THE USE OF *MORINGA OLEIFRA* LEAF POWDER IN THE MANAGEMENT OF SUB-CLINICAL PROTIEN ENERGY MALNUTRITION IN CHILDREN BETWEEN THE AGES OF 6 - 36 MONTHS.

A THESIS PRESENTED TO THE DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY OF THE KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE MASTER OF SCIENCE DECREE IN FOOD SCIENCE AND TECHNOLOGY

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ABSTRACT

Malnutrition is a major factor in the often high rates of infant mortality in the tropics and sub-tropics. Current treatment for children involves the use of special formulated foods which are either labeled as F-100 or F-75. These formulated foods are expensive and not sustainable in the long term. A study was undertaken to evaluate the potential of Moringa oleifera leaf powder in the management of malnutrition in children between the ages of 6 – 36 months. 25 selected malnourished children were fed 15 grams of dry Moringa leaf powder in their diets for 4 weeks. Anthropometric indicators were measured (weight to height ratio), haematological (haemoglobin and corrected white blood cell) and biochemical analysis (serum urea and serum albumin) were also carried out. Haematological results showed that daily addition of *Moringa oleifera* leaf powder to the diets of the children resulted in a significant ($p < 0.001$) linear increase in the haemoglobin levels of subjects. The individuals understudied had corrected white blood cell count that fell within the reference range of 4.5 – 13.5 K / µl both at the baseline studies and at the end of the study period. Biochemical results obtained showed that all the subjects had serum urea levels within the normal range of 1.4-6.8mmol/l and addition of Moringa leaf powder to the foods of the subjects resulted in a significant ($p < 0.001$) linear increase in serum urea levels of subjects. It was observed that none of the subjects fed on *Moringa oleifera* leaf powder had serum albumin level that was below the lower limit of the accepted range (38.0 - 51.0 g/ l) both at the baseline study and throughout the study period. Anthropometric results obtained showed that Moringa fed subjects obtained better weight gain than the F-100 fed subjects. Thus Moringa leaf powder has the potential to contribute significantly to the management of malnutrition in children between the ages of 6-36 months.

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CHAPTER ONE

1.0 INTRODUCTION

Malnutrition literally means 'bad nutrition' which is the cause of nutritional disease. This may be due to a deficiency or excess of nutrients or to the presence of poisonous substances (toxins) in the food. In Africa, most cases of malnutrition are caused by a deficiency of nutrients or by toxins, and protein energy malnutrition (PEM) is the most important deficiency disease. PEM is the range of nutritional disease arising from a simultaneous deficiency of protein and energy (calories). It is commonly associated with infections and occurs in all age groups but is most frequent in infants and young children (Parry, 1984).

Malnutrition causes a great deal of human suffering and impacts severely on the socio-economic development of a nation. This is because a work force that is stunted both mentally and physically may have a reduced work capacity (Pelletier, 1995). The interaction of poverty, poor health and poor nutrition has a multiplier effect on the general welfare of the population and also contributes significantly towards keeping a population in a downward trend of poverty and nutritional insecurity.

Malnutrition in its various forms (e.g. kwashiorkor, beri-beri, anaemia, scurvy, and marasmus) is a major factor in the often high rates of infant mortality in the tropics and sub-tropics. In the poorest countries, as many as one child in five will die during infancy (Population Reference Bureau, 1997). Worldwide, it is estimated that seven million

people die each year from hunger-related causes, and the vast majority of these deaths are caused by chronic undernutrition (Church World Service, 1999). Information obtained from the Ghana Demographic and health survey (1998), showed that as many as 28% of children born in the country are chronically under nourished and the three northern regions reported the highest number of cases, 15%.

Approaches made in the management and treatment of malnutrition included school lunch programs, nutrition education, introducing exotic vegetables, and even campaigns to periodically give children massive doses of vitamin A. A major drawback to these approaches is the dependence on imported solutions and outside personnel, and progress can quickly dissipate once the program funding dries up (GRAIN, 2000).

Current treatment (management) of malnutrition for children involves the use of special formulated foods which are either labeled as F-100 or F- 75 based on the degree of malnutrition being treated. These special formulae are made up of milk, sugar, cereal flour, vegetable oil and combined mineral and vitamin mix which are expensive and are out of reach of poor beneficiaries if offered commercially and thus not sustainable in the long term. There is therefore the need to find an inexpensive and local solution to the malnutrition menace in Ghana and Africa as a whole.

One potential approach for managing malnutrition at minimum cost is the use of *Moringa oleifera* leaves. "Ounce-for-ounce, Moringa leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas," and the protein quality of Moringa leaves

rivals that of milk and eggs (Fuglie, 1997). Thus the nutritional properties of Moringa make its consumption in situations where starvation is imminent very important.

Despite the numerous nutritional and health benefits of moringa it's rather unfortunate that its usage in prevention and management of child malnutrition is virtually limited in Ghana.

Work carried out by three non-governmental organizations; Trees for Life, Church World Service and Educational Concerns for Hunger Organization in Senegal showed that Moringa leaf powder can be used to combat malnutrition (Fuglie, 2000). Even though this work was done on pilot basis there were several limitations to the design of the work thus affecting the findings/observations of this research. Some of these limitations include:

- Lack of requisite data to support the chronological measurement of weight for age.
- The lack of laboratory data (hematological and biochemical investigations) to support the impact of moringa on the malnutrition management.

Thus the main objective of this study is to use *Moringa oleifera* leaf powder in the management of subclinical protein – energy malnutrition in children between 0 to 36 months old.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 MALNUTRITION

Malnutrition is often lost in discussions around the subject of hunger, especially in the context of the discourse to "end world hunger" or to; "feed the world". These blurred definitions help to perpetuate the inadequate response to malnutrition. It is crucial to distinguish between malnutrition and hunger as malnutrition requires responses that go beyond food aid. Hunger is usually taken to be a deficiency in caloric intake – any child between the ages of zero to five years whose daily dietary intake gives less than the defined minimum of 850 -180 Kcal per day is considered as suffering from hunger, or undernourished (Wardlaw, 1999). The typical response to hunger is food aid that supplements a person's daily caloric intake (Shepherd, 2006). Malnutrition however is not merely the result of too little food. It is a pathology caused principally by a lack of essential nutrients. Most food aid is an inadequate response to malnutrition as it either delivers insufficient amounts of essential nutrients or delivers them in a way that they are destroyed by cooking or not taken up properly by the body (Shepherd, 2006).

Children become malnourished when they do not receive the adequate nutrients their bodies require to resist infection and maintain growth. When nutritional deficiencies become too significant, a child will begin to 'waste' – to consume his/her own tissues to obtain needed nutrients. Wasting is a sign of acute malnutrition (Wardlaw, 1999).

The body's nutritional health is determined by the sum of its nutritional status with respect to each needed nutrient. Malnutrition literally means 'bad nutrition' which is the cause of nutritional disease. This may be due to a deficiency or excess of nutrients or to the presence of poisonous substances (toxins) in the food (Parry, 1984). The consequences of malnutrition in the community are shown in table 1 below.

Table 2.1. Effects of malnutrition in a community.

Deficiency of nutrients	**Excess nutrients**	**Toxins in food**
Low birth weight	Obesity	Liver disease
Poor lactation	Coronary arterial disease	Cancers(e.g. esophagus
High child mortality	Diabetes mellitus	Neuropathies
Retarded development in children		
Marasmus and kwashiorkor		
Loss of strength and productivity in adults		
Vitamin deficiency syndromes		

There are three general categories of nutritional status recognized; Desirable nutrition, under nutrition and over nutrition. The nutritional status for a particular person is desirable when body tissues have enough of the nutrients to support normal metabolic functions as well as surplus stores that can be utilized in times of increased need. This can be achieved by obtaining essential nutrients from a variety of foods. Under nutrition is caused by failing health that results from a long–standing dietary intake that does not meet nutritional needs. In over nutrition, there is prolonged consumption of more nutrients than the body needs. This can cause no signs or symptoms, but increase in some nutrients may result in serious diseases such as hypertension, obesity and kidney failures (Wardlaw, 1999).

The America Association of Clinical Endocrinologists (1997) assert that, good nutrition means eating foods each day that provide the vitamins, minerals and other nutrients that the body needs for proper development. It means eating foods that give enough calories to have a healthy body weight, getting enough to keep the body built up and repair any damage it may have. They further explain that, good nutrition means receiving a balance of both macronutrients and micronutrients from the food eaten in quantity that supports a healthy weight. Good nutrition is important because it can affect the overall health, energy level and emotional well-being.

Ideally, good nutrition is assured by a diet rich in meat, root, grain, fruit and vegetable foods. In reality, for a majority of the world's population such variety in food is unaffordable or seasonally unavailable. Within the sahelien region of Africa, for example, the dry seasons are marked by a heavy reliance on the staples of rice, millet and sorghum; during these months, fruits and greens can be found only in a few irrigated garden plots, and in virtually every year the wet season is a lean period where food stores have been exhausted one to three months prior to the new harvest. Within this region, infants in the weaning stage are the most vulnerable to malnutrition since their food intake is heavily reliant on millet (Fuglie, 2000).

Many host and environmental factors: lack of education, poverty, famine, parasites and impure drinking water interact to produce nutritional diseases. Ignorance and poverty are fundamental: insufficient food is produced, and what food there is, is unevenly distributed within the family and the community. A program which focuses on correcting

micro-nutrient deficiencies alone will not fully eradicate malnutrition until these other causes are addressed (Parry, 1984).

2.1.1 Undernutrition or Subclinical protein- energy malnutrition

Protein- energy malnutrition (PEM) is the range of nutritional disease arising from a simultaneous deficiency of protein and energy (calories). It is commonly associated with infections and occurs in all age groups but is most frequent in infants and young children (Alleyne *et al.* 1997). PEM is commonly found in children in the developing world such as Africa and in Asia. This is shown in children by a reduced growth rate and in adults by low weight- for -height and lack of normal amounts of fat. The highest incidence is at the time of weaning, when the change from breast milk to bulky staple results in a diet deficient in both calories and protein.

2.2 TYPES OF MALNUTRITION

There are different types of malnutrition, if dietary deficiencies are persistent, children will stop growing and become stunted (low height-for age). This is referred to as chronic malnutrition. If they experience weight loss or "wasting" (low weight-for-height), they are described as suffering from acute malnutrition. Both of these presentations of malnutrition may be further classified as moderate or severe (Shepherd, 2006).

Severe acute malnutrition includes two main clinical forms – severe wasting (called marasmus) and nutritional oedema (known as kwashiorkor). It is the clinical analysis that determines if treatment will be in a hospital or with therapeutic Ready to Use Feeds

(RUF) at home. Severe acute malnutrition has a case fatality of up to 21% without effective intervention. But any child with malnutrition is at an increased risk of developing complications leading to severe illness and death (Shepherd, 2006).

Kwashiorkor is a type of childhood malnutrition with controversial causes, but it is commonly believed to be caused by insufficient protein intake. Jamaican pediatrician Cicely D. Williams introduced the name into international scientific circles in her 1935 Lancet article. A large, protuberant belly is common. Skin conditions such as dermatitis, changes in pigmentation, thinning of hair and vitiligo are frequently seen. When a child is nursing, it receives certain amino acids vital to growth from its mother's breast milk. When the child is weaned, if the diet that replaces the milk is high in starches and carbohydrates, and deficient in protein (as is common in parts of the world where the bulk of the diet consists of starchy vegetables, or where famine has struck), the child may develop kwashiorkor (Parry, 1984).

Kwashiorkor, also called the wet, swollen, or edematous form of PEM, is associated with premature abandonment of breastfeeding, which typically occurs when a younger sibling is born, displacing the older child from the breast. It may also result from acute illness, often gastroenteritis or another infection, probably secondary to cytokine release, in a child who already has PEM. A diet that is more deficient in protein than energy may be more likely to cause kwashiorkor than marasmus. It tends to be confined to specific parts of the world, such as rural Africa, the Caribbean, and the Pacific islands (http://adam.about.com/encyclopedia/inectiousdiseases/Kwashiorkor).

Marasmus is a form of severe protein-energy malnutrition characterized by calorie and energy deficiency. Marasmus also called the dry form of PEM causes weight loss and depletion of fat and muscle. In the developing countries, marasmus is the most common form of PEM in children (America Association of Clinical Endocrinologist, 1997). Marasmic kwashiorkor is characterized by features of marasmus and kwashiorkor. Affected children have some edema and more body fat than those with marasmus (America Association of Clinical Endocrinologist, 1997)

2.2.1 Parameters for measuring sub-clinical protein – energy malnutrition (PEM)

Sub-clinical protein – energy malnutrition is detected by anthropometric and or biochemical measurements. Anthropometry (measurement of the body) measures the results of a series of nutritional events affecting the individual over a relatively long period and so it is less reliable to rapid fluctuations. It is best to relate these measurements to age but as this is seldom accurately known other ratios have been devised to try to overcome this difficulty (Waterlow, 1999).

► Mid-upper arm circumference. This is a very useful measurement of muscle mass in malnourished children and adults. In normal children the arm circumference changes little in the range of 1-4 years so it is only slightly affected by age (Parry, 1984).

► Triceps skinfold. This is a measure of subcutaneous fat and is useful in assessing energy deprivation (Parry, 1984).

► Weight for age. This accesses failure of growth and the degree of body wasting. In adults and children loss of weight is a sensitive index of energy deprivation.

► Height for age. This is the most useful measure of the long-term effects of PEM. The Harvard Standard of classifying malnutrition is based on this index.

► Weight for height. This index is becoming popular for the measurement of malnutrition in a community. This index is largely independent of age and race and also separates those children who are stunted (short children who are of low weight for their height) and the World Health Organization is currently using this as index for classifying malnutrition status. The World Health Organization has developed charts, currently used throughout West Africa, which enable health workers to compare a child's body length and weight and thereby determine to what degree the child deviates from the healthy median. According to where the child's length-weight ratio falls on the chart, he/she can be classified as normal, mildly or moderately malnourished (a Standard Deviation score of -1 to -2), or severely malnourished (-3 to -4 SD). Severe malnutrition in children is additionally defined as when there is "symmetrical oedema (fluid retention) involving at least the feet (World Health Organization, 1999).

When a child has reached this stage of severe malnutrition, there are very gross abnormalities physiologically, these include infections, impaired liver and intestinal function and problems related to imbalance of electrolytes. Intensive hospital care is required in these cases. The infections must be treated and new ones prevented, the electrolyte balance restored, and an intensive 24-hour feeding program instituted. Due to the physiological abnormalities, the severely mal-nourished child cannot tolerate iron or the usual amounts of dietary protein, fat and sodium. Until the child leaves this emergency phase and enters the rehabilitation phase (when the child's condition is stable

and his or her appetite has returned, normally after 2-7 days) his or her diet needs to be high in carbohydrates and contain potassium, magnesium and other essential minerals, but low in protein, fat and sodium and completely lacking in iron supplements (Fuglie, 2002). Thus Moringa leaves, with their high iron and protein contents, are not appropriate for use during initial treatment of the severely malnourished child.

However, mild or moderate malnutrition before this terminal stage is reached is a completely different situation: the physiological abnormalities are much less severe and successful recovery can be had through a fully balanced diet containing all 40 essential nutrients in the correct proportions.

Biochemical methods by comparison with anthropometrical methods are difficult and expensive but they are more sensitive to recent changes in nutrition and may give much clearer evidence of malnutrition or recovery.

► Urea excretion in urine. This falls rapidly if a person is on a low protein diet (Parry, 1984). It is also an indication that serum urea levels are low since there is reduction in protein metabolism secondary to low protein intake. Parry (1984), reported that urea excretion in urine falls rapidly if a person is on a low protein diet which is an indication that serum urea levels are low since there is a reduction in protein metabolism due to low protein intake (Parry, 1984). The normal kidney can excrete large amounts of urea. If the rate of production exceeds the rate of clearance, plasma urea concentrations rise even within the normal range. Cook reported in 2006 that the rate of urea production is accelerated by a high protein diet and other factors (Cook, 2006).

► Serum albumin. Levels start to fall below normal as protein deficiency becomes severe. A more sudden drop may occur after an infection like measles or severe gastro-enteritis (Parry, 1984). Robbins reports that there are two protein compartments in the body: the somatic protein compartment, represented by the skeletal muscles; and the visceral protein compartment represented by protein stores in the visceral organs, primarily in the liver. Interestingly, the visceral protein compartment which is presumably more precious and critical for survival is depleted only marginally and hence serum albumin levels are either normal or only slightly reduced. This is because the somatic protein compartment was being categorized sparing the visceral proteins and hence the loss of muscle mass observed in the subjects (Cotran *et al.*, 1999).

Cook (2006), reports that an abnormally high plasma concentration of albumin is found only artefactually in a sample taken with prolonged venous stasis (standing) or after loss of protein-free fluid (e.g. in the case of diarrhoea and vomiting).

2.3 Interrelationships between factors leading to malnutrition

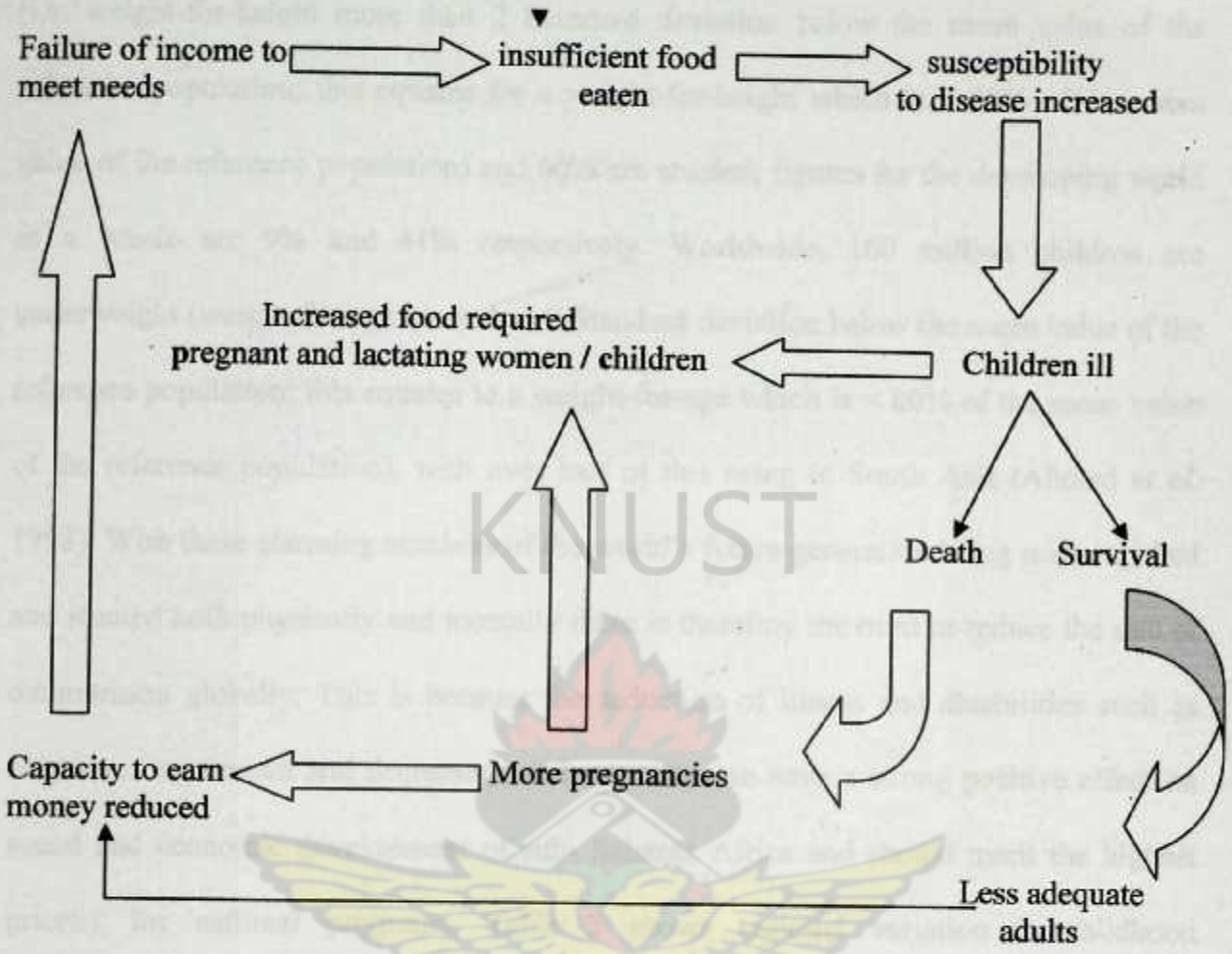


Fig 1
Source: Advisory committee on protein (1974)

2.4 The global burden of childhood malnutrition

It was estimated in 1998 that 168 million children under 5 years of age (27% of the world's children in this age group) are malnourished (as measured by weight-for-age); 76% of these children live in Asia, 21% in Africa and 3% in Latin America (World Health Organization, 1998). As many as 206 million children are stunted (i.e. height-for-age more than 2 Standard deviation below the mean value of the reference population; this equates to a height –for-age which is < 90% of the mean value of the reference

population). In South Asia as many as 17% of children under 5 years of age are wasted (i.e. weight-for-height more than 2 Standard deviation below the mean value of the reference population; this equates for a weight-for-height which is < 80% of the mean value of the reference population) and 60% are stunted; figures for the developing world as a whole are 9% and 41% respectively. Worldwide, 160 million children are underweight (weight-for-age more than 2 Standard deviation below the mean value of the reference population; this equates to a weight-for-age which is < 80% of the mean value of the reference population), with over half of this being in South Asia (Ahmed *et al.* 1998). With these alarming numbers of the world's future generation being malnourished and stunted both physically and mentally there is therefore the need to reduce the rate of malnutrition globally. This is because the reduction of illness and disabilities such as cognitive impairment and decreased work capacity can have a strong positive effect on social and economic development of sub- Saharan Africa and should merit the highest priority for national programs. Table 2 shows regional variation in childhood malnutrition.

Table 2.2 Regional variation in childhood malnutrition

Region	Low weight-for height † (Wasted; %)	Low height-for-age ‡ (Stunted; %)	Low weight-for-age § (Underweight; %)	Low birth weight ‖ (%)
South Asia	17.1	59.5	58.3	33
East and south-East Asia	5.2	33.3	23.6	11
Sub-Saharan Africa	7.0	38.3	30.2	16
Middle East North Africa	8.8	32.4	25.3	10
Latin America and Caribbean	2.6	22.7	12.0	11
Developing Countries	9.1	40.7	33.9	19

Source of data: United Nations (1994), United Nations International Children's Emergency Fund (1996), Food and Agriculture organization (1996).
† Weight-for-height more than 2 SD below mean for reference population.
‡ Height-for-age more than 2 SD below mean for reference population.
§ Weight-for-age more than 2 SD below mean for reference population.
‖ Small for gestational age: 10th centile expected weight for gestational age for reference population.

2.5 Recent trends in malnutrition in developing regions

Recent prevalence data compiled by ACC/SCN on levels of malnutrition based on indicators of vitamin A deficiency, anaemia, iodine-deficiency disorders, and childhood underweight revealed that Xerophthalmia affects around 1% of children in developing regions (West, 2002; Sommer and Davidson, 2002). Vitamin A deficiency affects some

25% to 35% of children (West, 2002). Anaemia is considered to affect 50% or more of women in developing regions (a higher percentage in pregnant women) and is becoming recognized as a serious problem in young children as well (World Health Organization, 2004). Iodine deficiency measured as goiter is known to have affected some 15% (World Health Organization, 1999), and measured as low urinary iodine excretion, the prevalence is estimated as around 35% (World Health Organization, 2004). Child underweight is probably the best known with overall developing region levels of 25%, decreasing by 0.5 percentage points per year (World Health Organization, 2004).

Malnutrition has been estimated to be the largest single risk factor in the global burden of disease (Ezzati *et al.* 2002) the contributions of different deficiencies, and the distinctions between these as risk factors and as disabilities have been calculated (Caulfield *et al.* 2004; Mason *et al.* 2003). Sub-Saharan Africa shows a generally unchanging or worsening situation, except for iodine-deficiency disorders, which are improving with expanding, iodized salt coverage. The overall prevalence of child underweight was decreasing slightly up to 2000, but this was reversed at least in Eastern and Southern Africa with drought (and the HIV/AIDS) epidemic after 2001 (UNICEF, 2004). Of particular concern are the prevalences of anaemia which are extremely high for children (> 70% is estimated). The general picture of static nutrition, or deteriorating nutrition in some countries, is in line with the discouraging health and economic situation in Africa.

The prevalence of anaemia is extremely high in Ghana (70%-80%) and is scarcely changing, in part probably because of low consumption of animal products, as well as the

extent of parasitic infection. In its report, the Ghana Demographic and Health survey (2003), showed that more than three-quarters of Ghanaian children 6-59 months old have some level of anaemia, including 23% of children who are mildly anaemic, 47% who are moderately anaemic and 6% who are severely anaemic. The prevalence of children 6-59 months old who are severely anaemic by region in 2003 showed that 8% of children in the Upper East region, 7% of children in the Brong Ahafo region, 7% in the Ashanti region and 9% of children in the Western region are severely anaemic and have anaemia figures above the national average. The Northern and Central regions had 6% each of 6-59 months old children who fell within the national average of being severely anaemic while Upper West, Eastern, Volta and Greater Accra regions had 3%, 4%, 2% and 4% respectively of children below the national average of being severely anaemia (Ghana Demographic and Health Survey, 2003).

2.6 Percentage of underweight or malnourished children in Ghana

Stunting, wasting, and being underweight are all indicators of malnutrition in children. All the ten regions in Ghana had a number of malnutrition cases to be managed. The decrease or increase in number of malnourished cases reported could be traced to the cultural practices, farming practices, economic status, location and the beliefs of the people. According to the 2003 Ghana Demographic and Health Survey, 30% of children under five are stunted and 11% severely stunted.7% of children under five are wasted and 1% severely wasted. Weight for age results show that 22% of children under five are underweight, 5% severely underweight. Children whose biological mothers were not in the household are more likely to be malnourished with 34% being stunted, and 25%

being underweight than children whose mothers were interviewed. It was observed that rural children showed increase percentages in being stunted, and underweight compared to urban children. 20% of urban children under five were stunted whiles 35% of children under five in the rural areas were stunted. In terms of underweight, whiles 15% of children in urban centres were underweight, 25% of rural children under five were underweight. In terms of wasting, both rural and urban children under five recorded the same percentage of 7 (Ghana Demographic and Health Survey, 2003).

The proportion of children under five who are stunted has increased from 26% in 1998 to 30 % in 2003. The proportion underweight decreased from 10% in 1998 to 7% in 2003. The proportion of children who are wasted also decreased from 25% in 1998 to 22% in 2003 (Ghana Demographic and Health Survey, 2003).

2.6.1 Malnutrition status in the Ashanti region

Ashanti region has 26 districts where malnutrition is managed. This includes Adansi North, Adansi South, Afigya Sekyere, Ahafo Ano South, Ahafo Ano North, Amansie Central, Amansie East, Amansie South, Asante Akim North, Asante Akim South, Atwima Nwabigya, Bosomtwe-Atwima kwawoma, Ejisu Juaben, Ejura –Seko, Obuasi, Offinso, Sekyere East and Sekyere West (Boateng, 2006).

The child welfare clinic covered from 67% in the Atwima Mponua to 17% in Tafo sub metro. However, the expectation is that each district should have covered 50% of the target population and only eight districts achieved this target out of the 26 districts. This

gives an indication that high population of children stand the risk of being malnourished. The incidence of malnutrition ranges from a high percentage of 11 in Amansie central to a lower percentage of 0.4 in the Adansi north. This is an indication that preventive interventions need to be strengthened. The rate of rehabilitation ranges from 86.3% in Bosomtwe Atwima Kwawoma to 5.3% in Atwima Mponoa district. Case fatality was highest in Amansie East with death rate of 7.3%. Though kwashiorkor cases were 8.3% of total cases registered, they accounted for 41.7% of total deaths (Boateng, 2006).

2.7 Malnutrition in relation to infection

Good nutrient status is required if the host is to combat infections effectively. Effective protection against invasion of the host by microorganisms requires an intact skin surface and intact linings of the naso-oesophageal, gastrointestinal and genito-urinary tracts since these produce a barrier to invasion (Bloem *et al.* 1990) This barrier also includes the ability of cells to produce mucous secretions and to maintain other physiological protective mechanisms (e.g. pH). Importantly, the cells in these linings are continually turning over, so that nutrient required for cell growth and replication must be available in order for the protection against infection to be maintained (Sommer *et al.* 1984). The immune response to infection involves a vast increase in cell differentiation and replication, in the production of immunoglobulins and acute-phase proteins and in the production of peptide-and-lipid-mediators (cytokines and eicosanoids respectively); this requires appropriate supply of nutrients to optimize the response. A component of the host response to infection is the production of damaging reactive O species; protection of host from this damage requires an appropriate status of antioxidant protective

mechanisms which include antioxidant enzymes (all of which include metal ions such as Fe, Zn, Cu, Mn, Se as an active component), antioxidant vitamins (e.g. vitamin E, vitamin C) and small peptides (e.g. glutathione). Thus, in order to maintain protection against infective agents and to mount a successful response if infected, the host requires a supply of a range of nutrients. Thus, there is a vast body of evidence that many infections are increased in prevalence or severity by specific nutrition deficiencies (Scrimshaw *et al.* 1986).

2.7.1 Infections and its role in malnutrition/ infections can cause malnutrition

Infections can alter nutritional status, mediated by changes in dietary intake, absorption, and nutrient requirements and losses of endogenous nutrients. Coupled with one or more existing nutrient deficiencies, the effect of infection can be particularly detrimental (Castaldo *et al.* 1996). Infection is characterized by anorexia: clearly a reduction in food intake will result in reduced intake of all macro-and micronutrients. This could lead to nutrient deficiencies even if the host was not already deficient and it could make existing borderline deficiencies apparent (Molla *et al.* 1983). Withdrawal of food from individuals with fever, diarrhoea, or other symptoms of infections is a common practice that will exacerbate the effects of anorexia. However, it is difficult to separate the effects of anorexia and food withdrawal but the combination could be devastating to the individual.

Infection is characterized by nutrient malabsorption and loss: the range of infections associated with nutrient malabsorption is wide and includes bacteria, viruses, protozoa and intestinal helminthes. Apparent protein absorption by children in Panama with

diarrhoea was generally reduced by 10-30% and sometimes by as much as 40% (Scrimshaw and San-Giovanni, 1997). Rates of absorption of macronutrients for healthy children would be expected to be > 90%. Infection blocks iron absorption (Cartwright *et al.* 1946). Vitamin A malabsorption also occurs during infections: children with acute diarrhoea or respiratory infections absorb only 30-70% of ingested vitamin A (Sivakumar and Reddy, 1972, 1975). Diarrhoea results in loss of zinc and copper (Castillo-Duran *et al.* 1988; Butler *et al.* 1993). Apart from malabsorption, nutrients may also be lost through the faeces as a result of damage to the intestinal wall.

Infection is characterized by altered metabolism and redistribution of nutrients: the acute phase response is the name given to the metabolic response to infections (and often to immunization) and it includes the onset of fever and anorexia, production of specific 'acute-phase reactants' and the activation and proliferation of immune cells. Such a catabolic response occurs with all infections even when they are subclinical (Beisel, 1972, 1975; Keusch &Farthing, 1986). This serves to cause redistribution of nutrients away from skeletal muscle and adipose tissue and towards the host immune response. This redistribution is mediated by production of pro-inflammatory cytokines by leucocytes and associated endocrine changes. The average loss of protein over a range of infections has been estimated to be 0.6g/kg per d (Powanda, 1977). The metabolic response to infection also results in increased oxidant stress (Grimble, 1999), which can potentially deplete reserves of cellular and plasma antioxidant vitamins (vitamin E, vitamin C, β-carotene; (Grimble, 1999).

One metabolic consequence of infection is a decrease in serum iron, zinc and vitamin A concentrations. There are contrasting views as to the role of this: one is that the nutrients are preferentially moved to tissue sites to promote host defence and the other is that they are cleared from circulation in order to deprive pathogens of nutrients that they need. These alternatives are important in understanding the meaning and role of changes in plasma concentrations and in designing appropriate nutritional interventions. Furthermore, an infection which causes diarrhoea and so results in vitamin A, iron, and zinc loss in the faeces, involves an interaction between redistribution of these nutrients within the body and the loss of these nutrients from the body (Mata *et al.*1977 & 1992).

2.8 Nutrition and immune function

Koster *et al.* (1987) reported that particular nutrients are required for an optimal immune response and that deficiency in one or more of these nutrients diminish immune function and provide a window of opportunity for infectious agents. It is logical that multiple nutrient deficiencies might have a more significant impart on immune function, and also resistance to infection, than would a single nutrient deficiency.

Protein deficiency diminishes immune responses and increases susceptibility to infection because immune defense are dependent upon cell replication and the production of protein with biological activities (immunoglobulins, cytokines, acute-phase protein) (Chandra *et al.* 1984). Practically all forms of immunity may be affected by protein-energy malnutrition, but non-specific defenses and cell-mediated immunity are more severely affected than humoral (antibody) responses; indeed Kuvibidila *et al* (1987) reported that circulating concentrations of immunoglobulins are often unaffected by

malnutrition. In addition to reduced protein availability, deficiencies in specific amino acids may affect immune function.

Deficiencies in many other micronutrients including magnesium, selenium, vitamin C, β-carotene, pyridoxine, folic acid, vitamin B_{12}, and vitamin E reduce immune function in experimental animals and in human populations (Scrimshaw and SanGiovanni, 1997; Stable and Spears, 1993; Bendich, 1993), and may play a role in increasing susceptibility to infections.

2.9 Recent interventions in malnutrition

Micronutrient deficiencies are important contributors to the global burden of disease. According to the WHO, 19% of the 10.8 million child deaths globally are attributable to iodine, iron, vitamin and zinc deficiencies. Recent estimates indicates that fortification or supplementation with iron, vitamin A, and zinc are among the most cost effective interventions available, even in areas that are poor or have high HIV infection rates, as is the case in much of sub-Saharan Africa (Black, 2003).

Iodine deficiency in pregnancy has long been linked to intra-uterine brain damage and possible fetal wastage. This has led to effective programs for making iodized salt available in iodine-deficient areas. Currently, while more than two billion people live in areas that used to be iodine- deficient, it is estimated that iodine deficiency is the attributable cause of only 0.2% of the global burden of disease (Black, 2003).

Iron deficiency affects about two billion people. However, interventions to control iron deficiency have been less successful. Research carried out in Nepal showed that antenatal folic acid and iron daily supplementation of pregnant women increased mean birth weight of new born babies and modestly reduced the risk of low birth weight babies (Christian P *et al.* 2003).

The importance of zinc deficiency is being increasingly recognized. Trials have shown that zinc supplementation results in improved growth in children, lower rates of diarrhoea, malaria and pneumonia, and reduced child mortality (Black, 2003).

Vitamin A deficiency (VAD) is one of the leading forms of micronutrient malnutrition in developing countries, ranking third after iron and iodine deficiency. Severe vitamin A deficiency has been associated with blindness, particularly childhood blindness. Approximately 300,000 blind children live in Africa (WHO Fact Sheet, 1999). In 1998, WHO recommended that in areas where VAD is prevalent, women be provided with a high-dose vitamin A supplement in the early postpartum. Postpartum dosing improves maternal vitamin A status and increases the vitamin A content of breast milk for at least six months. This means more vitamin A for the breast-feeding infant. Maternal postpartum dosing improves the vitamin A status of infants and enhances their vitamin stores up to six months when infants begin to be at a much higher risk of developing VAD. Postpartum dosing may also decrease infant morbidity and mortality while improving maternal health.

From all these current interventions what stands out clearly is the dependency on supplementation with vitamin A and iron, fortifications of salts with iodine, to the introduction of very expensive imported foods which are out of reach of poor beneficiaries if offered commercially, and are thus not sustainable in the long term. The world declaration and the plan on nutrition adopted by 159 countries during the international conference on nutrition in 1992 states that strategies to combat malnutrition should "ensure sustainable food base strategies are given the first priority, particularly for populations deficient in vitamin A and iron favouring locally available foods and taking into account local food habits"(Fuglie, 2000).

The problem with the vitamin and iron supplementation is that most of these vitamin supplements are not truly absorbed due to poor formulations. These vitamins are not truly bioavailable (absorbable and available for maximum effectiveness to the body). Alternatively, all humans need complex, natural vitamins provided by a nutritious diet consisting mostly of plants (leaves, fruits, roots, sprouts, mushrooms, etc.) (Marcu, 2005). Also while vitamin A is very important for health, an excess of it can lead to serious medical problems, but an excess of vitamin A can only be achieved by abusing vitamin A supplements. Since it is a liposoluble vitamin, it can accumulate in the body (liver) and lead to toxicity. It is therefore important to find other sources of vitamin A that will not lead to toxicity and this can be found in plants and a good example is Moringa. Moringa contains extremely rich amounts of vitamin A in the form of provitamin A or beta-carotene. The body produces vitamin A from beta-carotene, and if the beta-carotene is in excessive quantities, it can be eliminated or deposited in the fat

integrated with a primary health care activity, linked to nutrition education, and focused on the production of yellow and dark-green leafy vegetables such as Moringa significantly improved the vitamin A status of 2-5 year-old children in rural village in South Africa (Faber et al., 2003). Lockett *et al.* (2000) reported that commonly consumed species of edible wild barks, fruits, leaves, nuts, seeds, and tubers from Kuka bark (*Adansonia digitata*), Cediya (*Ficus thonningii*), dorowa (*Parkia biglobosa*) and zogale (*Moringa oleifera*) by Nigerian Fulani were rich sources of protein, fat, and carbohydrate and for minerals and can thus be used to combat malnutrition when added to everyday diet (Lockett et al. 2000).

2.10 The Moringa Tree

There are fourteen known species of trees belonging to the genus *Moringaceae.* However, the best known member of the genus is *Moringa oleifera*, a fast-growing, drought-resistant tree native to sub-Himalayan tracts of northern India but now distributed world-wide in the tropics and sub-tropics. *M. oleifera* is packed with so many vitamins and nutrients and has such a high nutritional value that it has been rightly dubbed by some as the miracle tree (Folkard and Sutherland, 1996).

All parts of *M. oleifera* are edible; the leaves can be eaten raw, cooked like spinach or made into powder that can be added to sauces, soups or chowders. The young, green pods can be eaten whole and are comparable in taste to asparagus. The older pods can be used for their seeds, which can be prepared as peas or roasted and eaten like peanuts. The flowers can be eaten fried and have the taste and texture of mushrooms (Fuglie, 2000).

2.10.1 Nutritional composition of *Moringa oleifera*

The leaves, flowers, roots and immature pods of the Moringa tree are edible and they form a part of the traditional diets in many countries of the tropics and sub-tropics. As a source of nutrition, Moringa leaves probably rank as the best of all tropical vegetables. They contain very strong concentrations of vitamins A & C, B-complex vitamins, iron, calcium, protein, zinc, selenium and unusual for a plant source, all of the essential amino acids (Booth and Wickens, 1988).

The leaves of the Moringa tree are an excellent source of vitamin A (four times the amount in carrots), the raw leaves are rich in vitamin C (seven times the amount in oranges), and they are also a good source of vitamin B and minerals. The leaves are also an outstanding source of calcium (four times the amount in milk), protein (twice the amount in milk), and potassium (three times the amount in bananas). The iron content of the leaves is very good and has been used for treating anaemia in the Philippines. The content of amino acids such as methionine and cystine is also high. Carbohydrates, fats and phosphorus content are low making this one of the finest plant foods to be found (Booth and Wickens, 1988).

Laboratory analysis of the vitamin and mineral content of fresh (raw) leaves and dried leaf powder done by Booth and Wickens in 1988 are provided in Table 3 below.

Table 2.3. Analysis of vitamin and mineral content of Moringa fresh (raw) and dried leaf powder per 100 grams of edible portion.

Component	Leaves	Leaf powder
Moisture (%)	75	7.5
Calories	92	205
Protein (g)	6.7	27.1
Fat (g)	1.7	2.3
Carbohydrate (g)	13.4	38.2
Fiber (g)	0.9	19.2
Minerals (g)	2.3	-
Calcium (mg)	440	2,003
Copper(mg)	1.1	0.57
Iron (mg)	7	28.2
Potassium(mg)	259	1,324
Magnesium (mg)	24	368
Phosphorus (mg)	70	204
Sulfer (mg)	137	870
Selenium (mg)	-	0.09
Zinc (mg)	-	3.27
Oxalic acid (mg)	101	1,600
Vitamin A (mg)	6.8	18.9

Component	Leaves	Leaf powder
Vitamin B (mg)	423	-
Vitamin B_1 (mg)	0.21	2.64
Vitamin B_2 (mg)	0.05	20.5
Vitamin B_3 (mg)	0.8	8.2
Vitamin C (mg)	220	17.3
Vitamin E (mg)	-	113
AMINO ACIDS		
Arginine (mg)	402	1,325
Histidine (mg)	141	613
Isoleucine (mg)	422	825
Leucine (mg)	623	1,950
Lysine (mg)	288	1,325
Methionine (mg)	134	350
Phenylanaline (mg)	429	1,388
Threonine (mg)	328	1,188
Tryptophan (mg)	127	425
Valine (mg)	476	1,063

Source: Booth and Wickens, (1988).
Campden and Chorleywood Food Research Association (1998)
Bactohem Laboratories (2000)

Table 2.4. gives an indication of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) 1996 Recommended Daily Allowance (RDA) of amino acids needed by a two (2) year old and the percentages of these amino acids that can be found in both the fresh leaves and leaf powder of *Moringa oleifera.*

Table 2.4. FAO/ WHO recommended daily allowance for a child aged 2 years

AMINO ACIDS (All values in mg)	RDA FOR 2 YEAR-OLD	LEAVES (100g)	LEAF POWDER (8g)
Arginine (mg)	159	402 (252%)	106 (66%)
Histidine (mg)	274	141 (51%)	49 (17%)
Isoleucine (mg)	403	422 (104%)	66 (16%)
Leucine (mg)	949	623 (65%)	156 (16%)
Lysine (mg)	832	288 (34%)	106 (12%)
Methionine (mg)	351	134 (38%)	28 (7%)
Phenylanaline (mg)	897	429 (47%)	111 (12%)
Threonine (mg)	481	328 (68%)	95 (19%)
Tryptophan (mg)	169	127 (75%)	34 (20%)
Valine (mg)	494	476 (96%)	85 (17%)

(Moringa leaves and leaf powder RDA equivalent expressed in parentheses).
Baker, 1996.

Table 2.5. gives an indication of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) 1996 Recommended Daily Allowance (RDA) of minerals and vitamins needed by a one –three (1-3) year old and the percentages of these

minerals and vitamins that can be found in both the fresh leaves and leaf powder of *Moringa oleifera.*

Table 2. 5. FAO/WHO recommended daily allowance for a child added 1-3 years old.

COMPONENT	RDA (mg)	LEAVES (100g)	LEAF POWDER (8g)
Calcium	400	440 (110%)	160 (40%)
Copper	0.8	1.1 (138%)	0.04 (5.7%)
Iron	10	7.0 (70%)	2.3 (22.6%)
Potassium	800	259 (32.4%)	106 (13.2%)
Magnesium	150	24 (16%)	29.4 (20.6%)
Phosphorus	800	70 (8.7%)	16.3 (2%)
Selenium	0.01	-	0.0072 (72%)
Zinc	3.9	-	0.26 (6%)
Vitamin A	1.5	6.8 (453%)	1.5 (100%)
Vitamin C	20	220 (1,100%)	1.4 (6.9%)

(Moringa leaves and leaf powder RDA equivalent expressed in parentheses).

2.10.2 Laboratory assessment of dried Moringa leaf powder

The laboratory reports done on Moringa's toxicity suggest nutritional composition are adequate for growth of children: they do not find evidence of any inherent toxins. In addition to several studies done in India, a study entitled "Toxicological and Some Pharmacological Assessment of Moringa Dried Leaf Powder," sponsored by Church World Service (CWS), was conducted by the Noguchi Memorial Institute for Medical

Research, and located in Ghana, in mid-2002. This study established that Moringa powder is practically non-toxic according to the classification of relative toxicity of chemical substances (Nyarko *et al.*, 2002).

2.10.3 Moringa in relation to nutrition

Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. Three non-governmental organizations in particular—Trees for Life, Church World Service and Educational Concerns for Hunger Organization—have advocated Moringa as "natural nutrition for the tropics." In their work which was carried out in Senegal using infants and nursing mothers, they showed that Moringa leaf powder can be used to combat malnutrition (Fuglie, 1999). Even though this work was done on pilot basis there were several limitations to the design of the work thus affecting the findings/observations of this research. Some of these limitations include

- the lack of empirical data to support the chronological measurement of weight for age.
- the lack of laboratory data (hematological and biochemical investigations) to support the impact of Moringa on the malnutrition management. It is therefore important to have controlled and well documented clinical studies to support the actual impart of Moringa in the management of malnutrition.

Moringa leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. Moringa is especially promising as a food source in the tropics because the tree is in full leaf at the

end of the dry season when other foods are typically scarce. Fuglie (1999), reports that "ounce-for-ounce, Moringa leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas," and that the protein quality of Moringa leaves rivals that of milk and eggs and that there are countless instances of lifesaving nutritional rescue that are attributed to. Moringa In fact, the nutritional properties of Moringa are now so well known that there seems to be little doubt of the substantial health benefit to be realized by consumption of Moringa leaf powder in situations where starvation is imminent. In many cultures throughout the tropics, differentiation between food and medicinal uses of plants (e.g. bark, fruit, leaves, nuts, seeds, tubers, roots, flowers), is very difficult since plant uses span both categories and this is deeply ingrained in the traditions and the fabric of the community (Lockett *el al*, 2000).

2.10.4 Moringa in relation to disease treatment and prevention

The benefits for the treatment or prevention of disease or infection that may accrue from either dietary or topical administration of Moringa preparations (e.g. extracts, decoctions, poultices, creams, oils, emollients, salves, powders, porridges) are not quite so well known (Palada, 1996). Although the oral history is voluminous, it has been subject to much less intense scientific scrutiny. A plethora of traditional medicine references attest to its curative power, and scientific validation of these popular uses is developing to support at least some of the claims. Moringa preparations have been cited in the scientific literature as having antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypo-cholesterolemic, and hypoglycemic activities, as well as having

considerable efficacy in water purification by flocculation, sedimentation, antibiosis and even reduction of Schistosome cercariae titer (Tsaknis *et al*, 1999). Unfortunately, many of these reports of efficacy in human beings are not supported by placebo controlled, randomized clinical trials. In many cases, published *in-vitro* (cultured cells) and *in-vivo* (animal) trials do provide a degree of mechanistic support for some of the claims that have sprung from the traditional medicine lore. For example, numerous studies now point to the elevation of a variety of detoxication and antioxidant enzymes and biomarkers as a result of treatment with Moringa or with phytochemicals isolated from Moringa (Fahey *et al,* 2004; Kumar and Pari, 2003; Rao *et al*, 1999). Antibiosis and cancer prevention are two examples of areas of Moringa research for which the existing scientific evidence appears to be particularly strong (Sampson, 2005).

Antibiotic Activity is clearly the area in which the preponderance of evidence—both classical scientific and extensive anecdotal evidence—is overwhelming. In the late 1940's and early 1950's a team from the University of Bombay (BR Das), Travancore University (PA Kurup), and the Department of Biochemistry at the Indian Institute of Science in Bangalore (PLN Rao), identified a compound they called pterygospermin (Anderson, Bell *et al*, 1986) a compound which readily dissociate into two molecules of benzyl isothiocyanate (Anwar and Bhanger, 2003). Benzyl isothiocyanate was already understood at that time to have antimicrobial properties. (They identified the tree from which they isolated this substance as "*Moringa pterygosperma,*" now regarded as an archaic designation for *Moringa oleifera.* Subsequent work published in 1964 by Bennie Badgett, identified a number of glyosylated derivatives of benzyl isothiocyanate (Anwar

and Bhanger, 2003) (e.g. compounds containing the 6-carbon simple sugar, rhamnose (Badgett, 1964). Seminal reports on the antibiotic activity of the primary rhamnosylated compound then followed from U Eilert and colleagues in Braunschweig, Germany (Eilert, 1978; Eilert, Wolters and Nahrstedt, 1981). They verified the activity of the latter compound against a wide range of bacteria and fungi. Extensive field reports and ecological studies claim efficacy of leaf, seed, root, bark, and flowers against a variety of dermal and internal infections. In this case, however, the *in-vitro* (bacterial cultures) and observational studies provide a very plausible mechanistic underpinning for the plethora of efficacy claims that have accumulated over the years. Moringa has also been found to have antibiotic effect against *Helicobacter pylori* (Abuye *et al*, 1999). *H. pylorus is* an omnipresent pathogen of human beings in medically underserved areas of the world, and amongst the poorest of poor populations worldwide. It is a major cause of gastritis, and of gastric and duodenal ulcers, and it is a major risk factor for gastric cancer (having been classified as a carcinogen by the W.H.O. in 1993).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 *Study Design*

To effectively study the effect of *Moringa oleifera* in the management of sub-clinical protein energy malnutrition in children under five (5) years, a protocol was designed. The study protocol was approved by the Committee on Human Research and Ethics of the School of Medical Sciences, KNUST. The inclusive criteria for the study in the protocol were as follows; a signed consent form and the establishment of the state of the malnourished child through physical medical examination, normal haematological and biochemical tests and medical history of the participants. A randomized complete block design was used in the study and the results obtained were subjected to statistical analysis using Statistical Analytical System (SAS).

3.2 *Selection of study site*

The Maternal and Child Health Hospital (MCHH) located at Kejetia-Pampaso Kumasi, in the Ashanti region of Ghana was used for this study. A letter was sent to Maternal and Child Health Hospital (MCHH) seeking permission to use their facility for the project. Permission was granted and the project commenced. The duration of the project was 8 weeks.

The Maternal Child Health hospital is a specialized children hospital and thus manages childhood diseases of various forms including sub-clinical protein–energy malnutrition. The hospital is demarcated for various activities which includes; The child welfare centre

where growth monitoring and immunization of children are done, the children's ward and the rehabilitation centre.

The inception of the rehabilitation centre was fulfilled as a result of the various relapse that occur when the children are discharged from the hospital after treatment. This problem caused the management of the hospital to set up the centre for the continuation phase of the treatment, after the children have been stabilized on the ward and they are due for discharge. Children sent to the centre are those suffering from kwashiorkor, marasmus, marasmic kwashiorkor, who have been stabilized on the ward and are due to be discharged. The main activities of the centre include; teaching mothers the importance of good nutrition, proper feed preparation, and personal hygiene.

3.3 *Selection of participants*

Mothers who had their children at the MCHH were first given a detailed briefing on the nature and benefits of the study and safety associated with the use of the test sample. Based on these, requests were made from mothers for volunteers. About 40 mothers then voluntarily applied to participate in the project. Using clinical observations and the WHO standard chart as guide, subjects selected were clerked into coded folders by the two (2) physicians and the nutritionist on the team (WHO, 1999). In all a total of 35 children between the ages of 6 months to 3 years were used as participants for the study. These were given special codes which were written on their folders and throughout the exercise they were addressed by their codes. Out of the 35 subjects 25 had *Moringa oleifera* leaf powder incorporated into their feeds while the remaining 10 were used as control and were fed with the WHO recommended food.

Pic. 1 Clinician taking patient's history

3.4 *Sample source*

The *Moringa oleifera* leaves were obtained from the Horticultural Department of the Faculty of Agriculture, KNUST. The WHO Standard feed, F-100 used was obtained from the Maternal and Child Health Department, MCHH.

3.4.1 *Sample preparation*

The Moringa leaves collected were cleaned, sorted out, thoroughly washed, and solar dried at a temperature of 40-45°C for two days. The dried leaves were then milled into powder using the plate mill (Fuglie, 1999). The leaf powder was packaged into polyethylene bags and then stored by refrigeration through out the study period.

The WHO Standard feed, F-100 is made up of full cream milk powder, sugar, oil, combined mineral and vitamin solution and water. The water was boiled for five (5) minutes and allowed to cool. 50 grams of sugar and 30 grams of oil were weighed and

mixed together. This was done to prevent the oil from separating out. 110 grams of full cream milk powder was weighed and then added to the sugar-oil mixture and this was well stirred to obtain a good mixture. 20 millilitres of combined mineral and vitamin solution was then added after which water (830 ml) was added up to the one (1) litre mark, and vigorously whisked. The prepared feed was covered and refrigerated. Unused feeds were discarded after twelve (12) hours.

3.5 *Feeding regime*

All children used for the research were given equal quantity of baseline feeds. A total of 35 subject were used. Subjects were put into two groups: the first group consisting of 25 subjects had Moringa leaf powder incorporated into their feeds after the first four weeks while the second group consisting of 10 subjects was given the F-100 in addition to the baseline feeds throughout the eight week period. The volume of F-100 fed subjects was calculated based on the weight of the subject (Appendix 2A and 3B). Children in the first group (i.e. A) were fed with baseline feeds and F-100 for the first four weeks and for the next four weeks they were feed baseline feeds incorporated with Moringa oleifera leaf powder in the absence of F-100. Fifteen grams (15g) of *M. oleifera* leaf powder per day was given to each of the children in group A after the first four weeks. This 15g was divided into 3 to cover the dose for the day. The leaf powder was added to both solid feeds (aprannsa, banku and groundnut soup, rice and beans stew) and semi- solid feeds (all types of porridge mixes e.g. weanimix) (MCHH, 2006).

Mothers were advised to add the leaf powder to small portions of the feed to ensure that the child ate all the leaf powder before giving the remaining portions of the food. In some cases the powder was added to their water or their fruit juices and some were even given the raw leaf powder to lick at specified intervals as the children tended to reject their porridge due to the color change.

Pic. 2. Mothers preparing baseline feeds for subjects.

3.6 *Nutritional analysis*

Proximate analysis of the *Moringa oleifera* leaves were carried out using the official methods of analysis of the AOAC (1990). Minerals analysis was carried out using the Atomic Absorption spectrophotometer (AAS) for both *M. oleifera* and F-100, WHO standard feed. Vitamins A and C were also determined on the samples using the official methods of analysis of the AOAC by Liquid chromatography.

3.7 *Anthropometric data collection*

The weight and height of each child was taken using Salter scale and infantometer respectively. This was compared with the WHO weight to height ratio chart to help establish the child's deviation from the expected standard deviation (WHO, 1999). The mid-upper arm circumference was also measured using the measuring tape for each child before the start of the research and during the research, anthropometric data on the subjects were recorded into the coded folders every other day (Parry, 1984).

Pic. 3. Two subjects on the Salter scale

3.8 *Blood collection, preparation and storage*

Before the start of the research five milliliters (mls) of venous blood was drawn from each of the thirty-five (35) subjects using sterilized and disposable syringes and needles by the six (6) laboratory technicians on the team. 2mls of the blood sample was then put into coded special bottles containing ethylenediaminetetraacetic acid (EDTA) for haematological analysis (i.e. Haemoglobin, and Corrected white blood cell count) (Van Assendelft, 1984). The remaining 3mls was put into coded special bottles for biochemical analysis (Urea, Creatinine, Albumin and Total proteins) (Ali, 1984). The blood samples on collection were put on cold storage in an ice chest and then sent immediately to the Haematology and Biochemistry laboratories of the Komfo Anokye Teaching Hospital

(KATH), Kumasi. These procedures were repeated fortnightly during the course of the research.

Pic.4. Lab. Technician taking blood sample

Pic.5. Specialized coded bottles

3.9 Haematological analysis

3.8.2 *Haemoglobin determination in blood*

The haemiglobincyanide method formerly called the cyanmethaemoglobin method was used. 0.02 millilitres of well mixed venous blood was measured and dispensed into 4 mls of Drabkin's neutral diluting fluid in a test tube. The test tube containing the diluted blood sample is then stopped, mixed by gently inverting the bottle and left at room temperature, protected from sunlight for 4 to 5 minutes for complete conversion of haemoglobin derivatives to haemiglobincyanide (Van Assendelft, 1984).

A yellow-green filter (eg. Llford 605) was placed in the colorimeter set at a wavelength of 540nm. The colorimeter was zeroed with Drabkin's fluid and the absorbance of the subject's blood sample read. The subject's haemoglobin value was determined from an already determined calibration graph (Lewis, 1984).

3.9.1 *Corrected White Blood Cell Count Determination in blood*

0.38ml of diluting fluid was pipetted into a test tube. The 0.02 ml pipette was then filled to the mark with blood and the outside of the pipette was wiped off. Contents of the pipette was then expelled into the diluting fluid, and washed out by drawing up the fluid and expelling into the tube a few times. The content of the test tube was then mixed for 2 minutes using a vortex (Sir John, 1984).

The haemocytometer was then set up with its cover glass in position. By means of a Pasteur pipette, some of the diluted blood was taken and both sides of the haemocytometer were filled. The haemocytometer was then allowed to stand undisturbed for 2 minutes for the cells to settle. Using the 10x objective and subdued light, cells in the four large squares on both sides of the haemocytometer were counted. The corrected white blood cells were then calculated on the basis of cells counted, areas counted and the dilution factor (Sir John, 1984). The formula below was used to calculate the corrected white blood cell count.

$$\text{Corrected White Blood Cells} = \frac{\text{Uncorrected White Blood Cells} \times 100}{(\text{Nucleated Red Blood Cells}/ 100\ \text{WBC}) \text{ on the film} + 100}$$

Where WBC - White Blood Cell

3.9.2 *Sickle cell determination*

The metabisulfite reduction method was used. One drop of well mixed venous blood was delivered on a slide marked 'P'. An equal volume of fresh reducing agent was added, mixed and covered with a cover glass. Care was taken to exclude any air bubbles.

A negative control was set up by delivering one drop of blood from a person who does not have sickle cell disorder on a slide marked 'Negative control'. An equal volume of fresh reducing agent was added, mixed and covered with a cover slide taking care to exclude any air bubbles. The slides were then placed in a container with a damp piece of blotting paper or tissue in the bottom to prevent drying of the prepared samples. The container was then closed and left to stand at room temperature for duration of 10 to 20 minutes after which microscopic examination for sickle cells in patient's preparation was done using the 10x and 40x objective lens. A positive test showed densely red cells varying in shape from oval to long elliptical (sickle shape) cells, all with pointed ends (Dacie , 1984).

3.9.4 *Haemoglobin (HB) Electrophoresis*

The procedure for performing Alkaline Cellulose Acetate Haemoglobin Electrophoresis using the Mylar- Backed supported Cellulose Acetate membranes referred to by Helena Bio Sciences as Titan III-H Acetate plates (*Cat. No.3022*, United Kingdom). The cellulose acetate membrane (Titan III Cellulose Acetate Plate) was prepared exactly as described in the Helena Bio Science Procedure. 100 ml of the Tris – EDTA – borate buffer was poured into each of the outer sections of the Zip – Zone electrophoresis chamber. Two wicks were wet in the buffer and one was draped over each support bridge, ensuring each makes contact with the buffer and that there were no air bubbles under the wicks. The chamber was covered to prevent evaporation. 0.05 ml of each haemolysate sample was transferred into the zip-zone plate. A cellulose acetate

membrane (plate) was then placed in the zip-zone aligning plate and applying the samples using the 8 unit applicator. The cellulose acetate membrane (plate) was immediately place in the electrophoresis chamber with the cellulose acetate side down. The chamber was connected to the zip-zone power supply and the plate was electrophoresed for 25 minutes at 350 volts. The relative mobilities of Haemoglobins A, F, S, D, E, C, and H after alkaline electrophoresis is then observed as shown in Picture 6 below. In alkaline buffer, HbS and HbD have similar mobility and HbC, HbA2, HbE, and HbO have similar mobility (Cheesbrough, 2000).

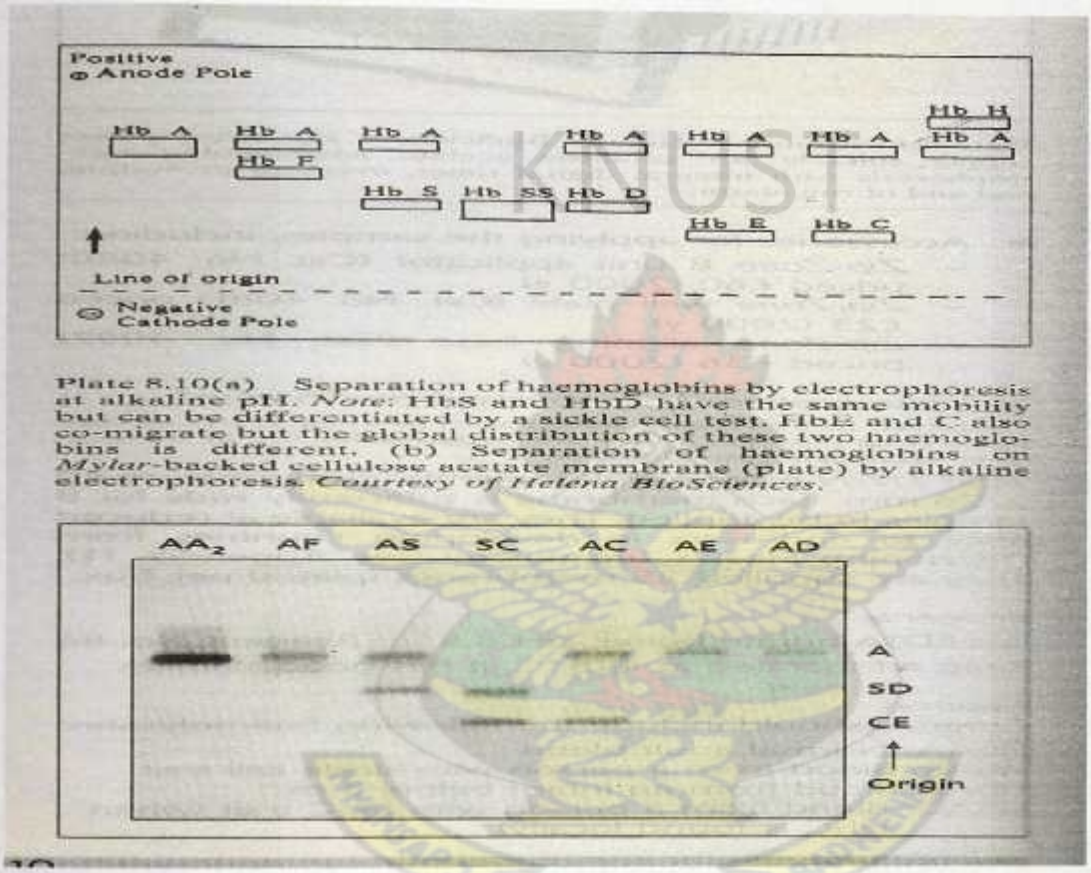


Plate 8.10(a) Separation of haemoglobins by electrophoresis at alkaline pH. *Note*: HbS and HbD have the same mobility but can be differentiated by a sickle cell test. HbE and C also co-migrate but the global distribution of these two haemoglobins is different. (b) Separation of haemoglobins on *Mylar*-backed cellulose acetate membrane (plate) by alkaline electrophoresis. *Courtesy of Helena BioSciences.*

Pic. 6. Electrophoresis in alkaline plate showing the relative mobilities of Haemoglobins A, F, S, D, E, C, and H.

3.9.5 *Glucose-6-Phosphate Dehydrogenase deficiency determination*

Three small glass tubes (5 ml capacity) were taken and labeled Test, Control and Deficient and the following reagents were pipetted into each tube:

Table 3.1. Glucose-6-Phosphate Dehydrogenase deficiency determination

Tube	Test	Control	Deficient
Fresh sodium nitrite-glucose reagent	0.1 ml	-	0.1 ml
Methylene blue reagent	0.1 ml	-	-
Subject's blood	2 ml	2 ml	2 ml

The tubes were then stopped and their contents mixed well by inverting the tube several times (gentle mixing). All three samples were incubated at a temperature of 35 °C– 37 °C for three hours.

Three large tubes (15 ml capacity) were taken and labeled as the previous ones i.e.; Test, Control and Deficient. 10 mls of distilled (deionized) water was pipette into each tube. 0.1 ml of well mixed sample was transferred from the Test, Control and Deficient tubes to the large tubes. The contents of each tube were then mixed and the colour of solution in each tube examined. The results was interpreted as follows : colour of test solution is similar to the red colour of the Control tube implied Normal G6PD activity while in Reduced G6PD activity (G6PD Deficiency in homozygote) the colour of the test solution is similar to the brown colour of the deficient tube (Evatt , 1983).

3.10 Biochemical analysis

3.10.1 *Measurement of serum urea*

The Diacetyl Monoxime method was used and the reagents used were Diacetyl monoxime, urea acid reagent, colour reagent, benzoic acid solution (1 g/l), trichloroacetic acid solution (50 g/l), stock urea solution (125 mmol/l) and working urea standard. The Diacetyl monoxime reagent was prepared by dissolving 2.0 grams of Diacetyl monoxime in distilled water and diluting it up to the 500 ml mark with distilled water in a volumetric flask. The colour reagent was prepared by mixing 50 ml of acid reagent with 50 ml of Diacetyl monoxime reagent in a small bottle. The urea stock solution was prepared by transferring 750 mg of urea into a 100 ml volumetric flask. 50 ml of benzoic acid solution was added and the resulting mixture was dissolved and diluted to 100 ml with benzoic acid solution. The working urea standard was prepared by diluting 4 ml of stock urea standard up to the 50 ml in a volumetric flask with benzoic acid (Wybenga, Di Gorgio and Pileggi, 1971).

0.5 ml of trichloroacetic acid solution was pipetted into labeled centrifuge tubes representing the Standard, Control serum and subject's blood. 0.05 ml of standard, control serum and subject's blood was added to the appropriate tube. The contents were mixed, and left at room temperature for 5 minutes after which centrifugation was repeated to obtain a clear supernatant.

Four (4) tubes were labeled; B (Reagent blank), S (Standard, 10 mmol/ l) C (Control serum) and sample tube was labeled (Sb). Four millilitres (4 ml) of freshly made urea colour reagent was pipetted into each tube and the following were added; B (20 µl (0.02

ml) distilled water), S (0.02 ml Standard), C (0.02 ml) Control serum), Sb (0.02 ml patient's serum). The contents of each test tube were well mixed and incubated at a temperature of 100 °C for 15 minutes, in a container of boiling water. The tubes were then removed and their contents cooled by placing them in a container of cold water for 5 minutes, making sure no water entered the tubes. The absorbencies of the solutions were then read in a spectrophotometer set at a wavelength of 530 nm. The concentration of urea in the control and patient's samples were calculated by using the formula below (Seaton & Ali, 1984).

$$\text{Urea mmol/ l} = \frac{AT}{AS} \times 10$$

Where; AT = Absorbance of test (s) or control

AS = Absorbance of 10 mmol/l standard

3.10.2 *Measurement Serum Albumin*

The Bromocresol green method was used and the following reagents were used; succinic acid solution (50 g/l), sodium hydroxide solution (10 g/l), "Bri j – 35' solution (250 g /l), working dye solution, succinate buffer solution, Albumin standard (30 g/ l) and sodium hydroxide solution. The Bri j – 35 solutions was prepared by warming 25 g solid Bri j – 35 in a small volume of distilled water to dissolve it and the solution was made up to 100 ml with distilled water. The working dye solution was prepared by dissolving 5.60 g of succinic acid, 58 mg of Bromocresol green and 100 mg of sodium azide in about 900

ml of distilled water. 1.0 g of sodium hydroxide was added and dissolved followed by the addition of 2.5 ml Bri j- 35 solution. The solution was then adjusted to a pH of 4.20 by using small volumes of sodium hydroxide solution. The resulting solution was transferred slowly into a 1 litre volumetric flask and made up to volume with distilled water. The solution was stored at 2 °C- 8 °C. The albumin standard was prepared by diluting 3.0 ml of the bovine albumin standard (100 g /l) with 3. 0 ml of sodium chloride solution. The standard is kept at a temperature of 2 °C- 8 °C (Webster, 1977).

Four (4) tubes were taken and these were labeled; B (Reagent Blank), S (Standard, 30 g/l) C (Control Serum) and the Subject's tube was also labeled St. Four millilitres (4 ml) of Bromocresol green reagent was pipette into each tube and to each tube, the following was added; B (20 µl (0.02 ml) distilled water), S (20 µl (0.02 ml)Standard 30 g/l), C (20 µl (0.02 ml) Control serum), and St (20 µl (0.02 ml) Subject's serum). The contents of the each test tube was well mixed care was taken to avoid frothing of the solutions as presence of air bubbles would result in incorrect absorbance reading. The absorbencies of the solutions were immediately read in a spectrometer, set at 623 nm. Serum albumin concentrations of control and subject's sample were then calculated by using the formula below (Spencer & Price, 1977).

$$\text{Serum Albumin} = \frac{\text{Absorbance of Test}}{\text{Absorbance of 30 g/l Standard}} \times 30$$

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Summary of the screening Results

Below is the summary of the screening results of the health status of the participants conducted by the medical officer involved with the research prior to the start of work.

Subject Code	Age (months)	Weight (Kg)	Height (Cm)	Clinical history	Clinical examination
0 01	15	6.8	69	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or Thalassaemia. •Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	• Subject was wasted with silky, thin Brownish and easily plaquable hair. • Had Ptyriasis Versicolor on face and trunk of the body
0 02	11	5.5	64	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Was delivered prematurely by caesarian section at 7 months • Exclusive breastfeeding for 6 months	• Subject was wasted with silky, thin brownish and easily plaquable hair. • Subject was not pale
0 03	18	8.1	77.5	• No history of any underlying infections, haemoglobinopathies	• Subject was wasted with silky, thin

				(G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	Brownish hair. • Subject was severely pale
0 04	10	5.8	64	• History of underlying infection of sepsis (cough, fever, poor feeding and vomiting) • No history of any haemoglobinopathies (G6PD deficiency), sickle cell disease or Thalassaemia • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 1 week.	Subject had oral thrush and was febrile •Had silky, thin and brownish hair.
0 05	16	8	77.5	• History of underlying infection and anaemia • No history of any haemoglobinopathies (G6PD deficiency), sickle cell disease or Thalassaemia • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	• Subject was slightly wasted with silky thin brownish hair. • Subject was not pale.
0 06	10	7.1	70	• History of underlying infection and anaemia • No history of any haemoglobinopathies (G6PD deficiency), sickle cell disease or Thalassaemia	• Subject was slightly pale and wasted • Skin and hair appeared normal and healthy.

				• Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 3 months	
0 07	30	7.8	74.9	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or Thalassaemia. • Had possible protein losing enteropathy • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	• Subject was pale and mildly wasted • Skin and hair appeared normal and he
0 08	36	9.6	81.5	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	• Subject was not pale but was slightly • Had silky, thin and brownish hair.
0 09	18	8.5	73.5	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • No exclusive breastfeeding	• Subject was pale, and wasted with sil thin brownish hair.
0 10	36	9.5	88.9	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium	• Subject was pale, and wasted with silky, thin grayish hair.

				• Exclusive breastfeeding for 3 months	
0 11	14	7.0	67.5	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 2 months	• Subject was not pale, • Subject wasted with silky, thin grayis hair.
0 12	12	6.0	68.5	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 4 months	• Subject was mildly pale and wasted • Had silky, thin and brownish hair.
0 13	16	6.6	72.4	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	• Subject was not pale but slightly wasted • Had silky, thin and brownish hair.
0 14	24	9.2	81.0	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	• Subject was pale and slightly waste • Had silky, thin grayish hair
0 15	15	6.6	70.0	• No history of any underlying infections, haemoglobinopathies	• Subject was pale and slightly wastec

				(G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	• Had silky, thin grayish hair
0 16	15	5.1	63.5	There was history of HIV • No history of any haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	• Subject was pale and slightly wastec • Had silky, thin grayish hair
0 17	14	8.0	72.5	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	• Subject was pale and slightly wastec • Had silky, thin brownish hair
0 18	16	7.2	71.0	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Subject was physiologically jaundiced at birth • Exclusive breastfeeding for 6 months	• Subject was pale and slightly wastec • Had silky, thin brownish hair
0 19	21	10.2	79	• No history of any haemoglobinopathies (G6PD deficiency), sickle	• Subject was febrile, pale and slightly

				cell disease or thalassaemia. • History of sepsis and urinary tract infection • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	wasted • Had silky, thin brownish hair
0 20	18	8.2	79.5	• No history of any haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • History of respiratory and urinary tract infection • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	• Subject was febrile, pale and severel wasted • Had silky, thin grayish hair
0 21	6	4.5	58.5	• No history of any haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • History of severe sepsis • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 1 month	• Subject was febrile, pale and wasted • Had silky, thin grayish hair • Had Ptyriasis Versicolor all over the body
0 22	15	7.1	78.0	• No history of any haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • History of respiratory tract infection • Uneventful pregnancy, delivery and pueparium	• Subject was febrile, moderately pale and slightly wasted • Had silky, thin brownish hair

				• Exclusive breastfeeding for 6 month	
0 23	24	7.9	105	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Subject was physiologically jaundiced 3 days after delivery • Exclusive breastfeeding for 6 months	• Subject was moderately pale and slightly wasted • Had silky, thin brownish hair
0 24	24	10.4	80.0	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 4 months	• Subject was not pale but was slightly wasted • Had silky, thin grayish hair
0 26	17	7.9	81.0	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 4 months	• Subject was not pale but was slightly wasted and febrile • Had silky, thin grayish hair
0 27	8	6.3	71.0	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 2 weeks	• Subject was not pale but was slightly wasted. • Had silky, thin brownish hair

0 28	18	7.1	75.5	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 4 months	• Subject was not pale but was slightly wasted. • Had silky, thin brownish hair.
0 29	12	6.1	70.0	• No history of any haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • History of sepsis and urinary tract infection • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 month	• Subject was febrile, pale and slightly wasted • Had silky, thin brownish hair
0 30	30	11.7	82.5	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Subject had physiological jaundice 3 days after birth. • Exclusive breastfeeding for 3 weeks	• Subject was not pale but was slightly wasted • Had silky, thin brownish hair • Had umbilical hernia
0 31	12	7.2	70.0	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	• Subject was pale and slightly waste • Had silky, thin brownish hair

0 33	12	5.9	68.5	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 6 months	• Subject was pale and slightly waste • Had silky, thin brownish hair
0 34	17	6.0	71.2	• No history of any haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • History of urinary tract infection • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 4 months	• Subject was not pale but slightly wasted • Had silky, thin brownish hair
0 37	18	7.9	74.0	• No history of any haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • History of respiratory tract infection • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 2 months	• Subject was pale and slightly waste • Had silky, thin brownish hair
0 38	18	8.3	77.2	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 4 months	• Subject was not pale but was slightly wasted • Had silky, thin grayish hair.

0 39	16	7.0	67.3	• No history of any haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • History of respiratory tract infection • Uneventful pregnancy, delivery and pueparium • No breastfeeding as mother died after birth	• Subject was not pale but was slightly wasted • Had silky, thin brownish hair
0 40	16	9.3	77.5	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 7 months	• Subject was pale and wasted • Had silky, thin brownish hair.
0 41	15	6.4	70.4	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 2 weeks.	• Subject was slightly pale and wastec • Had silky, thin brownish hair.
0 42	24	9.1	82.0	• No history of any underlying infections, haemoglobinopathies (G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 7 months	• Subject was not pale but was wastec • Had silky, thin brownish hair.
0 43	38	13.8	96.3	• No history of any underlying infections, haemoglobinopathies	• Subject was not pale but was wastec

				(G6PD deficiency), sickle cell disease or thalassaemia. • Uneventful pregnancy, delivery and pueparium • Exclusive breastfeeding for 7 months	• Had silky, thin brownish hair.

Prior to the start of the project screening was done by the medical officers and the nutritionist on the team on the participants who had volunteered to participate. The ages of day one and five years are as follows; A total of forty (40) subjects were screened for the exercise / project. Out of this number, five (5) subjects were suspended and their results were not used for the project because it was discovered during the screening process that one (1) subject with code 0 25 had nephroitic syndrome with symptoms and signs overlapping with that of malnutrition. Four of the subjects with codes 0 03, 0 35, 0 36 and 0 40 did not report after the initial screening process hence they did not participate in the feeding regime, and no haematological and biochemical analysis were done on them.

Thus, a total of thirty-five (35) subjects were used for the project. Out of this number, screening done by the medical officers revealed or showed that ten (10) subjects had history suggesting underlying infections ranging from Human Immuno deficiency Virus (HIV), Respiratory Tract Infection (RTI), Urinary Tract Infection (UTI) to Malaria. Of the 25 subjects who showed no history of underlying infection and haemoglobinopathies, it was observed that two of them with codes 0 18 and 0 30 between the ages of 12-18 months and 24-30 months respectively had physiological jaundice at birth. As a result further analysis was done on them after the

initial screening to find out if they had any haematological condition such as G6PD deficiency. These were found to be negative. A subject with code 0 07 had protein loosing enteropathy which is a chronic condition caused by an abnormality or pathology of the gut or intestines. The remaining 25 subjects had no history of underlying infection. The tables below show a summary of the number of subjects screened and the presence or absence of history indicating underlying infection with respect to their age groups, types of underlying infection and the numbers of subjects with particular infections, state of texture and colour of hair, haemoglobin status (Anaemic or non anaemic) of subjects, and number of subjects and the type of feeds given them respectively.

Table 4.1.0 Number of subjects screened and their state of infection or absence of it with respect to their age groups

Age group	Absence of infection	Presence of infection
6 – 12 months	6	3
12 – 18 months	12	6
18 – 24 months	4	1
24 – 30 months	1	-
30 – 36 months	2	-
Total	**25**	**10**

Table 4.1.1 Types of underlying infection and the numbers of subjects with particular infections

Type of Infection	Respiratory Tract Infection	Urinary Tract Infection	Sepsis	Malaria	HIV
Number of subjects	4	4	3	5	1

Table 4.1.2 State of hair texture and colour of subjects

Hair texture and colour	Normal black hair	Silky thin and grayish	Silky thin and brownish
Number of subjects	5	9	21

Table 4.1.3 Haemoglobin status (Anaemic or non anaemic) of subjects

Haemoglobin status	Anaemic	Non-anaemic
Number	22	13

Table 4.1.4 Number of subjects and the feeds given them

Type of feed	Baseline feeds+ F-100 only	Baseline feeds + F-100 only and then Baseline feeds +Moringa only
Number	10	25

The F-100 and baseline only subjects were later not used because their parents upon seeing the rapid changes in the Moringa fed subjects insisted that their children also be fed with Moringa and when they were refused they stopped coming to the rehabilitation centre because they felt they had been discriminated against. Thus there were no F- 100 fed subjects.

Pic.4.1.A. A subject at week zero

Pic. 4.1.B. At the week six of project

Pic. 4.2.A. A subject at week zero

Pic. 4.2.B. At the week six of project

4.2 RESULTS AND DISCUSSIONS

4.2.1 ANTHROPOMETRIC DATA ANALYSIS

Using the WHO Standard classification of daily weight gain which states ; Good daily weight gain of a child is when the child gains more than 10g /Kg/day, moderate daily weight gain is when a child gains 5g-10g /Kg/day and poor daily weight gain is when a child gains less than 5g /Kg/day (WHO, 1999). Anthropometric data analysis showed that all subjects had increase in weight gain by the end of the study. It was however observed that there were variations in the weight gained by subjects.

Anthropometric data analysis of subjects fed with baseline line feeds and F-100 for the first four weeks after which their feeds were incorporated with the *Moringa oleifera* leaf powder after which F- 100 was discontinued.

Table 4.2.1 Weight gain by subjects given baseline feeds and F- 100 for the first four weeks after which their baseline feeds were incorporated with Moringa leaf powder after which F- 100 was discontinued.

	Baseline feeds + F- 100		Baseline Feeds + Moringa powder	
Age in Months	Mean weight (kg)	Average Daily weight gain in g/kg/day	Mean weight (kg)	Average Daily weight gain in g/kg/day
6 – 12	5.8	2.1	6.6	4.7
12 – 18	7.1	1.5	7.7	4.0
18 – 24	7.5	2.8	8.3	6.8
24 – 30	7.7	1.8	8.4	3.2
30 – 36	8.0	2.3	9.1	4.3

It was observed that when subjects were fed with baseline feeds and F-100, they all exhibited poor daily weight gain (less than 5g/kg/day) with the lowest daily weight gain being 0.4g/kg/day

and the highest daily weight gain being 3.8g/kg/day recorded by subjects between the ages of 12-18 months and 30-36 months respectively. However, when the same subjects were fed baseline feeds incorporated with Moringa leaf powder, it was observed that 50% of them showed moderate daily weight gain (5-10g/kg/day) and the remaining 50% showed poor daily weight gain (less than 5g/kg/day)

With regards to average daily weight gain, it was observed that all subjects fed with baseline and F-100 showed poor average daily weight gain with the lowest value of 1.5g/kg/day recorded among subjects who were 12-18 months old (table 4.2.1). It was however observed that after subjects had been fed with baseline feeds and Moringa leaf powder their average daily weight gain was at least twice their average daily weight gain when they were fed baseline feeds and F-100 with 18-24 months old subjects exhibiting moderate average daily weight gain of 6.8g/kg/day. Data obtained also showed that although Moringa fed subjects had poor average daily weight gain values these values are higher and even closer to moderate average daily weight gain values then values obtained when subjects were fed baseline feeds and F-100 (Table 4.2.1).

It was also observed that, mean weight of subjects were higher when they were given baseline feeds and Moringa than when they were fed baseline feeds and F-100. In subjects who were 12-18 months old for instance, subjects mean weight was 5.8 kg when they were fed baseline feeds and F- 100 compared to their mean weight of 6.6 kg when they were fed baseline feeds and Moringa. Subjects thus gained a weight difference of 0.8 kg or 800g more when they were fed Moringa and baseline feeds.

These better increases in weight gained by subjects when they were fed with baseline feeds and Moringa leaf powder compared to when they were fed baseline feeds and F-100 although the energy content of F-100 for a child of 5.0 kg is 125Kcal is greater than the energy content of the 15 grams of Moringa leaf powder which is 76Kcal, may be attributed to the fact that subjects when fed baseline feeds and F-100 were always having diarrhoea and vomiting at regular intervals which may be attributed to the fact that mothers did not observe the necessary personal hygiene such as regular cleaning of milk containers, covering of milk, and serving of milk at wrong temperature that they ought to have observed. Diarrhoea and vomiting may have resulted in loss and malabsorption of important nutrients such as zinc and copper which the body needs for proper physical growth and development (Castillo-Duran *et al.* 1988; Butler *et al.* 1993). F-100, which is principally made up of milk impedes iron absorption and may cause lactose intolerance. All these factors may have contributed to the poor mean weight gained exhibited by subjects.

Moringa leaf powder contains all the nutrients in quantities that constitute a balanced diet (carbohydrates, proteins, vitamins, minerals and fats and oils) that the body needs for proper physical growth and development (Fuglie, 2002). The 15g of Moringa leaf powder provided subjects with 75.8Kilocalories and also provided subjects with other nutrients in appreciable quantities required by a child aged 1 to 3 years according to the recommended daily allowance of nutrients proposed by the WHO AND FAO (table 4.2.2), (WHO/FAO, 2004).

Table 4.2.2 FAO/WHO recommended daily allowance for a child aged 1-3 years old.

COMPONENT	RDA (mg)	% of daily requirement present in 15g of leaf powder
Proteins	400	25%
Carbohydrates	0.8	5%
Fibre	10	2.6%
Calcium	400	75%
Copper	0.8	10.7%
Potassium	800	13.2%
Iron	10	42.4%
Magnesium	150	36.6%
Phosphorus	800	3.8%
Selenium	0.01	(135%)
Zinc	3.9	(11.3%)
Vitamin A	1.5	187.5%

After feeding on the Moringa leaf powder, children started eating very well to the extent that mothers started complaining. This may mean that Moringa oleifera leaf powder had some attributes or constituents that increased the appetite of the children. Some of the appetite stimulating agents found in the drugs used in the treatment of anorexia (loss of appetite e.g. Harpenz, Zincovit, Vitafol) are ; vitamin C, vitamin A, minerals and trace element such as zinc, copper, calcium magnesium, potassium, phosphorous, selenium and manganese (BNF, 2001). All these constituents are found in Moringa leaf powder and that could explain the increase in appetite of the subjects (Fuglie, 2002). It was also observed that subjects were sleeping better after they were fed baseline feeds and Moringa leaf powder and this may have also contributed to the better weight gain as growth hormones are released faster during rest (Ganon, 1995).

4.3 HAEMATOLOGICAL RESULTS

4.3.1 HAEMATOLOGICAL ANALYSIS OF SUBJECTS FED WITH 15G OF MORINGA OLEIFERA LEAF POWDER

4.3.2 Haemoglobin (Hb)

A total of twenty-five (25) subjects were fed with the *Moringa oleifera* leaf powder over a four week period. Haematological results showed that daily addition of *Moringa oleifera* leaf powder to the diets of these children resulted in a significant ($p < 0.001$) linear increase in the haemoglobin (Hb) levels of subjects, from 9.32g/dl in week zero to 10.18g/dl in the fourth week (Fig.4.1.A). Based on the World Health Organization's classification of anaemia, the baseline Hb levels of subjects indicated that they were moderately anaemic (8.0g/dl - 9.4g/dl). Moringa supplementation to their diet during the study improved their haemoglobin status to being just mildly anaemic (9.0g/dl – 10.9g/dl) (Lewis *et al.* 2002).

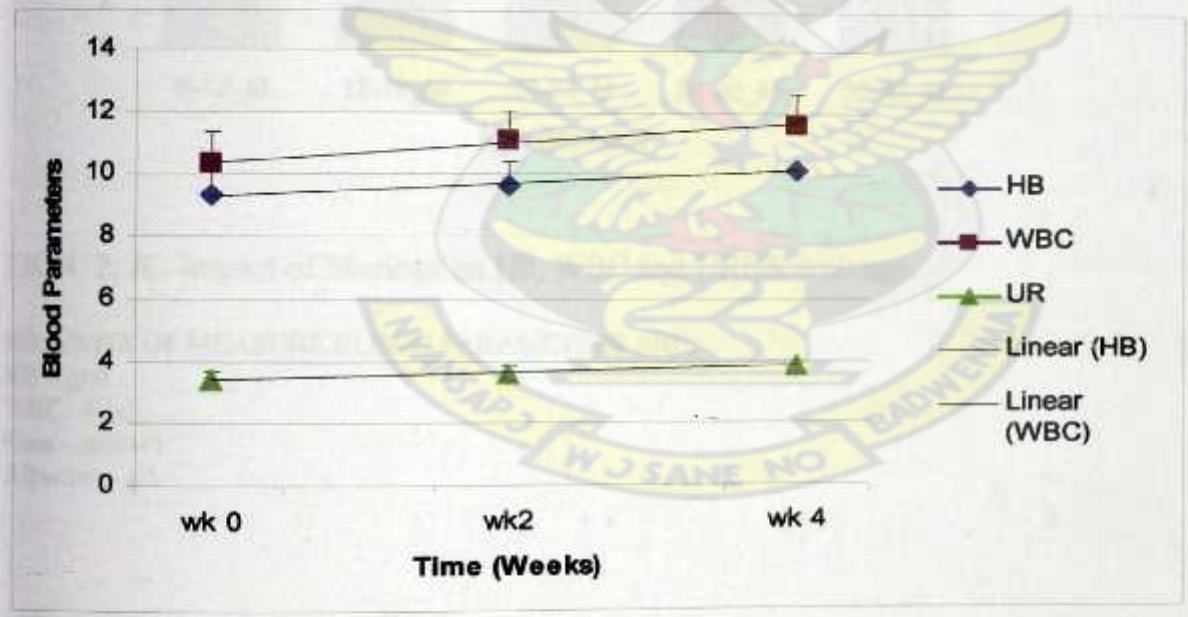


FIG. 4.1. A weekly impact of Moringa leaf powder on Hb, Wbc and urea.

With regards to the impact of Moringa leaf powder on age, it was observed that the haemoglobin level was highest in subjects who were between the ages of eighteen to twenty four (18-24) months old. They had mean haemoglobin level of 11.1g/dl and subjects who were between the ages of twenty four to thirty (24-30) months old had the lowest mean haemoglobin levels of 7.74g/dl (Fig 4.2.B). Results obtained from Moringa effect on Hb showed that there was a significant ($p < 0.001$) linear increase in haemoglobin level with time (weeks) in all the age groups.

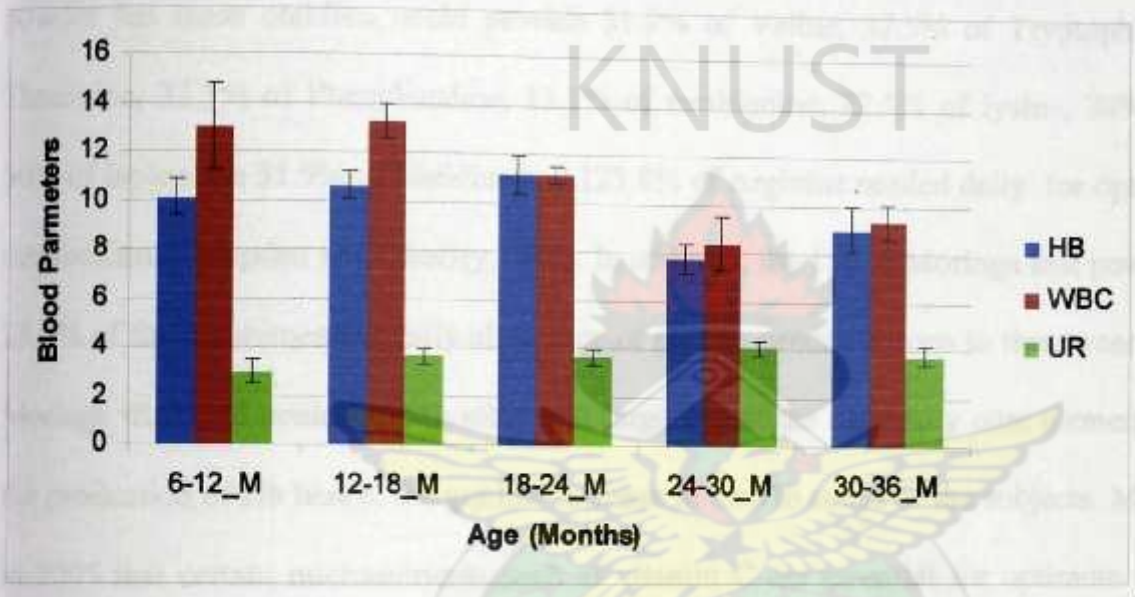


FIG.4. 2. A . Impact of Moringa on Hb, WBC and UREA with age.

NB: UNITS OF MEASURE BLOOD PARAMETERS ARE:
HB – g/dl
WBC –K/µl
Urea – mmol/l
Albumin – g/l

The linear increases in the Hb levels of the subjects by the end of the study compared to baseline values could be attributed to the high iron and protein content of the Moringa leaf powder (Fuglie, 2002). Hb is principally produced from two core elements; the haem and globin. The

haem portion of which iron is the core element and globin portion which is mainly proteins derived from the amino acids; Valine – Histidine – Leucine – Threonine – Proline- Glutamine-Glutamine – Lysine (Robbins, 1999; Guyton, 1991). These amino acids are all found in large quantities in the Moringa leaf powder (Booth and Wickens, 1988). Thus, the 15g of Moringa leaf powder taken daily provided about 42.4% of the recommended daily allowance of iron needed by a child between the ages of one and three years. Based on the World Health Organization's recommended daily allowance of amino acids for a two year old, the 15 grams of Moringa leaf powder fed these children could provide 31.9% of Valine, 37.5% of Tryptophan, 35.6% of Threonine, 22.5% of Phenylanaline, 13.1% of methionine, 22.5% of lysine, 30% of Leucine, 30% of Isoleucine 31.9% of histidine and 123.8% of Arginine needed daily for optimum protein metabolism (Campden and Chorley,1998). In addition, the 15g of Moringa leaf powder provided 25.8% of the recommended daily allowance of protein needed by one to three year old children. Moringa therefore could be providing to a large extent the necessary core elements needed for the production of Hb hence, the gradual increase in the Hb status of the subjects. Marcu reported in 2005 that certain micronutrients such as vitamin C are essential for optimum absorption of iron from a diet. Moringa leaf powder has very high vitamin C content and this may ensure that the high iron content in Moringa leaf powder was effectively absorbed for the production of Haemoglobin.

FIG.4. 3. A. Specific age groups and the impact of Moringa on blood parameters with increasing time

4.3.3 Corrected White Blood Cell Count (WBC)

The individuals understudied had corrected white blood cell count that fell within the reference range of 4.5 – 13.5 K / µl both at the baseline studies and at the end of the study period giving an indication that there was either the absence of infection or that the immune system of subjects were competent to fight off infection (immuno-competent). It may also mean the absence of any factor that could trigger high white blood cell production (leucocytosis) or the presence of powerful antioxidants such as vitamins A, C and E and rich protein content of the Moringa leaf powder helped maintained effective functioning of their immune system throughout the study period (Champe *et al.* 2005). However, the observed data showed some variations even though the corrected WBC count of subjects remained within the reference range throughout the study period.

Weekly results showed that addition of 15g of Moringa leaf powder to the food of the subjected resulted in a significant ($p < 0.001$) increase in corrected white blood cell count of subjects from 10.32 K/µl in week 0 to 11.63 K/µl in week 4.These increases were within the reference range of 4.5 – 13.5 K/µl (Fig. 4.1.A). The leucocytosis (increase in corrected WBC count) in the course of the study could be attributed to the presence of infections such as bacteria or fungi, parasitic infections such as malaria, and nutritional deficiencies such as inability to absorb proteins (protein loosing enteropathies) as was observed with one of the subjects (0 07) during the base line studies. Protein deficiency diminishes immune responses and increases susceptibility to infection because immune defense are dependent upon cell replication and the production of protein with biological activities (Chandra *et al.* 1984; Haslett, Chillverse *et al.* 2002).

The increase in leucocyte count may also be attributed to deficiencies in many other micronutrients including magnesium, selenium, β-carotene, pyridoxine, folic acid, vitamin B_{12}, - and vitamin E that reduce immune function (Scrimshaw and SanGiovanni, 1997; Stable and Spears, 1993; Bendich, 1993).

With regards to the impact of Moringa leaf powder on age, it was observed that corrected WBC count was highest in eighteen (18) months' old subjects. They had corrected white blood cell count of 13.23 K/µl followed by subjects who were twelve (12) months old with corrected white blood cell count of 12.97 K/µl. Subjects who were thirty (30) months old had the lowest corrected white blood cell count of 8.4 K/ul this was followed by thirty six (36) months old subjects with corrected white blood cell count of 9.33 K/ul (Fig. 4.2. A).

The decreased white blood cell count of 24 months, 30 months and 36 months old subjects may be due to the boosting of the immune system to an immuno- competent system which may be attributed to the high proteins and amino acids content of the Moringa leaf powder to combat infections. This is because the high proteins and amino acids in Moringa leads to increase in protein synthesis which can lead to increased antibody (immunoglobulins) production and thus making the immune system strong enough to fight off infections (Champe *et al.* 2005). In the absence of infections, there is thus the reduction of the white blood cell count to the accepted range (Champe *et al.* 2005). Moringa is also rich in vitamin E and this vitamin has the capacity to promote the ability of white blood cells to resist infectious diseases and could account for the white blood cell count being within the reference range (Fuglie, 2000).

The antioxidants properties of Moringa (Marcu, 2005) have the ability to boost effective functioning of the immune system as they donate electrons to electron- starved free radicals, thus rendering them tame and stable. This boost in the immune system of the subjects may also be attributed to the presence of beta- sitoseteral in the Moringa leaf powder as beta- sitoseteral are immunity boosters (Marcu, 2005).

With regard to specific age groups and the impact of Moringa on corrected WBC count with increasing time results obtained showed that there was an age by Week interaction. This was the result of the behavior of the 18 month age (Fig.4.4.A.). It was observed that 18 months subjectes had corrected WBC beginning at 13.2 K/µl, then decreasing to12.9 K/µl by week 2, then increasing to 13.6 K/µl by the end of week 4.

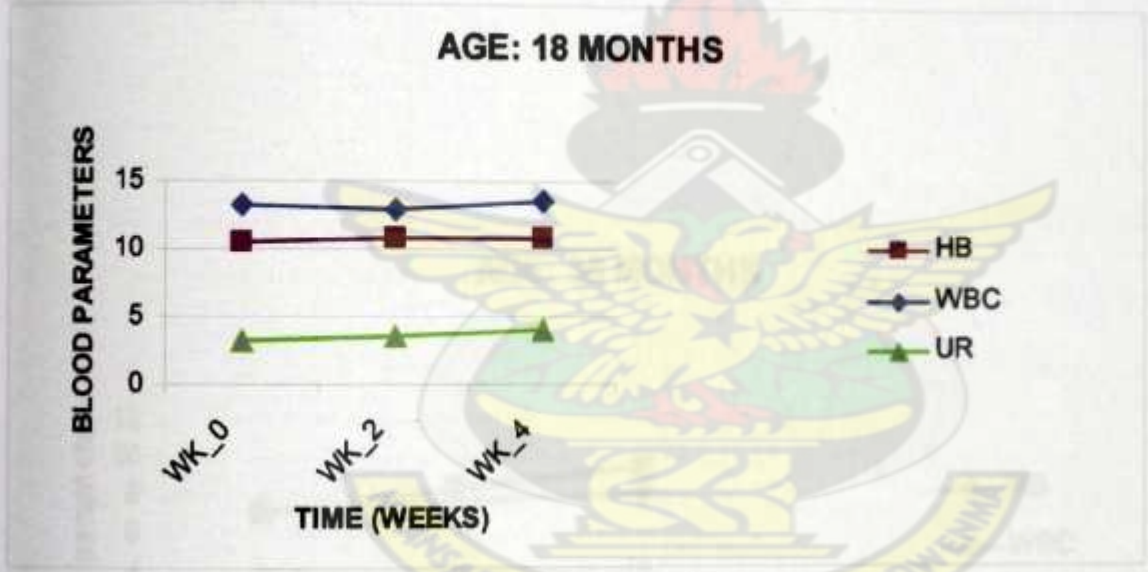


FIG.4. 4. A. Specific age groups and the impact of Moringa on blood parameters with increasing time.

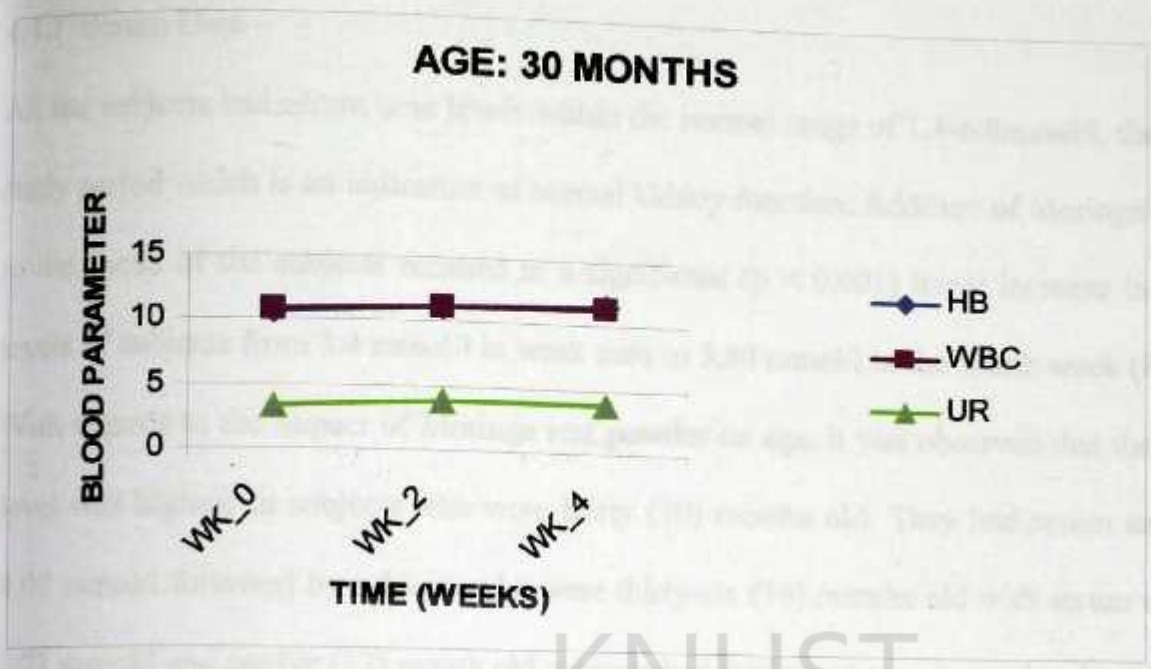
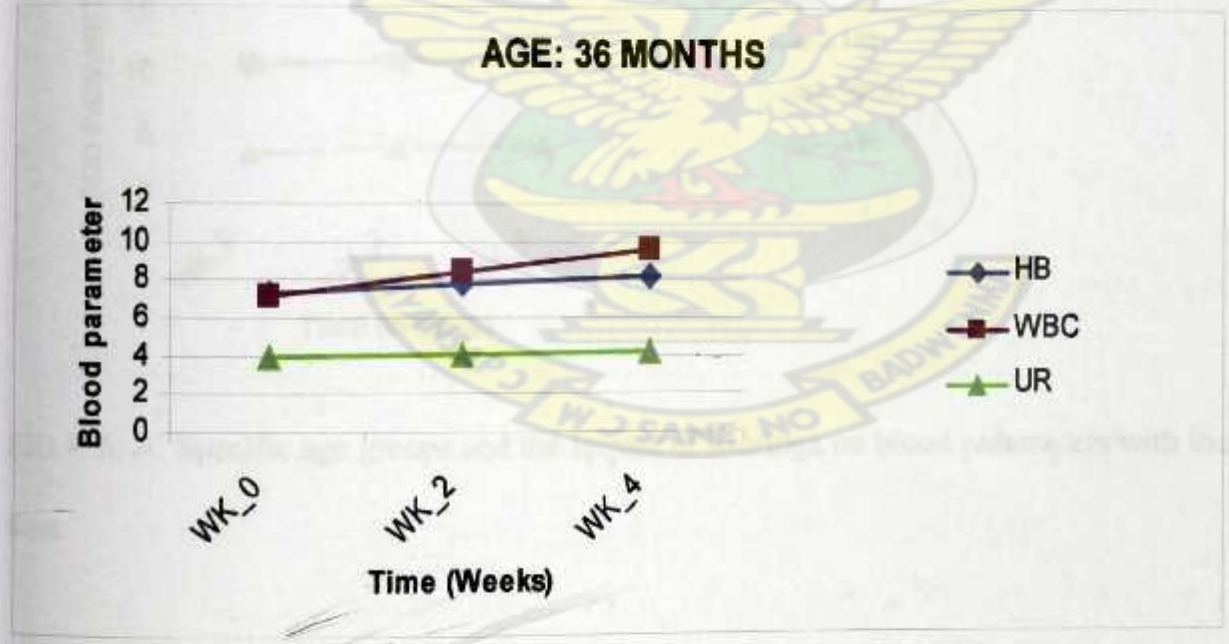


FIG. 4.6. A. Specific age groups and the impact of Moringa on blood parameters with increasing time



4.4.1. Serum Urea

All the subjects had serum urea levels within the normal range of 1.4-6.8mmol/l, throughout the study period which is an indication of normal kidney function. Addition of Moringa leaf powder to the foods of the subjects resulted in a significant ($p < 0.001$) linear increase in serum urea levels of subjects from 3.4 mmol/l in week zero to 3.80 mmol/l in the fourth week (Fig. 4.1. A.). With regards to the impact of Moringa leaf powder on age, it was observed that the serum urea level was highest in subjects who were thirty (30) months old. They had serum urea levels of 4.07 mmol/l followed by subjects who were thirty-six (36) months old with serum urea level of 3.71 mmol/l and twelve (12) month old subjects had the lowest serum urea levels of 2.97 mmol/l (Fig.4. 2. A).

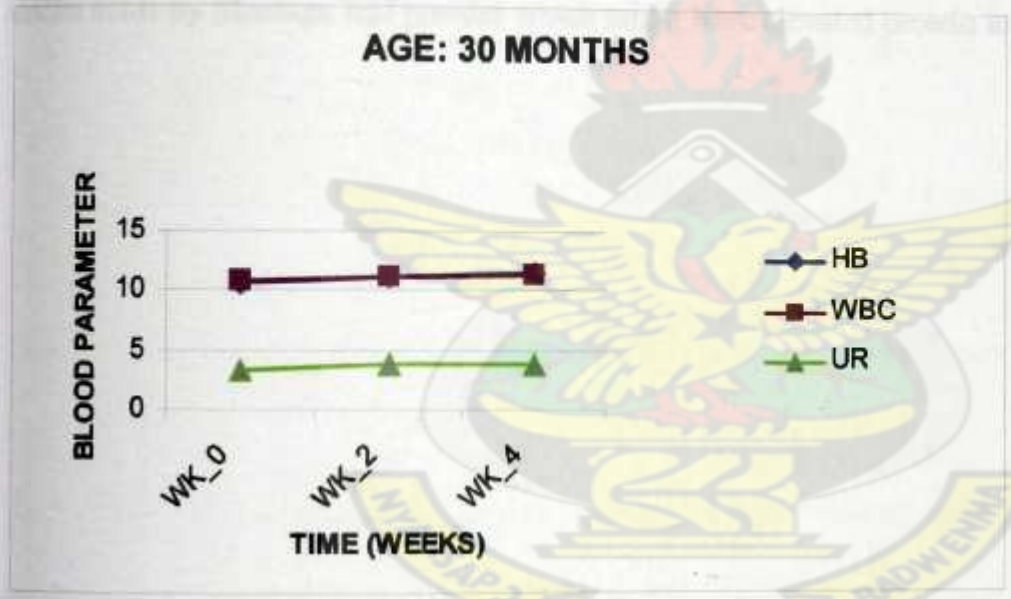


FIG.4. 6. A. Specific age groups and the impact of Moringa on blood parameters with increasing time

Parry (1984), reported that urea excretion in urine falls rapidly if a person is on a low protein diet which is an indication that serum urea levels are low since there is a reduction in protein

metabolism due to low protein intake (Parry, 1984) The increase in serum urea levels is therefore an indication that *Moringa oleifera* leaf powder being very rich in protein and amino acids supplied subjects with the requisite proteins needed by the body for optimum protein metabolism. Based on the World Health Organization's recommended daily allowance of amino acids for a two year old (WHO, 2002), the 15 grams of Moringa leaf powder fed these children could provide 31.9% of Valine, 37.5% of Tryptophan, 35.6% of Threonine, 22.5% of Phenylanaline, 13.1% of methionine, 22.5% of lysine, 30% of Leucine, 30% of Isoleucine 31.9% of histidine and 123.8% of Arginine needed on a daily bases for optimum protein metabolism as well as 25.8% of the recommended daily allowance of protein needed by a one to three year old children. The increased serum urea levels might have been due to the supply of proteins and amino acids by Moringa leaf powder which might have elevated protein levels.

4.4.2. Serum Albumin

It was observed that none of the subjects fed on *Moringa oleifera* leaf powder had serum albumin level that was below the lower limit of the accepted range (38.0 - 51.0 g/ l) both at the baseline study and throughout the four week period of study. Addition of Moringa leaf powder resulted in an increase in serum albumin levels of subjects from 53.1 g/l in week zero to 55.6g/l in the second week and 57.5g/l in the fourth week (Fig. 4.1.B.).

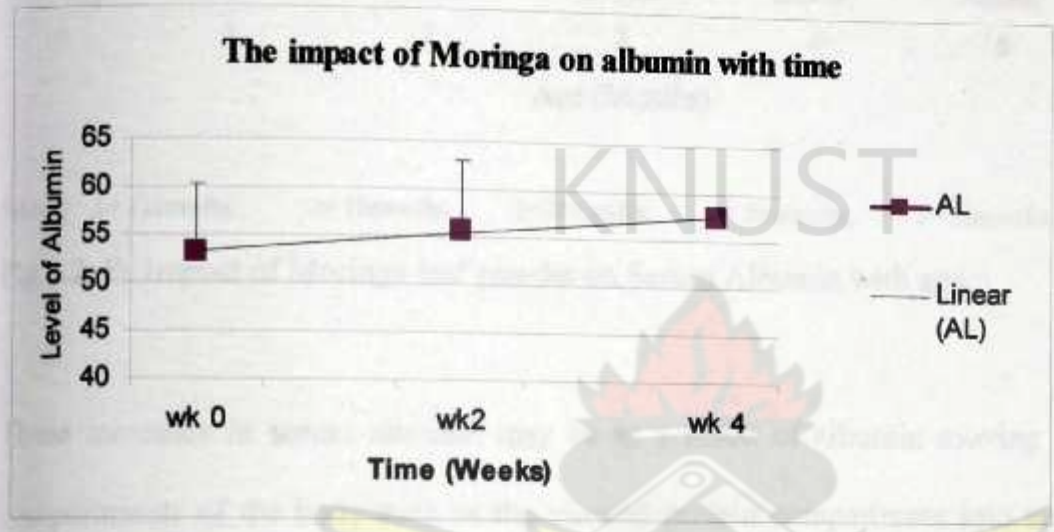
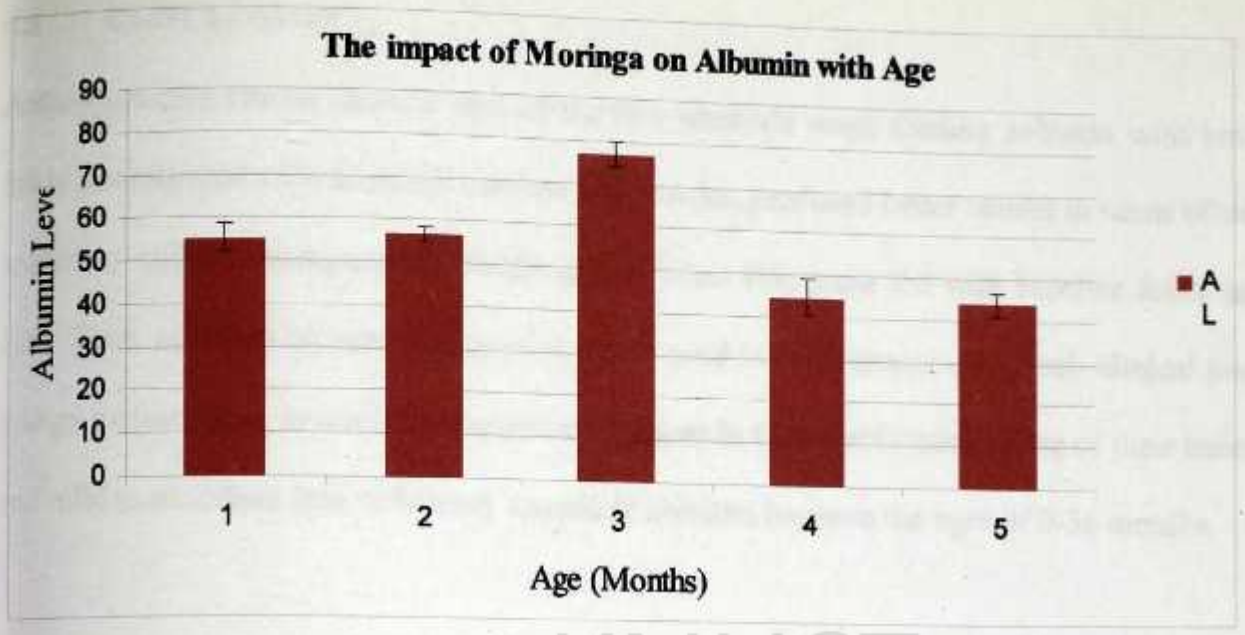


FIG. 4.1.B. Effect of Moringa leaf powder on Serum Albumin with time.

With regard to the impact of Moringa on serum urea with age, results showed that serum albumin levels were highest (76.7g/l) in 24 month, higher (56, 4 g/l) in 18 months and high (55.3 g/l) in 12 months old subjects. The lowest serum albumin level of 43.76 g/l was recoded in 36 month old subjects followed by 30 month old subjects with serum albumin value of 44.74 g/l (Fig. 4.2.B.). It is noted that the serum albumin increased from 6 months to 24 months and then decreased and this needs further investigation.



Where: 1= 12months, 2= 18months, 3=24 months, 4= 30 months, 5= 36months

Fig.4.2. B. Impact of Moringa leaf powder on Serum Albumin with age.

These increases in serum albumin may be as a result of albumin moving from other protein compartments of the body such as the visceral protein compartment into serum (Cotran *et al.*, 1999). Based on the World Health Organization's recommended daily allowance of amino acids for a two year old (WHO, 2002), the 15g of Moringa leaf powder fed this children could provide 31.9% of Valine, 37.5% of Tryptophan, 35.6% of Threonine, 22.5% of Phenylanaline, 13.1% of methionine, 22.5% of lysine, 30% of Leucine, 30% of Isoleucine 31.9% of histidine and 123.8% (more than all the percentage) of Arginine needed on a daily bases for optimum protein metabolism. Also the 15 grams of Moringa leaf powder provided 25.8% of the recommended daily allowance of protein needed by a child who is between the ages of one and three year old (Campden and Chorleywood Food Research Association, 1998). With the Moringa leaf powder providing high levels of proteins and amino acids there is thus an increase in albumin production, hence, the increase in serum albumin levels.

4.5 CONCLUSION

Anthropometric results showed that of the two methods used, feeding subjects with baseline feeds incorporated with *Moringa oleifera* leaf powder, produced better results in terms of weight gained by subjects compared to weight gained when they were fed with baseline feeds and F-100. Thus, *Moringa oleifera* leaf powder can be used in the management of sub-clinical protein-energy malnutrition, severely malnourished children in the rehabilitation phase of their treatment and mild to moderate iron deficiency anemia in children between the ages of 0-36 months.

4.6 RECOMMENDATIONS

♦.the duration of this research was short (eight weeks), It is recommended that subsequent studies should be conducted over a longer period of time (at least 4 months) to establish the impact of *Moringa oleifera* powder on the measured parameters.

♦. From literature, the biochemical analysis of urea and Creatinine should have been performed on a 24-hour urine collected samples instead of the serum used. Serum was used because it was difficult collecting the 24-hour urine sample as subjects were not housed at the rehabilitation centre. It is therefore recommended that subsequent research should take into consideration the housing of subjects to enable them have the 24 hour urine collection. This can be achieved by motivating the parents such as feeding the mothers.

♦. Considering the nutritious nature of Moringa oleifera leaf powder, it is recommend that, it should be introduced in the school feeding programme nationwide to help curb the malnutrition menace in Ghana. It is also recommended that public awareness be intensified about the nutritional attributes of Moringa oleifera leaf powder to encourage its usage in Ghanaian homes as it would helped reduce cases of malnutrition recorded in hospitals nationwide

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APPENDICES

APPENDIX 1A: Consent Form

TITLE OF PROJECT: The use of Moringa oleifera leaf powder in the management of sub-clinical Protein Energy Malnutrition in infants between the ages of 6-36 months.

INVESTIGATOR: Ms. Rosemary Asante (Postgraduate student)

DEPARTMENT: Biochemistry and Biotechnology – KNUST

Explanation of Procedures

You are being asked to participate in a study designed to assess the effect of *Moringa oleifera* leaf powder in the management of sub-clinical Protein Energy malnutrition in infants less than 5 years. Before you decide whether to take part in this study, we will like to explain the purpose of the study, the risks and the benefits to you and what is expected of you. Malnutrition is a serious problem affecting infants especially in sub-sahara Africa which includes Ghana. Several interventions are being undertaken to manage this problem, however the cost implications of these interventions and other factors have hindered the effectiveness of some of the programmes. Thus, this study is intended to use locally available resources to help control malnutrition. One of such resource is *Moringa oleifera*. If you decide to participate, your weight, height and mid-upper arm circumference will be measured and blood sample will be taken from you for hematological and biochemical analysis.

Risks and Discomforts

A single, small sample of blood will be taken. There may be mild discomfort and a small bruise because of the needle prick required to draw blood. However, only trained medical personnel will collect the blood sample for the study. All information collected during the study will be kept confidential.

Participant Signature / Initials / Thumbprint _____

APPENDIX 1 B: WEIGHT FOR HEIGHT REFERENCE TABLE

WHO/NCHS normalized reference weight-for-length (49-84 cm) and weight- for-height (85-110 cm), by sex

Boys' weight (kg)						Girls' weight (kg)				
-4SD	-3SD	-2SD	-1SD	Median	Length	Median	-1SD	-2SD	-3SD	-4SD
60%	70%	80%	90%		(cm)		90%	80%	70%	60%
1.8	2.1	2.5	2.8	3.1	49	3.3	2.9	2.6	2.2	1.8
1.8	2.2	2.5	2.9	3.3	50	3.4	3	2.6	2.3	1.9
1.8	2.2	2.6	3.1	3.5	51	3.5	3.1	2.7	2.3	1.9
1.9	2.3	2.8	3.2	3.7	52	3.7	3.3	2.8	2.4	2
1.9	2.4	2.9	3.4	3.9	53	3.9	3.4	3	2.5	2.1
2	2.6	3.1	3.6	4.1	54	4.1	3.6	3.1	2.7	2.2
2.2	2.7	3.3	3.8	4.3	55	4.3	3.8	3.3	2.8	2.3
2.3	2.9	3.5	4	4.6	56	4.5	4	3.5	3	2.4
2.5	3.1	3.7	4.3	4.8	57	4.8	4.2	3.7	3.1	2.6
2.7	3.3	3.9	4.5	5.1	58	5	4.4	3.9	3.3	2.7
2.9	3.5	4.1	4.8	5.4	59	5.3	4.7	4.1	3.5	2.9
3.1	3.7	4.4	5	5.7	60	5.5	4.9	4.3	3.7	3.1
3.3	4	4.6	5.3	5.9	61	5.8	5.2	4.6	3.9	3.3
3.5	4.2	4.9	5.6	6.2	62	6.1	5.4	4.8	4.1	3.5
3.8	4.5	5.2	5.8	6.5	63	6.4	5.7	5	4.4	3.7
4	4.7	5.4	6.1	6.8	64	6.7	6	5.3	4.6	3.9
4.3	5	5.7	6.4	7.1	65	7	6.3	5.5	4.8	4.1
4.5	5.3	6	6.7	7.4	66	7.3	6.5	5.8	5.1	4.3
4.8	5.5	6.2	7	7.7	67	7.5	6.8	6	5.3	4.5
5.1	5.8	6.5	7.3	8	68	7.8	7.1	6.3	5.5	4.8
5.3	6	6.8	7.5	8.3	69	8.1	7.3	6.5	5.8	5
5.5	6.3	7	7.8	8.5	70	8.4	7.6	6.8	6	5.2
5.8	6.5	7.3	8.1	8.8	71	8.6	7.8	7	6.2	5.4
6	6.8	7.5	8.3	9.1	72	8.9	8.1	7.2	6.4	5.6
6.2	7	7.8	8.6	9.3	73	9.1	8.3	7.5	6.6	5.8
6.4	7.2	8	8.8	9.6	74	9.4	8.5	7.7	6.8	6
6.6	7.4	8.2	9	9.8	75	9.6	8.7	7.9	7	6.2
6.8	7.6	8.4	9.2	10	76	9.8	8.9	8.1	7.2	6.4
7	7.8	8.6	9.4	10.3	77	10	9.1	8.3	7.4	6.6

7.1	8	8.8	9.7	10.5	78	10.2	9.3	8.5	7.6	6.7
7.3	8.2	9	9.9	10.7	79	10.4	9.5	8.7	7.8	6.9
7.5	8.3	9.2	10.1	10.9	80	10.6	9.7	8.8	8	7.1
7.6	8.5	9.4	10.2	11.1	81	10.8	9.9	9	8.1	7.2
7.8	8.7	9.6	10.4	11.3	82	11	10.1	9.2	8.3	7.4
7.9	8.8	9.7	10.6	11.5	83	11.2	10.3	9.4	8.5	7.6
8.1	9	9.9	10.8	11.7	84	11.4	10.5	9.6	8.7	7.7
7.8	8.9	9.9	11	12.1	85	11.8	10.8	9.7	8.6	7.6
7.9	9	10.1	11.2	12.3	86	12	11	9.9	8.8	7.7
8.1	9.2	10.3	11.5	12.6	87	12.3	11.2	10.1	9	7.9
8.3	9.4	10.5	11.7	12.8	88	12.5	11.4	10.3	9.2	8.1
8.4	9.6	10.7	11.9	13	89	12.7	11.6	10.5	9.3	8.2
8.6	9.8	10.9	12.1	13.3	90	12.9	11.8	10.7	9.5	8.4
8.8	9.9	11.1	12.3	13.5	91	13.2	12	10.8	9.7	8.5
8.9	10.1	11.3	12.5	13.7	92	13.4	12.2	11	9.9	8.7
9.1	10.3	11.5	12.8	14	93	13.6	12.4	11.2	10	8.8
9.2	10.5	11.7	13	14.2	94	13.9	12.6	11.4	10.2	9
9.4	10.7	11.9	13.2	14.5	95	14.1	12.9	11.6	10.4	9.1
9.6	10.9	12.1	13.4	14.7	96	14.3	13.1	11.8	10.6	9.3
9.7	11	12.4	13.7	15	97	14.6	13.3	12	10.7	9.5
9.9	11.2	12.6	13.9	15.2	98	14.9	13.5	12.2	10.9	9.6
10.1	11.4	12.8	14.1	15.5	99	15.1	13.8	12.4	11.1	9.8
10.3	11.6	13	14.4	15.7	100	15.4	14	12.7	11.3	9.9
10.4	11.8	13.2	14.6	16	101	15.6	14.3	12.9	11.5	10.1
10.6	12	13.4	14.9	16.3	102	15.9	14.5	13.1	11.7	10.3
10.8	12.2	13.7	15.1	16.6	103	16.2	14.7	13.3	11.9	10.5
11	12.4	13.9	15.4	16.9	104	16.5	15	13.5	12.1	10.6
11.2	12.7	14.2	15.6	17.1	105	16.7	15.3	13.8	12.3	10.8
11.4	12.9	14.4	15.9	17.4	106	17	15.5	14	12.5	11
11.6	13.1	14.7	16.2	17.7	107	17.3	15.8	14.3	12.7	11.2
11.8	13.4	14.9	16.5	18	108	17.6	16.1	14.5	13	11.4
12	13.6	15.2	16.8	18.3	109	17.9	16.4	14.8	13.2	11.6
12.2	13.8	15.4	17.1	18.7	110	18.2	16.6	15	13.4	11.9

Notes:

1. SD = standard deviation score or Z-score; although the interpretation of a fixed percent-of-median value varies across age and height, and generally the two scales cannot be compared, the approximate percent-of-the median values for -1 and -2SD are 90% and 80% of median, respectively (Bulletin of the World Health Organization, 1994, 72: 273-283).
2. Length is measured below 85 cm; height is measured 85 cm and above. Recumbent length is on average 0.5 cm greater than standing height, although the difference is of no importance to the individual child. A correction may be made by deducting 0.5 cm from all lengths above 84.9 cm if the standing height cannot be measured.

APPENDIX 1C: MEASUREMENTS OF PARAMETERS USING THE WORLD HEALTH ORGANIZATION'S REFERENCE VALUES

Anthropometric measurements

Weight and height

Biochemical measurements

Urea levels in serum	(1.4-6.8 mmol/l) – in Infants
Serum albumin levels	(38-55g/l) –All age groups

Haematological measurements

Haemoglobin	(11.3 – 14.8 g/dl) in infants
Corrected white blood cell count	(4.5-13.5k/μl) in children

APPENDIX 1D: MENU FOR THE REHABILITATION CENTRE WHEN SUBJECTS WERE FED WITH F-100

Mondays	Therapeutic Milk	W.S.B Porridge	Therapeutic Milk	Therapeutic Milk	• Banku with palm and green leave soup • Cut orange
Tuesdays	Therapeutic Milk	Corn and soy bean porridge	Therapeutic Milk	Therapeutic Milk	• Apapransa • Banana
Wednesday	Therapeutic Milk	Sorghum porridge	Therapeutic Milk	Therapeutic Milk	• Rice and beans stew • Cut orange
Thursday	Therapeutic Milk	W.S.B Porridge	Therapeutic Milk	Therapeutic Milk	• Rice balls with groundnut and green leaves soup • Banana
Friday	Therapeutic Milk	Sorghum porridge	Therapeutic Milk	Therapeutic Milk	• Kenkey with beans stew • Pineapples

APPENDIX 1E: MENU FOR THE REHABILITATION CENTRE WHEN SUBJECTS WERE FED BASELINE FEEDS AND MORINGA LEAF POWDER

Mondays	W.S.B Porridge + Leaf powder	Cut orange	• Banku with palm and green leave soup + Leaf powder
Tuesdays	Corn and soy bean porridge + Leaf powder	Banana	• Apapransa + Leaf powder
Wednesday	Sorghum porridge + Leaf powder	Cut orange	• Rice and beans stew + Leaf powder
Thursday	W.S.B Porridge + Leaf powder	Banana	• Rice balls with groundnut and green leaves soup + Leaf powder
Friday	Sorghum porridge + Leaf powder	Pineapples	• Kenkey with beans stew + Leaf powder

APPENDIX 2A: RANGE OF VOLUME FOR FREE-FEEDING WITH F-100

Weight of child (kg)	Range of volume per 4 hourly feeds		Range of daily volumes of F-100	
	Minimum (mls)	Maximum (mls)	Minimum(160ml/kg/day	Maximum(200ml/kg/day)
2.0	50	75	300	440
2.2	55	80	330	484
2.4	60	90	360	528
2.6	65	95	390	572
2.8	70	105	420	616
3.0	75	110	450	660
3.2	80	115	480	704
3.4	85	125	510	748
3.6	90	130	540	792
3.8	95	140	570	836
4.0	100	145	600	880
4.2	105	155	630	924
4.4	110	160	660	968
4.6	115	170	690	1012
4.8	120	175	720	1056
5.0	125	185	750	1100
5.2	130	190	780	1144
5.4	135	200	810	1188
5.6	140	205	840	1232
5.8	145	215	870	1276
6.0	150	220	900	1320
6.2	155	230	930	1364
6.4	160	235	950	1408
6.6	165	240	980	1452
6.8	170	250	1020	1496
7.0	175	255	1050	1540
7.2	180	265	1080	1588
7.4	185	270	1110	1628
7.6	190	280	1140	1672
7.8	195	285	1170	1716
8.0	200	295	1200	1760
8.2	205	300	1230	1804
8.4	210	310	1260	1848
8.6	215	315	1290	1892
8.8	220	325	1320	1936
9.0	225	330	1350	1980

9.2	230	335	1380	2024
9.4	235	345	1410	2068
9.6	240	350	1440	2112
9.8	245	360	1470	2156
10.0	250	365	1500	2200

APPENDIX 2B: RECIPES FOR F-100 (CATCH-UP FORMULAE)

Catch-up formula: Using skimmed milk powder

Dried skimmed milk powder	80g
Sugar	50g
Vegetable oil	60g
Mineral mix	20ml
Warm water(830 ml)	To 1000ml

Catch-up formula: Using full cream fresh or long-life milk

Dried whole milk	880ml
Sugar	75g
Oil	20g
Mineral mix	20ml
Water	To 1000ml

Catch-up formula: Using full cream fresh or long-life milk

Full cream milk powder	110g
Sugar	50g
Oil	30g
Mineral mix	20ml
Warm water(830 ml)	To 1000ml

APPENDIX 2.C: Composition **of Electrolyte /Mineral mix solution**

Ingredient	Amount (g)
potassium	224
Tripotassium citrate	81
Magnesium chloride	76
Zinc acetate	8.2
Copper sulphate	1.4
Water	To 2500ml

APPENDIX 2D: PERCENTAGE OF RECOMMENDED DAILY ALLOWANCE PROVIDED BY 15G OF MORINGA POWDER

15 grams of Moringa leaf powder added to an infant's food will give him roughly the following in terms of recommended daily allowance.

Proteins 25.2%

Calcium 75%

Magnesium 36.6%

Potassium 24.6%

Iron 42.6%

Vitamin A 163.2%

Vitamin C 13.2%

APPENDIX 3A: CALCULATION OF WEIGHT GAIN/KG PER DAY ON CATCH-UP DIET.

To find out how much per kilogram per day a child has gain over a number of days;

1. Calculate the weight gain in grams over a period by subtracting the first weight from the second weight.
2. Calculate the number of days between the two weights.
3. Divide the weight gain by the number of days to get the daily weight gain.
4. Calculate the average weight in kg over this period by adding the first and second weight and divide it by 2.
5. Divide the daily weight gain by the average weight to get the weight gain/kg per day

Example

1. Abu started catch-up formula on day 6 when he weighed 4.7kg (4700). On day 16 he weighed 5.3kg (5300) so he gained 5300-4700=600g
2. The period between the two weights was day 6 to 16=10 days.
3. So he gained 600g over 10 days=60g/day
4. His average weight gain in kg over this period was (4.7+5.3)/2=10.0/2=5.0kg
5. So Abu's weight gain /kg per day = (60 divided by 5)g/kg per day=12 g/kg per. This is a good rate of weight gain

APPENDIX 3B: CALCULATION OF VOLUME OF F-100 GIVEN TO SUBJECTS

Formula: $\frac{\text{Weight of subject} \times 130}{\text{Number of times to be fed}}$

Since subjects were fed six times in a day (in accordance with the normal feeding regime at the rehabilitation centre, the formula becomes

$$\frac{\text{Weight of subject} \times 130}{6}$$

Calculating the volume (ml) of F-100 given to a Child between the ages of 6-12months whose weight is 5.0 kg

$$\frac{5.0 \times 130}{6} = 108 \text{ ml}$$

This can be approximated to **110 ml** to make up for feeding waste.

APPENDIX 3C: CALCULATION OF ENERGY (Kcal) OF F-100 GIVEN TO SUBJECTS

Every 100ml of F-100 contains 100 Kcal of energy and 3 grams of proteins.

100 ml = 100 Kcal

Therefore the 110 ml of F-100 fed the 5.0 Kg subject provides

110 Kcal

If 100ml of F-100 provides 3.0 grams of proteins therefore the 110 ml of F-100 provides 3.3 grams of proteins.

Energy (Kcal) content of the 3.3 grams of proteins is calculated as:

If 1 gram of protein = 4.5 Kcal

Therefore 3.3 grams gives $\frac{4.5 \times 3.3}{1}$ = **15 Kcal**

Therefore total energy content of F-100 for a subject whose weight is 5.0 Kg is

110 Kcal + 15 Kcal = 125 Kcal

APPENDIX 3D: CALCULATION OF ENERGY (Kcal) OF THE 15 GRAMS OF *MORINGA OLEIFERA* LEAF POWDER GIVEN TO SUBJECTS

Nutritional constituents of Moringa and its energy (Kcal) equivalence.

Calories = 30.8

Proteins = 18.59

Carbohydrate = 23.25

Fat = 3.11

Therefore total energy (Kcal) in the 15 grams of *Moringa oleifera* leaf powder is

30.8 + 18.59 + 23.25 + 3.11 =**75.75 Kcal**

This is equivalent to 76 Kcal

APPENDIX 4A: DAILY WEIGHT GAIN OF SUBJECTS

Daily weight gain of subjects (grams/kg/day) when subjects were fed with baseline feeds and F-100

Variable	Age	Daily weight gained
1	6-12M	2.1
1	6-12	3.8
1	6-12	1.8
2	12-18	3.1
2	12-18	0.4
2	12-18	0.8

Daily weight gain of subjects (grams/kg/day) when subjects were fed with baseline feeds incorporated with Moringa leaf powder

Variable	Age	Daily weight gained
1	6-12M	5.5
1	6-12	6.7
1	6-12	6.7
2	12-18	3.8
2	12-18	3.0
2	12-18	3.2

APPENDIX 4B: RESULTS OF STATISTICAL ANALYTICAL SYSTEMS- STATISTICAL ARRANGEMENTOF DATA

Obs	Rep	Age	Week	HB	WBC	UR	CR	AL
1	1	12_M	0	9.80	11.60	2.10	90.40	52.40
2	1	18_M	0	10.60	12.80	3.40	91.20	54.60
3	1	24_M	0	10.00	10.50	3.40	94.80	76.20
4	1	30_M	0	7.40	7.40	3.80	86.00	48.40
5	1	36_M	0	8.90	8.50	3.30	82.40	40.10
6	2	12_M	0	9.00	11.80	3.10	93.00	54.10
7	2	18_M	0	9.70	13.10	3.30	90.00	56.30
8	2	24_M	0	10.60	10.90	3.30	93.50	71.30
9	2	30_M	0	7.10	6.90	4.10	83.10	40.30
10	2	36_M	0	8.50	8.80	4.10	87.40	40.00
11	3	12_M	0	10.80	10.70	3.10	93.35	53.10
12	3	18_M	0	11.00	13.60	3.20	90.10	54.10
13	3	24_M	0	11.20	11.00	3.20	93.10	73.60
14	3	30_M	0	7.25	7.15	3.95	82.95	40.55
15	3	36_M	0	8.00	10.00	3.70	82.65	41.20
16	1	12_M	2	9.90	12.70	2.50	82.30	50.30
17	1	18_M	2	10.80	11.60	3.40	90.40	55.00
18	1	24_M	2	10.60	10.90	3.60	98.70	78.70
19	1	30_M	2	7.50	8.70	3.90	87.00	49.40
20	1	36_M	2	9.30	8.70	3.20	82.10	45.30
21	2	12_M	2	9.10	12.30	3.00	94.00	59.10
22	2	18_M	2	10.10	13.40	3.80	92.10	57.30
23	2	24_M	2	10.80	11.20	4.10	93.70	74.30
24	2	30_M	2	7.30	8.20	4.40	83.70	41.00
25	2	36_M	2	8.50	9.20	4.30	88.00	47.10
26	3	12_M	2	10.40	15.20	3.00	98.15	54.65
27	3	18_M	2	11.30	13.80	3.60	92.10	56.00
28	3	24_M	2	12.00	11.40	3.60	97.30	78.30
29	3	30_M	2	8.40	8.45	4.00	85.85	44.70
30	3	36_M	2	8.40	10.30	3.80	84.90	43.15
31	1	12_M	4	10.50	13.10	3.00	80.10	57.10
32	1	18_M	4	10.30	13.10	3.70	90.20	58.10
33	1	24_M	4	11.00	10.90	3.70	99.30	79.80
34	1	30_M	4	7.80	9.90	3.60	88.80	51.00
35	1	36_M	4	11.00	8.70	3.30	86.20	46.30
36	2	12_M	4	10.10	12.80	3.80	96.30	59.00
37	2	18_M	4	10.40	13.60	4.00	93.40	58.10
38	2	24_M	4	11.10	11.30	4.00	94.10	79.70
39	2	30_M	4	8.00	9.30	4.60	89.40	41.70
40	2	36_M	4	8.50	9.40	4.20	89.10	47.10
41	3	12_M	4	11.20	16.50	3.15	96.55	58.25
42	3	18_M	4	11.40	14.10	4.20	94.00	58.40
43	3	24_M	4	12.60	11.80	3.80	98.90	78.00
44	3	30_M	4	8.90	9.60	4.25	87.70	45.65
45	3	36_M	4	9.90	10.40	3.95	85.05	43.60

APPENDIX 4C: EFFECTS OF MORINGA ON VARIOUS BLOOD PARAMETERS AT DIFFERENT AGES

Age=12_M

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	9	10.0888889	0.7321961	9.0000000	11.2000000
WBC	9	12.9666667	1.8165902	10.7000000	16.5000000
UR	9	2.9722222	0.4657730	2.1000000	3.8000000
AL	9	55.3333333	3.1657345	50.3000000	59.1000000

Age=18_M

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	9	10.6222222	0.5607535	9.7000000	11.4000000
WBC	9	13.2333333	0.7297260	11.6000000	14.1000000
UR	9	3.6222222	0.3345810	3.2000000	4.2000000
AL	9	56.4333333	1.6294171	54.1000000	58.4000000

Age=24_M

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	9	11.1000000	0.7810250	10.0000000	12.6000000
WBC	9	11.1000000	0.3741657	10.5000000	11.8000000
UR	9	3.6333333	0.3041381	3.2000000	4.1000000
AL	9	76.6555556	2.9921193	71.3000000	79.8000000

Age=30_M

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	9	7.7388889	0.5988415	7.1000000	8.9000000
WBC	9	8.4000000	1.0865657	6.9000000	9.9000000
UR	9	4.0666667	0.3092329	3.6000000	4.6000000
AL	9	44.7444444	4.1174510	40.3000000	51.0000000

Age=36_M

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	9	9.0000000	0.9367497	8.0000000	11.0000000
WBC	9	9.3333333	0.7348469	8.5000000	10.4000000
UR	9	3.7611111	0.4151640	3.2000000	4.3000000
AL	9	43.7611111	2.8639765	40.0000000	47.1000000

APPENDIX 4D: IMPACT OF MORINGA ON BLOOD PARAMETERS WITH TIME

Week=0

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	15	9.3233333	1.4206219	7.1000000	11.2000000
WBC	15	10.3166667	2.1619821	6.9000000	13.6000000
UR	15	3.4033333	0.5008802	2.1000000	4.1000000
AL	15	53.0833333	12.3222457	40.0000000	76.2000000

Week=2

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	15	9.6266667	1.4048216	7.3000000	12.0000000
WBC	15	11.0700000	2.1542151	8.2000000	15.2000000
UR	15	3.6133333	0.5235411	2.5000000	4.4000000
AL	15	55.6200000	12.3837134	41.0000000	78.7000000

Week=4

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	15	10.1800000	1.3549908	7.8000000	12.6000000
WBC	15	11.6333333	2.1985926	8.7000000	16.5000000
UR	15	3.8166667	0.4336995	3.0000000	4.6000000
AL	15	57.4533333	12.7446693	41.7000000	79.8000000

APPENDIX 4E: IMPACT OFMORINGA ON BLOOD PARAMETERS WITH RESPECT TO AGE AND TIME

Age=12_M Week=0

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	9.8666667	0.9018500	9.0000000	10.8000000
WBC	3	11.3666667	0.5859465	10.7000000	11.8000000
UR	3	2.7666667	0.5773503	2.1000000	3.1000000
AL	3	53.2000000	0.8544004	52.4000000	54.1000000

Age=12_M Week=2

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	9.8000000	0.6557439	9.1000000	10.4000000
WBC	3	13.4000000	1.5716234	12.3000000	15.2000000
UR	3	2.8333333	0.2886751	2.5000000	3.0000000
AL	3	54.6833333	4.4000947	50.3000000	59.1000000

Age=12_M Week=4

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	10.6000000	0.5567764	10.1000000	11.2000000
WBC	3	14.1333333	2.0550750	12.8000000	16.5000000
UR	3	3.3166667	0.4252450	3.0000000	3.8000000
AL	3	58.1166667	0.9569918	57.1000000	59.0000000

Age=18_M Week=0

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	10.4333333	0.6658328	9.7000000	11.0000000
WBC	3	13.1666667	0.4041452	12.8000000	13.6000000
UR	3	3.3000000	0.1000000	3.2000000	3.4000000
AL	3	55.0000000	1.1532563	54.1000000	56.3000000

Age=18_M Week=2

Variable	N	Mean	Std Dev	Minimum	Maximum

HB	3	10.7333333	0.6027714	10.1000000	11.3000000
WBC	3	12.9333333	1.1718931	11.6000000	13.8000000
UR	3	3.6000000	0.2000000	3.4000000	3.8000000
AL	3	56.1000000	1.1532563	55.0000000	57.3000000

Age=18_M Week=4

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	10.7000000	0.6082763	10.3000000	11.4000000
WBC	3	13.6000000	0.5000000	13.1000000	14.1000000
UR	3	3.9666667	0.2516611	3.7000000	4.2000000
AL	3	58.2000000	0.1732051	58.1000000	58.4000000

Age=24_M Week=0

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	10.6000000	0.6000000	10.0000000	11.2000000
WBC	3	10.8000000	0.2645751	10.5000000	11.0000000
UR	3	3.3000000	0.1000000	3.2000000	3.4000000
AL	3	73.7000000	2.4515301	71.3000000	76.2000000

Age=24_M Week=2

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	11.1333333	0.7571878	10.6000000	12.0000000
WBC	3	11.1666667	0.2516611	10.9000000	11.4000000
UR	3	3.7666667	0.2886751	3.6000000	4.1000000
AL	3	77.1000000	2.4331050	74.3000000	78.7000000

Age=24_M Week=4

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	11.5666667	0.8962886	11.0000000	12.6000000
WBC	3	11.3333333	0.4509250	10.9000000	11.8000000
UR	3	3.8333333	0.1527525	3.7000000	4.0000000
AL	3	79.1666667	1.0115994	78.0000000	79.8000000

Age=30_M Week=0

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	7.2500000	0.1500000	7.1000000	7.4000000
WBC	3	7.1500000	0.2500000	6.9000000	7.4000000
UR	3	3.9500000	0.1500000	3.8000000	4.1000000
AL	3	43.0833333	4.6060648	40.3000000	48.4000000

Age=30_M Week=2

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	7.7333333	0.5859465	7.3000000	8.4000000
WBC	3	8.4500000	0.2500000	8.2000000	8.7000000
UR	3	4.1000000	0.2645751	3.9000000	4.4000000
AL	3	45.0333333	4.2099089	41.0000000	49.4000000

Age=30_M Week=4

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	8.2333333	0.5859465	7.8000000	8.9000000
WBC	3	9.6000000	0.3000000	9.3000000	9.9000000
UR	3	4.1500000	0.5074446	3.6000000	4.6000000
AL	3	46.1166667	4.6675297	41.7000000	51.0000000

Age=36_M Week=0

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	8.4666667	0.4509250	8.0000000	8.9000000
WBC	3	9.1000000	0.7937254	8.5000000	10.0000000
UR	3	3.7000000	0.4000000	3.3000000	4.1000000
AL	3	40.4333333	0.6658328	40.0000000	41.2000000

Age=36_M Week=2

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	8.7333333	0.4932883	8.4000000	9.3000000
WBC	3	9.4000000	0.8185353	8.7000000	10.3000000
UR	3	3.7666667	0.5507571	3.2000000	4.3000000
AL	3	45.1833333	1.9775827	43.1500000	47.1000000

Age=36_M Week=4

Variable	N	Mean	Std Dev	Minimum	Maximum
HB	3	9.8000000	1.2529964	8.5000000	11.0000000
WBC	3	9.5000000	0.8544004	8.7000000	10.4000000
UR	3	3.8166667	0.4645787	3.3000000	4.2000000
AL	3	45.6666667	1.8339393	43.6000000	47.1000000

APPENDIX 4F: HB - AUTO REGRESSIVE

The Mixed Procedure

Model Information

Data Set	WORK.ROSEMARY
Dependent Variable	HB
Covariance Structures	Variance Components, Autoregressive
Subject Effect	Rep*Age
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
Rep	3	1 2 3
Age	5	12_M 18_M 24_M 30_M 36_M
Week	3	0 2 4

Dimensions

Covariance Parameters	4
Columns in X	24
Columns in Z	12
Subjects	1
Max Obs Per Subject	45

Number of Observations

Number of Observations Read	45
Number of Observations Used	45
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	79.56515478	
1	2	56.19772060	0.42060449
2	1	55.94923791	0.03124723
3	1	55.93611347	0.00008579
4	1	55.93607910	0.00000000

Convergence criteria met.

Estimated R Matrix for Rep*Age 1 12_M

Row	Col1	Col2	Col3
1	0.3355	0.2478	0.1830
2	0.2478	0.3355	0.2478
3	0.1830	0.2478	0.3355

Estimated R Correlation Matrix for Rep*Age 1 12_M

Row	Col1	Col2	Col3
1	1.0000	0.7385	0.5454
2	0.7385	1.0000	0.7385
3	0.5454	0.7385	1.0000

HB - AUTO REGRESSIVE

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Subject	Estimate	Standard Error	Z Value	Pr Z
Rep		0.1634	0.2167	0.75	0.2253
Rep*Week		0	.	.	.
AR(1)	Rep*Age	0.7385	0.1282	5.76	<.0001
Residual		0.3355	0.1365	2.46	0.0070

Fit Statistics

-2 Res Log Likelihood	55.9
AIC (smaller is better)	61.9
AICC (smaller is better)	62.9
BIC (smaller is better)	59.2

APPENDIX 4G: TYPE 3 TESTS OF FIXED EFFECTS

Effect	Num DF	Den DF	F Value	Pr > F
Age	4	7.71	21.79	0.0003
Week	2	19.2	16.51	<.0001
Age*Week	8	19.1	1.65	0.1770

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Week Linear	1	24	31.01	<.0001
Week LOF	1	15.5	2.07	0.1698
AGE Linear	1	7.71	30.59	0.0006
AGE Quad	1	7.71	4.85	0.0601
AGE Cubic	1	7.71	26.14	0.0010
AGE LOF	1	7.71	25.58	0.0011

Least Squares Means

Effect	Age	Week	Estimate	Standard Error	DF	t Value	Pr > |t|
Week		0	9.3233	0.2772	2.3	33.63	0.0004
Week		2	9.6267	0.2772	2.3	34.73	0.0004
Week		4	10.1800	0.2772	2.3	36.72	0.0003
Age	12_M		10.0889	0.3718	6.29	27.14	<.0001
Age	18_M		10.6222	0.3718	6.29	28.57	<.0001
Age	24_M		11.1000	0.3718	6.29	29.86	<.0001
Age	30_M		7.7389	0.3718	6.29	20.82	<.0001
Age	36_M		9.0000	0.3718	6.29	24.21	<.0001

Differences of Least Squares Means

Effect	Age	Week	_Age	_Week	Estimate	Standard Error	DF	t Value	Pr > |t|
Week		0		2	-0.3033	0.1160	19	-2.62	0.0170
Week		0		4	-0.8567	0.1538	24	-5.57	<.0001
Week		2		4	-0.5533	0.1160	19	-4.77	0.0001
Age	12_M		18_M		-0.5333	0.4092	7.71	-1.30	0.2300
Age	12_M		24_M		-1.0111	0.4092	7.71	-2.47	0.0397
Age	12_M		30_M		2.3500	0.4092	7.71	5.74	0.0005
Age	12_M		36_M		1.0889	0.4092	7.71	2.66	0.0297
Age	18_M		24_M		-0.4778	0.4092	7.71	-1.17	0.2778
Age	18_M		30_M		2.8833	0.4092	7.71	7.05	0.0001
Age	18_M		36_M		1.6222	0.4092	7.71	3.96	0.0045
Age	24_M		30_M		3.3611	0.4092	7.71	8.21	<.0001
Age	24_M		36_M		2.1000	0.4092	7.71	5.13	0.0010
Age	30_M		36_M		-1.2611	0.4092	7.71	-3.08	0.0158

APPENDIX 4H: WBC - AUTO REGRESSIVE

The Mixed Procedure

Model Information

Data Set	WORK.ROSEMARY
Dependent Variable	WBC
Covariance Structures	Variance Components, Autoregressive
Subject Effect	Rep*Age
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
Rep	3	1 2 3
Age	5	12_M 18_M 24_M 30_M 36_M
Week	3	0 2 4

Dimensions

Covariance Parameters	4
Columns in X	24
Columns in Z	12
Subjects	1
Max Obs Per Subject	45

Number of Observations

Number of Observations Read	45
Number of Observations Used	45
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	93.39554596	
1	4	82.13202229	.
2	1	82.04907418	0.00027906
3	1	82.04509839	0.00000254
4	1	82.04506399	0.00000000

Convergence criteria met.

Estimated R Matrix for Rep*Age 1 12_M

Row	Col1	Col2	Col3
1	0.5395	0.2428	0.1093
2	0.2428	0.5395	0.2428
3	0.1093	0.2428	0.5395

Estimated R Correlation Matrix for Rep*Age 1 12_M

Row	Col1	Col2	Col3
1	1.0000	0.4501	0.2026
2	0.4501	1.0000	0.4501
3	0.2026	0.4501	1.0000

WBC - AUTO REGRESSIVE

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Subject	Estimate	Standard Error	Z Value	Pr Z
Rep		0.1865	0.2549	0.73	0.2321
Rep*Week		0.01125	0.06600	0.17	0.4323
AR(1)	Rep*Age	0.4501	0.1979	2.27	0.0229
Residual		0.5395	0.1845	2.92	0.0017

Fit Statistics

-2 Res Log Likelihood	82.0
AIC (smaller is better)	90.0
AICC (smaller is better)	91.6
BIC (smaller is better)	86.4

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Age	4	8.38	46.64	<.0001

Week	2	2.92	12.11	0.0387
Age*Week	8	16.1	2.82	0.0368

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Week Linear	1	5.23	24.84	0.0037
Week LOF	1	1.98	0.28	0.6488
AGE Linear	1	8.38	148.43	<.0001
AGE Quad	1	8.38	0.43	0.5316
AGE Cubic	1	8.38	36.90	0.0002
AGE LOF	1	8.38	0.81	0.3929

Least Squares Means

Effect	Age	Week	Estimate	Standard Error	DF	t Value	Pr > |t|
Week		0	10.3167	0.3192	2.77	32.32	0.0001
Week		2	11.0700	0.3192	2.77	34.68	<.0001
Week		4	11.6333	0.3192	2.77	36.44	<.0001
Age	12_M		12.9667	0.4026	6.66	32.21	<.0001
Age	18_M		13.2333	0.4026	6.66	32.87	<.0001
Age	24_M		11.1000	0.4026	6.66	27.57	<.0001
Age	30_M		8.4000	0.4026	6.66	20.87	<.0001
Age	36_M		9.3333	0.4026	6.66	23.18	<.0001
Age*Week	12_M	0	11.3667	0.4957	13	22.93	<.0001
Age*Week	12_M	2	13.4000	0.4957	13	27.03	<.0001
Age*Week	12_M	4	14.1333	0.4957	13	28.51	<.0001
Age*Week	18_M	0	13.1667	0.4957	13	26.56	<.0001
Age*Week	18_M	2	12.9333	0.4957	13	26.09	<.0001
Age*Week	18_M	4	13.6000	0.4957	13	27.43	<.0001
Age*Week	24_M	0	10.8000	0.4957	13	21.79	<.0001
Age*Week	24_M	2	11.1667	0.4957	13	22.53	<.0001
Age*Week	24_M	4	11.3333	0.4957	13	22.86	<.0001
Age*Week	30_M	0	7.1500	0.4957	13	14.42	<.0001
Age*Week	30_M	2	8.4500	0.4957	13	17.05	<.0001
Age*Week	30_M	4	9.6000	0.4957	13	19.37	<.0001
Age*Week	36_M	0	9.1000	0.4957	13	18.36	<.0001
Age*Week	36_M	2	9.4000	0.4957	13	18.96	<.0001
Age*Week	36_M	4	9.5000	0.4957	13	19.16	<.0001

WBC - AUTO REGRESSIVE

Differences of Least Squares Means

Effect	Age	Week	_Age	_Week	Estimate	Standard Error	DF	t Value	Pr > |t|
Week		0		2	-0.7533	0.2223	2.65	-3.39	0.0515
Week		0		4	-1.3167	0.2642	5.23	-4.98	0.0037
Week		2		4	-0.5633	0.2223	2.65	-2.53	0.0961
Age	12_M		18_M		-0.2667	0.4442	8.38	-0.60	0.5641
Age	12_M		24_M		1.8667	0.4442	8.38	4.20	0.0027
Age	12_M		30_M		4.5667	0.4442	8.38	10.28	<.0001
Age	12_M		36_M		3.6333	0.4442	8.38	8.18	<.0001
Age	18_M		24_M		2.1333	0.4442	8.38	4.80	0.0012
Age	18_M		30_M		4.8333	0.4442	8.38	10.88	<.0001
Age	18_M		36_M		3.9000	0.4442	8.38	8.78	<.0001
Age	24_M		30_M		2.7000	0.4442	8.38	6.08	0.0002
Age	24_M		36_M		1.7667	0.4442	8.38	3.98	0.0037
Age	30_M		36_M		-0.9333	0.4442	8.38	-2.10	0.0672
Age*Week	12_M	0	12_M	2	-2.0333	0.4659	18.4	-4.36	0.0004
Age*Week	12_M	0	12_M	4	-2.7667	0.5648	27	-4.90	<.0001
Age*Week	12_M	0	18_M	0	-1.8000	0.5997	17.1	-3.00	0.0080
Age*Week	12_M	0	18_M	2	-1.5667	0.6059	19.9	-2.59	0.0177
Age*Week	12_M	0	18_M	4	-2.2333	0.6059	19.9	-3.69	0.0015
Age*Week	12_M	0	24_M	0	0.5667	0.5997	17.1	0.94	0.3579
Age*Week	12_M	0	24_M	2	0.2000	0.6059	19.9	0.33	0.7448
Age*Week	12_M	0	24_M	4	0.03333	0.6059	19.9	0.06	0.9567
Age*Week	12_M	0	30_M	0	4.2167	0.5997	17.1	7.03	<.0001
Age*Week	12_M	0	30_M	2	2.9167	0.6059	19.9	4.81	0.0001
Age*Week	12_M	0	30_M	4	1.7667	0.6059	19.9	2.92	0.0086
Age*Week	12_M	0	36_M	0	2.2667	0.5997	17.1	3.78	0.0015
Age*Week	12_M	0	36_M	2	1.9667	0.6059	19.9	3.25	0.0041
Age*Week	12_M	0	36_M	4	1.8667	0.6059	19.9	3.08	0.0059
Age*Week	12_M	2	12_M	4	-0.7333	0.4659	18.4	-1.57	0.1325
Age*Week	12_M	2	18_M	0	0.2333	0.6059	19.9	0.39	0.7042
Age*Week	12_M	2	18_M	2	0.4667	0.5997	17.1	0.78	0.4471
Age*Week	12_M	2	18_M	4	-0.2000	0.6059	19.9	-0.33	0.7448
Age*Week	12_M	2	24_M	0	2.6000	0.6059	19.9	4.29	0.0004
Age*Week	12_M	2	24_M	2	2.2333	0.5997	17.1	3.72	0.0017
Age*Week	12_M	2	24_M	4	2.0667	0.6059	19.9	3.41	0.0028

Age*week	12_M	2	30_M	0	6.2500	0.6059	19.9	10.31	<.0001
Age*week	12_M	2	30_M	2	4.9500	0.5997	17.1	8.25	<.0001
Age*week	12_M	2	30_M	4	3.8000	0.6059	19.9	6.27	<.0001
Age*week	12_M	2	36_M	0	4.3000	0.6059	19.9	7.10	<.0001
Age*week	12_M	2	36_M	2	4.0000	0.5997	17.1	6.67	<.0001
Age*week	12_M	2	36_M	4	3.9000	0.6059	19.9	6.44	<.0001
Age*week	12_M	4	18_M	0	0.9667	0.6059	19.9	1.60	0.1264
Age*week	12_M	4	18_M	2	1.2000	0.6059	19.9	1.98	0.0616
Age*week	12_M	4	18_M	4	0.5333	0.5997	17.1	0.89	0.3862
Age*week	12_M	4	24_M	0	3.3333	0.6059	19.9	5.50	<.0001
Age*week	12_M	4	24_M	2	2.9667	0.6059	19.9	4.90	<.0001
Age*week	12_M	4	24_M	4	2.8000	0.5997	17.1	4.67	0.0002
Age*week	12_M	4	30_M	0	6.9833	0.6059	19.9	11.53	<.0001
Age*week	12_M	4	30_M	2	5.6833	0.6059	19.9	9.38	<.0001
Age*week	12_M	4	30_M	4	4.5333	0.5997	17.1	7.56	<.0001
Age*week	12_M	4	36_M	0	5.0333	0.6059	19.9	8.31	<.0001
Age*week	12_M	4	36_M	2	4.7333	0.6059	19.9	7.81	<.0001
Age*week	12_M	4	36_M	4	4.6333	0.5997	17.1	7.73	<.0001
Age*week	18_M	0	18_M	2	0.2333	0.4659	18.4	0.50	0.6224
Age*week	18_M	0	18_M	4	-0.4333	0.5648	27	-0.77	0.4496
Age*week	18_M	0	24_M	0	2.3667	0.5997	17.1	3.95	0.0010
Age*week	18_M	0	24_M	2	2.0000	0.6059	19.9	3.30	0.0036
Age*week	18_M	0	24_M	4	1.8333	0.6059	19.9	3.03	0.0067
Age*week	18_M	0	30_M	0	6.0167	0.5997	17.1	10.03	<.0001
Age*week	18_M	0	30_M	2	4.7167	0.6059	19.9	7.78	<.0001
Age*week	18_M	0	30_M	4	3.5667	0.6059	19.9	5.89	<.0001
Age*week	18_M	0	36_M	0	4.0667	0.5997	17.1	6.78	<.0001
Age*week	18_M	0	36_M	2	3.7667	0.6059	19.9	6.22	<.0001
Age*week	18_M	0	36_M	4	3.6667	0.6059	19.9	6.05	<.0001
Age*week	18_M	2	18_M	4	-0.6667	0.4659	18.4	-1.43	0.1692
Age*week	18_M	2	24_M	0	2.1333	0.6059	19.9	3.52	0.0022
Age*week	18_M	2	24_M	2	1.7667	0.5997	17.1	2.95	0.0090
Age*week	18_M	2	24_M	4	1.6000	0.6059	19.9	2.64	0.0157
Age*week	18_M	2	30_M	0	5.7833	0.6059	19.9	9.54	<.0001
Age*week	18_M	2	30_M	2	4.4833	0.5997	17.1	7.48	<.0001
Age*week	18_M	2	30_M	4	3.3333	0.6059	19.9	5.50	<.0001
Age*week	18_M	2	36_M	0	3.8333	0.6059	19.9	6.33	<.0001
Age*week	18_M	2	36_M	2	3.5333	0.5997	17.1	5.89	<.0001
Age*week	18_M	2	36_M	4	3.4333	0.6059	19.9	5.67	<.0001
Age*week	18_M	4	24_M	0	2.8000	0.6059	19.9	4.62	0.0002
Age*week	18_M	4	24_M	2	2.4333	0.6059	19.9	4.02	0.0007
Age*week	18_M	4	24_M	4	2.2667	0.5997	17.1	3.78	0.0015
Age*week	18_M	4	30_M	0	6.4500	0.6059	19.9	10.65	<.0001
Age*week	18_M	4	30_M	2	5.1500	0.6059	19.9	8.50	<.0001
Age*week	18_M	4	30_M	4	4.0000	0.5997	17.1	6.67	<.0001
Age*week	18_M	4	36_M	0	4.5000	0.6059	19.9	7.43	<.0001
Age*week	18_M	4	36_M	2	4.2000	0.6059	19.9	6.93	<.0001
Age*week	18_M	4	36_M	4	4.1000	0.5997	17.1	6.84	<.0001
Age*week	24_M	0	24_M	2	-0.3667	0.4659	18.4	-0.79	0.4413
Age*week	24_M	0	24_M	4	-0.5333	0.5648	27	-0.94	0.3534
Age*week	24_M	0	30_M	0	3.6500	0.5997	17.1	6.09	<.0001
Age*week	24_M	0	30_M	2	2.3500	0.6059	19.9	3.88	0.0009
Age*week	24_M	0	30_M	4	1.2000	0.6059	19.9	1.98	0.0616
Age*week	24_M	0	36_M	0	1.7000	0.5997	17.1	2.83	0.0114
Age*week	24_M	0	36_M	2	1.4000	0.6059	19.9	2.31	0.0317
Age*week	24_M	0	36_M	4	1.3000	0.6059	19.9	2.15	0.0444
Age*week	24_M	2	24_M	4	-0.1667	0.4659	18.4	-0.36	0.7246
Age*week	24_M	2	30_M	0	4.0167	0.6059	19.9	6.63	<.0001
Age*week	24_M	2	30_M	2	2.7167	0.5997	17.1	4.53	0.0003
Age*week	24_M	2	30_M	4	1.5667	0.6059	19.9	2.59	0.0177
Age*week	24_M	2	36_M	0	2.0667	0.6059	19.9	3.41	0.0028
Age*week	24_M	2	36_M	2	1.7667	0.5997	17.1	2.95	0.0090
Age*week	24_M	2	36_M	4	1.6667	0.6059	19.9	2.75	0.0124
Age*week	24_M	4	30_M	0	4.1833	0.6059	19.9	6.90	<.0001
Age*week	24_M	4	30_M	2	2.8833	0.6059	19.9	4.76	0.0001
Age*week	24_M	4	30_M	4	1.7333	0.5997	17.1	2.89	0.0101
Age*week	24_M	4	36_M	0	2.2333	0.6059	19.9	3.69	0.0015
Age*week	24_M	4	36_M	2	1.9333	0.6059	19.9	3.19	0.0046
Age*week	24_M	4	36_M	4	1.8333	0.5997	17.1	3.06	0.0071
Age*week	30_M	0	30_M	2	-1.3000	0.4659	18.4	-2.79	0.0119
Age*week	30_M	0	30_M	4	-2.4500	0.5648	27	-4.34	0.0002
Age*week	30_M	0	36_M	0	-1.9500	0.5997	17.1	-3.25	0.0047
Age*week	30_M	0	36_M	2	-2.2500	0.6059	19.9	-3.71	0.0014
Age*week	30_M	0	36_M	4	-2.3500	0.6059	19.9	-3.88	0.0009
Age*week	30_M	2	30_M	4	-1.1500	0.4659	18.4	-2.47	0.0236
Age*week	30_M	2	36_M	0	-0.6500	0.6059	19.9	-1.07	0.2962
Age*week	30_M	2	36_M	2	-0.9500	0.5997	17.1	-1.58	0.1315

Age*Week	30_M	2	36_M	4	-1.0500	0.6059	19.9	-1.73	0.0986
Age*Week	30_M	4	36_M	0	0.5000	0.6059	19.9	0.83	0.4190
Age*Week	30_M	4	36_M	2	0.2000	0.6059	19.9	0.33	0.7448
Age*Week	30_M	4	36_M	4	0.1000	0.5997	17.1	0.17	0.8695
Age*Week	36_M	0	36_M	2	-0.3000	0.4659	18.4	-0.64	0.5276
Age*Week	36_M	0	36_M	4	-0.4000	0.5648	27	-0.71	0.4849
Age*Week	36_M	2	36_M	4	-0.1000	0.4659	18.4	-0.21	0.8324

APPENDIX 4I: UR - AUTO REGRESSIVE

The Mixed Procedure

Model Information

Data Set	WORK.ROSEMARY
Dependent Variable	UR
Covariance Structures	Variance Components, Autoregressive
Subject Effect	Rep*Age
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
Rep	3	1 2 3
Age	5	12_M 18_M 24_M 30_M 36_M
Week	3	0 2 4

Dimensions

Covariance Parameters	4
Columns in X	24
Columns in Z	12
Subjects	1
Max Obs Per Subject	45

Number of Observations

Number of Observations Read	45
Number of Observations Used	45
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	38.93747325	
1	2	15.41011221	0.00001479
2	1	15.40981739	0.00000000

Convergence criteria met.

Estimated R Matrix for Rep*Age 1 12_M

Row	Col1	Col2	Col3
1	0.05728	0.02735	0.01306
2	0.02735	0.05728	0.02735
3	0.01306	0.02735	0.05728

Estimated R Correlation Matrix for Rep*Age 1 12_M

Row	Col1	Col2	Col3
1	1.0000	0.4775	0.2280
2	0.4775	1.0000	0.4775
3	0.2280	0.4775	1.0000

UR - AUTO REGRESSIVE

Covariance Parameter Estimates

Cov Parm	Subject	Estimate	Standard Error	Z Value	Pr Z
Rep		0.06590	0.07275	0.91	0.1825
Rep*Week		0	.	.	.
AR(1)	Rep*Age	0.4775	0.2134	2.24	0.0252
Residual		0.05728	0.01934	2.96	0.0015

Fit Statistics

-2 Res Log Likelihood	15.4
AIC (smaller is better)	21.4
AICC (smaller is better)	22.3
BIC (smaller is better)	18.7

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Age	4	7.52	15.01	0.0011
Week	2	18.4	12.93	0.0003
Age*Week	8	18.3	1.68	0.1706

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Week Linear	1	26.7	26.02	<.0001
Week LOF	1	13	0.00	0.9497
AGE Linear	1	7.52	38.43	0.0003
AGE Quad	1	7.52	14.88	0.0054
AGE Cubic	1	7.52	0.09	0.7675
AGE LOF	1	7.52	6.63	0.0346

Least Squares Means

Effect	Age	Week	Estimate	Standard Error	DF	t Value	Pr > |t|
Week		0	3.4033	0.1606	2.27	21.19	0.0012
Week		2	3.6133	0.1606	2.27	22.50	0.0010
Week		4	3.8167	0.1606	2.27	23.77	0.0009
Age	12_M		2.9722	0.1806	3.68	16.46	0.0001
Age	18_M		3.6222	0.1806	3.68	20.06	<.0001
Age	24_M		3.6333	0.1806	3.68	20.12	<.0001
Age	30_M		4.0667	0.1806	3.68	22.52	<.0001
Age	36_M		3.7611	0.1806	3.68	20.83	<.0001

Differences of Least Squares Means

Effect	Age	Week	_Age	_Week	Estimate	Standard Error	DF	t Value	Pr > |t|
Week		0		2	-0.2100	0.06578	17.8	-3.19	0.0051
Week		0		4	-0.4133	0.08102	26.7	-5.10	<.0001
Week		2		4	-0.2033	0.06578	17.8	-3.09	0.0064
Age	12_M		18_M		-0.6500	0.1459	7.52	-4.46	0.0025
Age	12_M		24_M		-0.6611	0.1459	7.52	-4.53	0.0022
Age	12_M		30_M		-1.0944	0.1459	7.52	-7.50	<.0001
Age	12_M		36_M		-0.7889	0.1459	7.52	-5.41	0.0008
Age	18_M		24_M		-0.01111	0.1459	7.52	-0.08	0.9413
Age	18_M		30_M		-0.4444	0.1459	7.52	-3.05	0.0171
Age	18_M		36_M		-0.1389	0.1459	7.52	-0.95	0.3706
Age	24_M		30_M		-0.4333	0.1459	7.52	-2.97	0.0192
Age	24_M		36_M		-0.1278	0.1459	7.52	-0.88	0.4082
Age	30_M		36_M		0.3056	0.1459	7.52	2.09	0.0717

APPENDIX 4J: AL - AUTO REGRESSIVE

The Mixed Procedure

Model Information

Data Set	WORK.ROSEMARY
Dependent Variable	AL
Covariance Structures	Variance Components, Autoregressive
Subject Effect	Rep*Age
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
Rep	3	1 2 3
Age	5	12_M 18_M 24_M 30_M 36_M
Week	3	0 2 4

Dimensions

Covariance Parameters	4
Columns in X	24
Columns in Z	12
Subjects	1
Max Obs Per Subject	45

Number of Observations

Number of Observations Read	45
Number of Observations Used	45
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	160.01921773	
1	2	146.97608223	0.00040652
2	1	146.95565174	0.00001139

3	1	146.95511778	0.00000001
4	1	146.95511723	0.00000000

Convergence criteria met.

Estimated R Matrix for Rep*Age 1 12_M

Row	Col1	Col2	Col3
1	6.5163	4.3010	2.8388
2	4.3010	6.5163	4.3010
3	2.8388	4.3010	6.5163

Estimated R Correlation Matrix for Rep*Age 1 12_M

Row	Col1	Col2	Col3
1	1.0000	0.6600	0.4356
2	0.6600	1.0000	0.6600
3	0.4356	0.6600	1.0000

AL - AUTO REGRESSIVE

Covariance Parameter Estimates

Cov Parm	Subject	Estimate	Standard Error	Z Value	Pr Z
Rep		0	.	.	.
Rep*Week		0.06321	0.3109	0.20	0.4195
AR(1)	Rep*Age	0.6600	0.1309	5.04	<.0001
Residual		6.5163	2.1351	3.05	0.0011

Fit Statistics

-2 Res Log Likelihood	147.0
AIC (smaller is better)	153.0
AICC (smaller is better)	153.9
BIC (smaller is better)	150.3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Age	4	11.5	115.08	<.0001
Week	2	6.08	16.62	0.0034
Age*Week	8	17.7	1.04	0.4445

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Week Linear	1	12.6	33.22	<.0001
Week LOF	1	3.84	0.58	0.4917
AGE Linear	1	11.5	79.58	<.0001
AGE Quad	1	11.5	148.49	<.0001
AGE Cubic	1	11.5	9.14	0.0111
AGE LOF	1	11.5	223.11	<.0001

Least Squares Means

Effect	Age	Week	Estimate	Standard Error	DF	t Value	Pr > |t|
Week		0	53.0833	0.6749	14.6	78.65	<.0001
Week		2	55.6200	0.6749	14.6	82.41	<.0001
Week		4	57.4533	0.6749	14.6	85.13	<.0001
Age	12_M		55.3333	1.2377	11.6	44.71	<.0001
Age	18_M		56.4333	1.2377	11.6	45.60	<.0001
Age	24_M		76.6556	1.2377	11.6	61.94	<.0001
Age	30_M		44.7444	1.2377	11.6	36.15	<.0001
Age	36_M		43.7611	1.2377	11.6	35.36	<.0001

Differences of Least Squares Means

Effect	Age	Week	_Age	_Week	Estimate	Standard Error	DF	t Value	Pr > |t|
Week		0		2	-2.5367	0.5986	5.79	-4.24	0.0059
Week		0		4	-4.3700	0.7582	12.6	-5.76	<.0001
Week		2		4	-1.8333	0.5986	5.79	-3.06	0.0232
Age	12_M		18_M		-1.1000	1.7463	11.5	-0.63	0.5411
Age	12_M		24_M		-21.3222	1.7463	11.5	-12.21	<.0001
Age	12_M		30_M		10.5889	1.7463	11.5	6.06	<.0001
Age	12_M		36_M		11.5722	1.7463	11.5	6.63	<.0001
Age	18_M		24_M		-20.2222	1.7463	11.5	-11.58	<.0001
Age	18_M		30_M		11.6889	1.7463	11.5	6.69	<.0001
Age	18_M		36_M		12.6722	1.7463	11.5	7.26	<.0001
Age	24_M		30_M		31.9111	1.7463	11.5	18.27	<.0001
Age	24_M		36_M		32.8944	1.7463	11.5	18.84	<.0001
Age	30_M		36_M		0.9833	1.7463	11.5	0.56	0.5842