%) of respondents have had no formal education. The remaining 65% had been to school but only 51% completed (primary, JSS, middle school, SSS and vocational). This, they attributed mainly to teenage pregnancies, which forced them to be dropouts. The requirement for girls to stay at home to take care of siblings and share in the household chores and the responsibility on them to earn some money to supplement the family income were some other reasons given for the low admission and a higher drop out rates.

Table 4.1. Educational Level of Female Guardian (n=56)

No of years in school	Frequency	Percent (%)
None	20	35.7
Between 1 – 3	13	23.2
Between 4 – 10	15	26.8
More than 10	8	14.3

4.1.3 Maternal Employment

Farming and trading are the main income-generating activities in the community (Figure 4.1). Maize, cassava, pepper and tomatoes are cultivated for both sustenance and commercial. Most households kept domestic animals such as chickens, goats and sheep. A few (about 9%) of the respondents were housewives (unemployed): they were not engaged in any form of income-generating activities. A few of them were teenage mothers who were being trained to be self-employed in the areas of hairdressing and dress making. The professionals are made up of teachers, seamstresses, caterers and nurses. Almost half of the women had subsidiary occupation such as gari processing and stone quarrying and gathering, which brings in an additional income to supplement the house keeping money.

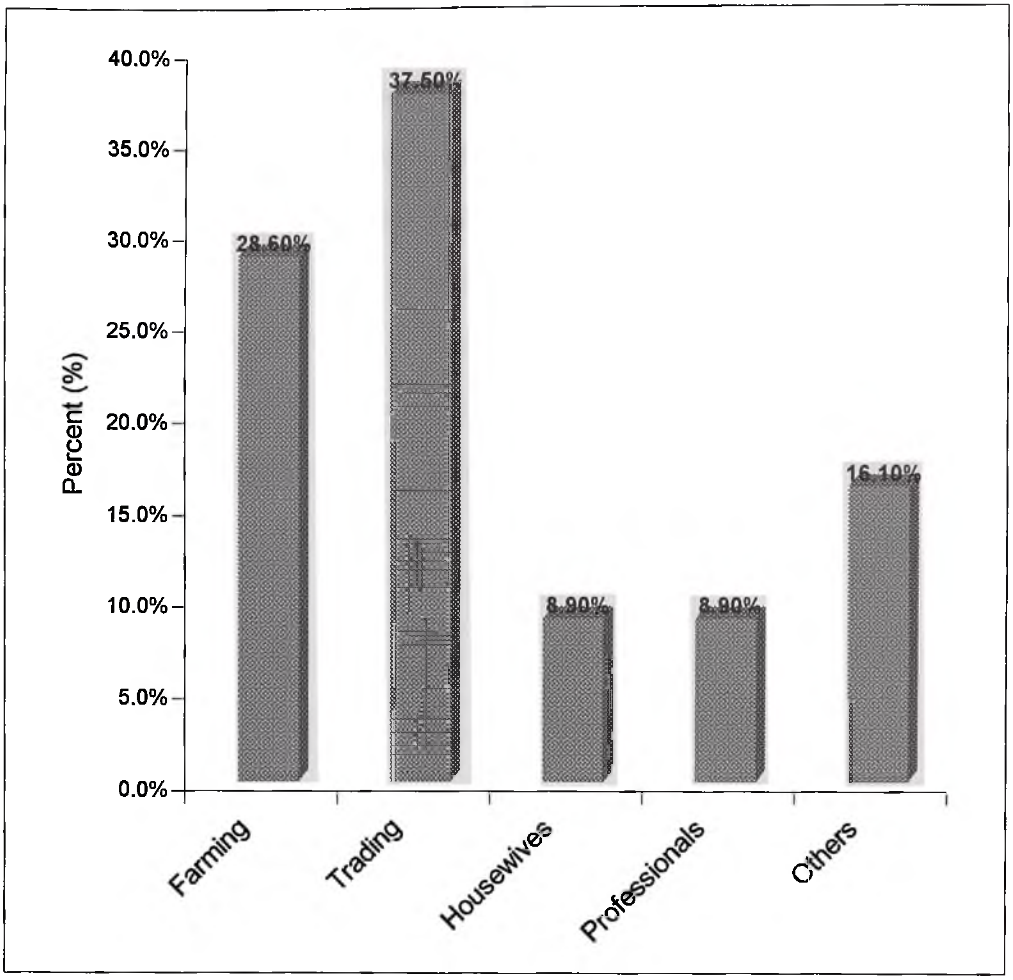


Figure 4.1 Occupation of female guardians (n=56)

4.2 BASELINE CHARACTERISTICS OF STUDY CHILDREN.

Twenty-six boys and thirty-four girls whose ages range between 6 and 18 months were recruited. The baseline characteristics of the households and infant feeding practices in the two groups were comparable (Table 4.2). There were no significance differences with respect to age of child, morbidity prevalence, age at supplementing breast milk, nor in maternal education and family size. Four children (2 from each group) did not complete the study. Reasons that were given for the dropping out included relocation (2), child refused project food (1) and mother did not feed project food (1).

Table 4.2 Baseline characteristics and infant feeding practices of children

Indicator	Iron-fortified (n=29)	Non iron-fortified (n=27)
Mean age of child (mo) [1]	11.10 (4.13)	11.37 (4.26)
Exclusive breastfeeding		
Yes	51.7% (15)	37.0% (10)
No	48.3% (14)	63.0% (17)
Age of supplementation (mo)[2]		
0 – 1	7.2% (2)	12.8% (2)
2 - 3 mo	71.4% (21)	46.3% (12)
> 4 mo	21.4% (6)	46.3% (12)
Maternal education (yrs)		
None	31.0% (9)	40.8% (11)
1 - 3	24.1% (7)	22.2% (6)
4 – 10	37.9% (11)	22.2% (6)
More than 10	6.9% (2)	14.8% (4)
Family size		
2 – 3	34.5% (10)	37.0% (10)
4 – 6	51.7% (15)	59.3% (16)
7 – 10	10.3% (3)	3.7% (1)
More than 10	3.5% (1)	0
Morbidity prevalence (%)[3]		
Yes	17.2% (5)	40.7% (11)
No	82.8% (24)	59.3% (16)

[1] Mean ± SD

[2] Age of supplementing breast milk

[3] Morbidity based on malaria/fever, diarrhoea and cough a month prior to commencement of intervention.

4.2.1 Infant Feeding Practices

Almost half (45%) of the children were fed solely on breast milk up to 3 months of age. The remaining children were fed breast milk and other liquids. Water was given several times a day, sometimes before breast-feeding. The age at which breast milk was supplemented is shown in Figure 4.2. There was a wide range in the age at which complementary foods were given. The most common was between 1 and 3 months, although some mothers were supplementing as early as the first month of the infants' life.

Majority of the respondents gave complementary foods as early as the first to third month. A few (about 6%) introduced it between birth and one month after delivery. About one-third supplemented the breast milk when their infants were aged more than 3 months old. Foods that were given include coconut water, sugar or glucose water, porridge/koko and purified water. Reasons given by mothers for supplementing breast milk are shown in Figure 4.3.

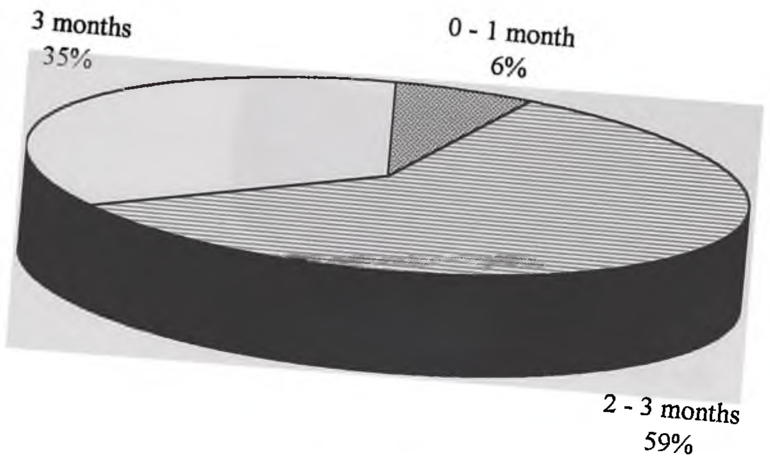


Figure 4.2 Age at which breast milk was supplemented (n=56)

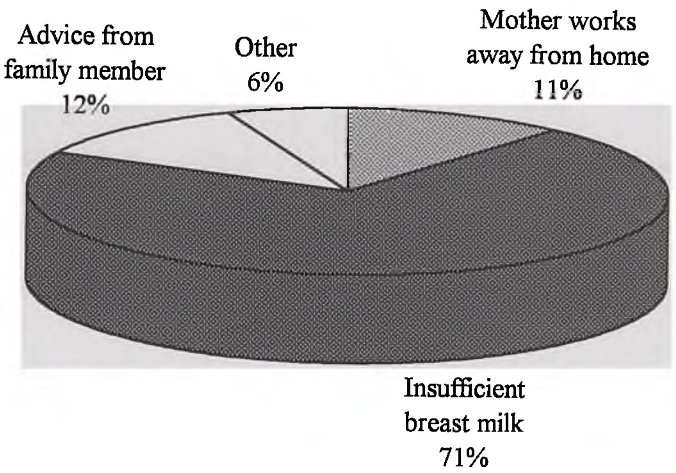


Figure 4.3 Reasons for supplementing breast milk (n=56)

Majority of the mothers reported that their babies cried a lot after breast-feeding or did not sleep well at night so they assumed that the breast milk alone did not satisfy them hence the giving of complementary foods. About 12% reported advice from family member - mothers and aunts insisted they gave complementary foods, as that had been the practice. Only a few (about 11%) of the mothers work away from home so they reasoned that in order for the child not to refuse complementary food when they are away they had to introduced it early for the children to get used to it. Others reported that their children refused breast milk, the mother was very ill; no milk in the breast or breast milk was very watery.

Majority (about 88%) of the children were still breast-feeding in addition to giving complementary foods. On the average, weaning age was given as between 18 and 24 months. Various reasons were given for cessation of breast-feeding at this age interval (Fig. 4.4).

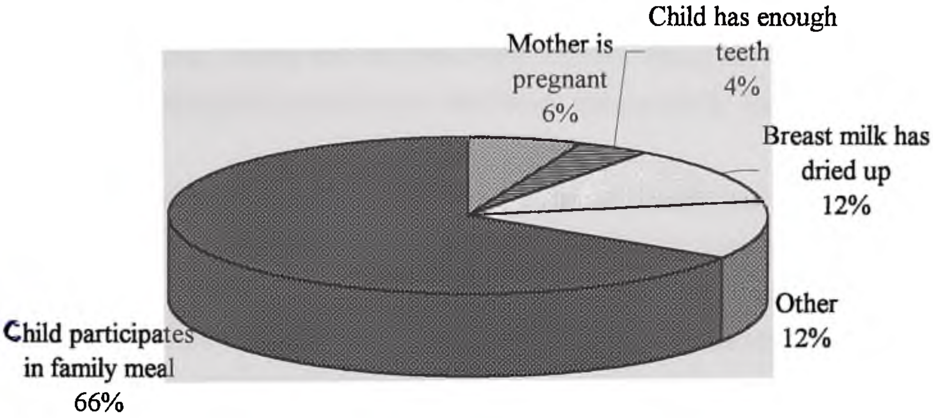


Figure 4.4 Reasons for cessation of breast-feeding (n=49)

4.2.2 Effect of maternal education and employment on infant feeding practices

Maternal education was found to be associated with age of introducing complementary foods, frequency of feeding, bottle-feeding (Table 4.3). Uneducated women gave complementary foods earlier than educated women. There was greater prevalence of bottle-feeding and high frequency of giving complementary foods by educated women. Duration of breast-feeding, feeding fish and meat were however, negatively correlated with maternal education. Uneducated women breast feed longer and frequently than educated ones.

Maternal employment was associated with age of introducing complementary foods, frequency of feeding, bottle-feeding (Table 4.3). Working mothers gave complementary foods earlier and more frequently than non-working women. The associations are however, weak. Only bottle-feeding was strongly associated with maternal employment. No association was observed between duration of breast-feeding and maternal employment.

Table 4.3 Relationship between maternal attributes and infant feeding practices (n=56)

Factor	Correlation (r)	P-value	Remarks
Maternal education versus bottle feeding	0.8175	0.0001	Significant
Maternal employment versus bottle feeding	0.5706	0.0001	Significant
Maternal education versus age of introducing complementary foods	0.5102	0.0034	Significant
Maternal employment versus age of introducing complementary foods	0.3582	0.0474	Significant
Maternal education versus frequency of feeding complementary foods	0.2982	0.0257	Significant
Maternal employment versus frequency of feeding complementary foods	0.2889	0.0308	Significant
Maternal employment versus duration of breast feeding	0.1157	0.4235	Insignificant
Maternal education versus duration of breast feeding	-0.0411	0.7769	Insignificant

4.2.3 Child Care Practices

Childcare practice was generally good in the community. About 46% of the mothers were sole providers of care. The rest had other care providers in addition to themselves. About 48% of them get assistance from grandmothers, 31.8% from older siblings, 15.9% day care attendant and 4.5% get assistance from their aunts.

4.2.4 Immunisation and Health

About 90% of the mothers attended child welfare clinics regularly. The study children were adequately immunised against childhood diseases. This was validated with growth monitoring cards that are issued by the Ministry of Health (MOH, Ghana). The health status of the children one-month prior to the intervention was studied. Less than one-third (about 29%) had suffered from a disease. Diseases reported by the mothers were fever/malaria, diarrhoea, cough and skin rashes. Fever/malaria had the highest frequency while skin rash is the least (Figure 4.5).

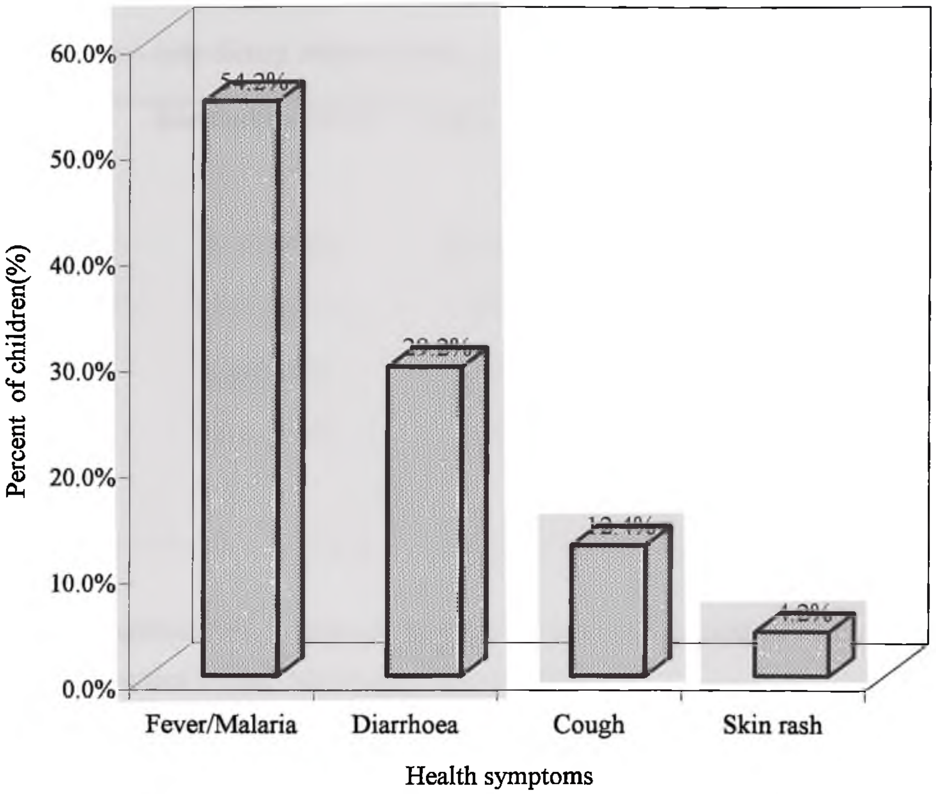


Figure 4.5 Health Symptoms of Study Children one Month Prior to Intervention (n=16)

4.3 DIETARY DATA

4.3.1 Dietary intakes of the study children at baseline

The dietary intakes of the children from complementary foods (non breast milk) were assessed. The results are summarised in Table 4.4.

Table 4.4. Mean daily dietary intakes of study children at baseline (n=56)*

	Iron-fortified (n=29)	Non iron-fortified (27)	P-value	RDA**
Energy (kcal)	327 (218.67)	272.56 (180.80)	0.3164	850 – 1300
Protein (g)	17.94 (13.15)	14.66 (16.89)	0.4138	14 – 16
Iron (mg)	8.15 (4.91)	7.04 (4.04)	0.3627	10
Calcium (mg)	196.3 (32.91)	199.88 (33.97)	0.9404	600 – 800

*Mean ± SD

** Food and Nutrition Board, National Academy of Sciences. National Research Council Recommended Dietary Allowances. Revised 1989.

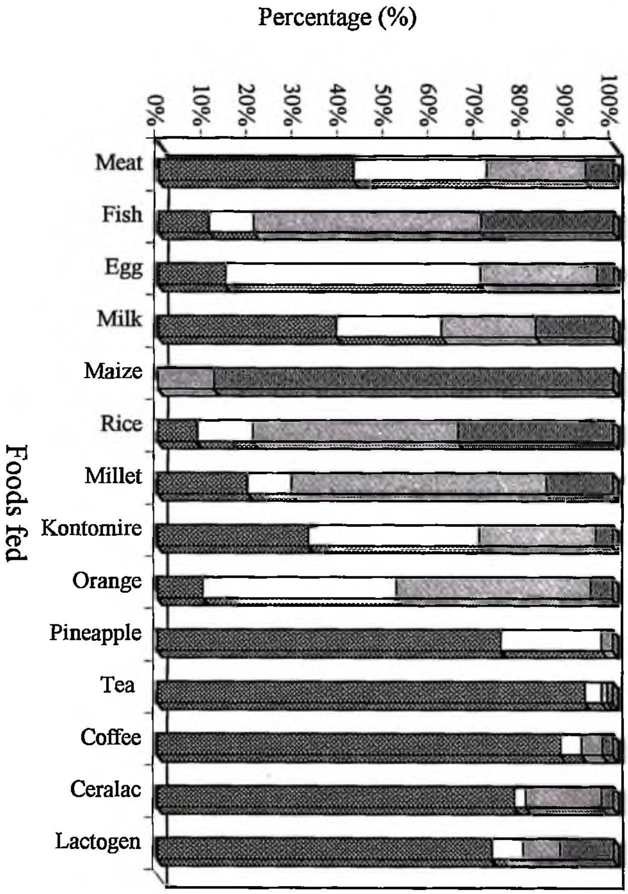
The dietary intakes of the children at the start of the intervention were comparable (Table 4.4). The intakes of protein and iron were within the range although a value as low as 0.74mg of iron intake/day was observed. The mean energy intake of the children was about 301 kilocalories, which was insufficient compared to the energy intake for the age group 6 – 18 months (Table 4.4). Calcium intake was also inadequate. Only a few of the mothers gave milk, which is a rich source of calcium. A relatively high percentage gave dried fish, anchovy, a good source of dietary calcium.

4.3.2 Dietary patterns of children

Mothers were asked to specify the frequency of giving certain foods to their children. A relatively high percentage of the mothers (more than 43%) had never given meat to their children, only 6% gave meat more than three times a week. Fish on the other hand, was frequently fed. Almost half of the respondents gave fish at least twice a week. Eggs and milk were seldom fed. Actually, beans are the second most frequently fed protein-rich food after fish. Maize, rice and millet were given regularly. Orange was given more often. Banana, pineapple and pawpaw were seldom given while mangoes were given only when in season. A few of the respondents gave infant formula and infant cereals. Consumption of tea and coffee among the children was negligible (Figure 4.6).

▩ No ☐ Occassional. ▨ 1- 3 wks ▩ >3wks

Figure 4.6 Frequency of feeding some common foods


Percentage (%)
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Meat
Fish
Egg
Milk
Maize
Rice
Millet
Kontomire
Orange
Pineapple
Tea
Coffee
Ceralac
Lactogen
Foods fed

4.3.3. Energy and Nutrient Composition of Diets

The nutrient and energy compositions of the test diets are shown in Table 4.5. The diets were similar in nutrients and energy content with the exception of iron.

Table 4.5 Nutrient Composition of Diets per kilogramme dry weight

	Iron-fortified	None iron-fortified
Energy (cal)	3660	3360
Protein (g)	124	124
Carbohydrate (g)	737	737
Total fat (g)	35	35
Iron (mg) [1]	184	75.7
Calcium (mg)	476	476
Thiamine (mg)	1.86	1.86

[1] Determined by AAS.

4.3.4. Energy and Nutrient Intake of Test Foods

Energy, protein and iron intakes from the test foods over the 6-month intervention period are shown in Table 4.6. The intake of energy and protein are comparable. As expected, intake of iron was significantly ($p<0.0001$) higher in the iron-fortified group.

Table 4.6 Ranges of Energy, Protein and Iron Intake from Test Foods

	Iron-fortified (n=29)	Non iron-fortified (n=27)
Energy (cal)	234.99 – 250.31	238.63 – 245.47)
Protein (g)	8.19 – 8.48	8.08 – 8.32
Iron (mg)	11.82 – 12.58	4.24 – 6.14

4.3.5. Relationship between Dietary Intake and Iron Status

Although there were marked difference in dietary iron intake at the start of the intervention, no relationship was observed between dietary intake of iron and iron status at baseline. A positively significant association between iron intake and haemoglobin concentration ($p<0.003$) and haemoglobin change ($p < 0.0008$) was found after controlling for protein and energy intake. There was however no association among other measures of iron status (serum iron, TIBC and percentage transferrin saturation).

4.4 BIOCHEMICAL DATA

4.4.1 Anaemia Prevalence and Iron Status

Table 4.7 shows biochemical measures of study children at the start of the intervention. There were no significant differences between intervention groups in terms of mean haemoglobin concentration, serum iron, total-iron-binding capacity and transferrin saturation.

Table 4.7 Baseline Biochemical Measures of Iron Status

Indicator	Iron-fortified group (n=29)	Non iron-fortified group (n=27)	P-value
Age (months) [1]	11.10 (4.13)	11.37 (4.26)	0.8127
Hb (g/dl) [1]	10.78 (1.23)	10.99 (2.03)	0.6825
% Anaemic	68.97	62.96	0.6825
Serum iron (µmol/L) [1]	17.18 (2.80)	17.39 (2.91)	0.7877
TIBC (µmol/L) [1]	63.05 (8.11)	61.46 (7.52)	0.6428
TS (%) [1]	28.19 (5.89)	28.34 (5.30)	0.9203

[1] Mean ± SD

Anaemia was assessed by measurement of haemoglobin concentration. Using WHO cut off points (Table 3.1), majority of the children were anaemic at the start of the intervention study. About 70% of the children were anaemic: 38% of the cases diagnosed indicated mild anaemia while about 32% were moderately anaemic. Only one-third of the children were of normal haemoglobin status (Table 4.8).

Table 4.8 Classification of anaemia at Baseline using Blood haemoglobin levels

	Iron-fortified group (n=29)	Non-iron-fortified group (n=27)	Total (n=56)
Non – anaemic	24.1% (7)	37.1% (10)	30.4% (17)
Mild anaemia	51.8% (15)	22.2% (6)	37.5% (21)
Moderate anaemia	24.1% (7)	40.7% (11)	32.1% (18)

4. 5 ANTHROPOMETRIC DATA

4.5.1 Anthropometric indices and Nutritional Status of Children

The anthropometric characteristics of the study children at baseline are summarised in Table 4.9. The two groups were comparable in all indicators. Using the interpretative guide in Table 3.3, the nutritional status of the children in the two groups at start and end of the intervention is presented in Table 4.10. The results revealed the presence of malnutrition regardless of the parameter used in the assessment.

Table 4.9 Baseline Anthropometric Indices of Children in Intervention Groups*

Indicator	Iron-fortified (n=29)	Non-iron-fortified group (n=27)	P-value
Age (months)	11.10 (4.13)*	11.37 (4.26)	0.8127
Weight (kg)	8.22 (1.41)	7.72 (1.56)	0.2132
Length (cm)	73.04 (5.61)	72.19 (6.39)	0.5971
Weight-for-length Z-score	-1.10 (1.15))	-1.45 (0.94)	0.2161
Weight-for-age Z-score	-1.01 (1.23)	-1.59 (1.11)	0.071
Length-for-age Z-score	-0.17 (1.19))	-0.60 (1.22)	0.1845
Mid upper arm circumference	14.27 (1.21)	13.96 (1.47)	0.3853

*Mean (Standard Deviation)

According to weight-for-length Z scores, 20.8% and 18.5% of the children were wasted. Relatively smaller percentages were stunted using length-for-age. Severe malnutrition was low. Only about 4% and 7% were underweight.

A relatively higher percentage of malnourished children was identified using mid-upper arm circumference (Table 4.11). Whereas about 20% (wasting) was reported using weight-for-height/length z-scores, more children (about 30%) were found wasted using mid-upper arm circumference.

Table 4.10 Nutritional Status of children after 6-month Intervention Period

	Baseline		End of intervention	
	Iron-fortified group	Non-iron-fortified group	Iron-fortified group	Non-iron-fortified group
Stunting[1]	10.3%	11.1%	6.9%	11.1%
Wasting[2]	20.8%	18.5%	17.2%	14.8%
Underweight[3]	3.5%	7.4%	3.5%	7.4%
Normal[4]	65.4%	63.0%	72.4%	66.7%

[1]Length-for-age < -2SD [2]Weight-for-length < -2SD [3]Weight-for-age <-2SD [4]Length-for-age, Weight-for-length, Weight-for-age >-2SD.

Table 4.11 Nutritional status of children after 6- month study period using MUAC

Intervention	Baseline			End of Intervention		
	Normal	Mild to moderate	Severe	Normal	Mild to moderate	Severe
Iron-fortified group	75.9%(22)	24.1% (7)	0	86.2%(25)	13.8%(4)	0
Non-iron-fortified group	63.0%(17)	29.6%(8)	7.4%(2)	66.7%(18)	33.3%(9)	0
Total	69.6%(39)	26.8%(15)	3.6% (2)	76.8%(43)	23.2%(13)	0

4.6 EFFECTS OF INTERVENTION ON GROWTH

The effect of intervention on growth of study children at baseline and end of study is shown in Tables 4.10, 4.11 and 4.12. Generally there was a decreasing trend of malnutrition among subjects. Although these were not statistically significant, stunting dropped from about 11% to approximately 9%. Wasting declined by about 4% (Table 4.10). However percentage of children who were both wasted and stunted remained the same after the six months intervention period. Large margins of change were observed in the iron-fortified group than in the non iron-fortified groups. The percentage of children who were stunted fell from about 10% to almost 7% in test group. Among the non iron-fortified group, there was no change. Wasting on the other hand dropped by virtually the same margin in both groups. Severe malnutrition as measured by MUAC declined from 7.4% to nil while mild to moderate malnutrition by about 3% in the non iron-fortified. In the iron fortified group, mild to moderate declined by about 10% at the end of intervention (Table 4.11). The results were comparable in both groups.

The mean change in weight of the two groups was comparable (Table 4.12), although a larger change was observed in the non iron-fortified group. However, mean changes in length and MUAC were higher in the iron-fortified group. Statistically significant differences were observed in length gains and MUAC but not in weight.

Table 4.12 Effects Of Intervention On Growth Of Pre-School Children after the six-month Intervention Period

	Iron-fortified group	Non iron-fortified group
Weight at baseline	8.22 (1.41)[3]	7.72 (1.56)
Weight at end	9.60 (1.79)	9.15 (1.85)
Weight gain	1.38 (0.70)	1.44 (0.71)
Length at baseline	73.04 (5.61)	72.19 (6.39)
Length at end	78.16 (4.93)	75.92 (5.94)
Length gain[1]	5.11 (2.13)	3.73 (2.18)
MUAC at baseline	14.27 (1.21)	13.96 (1.47)
MUAC at end	14.64 (1.07)	13.96 (1.26)
MUAC gain[2]	0.37 (0.93)	-0.004 (0.71)

[1] p=0.0201; [2] p=0.048; [3] Mean (Standard Deviation)

4.7 EFFECT OF INTERVENTION ON ANAEMIA PREVALENCE AND IRON STATUS

The changes in the biochemical measures of iron status at baseline and end of intervention are shown in Table 4.13. Children fed the iron-fortified food had a statistically higher haemoglobin concentration at the end of the intervention (Figure 4.7). Similarly the percentage of children who were anaemic decreased from about 69% to 28% in the iron-fortified group (Figure 4.8), but there was no significant decrease in anaemia rate in the non iron fortified group between baseline and end of intervention. Increases in haemoglobin concentrationwere higher in anaemic than among non-anaemic children. A significant negative correlation (r= - 0.5803; p=0.003) was observed between initial haemoglobin and the change in haemoglobin over the intervention period in iron-fortified group.

Table 4.13 Effect of Intervention on Biochemical Measures

Indicator	Iron-fortified group (n = 29)	Non iron-fortified group (n = 27)
Hb at baseline	10.78 (1.25)[3]	10.99 (2.03)
Hb at end	11.86 (1.49)	10.60 (1.37)
Hb change[1]	1.08 (1.43)	-0.40 (1.72)
Serum iron at baseline	17.18 (2.80)	17.39 (2.91)
Serum iron at end	16.87 (3.32)	16.18 (2.69)
Serum iron change	-0.024 (2.88)	-1.21 (4.61)
TIBC at baseline	63.05 (8.11)	61.46 (7.52)
TIBC at end	57.06 (13.03)	59.39 (11.53)
TIBC change	-5.98 (15.68)	-3.51 (14.22)
% TS at baseline	28.19 (5.89)	28.34 (5.30)
% TS at end	30.12 (10.18)	29.41 (6.96)
%TS change	2.11 (10.43)	1.50 (8.31)
% Iron deficient at baseline[2]	10.3	7.2
% Iron deficient at end	6.9	3.7
% change in iron deficiency	3.4	3.5

[1] p=0.0009; [2] % TS < 16%; [3] Mean (SD)

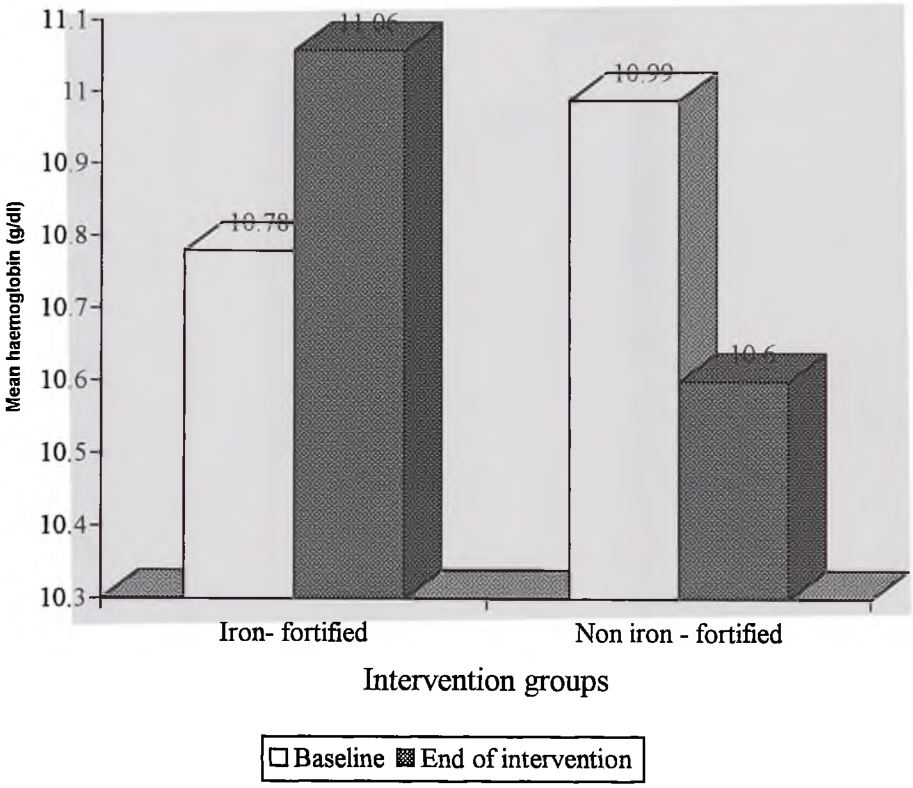


Figure 4.7 Mean Haemoglobin over Intervention Period

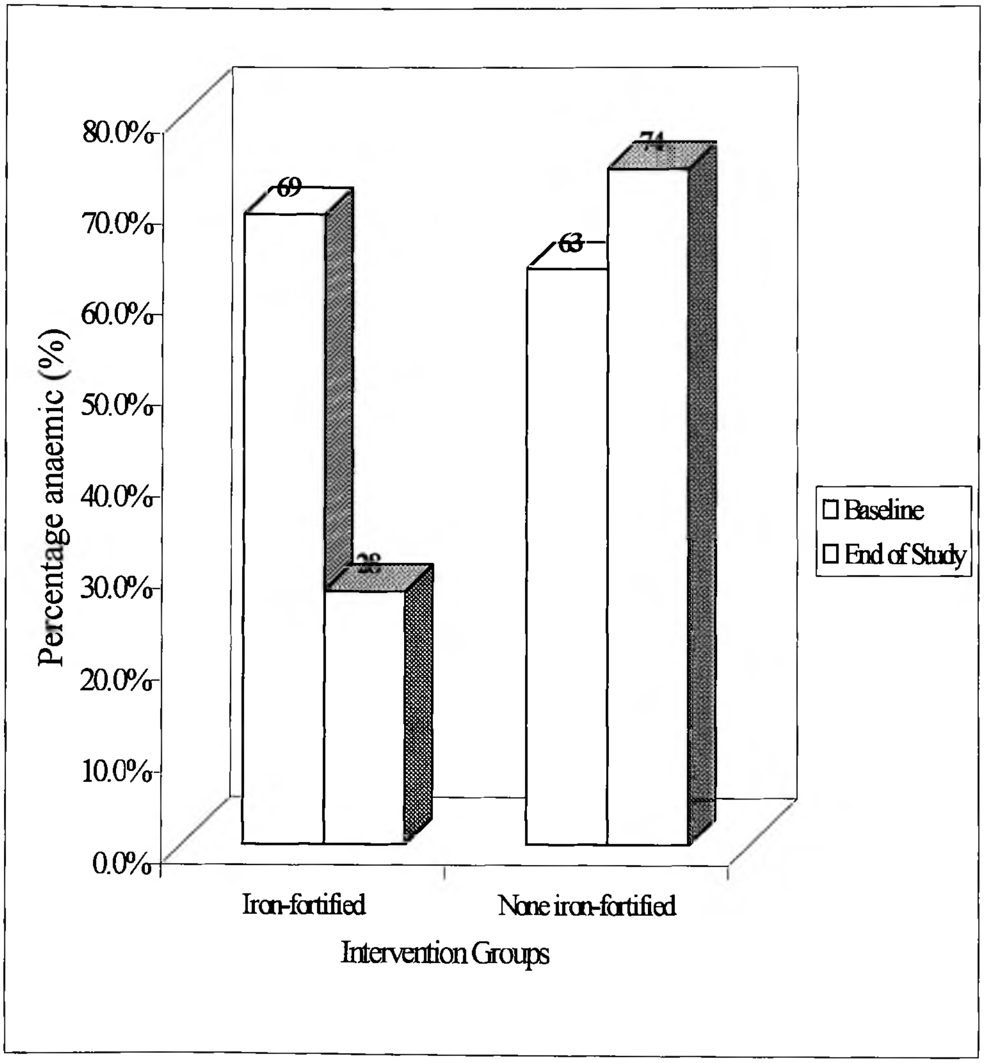


Figure 4.8 Anaemia Prevalence over the Study Period

Changes in other measures of iron status did not reach significant levels. There was a general decline in the means of serum iron at the end of intervention. A smaller change was however observed in the iron-fortified group than in the non iron-fortified group (Table 4.13). The changes in TIBC and percentage transferrin saturation were more pronounced in the iron-fortified group but were not significantly different among the two groups. There was a decrease in the percentage of iron deficiency. At the end of the intervention, number of people with low transferrin saturation declined in both groups but this was not significant. There were no differences between iron-fortified and non iron-fortified groups (Table 4.13). At baseline, the prevalence of iron deficiency was relatively low in both groups. Similar margins of change were observed at the end of the study.

Percentage children with moderate anaemia dropped drastically in the iron-fortified group from about 24% at baseline (Table 4.8) to about 7% (Table 4.14) at the end of intervention. Mild anaemia also declined by more than half the baseline figure. Although the number of children (in the non iron fortified group) with normal haemoglobin concentrations declined, more of the diagnosed anaemia was mild; even moderate anaemia dropped by about 3%.

Table 4.14 Classification of Anaemia at the end of Intervention

	Iron-fortified group (n=29)	Non-iron-fortified group (n=27)	Total (n=56)
Non – anaemic	69.0% (20)	22.2% (6)	46.5% (26)
Mild anaemia	24.2% (7)	40.7% (11)	32.1% (18)
Moderate anaemia	6.9% (2)	37.1% (10)	21.4% (12)

5.0 DISCUSSION

5.1. ANTHROPOMETRY

Growth of pre-school children is commonly used as an indicator of their nutritional status. Generally, the level of malnutrition was relatively low among the children at baseline. The mid-upper-arm circumference (MUAC) identified a higher percentage of malnourished children than weight-for height. This has been observed in other related studies (Velzeboer *et al.*, 1983; Zeitlin, 1986; Gayle *et al.*, 1988). MUAC has been shown to correlate well with clinical and other anthropometric indicators of nutritional status (Chen *et al.*, 1980; The Kasongo Project Team, 1989).

Stunting, which is an index of past chronic malnutrition was relatively low. In the Ghana Demographic and Health Survey conducted in 1988 (Ghana Demographic and Health Survey, 1989), 30% of children who were under five years were stunted and were considered chronically undernourished. The prevalence of stunting peaks between 18 and 23 months and does not increase thereafter. The lower prevalence observed in this study could therefore be due to the age range of children in the study.

Breast-feeding is one of the most effective means of reducing infant mortality and malnutrition, particularly in environments of poor sanitation. The growth rate of exclusively breast-fed infants in developing countries is comparable with that of infants in developed countries (Cohen *et al.*, 1994; Dewey *et al.*, 1992). It is after this period that infants in developing countries deviate from this satisfactory trend. This has been attributed to poor nutrient and energy quality of complementary foods (Rowland and Rowland, 1988).

Exclusive breast-feeding was practised by almost half of the women in the present study. Among those who did not, majority gave complementary foods very early in life, as early as three months of age (Fig.4.2).

Inappropriate weaning practices, including improper feeding and poor sanitary conditions are of critically importance in most cases of malnutrition among children under two years of age. For example, Armar-Klemesu and Wheeler (1991) observed that 30% of infants who were fed cereal porridge and adult foods as weaning foods were malnourished. It is likely that there was current acute, short-duration malnutrition as judged by relatively high wasting. Seasonal fluctuations in food supply and low-density, poor quality traditional porridge *(koko)* fed to infants could have contributed to the observed level. The observed mean intake of energy was much lower than RDA whereas that for protein was within (Table 4.4). The source of drinking water for majority of the people is a pond at the outskirts of the town. Surface water is a source of contamination leading to increased risk of diarrhoeal disease and under nutrition; wasting is almost twice as prevalent among households using an unprotected or surface water compared to those with a private supply of water (Guptill *et al.*, 1993).

If the energy intake from other sources is inadequate, then protein is used as an energy source and not for its primary function of growth and maintenance (Waterlow and Payne, 1975; Van Steenbergen *et al.*, 1978). Lack of adequate energy in the diet of these children (Table 4.3) may have jeopardised the use of protein for growth and maintenance.

A few of the women gave infant formula (lactogen) and infant cereal (cerelac) (Figure 4.6). These commercial weaning foods are expensive and may provide children with less energy and nutrients than they require when the foods are not properly reconstituted, thus increasing the risk of under nourishment. Considering the occupational patterns of the mothers (hence economic status), it is likely that the commercial foods may have been considerably diluted by the mothers to make them last longer.

5.2. IRON DEFICIENCY AND ANAEMIA AT BASELINE

The relatively high prevalence of anaemia observed is in accordance with findings by other researchers (Asibey-Berko *et al.*, 1999; Ministry of Health, 1998). A recent national survey reported that prevalence of anaemia is very high among pre-school children (6 – 59 months) (MOH, 1998). The Intercountry Workshop for National Programme Managers revealed that the figure ranges from 25% to 45% over the African continent (WHO/ICC/IDD, 1998). The highest rate of anaemia among pre-school children is found among 12 – 24 months of age, which could be explained by a rapid growth during the period. At this stage, there is an increase in the total number of red blood cells and in the muscle mass, which occur with normal development.

There is a relatively lower prevalence of iron deficiency (Table 4.13). A few of the children had transferrin saturation below 16%. Other indicators of iron deficiency could have given a different picture. Several physiological studies (FAO/WHO, 1970; Dallman *et al.*, 1980; Bothwell and Charlton, 1981; Cook and Bothwell, 1984; FAO/WHO, 1988; Oski, 1993) have shown that iron stores are more likely to be depleted by 6 months after birth and 2 – 3 years of age. Since there was a high prevalence of anaemia, it was expected that the level of iron deficiency should be high. The interesting observations could be due to the parameters used. Serum ferritin concentration is the most specific biochemical test indicator of body iron stores than transferrin saturation.

Dietary factors could have contributed to the high prevalence of anaemia in this population. A situation analysis of children in 1990 listed poor absorption and intake of iron resulting in negative iron balance as a primary cause of iron deficiency and anaemia among children (WHO/ICC/IDD, 1998).

There is a relatively low iron content in the diet. Mean iron intake at baseline was low (Table 4.4), and was mainly from non-animal sources. Many non-animal foods contain high levels of iron, but the non-heme form of this iron is not highly bioavailable. An overall decrease in consumption of animal protein was observed as evidenced by the low intake of meat, poultry and fish (Figure 4.6). The heme iron in meat is more bioavailable; the meat protein itself is a promotor of iron absorption because of its cysteine and methionine contents. Although mothers claimed they gave fruits, data obtained using food frequency tables revealed that only a few mothers gave oranges, a source of vitamin C, which is an enhancer of dietary iron absorption (Hazell and Johnson, 1987). Fish was fed frequently, but the quantities given were low or negligible as to contribute any significant amount of animal protein to the diet. Intake of dietary inhibitors of iron absorption such as tea and coffee (Hallberg *et al.*, 1987) are equally negligible and could not have contributed to the observed results. Consumption of cereals, particularly maize was high (Fig. 4.6). A study in Nigeria attributed the high rate of anaemia found in children to cereal-based diets among the population (WHO/ICC/IDD, 1998). Phytates and fibre in the maize-based products, which were consumed by the study children, may have contributed to the prevalence of anaemia.

Infections, example malaria and other infestations are important in the aetiology of iron deficiency and anaemia. Aside from dietary inadequacy, malaria is a major contributor to anaemia prevalence. Only a few of the subjects reported of malaria one month prior to the commencement of the intervention. Also worm infestation is not a problem among that age group. Actually, hookworm infestations and infection with *schistosoma haemotobium* did not contribute significantly to iron deficiency and anaemia in infants and young children (Ministry of Health, 1998).

5.3. EFFECT OF INTERVENTION ON BIOCHEMICAL MEASURES

The statistically higher haemoglobin concentration observed in children fed the iron-fortified food at the end of the intervention (Figure 4.7) confirms results from other related studies (Garby and Areekul, 1974; Nadiger *et al.*, 1980; Yeung *et al.*, 1981; Viteri *et al.*, 1981; Stekel *et al.*, 1986; Ballot *et al.*, 1989; Fairweather-Tait and Southon, 1989; Pizarro *et al.*, 1991; Bradley *et al.*, 1993; Walter *et al.*, 1993; Florentino and Pedro, 1998). Varying degrees in the decline of anaemia prevalence and improved iron stores were reported. Most of the findings were significant in terms of measured indicators.

Normal haemoglobin synthesis is dependent on adequate supply of iron, other haemopoietic nutrients as well as normal synthesis of heme and protein synthesis to form globin portion (which forms 97%). Most of the stages in the metabolism of iron are mediated by protein. Iron is transported to the developing red blood cells by transferrin, a plasma protein and stored in the liver as a complex with ferritin, a different protein. The test foods (maize-cowpea flour) were protein-enriched and could have contributed a greater amount of the protein needed for the synthesis of haemoglobin.

Dietary iron absorption depends on dietary iron content, physicochemical form of the food, other dietary constituents, iron status of the individual and food processing techniques (Fairweather-Tait, 1995). The iron dose is generally inversely related to the percentage that is absorbed, both with dietary iron and supplements. However the actual amount absorbed will rise progressively with increasing dietary intake provided that the iron is an assimilable form. Ferrous fumarate, which was added to test food, is highly bioavailable. The processing techniques used (dehulling and fermentation) could promote bioavailability. Phytates may be physically removed through extraction and dehulling of grains. These could have resulted in high bioavailability of iron and could explain the significant association between dietary iron intake test foods and haemoglobin concentration. At baseline no association was found between dietary iron and iron status. These findings are similar to other studies in which no association has been demonstrated between nutrient intake and iron status (Brault-Dubuc *et al.*, 1983; Arija *et al.*, 1990; Duggan *et al.*, 1991).

The greatest amount of the body's iron, about 70% occurs in red blood cells as a vital constituent of the heme portion of haemoglobin. A minute concentration of iron is found in the plasma bound to its transport carrier protein transferrin. Measuring serum ferritin concentration could have given a better indicator of iron in store. But due to financial constraints, ferritin was not measured.

Kruger *et al.*, (1996), observed that mean haemoglobin concentrations remained constant in children who received iron-fortified soup whereas a decreasing trend in haemoglobin concentration was observed in the group that received unfortified soup. Significant improvements in other indices of iron stores such as serum ferritin and mean corpuscular volume were observed. They did not find significant differences in total iron-binding capacity and transferrin saturation between iron-fortified and unfortified groups. They speculated that possible decrease in the rate of viral and bacterial infections had a greater effect on transferrin saturation than the additional iron intake. Infection and iron deficiency are both associated with decreased transferrin saturation but they have opposite effects on total iron-binding capacity. The slight increase in transferrin saturation in both iron-fortified and non iron-fortified groups in this study could be due to decrease in frequent infections.

Badenhorst *et al.*, (1991) reported significant improvements in serum ferritin but not in mean haemoglobin concentration over a 4-month period in a pilot study with the same fortified soup. They attributed this to the short intervention period. Significant differences in serum ferritin but not in serum transferrin saturation were reported in other related studies (Lartey *et al.*, 1998; Green-Finestone *et al.*, 1989). Green-Finestone *et al.*, (1989) reported that significantly fewer infants given iron-fortified cereal were at risk of reaching low haemoglobin values at the end of the trial than controls. Furthermore, infants who received iron-fortified cereal had lower haemoglobin and serum ferritin responses to ferrous sulphate supplement given at the end of the initial trial than controls. Thus, infants who received iron-fortified cereals had better iron status and greater storage than those who did not.

Anaemic subjects respond better and more significantly to iron therapy than no anaemic subjects (Chwang *et al.*, 1988; Soemantri *et al.*, 1985; Kruger *et al.*, 1996). Due to the numbers involved (17 nonanaemic, which is lower than the minimum of 26 subjects and 39 anaemic) in the present study, and the fact that the subjects were not grouped on their baseline haematological indices, it can not said with confidence that anaemic subjects in the study responded better. However the significant negative correlation ($r= - 0.5803$; $p=0.003$) observed between initial haemoglobin and the change in haemoglobin indicates that children with the lowest initial haemoglobin concentrations had the largest changes.

5.4. EFFECT OF INTERVENTION ON GROWTH

Iron is needed for a variety of physiological functions, apart from oxygen transport, that is necessary for growth. Iron deficiency has been associated with reduced appetite, although the mechanism for this effect is not yet understood. There are several reasons to believe that iron deficiency anaemia retards growth, but there is little evidence that iron therapy alone in anaemic children results in faster growth. Perhaps this is because iron is rarely the most limiting nutrient or when it is and the deficiency is corrected, other deficiencies (of protein, energy, or zinc for example) soon become limiting

In the present study, significantly faster gains were observed in length and MUAC over 6 months (Table 4.11). At the end of intervention, length gains were significant in the iron-fortified group. Other researchers in related studies reported similar gains in length and MUAC (Chwang *et al.*, 1988; Angeles *et al.*, 1993; Lawless *et al.*, 1994). Chwang *et al.*, (1988) reported faster gains in height and MUAC in treatment group than in control group over 12 weeks. Angeles *et al.*, 1993 observed a faster height gain in treatment than in controls. Faster height gain was reported by Lawless. Idjradinata *et al.*, (1994), observed that gains in length and MUAC did not differ significantly by treatment with iron in iron-replete children and that weight gains were similar in both iron-fortified and control groups

Iron supplementation however, resulted in growth retardation in iron-replete children in some studies. Idjradinata *et al.* (1994), found that there was an adverse effect of iron on weight gains of iron-replete young children but no reduction in linear growth occurred suggesting that any such negative effect was not significant. This finding had however, not been apparent in other small studies (Stephenson, 1994).

The development of cowpea-fortified food and its use in intervention studies has been well-documented (Sefa-Dedeh *et al.*, 1997). Maize-cowpea flour is an improved weaning food (Table 4.5) over the traditional *koko* and this could be responsible for the similar weight gains observed. Mothers were educated on good nutrition and environmental practices. It is likely that they practised proper child feeding procedures. The study protocol involved regular monitoring of the children. It is likely that mothers were more willing to report any health problems to the community health centre for immediate attention.

Habicht (1980) observed that increments in height are more sensitive than increments in weight in identifying the benefits of an improved diet in moderately malnourished children whereas weight is more useful in severe malnutrition than height. Some studies have also reported length increments with iron supplementation. Improved appetite and lower incidence of morbidity have been suggested as plausible explanation (Angeles *et al.*, 1993; Lawless *et al.*, 1994). However, these factors were not adequately explored in the current study.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSION

The following conclusions were drawn from the study:

The consumption of iron-fortified maize-cowpea flour, which is used as a weaning food, over the six-month intervention period, had a significant effect on haemoglobin levels of infants and young children. Fewer children were diagnosed as having low haemoglobin concentration at the end of the study.

The prevalence of anaemia among the children studied declined considerably in the test group. There were no significant effects of the intervention on serum iron, total iron-binding-capacity and transferrin saturation levels.

There was a positively significant effect on length gain and mid-upper-arm circumference. Length gains in subjects on iron-fortified flour were significantly higher than those on the non iron-fortified flour. There was however no effect on weight gain.

As dietary iron intake has been found to be a significant factor in the aetiology of iron deficiency anaemia, these results have important implications in the prevention and treatment of iron deficiency anaemia and in improving linear growth.

6.2 RECOMMENDATIONS

The following recommendations are made:

Mothers should be educated on good infant and child feeding practices. This will enable them to know the nutrient needs and some sources of iron-rich foods suitable for the children. There should be increased consumption of iron absorption enhancers such as meat, generous quantities of fish, fresh fruits and vegetables as these are rich in ascorbic acid and citric acid.

Considering that maternal iron deficiency is a contributory factor to iron deficiency in infants, young girls should be educated on the importance of increasing their dietary iron intakes. This will ensure that they enter pregnancy with adequate iron stores.

There should be adequate intake of other complementary nutrients, which are involved in iron absorption and metabolism, particularly vitamin A, folate, riboflavin, and vitamin B12.

Future studies assessing iron status should include the measurement of serum ferritin, which is a better indicator of sub-clinical iron-deficiency state alongside the measurement of an acute-phase protein (eg: C-reactive protein) to help identify false elevation due to infections.

REFERENCES

Abdalla, S. M. 1990. Iron and folate status in Gambian children with malaria. Annals of Trop Pediatr 10, 265-72.

ACC/SCN 1991. Statement on iron deficiency control. *In* Gillespie, S. R., Mason, J. B., Kevany, J. ed., Controlling iron deficiency. ACC/SCN, World Health Organisation, Geneva, Switzerland, state-of-the-art series, Nutrition Policy Discussion Paper 9.

ACC/SCN 1992. Second Report on the World Nutritional Situation. Volume 1: Global and regional results. ACC/SCN, WHO, Geneva, Switzerland.

Akenzua, G. I., Ihongbe, J. C., Imasuen, I. W. 1985. Haemapoietic responses of Nigerian village children to iron, folate supplementation and malaria prophylaxis. J Trop Paediatr, 31, 59 - 6

Allen, L. H. 1994. Nutritional influences on linear growth; a general review. Eur. J Clin Nutr 48 (suppl. 1 S75-S89)

Angeles, I. T., Schultink, W. J., Matulessi, P., Gross, R., Sastroamidjojo, S. 1993. Decreased rate of stunting among anaemic Indonesian pre-school children through iron supplementation. Am J Clin Nutr, 58 (3), 339-342.

Arija V., Salas, J., Fernandez, Ballart, J., Marti, Henneberg, C. 1990. Iron deficiency risk in children: discrepancy between dietary and biochemical assessments. Int J Vitam Nutr Res, 60, 150 – 155.

Armar-Klemesu, M. A., Wheeler, E. F. 1991. Weaning practices and their outcome: a critical look with special reference to Ghana. Bulletin of Noguchi Memorial Institute for Medical Research, 4, 3 – 24.

Artiss, J. D., Vinogradov, S., Zak, B. 1981. Colorimetric method for determining serum iron and unsaturated serum iron binding capacity. Clin Biochem, 14, 311 - 315.

Asibey-Berko, E., Zlotkin, S. H., Nti-Nimako, W., Faried, K. 1999. The impact of double-fortified salt on anaemia and iodine deficiency. *In* Asibey-Berko, E., Tayie, F. A. K. ed. Proceedings of the workshop on double-fortified salt. Ghana Universities Press, Accra. Pp 28 – 38.

Aukett, M. A., Parks, Y. A., Scotts, P. H., Wharton B. A. 1986. Treatment with iron increases the weight gain and psychomotor development. Arch Dis Child, 61: 849-57.

Badenhorst, C. J., Kruger, M., Benade, A.J.S., Mansvelt, E., Laubscher, J. A., Swart, R. 1994. Iron and vitamin C fortification in primary school children at risk of iron deficiency and anaemia: a cohort pilot field study. S Afri J Food Sci and Nutr 6 (suppl), 4.

Ballot D. E., McPhail A. P., Bothwell, T. H., Gillooly. M., Mayet, F. G. 1989. Fortification of curry powder with NaFe(II)EDTA in an iron-deficient population; a report of a controlled fortification trial. Am J Clin Nutr 49, 162-169.

Basta, S. S., Soekirman, Karyadi, D, Scrimshaw N. S. 1979. Iron deficiency anaemia and the production of adult males in Indonesia. Am J Clin Nutr 32, 916-925

Beard, J. L. and Borel, M. J. 1988. Thermogenesis and iron deficiency anaemia. Nutrition Today. 23: 41-45.

Beard, J. L., Borel, M. J., Derr, J. 1990. Impaired thermoregulation and thyroid function in iron deficiency anaemia. Am J Clin Nutr, 52 (5), 813-819.

Bhaskaram, C., Reddy, V. 1975. Cell-mediated Immunity in iron and vitamin deficient Children. Brit Med J, 3, 522.

Bhatia, D., Seshadri, S. 1993. Growth performance in anaemia and following iron supplementation. Paediatr 30, 195 - 200.

Boreham, P. F. L., Lenahan, J. K., Port, G. R., McGregor, I. A. 1981. Haptoglobin polymorphism and its relationship to malaria infection in the Gambian. Trans R Soc Trop Med Hyg, 75, 183-200.

Bothwell, T.H and Charlton, R.W. 1981. Iron deficieny in women: a report of the International Nutritional Anaemia Consultative Group (INACG). Nutr Foundation, Washington DC, USA.

Brabin, B. J. 1992. The role of malaria in nutritional anaemias. *In* Fomon, S. J., Zlotkin, S. ed. Nutritional anaemias. Vevey/Raven Press, Ltd., New York. Nestle Nutrition Workshop Series 30.

Bradley, C. K., Sherman, A. R., Leedy, D., Cordana, A. 1993. Evaluation of two iron-fortified, milk-based formulas during infancy. Pediatr, 91(5), 908-914.

Brault-Dubuc, M., Nadeau, M., Dickie, K. 1983. Iron status of French Canadian children: a three-year follow-up study. Hum Nutr Appl Nutr, 37A, 210 - -221.

Briend, A; Hoque, B.A and Aziz, K.M.A. 1990. Iron in tube well water and linear growth in rural Bangladesh. Arch Dis Child, 65, 224-225.

Brown, K. H., et al., 1995. Complementary feeding of infants and young children. WHO/AFRO/NUT96.2. Pp 38 - 44.

Calvo, E. B., Ginazzo N. 1990. Prevalence of iron deficiency in children aged 9 –24 months from a large urban area of Argentina. American Journal of Clinical Nutrition, 52, 534 –540.

Chan-Yip A., Gary- Donald K. 1978. Prevalence of iron deficiency anaemia in Chinese children aged 6 – 36 months in Montreal. Can Med Assoc J, 136, 373-378.

Charlton, R.W. and Bothwell T.H. 1983. Iron absorption. A Revue of Medicine, 34, 55-68.

Chen, L. C., Alauddin, Choudry, A. K. M., Huffman, S. L. 1980. Anthropometric assessment of energy-protein malnutrition and subsequent risk of mortality among preschool-aged children. Am J Clin Nutr 33, 1836 – 45.

Chwang, L. C., Soemantri, A. G., Pollitt, E. 1988. Iron supplementation and physical growth of rural Indonesian children. Am J Clin Nutr, 47 (3), 496-501.

Clark, M., Royal, J. Seeler, R. 1988. Interactions of iron deficiency and lead and the haematological findings in children with severe lead poisoning. Paediatr, 81, 247-254.

Cohen, R. J., Brown, K. H., Canahuati, J., Landa-Rivera, L., Dewey, K. G. 1994. Effects of age of introducing complementary foods on infant breast milk intake, total energy and growth: a randomized intervention study in Honduras. Lancet, 344, 288-93.

Cook, J. D. and Reusser, M. E. 1983. Iron fortification: an update. Am J Clin Nutr, 38. 648-659.

Cook, J. D., Bothwell, T.H. 1984. Availability of iron from infant foods. In: Stekel, A., Iron nutrition in infancy and childhood. New York: Nestle/Raven Press, 119 –45.

Dallman, P. R., Siimes, M. A., Stekel, A., 1980. Iron deficiency in infancy and childhood. Am J Clin Nutr 33, 86-118.

DeMaeyer,E. M., Dallman, P., Gurney, I. M., Hallberg, L., Sood, S. K., Srikantia, S. G. 1989. Preventing and controlling iron deficiency anaemia through Primary Health Care. Geneva: WHO 1989.

Dewey, K. G., Peerson, M. J., Heining, M. J., et al., 1992. Growth patterns of breast-fed infants in affluent (United States) and poor (Peru) communities: implications for timing of complementary feeding. Am J Clin Nutr, 56, 1012-8.

Dillman, E., Gale C., Green W. 1982. Hypothermia in iron deficiency due to altered triiodothyronine metabolism. Am J Physiol, 239, R377 - R381.

Draper, C. C. 1960. Effect of malaria control on haemoglobin levels. Br Med J 1960; i 11480-3.

Dreyfuss, M. L., Shrestha, J. B., Khatry, S. K. et al. 1996. Relationships between iron status and helminth infection among pregnant women in Nepal. Federation of American Societies for Experimental Biology Journal , 10(3), A730, 4219.

Duggan, M. B., Steel, G., Harbottle, L., Noble, C. 1991. Iron status, energy intake and nutritional status of healthy young Asian children. Arch Dis Child, 1386 – 1389.

Edgerton, V.R. 1981. Elevation of haemoglobin and work performance in non-iron-deficient subjects. Journal of Nutritional Science, 27:77-86.

Enwonwu, C. O., ed., 1990. Functional significance of iron deficiency. Merry Medical College, Nashville, Tenn, USA. Pp 1 – 13.

Eyeson, K., Ankrah, E. K., Sundarnrajan, A. R., Karinpaa, A., Rudzka, J. M. 1975. Composition of foods commonly used in Ghana. Accra, Ghana: Food Research Institute. Council for Scientific and Industrial Research.

Fairweather-Tait, S. J. 1995. Bioavailability of iron. *In* Nestel, P., ed. Iron intervention for child survival: proceedings of the 17 – 18 May 1995 Conference, London. Opportunities for Micronutrient Interventions, Washington, D.C., USA. Proceedings.

Fairweather-Tait, S. J., Southon, S. 1989. Studies of iron:zinc interactions in adult rats and the effect of iron fortification of two commercial infant weaning products on iron and zinc status of weanling rats. J. Nutr., 119 (4), 599-606.

FAO/WHO 1970. Requirements of ascorbic acid, vitamin D Vitamin B12, folate and iron. WHO, Geneva, Switzerland. Technical Report Series 452.

FAO/WHO 1988. Requirements of vitamin A, iron, folate and vitamin B12. FAO, Rome, Italy. Food and Nutrition Series 23.

FAO/WHO/UNU 1985. Energy and protein requirements. WHO, Geneva, Switzerland. Technical Report Series 724.

Fleming, A. F. 1998. Haematological Diseases. In Strickland, G.T. ed., Hunter's Tropical Medicine, 8th edition. Saunders, W.B. Philadelphia, PA, USA.

Florentino, R.F., Pedro, R. 1996. Update on rice fortification. Philippines Food and Nutrition Research Institute, Manila, Philippines.

Food and Nutrition Board, National Academy of Sciences. National Research Council Recommended Dietary Allowances, Revised, 1989.

Fulton, J. D., Maegraith, B. G. 1949. The Physiologic Pathology of malaria. In: Boyd M. F., ed., Malariology. Philadelphia: Saunders, W. B.

Garby, L. and Areekul, S. 1974. Iron supplementation in Thai fish sauce. Annals of Medicine and Parasitology, 467-476.

Garn S. N., Ridella, S. A., Petzold, A. S and Falkner, F. 1981. Maternal haematologic levels and pregnancy outcomes. Seminars in Perinatology, 5, 155 - 162.

Gayle, H. D., Binkin, N. J., Staehling , N. W., Trowbridge, F. L. 1988. Arm circumference versus weight-for-age in nutritional assessment: are the findings comparable? J Trop

Ghana Demography and Health Study. 1989. Ghana Statistical Institute for Resource Development/Macro Systems Inc. Columbia, Maryland, USA.

Gleerup, A., Rossander-Hallen, L., Gramatkovski, E., Hallberg, L. 1995. Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. Am J Clin Nutr, 61, 97 – 104.

Goodwin, J. F., Murphy, B., Guillette, M. 1966. Clin Chem 12, 47.

Greene-Finestone, L., Feldman, W, Heick, H., Luke, B. 1989. Infant feeding practices and socio-demographic factors in Ottawa-Carlton. Can J Public Health, 80:173-6.

Greenham, R. 1978. Anaemia and *schistosoma haematobium* infection in the northeastern province of Kenya. Trans R Soc Trop Med Hyg, 72:72-75.

Guptill, K. S., Esrey, S. A., Oni, G, A., Brown, K. H. 1993. Evaluation of a face-to-face weaning food intervention in Kwara State, Nigeria: knowledge, trial and adoption of a home-prepared weaning food. Soc Sci Med, 36, 665-72.

Guthrie, H.A. 1986. Introductory Nutrition. 6th edition. Times Mirror/Mosby College Publishing. Pp 526-553.

Habicht, J. P. 1980. Some characteristics of indicators of nutritional status for use in screening and surveillance. Am J Clin Nutr33, 531 – 35.

Hahn, P. F., Carothers, E. E., Darby, W.J., Martin, M., Sheppard, C. W., et al., 1951. Iron metabolism in early pregnancy as studied with the radioactive isotope 59Fe. Am J Obstetr and Gynecol, 61, 499 – 486.

Hallberg, L., Rossander, L., Skanberg, A. B. 1987. Phytates and the inhibitory effect of bran on iron absorption in man. Am J Clin Nutr, 45(5), 988 – 996.

Hallberg, L., Rossander-Hulten, L., Brune, M., Gleerup, A. 1992. Calcium and iron absorption: mechanism of action and nutritional importance. Eur J Clin Nutr, 46(5, 317 – 327).

Hazell, T., Johnson, I. T. 1987. In vitro estimation of iron availability from a range of plant foods: influence of phytates, ascorbate and citrate. Brit J Nutr, 57 (2), 223 – 233.

Heldenberg, D., Tenembaum, G., Weisman, Y. 1992. Effect of iron on serum 25-hydroxyvitamin D and 24, 25-dihydroxyvitamin D concentrations. Am J Clin Nutr, 56, 533 – 536.

Hurrell, R. F., Lynch, S. R., Trinidad, T. P. 1989. Iron absorption in humans as influenced by bovine milk proteins. Am J Clin Nutr. 49. 546-552.

Hussaini M. A; Karyadi H. D and Gunadi H. 1981. Evaluation of nutritional anaemias intervention among anaemic female workers on a tea plantation. *In* Hallberg L, Scrimshaw N.S. ed. Iron deficiency and work performance. Nutrition Foundation, Washington, DC, USA. Pp 72-85

Hussein, M., Hassan, H. A., Abdel-Ghaffer, A. A. 1988. Effect of iron supplements on the occurrence of diarrhoea among children in rural Egypt. Food and Nutrition Bulletin, 10(2), 35 – 39.

Idjradinata, P., Watkins, W. E. and Pollitt, E. 1994. Adverse effect of iron supplementation on weight gain of iron-replete young children. Lancet, 343 (8908), 01252-1254.

INACG (International Nutritional Anaemia Consultative Group). 1986. Combating iron deficiency in Chile: a case study. INACG, Nutrition Foundation, Washington, DC, USA.

Judisch, J. M., Naian, J. L., Oski, F. A. 1986. The fallacy of the fat iron-deficient child. Paediatr 37, 987 - 992.

Kariks, J. 1969. Iron deficiency- a widespread cause of anaemia in coastal areas of New Guinea. Med J Aust, 2, 1289-95.

Koblinsky, M. 1995. Beyond maternal mortality- magnitude, interrelationship and consequences of women' health, pregnancy related complications and nutritional status on pregnancy outcomes. Int J Gynaecol and Obstetr, 48(supplement), S21-S32.

Kruger, M., Badenhorst, C. J., Mansvelt, E. P. G., Laubscher, J. A., Spinnler, Benade, J. A. 1996. Effects of iron fortification in a school feeding scheme and anthelmintic therapy on the iron status and growth of six- to eight- year old school children. Food and Nutrition Bulletin, 17(1), 11-21.

Lartey, A., Manu, A., Brown, K. H., Peerson, J. M., Dewey, K. G. 1999. A randomised, community-based trial of the effects of improved centrally processed complementary foods on growth and micronutrient status of Ghanaian infants form 6 to 12 mo of age. Am J Clin Nutr., 70, 391-404.

Latham, M. C., Stephenson, L. S., Kinoti, S. N., Zaman, M. S., Kurk, K. M. 1990. Improvements in growth following iron supplementation in Kenya children. Nutrition. 1990. 6: 159-65.

Lawless, J. W., Latham, M. C., Stephenson, L. S. Iron supplementation improves appetite and growth in anaemic Kenyan primary school children. J Nutr, 1994; 124: 645-654.

Layrisse, M., Garcia-Casal, M. N., Solano, L., Baron, M. A., Arguello, F. et al., 1998. Vitamin A reduces inhibition of iron absorption by phytates and polyphenols. Food and Nutrition Bulletin, 19 (1), 3.

Levin, H. M. 1986. A benefit-cost analysis of nutritional programs for anaemia reduction. Research Observer, 1, 219 – 246.

Li, R., Chen, X., Yan, H., Deurenberg, P., Garby, L., Hautvast, J. G. 1994. Functional consequences of iron supplementation in iron-deficient female cotton mill workers in Beijing, China. Am J Clin Nutr, 59(4), 908 – 913.

Lonnerdal, B. 1991. Iron intake and requirements: interactions with other trace elements. Carnation nutrition education series, 2, 199-211.

Lozoff, B. 1990. Has iron deficiency been shown to cause altered behaviour in infants? In Dobbing, J., ed., Brain, behaviour and iron in the infant diet. Springer-Verlag, London, UK. Pp 107 – 131.

Lozoff, B., Jimenez, E., Wolf, A. W. 1991. Long-term developmental outcome of infants with iron deficiency. New Engl J Med, 325, 687 – 694.

Martinez-Torres, C., Cebeddu, L., Dillman, E. 1984. Effective exposure to low temperature on normal iron-deficient subjects. Am J Physiol, 246, R380 – R383.

Martorell, R. 1997. Undernutrition during pregnancy and early childhood and its consequences for cognitive and behavioural development. *In* Young, M. E. ed. Early childhood development: investing in our children's future. Elsevier Science, Amsterdam, Netherlands. Pp 39 – 83.

McGregor, I. A. 1988. Malaria and nutrition. *In* Wernsdorfer, W. H., McGregor, I. A., ed. Malaria. Principles and practice of malariology, Vol 1. Edinburgh: Churchill Livingstone, 753-67.

McGregor, M. W. 1963. Maternal anaemias as a factor in prematurity and prenatal mortality. Scottish Medical Journal, 8, 134.

Mejia, L. A., Arrovaye, G. 1982. The effect of vitamin A fortification of sugar on iron metabolism in preschool children in Guatemala. Am J Clin Nutr, 36, 87 – 93.

Ministry of Health, Nutrition Division. 1998. Abstract from workshop on prevalence of anaemia and vitamin A deficiency.

Molyneux, M., Looareesuwan, S., Menzies, I. S. *et al.* 1989. Reduced hepatic blood flow and intestinal malabsorption in severe falciparum malaria. Am J Trop Med Hyg, 40, 470-6.

Monsen, E. R., Hallberg, L., Larrisse, M., Hegstead, D. M., Cook, J. D., Mertz, W., Finch, C. A. 1978. Estimation of available dietary iron. Am J Clin Nutr, 31, 134 – 41.

Murphy, J. F., O'Riordan, J., Newcombe, R. G., Coles, E. C., Pearson, J. F. 1986. The effect of haemoglobin levels in the first and second trimesters to outcome of pregnancy. Lancet, 1, 1992 – 995.

Nadiger, H. A. *et al* 1980. The use of common salt (NaCl) fortified with iron to control anaemia: Result of a preliminary study. Brit J Nutr 43, 45

Nagel, R., Fleming, A. F. 1992. The genetic epidemiology of the BS genes. Baillieres Clinical Haematology, 5 331 – 365.

Naiman, J. L., Oski, F. A., Diamond, L. K. 1964. The gastrointestinal effects of iron deficiency anaemia. Paediatr, 33, 83 – 99.

National Centre for Health Statistics. NCHS growth curves for children from birth to 18 years. US Department of Health, Education, And Welfare, Washington, DC: Government Printing Office, 1975.

Ohira, Y., Edgerton, V.R., Gardner, G.W., Gunawardena, K. A., Senewiratne, B.,Ikawa, S. 1981. Work capacity after iron treatment as a function of hemoglobin and iron deficiency. Journal of Nutritional Science and Vitaminology, 27(2), 87 – 96.

Ohira, Y., Edgerton, V.R., Gardner, G.W., Senewiratne, B., Barnard, R.J., Simpson, D.R. 1979. Work capacity, heart rate and blood lactate responses to iron treatment. Brit J Haematog, 41(3), 365 – 372.

Osborne, D. R., Voogt, T. 1978. The analysis of nutrient of foods. Academic Press London.

Oski, F. A. 1993. Iron deficiency in infancy and childhood. N Engl J Med, 329, 190-3.

Passmore, R., Eastwood, M. A. 1986. Human Nutrition and Dietetics. Longman Group Limited, Churchill Livingstone, New York.
Pediatr 34, 213 - 17.

Perry, G. S., Byers, T., Yip, R., Margen, S. 1992. Iron nutrition does not account for haemoglobin differences between blacks and whites. J Nutr, 122, 1417 - 1424.

Powers, H. J., Bates, C. J., Prentice, A. M. 1983. The relative effectiveness of iron and iron with riboflavin in correcting a microcytic anaemia in men and children in rural Gambia. Human Nutrition and Clinical Nutrition, 37C, 413 – 425.

Punnonen, K., Irjala, K., Rajamaki, A. 1944. Iron deficiency anaemia is associated with high concentrations of transferrin receptor in serum. Clin Chem 40, 774 - 776.

Ross, J. S., Thomas, E. L. 1996. Iron deficiency anaemia and maternal mortality. Academy of Educational Development, Washington, DC, USA. PROFILES 3, Working Notes Series 3.

Rowland, R. G. M., Rowland, S. G. J. 1988. Impact of infection on growth of children from 0 - 12 years in an urban West African community. Am J Clin Nutr., 47, 138 - 40.

Ruff, H. A., Bijur, P. R., Markovitz, M., Ma, Y. C., Rosen, J. F. 1993. Declining blood lead levels and cognitive changes in moderately lead-poisoned children. J Am Med Assoc 269, 1641 1646.

Rush, D., Horvitz, D. G., Seaver, W. B., Alvir, J. M., Garbowski, G. C., et al., 1988. The national WIC evaluation: evaluation of the special supplemental food program for women, infants and children: I- Background and introduction. Am J Clin Nutr, 48(supplement 2), 389 - 393.

Scholz, B. D., Gross, R., Schultink, W., Sastroamidjojo, S. 1997. Anaemia is associated with reduced productivity of women workers even in less-physically-strenous tasks. Brit J Nutr, 77(1), 47 – 57.

Scrimshaw, N. S. 1991. Iron deficiency. Scientific America. October edition. Pp 46 – 52.

Scrimshaw, N. S. 1996. International workshop on iron deficiency anaemia. Food and Nutrition Bulletin, 17 (1), 1 – 2.

Sefa-Dedeh, S., Amegatse, W. and Lartey, A. 1997. Food production, nutrition and family health. A case study of the nutritional status of cowpea farming families in the Ga Rural District.

Soemantri, A. G., Pollitt,E., Kim, I. 1985. Iron deficiency anemia and educatiional achievement. Am J Clin Nutr, 42, 1221 - 8.

Sosi, S. 1999. Effects of iron fortification and fermentation on processing, functional, microbiology and storage properties of maize blend-weaning foods. M Phil thesis. Department of Nutrition and Food Science, University of Ghana, Legon.

Stekel, A., Beyda, V., Monckeberg, F. 1986. Combating iron deficiency in Chile:a case study. International Nutritional Anemia Consultative Group (INACG), Washington, DC, USA.

Stephenson, L. S. 1987. Impact of helminth infections on human nutrition. Taylor and Francis, New York, N. Y. USA.

Stephenson, L. S., Latham, M. C., Kurz, K. M. 1985. Relationships of schistosoma haematobium, hookworm and malarial infections and metrifonate treatment to haemoglobin level in Kenyan school children. Am J Trop Medicine and Hygiene, 4(3), 519 - 528.

Stephenson, L. S. 1995. Possible new developments in community control of iron deficiency anaemia. Nutrition Reviews, 53 (2), 23 – 30.

Stephenson, L. S., Kinoti, S. N., Latham, M. C. *et al.* 1989. Single dose metrifonate or praziquantel treatment in Kenyan children- i: Effects of schistosoma haemotobium, hookworm, haemoglobin levels, splenomegaly and hepatomegaly. Am J Trop Med Hyg, 41, 436 - 444.

Stinnert, J. D. 1983. Nutrition and the immune response. CRC Press, Boca Raton, FL, USA.

Stoltzfus, R. J., Dreyfuss, M. L. 1997. Guidelines for the use of iron supplements to prevent and treat iron deficiency anaemia: a draft document prepared for the INACG, 17th July 1997. INACG Nutrition Foundation, Washington, DC. USA Mimeo.

The Child Cannot Wait. A National Programme of Action on the follow-up to the World Summit for Children. Accra, June 1992. Pp 9 – 14.

The Kasongo Project Team. 1989. Anthropometric assessment of young children's nutritional status as an indicator of subsequent of dying. J Trop Pediatr 29, 77 - 83.

Tulchinsky, T. H., Ebweini, S. E., Ginsberg, G. M., Abed, Y., Montano-Cuellar, D., Schoenbaum M., Zansky, S. M., Jacobs, S., Tibbi, A. J., Abu Sha'aban, Koch, J., Melnick, Y. 1994. Growth and nutritional patterns of infants associated witrh a nutrition education and supplementation programme in Gaza, 1987 - 1992. WHO Bull, 72(6), 869 - 875.

Tumbi, Z. and Dodd, N. S. 1990. Effect of ferrous fumarate on the iron status and physical work capacity of women. Nutr Res, 10(12), 1375 - 1384.

Van Schenck, H., Falkensson, M., Lundberg, B. 1986. Evaluation of "Hemocue", a new device for determining haemoglobin. Clin Chem, 32, 526 – 529.

Van Steenbergen, W. M., Kusin, J. A., Voorhoeve, A. M., Jansen, A. A. J. 1978. Machakos Project Studies: Agents affecting health of mother and child in a rural area of Kenya. Food intake, feeding habits and nutritional state of the Akamba infant and toddler. Tropical and Geographical Med, 30, 505 - 522.

Velez, H., Restrepo, A., Vitale, J. J., Hellerstein, E. E. 1966. Folic acid deficiency secondary to iron deficiency in man: remission with iron therapy and a diet low in folic acid. Am J Clin Nutr, 19, 27 - 36.

Velzeboer, M. I., Selwyn, B. J., Sargent, I. I. Fi., Pollitt, E., Delgado, H. 1983. Evaluation of arm circumference as a public health index of protein-energy-malnutrition in early childhood. J Trop Pediatr 29, 135 - 43.

Villar, J., de Onis, M., Kesler, E., Bolanos, F., Cerezo, R., Berneded, H. 1990. The differential neonatal morbidity of the intrauterine growth retardation syndrome. Am J Obstetr and Gynecol, 163 (1,part 1), 151 - 582.

Viteri, F. E., Alvarez, E., Bulux, J., Pineda, O., Bartres, R. 1981. Iron fortification in developing countries: In Harper, A. E., Davis, G. K., ed., Nutrition in health and disease and international development: Symposia from the 12th International Congress of Nutrition. Alan R. Liss, New York, N Y, USA. Progress in Clinical and Biological Research, vol. 77. Pp 345 - 354

Walt, F., Holman, S., Hendrickse, R. G. 1956. Megaloblastic anaemia of infancy in Kwashiorkor and other diseases. Br Med J, I, 1199 - 203.

Walter, T., Dallman, P. R., Pizarro, F., Velozo, L., Pena, G., *et al.*, 1993. Effectiveness of iron-fortified infant cereal in prevention on ron deficiency aneima. Pediatr, 91(5), 976 - 982.

Waterlow, J. C., Payne, P. R. 1975. The protein gap. Nature, 258, 113-117.

Watson, W. S., Hume, R. M., Moore, M. R. 1980. Oral absorption of Pb and Fe. Lancet, ii, 236 - 237.

WHO, 1968. Nutritional anaemias: report of a WHO research group. WHO, Geneva, Swwitzerland. Technical Report Series 405.

WHO, 1991. National strategies for overcoming micronutrient malnutrition. WHO, Geneva, Switzerland Doc. EB89/27.

WHO, 1996. Control of iron deficiency anaemia in South-East Asia: report of an Intercountry Workshop, Mahidol University, Salaya, Thailand 11-14 December 1995. WHO, Geneva, Switzerland.

WHO/ICC/IDD. 1998. WHO Intercountry Workshop for National Program Managers: Micronutrient deficiencies in Africa.

WHO/UNICEF/UNU 1996. Indicators for assessing iron deficiency and strategies for its prevention (draft based on a WHO/UNICEF/UNU Consultation 6 – 10 December 1993). WHO, 20 Avenue Appia, CH-1211, Geneva 27, Switzerland.

Yeung, D. L., Penell, M., Hall, J., Anderson, G. H. 1981. Iron intake of infants: the importance of infant cereals . Can Med Assoc J, 125, 99-1002.

Yip R. 1990. Multiple interactions between childhood iron deficiency and lead poisoning: evidence that childhood lead poisoning is an adverse consequence of iron deficiency. In: Hercberg, S., Galan, P., Dupin, H. ed., Recent knowledge on iron and folate deficiencies in the world. Istitut national de la sante et de la re cherche medicale (INSEM), Paris, France, Colloque, 197.

Yip, R. 1989. The changing characteristics of childhood iron nutritional status in the United States. In: Filer, L. Jr., ed., Dietary iron: birth to 2 years. New York: Raven Press. Pp 37 - 61.

Yip, R. 1994. Iron deficiency: contemporary scientific issue and international programmatic approaches. J Nutr, 124, 1479S-1490S.

Yip, R., Johnson, C., Dallman, P. R. 1984. Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. Am J Clin Nutr ,39 426 - 36.

Yip, R., Stolzfus, R., Simmons, W. K. 1996. Assessment of the prevalence and the nature of iron deficiency for populations: the utility of comparing haemoglobin distributions. *In* Hallberg, L., Asp, N. G. ed. Iron nutrition in health and diseases. John Libbey and Co. Ltd., London, UK.

Zeitlin, M. F. 1986. Comparison of malnourished children selected by weight-for-age, mid upper arm circumference and maximum thigh circumference. J Trop Pediatr 32, 190 - 95.

Zhu, Y. I., Haas, J. D. 1998. Altered metabolic response in iron-depleted nonanemic young women during a 15-Km time trial. J Appl Physiol, 84(5), 1768 - 1775.

APPENDICES

APPENDIX I Questionnaire and consent form

Department of Nutrition and Food Science

University of Ghana

CONSENT FORM

Name of Child:.. ID. No..............

The Department of Nutrition and Food Science, University of Ghana, in collaboration with other organisations, wish to conduct an investigation in your community. The objective of the trial is to study the efficacy of iron – fortified food in improving iron nutrition. Mothers and their pre-school children between 6 and 18 months of age are invited to participate in our study.

Questions will be asked on socio – economic status of female guardians, health and feeding practices. Mothers will be given either iron – fortified or non–iron-fortified flour to prepare for their children at home. Food that will be given meets all standards for human consumption. Before and after the feeding period, a number of examinations and laboratory tests on blood samples will be performed in the same manner as done in a clinic or hospital. Feeding will be done for six months.

If you decide some reason to exclude your child from the trial, you are free to do so.
All findings of the trial will be confidential, and used for the benefit of the child and the purpose of the study.
I understand these conditions and agree to participate in the study.

____
Signature/Right Thumb print
of Parent/Guardian of child.
Date ....

____
Signature of Enumerator

DEPARTMENT OF NUTRITION AND FOOD SCIENCE
UNIVERSITY OF GHANA

QUESTIONNAIRE FOR COLLECTING DATA ON THE EFFICACY OF IRON – FORTIFIED WEANING FOOD IN IMPROVING IRON STATUS OF INFANTS AND YOUNG CHILDREN

SECTION A: PERSONAL DATA

1. Name of female guardian ....

2. Name of child ....

3. Sex of child [ ] Male [ ] Female

4. Date of birth / Age..months

5. Relationship of female guardian to child.

   [ ] Mother [ ] Grandmother [ ] Aunt [ ] Other (specify)

6. Ethnic origin [ ] Ewe [ ] Fanti [ ] Ga

   [ ] Hausa [ ]Twi [ ] Other (specify)

7. Name of male guardian

8. Relationship of male guardian to child

   [ ]Father [ ]Grandfather [ ] Uncle [ ] Other (specify)

SECTION B: SOCIO – ECONOMIC STATUS OF FEMALE GUARDIAN

9. Level of education attained

   [ ] None [ ] Primary [ ] Middle / JSS [ ] Sixth form[ ]

   [ ] Vocational / Polyclinic [ ] University [ ] Informal education

10. Did you complete the level ticked? [ ] Yes [ ] No

11. If no, at what stage did you stop?

12. Main occupation

[ ]Unemployed [ ]Farmer [ ]Clerk [ ]Teacher [ ]Street food vendor

13. Subsidiary

[ ] None [ ] Farmer [ ] Petty trader [ ] Other (specify)

14. Animals reared

[ ] Poultry [ ] Goat [ ] Sheep [ ] Pig [ ] Other (specify)

15. Crops grown

[ ] Cassava [ ] Yam [ ] [ ] Cocoyam [ ] Plantain [ ] Other (specify)

16. Vegetables grown

[ ] Pepper [ ] Garden eggs [ ] Tomatoes [ ] Okro [ ] Other (specify)

17. Family size

[ ] 1 – 3 [ ] 4 – 6 [ ] 7 – 10 [ ] More than 10

18. Type of residence

[ ] Owned [ ] Rented [ ] Family house [ ] Other (specify)

19. (To viewer) Observe major building materials. [ ] Straw

[ ] Clay [ ] Wood [ ] Bricks [ ] Cement

20. Number of rooms

[ ] One [ ] Two [ ] Three [ ] Four [ ] More than four

21. Toilet facilities

[ ] Water closet [ ] KVIP/Pit latrine (private) [ ] KVIP/Pit latrine (public)

[ ] Pan latrine [ ] None

22. Source of drinking water

[ ] Tap water [ ] Borehole [ ] Stream [ ] Well [ ] Other (specify)

23. Evidence of wealth

SECTION C: INFANT FEEDING PRACTICES AND HEALTH

24. Was subject exclusively breast – fed for the first 4 – 6 months after birth?

[ ]Yes [ ]No

25. If no, at what age did you introduce complementary foods?

[ ] 0 – 1 months [ ] 1 – 3 months [ ] 3 – 6 months

26. Why did you give other foods to your child?

[ ]Breast-milk is insufficient [ ]Mother works away from away

[ ]Advice from health worker [ ]Advice from family member [ ] Other

27. Is subject still breast – feeding?

[ ]Yes [ ]No

28. If yes, at what age will you stop breast-feeding?

[ ] 6 – 9 months [ ] 9 – 10 months [ ] 10 – 12 months [ ] After 12 months

29. Why at that age?

[ ]Child has enough teeth [ ]Breast – milk has dried up

[ ]Mother is pregnant [ ]Child participates in family meals [ ]Other

30. How many times do you give complementary food to your child in a day?

[ ] Once [ ] Twice [ ] Thrice [ ] Four [ ] More than four

31 Do you give any of these foods to your child? (To interviewer)

Food Item	No	Yes	If yes, state frequency						
			O	1/W	1–2/W	2-3/W	>3/W	1/D	2/D
Egg									
Meat									
Fish									
Beans									
Groundnuts									
Milk									
Yaa Asantewa									

32 Do you give foods containing any of the following to your child?

Food Item	No	Yes	If yes, state frequency						
			O	1/W	1–2/W	2-3/W	>3/W	1/D	2/D
Maize									
Rice									
Millet									
Sorghum									
Cassava									
Cocoyam									
Yam									
Infant formula									
Fruits (specify)									
Tea									
Coffee									

33 Immunisation status (please tick)

	Measles	Yel. fever	DPT	BCG	Polio	Whooping Cough
Fully immunised	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Partially immunised	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Not immunised	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

34 Do you attend child welfare clinics? [ ] Yes [ ] No

35 Has your child been sick during the past one-month? [ ] Yes [ ] No

36 If yes, of what disease?

[ ] Diarrhoea [ ] Fever/Malaria [ ]Vomiting [ ]Other (specify)

37 Do you take care of the child all the time? [ ] Yes [ ] No

38 If no, who takes care of the child apart from you?

[ ] Grandmother [ ] Aunt [ ] Older sibling [ ] Other (specify)

SECTION E: DIETARY EVALUATION USING 24 HOUR RECALL

	Food Eaten	Estimated Quantity
This Morning		
Yesterday Evening		
Yesterday Afternoon		

SECTION F: ANTHROPOMETRIC MEASUREMENT

Measure length of child and record

1. ……………………..cm.
2. ……………………..cm.

Measure weight of child and record

1. ……………………..cm.
2. ……………………..cm

Measure left mid – upper arm circumference of child and record

1. ……………………..cm.
2. ……………………..cm.

SECTION G: HAEMATOLOGICAL INDICES

Haemoglobin concentration

1. ……………………g/dL
2. ……………………g/dL

Serum iron concentration

1. ……………………µmol/L
2. ……………………µmol/L

Total iron binding capacity

1. ……………………µmol/L
2. ……………………µmol/L

Transferrin saturation

1. ……………………….%
2. ……………………….%

APPENDIX II

Results of Biochemical analysis

ID	HBB	HBF	SIB	SIF	TIBCB	TIBCF	TSB	TSF
	g/dl		µmol/L		µmol/L		%	
1	10.4	10.7	18.12	19.30	59.44	45.84	30.5	42.1
2	10.2	12.6	17.8	17.50	54.54	53.06	32.6	33.0
3	10.8	10.2	18.5	19.86	65.46	55.96	28.3	35.5
4	10.6	11.6	22.7	16.73	67.41	51.30	33.7	32.6
5	11.4	12.2	20.47	15.30	63.33	63.66	32.3	24.0
6	10.8	13.3	12.51	15.76	79.00	31.38	15.8	50.2
7	10.9	10.7	18.25	16.35	60.47	39.27	30.2	41.6
8	11.0	11.6	19.20	16.95	56.16	39.40	34.2	43.0
9	14.3	11.9	17.24	12.77	62.16	79.90	27.7	15.9
10	16.0	14.1	17.6.	17.33	69.20	38.71	25.4	44.8
11	9.1	10.5	19.50	16.12	59.52	69.74	32.8	23.1
12	17.3	14.4	18.20	16.81	57.84	52.23	31.5	32.2
13	10.6	13.9	19.58	8.56	60.57	54.14	32.3	15.8
14	9.8	9.7	15.80	18.47	59.84	53.73	26.4	34.4
16	10.1	12.4	20.48	16.94	68.16	55.12	30.0	30.7
18	12.7	10.2	25.40	18.94	75.88	69.80	33.5	27.1
19	10.2	10.2	19.72	17.24	60.40	55.92	32.6	30.8
20	11.4	11.0	17.62	17.77	58.44	57.98	30.2	30.6
21	10.6	9.6	18.50	16.77	66.97	54.73	27.6	30.6
22	10.1	12.9	18.20	19.60	62.02	64.42	29.3	30.4
23	12.1	9.0	15.42	18.30	49.63	71.54	31.1	25.6
24	10.5	12.3	17.89	15.37	58.74	57.26	30.5	26.8
25	11.5	11.0	20.06	20.06	74.18	65.39	27.0	30.7
26	11.5	9.7	18.96	15.48	59.17	55.06	32.0	28.1
28	9.6	10.3	16.25	10.45	56.79	56.99	28.6	18.3
29	10.2	9.2	16.84	15.63	56.85	66.83	29.6	23.4
30	10.1	10.9	17.23	16.13	61.33	37.96	28.1	42.5
31	10.5	11.6	18.52	8.95	62.03	52.15	29.9	17.2
32	9.8	10.7	11.50	21.48	84.60	74.58	13.6	28.8
33	9.8	12.3	11.93	19.52	68.14	39.94	17.5	48.9
34	11.5	8.7	17.29	18.40	56.33	57.68	30.7	31.9
36	9.8	10.0	18.36	17.20	57.27	55.69	32.1	30.9
37	10.4	8.9	18.92	16.50	61.27	50.40	30.9	32.7
38	9.8	11.8	16.90	16.90	70.33	38.93	24.0	43.4
39	9.8	12.0	17.48	16.45	56.40	35.58	31.0	46.2
40	11.0	11.7	20.54	20.80	65.86	44.88	31.2	46.3
41	14.4	10.0	9.88	12.80	74.50	54.82	13.3	23.3
42	9.2	10.1	16.54	15.84	56.69	60.97	29.2	26.0
43	10.2	11.4	18.32	17.74	57.57	50.98	31.8	34.8
45	9.4	9.0	17.42	18.60	57.74	62.58	30.2	29.7
47	10.3	11.9	17.42	16.99	57.37	50.97	30.4	33.3

48	11.3	12.1	10.38	15.90	71.96	58.51	14.4	27.2
49	11.4	11.9	18.62	19.89	58.89	79.86	31.6	24.9
50	9.6	10.4	16.98	16.52	57.36	66.34	29.6	24.9
51	11.8	13.2	17.36	11.83	56.07	78.40	31.0	15.0
52	9.7	10.5	17.01	19.72	60.19	70.79	28.3	27.9
53	9.8	10.2	18.52	15.62	57.57	57.44	31.8	27.2
54	14.3	14.7	16.92	14.55	55.66	55.36	30.4	26.3
55	10.5	10.0	16.98	21.10	50.81	74.43	33.4	29.1
56	9.2	11.6	17.32	18.14	57.14	68.43	30.3	26.5
57	11.3	9.0	17.90	22.09	59.94	80.18	29.9	27.6
58	9.5	10.8	17.29	17.30	71.21	58.32	24.3	29.7
59	9.5	10.8	15.49	9.86	69.61	72.20	22.3	13.7
60	9.6	12.4	14.58	13.64	56.28	53.48	25.9	25.5
61	12.9	15.7	11.04	18.63	85.30	57.57	12.9	32.4
62	9.5	10.5	12.60	12.85	57.24	60.83	22.0	21.1

ID= Identification number HBB= Haemoglobin concentration at baseline HBF= Haemoglobin concentration at the end of intervention SIB= Serum iron concentration at baseline SIF= Serum iron concentration at end of the intervention TIBCB= Total iron binding capacity at baseline TIBCF= Total iron binding capacity at end of the intervention TSB= Transferrin saturation at baseline TSF= Transferrin saturation at the end of the intervention

APPENDIX III

Results of Anthropometric assessment (Z-scores)

ID	WAB	WAF	WLB	WLF	LAB	LAF
1	-1.60	-0.78	-0.68	-1.56	0.00	0.43
2	-2.24	-2.11	-0.77	-1.10	-2.89	-2.33
3	-3.47	-3.40	-3.95	-2.59	-0.89	-0.67
4	-1.50	-1.16	-0.58	-1.36	-1.51	-0.30
5	-1.06	-1.40	-2.58	-1.68	0.35	1.66
6	0.50	-0.13	0.54	-0.60	0.24	0.78
7	-3.44	-3.11	-2.25	-2.00	-2.92	-2.07
8	-3.17	-2.52	-1.34	-2.02	-2.92	-2.64
9	0.05	-0.70	-0.61	-0.55	1.20	-0.46
10	-0.44	-0.36	-0.58	-1.28	0.06	1.43
11	-0.15	0.38	0.13	-0.51	-0.33	1.30
12	-1.30	-1.96	-0.50	-1.96	-1.48	-0.58
13	-0.59	-1.71	-0.34	-1.45	-0.63	-0.91
14	-2.46	-2.18	-2.26	-1.92	-1.32	-0.79
16	-2.27	-1.50	-2.02	-2.35	-1.13	0.50
18	-3.11	-2.55	-2.49	-1.49	-1.76	-1.04
19	-1.02	-1.00	-0.96	-0.79	-0.52	-0.70
20	0.54	-0.92	0.33	-1.86	0.69	1.15
21	-2.18	-1.51	-1.66	-2.08	-0.39	-0.74
22	-1.95	-0.46	-2.04	-1.01	-0.45	0.34
23	-1.02	-1.17	-0.53	-1.53	-1.17	0.10
24	-0.92	-0.37	-0.95	-0.85	-0.33	0.71
25	-2.26	-2.31	-1.75	-1.28	-2.42	-1.98
26	-0.79	-0.15	-0.64	0.07	-0.35	-0.25
28	-2.34	-2.26	-0.74	-1.24	-3.07	-1.74
29	-1.18	-0.50	-1.08	-0.29	-0.47	-0.28
30	1.75	0.03	0.49	-0.79	0.88	1.35
31	-1.60	-0.72	-2.28	-1.61	0.57	0.94
32	-1.47	-0.80	-1.57	-1.55	-0.29	0.75
33	-1.24	-0.22	-1.60	-0.60	0.23	0.18
34	0.92	-1.00	0.74	0.51	0.50	0.38
36	-1.79	-1.49	-0.41	-0.30	-2.21	-1.68
37	-2.25	-2.43	-2.04	-2.48	-1.14	-0.46
38	-2.76	-2.90	-2.99	-3.32	-1.07	-0.50
39	-0.61	-0.50	-0.41	-1.23	-0.50	1.01
40	1.00	1.20	0.93	0.37	0.55	1.92
41	-2.49	-2.15	-1.36	-1.79	-2.18	-1.54
42	-1.54	-1.35	-0.31	-1.17	-2.45	-0.59
43	-1.87	-1.87	-0.58	-0.72	-2.67	-0.73
45	-3.42	-3.23	-2.04	-1.86	-3.14	-1.41
47	-.2.43	-2.23	-0.41	-0.23	-3.21	-2.66
48	-0.19	0.02	0.06	0.84	-0.40	-0.81

49	0.47	-1.28	0.88	-1.43	-0.65	-0.33
50	-2.27	-1.06	-0.56	-0.43	-2.63	-1.05
51	0.19	-0.51	1.02	-0.73	-1.09	-1.49
52	-2.34	-1.46	-1.47	-1.77	-1.69	1.16
53	-3.11	-2.54	-2.22	-2.06	-2.01	-1.11
54	-2.34	-2.12	-2.22	-2.18	-1.22	0.26
55	-2.66	-1.94	-1.33	-1.48	-2.70	-0.90
56	-1.20	-0.21	-1.32	0.55	-0.16	-0.76
57	-0.15	-0.35	1.58	-1.03	-0.92	0.44
58	0.41	-0.35	1.50	-1.03	-1.22	0.44
59	-1.49	-0.37	-0.80	-0.20	-1.29	-0.25
60	0.32	1.23	0.33	1.06	0.25	0.40
61	0.57	1.50	0.76	1.55	0.11	0.25
62	-2.17	-0.98	-1.84	-1.93	-3.18	-0.51

ID= Identification number; WAB= weight-for-age at baseline; WLB= weight-for-length at baseline LAB= length-for-age at baseline; WAF= weight-for-age at end of intervention; WLF= weight-for-length at end of intervention; LAF= length-for-age at end of intervention

APPENDIX IV ANALYTICAL SETTINGS FOR DIETARY IRON

Current	5.0mA
Wavelength	248.3nm
Slit	0.15mm
Sensitivity	0.4ppm