

**EFFECT OF CONSUMPTION OF ROSELLE JUICE ON HAEMOGLOBIN  
CONCENTRATION, APPETITE AND VITAMIN C SATURATION IN  
CHILDREN AGED BELOW 24 MONTHS**

**BY**

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## ABSTRACT

Malnutrition rates of over 38% among children of age below 5 years have been addressed by interventions like growth promotion and monitoring. The study sought to further address poor nutritional status among children of age below 2 years by supplementing their diet with Roselle juice. Roselle calyx from which the juice was made contains high concentrations of vitamin C, iron and zinc which are highly deficient in diets of children in Magubike village. A randomised single blind design with one time dietary assessment was used in this study. Household characteristics, appetite and morbidity data of children were obtained. Anthropometric (weight and height) and biochemical indicators were determined. Children were supplemented with Roselle and placebo juice and followed up for four months. Results revealed that there was low energy, fat, zinc, vitamin C and high fibre content in the common complementary foods given to children. Consumption of Roselle juice significantly increased ( $p < 0.05$ ) vitamin C intake from 28 to 60% of RDA. The prevalence of underweight, a composite indicator of stunting and wasting was 9% among the children. Haemoglobin concentration increased ( $p < 0.05$ ) by  $1 \text{ g dl}^{-1}$  among children in the intervention group. The morbidity rate among children in the intervention group significantly decreased by 27% ( $P < 0.05$ ) from 81% to 54% after four months of supplementation. The concentration of vitamin C in urine for the children in the placebo and intervention group increased by 0.008 and  $0.015 \text{ mg kg}^{-1}$  respectively after four months of supplementation. The proportion of children in the intervention group with urine zinc concentration outside normal range increased by 4% compared to 14% in placebo group after 4 months of supplementation. Promoting consumption of Roselle juice is important in areas with high prevalence of anaemia.

**DECLARATION**

I, RICHARD BUKENYA, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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Date

The above declaration is confirmed by:

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Prof. Joyce Kinabo  
(Supervisor)

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Date

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**DEDICATION**

I dedicate this work to my beloved wife, Flora and daughter, Faith. I love you ladies.

## TABLE OF CONTENTS

ABSTRACT	i
DECLARATION	ii
COPYRIGHT	iii
ACKNOWLEDGEMENTS.....	iv
DEDICATION	vi
<p>I dedicate this work to my beloved wife, Flora and daughter, Faith. I love you ladies.</p>	
	vi
TABLE OF CONTENTS.....	vii
LIST OF TABLES .....	xvi
LIST OF FIGURES.....	xvii
LIST OF APPENDICES.....	xvii
LIST OF ABBREVIATIONS AND ACRONYMS.....	xviii
CHAPTER ONE	1
1.0 INTRODUCTION.....	1
1.1 Background.....	1
1.2 Problem Statement.....	3
1.3 Justification of the Study.....	3
1.4 Objectives of the Study.....	5
1.4.1 Overall objective.....	5
1.4.2 Specific objectives.....	5
CHAPTER TWO	6
2.0 LITERATURE REVIEW.....	6
2.1 Overview of the Nutritional Status in Tanzania.....	6
2.1.1 Morbidity and mortality.....	6



2.1.2 Nutritional status.....	7
2.2 Growth Pattern and Effect of Micronutrient Supplementation.....	8
2.2.1 Growth pattern of infants and young children.....	8
2.2.2 Growth faltering.....	10

Growth faltering is failure to attain desired increments in height or weight (Shrimpton et al., 2001; WHO, 2005). In times of deficiency, clinical symptoms tend not to emerge, but growth faltering rapidly occurs (Branca and Ferrari, 2002). Faltering of weight among children is regularly observed to start at 3 to 4 months of age and ends at 24 to 36 months of age (Shrimpton et al., 2001 and Schoeman et al., 2006). Faltering of height immediately starts at birth and ends around one year. Growth faltering has been estimated between 10% and 50% of the children below 5 years of age (Schoeman et al., 2006). Growth faltering yields high prevalence of poor nutritional status among children with age of below 2 years. The cause of faltering in growth is regular intake of nutrient deficiency diets that are required for growth and development such as energy, carbohydrates, fats, proteins and micronutrients (Garner et al., 2000). Deficient nutrient intake levels are as a result of inadequate food intake, infections and increased malabsorption (WHO, 2005). Episodes of diarrhoea result in growth faltering and stunting and also make the child more susceptible to a range of other infectious diseases. Contamination of food has been estimated to be the cause of up to 70% of episodes of diarrhoea in children under the age of 5 years. WHO has estimated that worldwide there are 1 500 million children with such episodes resulting in the deaths (usually from dehydration) of over 3 million children a year (Shrimpton et al., 2001). Studies conducted in America, Europe and Africa observed that children having HIV below 18 months of age are 0.7 kg lighter and 2.2 cm shorter than their counterparts of the same age

(WHO, 2005). Faltering in height among children is due to long term factors like poor maternal nutrition, poor eating habits during pregnancy and continued low intake of micronutrients like zinc by the children after birth (Shrimpton et al., 2001; Branca and Ferrari, 2002). Other causes of growth faltering include social deprivation due to poverty and child abuse. Growth faltering can be detected early by growth monitoring to allow timely remedial interventions and prevention of further growth failure (Garner et al., 2000). Factors before birth of child that contribute to faltering of growth are hard to manage especially when growth deficits have already happened during conception (Shrimpton et al., 2001). Special emphasis should be given to the development of effective interventions to stop the critical faltering that occurs from birth to 24 months (Garner et al., 2000). Other interventions for children with growth faltering include counselling of the mother, caregiver or guardian of the child; providing nutritional supplements to the child; early detection and treatment of concurrent diseases such as diarrhoea; and social support through promoting livelihood programs (Branca and Ferrari, 2002; Schoeman et al., 2006).....	11
2.2.3 Effect of micronutrient supplementation on weight and height of children.....	12
2.3 Roselle and Its Importance.....	13
2.3.1 Food products made from Roselle and importance .....	13
2.3.2 Chemical and nutrient composition of Roselle calyces.....	14
Nutrient	16
measure	16
Content	16
Protein	16
%	16
5.8	16

69.5	16
3.0	16
Fibre	16
9.5	16
Ash	16
12.2	16
Vitamin C	16
Mg/100g	16
141.1	16
Iron	16
3.2	16
Zinc	16
12.2	16
β-carotene	16
1.9	16
Lycopene	16
0.2	16
Phytic acid	16
%	16
0.3	16
Tannic acid	16
6.2	16
Hydrocyanic acid	16
0.2	16
Oxalate	16

## 0.7 16

Source: (Morton, 1987; Wong et al., 2002; Ojokoh et al., 2002; Fasoyiro et al., 2005)

## 16

## 2.4 Importance of Vitamin C and Implication of the Ascorbic Acid Test .....16

## 2.4.1 Uses of vitamin C .....16

## 2.4.2 Ascorbic acid saturation test and its implication.....17

## 2.5 Plasma and Urinary Zinc.....20

Rapid decrease in endogenous faecal and urinary zinc is obtained with low zinc diets while there is limited drop in plasma zinc concentration (King et al., 2000). When dietary zinc is reduced from 241 to 4  $\mu\text{mol/ day}$ , urinary zinc losses drop by 75% by the end of the second week, whereas the plasma zinc concentrations are unchanged (King et al., 2000). Plasma zinc which is the most widely used biomarker of zinc status has poor sensitivity and imperfect specificity (Hambidge, 2003). While urine zinc measurements are poor indicators of tissue zinc status, they are in clinical practice the means by which judgements are made about the adequacy of zinc supplementation during intravenous nutrition. Low urinary zinc concentrations of less than 2  $\mu\text{mol/ day}$  depict zinc depletion while those above 80  $\mu\text{mol/ day}$  depict catabolism which calls for zinc supplementation (Hambidge, 2003). The normal concentration of zinc that has been used ranges from 0.76-7.6  $\mu\text{mol/L}$  (49-490  $\mu\text{g/L}$ ). Urinary zinc losses in children are 9  $\mu\text{g Zn/kg/day}$  (Menary et al., 2002).....20

## 2.6 Nutrient Composition of Breast Milk and Intake.....20

## 2.6.1 Nutrient composition of breast milk.....20

## 2.6.2 Breast milk intake.....21

## 2.7 Complementary Foods.....22

## 2.7.1 Complementary feeding.....22

2.7.2 Energy and nutrient intake among children in Tanzania .....	23
2.7.3 Micronutrient availability in complementary foods.....	25
2.8 Appetite Assessment.....	26
CHAPTER THREE.....	27
3.0 MATERIALS AND METHODS.....	27
3.1 Study Area and Population.....	27
3.2 Study Design and Sample Selection.....	28
3.2.1 Study design	28
3.2.2 Inclusion and exclusion criteria.....	29
3.2.3 Sample size and sampling.....	30
3.3 Data Collection .....	31
3.3.1 Socio-demographic information.....	31
3.3.2 Feeding and morbidity information.....	31
3.3.3 Anthropometric measurements.....	33
3.3.4 Biochemical assessments .....	33
3.3.4.1 Determination of haemoglobin concentration .....	33
3.3.4.2 Ascorbic acid saturation test .....	34
3.3.4.3 Breast milk analysis.....	36
3.3.5 Dietary assessment.....	37
3.3.6 Preparation and analysis of nutrient composition of the juice.....	37
3.3.6.1 Preparation and nutrient analysis of Roselle juice .....	37
3.3.6.2 Preparation of the placebo juice.....	38
3.3.6.3 Administration of the juice.....	39
3.4 Data Entry and Analysis.....	39
3.5 Ethical Considerations.....	40

3.6 Quality Control.....	40
3.7 Study Limitations.....	41
CHAPTER FOUR	42
4.0 RESULTS	42
4.1 Demographic Characteristics.....	42
4.1.1 Household characteristics.....	42
4.2 Dietary Assessment.....	44
4.2.1 Breast feeding frequency and practices.....	44
4.2.2 Feeding frequency.....	44
4.2.3 Food consumption .....	45
4.2.3.1 Foods from animal and plant legumes.....	45
4.2.3.2 Starchy, sugars and oily foods.....	46
4.2.3.3 Consumption of fruits.....	47
4.2.3.4 Cooked leafy vegetables and fruits.....	48
4.2.4 Nutrient composition of the major complementary meals.....	49
4.2.5 Nutrient composition of the Roselle juice.....	50
4.2.6 Estimated nutrient intake.....	51
4.3 Nutritional Status of the Children.....	52
The mean baseline measurements for body weight and height of the children in the study group are presented in Table in Appendix 7.....	52
4.3.1 Prevalence of underweight .....	52
4.3.2 Prevalence of wasting.....	53
4.4 Iron Status	54
4.5 Appetite Status	56
4.6 Morbidity among Children in the Study Group.....	57

60

4.7 Vitamin C Saturation Test.....	60
4.8 Urinary Zinc Concentration.....	62
The proportion of children with zinc concentration outside the normal range of urine zinc concentration (49-490 µg/ L) increased by over 8 µg/ L from 26 to 35% (Table 14). The proportion of children in the intervention group with urine zinc concentration outside normal range increased by 4% from 26% at baseline to 30% after 4 months of supplementation. The proportion of children in the placebo group with urine zinc concentration outside normal range increased by 14% from 26% at baseline to 40% after 4 months of supplementation. ....	62

## CHAPTER FIVE 62

## 5.0 DISCUSSION 62

5.1 Household Characteristics.....	62
5.2 Dietary Assessment.....	64
5.2.1 Breast feeding frequency.....	64
5.2.2 Frequency of feeding complementary meals.....	65
5.2.3 Food frequency and adequacy.....	66
5.2.4 Nutrient composition of Roselle juice.....	68
5.3 Appetite of the Children.....	69
5.4 Vitamin C Saturation.....	71
5.5 Iron Deficiency Anaemia.....	73
5.6 Illness among Children.....	74
5.7 Urinary Zinc Concentration.....	75
5.8 Nutritional Status of the Children.....	76

## CHAPTER SIX 78

6.0 CONCLUSIONS AND RECOMMENDATIONS.....	78
6.1 Conclusions	78
6.2 Recommendations.....	79
REFERENCES	80
Branca, F. and Ferrari, M. (2002). Impact of Micronutrient Deficiencies on Growth: The Stunting Syndrome. <i>Annals of Nutrition and metabolism</i> 46 (1): 8 - 17.....	82
Garner, P., Panpanich, R. and Logan S. (2000). Is routine growth monitoring effective? A systematic review of trials. <i>Archives of Disease in Childhood</i> 82 (3): 197-201.....	86
Schoeman, S. E., Hendricks, M. K., Hattingh, S. P., Benade, A. J. S., Laubscher, J. A. and Dhansay, M. A. (2006). The targeting of nutritionally at-risk children attending a primary health care facility in the Western Cape Province of South Africa. <i>Journal of Public Health Nutrition</i> 9 (8): 1 007–1 012.....	94
Shrimpton, R., Victoria, C. G., Onis, M., Costa Lima, R., Bloëssner, M. and Clugston, G. (2001). Worldwide Timing of Growth Faltering: Implications for Nutritional Interventions. <i>Journal of Pediatrics</i> 107 (5): 1-7.....	95
APPENDICES	98



### LIST OF TABLES

Table 1: Nutritional value of Roselle calyces.....	15
Table 2: Effect of maternal deficiency on breast milk micronutrient content.....	21
Table 3: Breast milk intake by children in developing countries.....	22
Table 4: Recommended nutrient intakes used in the WHO/UNICEF 1998, new DRI and WHO 2002	24
Table 5: Classification of malnutrition.....	33
Table 6: Household Characteristics at baseline .....	43
Table 7: Mean daily breast feeding frequency.....	44
All values are mean $\pm$ SD , * significantly different from baseline value ( $p < 0.05$ )....	44
Table 8: Animal and plant legume foods.....	46
Table 9: Consumption of Starchy, sugar and oily foods.....	47
Table 10: Fruits eaten without cooking.....	48
Table 11: Vegetable, plant leaves and cooked fruits.....	49
Table 12: Nutrient composition of the major foods given to the children.....	50
Table 13: Average Nutrient intake from home prepared complementary foods.....	52
Table 14: Urinary zinc concentration of the children .....	62

## LIST OF FIGURES

Figure 1: Infection- malnutrition interaction and impact.....	7
Figure 2: Increase in weight with age of a normal child.....	9
Figure 3: Plasma concentrations in rabbits after ascorbic dose administration.....	19
Figure 4: Energy required from the breast milk and complementary foods.....	23
Figure 5: Prevalence of anorexia against age of the children.....	27
Figure 6: Study design, sampling and follow up of children in both feeding regimens	
32	
45	
Figure 7: Frequency of feeding children on complementary meals.....	45
Figure 8: Prevalence of underweight among children during baseline.....	53
54	
Figure 9: Prevalence of Global acute malnutrition (wasting) .....	54
56	
Figure 10: Prevalence of anaemia at baseline and the fourth month .....	56
Figure 11: Prevalence of illnesses in the previous month.....	58
Figure 12: Illnesses among children .....	59
Figure 13: Prevalence of malaria among children.....	60
Figure 14: Concentration of vitamin C in urine after 4-6 hours of dose administration	
61	

## LIST OF APPENDICES

Appendix 1 Questionnaire used for interviews.....	98
Appendix 2 Determination of vitamin C.....	103
Appendix 3 Determination of proximate composition in Roselle and food samples	
104	

Appendix 4 Standard curve of ascorbic acid concentration using folin's reagent....	106
Appendix 5 Sample size determination.....	107
Appendix 6 Consent form.....	108
Appendix 7 Baseline anthropometric characteristics of the children.....	111

#### **LIST OF ABBREVIATIONS AND ACRONYMS**

µg	-	microgram
Ca	-	Calcium
CDC	-	Centre for Disease Control
ENA	-	Emergency Nutrition Assessment
HAZ	-	Height for age Z-scores
GPM	-	Growth Promotion and monitoring
mg	-	milligram
NIMR	-	National Institute for Medical Research
PANTIL	-	Program for Agriculture and Natural resources Transformation for improved Livelihood
PLWHA	-	People Living with HIV/AIDS
RCHC	-	Reproductive Child Health Clinic
rpm	-	revolution per minute
SUA	-	Sokoine University of Agriculture
TCA	-	Trichloro- acetic acid
TDHS	-	Tanzania Health and Demographic Survey
UK	-	United Kingdom
UNICEF	-	United Nations Children's Fund
WAZ	-	Weight for age Z scores
WHZ	-	Weight for height Z scores

WFP	-	United Nations World Food Programme
WHO	-	World Health Organization
Zn	-	Zinc

## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Background

Malnutrition is a condition manifested by the body due to over or inadequate intake of the required nutrients. Under nutrition, the most prevalent form among children in the rural and urban areas of Tanzania can be addressed by providing adequate nutrients. Adequate nutritional status and growth of children with ages between 6 and 24 months can be achieved by proper complementary feeding practices, deworming, growth monitoring and nutrient supplementation (Dewey and Adu-Afarwuah, 2008). Continued breast feeding is also important to provide the immune factor, the secretory immunoglobulin A, which protects children from infections (Allen, 2001). Inadequate feeding practices during the first 2 years of life culminates into malnutrition that leads to increased morbidity, mortality, poor cognitive development and poor school performance during later ages (World Bank, 2006).

The most common type of malnutrition affecting children below five years of age in Tanzania include stunting, underweight, wasting and anaemia. Apart from vitamin A deficiency, Iodine deficiency disorders and Iron deficiency anaemia, there are other micronutrient deficiencies in Tanzania that have not been well documented. Stunting and underweight prevalence rates are 38 and 22% respectively among children 0-59 months as reported by TDHS (2005) are regarded as serious public health problems according to WHO while anaemia is severe (CDC and WFP, 2005). Over 70% of children 0-59 months in Tanzania are anaemic. Furthermore, 36 and 16% of the children 0-59 months in Morogoro Region are stunted and underweight respectively (TDHS, 2005). In addition, 78% of the children aged 0-59 months are anaemic. In

Magubike village, 38 and 28% of the children (0-59 months) are stunted and underweight respectively while 78% are anaemic (Jumbe, 2007).

The causes of malnutrition have been explained using the UNICEF/TFNC conceptual frame for the causes of malnutrition (CDC and WFP, 2005). The immediate causes of malnutrition among children below five years are poor diets and frequent infections. Furthermore, poor diets and infections may be attributed to household food insecurity; inadequate child care; poor sanitation and inadequate health care. The above factors are exacerbated by inadequate and poor distribution of potential resources and poor socio-cultural practices (CDC and WFP, 2005).

High malnutrition rates and poor growth pattern have been addressed through Growth Promotion and Monitoring Program (GPMP) in rural and urban areas of Tanzania. Several researchers have perceived GPMP as beneficial in reducing the prevalence of malnutrition and mortality. Growth promotion and monitoring may help to reduce growth faltering, increase nutrition awareness and improve health and nutrition information management systems (Ljungquist, 1993; Maletnema, 2002; Ann *et al.*, 2008). However, the program has been weakened by low participation rates, poor health worker performances and inadequacies in health system infrastructure that have constrained effective growth-promoting action (Ann *et al.*, 2008). In addition, good nutrition counselling is paramount for growth promotion and is often done badly. Integration of GPMP with other interventions would improve the situation. There has been increased promotion of consumption of fruits and vegetables to reduce the high prevalence of micronutrient deficiencies like iron and vitamin deficiencies. The effectiveness of providing micronutrient rich foods

together with implementation of GPMP to avert poor nutrition needs more exploration. Supplementation with micronutrient rich foods like Roselle and its products to children below 2 years of age as one of the interventions can reduce prevalence of micronutrient deficiencies, which in turn reduce incidences of diseases, poor nutritional status and growth faltering among children of age below 2 years.

### **1.2 Problem Statement**

Despite Growth Promotion Monitoring Program being implemented in the urban and rural areas of Tanzania, such as Magubike village, there still exists a high rate of malnutrition among children below 2 years of age. Studies by Jumbe *et al.* (2007) and Jumbe (2007) identified high rates of malnutrition and morbidity among children in Magubike village, especially anaemia and underweight. From these observations it was recommended that nutritional interventions be introduced to address these problems. Supplementation with micronutrient rich foods like Roselle could reduce the high rates of malnutrition such as underweight, wasting, anaemia, inadequate weight gain and infectious diseases but less has been documented on its effectiveness to reduce such problems in children.

### **1.3 Justification of the Study**

The first two years of human life presents a single opportunity to provide a strong nutritional and immunological foundation (World Bank, 2006). During this period, adequate nutrition is important for intellectual and physical growth (Dewey and Adufarwuh, 2008). Also high prevalence of infections poses a high risk of acquiring micronutrient deficiencies like anaemia in the later ages (Henrik, 2002). About 74% of the children (0-59 months) in Magubike are affected by different diseases each

month (Jumbe, 2007). Infections such as malaria among children of age between 0 and 59 months affect food intake by developing nausea leading to appetite loss (Henrik, 2002). In Magubike village, 60% of children above 2 years of age are anaemic (Jumbe, 2007). Furthermore, there is poor infant and young child feeding practices and inadequate maternal nutrition. It has been observed that 87% of lactating mothers do not continue breast feeding their children during disease episodes (Jumbe, 2007).

Foods rich in micronutrients such as Roselle, one of the high value crops presents many nutritional and health advantages. For example, 100g of the Roselle inflorescence, contains 51.7 calories, 86.2 % water, 1.6 g protein, 0.1 g fat, 11.1 g total carbohydrates, 2.5 g fibre, 1.0 g ash, 160 mg calcium, 60 mg phosphorus, 3.8 mg iron, 300 µg β-carotene equivalent, 12.5-31.33 mg ascorbic acid, 0.04 mg thiamine, 0.6 mg riboflavin, 0.5 mg niacin, 12 mg zinc and anthocyanin as summarised in Table 1. Vitamins like thiamine, riboflavin and niacin are essential for carbohydrate and energy metabolism. Ascorbic acid increases the bioavailability of plant iron and folate while β-carotene and zinc in Roselle boost the immunity and promote growth. Anthocyanins are good antioxidants that quench free radicals in the body (Henrik, 2002). Foods like maize meal, which constitute 61% of the children's major diet in Magubike (Jumbe, 2007) have inadequate micronutrients to meet the nutrient demand.

Apart from the above properties, Roselle calyces and products like juice and wine have been widely sold in several local markets in Tanzania. These include several supermarkets, groceries, retail shops, restaurants and entrepreneurship business



projects. So much has been claimed to be achieved if the Roselle and its products are consumed. This includes improving iron status, lowering high blood pressure and improving appetite among adults. However, there is less information about the effectiveness of Roselle juice on improving nutritional, iron and appetite status of children aged below 2 years. Therefore this study investigated the effect of consumption of Roselle juice on haemoglobin concentration appetite and vitamin C status of children aged between 5 and 25 months.

#### **1.4 Objectives of the Study**

##### **1.4.1 Overall objective**

To investigate the effect of supplementing local complementary foods with Roselle juice on haemoglobin concentration, appetite and vitamin C status of children aged below 24 months in Magubike village.

##### **1.4.2 Specific objectives**

1. To determine the nutrient composition of Roselle calyces and juice.
2. To assess the nutritional status and dietary intake of children below 24 months of age.
3. To determine the effect of intake of Roselle juice on appetite score.
4. To examine the effect of intake of Roselle juice on incidence of infections, body vitamin C saturation and haemoglobin concentration.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Overview of the Nutritional Status in Tanzania

##### 2.1.1 Morbidity and mortality

The expenditure on health over the years has increased whereby 11% of the developed countries' GDP and 4.7% of the developing countries' GDP is spent on health (WHO, 2007). The Tanzanian government recently increased her 2009/2010 budget by 5.7% of the 2008/2009 expenditure on health (Guardian Reporter, 2009). The major illnesses threatening the Sub Saharan region in Africa and affecting children include the pneumonia, malaria and diarrhoea which contribute to 29, 22 and 18% respectively of child deaths (Bruce *et al.*, 2005; Mulholland and Adegbola, 2005). In Tanzania, malaria, diarrhoea and ARI are among the major causes of morbidity and mortality among children below five years. They comprise 40, 12.6 and 8.1% of the children deaths respectively (TDHS, 2005). Further, the prevalence of malaria among children below 5 years in rural and urban Morogoro is between 25 and 50% (TDHS, 2005; Mamiro *et al.*, 2005). In Magubike, Kilosa district the prevalence of malaria is 31.6% among children with age of 0-24 months (Jumbe *et al.*, 2007). The consequences of having frequent illnesses such as nausea, vomiting, dehydration, appetite loss and alteration of nutrient use (Black and Sazawal, 2001; Schaible and Kaufmann, 2007) exacerbates the already poor nutritional and health status of the children (Qureshi *et al.*, 2002). The cycle between malnutrition and development of diseases is summarized in Fig. 1. Infection causes energy loss in individual, which reduces productivity at community level and perpetuates malnutrition, infection, disease, and poverty (Schaible and Kaufmann, 2007).

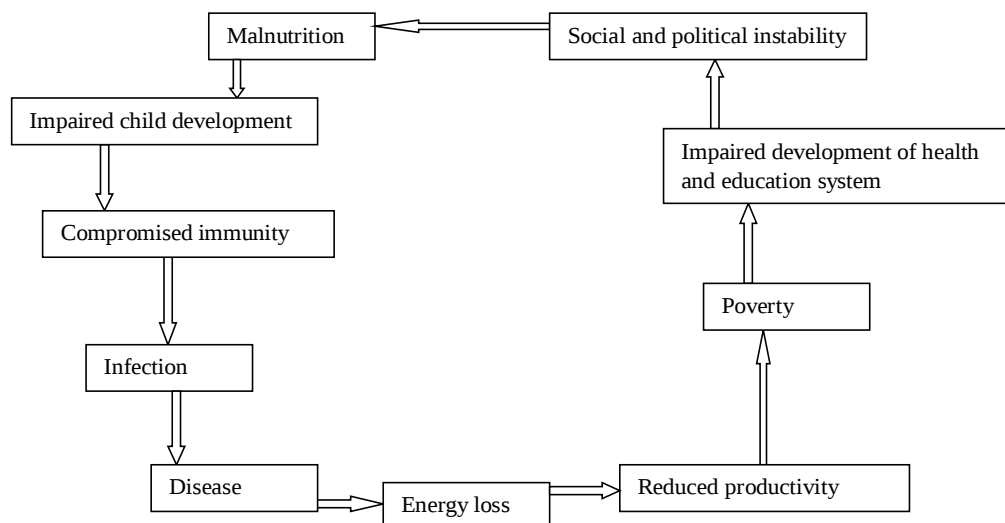


Figure 1: Infection- malnutrition interaction and impact

Source: Schaible and Kaufmann (2007)

### 2.1.2 Nutritional status

As for illnesses and infection, malnutrition has also been implicated directly or indirectly for causing more than 50% of the world deaths (Schaible and Kaufmann, 2007). The major forms of malnutrition include protein energy malnutrition (PEM) and micronutrient deficiencies. The major forms most widely documented include wasting, underweight, stunting, vitamin A, zinc, and iron (anaemia) deficiencies. Their prevalence in Africa have been estimated to be over 10, 31, over 40, 35, 62, and 60% respectively (Caulfield *et al.*, 2004; Onis *et al.*, 2006). It is reported in the Demographic and Health survey of Tanzania (2005) that 51% of the children below five years of age are stunted, 26% are underweight, 3% are wasted and 75% are anaemic. Various studies conducted in Morogoro region show high prevalence of stunting, underweight and anaemia among children below 5 years. The demographic and health Survey of 2005; Mselle *et al.* (2006) and Jumbe *et al.* (2007) show

stunting rates between 22-59%, underweight 23-28%, wasting 3-16% and anaemia 43-78%. The high rates of malnutrition have caused more harm to the children below 5 years of age and the communities where they manifest widely. Figure 1 further summarizes the consequences of poor nutrition in the community. Under nutrition is considered to be the underlying cause of more than 50% of all childhood deaths in the world (Caulfield *et al.*, 2004). Under nutrition diminishes the ability of all systems of the body to perform properly, with particularly grave consequences in young children (Schaible and Kaufmann, 2007). Malnourished children have increased risk of infections like malaria (Caulfield *et al.*, 2004). This further leads to low productivity, increased expenditure on diseases and reduced efficiency of the health service delivery (Schaible and Kaufmann, 2007).

## **2.2 Growth Pattern and Effect of Micronutrient Supplementation**

### **2.2.1 Growth pattern of infants and young children**

Child growth and development during the first 2 years of life occurs in a sequence, involving physical, cognitive, and emotional changes (Allen, 2001). Physical changes include increase in bone thickness, size, gross motor, fine motor, vision, hearing and perceptual development (Arvedson, 2006). Weight of a normal child increases by 1 kg in the first three months of age and by 500 g from 4 to 6 months (Fig. 2). The child aged 6-12 months starts to follow simple directions, imitates simple words, crawls on four limbs, pulls to stand, gains average height of 1.5 cm and weight of 300 g per month (Fig. 2). The child aged 12-24 months holds own cup or bottle, walks and runs independently, climbs stairs, gains an average height of 0.8cm and weight of 200g per month (Arvedson, 2006; WHO, 2006).

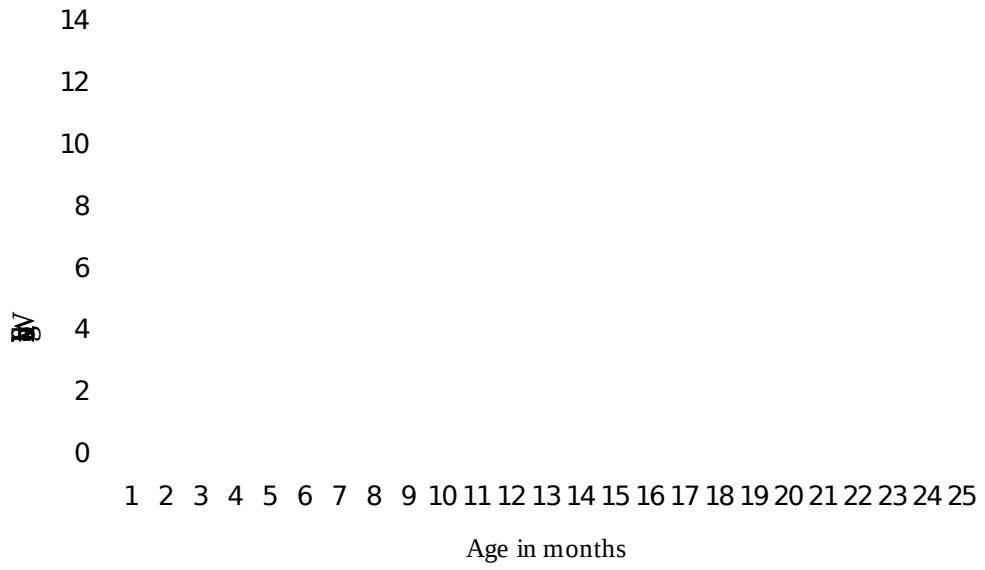


Figure 2: Increase in weight with age of a normal child

Source: WHO (2006)

### **2.2.2 Growth faltering**

Growth faltering is failure to attain desired increments in height or weight (Shrimpton *et al.*, 2001; WHO, 2005). In times of deficiency, clinical symptoms tend not to emerge, but growth faltering rapidly occurs (Branca and Ferrari, 2002). Faltering of weight among children is regularly observed to start at 3 to 4 months of age and ends at 24 to 36 months of age (Shrimpton *et al.*, 2001 and Schoeman *et al.*, 2006). Faltering of height immediately starts at birth and ends around one year. Growth faltering has been estimated between 10% and 50% of the children below 5 years of age (Schoeman *et al.*, 2006). Growth faltering yields high prevalence of poor nutritional status among children with age of below 2 years. The cause of faltering in growth is regular intake of nutrient deficiency diets that are required for growth and development such as energy, carbohydrates, fats, proteins and micronutrients (Garner *et al.*, 2000). Deficient nutrient intake levels are as a result of inadequate food intake, infections and increased malabsorption (WHO, 2005). Episodes of diarrhoea result in growth faltering and stunting and also make the child more susceptible to a range of other infectious diseases. Contamination of food has been estimated to be the cause of up to 70% of episodes of diarrhoea in children under the age of 5 years. WHO has estimated that worldwide there are 1 500 million children with such episodes resulting in the deaths (usually from dehydration) of over 3 million children a year (Shrimpton *et al.*, 2001). Studies conducted in America, Europe and Africa observed that children having HIV below 18 months of age are 0.7 kg lighter and 2.2 cm shorter than their counterparts of the same age (WHO, 2005). Faltering in height among children is due to long term factors like poor maternal nutrition, poor eating habits during pregnancy and continued low intake of micronutrients like zinc by the children after birth (Shrimpton *et al.*, 2001; Branca and Ferrari, 2002). Other causes of growth faltering include social

deprivation due to poverty and child abuse. Growth faltering can be detected early by growth monitoring to allow timely remedial interventions and prevention of further growth failure (Garner *et al.*, 2000). Factors before birth of child that contribute to faltering of growth are hard to manage especially when growth deficits have already happened during conception (Shrimpton *et al.*, 2001). Special emphasis should be given to the development of effective interventions to stop the critical faltering that occurs from birth to 24 months (Garner *et al.*, 2000). Other interventions for children with growth faltering include counselling of the mother, caregiver or guardian of the child; providing nutritional supplements to the child; early detection and treatment of concurrent diseases such as diarrhoea; and social support through promoting livelihood programs (Branca and Ferrari, 2002; Schoeman *et al.*, 2006).

### **2.2.3 Effect of micronutrient supplementation on weight and height of children**

The effect of micronutrient supplementation on growth is contradicting; some studies claim affirmative effect while others are negative. In a poor population, the effects of maternal micronutrient supplementation on the foetus persist into childhood, with increases in both weight and body size (Vaidya *et al.*, 2008). Supplementation of HIV positive mothers with multi-vitamins during pregnancy and lactation improves weight gain in children (Eduardo *et al.*, 2005). There is increase in height for age Z-scores of stunted children on micronutrient supplementation (Bui *et al.*, 1999). Meta-analysis of zinc supplementation trials show that zinc has a significant impact on length gain in children 0-13 years of age (Bhandari *et al.*, 2001). Micronutrient supplementation improves iron status and reduces growth faltering (Le Thi and Jacques, 2005). Feeding children with food fortified with micronutrients has been shown to have positive effects on milestone acquisition (Seth *et al.*, 2007). Juan *et*



*al.* (2003) reported that growth faltering was more prevalent in vitamin A deficient children. On the other hand, Untoro *et al.* (2005) found no significant effect of micronutrient supplementation on growth, morbidity and growth faltering. Supplementation of children with a single micronutrient often lacks effectiveness (Bui *et al.*, 1999). Vitamin A supplementation does not increase growth, rather reduces morbidity and mortality (Kirkwood *et al.*, 1996). Therefore, a combination of micronutrient in a natural state may provide effective results.

## **2.3 Roselle and Its Importance**

### **2.3.1 Food products made from Roselle and importance**

Roselle (*Hibiscus sabdariffa*) belongs to the family Malvaceace and is a popular vegetable in Indonesia, India, West Africa and many tropical regions. Infusions of the calyces are used as a caffeine-free drink, preservatives and flavourings (Mounigan and Badrie, 2006; Adebayo-tayo and Samuel, 2009). Roselle calyx is used for production of fruit drink in the tropics (Daramola and Asunni, 2006; Bolade *et al.*, 2009). Demand is on the increase because of nutraceutical properties associated with its natural drink. Also Roselle pizza is one of the deep-fat-fried products commonly consumed in Nigeria (Daramola and Asunni, 2006).

Roselle has been used in traditional medicine as a digestive and purgative agent and a folk remedy for abscesses, billows, cancer, hypertension, debility, diabetes, fever scurvy, cough and dysuria (Dahiru *et al.*, 2003; Egberé *et al.*, 2007).

The characteristics of Roselle like absence of mutagenicity to some organisms, antiviral, antibacterial, antioxidant activity (attributed to protocatechuic acid),

diuretic, emollient, anti-inflammatory, sedative, laxative and tonic activity, and amelioration of many common ailments and injuries make Roselle a medicinal crop (Mounigan and Badrie, 2006; Adebayo-tayo and Samuel, 2009). The antioxidative attributes of anthocyanins and their aglycons against peroxy radicals has been confirmed (Prenesti *et al.*, 2005; Egberé *et al.*, 2007).

### **2.3.2 Chemical and nutrient composition of Roselle calyces**

The chemical composition of Roselle calyx extract is presented in Table 1. It has a high amount of zinc, iron and vitamin C. The anti-nutritional factors found in Roselle calyces include phytic acid, tannic acid, hydrocyanic acid and oxalate. The level of phytic acid is far below the level reported in adult meal to avoid negative effects on zinc and iron absorption (Tsai *et al.*, 2005; Adanlawo and Ajibade, 2006). The hydro cyanide content in the Roselle calyx extract is less than the toxic dose for man of 10 mg /kg of cyanide equivalent. The oxalate in beverage is lower than that found in raw amaranths (280.6 mg/100 g), soy milk (10.9 mg/100 g) and 48.6 mg/100 g in sweet potato leaves (Tsai *et al.*, 2005). The levels of these antinutrient factors may further be reduced by cooking. Also the oxalic acid effect of binding with ferrous iron and therefore inhibiting its absorption can be counteracted by intake of high vitamin C content (Lachat *et al.*, 2006).

Table 1: Nutritional value of Roselle calyces

Source: (Morton, 1987; Wong *et al.*, 2002; Ojokoh *et al.*, 2002; Fasoyiro *et al.*, 2005)

<b>Nutrient</b>	<b>measure</b>	<b>Content</b>
Protein	%	5.8
Carbohydrate		69.5
Fat		3.0
Fibre		9.5
Ash		12.2
Vitamin C	Mg/100g	141.1
Iron		3.2
Zinc		12.2
$\beta$ -carotene		1.9
Lycopene		0.2
Phytic acid	%	0.3
Tannic acid		6.2
Hydrocyanic acid		0.2
Oxalate		0.7

## **2.4 Importance of Vitamin C and Implication of the Ascorbic Acid Test**

### **2.4.1 Uses of vitamin C**

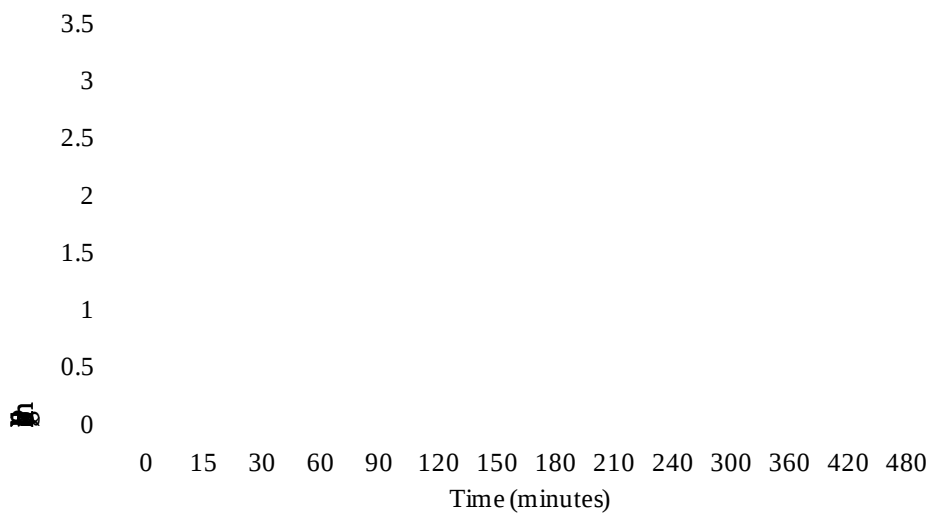
Many studies have not reported direct relationship of vitamin C with growth in children, rather characteristics like reduced morbidity to diseases, appetite gain,

improving lipid metabolism, improving the CD4 count and appetite (Tang and Smit, 2002; Nicolle *et al.*, 2003). Also vitamin C has been shown to decrease the viral load *in vitro*. It also provides main defence against free radicals that are produced continually in the body by scavenging and cleans up waste products (Tang and Smit, 2002). Vitamin C improves iron and copper bioavailability in foods (Gibson *et al.*, 2006); reduce inflammations and increase production and activity of lymphocytes (Rössig *et al.*, 2001; Tang and Smit, 2002); helps in the assembly of a three dimensional stranded structure with amino acids, glycine and proline as principle component of the pro-collagen precursor. It further helps during the conversion of the pro-collagen precursor to collagen (Tang and Smit, 2002).

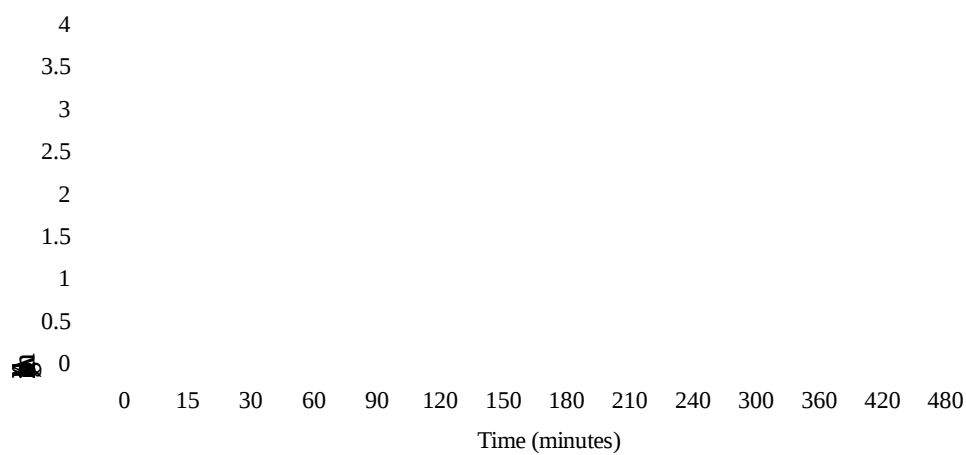
#### **2.4.2 Ascorbic acid saturation test and its implication**

The determination of plasma or urinary levels of ascorbic acid may not always indicate ascorbic acid status of an individual. A satisfactory method for assessing it is by using the ascorbic acid saturation test (Ramakrishnan *et al.*, 2004). A saturation of the ascorbate uptake into endothelial cells which might occur between 100 and 200  $\mu\text{M}$  is equal to the concentration of the vitamin in plasma of healthy individuals (Heller *et al.*, 1999). The orally or intravenously administered ascorbic acid is taken up by the tissues which get saturated 4-6 hours after administration (Ramakrishnan *et al.*, 2004). Donati (2005) demonstrated the pharmacokinetics of vitamin C after oral administration. The maximum concentrations of vitamin C concentration in blood plasma were obtained at 3 and 4 hours after oral administration of 60 and 100mg (Fig. 3). When the cells in the body are saturated with the vitamin excess is lost in urine. Excretion of the vitamin in urine increases with the dosage given. The concentration excreted in urine 4 hours after dose administration should not be less

than 3 and 50mg for healthy individuals on the first and subsequent days. The test is therefore useful in measuring the sub clinical scorbutic state (Ramakrishnan *et al.*, 2004).



A. Plasma concentration of ascorbic acid after oral administration of 60mg in rabbits



B. Plasma concentration of ascorbic acid after oral administration of 100 mg in rabbits

Figure 3: Plasma concentrations in rabbits after ascorbic dose administration

Source: Donati (2005)

## **2.5 Plasma and Urinary Zinc**

Rapid decrease in endogenous faecal and urinary zinc is obtained with low zinc diets while there is limited drop in plasma zinc concentration (King *et al.*, 2000). When dietary zinc is reduced from 241 to 4  $\mu\text{mol/ day}$ , urinary zinc losses drop by 75% by the end of the second week, whereas the plasma zinc concentrations are unchanged (King *et al.*, 2000). Plasma zinc which is the most widely used biomarker of zinc status has poor sensitivity and imperfect specificity (Hambidge, 2003). While urine zinc measurements are poor indicators of tissue zinc status, they are in clinical practice the means by which judgements are made about the adequacy of zinc supplementation during intravenous nutrition. Low urinary zinc concentrations of less than 2  $\mu\text{mol/ day}$  depict zinc depletion while those above 80  $\mu\text{mol/ day}$  depict catabolism which calls for zinc supplementation (Hambidge, 2003). The normal concentration of zinc that has been used ranges from 0.76-7.6  $\mu\text{mol/L}$  (49-490  $\mu\text{g/L}$ ). Urinary zinc losses in children are 9  $\mu\text{g Zn/kg/day}$  (Menary *et al.*, 2002).

## **2.6 Nutrient Composition of Breast Milk and Intake**

### **2.6.1 Nutrient composition of breast milk**

The protein and fat content of human milk is between 8-9 g/L and 3.8% respectively (Allen, 2001). The fat content, which is supposed to contribute 45% of the energy in breast milk, is readily affected by the maternal fat intake (Greenberg and Smith, 1996; Jensen *et al.*, 2001). Table 2 provides the content of micronutrients in breast milk and how they are affected by deficiency of the respective nutrients in the mother's body.



Table 2: Effect of maternal deficiency on breast milk micronutrient content

Nutrient	Concentration in normal milk	Effect of maternal deficiency
Vitamin A ( $\mu\text{g/L}$ )	500	Decrease to 170
Vitamin D ( $\mu\text{g/L}$ )	0.55	Decrease to 0.25
Thiamine (mg/L)	0.21	Decrease to 0.11
Riboflavin (mg/L)	0.35	Decrease to 0.2
Vitamin B6 (mg/L)	0.93	Decrease to 0.9
Folate ( $\mu\text{g/L}$ )	85	No change
Vitamin B12 ( $\mu\text{g/L}$ )	0.97	Decrease to less than 0.5
Ascorbic acid(mg/L)	40	To 25
Calcium (mg/L)	280	Decrease to 215
Iron (mg/L)	0.3	No change
Zinc (mg/L)	1.2	No change
Copper (mg/L)	0.25	No change
Iodine ( $\mu\text{g/L}$ )	110	Slight change
Selenium ( $\mu\text{g/L}$ )	20	Decrease to less than 10

Source: Allen (2001)

### 2.6.2 Breast milk intake

Infants' intake of breast milk varies with breastfeeding practices. In many African societies the colostrum is expressed and discarded or withheld until the secretion of mature milk begins, which can be 6–10 days after delivery (Ross and Harvey, 2003). Children are given prelacteal feeds especially cereals and cow's milk which increases risks to infections and reduced breast milk intake as well as nutrients like vitamin A (Ross and Harvey, 2003). This increases the risk of malnutrition since the prelacteal and complementary feeds have unbalanced quantities of nutrients that may not meet the children's recommended dietary requirements (Hotz and Gibson, 2007). Also the complementary foods given do not necessarily increase the energy intake, which is needed for growth (Mamiro, 2003). Further most of the mothers in developing countries have poor dietary intake of fat and protein which reduces the energy in the breast milk (Owino *et al.*, 2007). Children with malnourished mothers, consume more breast milk to meet their energy needs than those born to nourished mothers (Galpin *et al.*, 2007). The World Health Organization has published the breast milk intake of children in developing countries as shown in the Table 3 for the children 6-

23 months. The lowest and highest values of breast milk intake have been represented by computing -2 and +2SD deviation respectively.

Table 3: Breast milk intake by children in developing countries

Age group (months)	Breast milk intake (g/ day)		
	Low (-2 SD)	Mean	High (+2 SD)
6-8	371	660	949
9-11	271	616	960
12-23	175	549	922

Source: Ross and Harvey (2003)

## 2.7 Complementary Foods

### 2.7.1 Complementary feeding

Complementary feeding means giving other foods to children in addition to breast milk. These other foods prepared for and given to children are called complementary foods. There are two kinds of complementary foods which include specially prepared foods and usual family foods that are modified to make them easy to eat and can provide enough nutrients (WHO, 2000). During the period of complementary feeding, a baby gradually becomes accustomed to eating family foods (WHO, 2000). Complementary foods are of lesser nutritional quality than breast milk (Faber, 2004). Therefore, they should not be aimed at replacing breast milk completely; rather it should contribute to the nutrient (energy) deficit left by intake of breast milk (Fig. 4).

Figure 4 shows the energy needed as the child grows older and becomes more active. It also shows how much of this energy is supplied by breast milk if a mother breastfeeds frequently (the shaded area). From the age of 6 months onwards, the gap between the total energy needs and the energy provided by breast milk widens. This implies that the energy deficits are to be provided by the complementary foods, and

therefore quantity of food increases as the child becomes older and failure to meet these needs the child's growth velocity slows down.

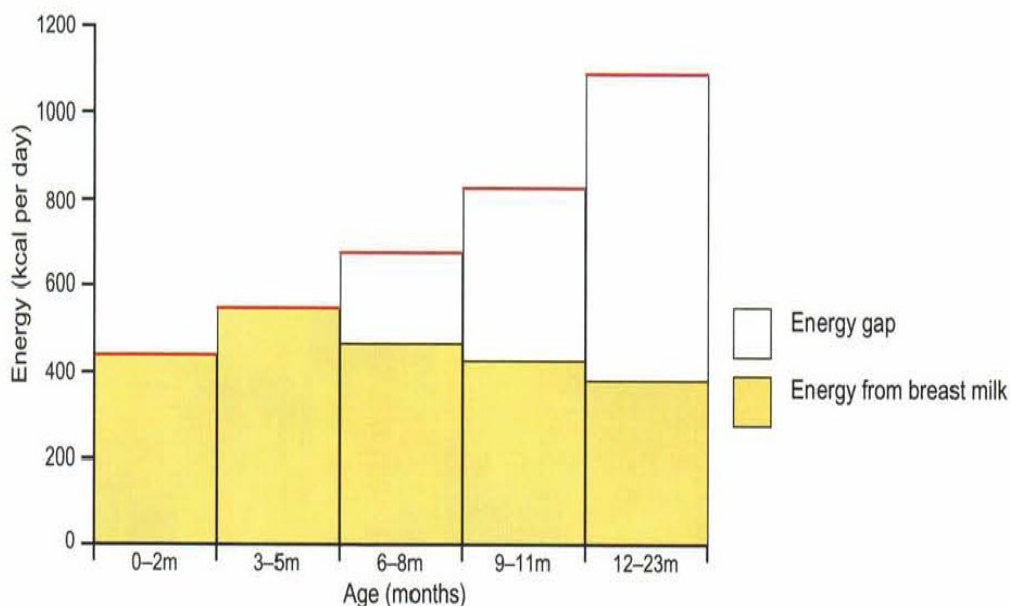


Figure 4: Energy required from the breast milk and complementary foods

Source: WHO (2000)

### 2.7.2 Energy and nutrient intake among children in Tanzania

The major complementary foods in Tanzania are starchy and usually cereals such as maize, sorghum, millet, rice and non cereals like cassava, sweet potatoes, plantains and bananas. Most of these foods do not have high nutrient density and may not provide for the requirements of the child (Nestel *et al.*, 2003). They also contain high densities of anti-nutritional like phytates, thiaminases, goitrogens and oxalic acids, which make meeting the micronutrients requirement the greatest challenge (Hotz and Gibson, 2007). The requirements in Table 4 below can be attained by processing, fortification, supplementation and consumption of animal foodstuffs. Many projects like the Joint Support Nutrition Project and the Child Survival Development Program were introduced to avert some of the above problems. The

projects identified local complementary foods with high nutrient densities to reduce malnutrition. However, they focused at reducing dietary bulk and enriching complementary foods with nutrients. Further blending ratios were lacking, which resulted into inadequate intake of nutrients to the children (Mamiro, 2003).

Table 4: Recommended nutrient intakes used in the WHO/UNICEF 1998, new DRI and WHO 2002

Nutrient	Recommended nutrient intake								
	6–8 mo			9–11 mo			12–23 mo		
	WHO/ UNICEF 1998	New DRI	WHO 2002	WHO/ UNICEF 1998	New DRI	WHO 2002	WHO/ UNICEF 1998	New DRI	WHO 2002
Protein (g/day)	9.1	NA	NA	9.6	NA	NA	10.9	NA	NA
Vitamin A (µg RE/day)	350	500 <sup>b</sup>	400	350	500 <sup>b</sup>	400	400	300	400
Folate (µg/day)	32	80 <sup>b</sup>	80	32	80 <sup>b</sup>	80	50	150	160
Niacin (mg/day)	4	4 <sup>b</sup>	4	5	4 <sup>b</sup>	4	8	6	6
Pantothenic acid (mg/day)	1.7 <sup>c</sup>	1.8 <sup>b</sup>	1.8	1.7 <sup>c</sup>	1.8 <sup>b</sup>	1.8	1.7 <sup>c</sup>	2.0 <sup>b</sup>	2.0
Riboflavin (mg/day)	0.4	0.4 <sup>b</sup>	0.4	0.4	0.4 <sup>b</sup>	0.4	0.6	0.5	0.5
Thiamine (mg/day)	0.2	0.3 <sup>b</sup>	0.3	0.3	0.3 <sup>b</sup>	0.3	0.5	0.5	0.5
Vitamin B <sub>6</sub> (mg/day)	0.3	0.3 <sup>b</sup>	0.3	0.4	0.3 <sup>b</sup>	0.3	0.7	0.5	0.5
Vitamin B <sub>12</sub> (µg/day)	0.4	0.5 <sup>b</sup>	0.5	0.4	0.5 <sup>b</sup>	0.5	0.5	0.9	0.9
Vitamin C (mg/day)	25	50 <sup>b</sup>	30	25	50 <sup>b</sup>	30	30	15	30
Vitamin D (µg/day)	7	5 <sup>b</sup>	5	7	5 <sup>b</sup>	5	7	5 <sup>b</sup>	5
Vitamin K (µg/day)	10 <sup>c</sup>	2.5 <sup>b</sup>	10	10 <sup>c</sup>	2.5 <sup>b</sup>	10	10 <sup>c</sup>	30 <sup>b</sup>	15
Calcium (mg/day)	525	270 <sup>b</sup>	400	525	270 <sup>b</sup>	400	350	500 <sup>b</sup>	500
Chloride (mg/day)	500	NA	NA	500	NA	NA	800	NA	NA
Copper (mg/day)	0.3	0.2 <sup>b</sup>	NA	0.3	0.2 <sup>b</sup>	NA	0.4	0.3	NA
Fluoride (µg/day)	0.05 <sup>c</sup>	0.5 <sup>b</sup>	NA	0.05 <sup>c</sup>	0.5 <sup>b</sup>	NA	0.05 <sup>c</sup>	0.7 <sup>b</sup>	NA
Iodine (µg/day)	21	130 <sup>b</sup>	90	21	130 <sup>b</sup>	90	12	90	90
Iron (mg/day) <sup>d</sup>	11	11	9.3	11	11	9.3	6	7	5.8
Magnesium (mg/day)	75	75 <sup>b</sup>	54	80	75 <sup>b</sup>	54	85	80	60
Manganese (mg/day)	0.02 <sup>c</sup>	0.6 <sup>b</sup>	NA	0.02 <sup>c</sup>	0.6 <sup>b</sup>	NA	0.02 <sup>c</sup>	1.2 <sup>b</sup>	NA
Phosphorus (mg/day)	400	275 <sup>b</sup>	NA	400	275 <sup>b</sup>	NA	270	460	NA
Potassium (mg/day)	700	NA	NA	700	NA	NA	800	NA	NA
Selenium (µg/day)	10	20 <sup>b</sup>	10	10	20 <sup>b</sup>	10	15	20	17
Sodium (mg/day)	320	NA	NA	350	NA	NA	500	NA	NA
Zinc (mg/day)	2.8 <sup>e</sup>	3	4.1 <sup>f</sup>	2.8 <sup>e</sup>	3	4.1 <sup>f</sup>	2.8 <sup>e</sup>	3	4.1 <sup>f</sup>

a. Shaded areas are cases in which at least two of the reference values differ by more than 20%. NA, Not yet available.

b. Based on adequate intake (AI) estimates.

c. Based on "safe nutrient intake" from British dietary reference values.

d. Assuming medium bioavailability (10%).

e. Based on Annex III of the 1998 report.

f. Assuming moderate bioavailability (30%).

Source: Dewey and Brown (2003)

### 2.7.3 Micronutrient availability in complementary foods

Iron, zinc and vitamin C deficiencies have long been manifested in the African communities due to low consumption of animal products, fresh vegetables, fruits and consumption of foods with high fibre, oxalates, polyphenols and phytates (Hotz and Gibson, 2007). Techniques like germination, microbial fermentation or soaking to reduce the phytate and polyphenol content of unrefined cereal porridges used for young child feeding. Addition of ascorbic acid-containing fruits to enhance non-haem iron absorption; heating to destroy heat-labile anti-nutritional factors (e.g.

goitrogens, thiaminases) or disrupt carotenoid–protein complexes have been employed in` research and at community levels (Gibson *et al.*, 2006). Vitamin C act mainly in the stomach and duodenum as both a solubilising ligand and reducing agent. It reduces ferrous iron to ferric ( $\text{Fe}^{3+}$ ) and zinc to  $\text{Zn}^{2+}$  thus increasing their solubility (Mamiro, 2003; Lachat *et al.*, 2006). Although vitamin C increases the bioavailability of minerals like iron and zinc, most of the diets of the children have inadequate vitamin C due to over cooking of the vegetables and poor storage of fruits (Faber, 2004).

## **2.8 Appetite Assessment**

The council on nutrition appetite questionnaire (CNAQ), simplified nutrition appetite questionnaire (SNAQ) and subjective questions for children’s disliking to eat (anorexia) have been developed to assess appetite and weight loss (Wilson *et al.*, 2005). The tools have been used to estimate energy intakes of the children from the meals eaten, which have been theoretically related to food availability. However, reductions of children’s appetite due to infection, nutrient imbalance, or other intrinsic characteristics may provide alternative explanations (Brown *et al.*, 1995).

Different studies have been conducted to assess appetite. In their study on adults using CNAQ and SNAQ, Wilson *et al.* (2005) found out that more than 11% of the adults lose their weight with subjective reporting of loss of appetite. In yet another study among patients with haemodialysis, Burrowes *et al.* (2005) found out that 43% of the patients reported to have lost appetite which significantly correlated with weight loss. There are few studies that have been done in developing countries particularly for children. Brown *et al.* (1995) showed that prevalence of anorexia among children below one year increased with age (Fig. 5). In the same study,

appetite increase was inversely associated with consumption of breast milk. Appetite loss was related to infections.

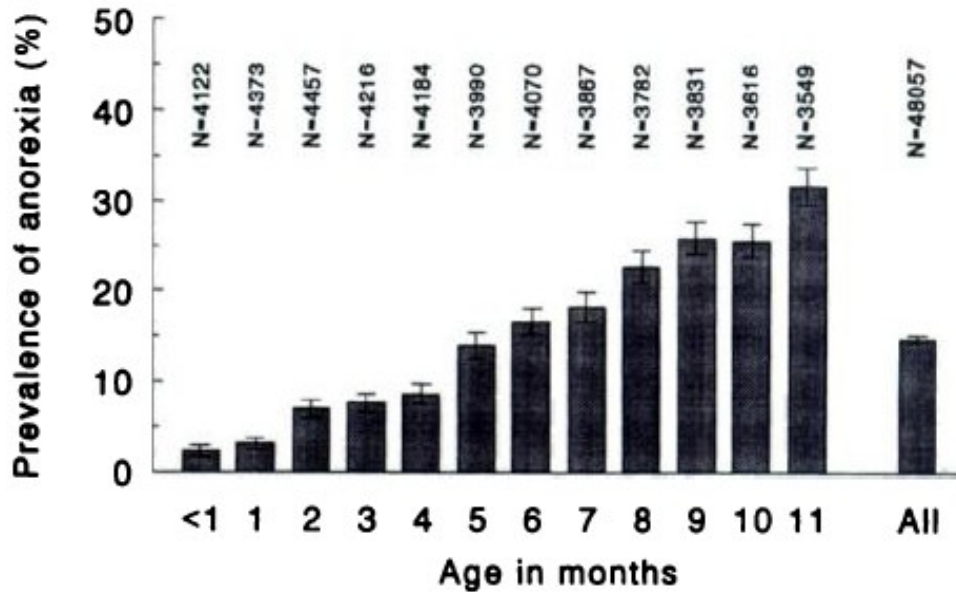


Figure 5: Prevalence of anorexia against age of the children

Source: Brown *et al.* (1995)

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Study Area and Population

The study was conducted in Magubike Village in Magubike ward, Kilosa District, Morogoro Region in Tanzania. Morogoro region lies between latitude 5° 58" and 10° 0" to the South of the Equator and longitude 35 ° 25" and 35° 30" East. The district is

bordered by the following regions: Tanga in the North, Dodoma and Iringa in the West. It is bordered by districts of Kilombero in the South; Mvomero, Morogoro urban and rural in the east. Kilosa is located 300 km west of Dar es Salaam. It has a population of 489 513 and 105 635 households in 135 registered villages (CSPD, 2005). The average household size of the district was 4.6. The district has one government run hospital and one missionary hospital (Berega).

Magubike village is located 82km from Morogoro town and has a total population of 7 501. There were 1 500 children with ages 0-59 months at the period of this study. The village has ten hamlets namely; Makangalawe, Mgalai, Ipondelo, Lugofu, Chimale A, Chimale B, Mjini, Sokoni, Mazimbo and Mtunye. Each hamlet is headed by a Chairperson. Most villagers are subsistence farmers growing maize, cassava and sorghum. There is increasing number of individuals involved in petty trade. Food shortage months are from February to March while harvest periods are July to September. The health facilities available in Magubike include a health centre and home based care services for people living with HIV/AIDS (PLWHA). The most common illnesses that affect Magubike dwellers are malaria, diarrhoea, acute respiratory infections and anaemia (Jumbe, 2007).

### **3.2 Study Design and Sample Selection**

#### **3.2.1 Study design**

Randomized control trial design, single blind was used, in which two types of juices with a similar colour i.e. placebo (red coloured drink) and intervention (Roselle) juice were given to the children in the two respective groups as snacks daily. Mothers and village nutrition assistants were not informed of the difference between the two types of juices given to children during the supplementation period. The investigator



was not blinded because of the extra resources that were required and to ensure that the quality of juices was maintained. The children were recruited in a single phase due to the short time available for accomplishing the study. Children were randomly assigned to intervention (Roselle juice) and placebo feeding regimen as shown in Fig. 6. Mothers were free to give any food of their choice apart from the juices given. Children in each group were given 100 ml of intervention and placebo juices each day for four months (12 litres each). PANTIL nutrition assistants in the village delivered juice to the children homes every day and ensured that all the juice was consumed by the target child. Anthropometric measurements were taken at baseline, each month during the supplementation period and 2 months after supplementation. Other information in section 3.3 was obtained at baseline, fourth month of feeding regimen period and 2 months after supplementation. The wash-out period of one month was given to excrete all the vitamin C that was consumed during saturation and for the body to return to usual status. Routine vaccination was also ensured among all children by encouraging caregivers to take the children for timely vaccination. A dietary assessment was done once during collection of baseline information.

### **3.2.2 Inclusion and exclusion criteria**

All children with ages 6-21.9 months who were still breast feeding were included in this study. Children with ages 6-21.9 months with acute and chronic illnesses, history premature, low birth weight and multiple foetus birth status were excluded from the study. All selected children were dewormed; examined, treated against any infections; and given a washout period of one month before the four months feeding regimen period.

### **3.2.3 Sample size and sampling**

A total of 274 children with ages 6-21.9 months were obtained from the list of the Reproductive Child Health Clinic (RCHC) program register. Using the procedure in section 3.2.2, three children never fitted the criterion and were excluded by consulting the hamlet leaders. Eighty seven children were removed from the list because the hamlet leaders failed to locate their households. Therefore, sampling frame was 184 children as shown in Fig. 6. The equation by CDC and WFP, (2005) in appendix 5 was used to calculate sample size. According to Jumbe, (2007) the prevalence of stunting in Magubike was 37% thus growth faltering was assumed to be 40%. It was assumed that growth faltering would be eradicated. The level of significance used was 5% with power of 95%. Forty (40) children were obtained in each group making a total of 80 children. Allowing an average of 10% dropout in each month, then the sample size was estimated at 54 children for each group. From the sampling frame, 120 children with ages 6-21.9 months were randomly selected for this study. They were randomly assigned to intervention and placebo feeding regimens. Names of children were arranged in ascending (alphabetical) order in their hamlets and assigned numbers 1 and 2 corresponding to intervention and placebo group respectively. The sample sizes obtained in each month are given in Fig. 6. However, not all children that had not dropped out were obtained at each follow up visit. The reasons for not reporting at each month are given in Fig. 6. The major reason was unavailability of their parents because of farming and business commitments. Although the parents were unavailable, children continued to take the juice.

### **3.3 Data Collection**

#### **3.3.1 Socio-demographic information**

Parent and household characteristics were obtained using a structured questionnaire (Appendix 1). Children's characteristics like age, sex, breast feeding and infant and young children feeding practices were obtained using the structured questionnaire.

#### **3.3.2 Feeding and morbidity information**

Structured questionnaire in appendix 1 was used to collect information on health status, and feeding practices of the children. History of immunization, use of vitamin supplements and reason for supplementation was ascertained to ensure that children were on schedule.

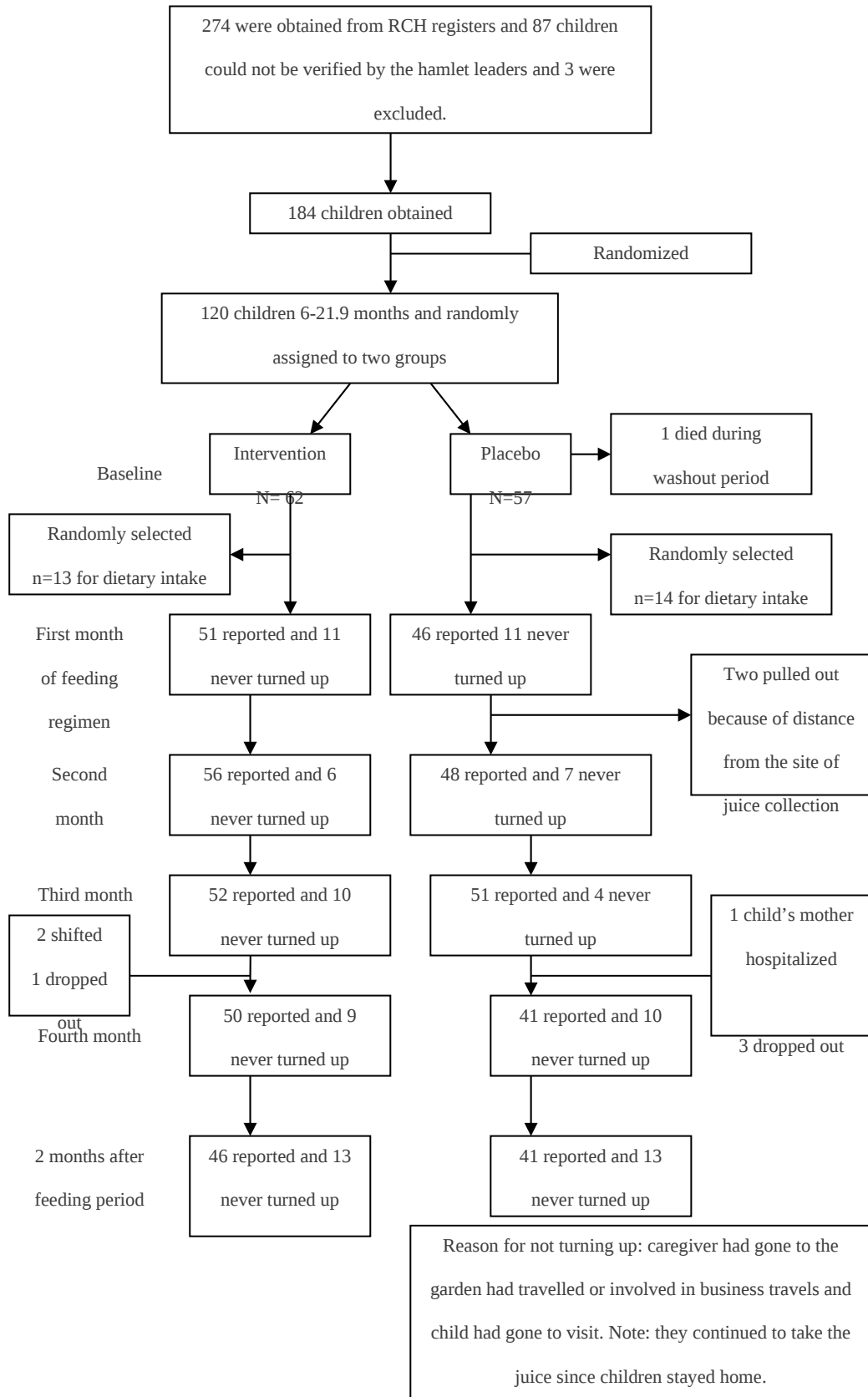


Figure 6: Study design, sampling and follow up of children in both feeding regimens

### 3.3.3 Anthropometric measurements

The anthropometric measurements were conducted by the investigator with the help of PANTIL nutrition research assistants. Body length (cm) and weight (Kg) were taken using Seca weighing scales (model: 881 1021659, seca gms & Co., Germany) and length or height board (Shorr Production, 17802 Shotley Bridge Pl. Olney, Maryland, USA, 20832) respectively. The Z- scores of different indices were obtained using WHO Anthro v.3.0.1, 2009 software. The WHO 2006 reference population was used to compute anthropometric indices. Table 5 summarizes how the Z-scores were used to categorize children into the different nutritional status categories. Children (6-24 months) with stable weight, weight loss and those with a weight gain of less than 300 g in three months were regarded as having faltered growth.

Table 5: Classification of malnutrition

<b>Level of malnutrition</b>	<b>Weight-for-height (wasting)</b>	<b>Height-for-age (stunting)</b>	<b>Weight-for-age (underweight)</b>
Moderate	between - 2 and -3 Z scores	between - 2 and -3 Z- scores	between - 2 and -3 Z- scores
Malnutrition	less than -3 Z-scores	less than -3 Z-scores	less than -3 Z-scores
Severe Malnutrition	less than -2 Z-scores	less than -2 Z-scores	less than -2 Z-scores
Global Malnutrition	less than -2 Z-scores	less than -2 Z-scores	less than -2 Z-scores

Source: CDC and WFP (2005)

### 3.3.4 Biochemical assessments

#### 3.3.4.1 Determination of haemoglobin concentration

Haemoglobin concentration in the blood was determined by the hemocue (HemoCue, AB, Angelhom, Sweden) technique. The erythrocyte membranes are disintegrated by

sodium deoxycholate, releasing the haemoglobin. Sodium nitrite converts the haemoglobin iron from the ferrous to the ferric state to form methemoglobin, which then combines with azide to form azidemetemoglobin (Robinett *et al.*, 2000). The analyzer was turned on and the cuvette holder pulled outwards and until the screen displayed "READY." The standard cuvette was inserted in the machine to check whether the Hemocue was functioning well and calibrated before the haemoglobin concentration of the child was taken. The puncture site (middle finger) was cleaned with a spirit (disinfectant) and allowed to dry. A gentle pressure was applied, pricked and the first 2-3 drops of blood at the pricked site were wiped away. The cuvette was filled with capillary blood in continuous process. Any excess blood was wiped off the outer side of the cuvette without touching the tip. An adhesive bandage was placed at the pricked area to stop bleeding. The filled cuvette was placed into the cuvette holder and immediately put in the measuring position. The haemoglobin concentration was read off the screen and recorded in  $\text{g dl}^{-1}$ . Children with haemoglobin concentration of less than  $11 \text{ g dl}^{-1}$  were categorized as anaemic while those with less  $7 \text{ g dl}^{-1}$  were severely anaemic. Children with haemoglobin concentration between  $7\text{-}10.99 \text{ g l}^{-1}$  were categorized as being moderately anaemic (CDC and WFP, 2005). Determination of haemoglobin concentration of the children was conducted at baseline and at the end of the fourth month of intervention.

#### **3.3.4.2 Ascorbic acid saturation test**

The principle behind the saturation test is that the administered ascorbic acid is taken up by the body tissues until they are saturated and after 3-4 hours there is a sharp rise in the urinary ascorbic acid excretion (Ramakrishnan *et al.*, 2004).

The children were orally given 50 mg of ascorbic acid that was obtained from the pharmacy. It was dissolved in 50 ml of water and given to the child at 0700-0800 h before giving the child any other food. The child was left without giving any food for one hour. The urine that was excreted by the child between 4 and 6 hours (1200-1400 h) after administration of vitamin C was collected using paediatric urine collectors (Lot 20070825, manufacture date: 08 2007; expiry date 08 2012). The volume of urine excreted by the child after 4-6 hours was measured using a measuring cylinder. The urine was quickly transferred into three vials of 1.5 ml each and stored at  $-18^{\circ}\text{C}$  in a freezer. The urine samples were taken to SUA laboratory for analysis of vitamin C. Dichlorophenoindolphenol was used as the principle reagent. It reacts with ascorbic acid giving a colourless compound. One (1) ml of the urine sample was diluted with 9 ml of 0.1 M citric acid buffer in a test tube. Then 1 ml of 0.1 M dichlorophenoindolphenol (DCPIP: B.NO. 103028.0005, Merck, Germany) was added. The contents were mixed thoroughly and quickly transferred into translucent cuvette to the mark. The cuvette was inserted into the spectrophometer (Wagtech, Cecil International, House of Tumors, Birkshire, UK) and absorption was read off at 520 nm. The concentration (mg/ml) was determined by comparing the absorption of standard ascorbic acid (B.No. 1.0127-250 Merck, Germany) solution of  $2\ \mu\text{g ml}^{-1}$ . The concentration ( $\text{mg ml}^{-1}$ ) of ascorbic acid was computed using equation (i) below. A child was saturated when the urinary ascorbic acid secretion during the 4-6 hours following an oral dose of 50 mg vitamin C exceeded 280 mol or 0.8 mg/ kg body weight. Children with total vitamin C of 3 mg or less were considered deficient of vitamin C. The saturation test was conducted at baseline and at the fourth month of supplementation.

$$\text{Concentration (mg/ ml)} = \frac{\text{Blank - Test reading}}{\text{Blank - standard reading}} \times \text{DF} \times 2 \mu\text{g} \dots\dots \text{(I)}$$

Where; DF is the Dilution Factor which was 10 for urine

### 3.3.4.3 Breast milk analysis

Five mothers with children of ages 8, 10, 14, 15 and 16 months after consenting, manually expressed 10 to 40 ml of milk from the right breast into sterilized dark plastic tubes, which were sealed and stored at -18°C. The breast milk samples were expressed and collected between 0800-1200 h. The procedures in Appendix 3 were used to determine the proximate and mineral composition in the breast milk samples (40 ml tubes).

Preparation and analysis procedure of vitamin C in breast milk explained by Ahmed *et al.* (2004) was used. Milk samples (10 ml) were centrifuged at 3000 rpm for 20 minutes. On centrifugation, the fat component of milk separates from the fluid part and the cells settle at the bottom. From the clear mid-zone, 0.3 ml sample was taken in a test tube to which 1.2 ml of 0.1 M trichloro acetic acid (TCA- B.No. 2976, Pharmacos Ltd, Essex, England) was added and mixed well for 15 seconds. The mixture was then centrifuged at 3000 rpm for 10 minutes. From this, 0.9 ml supernatant was taken and 0.4 ml Folin-ciocalteaus' phenol (folin's) reagent (B.No. 0695-895-2206x) was added. It was then covered with aluminium foil and incubated at 60°C for 60 minutes in a water bath. Immediately after incubation, the sample was chilled in ice-cold water and 1.6 ml of 65% sulphuric acid was added gradually. Finally the treated sample was stored at room temperature for 30 minutes. Absorbance was measured against a reagent blank at 760 nm in a spectrophotometer



(Wagtech, Cecil International, House of Tumors, Birkshire, UK). All samples were analysed in duplicates. Equation (ii) from the standard curve of ascorbic acid in appendix 4 was used to calculate the concentration (mg/ml).

$$\text{Concentration}(mg / ml) = (\text{Absorbance}(y)) \times 5.3838 \dots\dots\dots(ii)$$

### **3.3.5 Dietary assessment**

A weekly food frequency and 24 hour interactive dietary recall questionnaire in appendix 1 for three non consecutive days in a week was administered. Quantities of the food consumed were estimated with the help of household measures and TANITA cooking scales (Model: 1150, Japan). Cooked food samples were collected in plastic food containers and stored in a cool box and carried to the Departments of Food Science and Technology and Animal Science, Sokoine University of Agriculture (SUA) for analysis of nutrient composition using procedures explained in appendix 2 and 3. Vitamin C was determined using the procedure explained by Dashman *et al.* (1997). Proximate composition and mineral analysis were conducted using the AOAC, (1990). Appetite assessment tool in appendix 1 was used to determine children's appetite score. The dietary assessment was only conducted at baseline between 16<sup>th</sup> and 30<sup>th</sup> October 2008.

### **3.3.6 Preparation and analysis of nutrient composition of the juice**

#### **3.3.6.1 Preparation and nutrient analysis of Roselle juice**

Roselle juice was made using the locally available recipes. Fresh Roselle calyces were bought from a local farmer and dried using solar vegetable driers to maintain product uniformity and composition. The ingredients used in the preparation of the

juice included 100 g of solar dried Roselle calyces, 500 g of sugar and 5 litres of boiling water. Materials used include two saucepans, muslin cloth and sterilized polythene (plastic) bags for packaging.

The juice was prepared in the Department of Food Science laboratory, at SUA Tanzania. Five (5) litres of distilled water were left to boil to 98°C in a saucepan for 5 minutes. Then 100 g of Roselle calyces (solar dried) and 500 g of sugar were added, stirred and then covered. The mixture was left to boil for another 5 minutes. The saucepan was put off the heater and the juice was left to cool to a temperature of 30-40°C. The juice was filtered using a muslin cloth into a clean saucepan. The juice was allowed to cool to a temperature of 20-30°C, and then packed in sterilized 100 ml polythene bags, sealed and stored in a freezer at -18°C. The packed juice was delivered to children in cool boxes and served while chilled. Three days' batches were delivered to Magubike PANTIL office. Samples from weekly batches were collected for proximate, vitamin C and mineral analysis. The nutrient composition of Roselle juice was determined using procedures explained in Appendix 2 and 3. Vitamin C determination was conducted using the procedure explained by Dashman *et al.* (1997). Proximate composition and mineral analysis were conducted using a procedure explained by AOAC (1990).

### **3.3.6.2 Preparation of the placebo juice**

The ingredients used included one litre of *anjari* concentrate (made in Tanzania), 20 litres of distilled water and 1.5 kg of sugar. Materials used include two saucepans, muslin cloth and polythene (plastic) bags for packaging.

The 20 litres of water in a saucepan were left to boil for 10 minutes and then one litre of anjari concentrate, 1.5 kg of sugar, were added, mixed well and let to boil for 10 minutes. The juice was left to cool to a temperature of 30-40°C and sieved using a muslin cloth into a clean saucepan. The juice was cooled at room temperature and packaged in sterilized 100 ml polythene bags, sealed and stored in a freezer at -18°C. The nutrient composition of placebo juice was determined using procedures explained in Appendix 2 and 3. Vitamin C determination was conducted using the procedure explained by Dashman *et al.* (1997). Proximate composition and mineral analysis were conducted using a procedure explained by AOAC (1990).

### **3.3.6.3 Administration of the juice**

PANTIL nutrition assistants in the village distributed the packed juice (100 ml) to the homes of the selected children each day and ensured the juice was consumed by the targeted child. The children were free to take their usual meals and complemented with juices. The juice was administered to the children orally by the PANTIL nutrition assistants with the help of the caregivers. The juice was served from 1200 hours to capture the time after lunch.

### **3.4 Data Entry and Analysis**

Nutrient composition of the food items were computed with the help of Nutri Survey (2007) software. Food weights were entered into Nutri Survey (2007) to estimate nutrient intake. Anthropometric measurements were entered into WHO Anthro, v.3.0.1, software to generate anthropometric indices.

The rest of the data was directly entered into Statistical Package for Social Sciences (SPSS-12.5). Data from dietary intake and anthropometric data was exported to SPSS for analysis. The descriptive statistics like mean and standard deviations were obtained. The analysis of variance, confidence intervals at 95% and student t-tests separated the mean Z scores of weight for age, weight for height and height for age of the children in the placebo and intervention groups. The difference in the mean nutrient intake of the children in the placebo and intervention groups was determined by the student t- test. Chi square test ( $\chi^2$ ) at 5% significance was used to determine relationships of categorical variables. Bivariate correlation analysis was used to establish associations between different continuous variables. Duncan's rank analysis was used to compare means of nutrients contained in the different food stuffs.

### **3.5 Ethical Considerations**

The Principal investigator sought ethical clearance from the National Institute of Medical Research (NIMR) Dar es Salaam, Tanzania. Permission to conduct the study was sought from Sokoine University of Agriculture (SUA), Morogoro Region, Kilosa district and Magubike village administration. Selected mothers or caregivers were asked to sign a translated written consent form (Appendix 6) to participate in this study. At the end of the study households of all children involved in the study were given Roselle seeds to plant.

### **3.6 Quality Control**

Prompt follow up of the mothers by the research assistants was done to ensure that the urine bags were inserted in the right range of time. Each assistant followed up 10 children each day to increase efficiency. The administration of vitamin C was done at

the PANTIL offices in the village to ensure that children consumed the vitamin C tablet. In addition, the PANTIL nutrition assistants were trained on proper taking of anthropometric measurements. The juice was carried in frozen conditions and calyces were obtained from a single farmer to ensure that quality was maintained. The vitamin C concentration, proximate and analysis were conducted three times to check on variation of the nutrient content of juices. The same preparation procedure of the juices was maintained throughout the feeding time.

### **3.7 Study Limitations**

The study employed a single blinded trial design due to minimum resources like funding and trained human resource to prepare the juice.

In this study, follow up dietary assessments were not done due to inadequate time and financial resources, which made it hard to establish impact of consuming other foods.

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Demographic Characteristics

##### 4.1.1 Household characteristics

The results of the different household characteristics are summarized in Table 6. The mean size of the households from where the children in the study groups were obtained was five (5) and average number of children below five years of age was one (1). The average proportion of children below five years from the households in the study groups was 30%. The male to female ratio in households from where the children in both feeding regimens were obtained was 0.9. The age of mothers for most children (68%) was 27 years. In addition, about 19% of all the children had mothers of age below 20 years and 13% had mothers of age above 45 years. The age of fathers ranged between 20-35 years for 59% of the children. Results further revealed that 20% of the children were obtained from female headed households. The average monthly household income was Sh. 46 598.99. The average contribution of the mothers to the household income was 45%. Parity significantly ( $p < 0.05$ ) increased with age, with children's mothers from both feeding regimens. More results on education status of the parents and marital status are summarized in Table 6.

Table 6: Household Characteristics at baseline

Characteristics of Households	Means or %
Household size	5.1±2.0 (N=119)
Number of children below 5yrs	1.4±0.6 (N=119)
Average proportion of children less than five years of age	29.8±11.5(N=119)
Male: Female ratio in household	0.9
Sex ratio, (female: male) of sampled children	119, (1.01)
Ages of the female parents	
<20years	17.6±1.1 (n=22)
20-35 years	27.4±4.9 (n=80)
>35 years	39.3±3.4 (n=15)
Ages of the male parents	
<20 years	18.5±0.7 (n=2)
20-35 years	29.1±4.3 (n=56)
36-50 years	40.5±4.3(n=35)
>50 years	62.5±7.7 (n=2)
Female Headed households	20.4%
Average monthly household income (T. Sh.)	46 598.99±30 928.08 (N=89)
Per capita income (T.Sh.)	353.74±218.1 (N=89)
Average contribution of income by mother (%)	44.9±33.0 (N=89)
Parity of women	
<20 years	1.2±0.7 (n=22)
20-35 years	3.2±1.6 (n=80)
Above 35 years	5.8±1.5 (n=15)
Female Marital Status	
Married	45.1%
Cohabiting	33.1%
Divorced/Separated	1.7%
Single parent	20.1%
Mother never gone to school N, (%)	98, (16.5)
Mother attended less than secondary N, (%)	98,( 79.9)
Father never gone to school N, (%)	89, (13.3)
Father less than secondary. N, (%)	89, (77.9)
Values are Means ±SD or %	

## 4.2 Dietary Assessment

### 4.2.1 Breast feeding frequency and practices

About 82% of the children aged less than 12 months and 63% of the children aged 12-21.99 months were breast fed for at least 8 times within the previous 24 hours prior to the survey. Table 7 shows the mean daily breast feeding frequency of the children in the study groups at baseline and after four months of supplementation. The mean breast feeding frequency for all children was 9 times (Table 7). The frequency of breast feeding of children with age group between 14-22 months significantly decreased ( $p < 0.05$ ) from 9 to 6 times a day after 4 months of supplementation. Further more, only 33% of children who were regularly changed from one breast to another before emptying in a single breast feeding session.

Table 7: Mean daily breast feeding frequency

Age group (months)	Month of visit	Placebo	n	Intervention	n	P value	Combined	N
6-14	Baseline	8.9±2.4	3	8.8±2.5	3	$p > 0.05$	8.8±2.4	67
			1		6			
	Fourth	7.2±2.3	2	8.0±2.4	2	$p > 0.05$	7.6±2.4	54
14.01-22	Baseline	9.5±3.7	1	7.2±3.0	1	$p > 0.05$	8.4±3.5	26
			3		3			
	Fourth	*2.0±3.5	1	*3.3±4.4	9	$p > 0.05$	*2.6±3.8	20
6-22	Baseline	9.1±2.8	4	8.5±2.7	4	$p > 0.05$	8.7±2.8	93
			4		9			
	Fourth	*5.5±3.6	3	*6.9±3.6	3	$p > 0.05$	*6.2±3.6	74
			6		8			

All values are mean  $\pm$ SD , \* significantly different from baseline value ( $p < 0.05$ )

### 4.2.2 Feeding frequency

The frequency of feeding children did not significantly ( $p > 0.05$ ) change throughout the study period (Fig. 7). The mean feeding frequency of children was 2.5 times a



day at baseline and 3 times at the fourth month of supplementation. There was no observed significant differences ( $p>0.05$ ) in the mean feeding frequencies of children between the placebo and intervention groups at baseline and at the fourth month of supplementation (Fig. 7).

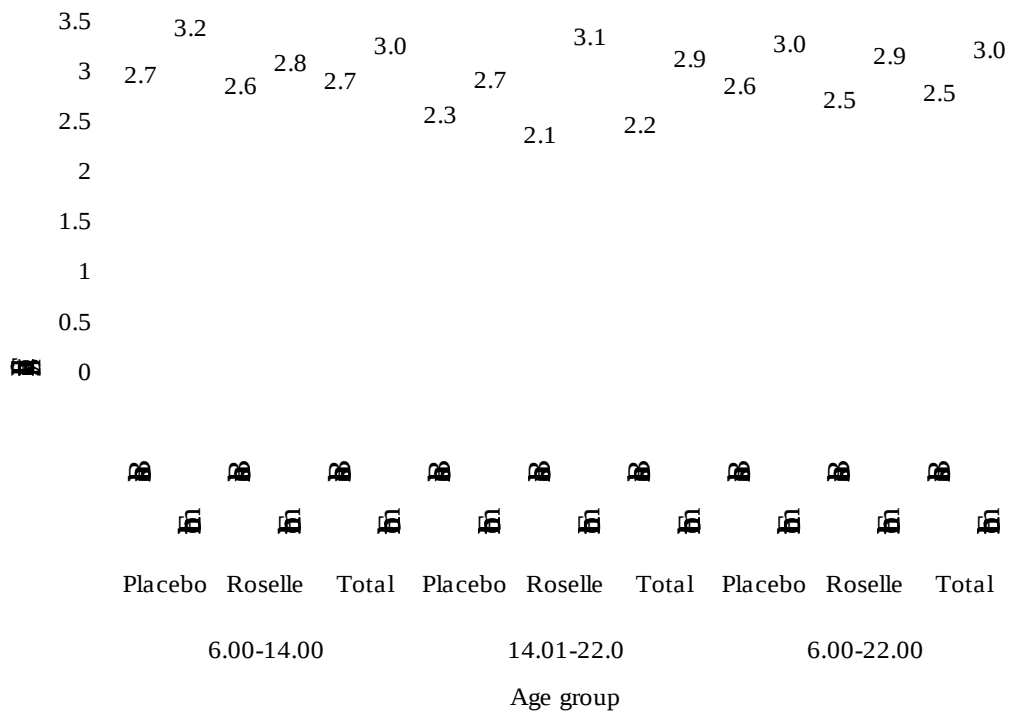


Figure 7: Frequency of feeding children on complementary meals

### 4.2.3 Food consumption

#### 4.2.3.1 Foods from animal and plant legumes

The proportion of children who consumed particular food stuffs within the period of one week prior to the survey is presented in Table 8. There was low consumption of animal and legume food stuffs. Only 9, 7, 3, 2 and 1% of the children consumed sardines, cow’s milk, liver, formula milk and eggs respectively daily. There was only

one type of legume consumed by the children, i.e. beans. This was part of the composite flour for making porridge. About 13% of the children consumed beans.

Table 8: Animal and plant legume foods

Food s Eaten	Number of days reported consuming the food in the previous week							
	1	2	3	4	5	6	7	Never
	<b>Proportion of all children (%) N=119</b>							
Beef	24.4	8.4	5.9	0.8	0.0	0.0	0.0	60.5
Goat meat	16.0	5.0	2.5	0.8	0.0	0.0	0.0	75.6
Pork	8.4	0.8	0.0	1.7	0.0	0.0	0.0	89.1
Chicken	16.0	10.1	0.8	0.0	0.0	0.0	0.0	73.1
Eggs	15.1	5.0	4.2	0.8	0.0	0.0	0.8	73.9
Liver	8.5	0.0	0.0	0.0	0.0	0.0	3.4	88.1
Sardines	21.0	21.8	11.8	5.9	2.5	4.2	9.2	23.5
Cow's milk	5.0	4.2	1.7	1.7	1.7	0.8	6.7	78.2
Formula milk	0.8	0.8	0.8	0.0	0.8	0.0	2.5	94.1
Beans	16.8	21.0	12.6	10.9	5.0	1.7	1.3	18.5
Ground nuts	8.4	20.2	8.4	3.4	2.5	1.7	12.6	42.9

#### 4.2.3.2 Starchy, sugars and oily foods

Maize (76.5%) was the major food consumed daily by the children of age between 6-22 months (Table 9). A large proportion of children had consumed maize (83%) and cooking oil (75%) that was added to their food at least once in a week (Table 9). Other foods consumed by the children included sweet potatoes (16 %), sorghum in porridge (12%), plantain (7%), sugar cane (7%), cassava (4%) and rice (4%).

Table 9: Consumption of Starchy, sugar and oily foods

Food Eaten	Number of days reported of consuming the food in the previous week							
	1	2	3	4	5	6	7	Never
	Proportion of all children (%)							
Maize meal	1.7	0.0	1.7	0.8	0.0	2.5	76.5	16.8
Sorghum meal	0.0	0.8	0.0	0.0	0.0	0.0	11.8	87.4
Plantain	8.4	6.7	0.8	0.0	0.0	0.8	6.7	76.5
Sweet potatoes	10.9	8.4	5.0	4.2	0.0	2.5	16.0	52.9
Cassava	13.4	15.1	4.2	0.8	1.7	2.5	4.2	58.0
Rice	13.4	11.8	7.6	3.4	2.5	0.8	4.2	56.3
Honey	2.5	1.7	3.4	0.0	0.8	0.8	0.0	90.8
Sugarcane	7.6	11.8	8.4	6.7	3.4	0.0	6.7	55.5
Coconut	0.8	3.4	0.0	1.7	0.0	0.8	0.8	92.4
Cooking oil	3.4	10.1	5.0	2.5	1.7	2.5	49.6	25.2

#### 4.2.3.3 Consumption of fruits

Bananas (74%) and pawpaws (53%) were the major fruits consumed by the children for at least a day in a week. Other fruits that were frequently consumed during the week include tamarind (30%), grape fruit (16%) and baobab (12%). Bananas, pawpaw, grape fruit and passion fruits were most consumed on daily basis. The least consumed fruits include syzygium, annona sweetsop and saba sp (Table 10).

Table 10: Fruits eaten without cooking

Food Eaten	Number of days reported consuming the fruit in the previous week							
	1	2	3	4	5	6	7	Never
	Proportion of all children (%)							
Mango	0.0	0.8	1.7	0.0	0.0	0.0	0.0	97.5
Banana	17.6	15.1	15.1	5.9	0.0	1.7	18.5	26.1
Pawpaw	21.8	8.4	5.0	2.5	0.0	0.8	14.3	47.1
Oranges	4.2	2.5	0.0	0.8	0.0	0.0	0.0	92.4
Lemon	1.7	3.4	0.8	0.8	0.8	0.0	5.0	87.4
Grape fruit ( <i>Mandarazi</i> )	0.0	2.5	0.0	0.8	0.0	0.0	12.6	84.0
Bilimbi ( <i>Mbilimbi</i> )	0.0	0.0	0.0	0.8	0.0	0.0	0.8	98.3
Syzygium ( <i>Zambalau</i> )	0.0	0.0	0.0	0.8	0.0	0.0	0.0	99.2
Avocado	0.0	0.0	0.0	0.0	0.0	0.0	0.8	99.2
Guava	2.5	0.0	0.0	0.0	0.0	0.0	0.0	97.5
Apple	0.0	0.8	0.0	0.0	0.0	0.0	0.8	98.3
Grape	0.0	0.9	0.0	0.0	0.0	0.0	0.9	98.3
Passion fruit	1.7	0.0	0.8	1.7	1.7	0.8	8.4	84.9
Pineapple	1.7	0.8	0.8	0.0	0.0	0.8	0.8	95.0
Tamarind ( <i>Ukwaju</i> )	7.6	6.7	7.6	2.5	2.5	0.0	3.4	69.7
Baobab	4.2	3.4	2.5	0.8	0.0	0.0	0.8	88.2
Annona ( <i>Mastafeli</i> )	0.8	0.0	0.0	0.0	0.0	0.8	0.8	97.6
Annona Sweetsop ( <i>Topetope</i> )	0.8	0.0	0.0	0.0	0.0	0.0	0.0	99.2
Vitex sp ( <i>Fulu</i> )	0.0	0.8	0.0	0.0	0.0	1.7	0.8	96.6
Saba sp ( <i>Mabungo</i> )	0.0	0.8	0.0	0.0	0.0	0.0	0.0	99.2
Ginger	2.5	0.8	0.8	0.0	0.0	0.0	1.7	94.1
Water melon	0.8	0.0	0.8	0.0	0.0	0.0	0.0	98.3
Cucumber	0.8	0.8	0.0	0.0	0.0	0.8	0.8	96.8

#### 4.2.3.4 Cooked leafy vegetables and fruits

Consumption of cooked leafy vegetables and fruits was relatively higher compared to fruits eaten while raw. The foods that were most consumed on a weekly basis include tomatoes, onions, amaranths, potato leaves, French beans and bitter tomatoes (Table 11). Tomatoes and onions were consumed most frequently (daily) by 50 and 43% of the children respectively. Other vegetables consumed on a daily basis included cow

pea leaves (13%), amaranths (12%), sweet potato leaves (12%), pumpkin leaves (11%) and French beans (10%) (Table 11).

Table 11: Vegetable, plant leaves and cooked fruits

Food Eaten	Number of days reported consuming vegetables within a week							
	1	2	3	4	5	6	7	Never
	Proportion of all children (%)							
Amaranths	20.2	19.3	6.7	3.4	1.7	1.7	11.8	35.3
Potato leaves	13.4	18.5	14.3	5.9	0.0	0.8	11.8	35.3
Egg plants	0.8	1.7	0.0	0.0	0.8	0.8	2.5	93.2
Pumpkin seeds ( <i>Tetele</i> )	5.0	1.7	0.0	0.0	0.0	0.0	4.2	89.1
Bitter tomatoes	15.1	12.6	8.4	1.7	1.7	0.0	6.7	53.8
Cassava leaves	14.3	5.9	0.8	0.8	0.8	2.5	5.0	69.7
French beans	5.0	8.4	10.9	4.2	2.5	0.8	10.1	42.0
Spinach	5.0	0.0	0.8	0.8	0.0	0.0	0.8	92.4
Chinese cabbage	9.2	2.5	1.7	0.0	0.0	0.0	0.8	85.7
<i>Brasica carinata</i> ( <i>Sukuma wiki</i> )	0.0	0.8	0.0	0.8	0.0	0.0	0.8	97.5
Pumpkin	4.2	3.4	0.8	0.0	0.8	0.8	3.4	86.6
Pumpkin leaves	16.8	15.1	9.2	2.5	0.8	0.8	10.9	43.7
Onions	2.5	3.4	3.4	3.4	8.4	2.5	42.9	33.6
Tomatoes	1.7	3.4	5.0	3.4	7.6	4.2	50.4	24.4
<i>Asystasia</i> ( <i>Mwidu</i> )	3.4	5.0	0.0	0.0	0.0	0.0	0.0	91.6
Milk weed ( <i>Chunga</i> )	1.7	2.5	0.8	0.8	0.0	0.0	0.8	93.3
Black night shade ( <i>Munavu</i> )	2.5	2.5	0.8	0.0	0.8	0.8	3.4	89.1
Cow peas leaves	11.8	13.4	8.4	0.0	3.4	0.0	13.4	49.6
Roselle juice	0.8	0.8	1.7	0.0	0.8	0.0	0.0	95.8

#### 4.2.4 Nutrient composition of the major complementary meals

The mean nutrient content of the foods given to the children of age below 24 months in Magubike village is presented in Table 12. Doughnut, rice and stiff porridge (*ugali*) had the highest amount of energy with 323.16, 144.05 and 106.86 Kcal/100 g of food respectively. The major sources of carbohydrate in the children's diets were doughnut (51.43 g/100 g), rice (29.89 g/100 g), stiff porridge (23.3 g/100 g) and banana (20.2 g/100 g). Sardines, doughnut and beans were the major sources of proteins in the children's diets. The crude protein concentration for sardines was 8.95

g/100g, doughnut (6.68 g/100 g) and beans (6.32 g/100 g). The major sources of fat were doughnut (10.08 g/100 g), sardines (5.55 g/100 g), sweet potato leaves (5.39 g/100 g), amaranth (4.58 g/100 g) and cassava leaves (4.54 g/100 g) cooked with oil. Fibre was only absent in cow's and human milk among all the foods given to the children. Pumpkin and cassava leaves had the highest amount of fibre (3.98 g/100 g). Iron concentration was highest in amaranths (6.52 mg/100 g) followed by sardines (4.19 mg/100 g), cassava leaves (3.85 mg/100 g) and pumpkin leaves (3.81 mg/100 g). Zinc concentration was low in all foods eaten by the children. Nevertheless, sardines had the highest zinc concentration (1.07 mg/100 g).

#### **4.2.5 Nutrient composition of the Roselle juice**

Roselle juice contained significant ( $p < 0.05$ ) concentration of vitamin C compared to other foods consumed by the children. All other nutrients were similar to the other family food eaten by the children (Table 12). Also Roselle powder had significant ( $p < 0.05$ ) concentration of iron (14 mg/ 100 g), zinc (2 mg/ 100g) and vitamin C (96 mg/100g) compared to other foods consumed by the children.

Table 12: Nutrient composition of the major foods given to the children

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**Cooked Food with I    Mean Nutrient content per 100g of cooked edible portion**

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ingredients	Energy (Kcal)	CHO (g)	Crude protein (g)	Fat (g)	Fibre (g)	Iron (mg)	Zinc (mg)	Vit. C (mg)
Cereal porridge	50.87 <sup>abcd</sup>	11.14 <sup>de</sup>	1.16 <sup>b</sup>	0.18 <sup>a</sup>	0.20 <sup>a</sup>	0.54 <sup>abc</sup>	0.08 <sup>a</sup>	0.00 <sup>a</sup>
Stiff porridge ( <i>Ugali</i> )	106.86 <sup>h</sup>	23.30 <sup>g</sup>	2.69 <sup>d</sup>	0.32 <sup>a</sup>	1.99 <sup>ab</sup>	1.52 <sup>abcde</sup>	0.05 <sup>a</sup>	0.01 <sup>a</sup>
Cow's Milk	57.2 <sup>bcde</sup>	7.60 <sup>bcd</sup>	1.98 <sup>bcd</sup>	2.40 <sup>ab</sup>	0.00 <sup>a</sup>	0.40 <sup>ab</sup>	0.36 <sup>ab</sup>	0.28 <sup>a</sup>
Human milk	60.10 <sup>bcdef</sup>	7.78 <sup>bcd</sup>	1.52 <sup>bc</sup>	2.54 <sup>ab</sup>	0.00 <sup>a</sup>	0.39 <sup>ab</sup>	0.25 <sup>ab</sup>	2.27 <sup>a</sup>
Beans	97.09 <sup>gh</sup>	15.80 <sup>ef</sup>	6.32 <sup>f</sup>	0.96 <sup>a</sup>	3.40 <sup>ab</sup>	1.98 <sup>bcdef</sup>	0.15 <sup>a</sup>	0.00 <sup>a</sup>
Potato leaves	74.49 <sup>defg</sup>	4.11 <sup>abc</sup>	2.38 <sup>cd</sup>	5.39 <sup>c</sup>	2.64 <sup>ab</sup>	2.22 <sup>defg</sup>	0.19 <sup>a</sup>	0.01 <sup>a</sup>
Cassava leaves	69.83 <sup>defg</sup>	2.94 <sup>ab</sup>	4.30 <sup>e</sup>	4.54 <sup>bc</sup>	3.98 <sup>ab</sup>	3.85 <sup>gh</sup>	0.03 <sup>a</sup>	0.09 <sup>a</sup>
Rice	144.05 <sup>i</sup>	29.80 <sup>h</sup>	2.59 <sup>d</sup>	1.61 <sup>a</sup>	1.65 <sup>ab</sup>	1.75 <sup>cdef</sup>	0.02 <sup>a</sup>	0.01 <sup>a</sup>
Amaranth cooked	57.23 <sup>bcde</sup>	1.57 <sup>a</sup>	2.44 <sup>cd</sup>	4.58 <sup>bc</sup>	0.04 <sup>a</sup>	6.52 <sup>j</sup>	0.01 <sup>a</sup>	0.04 <sup>a</sup>
with oil								
Banana	85.77 <sup>efgh</sup>	20.21 <sup>fg</sup>	1.24 <sup>b</sup>	0.10 <sup>a</sup>	0.88 <sup>ab</sup>	0.90 <sup>abcd</sup>	0.01 <sup>a</sup>	8.56 <sup>b</sup>
Sardines	91.55 <sup>gh</sup>	1.44 <sup>a</sup>	8.95 <sup>g</sup>	5.55 <sup>c</sup>	1.98 <sup>ab</sup>	4.19 <sup>hi</sup>	1.07 <sup>c</sup>	0.01 <sup>a</sup>
Doughnut( <i>Mandazi</i> )	323.16 <sup>k</sup>	51.43 <sup>i</sup>	6.68 <sup>f</sup>	10.08 <sup>d</sup>	3.68 <sup>ab</sup>	2.72 <sup>efgh</sup>	0.57 <sup>b</sup>	0.03 <sup>a</sup>
Pumpkin leaves	28.16 <sup>a</sup>	1.10 <sup>a</sup>	2.16 <sup>d</sup>	1.68 <sup>a</sup>	3.98 <sup>ab</sup>	3.81 <sup>fgh</sup>	0.01 <sup>a</sup>	0.00 <sup>a</sup>
Roselle powder	263.58 <sup>i</sup>	56 <sup>i</sup>	8.30 <sup>g</sup>	0.52 <sup>a</sup>	13.2 <sup>c</sup>	13.57 <sup>k</sup>	1.67 <sup>d</sup>	96.57 <sup>d</sup>
Roselle juice	31.37 <sup>ab</sup>	7.57 <sup>bcd</sup>	0.04 <sup>a</sup>	0.104 <sup>a</sup>	0.00 <sup>a</sup>	0.43 <sup>ab</sup>	0.11 <sup>a</sup>	15.69 <sup>c</sup>
Placebo juice	31.37 <sup>ab</sup>	7.84 <sup>cd</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.15 <sup>a</sup>	0.04 <sup>a</sup>	1.32 <sup>a</sup>

Values in a single column that have the same superscript letters are not significantly different ( $p > 0.05$ )

#### 4.2.6 Estimated nutrient intake

The mean values of nutrient intake and average %RDA for the children in this study are presented in Table 13. During the supplementation period, energy intake was estimated to increase by 3% of RDA in both groups. The crude protein intake of 19 g among the children did not change during the supplementation period. There was a high intake of fibre among all the children ( $5.2 \pm 4.2$  g). The level of iron intake among the children was 98% of RDA during the intervention period. Iron intake increased by 4% of RDA for all the children during the supplementation period. Zinc intake increased by 2% of RDA during the intervention period.

The mean vitamin C intake of the children significantly ( $p<0.05$ ) increased to 49% of RDA. There was a significant difference ( $p=0.0001$ ) in the mean vitamin C intake of the children in the placebo and intervention group observed during the supplementation period.

Table 13: Average Nutrient intake from home prepared complementary foods

Nutrient	Nutrient intake		p-value
	Baseline n=27	During intervention n=27	
Energy (Kcal)	626.4±148.9	656±148.9	$p>0.05$
% RDA- Energy	58.7	61.7	
Protein (g)	18.5±5.8	18.5±5.8	$p>0.05$
% RDA-protein	123.20	123.2	
Fat (g)	17.2±2.7	17.3±2.7	$p>0.05$
% RDA- fat	37.8	37.9	
CHO (g)	106.4±31.7	114.1±31.6	$p>0.05$
%RDA CHO	74.1	79.7	
Fibre (g)	5.2±4.3	5.1±4.3	$p>0.05$
Vitamin C(mg)	13.1±2.7	21.4±8.1	$p<0.05$
%RDA vitamin C	26.8	48.9	
Iron (mg)	7.5±3.5	7.8±3.8	$p>0.05$
% RDA- Iron	94.3	97.8	
Zinc (mg)	1.8±0.5	1.9±0.5	$p>0.05$
% RDA Zinc	37.4	38.9	

The results include mean of the lower boundary of 95% confidence for mean breast milk intakes from different studies in developing countries. Onyango *et al.* (2002); Albernaz *et al.* (2003); Islam *et al.* (2006); Owino *et al.* (2007); Galpin *et al.* (2007). Assumed that breast milk intake is high since over 80% of the children breast feed at least 6 times a day.

### 4.3 Nutritional Status of the Children

The mean baseline measurements for body weight and height of the children in the study group are presented in Table in Appendix 7.

#### 4.3.1 Prevalence of underweight

The prevalence of underweight among all children was 9% at baseline (Fig. 8). About 8% were moderately underweight and 1% of the children that were included



in the study were severely underweight. About 10% of the girls in this study were underweight while 7% of the boys were under weight. The mean WAZ of all children included in this study was  $-0.6\pm 1.1$  and that of the girls was  $-0.7\pm 1.0$  whereas that of the boys was  $-0.6\pm 1.1$ . Results showed a strong association with increased WAZ and weekly consumption of goat's meat ( $r=0.25$ ,  $p=0.01$ ), honey ( $r=0.27$ ,  $p=0.00$ ), spinach ( $r=0.26$ ,  $p=0.00$ ) and pineapple ( $r=0.20$ ,  $p=0.03$ ).

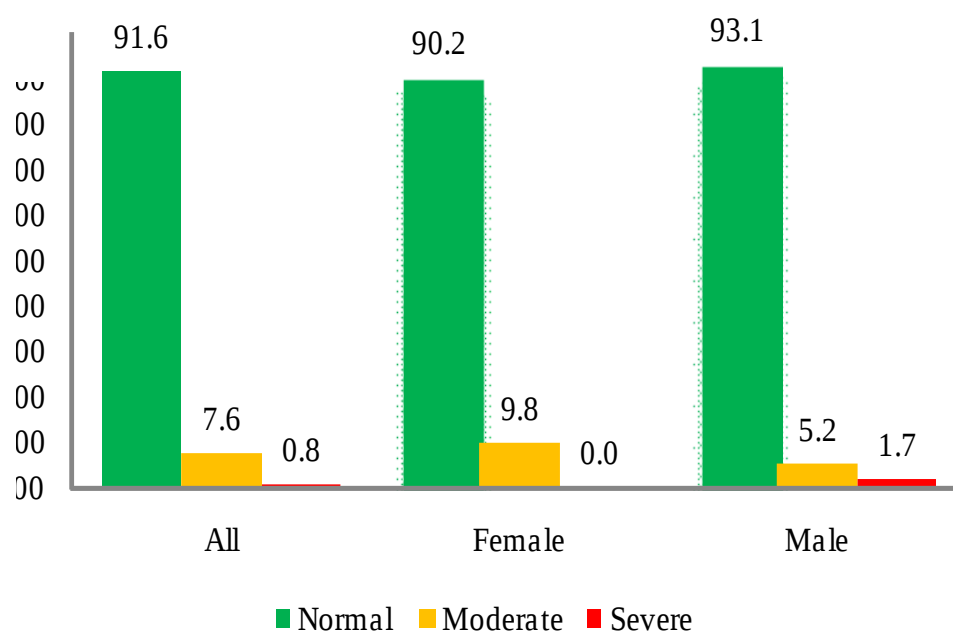


Figure 8: Prevalence of underweight among children during baseline

#### 4.3.2 Prevalence of wasting

The prevalence of Global acute malnutrition (wasting) among children with less than 2 years of age was 2% (Fig. 9). The mean WHZ of all the children at baseline was  $0.2\pm 1.1$ . The mean WHZ of the female children with age less than 2 years was  $0.1\pm 1.0$  while that of the male children was  $0.2\pm 1.2$ . Further, decreased weight for height Z scores among children were ( $r=0.23$ ,  $p=0.01$ ) associated with having single mothers. Also high WHZ scores at baseline were strongly associated with increased

monthly household income ( $r= 0.29$ ,  $p=0.01$ ) and per capita income ( $r= 0.29$ ,  $p=0.01$ ).

Increased WHZ scores was also strongly associated ( $r=0.23$ ,  $p=0.02$ ) with increase in appetite score of the children. Also increased WHZ was strongly associated with increased weekly consumption of goat's meat ( $r=0.21$ ,  $p=0.02$ ), pork ( $r=0.20$ ,  $p=0.02$ ), honey ( $r=0.24$ ,  $p=0.01$ ), pineapple ( $r=0.20$ ,  $p=0.02$ ).

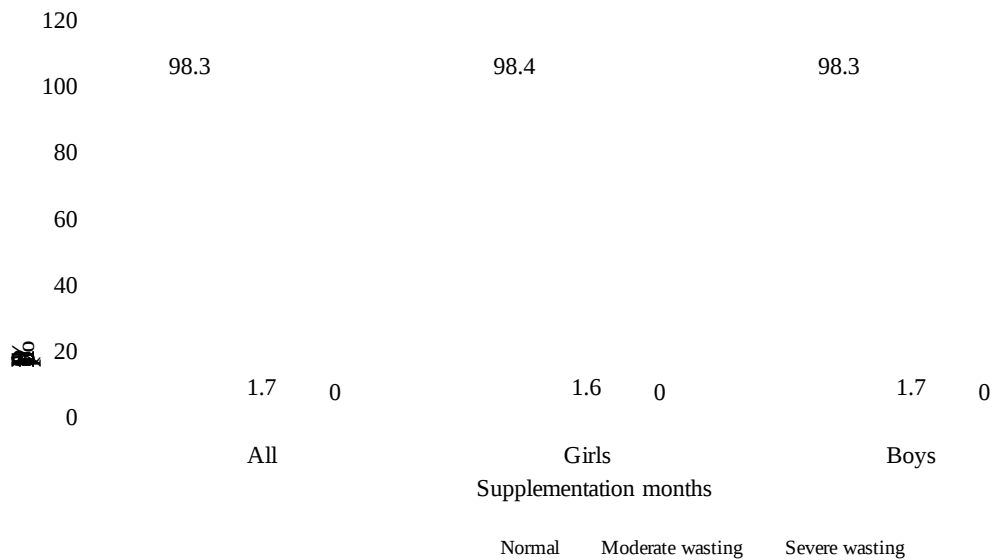


Figure 9: Prevalence of Global acute malnutrition (wasting)

#### 4.4 Iron Status

There was improvement in haemoglobin levels in both groups as presented in Fig. 10. The prevalence of anaemia among all children significantly ( $\chi^2=22.92$ ,  $p=0.00$ ) decreased from 88.2% at baseline to 59.8% at the end of the four months of supplementation. The prevalence of anaemia 52 days after the feeding regimen among all children was 58.7%. The mean haemoglobin (Hb) concentration of all the children increased significantly ( $p<0.05$ ) from 9.6 g/dl (CI 95% 9.4-9.8 g/dl) at baseline to 10.5 g/dl (CI 95% 10.3-10.8 g/dl) at the fourth follow up month. There

was a significant difference ( $\chi^2=4.36$ ,  $p=0.04$ ) between the proportion of children with haemoglobin level less than 11.0 g/dl in the placebo (71.4%) and intervention (50%) group at the fourth follow up month. Also a significant difference ( $p<0.05$ ) was observed between the mean haemoglobin concentration of the children in the intervention group at baseline (9.8 g/dl CI 95% 9.5-10.1 g/dl) and the fourth follow up month (10.8 g/dl CI 95% 10.5-11.2 g/dl). There was no significant difference ( $p>0.05$ ) between the haemoglobin concentration of the children in the placebo and intervention group at the baseline and fourth follow up month. The mean haemoglobin concentration of the children in the placebo group increased ( $p>0.05$ ) from 9.5 g/dl (CI 95% 9.2-9.8 g/dl) at baseline to 10.1 g/dl (CI 95% 9.8-10.5 g/dl) at the fourth month of supplementation. In addition, the mean haemoglobin concentration of the children in intervention group increased ( $p<0.05$ ) from 9.8g/dl (CI 95% 9.4-10.1 g/dl) at baseline to 10.8 g/dl (CI 95% 10.5-11.2 g/dl) at the fourth follow up month. Increase in Hb was strongly ( $r=0.22$ ,  $p=0.01$ ) related to increased number of days for consumption of beef in a week.

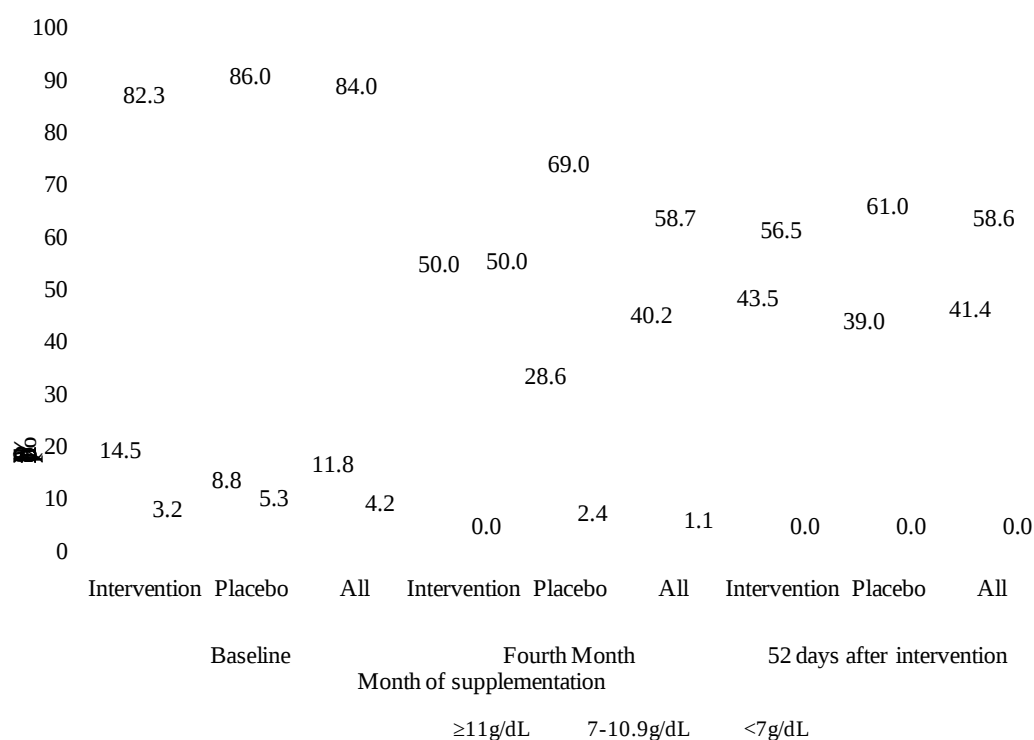


Figure 10: Prevalence of anaemia at baseline and the fourth month

#### 4.5 Appetite Status

There was a general increase ( $P > 0.05$ ) in appetite score for both children in the placebo and intervention group. The mean appetite score for children in the study increased from 13.6 (CI 95% 13.1-14.2) at baseline to 14.6 (CI 95% 14.0-15.2) at the fourth month of supplementation. There was no significant differences ( $p > 0.05$ ) observed between the mean appetite score of the children of different age groups in the placebo and the intervention group at baseline and fourth follow up month. However, a significant increase ( $p < 0.05$ ) in the mean appetite score for children with ages of at least 12 months in the intervention group was observed. The mean appetite score at baseline for children of ages 12 months in the intervention group was 13.3

(CI 95% 12.2-14.4) while that at the fourth month of supplementation was 15.6 (CI 95% 14.5-16.8).

The mean appetite score of the children in placebo group 52 days after the feeding regimen was 14.3 (CI 95%13.4-15.2) and that of the children in the intervention group was 14.4 (CI 95% 13.5-15.3). There was no significant difference ( $p>0.05$ ) observed between the mean appetite score of the children of different age groups in the placebo and intervention groups throughout the study period. Increase in appetite score was strongly associated with increase in weight for height ( $r=0.19$ ,  $p=0.001$ ) and weight for age ( $r=0.16$ ,  $p=0.007$ ).

## **4.6 Morbidity among Children in the Study Group**

### **4.6.1 Prevalence of illnesses**

The proportion of children with any illnesses in the previous month prior to the survey at baseline was 80% and it significantly ( $\chi^2= 8.2$ ,  $p=0.00$ ) decreased to 62% after four months of supplementation. The morbidity rate among children in the intervention group significantly decreased by 27% ( $P<0.05$ ) from 81% at baseline to 54% after four months of supplementation (Fig. 11). The morbidity rate among children in the placebo group decreased by 8% ( $p>0.05$ ) from 79% at baseline to 71% after four months of supplementation. There was no significant difference ( $\chi^2= 2.92$ ,  $p=0.09$ ) between the prevalence of illnesses among children within the previous month prior to the survey in the placebo (71%) and the intervention (54%) group after four months of supplementation. The prevalence of illnesses among children in the placebo group of age less than 12 months increased from 77% at baseline to 83% at fourth month of supplementation. The prevalence of illnesses among children of

age from 12 months decreased in both feeding groups. The prevalence of illnesses among children with the age of at least 12 months in the placebo decreased by 24% ( $\chi^2=3.06$ ,  $p=0.08$ ) from 82% at baseline to 57.9% at the fourth month of supplementation. Also the prevalence of illnesses among children of age 12 months in the intervention group significantly decreased by 48% ( $\chi^2=13.51$ ,  $p=0.00$ ) from 86.2% at baseline to 38.5% at the fourth month of supplementation.

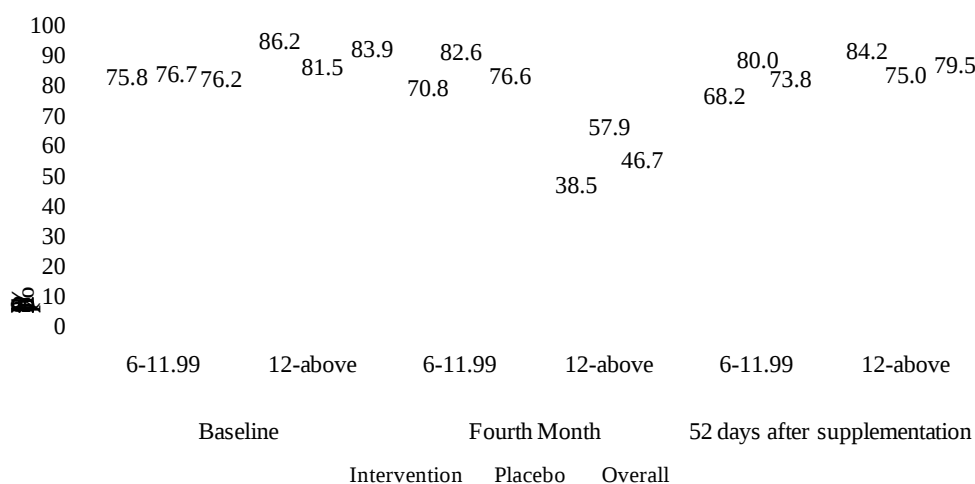


Figure 11: Prevalence of illnesses in the previous month

#### 4.6.2 Causes of morbidity

The major causes of morbidity were malaria, cough, diarrhea and influenza (Fig. 12). The prevalence of illnesses among children in the study group had been reduced after four months of supplementation. However, a significant difference ( $\chi^2=4.83$ ,  $p=0.03$ ) was observed between the proportions of children reported with malaria at baseline (55.5%) and after four months of supplementation (40.2%). A significant decrease ( $\chi^2=10.0$ ,  $p=0.00$ ) in the prevalence of malaria was observed among children in the intervention group of age between 12-21.99 months (Fig. 13). The proportion of children of age from 12 months in the intervention group reported with malaria was

66% at baseline and 23% after four months of supplementation. There was also a significant difference ( $\chi^2=5.66$ ,  $p=0.02$ ) between the proportion of children of age between 12-21.99 months reported with malaria in the placebo (57.9%) and intervention (23.1%) groups after four months of supplementation.

The proportion of children of age between 6-11.9 months reported with malaria at 52 days after the supplementation period was 43 and 32% ( $p>0.05$ ) for those in the placebo and intervention groups respectively. Furthermore, 55.0% of the children of age between 12-21.9 months in the placebo group and 50.0% in the intervention group were reported to have malaria at 52 days after the supplementation period.

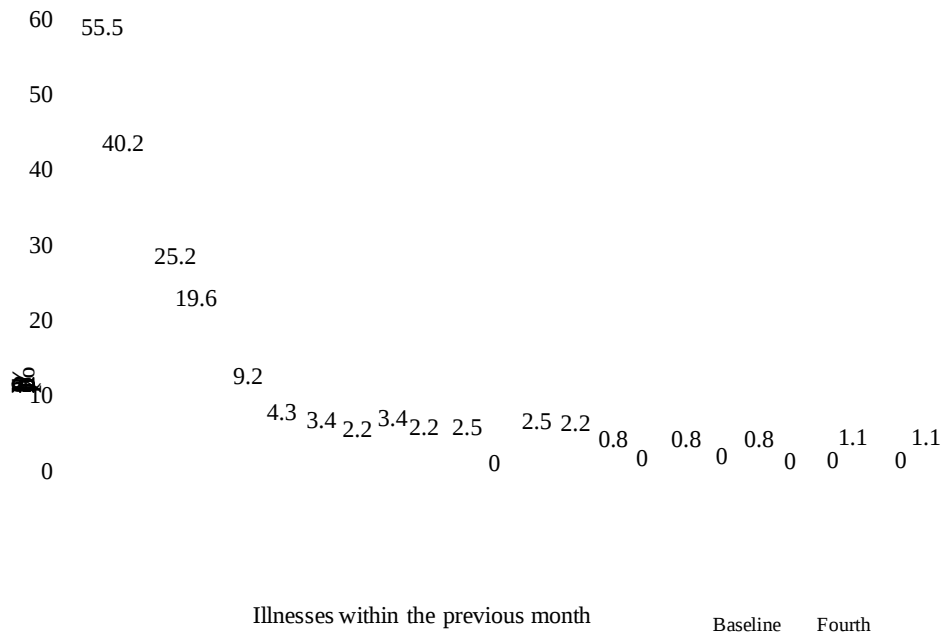


Figure 12: Illnesses among children

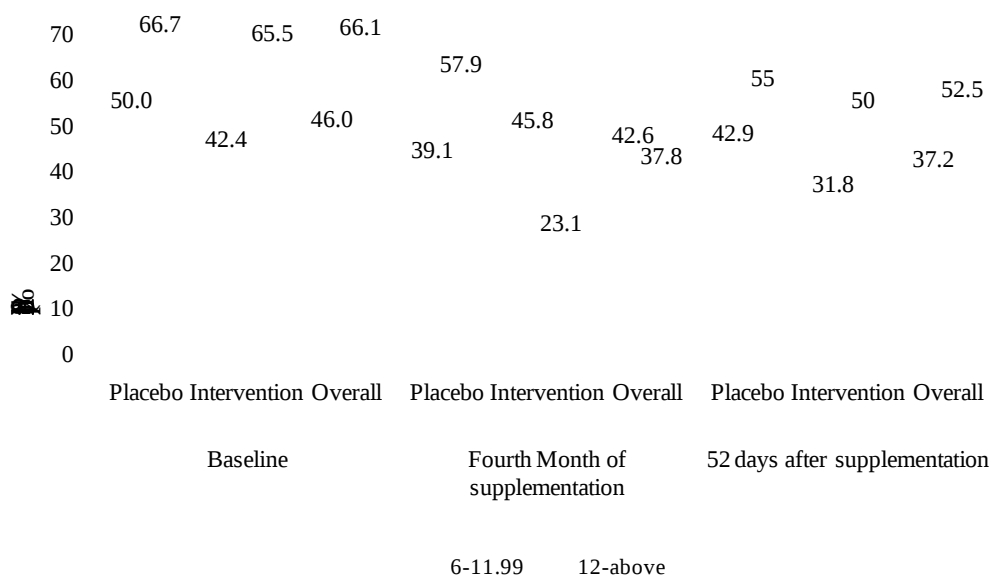


Figure 13: Prevalence of malaria among children

#### 4.7 Vitamin C Saturation Test

The mean concentration of vitamin C excreted in urine after 50 mg of vitamin C administration was 0.38 mg (CI 95% 0.28-0.49 mg) for the children in the placebo group and 0.36 mg (CI 95% 0.26-0.46mg) for the children in the intervention group at baseline (Fig. 14A). The concentration of vitamin C in urine of the children in the placebo group increased ( $p>0.05$ ) by 0.12 mg and that of children in the intervention group increased ( $p>0.05$ ) by 0.17 mg after four months of supplementation. The mean concentration of vitamin C per kg body weight increased in the same trend (Fig. 14B). The mean vitamin C concentration in urine per kg body weight was 0.044 mg/kg (CI 95% 0.03-0.06 mg/kg) for children in the placebo and 0.043 mg/kg (0.32-0.054 mg/kg) for children in the intervention group at baseline. The concentration of vitamin C in urine for the children in the placebo and intervention group increased by 0.008 and 0.015 mg/ kg respectively after four months of supplementation. There



was no significant difference ( $p>0.05$ ) between the mean urinary vitamin C concentration for the children in the placebo (0.05 mg/kg, CI 95% 0.04-0.07 mg/kg) and intervention group (0.058, CI 95% 0.04-0.07 mg/kg) after four months of supplementation.

A.

B.

Figure 14: Concentration of vitamin C in urine after 4-6 hours of dose administration

#### 4.8 Urinary Zinc Concentration

The proportion of children with zinc concentration outside the normal range of urine zinc concentration (49-490 µg/ L) increased by over 8 µg/ L from 26 to 35% (Table 14). The proportion of children in the intervention group with urine zinc concentration outside normal range increased by 4% from 26% at baseline to 30% after 4 months of supplementation. The proportion of children in the placebo group with urine zinc concentration outside normal range increased by 14% from 26% at baseline to 40% after 4 months of supplementation.

Table 14: Urinary zinc concentration of the children

Feeding Regimen	Proportion (%) of children with urine zinc concentration outside normal range (49-490 µg/ L)			
	Baseline	p- value	Fourth Month	p- value
Intervention	26.4	1.00	30.4	0.48
Placebo	26.1		40.0	
All	26.3		34.6	

## CHAPTER FIVE

### 5.0 DISCUSSION

#### 5.1 Household Characteristics

The average size (5.0±2.0) of the households in Magubike village from where the children were sampled was higher than that of Kilosa district of 4.6 (Population and

Housing census of 2002). Large household sizes pose challenges in intra-household food distribution leading to low food intake and culminating into nutritional deficiencies. Mamiro (2003) reported a high average household size of 5.8 in Kilosa District. Further, the average proportion of children of age below 5 years in the households of 29% was higher than that of Tanzania 2002 census of 20%. High proportion of children of age below 5 years increases the dependency ratio, expenditure of the income on care of household members, and perpetuates poverty (low per capita income). Low per capita income increases the risk of having children with poor nutritional status. In this study low per capita income was associated ( $p < 0.05$ ) with low weight for age and weight for height. The large household size might be attributed to high birth rate and inadequate access to family planning services. Contraceptives like condoms, hormonal oral pills and injections, which are available at the only Health Centre in the village effectively, reduce unwanted pregnancies and hence decrease the number of household dependants. The parity of the mothers significantly increased with age. At an average age of 17.6 years a woman had already given birth to the first child while at 39 years the mother had 6 children. High parity increases the risk of having maternal malnutrition and decreases the household per capita income. High parity might reduce the duration of breast feeding, enhancing the risk of malnutrition among children. High parity further might explain the high proportion of children in the households. High parity is an indication of low or poor accessibility to contraceptive services by women (Chan and Lao, 1999; Santelli *et al.*, 2006). However women in Magubike equate “love for the husband” by the number of children they bear. The household male to female ratio was comparable to that observed by Jumbe (2007) of 0.92:1 for children aged 0-59 months.

## **5.2 Dietary Assessment**

### **5.2.1 Breast feeding frequency**

Breast feeding prevalence and frequency was high in the study population. Children were breast fed frequently for more than 8 times a day. This was expected in a population which consumes diets containing low fat. The average fat intake was estimated at 37% of RDA. Galpin *et al.* (2007) showed that low fat intake among mothers was related to reduced energy intake among children, which increased their hunger and therefore frequent demand to breast feeding. The contribution of fat in milk to the overall energy intake was 38% compared to the recommended 45-50% (WHO, 2000). Also fat is known to have satiating effect. Ingestion of fats leads to the release of endogenous cholecystokinin (CCK) in humans, a peptide that is involved in mediating the satiating effects of food. Fat in the body provides large quantities of energy compared to the carbohydrates hence extending the time the children would have needed another feeding session. The foods that were consumed by the children contained less quantities of fat increasing risk of developing energy deficiency. In this study, over 30% of the children never emptied milk in one breast before switch to another. This practice reduces the production of breast milk and intake of nutrients since the last (hind) milk is richer in nutrients. This further explains the reduction in breast feeding frequency by the mothers claiming that the breast milk production was not enough.

High breast feeding frequency might have been attributed to household food shortages and inadequate time to prepare children's food. This increased the dependency of the children on readily available breast milk as a major source of

food. Increased dependency of the children on breast milk as a major food decreases the intake of energy. This increases the risk of developing malnutrition among the children with age of below five years.

### **5.2.2 Frequency of feeding complementary meals**

The average feeding frequency of complementary foods was two meals per day. This decreased the chances of attaining the recommended energy intake among children. The World Health Organization recommends that breast feeding children be given three complementary feeds and two snacks each day to achieve the RDA for energy (WHO, 2000). The low feeding frequency might be attributed to widespread food shortage due to the 2007/2008 drought that caused low food production and hence decreased food availability in the households (Jumbe, 2007) but also to inherent food habit of consuming two to three meals a day. In addition, increased prevalence of diseases like malaria, cough and diarrhoea might have caused reduced appetite among children and hence reduced food demand. These diseases are known to increase vomiting and food aversion (Semba and Tang, 1999). Another factor that might have contributed to reduced feeding frequency was inadequate care and time accorded to the children during the feeding sessions. Most mothers were either involved in other household chores, businesses in the market or in the fields in preparation for planting. Also labour demanding crops like rice kept the mothers in the fields for a long time to scare birds from eating the rice grains. The long distances from home to the fields prevented the mothers from ensuring that their children were fed frequently. In addition, the dietary diversity of the foods consumed by children was low increasing risk of developing nutrient deficiencies.

### 5.2.3 Food frequency and adequacy

Energy intake of the children was lower than the 1000-1200 Kcal recommended by WHO, which was due to low intake of energy rich foods and low feeding frequency. Consumption of foods from animal sources was low, which might have been one of the causes of high malnutrition rates. The availability and density of nutrients are higher in food from animal sources than from plants sources. For example, iron from animal flesh is readily available because it contains haeme component, which is always well absorbed, and it promotes the absorption of other (non-haeme) dietary iron. Low consumption of foods from animal sources increases the risk of developing various nutrient deficiencies like energy, proteins, iron, calcium and zinc. Also the mentioned nutrients are regularly needed to be provided by diet for growth and replenishment in the body as they cannot be synthesised by the body. It has been observed that there are significant relationships between consumption of animal foodstuffs and nutritional status in developing countries (Popkin and Du, 2003; Hu, 2008; Popkin, 2008). The average per capita income of the households from where the children in the study group were obtained was less than T.Sh.400/-. The price of animal food-stuffs ranged from T.Sh.500/- for each litre of milk to T.Sh.2000/- for goats milk. The price of beef, which is a rich source of iron and protein was T.Sh. 3000- 4000/-. The high market price compared to the low per capita income increased the dependency on legumes as alternative sources of proteins. Legumes were mainly obtained from the gardens from the previous seasons' harvests and also could be bought from the village market at Sh.800 per kg of beans.

In addition beans were not regularly eaten which increased the risk of developing protein malnutrition. This might be attributed to the long spell of drought that led to

exhausting all the food stores from previous season's harvest in the households. The high price of fuel for cooking made the households avoid food items that require a lot of energy for cooking.

Maize porridge which was the daily complementary meal for all children was the major source of carbohydrates and energy. Most children's relish (family sauce) was cooked with oil although that was not high enough to fill the energy gap. The recommended dietary intake of energy would not be reached under such a feeding regime.

Fruits and vegetables are good sources of vitamins especially the thermal labile vitamins and minerals that need to be consumed daily for the healthy function of the body. However, fruit consumption was low and could be a contributing factor to increased risk of micronutrient deficiencies like iron, vitamin A and C. The low consumption of fruits might be attributed to dependence on seasonal fruits like mangoes and oranges. In addition, vegetables such as tomatoes, egg plants and bitter tomatoes were not purchased because of high market prices compared to the low income levels.

Generally, the low consumption of vegetables might be attributed to low availability of vegetables in the village because they are not regularly cultivated by the households. Also there was a poor attitude among women of not attaching good feeding practice to consumption of vegetables during a meal. In addition, the cooked vegetables had inadequate vitamin C, low quantities of iron and zinc. Vegetables like sweet potato leaves, cassava leaves, amaranths, onions and tomatoes, which would

be the alternative sources of iron, vitamins and minerals were overcooked. The average cooking time for cassava leaves was 25-30 minutes, and amaranth was 20-23 minutes. Also the procedures for cooking the vegetables were poor. For example cassava leaves were boiled in excess water that was discarded. This procedure often leads to increased losses of minerals and water soluble vitamins. The water soluble nutrients usually tend to leach into the cooking water, therefore by discarding the water, much of the water soluble nutrients like folic acid are lost (McKillop *et al.*, 2002). All vegetables have high fibre content, which further decreases their reliability as good sources of minerals like zinc, copper and iron.

#### **5.2.4 Nutrient composition of Roselle juice**

Roselle juice is a local beverage made from the reddish purple, acid-succulent calyces from the flower of *Hibiscus Sabdariffa*. The juice has high nutritional value with high vitamin C content, immune function and medicinal properties.

Roselle juice contained high ( $p < 0.05$ ) concentration of vitamin C (16 mg/100 g) compared to other complementary foods. This contributed to the increased ( $p < 0.05$ ) vitamin C intake among children who consumed Roselle juice. This might explain the increased haemoglobin (Hb) levels observed among the children who consumed Roselle juice. Daily supplementation of children with vitamin C has been shown to increase haemoglobin concentration in children (Bukonya, 2003; Donati, 2005). The high concentration of the vitamin C in Roselle beverages has been reported by Mounigan and Badrie (2006) of 23 mg/100 g and Bolade *et al.* (2009) with concentration between 17 and 31 mg/100 g. The lower concentration of vitamin C that was observed in the present study than those reported in literature might have



been attributed to dilution of the calyces extract and the heat treatment given to the extract during preparation to destroy microbial organisms. Other nutrients like protein, carbohydrates, fat, zinc and iron in Roselle juice were not significantly different ( $p>0.05$ ) from those in other complementary foods. Protein, carbohydrates, fats and zinc are needed by the body for growth and development of children. This might explain lack of significant increments in weight and height of the children in the study group. Dilution of Roselle extract during preparation might have lowered the concentration of the mentioned nutrients. Fibre was lost during the process of sieving the extract using the muslin cloth. Fibre plays a great role in churning of the ingested food in the stomach hence preventing constipation in children. However, high intake of fibre among children in the study group (6-24 months) is not desirable as it lowers availability of iron and other minerals which increases the risk of micronutrient deficiencies. Therefore, consumption of Roselle juice relatively decreased intake of fibre by the children hence improved availability of nutrients.

### **5.3 Appetite of the Children**

Appetite refers to the complex desires in humans for food and drink that are often conditioned or influenced by previous experiences or cultural factors as well as by a person's present health status (Matteri, 2001). Food intake is regulated by the central nervous system depending on macronutrients and environmental changes. The hypothalamus is the target of hunger and satiety signals arising from the peripheral organs and the brain. Noradrenaline-neuropeptide and opioid-galanine are involved in carbohydrate and fat intake, respectively and therefore their secretion is stimulated by the presence of these nutrients in the diet (Matteri, 2001). During meals, signals such as cholecystokinin arise primarily from the gastro intestinal tract to cause

satiation and meal termination where signals secreted in proportion to body fat such as insulin and leptin interact with satiation signals and provide effective regulation by dictating meal size to amounts that are appropriate for body fatness, or stored energy (Matteri, 2001). Stressors due to diseases lead to production of free radicals in the body that further stimulate satiating effect. The increase of stressors leads to low macronutrient intake especially fat and carbohydrates, which increases risk of having under nutrition. Roselle juice is rich in vitamin C and other anti oxidants which were not investigated in this study. They are known to increase diuresis and hence reducing the free radicals in the body. Therefore, this might explain significant increase in appetite among children in the intervention group.

During this study, there was a general increase in the appetite of the children in both the placebo and intervention group during the period between the baseline and four months of supplementation. As age of the children increases, their liking to eat other foods increases. This is due to the increased quantities of macronutrients needed by the body and developed gastro intestinal tract system, which increases absorption and utilization of the nutrients in the body. Brown *et al.* (1995) observed that increase in appetite was related to age of the children. Children with higher ages had low incidences of infections, which are known to produce free radicals in the body. Free radicals in the body increase stress, which enhances the satiating effect (Matteri, 2001).

The appetite scores for the children aged below 12 months between those in the placebo and intervention group did not differ significantly ( $p>0.05$ ) by the fourth month. This might be attributed to high prevalence of illnesses like malaria among

children below 12 months. Malaria and other infections are known to increase nausea, vomiting and food aversion which aggregately reduce desire to eat (Wilson *et al.*, 2005). Also increased Hb meant increased iron concentration in the blood. With high prevalence of illnesses among children, iron availability in the body might have increased multiplication of the micro organisms. High iron concentration in the blood favour replication of viruses (Weinberg *et al.*, 2002).

There was a significant association between appetite and WHZ and WAZ scores. Increased appetite increases intake of food quantities and hence increasing chances of nutrient intake like carbohydrates, proteins and fats and minerals, which are needed for proper functioning of the child's body and hence increase in weight. The significant ( $p < 0.05$ ) association between appetite and increased Z scores (weight for age, weight for height) observed in this study was also observed earlier by Burrowes *et al.* (2005). Their study reported a significant association of weight loss with reduced appetite.

#### **5.4 Vitamin C Saturation**

Vitamin C saturation test showed that children's body tissues in both groups were not saturated with vitamin C. It might have been attributed to vitamin C deficiency that was prevalent among the children in the study group. The level recorded in the present study was 0.043mg/kg body weight/ day at baseline, which is lower than what has been observed in other studies. Review of literatures showed vitamin C saturation of 0.06-0.1 mg/ kg body weight per day (Heller *et al.*, 1999; Ramakrishnan *et al.*, 2004). The levels obtained in this study were far below 0.8 mg/ kg body weight, which are levels of adequate vitamin C status in the body. The dosage of

50mg that was administered at the Health Centre to children of ages below 2 years was low to cause vitamin saturation in such a community with vitamin C deficient diets in a single day. Complementary and family foods like porridge, stiff porridge (*Ugali*), sweet potato leaves and sardines had insufficient quantities of vitamin C. Also all the children were highly vitamin C deficient at baseline (0.044 mg/kg) and four months after supplementation (0.055 mg/kg). Roselle juice led increased the vitamin C intake of the children in the intervention group to 60% of RDA indicating that there was still high deficiency in the diet of the children.

Low saturation values, were also attributed to high disease prevalence among the children in both groups. Diseases such as malaria are known to cause nausea and vomiting which are related to reducing food intake.

However, increased saturation was more registered among children in the intervention group than in the placebo group. The vitamin C concentration was significantly ( $p < 0.05$ ) higher in Roselle than in the placebo juice. Nevertheless, there was no significant difference in vitamin C content of the urine because of the high demand of the vitamin in the body. Vitamin C in the diet has been shown (Peneau *et al.*, 2008) to increase the solubility of minerals like zinc and iron in the body. It could have been used for removing redox radicals due to sickness and the antimicrobial activity; and use in collagen formation (Tang and Smit, 2002). With such high demand for better health yields, high fibre diets and low dietary vitamin C, supplementation of the vitamins would be desirable in large densities or frequent intake of vitamin C dense foods. Roselle juice contained only 15 mg/100 of vitamin C and was administered once a day. This concentration however, was enough to

avoid scorbutic symptoms. The minimum vitamin C intake to prevent the development of scurvy is 10 mg/day (Elmadfa and Koenig, 2001).

### **5.5 Iron Deficiency Anaemia**

An adequate supply of dietary iron during the first 24 months of life is essential for preventing iron deficiency with its attendant negative effects on mental, motor and emotional development as well as later cognitive performance. The prevalence of anaemia significantly ( $p < 0.05$ ) decreased by 36% among children in the intervention group and was maintained high two months after the supplementation period. The haemoglobin concentration significantly increased ( $p < 0.05$ ) during supplementation period by 1 g/ dl in children in the intervention group. The reduced prevalence of anaemia could be due to combined effects of increased ( $p < 0.05$ ) intake of vitamin C and iron rich Roselle among the children in the intervention group. Vitamin C improves the bioavailability of non-haem dietary iron by reducing its iron in ligand form bound to compounds like phytic and oxalic acid to readily absorbed iron ( $\text{Fe}^{2+}$ ). Increased iron absorption, enhances production and regulation of red blood cells (erythropoiesis) (Weinberg *et al.*, 2002). Infections like malaria, which was more prevalent among children in the placebo group and inflammatory diseases tend to decrease iron absorption in the small intestine, that induce iron sequestration in macrophages, the hallmark of anaemia of inflammation (Crawley, 2004). Iron in the red blood cells is a component of haemoglobin. In vitamin C deficient individuals, the process of manufacturing haemoglobin is slower because the iron in the body is kept in body stores (Fishbane, 2006). Vitamin C deficiency exacerbates cell damage (Rössig *et al.*, 2001) including red blood cells.

Anaemia in infants and children may be as a result of cytokine mediated inflammation, causing iron sequestration (insoluble precipitates of iron) in macrophages and decreased absorption in the small intestines due to repeated illness among the children (Crawley, 2004). The Hb concentrations of the children in both feeding regimens were high even after the feeding period. This can be explained by the life span of red blood cells which is 100-120 days (Bosman *et al.*, 2005).

### **5.6 Illness among Children**

The high morbidity rates observed among the children at baseline and throughout the study were significantly ( $p < 0.05$ ) due to malaria. Malaria remained number one cause of morbidity among children according to the health statistics at the Health Unit in Magubike village. Through personal communication with health centre staff and the village leaders, it was found out that mosquito bed nets had been distributed to children aged below 5 years during their clinic days as part of the national campaign against malaria. However, the nets are not properly used, consequently the prevalence of malaria in Morogoro region is still very high, 54% (Jumbe, 2007), which was also observed in the present study.

Roselle juice or extract has been shown to have anti-microbial, diuretic, antioxidant, and anti-inflammatory activity (Mounigan and Badrie, 2006). In addition, the anti-oxidative attributes of Roselle anthocyanins and their aglycons that quench peroxy radicals formed during infections have been confirmed (Prenesti *et al.*, 2005; Egbere *et al.*, 2007). Therefore these attributes might explain the significant ( $p < 0.05$ ) decline in the prevalence of malaria among children who were fed Roselle juice for four months (intervention group). Similarly the high concentrations of vitamin C

present in the Roselle juice, which is known to boost immunity (Tang and Smit, 2002) might further explain for the reduced prevalence of malaria among children by the fourth month.

### **5.7 Urinary Zinc Concentration**

The proportion of children with urinary zinc concentration outside the normal range was lower in the intervention group (30%) than in the placebo group (40%). This might be attributed to higher disease prevalence among the children in the placebo group than in the intervention group. High urinary zinc concentrations of beyond 490  $\mu\text{g}/\text{L}$ , which was more prevalent among children in the placebo group shows increased muscle activity and protein breakdown as the body produces more energy to fill the energy demand during illness. This is mainly observed in the acute inflammation response phase of infection which is characterised by increase exogenous loss of zinc due to the above activities (Prenesti *et al.*, 2005; Egbere *et al.*, 2007).

Higher proportion of children observed as having the low zinc concentrations after 4 months of supplementation might be explained by continued low intake of zinc in the diets. The mean zinc intake among the children was only 40% of the RDA throughout the supplementation period. The zinc intake was never increased with age of the children hence reduced urinary zinc of less than 49  $\mu\text{g}/\text{L}$ . However, urinary zinc is highly dependent on the dietary zinc intake and therefore not often used to estimate zinc status of individuals.

### **5.8 Nutritional Status of the Children**

Childhood malnutrition is a major health problem affecting over 30% of children in Tanzania. According to WHO (2007), a mean Z score significantly lower than zero means that the entire distribution has shifted downwards, suggesting that most, if not all individuals have been affected. Weight for age is a composite index of weight for height and height for age. The mean WAZ scores of this study population shifted towards the negative side suggesting that all the population was affected. The poor nutritional status might be due to low intake of energy (60% of RDA) among the children during the study period. It has been observed that as age of the child increases breast milk alone can no longer satisfy the energy needs of the growing child (WHO, 2000). Therefore, complementary foods of adequate nutritional quality and quantities have to be provided at infancy and to young children. However, in the present study it was observed that the frequency of feeding of breast milk and complementary foods did not increase with age. This predisposed the children to developing malnutrition as they grew older. Some children were given more than three meals each day but the energy density of the foods was low for example porridge (51 Kcal) and cow's milk (57 Kcal) consumed by children, which further impacted on their growth and development. Fat content in the major food items like breast milk and porridge fed to the infants and young children was low. Fat has higher energy densities (9 kcal/g) than any other nutrients. Other factors like increased infection due to malaria, reduced appetite, reduced intake of zinc and vitamin C, inadequate care, and inadequate income exacerbated the severity of malnutrition among children. Infections like malaria increase body's activities and therefore demand for energy. Failure to cope with the demand by intake of more



nutrients during illnesses, increase the use of body fat reserves and muscles as alternate sources of energy (Schaible and Kaufmann, 2007).

Continued low zinc intake among children in both groups might have increased prevalence of stunting hence low weight for age. Umeta *et al.* (2000) showed that diets rich in zinc improved growth (increase in weight and height) of stunted children in Ethiopia and this was attributed to the ability of zinc to reduce morbidity due to infection and to increase appetite which all have positive effect on growth in terms of weight and height.

Deteriorating nutritional status can further be explained by the UNICEF conceptual framework for the causes of malnutrition (CDC and WFP, 2005). Apart from having diseases and inadequate nutrient intake, care to the children by caregivers (mothers) was lacking due to commitment in the rice fields. Also food insecurity was earlier reported in the village during the previous season. There was low level of education among women (17%) that decreased access to nutrition information. In addition, most of the households earned less one dollar per day hence accessibility to adequate foods was limited.

## CHAPTER SIX

### 6.0 CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 Conclusions

The dietary intake (nutrient intake) of the children from consumption of family complementary foods was heavily insufficient. Roselle juice contained significant concentration of vitamin C. It also contained substantial amounts of zinc and iron. The energy, protein, fat and carbohydrate concentrations were comparable to most of the complementary family foods like cereal porridge given to children in this age group. Consumption of Roselle juice in this study led to significant increase ( $p < 0.05$ ) intake of vitamin C although not attaining the desired intake levels in food. Consumption of Roselle juice also led to increased intake of other nutrients in the diet. Frequent consumption of Roselle juice with meals by the children for example 3 to 4 times could contribute to improvement of nutrient intake in the diet.

Consumption of Roselle juice led to improved appetite of the children. Improvement in appetite was mainly observed among children of age above 12 months who consumed Roselle juice. However, the attributes in Roselle juice that improved appetite need further investigation.

Although consumption of Roselle juice significantly improved dietary intake of vitamin C, children's bodies could not be saturated with the vitamin. The 100ml of Roselle supplemented to the children could not lead to vitamin C saturation. Consumption of Roselle juice did not significantly improve dietary intake of iron among the children. Supplementing a community like Magubike with high prevalence of vitamin C deficiency would require more volumes of Roselle juice.

Consumption of Roselle juice led to significant increase of haemoglobin concentration of the children with age below 2 years by 1 g/dl. However, consumption of Roselle juice did not significantly improve zinc status of the children in this age group.

During this study, Roselle juice supplementation led to reduction in morbidity among children. Consumption of Roselle juice by children reduced morbidity caused by malaria. Consumption of Roselle juice should be promoted in this age group to reduce morbidity. However, the attributes in the juice that are responsible for reduction of morbidity need further investigation.

## **6.2 Recommendations**

- Consumption of Roselle juice should be promoted in Magubike village to reduce the high prevalence anaemia and morbidity. Also consumption should be promoted among children to improve appetite.
- Attributes in Roselle responsible to improve appetite, reduce illnesses need to be further investigated.
- Another study is recommended by supplementing children with increased quantities (More than 100g) of Roselle juice supplemented to the children to explore any significant changes in nutritional status.

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## APPENDICES

### Appendix 1 Questionnaire used for interviews

Serial Number.....Date..... Baseline/Follow-up.....

Data collector's name.....

#### Demographic Information

Household size.....Children (<5 years)..... Individuals  $\geq$ 5years.....

Head of household.....How many are: male?.....female?.....

#### Information of the Parents

1. Age of female caregiver/mother.....Birth order if mother of the child.....

2. Age of the father..... Marital status of mother .....

3. Days per week of work if employed of: Mother.....  
Father.....

4. Average income/day of the mother.....Average income/ day of the father.....

#### Information of child

5. Age.....Weight.....Height.....Sex.....Haemoglobin.....

6. Oedema yes/no

7. Any illness in the last two weeks yes/no? Which one.....

8. Any illness in a month yes/no? Which one.....

9. If cured, how long the illness did last with the child?..... Days.

10. How much (T.Sh.) did you spend on the child while sick if well now?.....

11. While the child was sick did you maintain the normal food portion? Yes/No

12. Give reason for yes or no.....
13. Does this child demand for food always? Yes/No. *It can be through crying or asking for food*
14. How frequent does the child ask or cry for food?.....try to estimate times per day
15. Frequency of feeding the child with; a) breast milk..... b) other foods.....yesterday
16. Did the child finish all the food given to her at the different times yesterday?
17. Why do you breast feed less than 6 times a day?.....
18. Do you empty one breast before moving to another while breast feeding?
19. Has this child been supplemented with any vitamins or minerals within the last month?
20. If yes, which ones? .....
21. List foods given to the child yesterday and estimate quantities using household measures

**Food frequency questionnaire**

How many times did the child eat any of the under listed food in the previous seven days

List of foods consumed	Days in a week (day 1 is Monday up to day 7 =Sunday (tick when child ate the food)		Never
------------------------	--	--	-------





Mwidu									
Chunga									
Mustafali									
Topetope									
Jack fruit									
Fulu									
Munavu									
Mabungo									
Misasati									
Strawberry									
Cucumber									
Tomato									
Cow peas leaves									

**ii.) 24 hr Food recall**

From waking up time in the morning yesterday to morning wake up time today, how many times did you give your child other foods or drinks other than breast milk?

State the time

Time given the food	Yes	No
a)		
b)		
c)		
d)		
e)		
f)		
g)		
<b>TOTAL</b>		

*NOTE: Ask about each any possible food or snack. People often leave out or forget certain meal times. Record the total number of mealtimes for the household (number of “yes” responses).*

What were the foods given from the time the child wake up in the morning yesterday and waking up time today?

Time	Type of dish	Description	Ingredients	Amount of ingredients	Amount of dish	Amount served	Amount eaten	Amount left

### iii.) Questions on appetite assessment

Complete these questions with the help of the caregiver or mother and circle out the choices given, then tally the results based upon the following numerical scale: a = 1, b = 2, c = 3, d = 4, e = 5. The sum of the scores constitutes the SNAQ score.

1. Generally how is the child's liking to eat?
  - a. very poor
  - b. Poor
  - c. average
  - d. good
  - e. very good
2. When the child is given food on usual plate, ...
  - a. refuses to continue eating after a few mouthfuls
  - b. refuses to continue eating after third of a meal
  - c. refuses to continue eating after over half a meal
  - d. refuses to continue eating after most of the meal
  - e. continues to cry/demand for more food after finishing
3. Does the child like the food given?
  - a. does not like food completely
  - b. does not like the food
  - c. the child fairly likes eating the food
  - d. the child likes eating the food
  - e. eats the food very well
4. Normally how many times does the child eat

- a. less than one meal a day
- b. one meal a day
- c. two meals a day
- d. three meals a day
- e. more than three meals a day

What kind of food does the child like eating? .....

## **Appendix 2 Determination of vitamin C**

The reagents used include: Standard ascorbic acid (B.No. 1.0127-250 Merck, Germany) which was used to prepare 2µg/ml standard ascorbic acid solution; Citrate buffer (0.1M, pH 4) prepared from 330ml 0.1M citric acid (Merck KGaA, 64271D, Kebo Lab, Germany, R:36 S:24/25) and 170ml 0.1M sodium citrate (B.No. S-3265 FINKEM Laboratory Reagent, Mfg: 01/2006, Exp.2010); 2,6 Dichlorophenolindophenol (DCPIP-B.NO. 103028.0005, Merck, Germany) dye solution (45%) and working solution (1:18 water); and distilled water. The food sample was mixed well in the food container and 5g measured using a weighing balance (BD125-10 model A60, Wagtech UK). The weighed sample in a 100 ml beaker was macerated and homogenized in 65ml of citrate buffer i.e. ratio of 1:13 w/v. The filter paper (Whatman International, England) and funnel were used to filter extract into the conical flask. The filtrate was diluted with 50ml citrate buffer, that is, a 1:25 dilution (w/v). In the test tube, 3ml of citrate buffer, 2ml of the diluted food

extract and 1ml of DCPIP were added. The mixture was quickly transferred into a cuvette and inserted into the spectrophotometer (Wagtech, Cecil International, House of Tumors, Birkshire, UK) to read the absorption (nm) at 520nm. If the red colour of the mixture disappeared before 30 second, 5 ml of diluted food extract was further diluted by 5ml of citrate buffer and the procedure of determining the absorption repeated. The dilution then was 1:50 (w/v). The concentration (mg/ml) of ascorbic acid in the food sample was determined by comparing the absorption of 2µg/ml standard ascorbic acid solution. The equation below was used to determine the concentration (mg/ml) in the weighed food sample. The concentration in 100g of the food sample was computed.

$$\text{Concentration (mg/ml)} = \frac{\text{Blank - Test reading}}{\text{Blank - standard reading}} \times \text{DF} \times 2 \mu\text{g}$$

Where; DF is the Dilution Factor which was 25 or 50 accordingly.

### **Appendix 3 Determination of proximate composition in Roselle and food samples**

Moisture and dry matter content was determined by drying approximately 20g of the Roselle powder or food sample in a porcelain dish at 50°C for 12 hours and then 105°C for 24 hours in a temperature controlled oven until constant weight was obtained. Sand was added to milk to avoid over flowing.

The equation below was used to obtain the proportion of water:

$$\%MC = \left( \frac{W_1 - W_2}{W_1} \times 100 \right)$$

Where:

%MC = Percentage moisture content

$W_1$  = Weight of sample before drying

$W_2$  = Weight of sample after drying

Crude protein in both food samples and role was determined by the Kjeldahl method using 6.25 (conversion factor) as the coefficient of conversion of total nitrogen to protein;

$$\%N = \frac{14.01 \times (\text{Titre} - \text{Blank}) \times NA}{W \times 10}$$

Where:

$\%N$  = Percentage nitrogen content

$NA$  = Normality of acid

$W$  = Weight of sample

Therefore  $\% \text{ Protein} = \%N \times 6.25$

Crude fat was determined by Soxhlet apparatus. The following equation was used

$$\%EE = \frac{W_e}{W} \times 100$$

Where:

$\%EE$  = Ether extract (crude fat)

$W_e$  = Weight of extract

$W$  = Weight of sample

Crude fibre was determined by acid and alkali digestion. The following equation was used to calculate the proportion of fibre.

$$\%CF = \frac{W_r - W_a}{W} \times 100$$

Where:

$\%CF$  = Percentage crude fibre

$W_r$  = Weight of residue

Wa = Weight of ash

W = Weight of sample

Total ash was determined by heating the sample in a muffle furnace at 600°C for 2-4 hours; and the equation below was used to determine the ash content.

$$\%A = \frac{Wa}{Ws} \times 100$$

Where:

%A = Percentage ash

Wa = Weight of ash

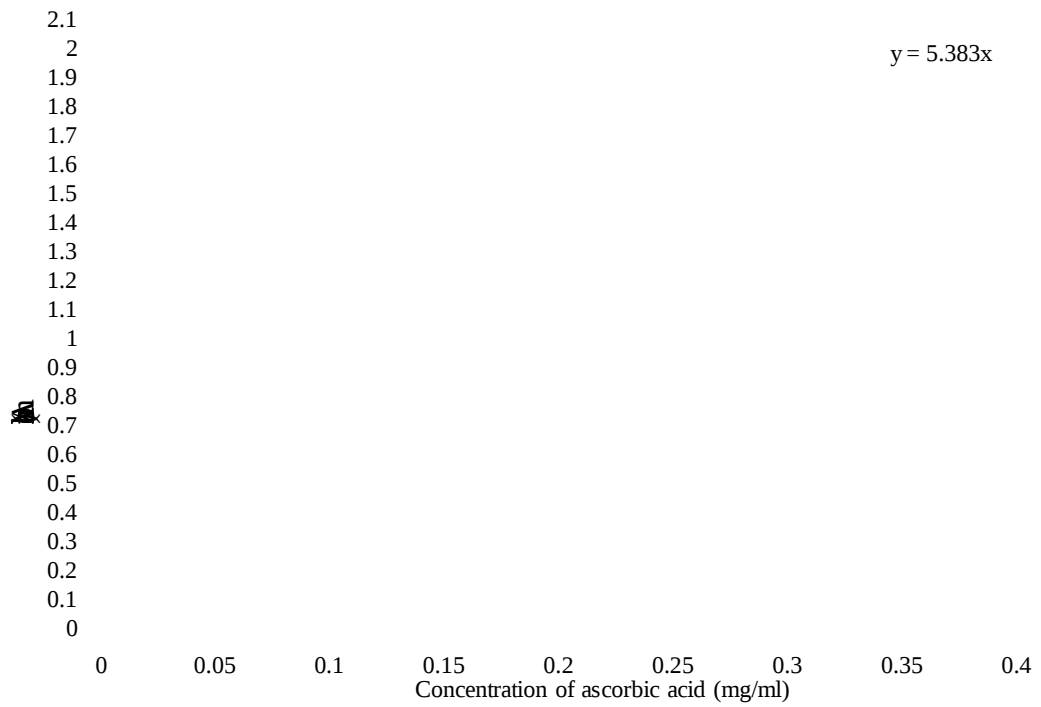
Ws = Weight of sample

- ❖ Carbohydrate was estimated by subtracting the sum of contents (%) of moisture, protein, fibre, fat and ash in the sample from 100%.
- ❖ Energy was calculated using Atwater factors equation

$$\text{Energy content} = \sum [(\text{Carbohydrate} \times 4) + (\text{Fat} \times 9) + (\text{protein} \times 4)]$$

Iron and Zinc concentrations (mg/L) were determined with using atomic absorption spectrophotometer (AAS UNICAM 919, Type: 991171; NC: 942339100071; GE: 420556; England) read at 248.5 and 213.2nm respectively.

#### **Appendix 4 Standard curve of ascorbic acid concentration using folin's reagent**



### **Appendix 5 Sample size determination**

The sample size was obtained by using an assumed rate of growth faltering of 40% since the chronic malnutrition rate is 37% (Jumbe, 2007). The equation will be used to determine the sample size. (CDC and WFP, 2005)

The assumptions you need to make before calculating the sample size include:

$p_1$  The estimated proportion derived from survey 1 (40%)

$p_2$  The estimated proportion derived from survey 2 (0%)

$\alpha$  Level of significance (“alpha”), usually 0.05 or 5% (corresponds with 95% confidence interval); therefore,  $Z_{\alpha/2}$  usually equals 1.96.

$1 - \beta$  Power, usually 0.95 (95%); therefore,  $Z_{1-\beta}$  usually equals  $-1.96$ .

DEFF Design effect resulting from cluster sampling which is 1

The formula is:

$$n = DEFF \times \frac{\left[ Z_{\alpha/2} \sqrt{2\bar{p}(1-\bar{p})} - Z_{1-\beta} \sqrt{p_1(1-p_1) + p_2(1-p_2)} \right]^2}{(p_1 - p_2)^2}$$

Where,

$$\bar{p} = \frac{p_1 + p_2}{2}$$

$p_1 - p_2 =$  the smallest difference between the prevalence rates derived from each survey which should be statistically significant.

$Z_{\alpha/2}$  the z value for the level of significance (usually 1.96)

$Z_{1-\beta}$  the z value for the power (usually =  $-1.96$ )

## Appendix 6 Consent form

- I. The effect of supplementing Roselle juice to children 6-18 months on health and growth in Magubike village.



- II. **Aim of the study:** This study aims at determining the effect of supplementing the juice on growth and nutritional status of children aged 6-18 months. Also the study aims at effect of supplementing the juice on reducing morbidity rates due to the common infections like malaria, diarrhoea, and ARI. It is envisaged that the juice will improve appetite status, haemoglobin levels, vitamin C status and zinc status. This study will be conducted with collaboration by Department of Food Science and Technology, Sokoine University of Agriculture; Magubike village administration and Health Centre; and the PANTIL Nutrition Intervention project.
- III. **Data to be collected:** On obtaining consent from the guardian and explaining the aim of the study, a structured questionnaire will be administered to obtain children health and nutrition information. The guardian or caregiver of the child will be requested to obtain the following measurements: weight, height, arm circumference and head circumference each month. Haemoglobin levels will be obtained from capillary blood as well as Zinc status and Vitamin C saturation from urine at the beginning and end of supplementation period.
- IV. **Anticipated Dangers:** There will be no dangers in taking measurements except during pricking to obtain capillary blood. The juice will be made using the local recipes in Morogoro.
- V. **Importance of this study:** This study offers cost effective opportunity to reduce high rates of anaemia, morbidity and consequently reducing poor growth and poor nutritional status.
- VI. **Incentives:** No incentives in any form will be given to the participants.

VII. **Confidentiality:** Confidentiality of the information collected will be maintained among the stakeholders and information will be used to generate a report to be used by the community. No names will be used in the report. The ingredients in the juices will be communicated in the report but will not be given during the study.

VIII. **Compliance to the consent:** The caregiver and the child who will volunteer, has the right to stop taking part in the study at any time because of any reason. No legal action will be taken for those who wish to stop taking part in the study

IX. **Contact Person:**

Bukenya, Richard

Sokoine University of Agriculture, Department of Food Science and Technology,  
P.O. Box 3006, Morogoro- Tanzania.

**Agreement:** I have read all the above information and was given a chance by the researcher to respond to all my questions. I satisfied to all what was explained and on behalf of my child I agree to take part in this study.

**Agreement to take part in the study:**

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<b>Name of caregiver of child</b>	<b>Signature/thumb print</b>	<b>Date</b>
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<b>Name of researcher</b>	<b>Signature</b>	<b>Date</b>
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### Appendix 7 Baseline anthropometric characteristics of the children

Age group	Age (months)		Weight (Kg)		Height (cm)		MUAC (cm)		HC (cm)	
	Placebo	Intervention	Placebo	Intervention	Placebo	Intervention	Placebo	Intervention	Placebo	Intervention
6-7.99	7.1±0.8	7.3±0.7	7.4±1.1	7.8±1.2	65.9±1.9	66.4±3.1	14.1±1.2	14.2±1.6	43.9±1.8	44.5±1.7
8-9.99	9.0±0.6	9.2±0.6	8.6±1.2	7.8±0.8	68.8±3.4	68.8±2.2	14.7±1.0	13.8±1.1	45.8±1.4	44.8±0.9
10-11.99	11.1±0.6	11.2±0.6	9.1±1.4	7.9±0.7	71.6±2.2	68.9±1.6	14.7±1.3	14.0±1.1	46.3±0.8	45.5±1.6
<b>6-11.99</b>	<b>9.0±1.7</b>	<b>8.9±1.6</b>	<b>8.4±1.2</b>	<b>7.8±0.9</b>	<b>68.8±3.4</b>	<b>67.9±2.7</b>	<b>14.5±1.1</b>	<b>14.0±1.3</b>	<b>45.4±1.7</b>	<b>44.8±1.4</b>
12-13.99	13.3±0.5	13.1±0.6	9.0±1.3	9.1±0.9	72.5±2.4	72.5±2.5	14.7±1.7	14.7±0.9	47.2±1.4	46.3±1.5
14-15.99	14.9±0.7	14.9±0.7	9.4±1.2	9.7±1.2	74.9±4.3	74.1±2.6	14.4±1.0	15.2±0.8	47.4±2.3	47.0±2.3
16-17.99	16.8±0.6	16.8±0.7	9.2±0.8	9.8±0.9	75.1±2.1	76.8±0.8	15.0±1.0	15.2±1.1	46.3±1.5	48.2±1.5
18-21.99	19.5±1.4	20.1±0.9	8.9±0.8	8.6±1.3	74.3±3.6	77.1±2.2	14.5±0.9	14.9±1.0	47.6±1.4	47.2±0.7
<b>12-21.99</b>	<b>15.8±2.4</b>	<b>15.8±2.7</b>	<b>9.2±1.0</b>	<b>9.5±1.0</b>	<b>74.1±3.2</b>	<b>74.7±2.9</b>	<b>14.7±1.2</b>	<b>15.0±0.9</b>	<b>47.1±1.7</b>	<b>47.0±1.7</b>
<b>6-21.99</b>	<b>12.2±4.0</b>	<b>12.1±4.1</b>	<b>8.8±1.3</b>	<b>8.6±1.3</b>	<b>71.3±4.2</b>	<b>71.1±4.4</b>	<b>14.6±1.2</b>	<b>14.5±1.2</b>	<b>46.2±1.9</b>	<b>45.9±1.9</b>

