ASSESSMENT OF THE POTENTIAL OF COMFREY

(*SYMPHYTUM ASPERRIMUM*) AS A SOURCE OF

VITAMIN A IN A SCHOOL FEEDING

PROGRAMME

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DECLARATION

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DEDICATION

This work is dedicated to my loving parents, Mr. and Mrs. Wachira Chania for their endless support and prayers through out the course of my work.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

3. Social background

4. Dietary history

5. Information on daily dietary intake

6. Information sheet for serum ß-carotene and retinol estimates

7. Stool specimen sheet

ACRONYMS

1.	FAO	Food and Agricultural Organization
2.	ACC/SCN	Administrative Committee on Co-ordination/
		Sub-Committee on Nutrition
4.	CCD	Comfrey containing diet
5.	CFD	Comfrey free diet
6.	ß-C	Beta-Carotene
7.	Dm	Dry matter
8.	GLV	Green Leafy Vegetables
9.	GoK	Government of Kenya
10.	HB	Haemoglobin
11.	HPLC	High Performance Liquid Chromatography
12.	ILSI	International Life Sciences Institute
13.	IPGRI	International Plant Genetic Resources Institute
14.	IU	International Units
15.	IVACG	International Vitamin A Consultative Group
16.	KARI	Kenya Agricultural Research Institute
17.	KEMRI	Kenya Medical Research Institute
18.	MRC	Medical Research Center
19.	μG	Micrograms
20.	NRC	National Research Council
21.	PEM	Protein - Energy - Malnutrition
22.	RBP	Retinol Binding Protein
23.	RE	Retinol Equivalent
24.	S-ß-C	Serum Beta-carotene
25.	S-R-L	Serum Retinol Levels

26. S.mansoni   Schistosome mansoni

27. TFNC   Tanzania Food and Nutrition Center

28. URI   Upper Respiratory Infections

29. VA   Vitamin A

30. VAD   Vitamin A Deficiency

31. Yrs   Years

ACKNOWLEDGEMENT

ABSTRACT

The study was carried out in Gitathuru Approved School, Nairobi, Kenya. A sample of 85 children (8-13 years) whose haemoglobin level was <14 gms/dl were identified from a total of 106 children. All the study subjects were examined for the presence of parasitic infestation and later dewormed with mebendazole. Clinical examinations were carried out. The subjects were randomly allocated into an experimental group (CCD) whose diet was supplemented with 10 gms of comfrey powder/day and a control group (CFD) whose diet was not supplemented. The serum ß-carotene and serum retinol levels at the baseline and after 21 days of intervention were determined to assess the potential of comfrey as a source of vitamin A. Both pre- and post-intervention haemoglobin levels were not significantly different (P > 0.05).

Analysis of ß-carotene content of fresh comfrey, blanched-oven dried comfrey powder, and solar dried comfrey powders were carried out. Storage studies on ß-carotene stability, sieve analysis and adsorption capacity of comfrey powder were also carried out. The ß-carotene content of whole unblanched, oven dried comfrey powder was significantly higher (49.03±0.14mg/100gms than solar dried comfrey powder (34.3±1.9mg/100gms) by 30% with P<0.05. The content of whole steam blanched, oven dried powder was higher than that of chopped steam blanched powder.

The loss of ß-carotene after 5 minutes of cooking comfrey powder was 4.5% and 21.7% after 7 minutes of cooking.

After 2 weeks of storage of comfrey powder in 200 gauge black polythene bags at 25°c under CO_2, the loss was 27.8% (i.e. from 49.03±0.14 mg/100gms to about 35.4±0.24 mg/100gms) and fell gradually to about 55% after five weeks of storage.

There were no significant differences in base levels of serum **B** carotene and retinol concentrations between the two groups of children. However, the serum ß-carotene levels of the CCD group (4.99±1.28μmol/l) was significantly higher than those of the CFD group post intervention (P<0.05). On the other hand post intervention serum retinol levels for CCD group (0.29±0.18μmol/l) and those of the CFD group (0.37±0.17μmol/l) were not significantly different.

Analyses of data and storage studies shows that appreciable amounts of ß-carotene remained, indicate that comfrey powder has a potential for use in institutions where it can be stored for a relatively long period of time. The study indicates that further investigation on the bio-availability and bio-conversion of ß-carotene into active form of vitamin A is needed. Nevertheless, it should be noted increasing ß-carotene levels above baseline levels 4.39±1.28μmol/l) by supplementation is desirable.

CHAPTER ONE

1. *INTRODUCTION*

Malnutrition in form of Protein-Energy-Malnutrition (PEM) is prevalent in many developing countries. This PEM is always accompanied by micronutrient deficiency. It is also the most widely researched on form of malnutrition. Recently, however, attention has been focused on Vitamin A, iodine and zinc deficiencies which are now public health problems in many countries.

Vitamin A is of particular importance because it is required for immunocompetence and cell differentiation and consequently for epithelial cell turnover. If not addressed it could lead to protein-energy-malnutrition. A lot of work has been done on vitamin A deficiency (VAD), on young children (0-5 years) and on those with parasitic infestations and diarrhoea. There is a feeling in a number of studies that dietary intervention could be a long-term solution to vitamin A deficiency.

Preservation and processing of vegetables rich in ß-carotene is seen as one way of modifying the risk of VAD especially because of seasonality.

In Kenya, VAD is a major health issue. One of the causes is seasonality. The problem of vitamin A availability is more severe in marginal areas where vegetables and fruits are not common. The areas in Kenya with vitamin A deficiency include parts of Nyeri, Meru and Nakuru with moderate vitamin A deficiency of 10-20%. Mombasa, Kwale, Kitui, Kisii, Kisumu, Bungoma, Baringo, Garrisa, parts of Kiambu, and South Nyanza have severe VAD of > 20% (UNICEF, 1994).

While poverty, socio-cultural and socio-demographic factors can cause micronutrient deficiencies, ecological and climatic conditions also contribute to the changes because of decreased availability of foods rich in the aforementioned nutrients (VA, Iodine and Zinc). Another problem is that of plant pro-vitamin A substance mainly ß-carotene which is of low bioavailability. This is aggravated by low fat intake which decreases its absorption. Further, it is possible preparation into a vegetable other than a mercerate may limit the absorption and availability of provitamin A which is embedded in the polysaccharide and protein matrix.

It has, however, been reported, that the conventional belief that in areas where green vegetables and fruits are available in abundance the prevalence of vitamin A deficiency is low is wrong; UNICEF has drawn our attention to the fact that VAD may be wide spread even in areas where there is abundance of foods rich in pro-vitamin A substances (UNICEF, 1994).

In a number of countries, fighting vitamin A deficiency has been achieved by food fortification. This has met with a number of social, economic, political and logistical problems. The next alternative involving administration of vitamin A capsules is wrought with problems since excessive dosage of VA capsules can lead to hypervitaminosis A. However, excessive carotene from GLV is not known to have any adverse effects other than causing carotenaemia, an orange-yellow color on the skin (Dekker, 1994). Moreover, megadose vitamin A capsule distribution is more expensive than the promotion of GLV as a control of vitamin A deficiency.

With the above observations vegetables appear to be the best alternative. Therefore methods for their utilization as vitamin A source should be sought. These vegetables are the most feasible food stuffs in use for prevention of vitamin A deficiency and are readily available for a majority of the people who are of low economic status.

A number of traditional vegetables are extremely rich in ß-carotene. If they are dried with little vitamin A loss, they could be used during the dry season. A vegetable powder could be used for soups and even in infant gruels as is done in Zimbabwe. Here, the powders are sieved to decrease fibre content (Muroki, 1995). The availability of ß-carotene is most likely increased when powders other than whole green vegetables are used.

Comfrey is one vegetable which has a high carotene content and is low in fibre content. It is consumed as a vegetable in Papua New Guinea and has for a long time been used to prepare a tea often referred to as "comfrey tea" in Europe and Japan. In Kenya, it was introduced as animal fodder, but is now commonly used to make "irio" (potato mash). Abdilla, (1976) reported that it can form part of diabetic diet to prevent blood sugar glucose excursion. Preliminary studies by Muroki (1995) showed that comfrey is a rich source of vitamin A and C, calcium, iron and phosphorus. The contents of these nutrients may however change with seasons (Hill, 1976).

A women project in Guatemala showed that solar dried vegetables such as kales and fruits which have lower ß-carotene content than comfrey are a feasible means for alleviation of vitamin A deficiencies. Hence if inexpensive methods of drying comfrey can be made, comfrey powder can then be easily incorporated in infant foods and can be a source of the other nutrients mentioned above as well as protein which is reported to be about 30% dry matter (Abdilla, 1976).

1.2 *STUDY OBJECTIVES*

1.2.1: *Overall objective*

The overall objective of this study was to assess the potential of Comfrey powder as a source of vitamin A for school children of ages 8-13 years.

1.2.2: *Specific objectives*

1. To determine the content of ß-carotene in comfrey.

2. To determine the stability of ß-carotene content in comfrey during storage.

3. To determine the adsorption capacity of various particle sizes of comfrey powder.

4. To determine the effect of blanching and cooking on ß-carotene levels in comfrey preparations.

5. To determine the effectiveness of comfrey in raising serum ß-carotene levels of school children.

6. To determine the effectiveness of comfrey in raising serum retinol levels of school children.

1.3 *Hypotheses*

1. There is no difference between serum ß-carotene levels of children fed on diet supplemented with comfrey powder and those children fed on a diet without the comfrey powder.

2. There is no difference in serum retinol levels of children fed on diet supplemented with comfrey powder and those children fed on a diet without the comfrey powder.

3. There is no difference in baseline serum ß-carotene levels and the post-intervention serum ß-carotene levels of the children.

4. There is no difference in baseline serum retinol levels and the post-intervention serum retinol levels of the children.

1.4 *Potential benefits of the study*

The findings of this research are expected to be used:

a. By the nutrition fraternity with special interest in vitamin A and ß-carotene supplementation studies.

b. To provide data for effective planning of vitamin A interventions and use of green leafy vegetables especially comfrey powder as a source of vitamin A for the general population.

CHAPTER TWO

2.   *LITERATURE REVIEW*

2.1 *Overview of Malnutrition*

Poor nutrition is a powerful constraint to realizing human potential in societies. Operating in synergism with diarrhoeal, respiratory diseases, micronutrient deficiencies, and other infections, poor diets in early childhood lead to growth failure, delayed motor and mental development, impaired immunocompetence, blindness and increased risk of complications and death from infections (Waterlow, 1992). Children who grow up in environments of poverty and malnutrition in developing countries lead a life of diminished capacity.

Although malnutrition has been studied for many years, most of the attention was mainly focused on macronutrients (PEM) and not micronutrients. Recently after observing the devastating effects of the micronutrient deficiencies there has been increased attention to alleviate these deficiencies.

Single nutrient deficiencies particularly VAD, iron and iodine deficiency presenting in form of xerophthalmia, nutritional anaemia and IDD respectively are common at all ages. These diseases are also of regional public health importance (Stephenson, 1987).

Decreased food intake is reportedly the main cause of PEM. However a number of factors account for decreased food intake. These include: Poor economic status, poor health status (infections and parasitic infestations). The poor health status may be due to decreased nutrient intake, poor digestion, absorption and utilization of nutrients due to illnesses (cromption and Nesheim, 1984).

Cultural and socio-economic factors especially poverty, lead to low food production and consumption which may pre-dispose individuals to nutrient deficiencies. Ecological factors such as seasonality (of foodstuffs rich in certain nutrients like vitamin A), soils which may influence content of nutrient such as iodine of food could all pre-dispose individuals to malnutrition. Methods of processing and preservation of these foodstuffs are other factors which can affect nutrient content of food.

In general micronutrient deficiencies, result in losses of human potential, the social and economic costs of which communities can not afford. The impact of deficiency is not limited to a single organ but affect multiple organ systems (UNICEF, 1994). Thus iron is not just responsible for nutritional anaemia, nor iodine deficiency for goiter, nor Vitamin A just responsible for xerophthalmia. Hence the roles of these micronutrients in growth, development and immunity are more fundamental than has been formerly believed (Ziegler, 1997; UNICEF, 1994).

2.2 *Micronutrient deficiencies*

It is now recognized that micronutrient deficiencies have severe implications for the economic and social development of individuals, communities and Nations (UNICEF, 1994).

Micronutrients are major contributors to child health, survival and development. Their role in preventing morbidity and mortality has been extensively documented. Abundant evidence on morbidity effect is also accumulating (UNICEF, 1994).

In institutions, micronutrient deficiency are common due to limited dietary intake of a variety of foodstuffs. One of the most common deficiencies is Vitamin A deficiency. Vitamin A is of great importance because it interacts with many nutrients and its deficiency is much more common than the other three major micronutrient deficiencies (i.e iodine, zinc and iron). Its function in the synthesis of the epithelia cells is critical in disease prevention and in absorption and utilization of nutrients.

2.3 *Vitamin A deficiency*

Vitamin A deficiency is a serious health issue, it threatens the productivity of more than 2 billion people world wide (FAO/WHO, 1989). VAD is a single major cause of childhood blindness (UNICEF, 1998) and is associated with increased morbidity and mortality in young children under 5 years (FAO/WHO, 1992).

WHO estimates that between 250,000-500,000 pre-school children go blind from VAD, two thirds of these die within months of going blind (WHO, 1992). Other groups at risk include old people, school-age children, pregnant adolescent girls; refugees and displaced people (Savage, 1993; UNICEF, 1998).

VAD is also a common feature in institutions because of limited availability of VA-rich foods and also due to the use of dietary menus in schools which are not flexible.

VAD though known for a long time, has only received growing attention as a preventable problem of public health significance in the last 2 decades. It should be known that VAD with or without clinical signs is of a significant public health importance (UNICEF, 1994).

Reasons advanced to explain the lack of awareness of the true magnitude of the problem and its cost to society are given by Mckingney (1983).

A world wide survey in 1962-63 enabled WHO to document the widespread geographic distribution and high mortality rates associated with xerophthalmia. Subsequently WHO prepared a tentative list of 72 countries where VAD is suspected to be a significant public health problem but information is lacking (UNICEF, 1994).

In Tanzania, the Tanzania Food and Nutrition Center (TFNC) estimates that 1.36 million Tanzanians or 6.1% of the population are VAD deficient, 98% of whom are children under 6 years of age (Temalilwa, 1993). It is further estimated that there are 10,000 children suffering from xerophthalmia at any one time, with 2000-4000 new cases of blindness every year. This is very severe in drought stricken areas of the country. However, VAD has also been observed in areas where VA rich foods are plentiful such as Iringa, Kagera and Kilimanjaro (Temalilwa et al.,1993).

2.4 *Etiology of VAD*

Vitamin A is often inadequate because of the seasonality of VA-rich food sources and other food sources too (Stephenson et al., 1987). Inadequacy in food sources may lead to PEM which in turn may cause VAD particularly because of deficiency of retinol-binding-protein (Ziegler, 1997; Bender, 1993).

In under five year olds, early and abrupt stoppage of breast feeding will cause VAD. For example VAD is very common in India, Bangladesh and Indonesia, especially in children due to inadequate food intake, early and abrupt weaning. Even when rich sources of vitamin A rich foods are widely produced in a country there may still be VAD. In West Africa for instance the red palm which is rich in VA is not marketed widely such that VAD is still observed. In this region there is variation in VAD, with some areas having marginal deficiency.

Diarrhoea and deficient fat ingestion are other factors which may impair carotene absorption. Habitual intake of liquid paraffin increases VA requirement (Antia, 1973). Other factors include infections, diseases, parasitic infestations, poor absorption, transportation and utilization of nutrients.

Other major causes of VAD are poor economic status and lack of knowledge on the utilization of locally available resources and socio-cultural factors. Affordability of these foods is also an issue to reckon with. Poor living conditions may encourage parasitic infections and worm infestation may cause VAD among other deficiencies especially among inmates.

2.5 *Sources of vitamin A*

Vitamin A is a fat soluble vitamin. It is obtained from animal products and also plant sources. The VA-rich foods are cheap to obtain and are widely spread ecologically. ß-carotene provides 90% of all vitamin A in the diet in many developing countries.

Vitamin A in animal products is preformed (retinol). The sources of preformed form include milk, liver oils of shark, oils of marine fish e.g. halibut and marine mammals e.g. polar bear (Machlin, 1991). The provitamin A form (carotenoid) are mainly from plants. There are over 500 different carotenoids in nature but only a few have VA activity. These are α-carotene, ß-cryptoxanthin, ß-apo-8'-carotene, and ß-carotene (Simpson, 1986). ß-carotene is the most important carotenoid because it is nutritionally and biologically the most active. It is converted into retinol (the active form of VA) in a ratio estimated as 6:1 (Machlin, 1991). Sources of ß-carotene include dark green leafy vegetables, yellow and red fruits and red palm oil. There are traditional vegetables such as amaranths, cassava, sweet potato and pumpkin leaves, spinach and carrots with large amounts of ß-carotene (Antia, 1973). Yellow maize has VA-active carotenoids although it is not popular in many parts of Africa (Savage, 1993).

Among the richest sources of carotenoids is the red palm oil which has about 0.5 mg of mixed α and ß-carotene per ml. Thus about 7 mls of red palm oil per day should meet daily requirement of a pre-schoolar. Comfrey powder is also very rich in ß-carotene and it has a ß-carotene content of about 49 mg/100gms on dm basis (Muroki, 1995).

2.6 *Functions of vitamin A*

2.6.1 *Vision*

Vitamin A is best known for its role in vision (Guthrie et al.,1995). Retinol, supplied to the retina in blood, is converted to retinal. The retina of most vertebrates, man included contains 2 kinds of light receptors, rods for vision in dim light and cones for vision in bright light and color vision.

Retinal combines with a protein called opsin to produce the purple pigment called rhodopsin. Rhodopsin is located in the light sensitive rod cells of the retina. When light strikes the retina, the rod cells are bleached as the rhodopsin splits to form retinal and opsin. As the cycle continues a small amount of retinol is converted to retinoic acid or another inactive compound and is lost from the rod cells. This lost retinol must continually be replaced by fresh supplies of retinol brought by the blood.

Thus the amount of retinol in the blood determines the rate at which rhodopsin is regenerated. If regeneration of rhodopsin is slow, vision in dim light is poor as is the case in VAD (Guthrie, et al.,1995).

2.6.2   *Growth of bones*

Normal children's bones grow longer, and children grow taller by remodelling of each old bone into a new bigger version. Vitamin A is needed in the dismantling of the old bone structures and replacing them with new bone parts (Antia, 1973).

By helping restructure the jaw bone as it develops, Vitamin A permits normal tooth spacing. Crooked teeth and poor dental health can result from a deficiency in prenatal or early post-natal life (Passmore, 1986).

2.6.3   *Cell differentiation and gene expression:*

VA is not only involved in the development or differentiation of immature bone cells into different types of mature cells but also in the integrity of epithelial tissue (Guthrie et al.,1995; UNICEF, 1998).

Epithelial cells within the body normally secrete mucus and are covered in hair like cilia. The cilii on the lining of the genitourinary and respiratory systems prevent the accumulation of foreign material on the surface of the epithelial cells by their constant motion. The action of the cilia is involved in protecting the body against infection by sweeping the cell surfaces clear of invading micro-organisms.

Epithelial cells are continually shed and replaced, and because of this VA is required for their formation, a constant supply of VA is required for normal health.

The intestinal mucosa cell turnover and state which determines digestion and absorption of nutrients is dependent on sufficient supply of VA (Gibney, 1986; Passmore et al.,1986; Guthrie, 1989). Both retinol and retinoic acid are active in the promotion of growth, development and tissue differentiation (Bender, 1993). They have different actions in different tissues. Retinol and retinoic acid bind to intracellular receptors, and regulate the transcription of genes in different tissues and at different stages in development (Guthrie, 1995; Machlin, 1991).

2.6.4   *Immunocompetence*

The body's defences against infection and diseases depend on vitamin A among other factors; The immune mechanism protective cells require VA to function (Guthrie et al.,1995). Although it is not well known how it happens, Vitamin A precursor carotenoids and those that are not VA precursors stimulate the immune system (Dennet, 1984; Guthrie et al.,1995). This may be as the result of vitamin A's role as antioxidant (Savage, 1993; Bender, 1993).

2.7   *Interactions of vitamin A with other nutrients, diseases*
        and infections

2.7.1   *Vitamin A - iron interactions*

It has been known that VAD causes impaired hematopoieses (Koessler et al.,1926). Mejia in 1977 reported that low plasma retinol levels in children were correlated to low hemoglobin, serum iron and transferrin saturation values (Mejia et al.,1977; Keith et al.,1993; Surhano et al.,1993; Wolde et al.,1993). Hodges in his experimental study of VAD in human volunteers, found that hemoglobin values decreased in a pattern similar to that of plasma VA and that it increased with plasma VA.

In VAD, the intestinal absorption of iron is not affected but plasma iron levels fall, tissue iron deposits increase and the incorporation of iron into hemoglobin decreases (Machlin, 1991). Therefore VAD inhibits iron utilization and accelerates the development of anaemia.

2.7.2   *Vitamin A, diseases and infections*

Diseases influence VA by altering its absorption, storage tissue utilization and conservation or recycling. Absorption of both carotenoids and VA depends on the formation of bile salts containing micelles, on the cleavage of esters with pancreatic lipases or esterases and on the transfer of lipid moieties of the micelle into the mucosal cell.

Chronic lipid malabsorption syndromes will reduce the intestinal absorption of carotenoids and to a some what lesser extent the preformed VA (Olson, 1994). These syndromes include pancreatitis, biliary cirrhosis and sprue. Chronic diarrhoea and severe protein energy malnutrition also reduce the absorption of carotenoids and VA (WHO, 1982).

The efficient storage and transport of VA depends largely on the integrity of the liver. Liver diseases such as hepatitis, cirrhosis and liver cancer, all reduce both absorption and storage.

In cystic fibrosis both the plasma levels of vitamin A and the liver stores are reduced markedly (Olson, 1994). This condition is characterized by poor lipid absorption and impaired hepatic function.

Susceptibility to infection increases risk of VAD (Sommer, 1987; Dekker, 1994). Various infections affect transport and utilization of VA. Hepatitis, measles and URI reduce VA and Retinol-binding-protein levels of the plasma to a significant degree. Severe diarrhoea tend to raise plasma retinol and RBP values probably due to marked dehydration and hemoconcentration that occurs in that disease (Machlin, 1991). It has been observed that supplementation with VA may reduce the impact and severity of the aforementioned infections (Sommer, 1986). On the other hand VAD predisposes children to infections and diseases due to diminished immunity (Scrimshaw, 1968). This is because children with infections are at increased risk of having VA malabsorption, decreased mobilization of VA reserves and increased use of VA by immune systems. Thus a vicious cycle can develop and may lead to increased mortality (Bahl et al.,1995). Needless to say many other diseases also affect VA levels both in plasma and the liver.

2.7.3   *Vitamin A and parasitic infestation*

Parasitic infestations influence both plasma retinol values as well as overall VA status (Machlin, 1991). Human adults infested with intestinal flukes have mean plasma values only 1/3 of those of normal adults. Hookworm and liver fluke infestations lower plasma retinol levels by 1/4 (Machlin, 1991). Children and adults suffering from ascariasis or giardiasis show an impaired absorption of Vitamin A. Adults who suffer from liver schistosomiasis often associated with chronic salmonella infection, absorb VA poorly and have very low liver stores (Machlin,1991). Intestinal schistosomiasis and malarial parasitemia lower serum retinol levels in children and exacerbate VAD in rats.

Fat absorption which is necessary in VA metabolism can be impaired by parasitic infections of the intestine (Mahalanabis et al.,1979; Sivakumar; Reddy and Sivakumar 1972). Conclusively intestinal parasites seem to act mainly by reducing the absorption of VA and carotenoids, whereas liver flukes and schistosomes impair liver function and VA storage (Machlin, 1991).

2.8 *Vitamin A metabolism*

Dietary forms of vitamin A are retinyl esters, derived entirely from animal sources, and provitamin A carotenoids, derived largely from plant foods. After food is ingested, preformed vitamin A of animal tissues and the provitamin A carotenoid substances mainly of vegetables and fruits are released from proteins by the action of pepsin in the stomach and proteolytic enzymes in the small intestine (Machlin 1991).

In the stomach the carotenoids and retinol esters tend to congregate in fatty globules, which then enter the duodenum. In the presence of bile salts, the globules are broken up into smaller lipid congregates, which can be digested by pancreatic lipase, retinyl ester hydrolase, and cholesteryl ester hydrolyse (Machlin, 1991; Goodman, 1984; Dekker, 1994). The resultant mixed micelles, which contain retinol, the carotenoids, sterols, some phospholipids, mono-and diglycerides and fatty acids, diffuse into the glycoprotein layer surrounding the microvillus and contact with the cell membranes. Various components of the micelles, except for the bile salts are then readily absorbed into the mucosal cell, in the upper half the intestine.

The bioavailability and the digestion of vitamin A and carotenoids, are affected by the overall nutritional status of the individual and the integrity of the intestinal mucosa (Dekker, 1994). Nutritional factors of importance are protein, fat, vitamin E, zinc and probably iron (GoK-UNICEF, 1992; Erdman, 1988; Kuhnlein, 1992). Some fibre e.g. highly methoxylated pectins markedly reduce carotenoid absorption (Erdman et al.,1986). Carotenoid absorption has an absolute requirement for bile salts independent in its dispersion in its micelle (Olson, 1994), whereas VA in any of its solubilized form is readily absorbed.

Within mucosal cells provitamin A carotenoids are oxidatively cleaved to retinal (Dekker, 1994; Olson, 1965; Goodman, 1965). The retinal formed from carotenoids is mainly reduced to retinol by retinal reductase of the intestinal mucosa, and merges with retinol derived from dietary preformed vitamin A. Intracellular retinol is then largely esterified (70-80%) within long-chain fatty acids in the mucosa cell (Machlin, 1991). Retinol is circulated within the plasma by Retinol-binding-protein (RBP). The RBP serves as vehicles to solubilize and transport retinoids. The synthesis of RBP is reduced in protein deficiency and hence aggravates vitamin A deficiency (Antia, 1993). The secretion of RBP from the liver is also affected by estrogen levels and zinc deficiency hence reducing the circulating levels of retinol (Guthrie, 1995).

The overall absorption of VA is approximately 80-90%, with somewhat less efficient absorption at very high doses. The efficiency of absorption of carotenoids from foods is 50-60% depending on its bioavailability (Oslon, 1987; Antia, 1973). Absorption efficiency of carotenoids decreases markedly at high intakes or doses (Machlin, 1991; Olson, 1987).

2.9 *Vitamin A toxicity*

The fat soluble vitamin A, is not excreted in the urine. It is stored in the liver, hence deficiency symptoms are slow to appear. Since VA is stored in the body, it can be toxic if taken in excess. The toxicity of the vitamin depends on the age of the patient, the dose and the duration of administration. Hypervitaminosis A can be due to either acute or chronic ingestion of retinol. High intakes may result from prophylactic vitamin A therapy. Signs and symptoms of chronic VA intoxication include irritability, vomiting, loss of appetite, headache, dry and pruritic skin desquamation and erythematous dermatitis (Olum et al.,1990).

2.10 *Intervention programs to prevent VAD*

Researchers have tried many intervention studies to curb the spread and to treat reversible manifestations of VAD. VA prophylaxis has been commonly and widely used (Olum,et al.,1990). The use of massive doses of VA to prevent VAD is now feasible due to several unique physiological and practical considerations: (1) the high efficiency of absorption and storage which is largely independent of dose, (2) the ability of the liver to store efficiently very large amounts of VA and to release it at a relatively slow rate (3) the infrequency of manifestations of hypervitaminosis A at the dosage given (4) the relatively low cost of a large dose of VA and (5) its ease of administration (Machlin, 1991).

A number of intervention programs have involved vitamin A administration. Large doses of vitamin A delivery in the community are reported to offer a practical, effective approach to short-term control (Dekker, 1998). A study on semi-annual supplements of 200,000 International units (IU) of vitamin A, however, only marginally reduced the risk of xerophthalmia in Sudanese children (Annon, Vit A⁺ Sieve, 1994). Large doses of VA supplements have been used in Bangladesh, Sudan, Hyderabad, Zambia, Mozambique and Kenya (FAO/WHO, 1992). These doses of vitamin A capsule are supposed to be given every 4-6 months. However this is often impossible in practice due to logistical problems.

There is also need to provide an increased number of 100,000 IU doses or even 25,000 IU doses to infants, This becomes quite difficult to accurately dose children with 100,000 IU VA or less.Therefore, use of vitamin A Capsules may not be sustainable in the long run.

Vitamin A fortification is another approach which has been used as an intervention measure. In the Philippines, VA was fortified with monosodium glutamate (MSG) (Solon et al., 1985). This was because MSG is an important ingredient in traditional Philippine cooking. In Latin America and the Caribbean, fortifications of sugar with VA (palmitate) was carried out. In Guatemala within 2 years of fortification, out of 21.5% children with low VA levels only 8.9% had it (Arroyave et al.,1979).

In 1975 fortification program by the Institute of Nutrition of Central America and Panama (INCAP) fortified sugar with VA for children and lactating mothers for 5 years. This study showed a 3-fold increase in average daily intake of retinol equivalents (Mejia, 1985). In Kenya cooking fat (* Kimbo and Chipo brands) have also been fortified with vitamin A.

* Shortening cooking fat

Needless to say, fortification is a good method of fighting VAD but it is very expensive and sensitive in that before fortification proper evaluation of the safety of fortification for human consumption, technical feasibility of incorporating the nutrient, interaction of nutrients are issues to be addressed.

According to FAO, people will increase their dietary intake of vitamin A only if they understand the link between the vitamin and nutritionally induced ailments (UNICEF, 1994).

They need information about which plant and animal foods are rich in vitamin A, how to grow or obtain them, and how to store and prepare them without loosing the vitamin content. A good diet can be a reliable prevention measure of VAD. This is however, limited by availability of vitamin A or provitamin A rich sources because of such factors as socio-economic status and seasonality of the foodstuffs as vegetables.

With such observations as made here, increased dietary intake from local foods would be preferred. The current thinking is that most communities should be encouraged to use natural products to meet their vitamin A needs.

studies conducted by various scholars on how to alleviate vitamin A deficiency are quite exhaustive, and proximate analytical studies have identified most of the very rich vitamin A sources like green leafy vegetables, red and yellow fruits. Unfortunately these foods do not always find their way into the diet of the most vulnerable section of population.

Food based strategies to prevent VA deficiency emphasizes the production, processing, preservation and consumption of beta-carotene rich foods. A number of presentations advanced the premise that the food based approach is a strategy whose time has come since it aims to correct the underlying dietary causes of VAD. It is also a long term solution to fighting VAD.

The importance of this approach is underscored by the recent commitment strategies to meet micronutrient needs as set forth in the world declaration and plan of Action that was adopted at ICN and embodied in the 1991 policy statement of FAO on the use of nutrient-rich foods to prevent micronutrient deficiencies.

Several papers have reported findings from various food based intervention projects. In Tamil Nadu, India regular provision of small amounts of papaya and drumstick leaves coupled with nutrition education over a period of 1 year, measurably

improved child, mother and general family diets with respect to ß-carotene intake (XV IVACG, 1993). In Guatemala women have used dried carrots and papaya to control VAD successfully.

Another study carried out in Tamil Nadu in 20 schools participating in a state-wide 9 million Child school meal program, evaluated the effects of 12 months of nutrition education that emphasized VA rich foods. The prevalence of xerophthalmia decreased from 7% to 2% and mothers of children increased their awareness scores from 56% to 70%.

Increased attention is being given to raising VA intake through sustainable and effective kitchen gardening initiatives. A new four-province FAO-government project in Vietnam will identify, propagate and promote the production and consumption of suitable species and varieties of horticultural crops for home gardening. Advances are also reported on local ways to process and preserve VA-rich foods.

In Guatemala, instantized sweet potato buds have been locally processed, analyzed and tested for acceptability in the forms of gruel, cakes, puree and other dishes and although rural urban progress existed with respect to recipes, overall acceptance was 90% high (Noel, 1993). Solar drying has long been recognized as a local means to preserve and extend the availability of seasonal fruits and vegetables.

The VA field support project (VITAL) unveiled several solar dryers of different sizes, all constructable from local materials at low cost that minimizes drying time by their heat conductance and air convection properties and achieve up to 50%-80% VA retention.

In Senegal, mangoes, that were solar dried in a direct-indirect model dryer for 24-30 hours achieved a lower moisture content (8%-10%) and a similar level of VA retention, 8%-10% (2700 RE/100g) as well as appreciable retention of vitamin C (Rankins, 1993; Morris, 1993).

A lot of traditional vegetables will retain appreciable amounts of ß-carotene even after solar drying (Yadar et al.,1996). However these methods of processing and preservation of vegetables and fruits are not carefully evaluated and carried out to determine the retention of vitamin A and its availability.

Availlability can be greatly reduced by interaction with other food components. ß-carotene embendment in leaf matrix as aforementioned may decrease its availability unless broken either physically or by enzymatic action. Vegetables can be broken by grinding after drying inorder to increase their surface area which in turn increases the adsorptive capacity of the foods (Gregg, 1967). Effort should be made to also look

into resolving issues related to packaging, reconstitution ability, shelf life and marketing of the processed food to improve availability and consumption of a local ß-carotene rich food.

2.11 *The role of traditional vegetables*

More than 70% of vitamin A in developing countries is derived from plant sources (ACCN/SCN, 1992; Olson, 1996). In 1967 Rao indicated that 90% of all vitamin A in human diet is derived from ß-carotene (Rao, 1967). Traditional vegetables are among the most important sources of vitamin A in developing countries where animal sources of the vitamin are unaffordable (Mason et al., 1993). Most traditional vegetables such as amaranths, pumpkin and cowpeas leaves grow easily on the farms. However these vegetables are quite seasonal.

The loss of ß-carotene from these foods during preparation can be minimized by use of moderate temperatures and covering the pots when cooking (Imungi, 1996) while drying, processing and preservation will guarantee supplies during the dry period.

2.12   *Comfrey*

Comfrey which can be considered exotic is another vegetable with potential for alleviation of VAD (Muroki, 1995). It was introduced in Kenya as fodder crop. Comfrey has been found to have appreciably high level of pro-vitamin A (Muroki,1995). It is possible that the availability of ß-carotene in comfrey is high because it is low in fibre content.

Comfrey consumption would be an appropriate alternative to administration of vitamin A capsules and other vegetables because it is cheaply and readily available in Kenya. The fresh leaves of Russian comfrey are expected to have more vitamin A than the dry one (Muroki, 1995).

The Russian comfrey which was the form introduced in Kenya as animal feed is different from 'plain comfrey' which is *Symphytum asperrimum*. Comfrey has high adaptability and can therefore grow in a wide range of ecological zones. It is cheap and easy to grow (Hill, 1976).

Experience shows that the crop can thrive very well in kitchen gardens, school farms and can provide enough Vitamin A for a household and school children. Research has shown that it is more advantageous to grow the crop on large scale than small scale (Hill, 1976).

Russian comfrey has been used as human food for many years. This green leafy vegetable can be consumed in different ways. Research has shown that it can be consumed in tea in form of powder (Hill, 1976). Experience shows that it can be eaten with Ugali (a semi-solid cake prepared from cereal or cereal root-crop flour) or be mashed with solid or semi-solid gruels/cakes of cereal, legume or rootcrops or a mixture of one or more of these foodstuffs. This vegetable could also be consumed as soup made from the powder (Hill, 1976).

Russian comfrey and the *Symphytum asperrimum* contain less dry matter than any other fodder crop except drum head cabbage, but dry matter yield per year is more than that of other crops e.g. marrowstem, Kale, green maize, drumhead cabbage.

Comfrey is among the fastest protein and carbohydrates builders and at one time it has been recommended for the undernourished children of the developing Nations (Abdilla, 1976).

It leads in crude protein productivity content which can be as high as 3.6 tons per year which is more than that of soy beans which is 1.3-1.8 tons (Hill, 1976).

The protein and carbohydrates production per year are seven and eight times those of soya bean respectively. It is also a rich source of vitamin B_{12} and as can be seen in Table 1 dried comfrey has higher or comparable amounts of four essential amino-acids compared with legumes as well as dairy products.

Table 1: Comparative analysis of Protein, Methionine, Lysine and Isoleucine content in comfrey and other foods.

	Total Protein %	Methionine %	Tryptophan %	Lysine %	Isoleucine %
Comfrey Tea	34.6	0.58	0.64	1.41	1.15
Soya beans whole	34.9	0.51	0.53	2.41	2.05
Butter beans (dried)	21.34	0.31	0.18	1.25	1.28
Cashew nuts	18.93	0.31	0.41	0.68	1.06

Source:D. Hill, Analysis of Russian Comfrey London.

The table does not suggest that comfrey tea, as drunk with milk and sugar in Britain and with lemon juice or dried pepper nut in U.S.A. has this percentage of amino acid. It was simply used as a source of dried comfrey to show the proportions of them there would be in a dried flour made from the foliage.

In Universities of California, New Zealand and Kenya the crop is cut all year round. Comfrey is established from root cuttings and will last for 20 years from planting.

CHAPTER THREE

3.   *STUDY SETTING AND METHODOLOGY*

3.1   *Study area and study subject*

The study was conducted at Gitathuru Approved School in Lower
Kabete, Nairobi, Kenya. This is a rehabilitation school and
the only reception center for male juvenile delinquents in the
country. It is a government owned institution under the Child
Welfare Department, Ministry of Home Affairs.

The study subjects were enrolled in the school between January
and April 1997 from the juvenile ccurts all over the country.
The children remain in the school for three months before
being distributed to the various approved schools in the
country.

Gitathuru Approved School has nine levels of classes, (i.e.
from nursery up to standard eight). Children are distributed
into these classes as they join the school according to their
previous level of education before conviction.
This is done regardless of their age. The study subjects were
distributed as in the following proportions: Nursery school
14.5%, Standard one 30.1%, Standard two 15.7%, Standard three
13.3%, Standard four 3.6% and Standard five 3.6%.

At the time of the study, the pupils age ranged between 8 and 16 years. The school had 320 male pupils of whom 85 (8-13 years) were enrolled as the study subjects. These were mainly from Nairobi, Central Province (Nyeri, Murang'a, Kiambu), Eastern Province (Meru, Machakos, Kitui, Embu), North Eastern, Western and Nyanza Provinces. Nairobi Province made the highest proportion of children which was 8.4%. About a quarter (24%) of the children had been in custody for more than 2 weeks while 16% were there for 2 weeks.

3.2   RESEARCH METHODOLGY

3.2.1   Study design

The study was experimental and interventional in nature (Figure 1). The baseline and post-intervention serum ß-carotene and retinol status of children whose diet was supplemented with comfrey powder (CCD group) and that of those children who were on a comfrey free diet (CFD group) were determined using HPLC biochemical method (IVACG, 1982). Incorporated is laboratory analyses of fresh comfrey, comfrey powder processed in different ways, cooked comfrey powder and comfrey powder stored under CO_2 (as shown in figure 2) and described in sections 3.6.1-3.6.6.

The adsorption capacity of comfrey powder of various sizes were determined as this has bearing on absorption and availability of nutrients (Gregg, 1967). The comfrey powder was incorporated into the meals of the experimental subjects on a daily basis for twenty one days beginning May, 1997.

For the purpose of the study, it was necessary to deworm the children. Clinical examination for any indication of vitamin A deficiency, any infections and parasitic infestations were also carried out. The latter two may have potential to influence the absorption and bioavailability of vitamin A.

The haemoglobin (Hb) level of the children was also determined since this is found to be associated with vitamin A status (Hodges et al.,1978; West et al.,1993; Mejia,1985).

Figure:1 Study Design and Methodology
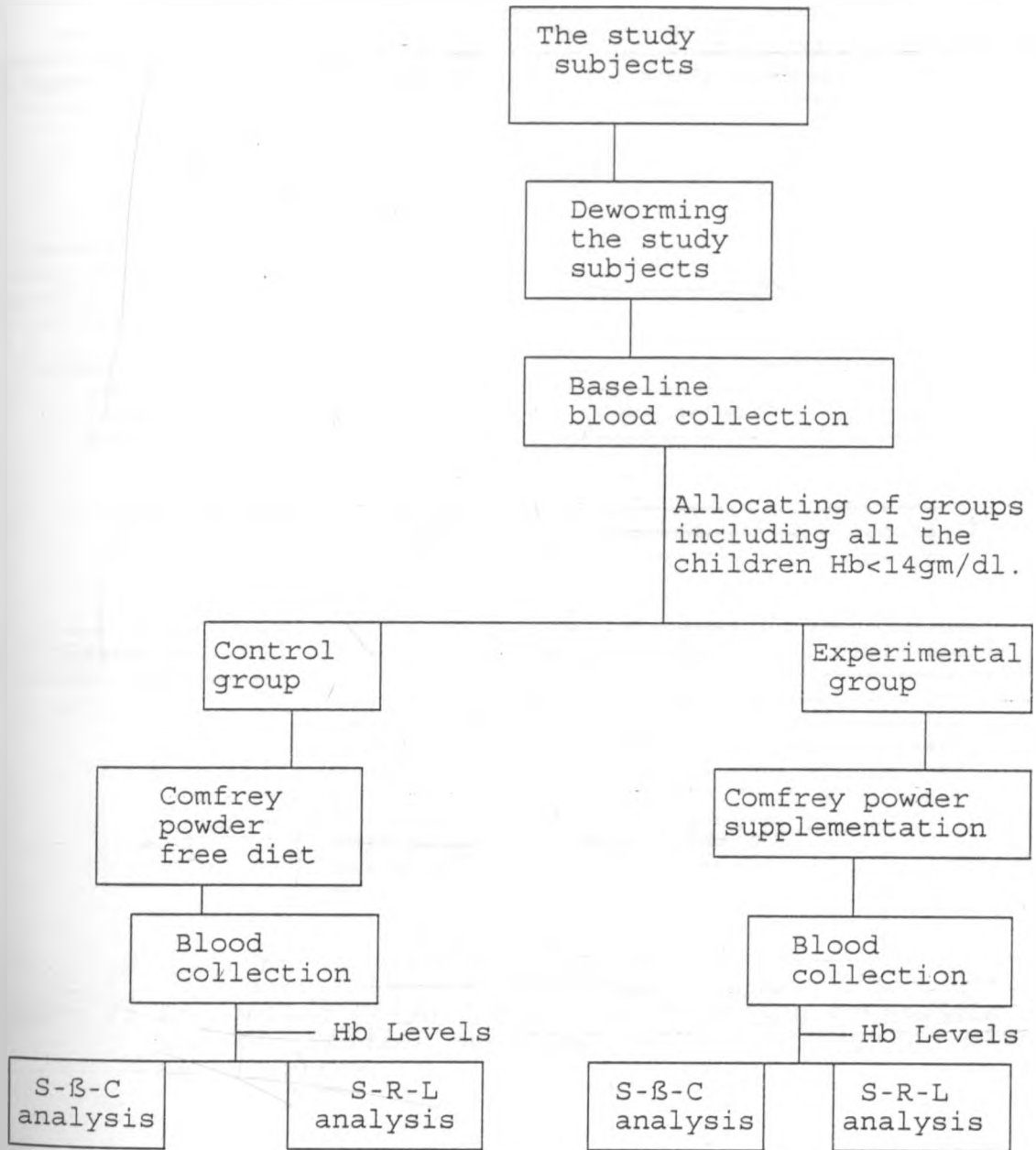
S-ß-4C: Serum ß-Carotene
S-R-L: Serum Retinol Levels
Hb   :Haemoglobin levels
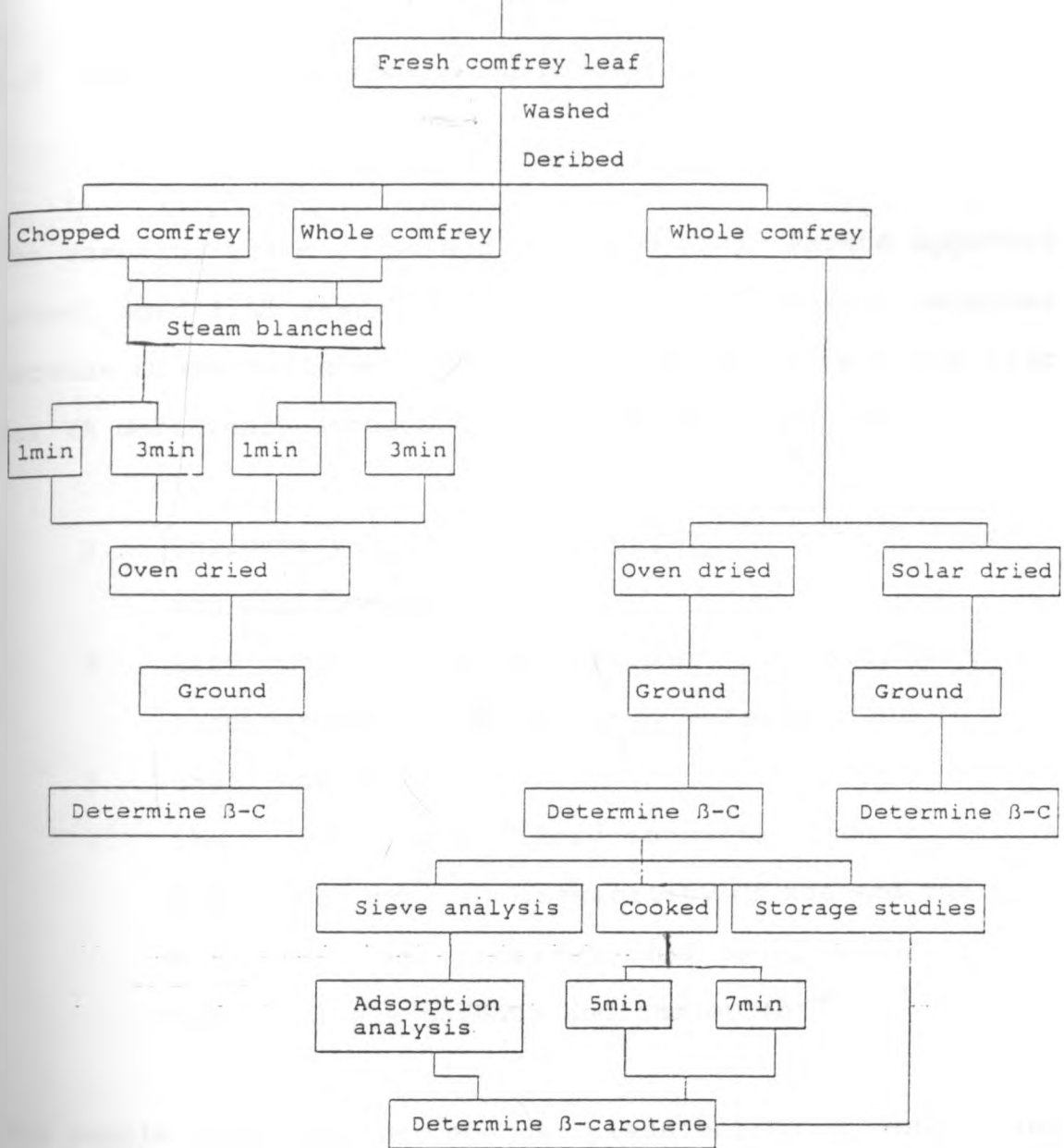
Figure 2: *Processing Fresh Comfrey to Determine ß-carotene and for Studies on Effect of Processing and Storage on ß-carotene*

3.3 *Sample size   and sampling procedures*

The sampling frame comprised of 320 boys in Kabete Approved School aged 8-16 years. The school was purposively selected because it was believed that the children would have high risk for VA deficiency among other reasons which include:

1. Uniformity in the feeding pattern of the control and experimental group.
2. Easy control of conditions and avoidance of interference of the study by external factors.
3. Small age variation.
4. The children were likely to suffer from vitamin A deficiency due to vulnerability of age and the fact that they came from deprived backgrounds (broken homes, single parents and the streets).

The sample size took into account plausibility of results and the financial constraints. The sampling procedure is presented in Figure 3. From the sampling frame all the children (106) aged 8-13 years were registered as the study population. Haemoglobin determination was carried out. All the children (85) whose haemoglobin levels (Hb) were less than 14gms/dl were included in the study.

These were then randomly allocated into two groups, i.e. an experimental group of 44 children whose diet was supplemented with comfrey (CCD) and a control group of 41 whose diet was without the comfrey powder (CFD). All the 85 children were dewormed by giving a full dose of 'Mebendazole'.

Exclusion criteria:

- Age greater than 13 years.
- Suffering any type of morbidity except for skin diseases which practically all the children had.
- Haemoglobin status greater than 14 gms/dl.

Figure 3: Sampling Procedure
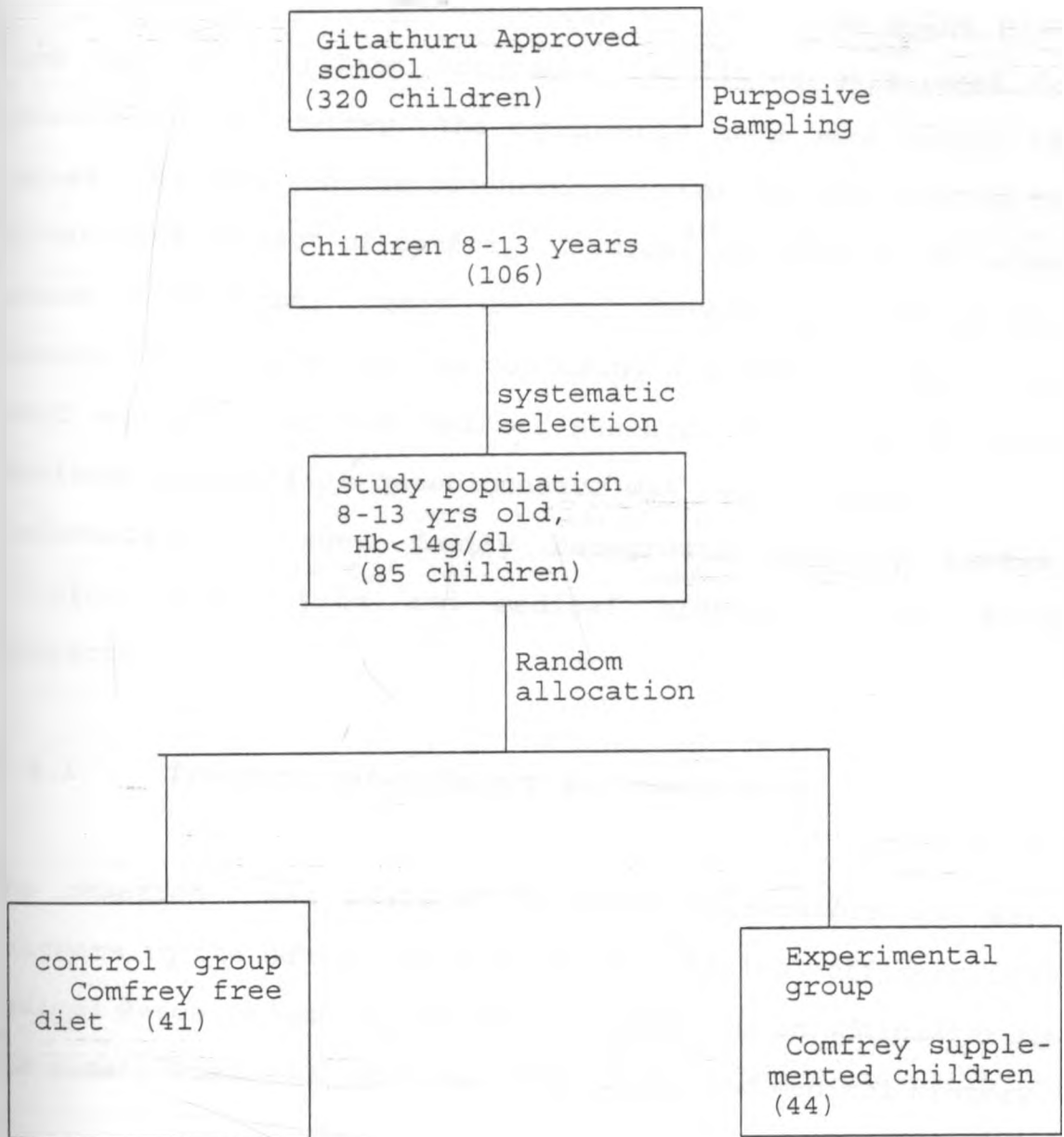
3.4 *STUDY INSTRUMENTS AND DATA COLLECTION*

The department of Food Technology and Nutrition pilot plant and Applied Nutrition Programme facilities were used for processing of comfrey. The equipments used were mainly the drier, the mill and the spectrophotometer for determining the ß-carotene content of comfrey. Chemical analyses to determine serum ß-carotene, serum retinol levels and haemoglobin determination were carried out using a spectrophotometer and HPLC equipment at the Medical Research Center at Kenyatta National Hospital. A questionnaire was used to seek information on age, family background, dietary intake, clinical examination and medical history of the study subjects.

3.4.1 *Training interviewers and assistants*

The researcher was assisted by three enumerators who were teachers in the school and also by two clinical officers from Medical Research Center, Nairobi who assisted in administering the questionnaire on clinical examination and medical history.

The teachers were intensively trained on interpretation of the questionnaire. They were quite conversant with the methods of interaction with the respondents.

There was also intensive training of research assistants in the processing of the comfrey powder. The assistants were trained on how to transport the fresh vegetable from the farm, remove the mid rib, spread the leaves on the stainless steel wire mesh trays for drying in a Fessman (Switzerland) air ciculating oven dryer, milling using a Sanyo (Japan) blender, cooking, preserving under CO_2 and packaging and storing the ready flour.

3.4.2   *Questionnaire*

A structured questionnaire (Appendix 1) consisting of questions on, level of education of the child, dietary history, medical history and length of stay in the approved school was administered to the children.

Teachers responded to questions regarding children for further verification of details. Information on the date of admission was obtained from the admission registers, and medical history data from the school sanatorium.

3.4.3   *Child age determination*

Information on age was obtained from the school register. However, to confirm the age beyond reasonable doubt, a clinical examination using the level of pubic hair of the child and dental count was carried out by clinical officers from the Medical Research Center (MRC), Nairobi.

3.4.4   *Food intake*

The amount of food consumed by the children was weighed using a dietary scale for all the meals eaten in a day. These measurements were done for a weekend day and normal day. The amount of vitamin A, protein and calories of each ingredient consumed by the child was calculated using National Food Composition Tables prepared by Sehmi (1993).

Determination of body mass index (BMI)

Anthropometric measurements, namely: weight and height were taken after it was observed that the caloric and protein intake were very low and not even sufficient for basal metabolic requirement. The weights were taken using a Salter scale to the nearest 0.1 kg. while the heights were taken using a stadiometer to the nearest one centimeter.

3.4.5 *Implementation of research activities*

In December, 1996, the researcher obtained a research permit from the Office of the president (OP). The study objectives and methodology were tabled before the National Ethical Committee at Kenyatta National Hospital. These were then communicated through the director of Children's Welfare Department, Ministry of Home Affairs, (OP) to the institution in early January, 1997.

Part of the pilot study followed shortly but the actual study did not begin until early May, 1997.

General information about the study area was collected from the available literature, the school manager and local people.

The logistics of how to supplement the diet with cooked comfrey powder was tested on 10 children.

Over the duration of the field study, the questionnaires completed each day were checked by the researcher for completeness of data, clarity and consistency of answers. Data from laboratory tests were scrutinized immediately a sample was analyzed. Data was entered and cleaned in Dbase III+ software and the Statistical package for social scientists (SPSS) both at the Applied Nutrition Program unit (ANP) and Medical Research Center (MRC), Nairobi.

The statistical tests included t-Tests, Pearson Correlation, one-way ANOVA, Levene's test for equality, Fisher's exact test and Mann-Whitney test (non parametric) statistics (Daniel, 1991; Steel, 1980).

3.4.6   *Validation and reliabity*

The researcher closely supervised the data collection, process of comfrey meal preparation and administration and blood collection.

3.5 *Processing and packaging of comfrey*

Fresh comfrey leaves which were grown during the wet season were obtained from Nairobi show ground. These were immediately transported in polythene bags at a temperature of about 22°C-25°C to the Department of Food Technology and Nutrition (DFTN) pilot plant cold room (2-4°C) within an hour of harvest and processed using different methods as described below.

3.5.1 *Processing of unblanched comfrey powder for chemical analyses and sieve analysis*

The fresh comfrey was washed and excess water allowed to drain for 20-30 minutes. The midribs were removed and the vegetable finally dried on stainless steel wire mesh trays of the Fessmann (Zurich) air circulating oven. The drying at 60°C for 3 hours, followed by milling in a Sanyo food mix blender was as follows: About 50 gms of leaves were broken by adjusting a Sanyo blender to the "blending" operation for two minutes.

The blender was then adjusted to the "liquefying" operation and the vegetable processed for another 2 minutes. The vegetable was further milled to increase the surface area by adjusting the blender to the "flash" position also for 2 minutes. The powder was sieved through a 0.125 nanometer (nm) sieve. This was poured in 200 gauge black polythene bags in 500g packs, flashed with carbon dioxide gas to expel air, then tightly sealed. These were then stored under refrigeration at -8°C until analysis was carried out and for use in feeding the children.

3.5.2 *Preparation of cooked comfrey powder for determination of ß-carotene*

The unblanched, and oven dried powders were cooked as follows: About 10 gms of powder was fried in 20 gms of fat and 50 mls of water added and cooking done for 5 minutes from boiling for one sublot of the sample and for 7 minutes for another sublot of the sample. These were then allowed to cool at room temperature (25°C) for 15 minutes and analyzed for ß-carotene content.

3.5.3 *Preparation of cooked comfrey powder and meal administration*

Unblanched, oven dried comfrey powder cooked as described above was used for the intervention, which lasted twenty one days. Five hundred grams of the comfrey powder was obtained as described in section 3.5.1 then fried for 5 minutes. Two hundred and twenty grams of fat (*Kapa fat, a Kenyan brand) were melted in a cooking pot, One and a half liters of water was added into the fat and a table spoonful of '** Mchuzi mix' flavoring mixture (from East African Industries, Nairobi, Kenya) was added. This was brought to boil and then the 500 gms of powder was introduced with constant stirring for two minutes at which it was well mixed.

*Vegetable fat; **Seasoning agent made of beef flavourants, Yeast, starch and MSG.*

This was covered and left to cook for 5 minutes before being removed from the fire. The cooked comfrey which was now in form of a cake was allowed to cool at room temperature (25°c) for about 5 minutes.

A bowl of maize and beans, ugali and beans or rice and beans were supplemented with comfrey cake (85 ± 1.9 gms) for each child in the experimental group. It was then well mixed with the rest of the food.

The children were then assembled for the meal after all the food in the bowls was well mixed and close supervision by the researcher to ensure that the children ate all the food (no remains).

The intervention was carried out once per day (at lunch time) for 21 days without a break. After the supplementation period was over, blood samples (5 ml) were drawn from each study subject for haemoglobin, serum retinol and ß-carotene level analysis.

3.5.4   *Processing of blanched comfrey material*

Whole and chopped comfrey (both without the midribs) were steam-blanched. The sublot of whole comfrey leaves without the midrib was steam blanched for one minute while another was steam blanched for three minutes. These were quickly cooled with clean cold water while still in the blancher.
Excess water was allowed to drain on stainless steel wire mesh trays for 15-20 minutes and then dried in an air circulating oven at 60°c, milled, packaged and stored as described in section 3.5.1 for unblanched comfrey powder (section 3.5.1).

Another lot, also without the midribs was chopped quickly into pieces of approximately 2cm x 2cm, and divided into two sublots, one sublot was blanched for one minute and the other for three minutes. These were processed as described for the unchopped comfrey and packaged, stored in a similar manner.

3.5.5   *Processing of sun dried comfrey*
The whole comfrey was washed and excess water allowed to drain for 20-30 minutes. The midribs were removed and the vegetable dried in a direct solar drier for 24 hours with mean temperature recorded as 25°C. The leaves after crushing between the palms were milled in lots of 50 gms at a time in the Sanyo blender, packaged and stored as described above (section 3.5.1).

3.6   *Analysis of comfrey*

3.6.1   *Moisture content of comfrey powder*

The moisture content of the comfrey powder was determined as follows: Five grams of powder were weighed, into an aluminium dish. This was put in an air oven maintained at 105°c for four hours.

It was then cooled in a desiccator and weighed. The powder was returned in the oven, dried for another thirty minutes, cooled in a desiccator and weighed again. This operation was repeated until constant weight was observed. The moisture content and dry matter were then calculated.

3.6.2   *Water content of comfrey leaves*

Fresh comfrey leaves were cut into small pieces and 250 gms were weighed into a porcelain dish. This was allowed to dry overnight at 60°c in an air circulating oven and then cooled in a desiccator. The dry comfrey leaves were then weighed and the percentage water loss calculated. The total water content was then calculated as; water loss at 60° and moisture content determined as above (section 3.6.1).

3.6.3 *Beta-carotene analysis of comfrey*

The content of ß-carotene in comfrey was determined in samples that were subject to different treatments (ie unblanched oven dried comfrey powder, steam blanched comfrey powder, sun dried comfrey powder, cooked comfrey powder and unprocessed fresh comfrey materials) as follows.

Two grams of powder was transferred into a mortar, then adding acetone as the solvent was crashed using a pestle. The extraction was repeated several times until there was practically no green colour. The extract was then filtered into a 100 ml volummetric flask and made up to the mark with acetone. Twenty five milliliters of the extract was then transferred into a 50 ml round bottomed flask and evaporated to dryness in a Heildoph vacuum evaporator.

The yellow ß-carotene pigment was eluted through the silica gel-petroleum ether column and collected into a 25 ml volummetric flask and then made to the mark with petroleum ether. Absorbance was read at 450 nm using a spectrophotometer and ß-carotene content determined using a ß-carotene standard curve.

3.6.4   *Fresh comfrey leaves*

The vegetable was washed as aforementioned. About a 100 gms of the whole fresh leaf was ground by use of mortar and pestle using acetone as the solvent. The ß-carotene dissolved in petroleum ether was analyzed as described above (section 3.6.3).

3.6.5   *Storage studies*

Studies to determine loss of ß-carotene in comfrey powder stored in black polythene bags under CO_2 were conducted. Comfrey powder was processed and packaged as described earlier. This was then left at room temperature and analyzed after two weeks, three, four and five weeks for change in ß-carotene.

3.6.6   *Analysis of adsorption capacity of comfrey powder*

The adsorption and surface area analysis was conducted using the methylene blue dye stuff. About 0.05g of comfrey powder was put in a tube and 10ml of distilled water added. The mixture was shaken and mixed for three minutes on a roller (Coulter mixer, Coultronics. France SA 14, Rue E.Legendre MARGENCY 95580 ANDILLY).

This was then centrifuged for three minutes at 3000 revolutions per minute (RPM) using MSE Minor (London, England) centrifuge. The supernatant was discarded using a pasteur pipette carefully leaving behind the comfrey powder residue. 0.05g/500ml of Methylene blue dye stuff was diluted into 20/100ml of the dye solution. Then 10ml of the diluted dye solution was added to the comfrey powder residue. It was then shaken and mixed on Coulter mixer as mentioned above for three minutes and centrifuged for three minutes at 3000 RPM using MSE Minor (London England) centrifuge.

Five milliliters of the supernatant was taken using a pasteur pipette into a clean tube. Two milliliters of the 5ml was put in a cuvette and absorbance read at 661.6nm using PERKIN ELMER Labda 15 uv/vis Spectrophotometer. The adsorption capacity was then calculated for the various particle sizes.

3.7   *Analysis of serum ß-carotene and retinol levels and determination of haemoglobin level*

3.7.1   *Blood collection*

Blood collection and sample preparation were carried out with the assistance of laboratory technician from Medical Research Center, Nairobi.

Five milliliters of venous blood was collected after a clean venipuncture using a microlance 21g needle and a 5ml sterile disposable syringe both from BECTON DICKINSON (B-D).

The blood was removed from antecubital vein after cleaning the area with surgical spirit swab. An aliquot of one ml. of blood was put into a bottle with Ethylene Diamine Tetracetic Acid (EDTA) for haemoglobin determination.

The remaining 4 mls of blood was put in sterile screw cap bottles covered with aluminium foil and kept in a cool box maintained at 8-10°C with ice packs. The labelled samples were then transported to the laboratory where full Hb level was determined immediately and the serum was separated from the clotted blood.

3.7.2   *Determination of haemoglobin levels*

Haemoglobin levels were determined using haemoglobin cyanide (cyanomethemoglobin) method of biochemical analysis using venous blood (Drabkin & Austin,1932). The analysis was carried out using electronic counter (Coulter Electronics Ltd).

Procedure:

A 1:500 dilution of whole blood from the EDTA bottle for Hb determination was made in Isotone II (Coulter Electronics Ltd) using a Double Diluteur III (Coulter Electronics Ltd).

This was made by taking 0.04 mls of blood and mixed in 20 mls of Isotone II, six drops of Zap-oglobin (coulter Electronics Ltd) was added to lyse the erythrocytes and release the haemoglobin pigment which is equivalent to the haemoglobin cyanide which is measured at 525 nm using Coulter Counter Model M530. The Coulter Counter Model M530 was calibrated using 4C Normal and checked by 4C Abnormal.

3.7.3 Separation of Serum

The clotted blood was centrifuged at 3000 revolutions per minute using an MSE model centrifuge. The clear serum was separated using a pasteur pipette and put into a sterile cryotube, labelled by name and study number using a permanent marker.

The serum was flushed with nitrogen gas, covered with aluminium foil and kept at a -40°C deep freezer until analyzed for retinol and ß-carotene which was done within three months using High performance liquid chromatography (HPLC) method of biochemical assay as described below.

3.7.4 : *Analysis of serum ß-carotene levels*

Principle: Beta-carotene was extracted with petroleum ether and measured at 450 nm using a spectrophotometer against a petroleum ether blank (Neeld, et al., 1963).

Procedure:

An aliquot of 0.25 ml of serum was pipetted into glass stoppered test tube. Then 0.25 ml of ethanol (95%) was added and vortexed for 20 seconds, petroleum ether (1.5 ml) was then added. The tube was stoppered and the contents mixed vigorously on the vortex mixer for 2 minutes to ensure complete extraction of ß-carotene.

This was then centrifuged at 3000 revolutions per minute for 10 minutes in an MSE centrifuge to obtain a clear separation of phases. One milliliter of the upper layer of petroleum ether extract was pipetted into a cuvette and absorbance measured at 450 nanometer (nm) against petroleum ether blank and the ß-carotene determined using ß-carotene standard curve.

3.7.5 *Serum retinol analysis*

Serum retinol levels were determined using High performance liquid chromatography (HPLC) biochemical method of analysis (IVACG, 1982).

Procedure:

The frozen serum samples were removed from the deep freezer and thawed at ambient temperature (25°C) and vortexed. One hundred micro-liters of serum was pipetted into a conical centrifuge tube with a screw-cap using a 40-200μl Finn pipette. Five micro-liters of 100ug/ml of the internal standard (Retinyl Acetate) was added using Hamilton pipette, A 100μl of methanol was added and vortexing done for 20 seconds to denature the proteins. Two hundred micro-liters of diethyl ether: dichloromethane: 2 propanol in the ratio of 80:19:1 respectively was added then vortexed for 1 minute.

This was then centrifuged at 3000 rpm for two minutes, removed from the centrifuge and a 100 μl of the upper layer of supernatant was taken and evaporated in a waterbath at 50°C under a gentle stream of nitrogen. The residue was then reconstituted with 50 μl of mobile phase consisting of 95 parts methanol and 5 parts distilled water.

Twenty microliters of the analyte was then injected into the HPLC column using Hamilton (50μl) gastight syringe no. 1705. The retinol peak was detected at 328 nm using a Hitachi L-4000H UV detector. The peaks and the peak area counts were recorded by Hitachi D 2520 the GPC intergrator. A 10μm particle U Bondapak C18 3.9 X300mm steel column (waters) was used. A C18 guard column was used to protect the main column.

CHAPTER FOUR

4.   *RESULTS*

This chapter presents data on the characteristics of the children at Gitathuru Approved School. The chapter also presents data on dietary intake, haemoglobin status, and prevalence of parasitic infections, all of which may affect the vitamin A status of the children.

Also presented are data on sieve analysis and adsorption capacity determination, the ß-carotene content of comfrey, data on the effect of storage on ß-carotene levels of the comfrey powder and data on the effect of cooking of comfrey as these would determine the potential for utilization of comfrey in supplementation of diets of large institutions. Results of serum ß-carotene and retinol levels before and after supplementation of the children's diet with comfrey powder are also presented.

4.1 *characteristics of the study subjects*

The social background of the children differed greatly. Some of the children came from stable, broken and single family backgrounds (32.5%), while about two thirds (63.9%) were street children.

The age distribution in the two groups (Comfrey containing diet group and Comfrey free diet group) was as shown in Figure 4. There was no significant difference between them.

4.2 *Dietary intake and body mass index*

The average daily intake of foods is presented in Table:2. The children consumed a diet that was grossly deficient in green leafy vegetables. The diet contributed to less than 20% of RDA for energy and 15% of RDA for protein. The contribution to vitamin A was negligible (i.e. only 0.14%) of RDA).

The mean body mass index (BMI) was 15.06 ± 0.13 which is well below the cut-off point i.e BMI=18 (Gibson, 1993; WHO 1995).

4.3 *Haemoglobin status*

There was no significant difference between Hb levels of the two groups at baseline and even after intervention neither was there any significant changes between the two groups (Table 3:). The average Hb level in both groups was 12.6 gms/dl.

4.4 *Parasitic infestation*

There was no significant difference in parasitic infestation rates between the two groups. The children were mainly infested with trichuris, Ascaris, hookworm and Schistosomes-mansoni (Table 4:) all of which are reported to affect vitamin A status adversely (Kjolhede, 1990).

Table 2: Mean daily intake of nutrients prior to supplementation

	Daily intake	RDA	Daily intake (as % of RDA)
Kilo calories	426	2200	19.4
Protein (gms)	5.3	34	15.6
Fat (gms)	9.3	5	186
Total Vit.A (R.E.)*	0.81	600	0.14

*R.E. - retinol equivalent

Table 3: Haemoglobin levels of the study children

Study group	Sample size	Haemoglobin levels (gm/dl)	
		Baseline	Post-Intervention
Control	41	12.5 ± 1.1	12.8 ± 1.2
Experimental group	44	12.4 ± 1.1	12.5 ± 1.2
P-value*		0.24	0.35

* The test used was Levene's test for equality of variances

Table 4: Parasitic infestations among the study children

	Type of treatment				
Type of infestation	Experimental (n=44)		Control (n=41)		P-Value*
	Yes	No	Yes	No	
Trichuris	27 (61.4)	17 (38.6)	19 (47.5)	21 (52.5)	0.2
Ascaris	20 (45.5)	24 (54.5)	19 (47.5)	21 (52.5)	0.9
Hookworm	26 (59.1)	18 (40.9)	23 (57.5)	17 (42.5)	0.9
S.Mansoni	36 (81.8)	8 (18.2)	38 (95.0)	2 (5.0)	0.1**

* The test used was Chi sq.
** The test used was Fisher's Exact test
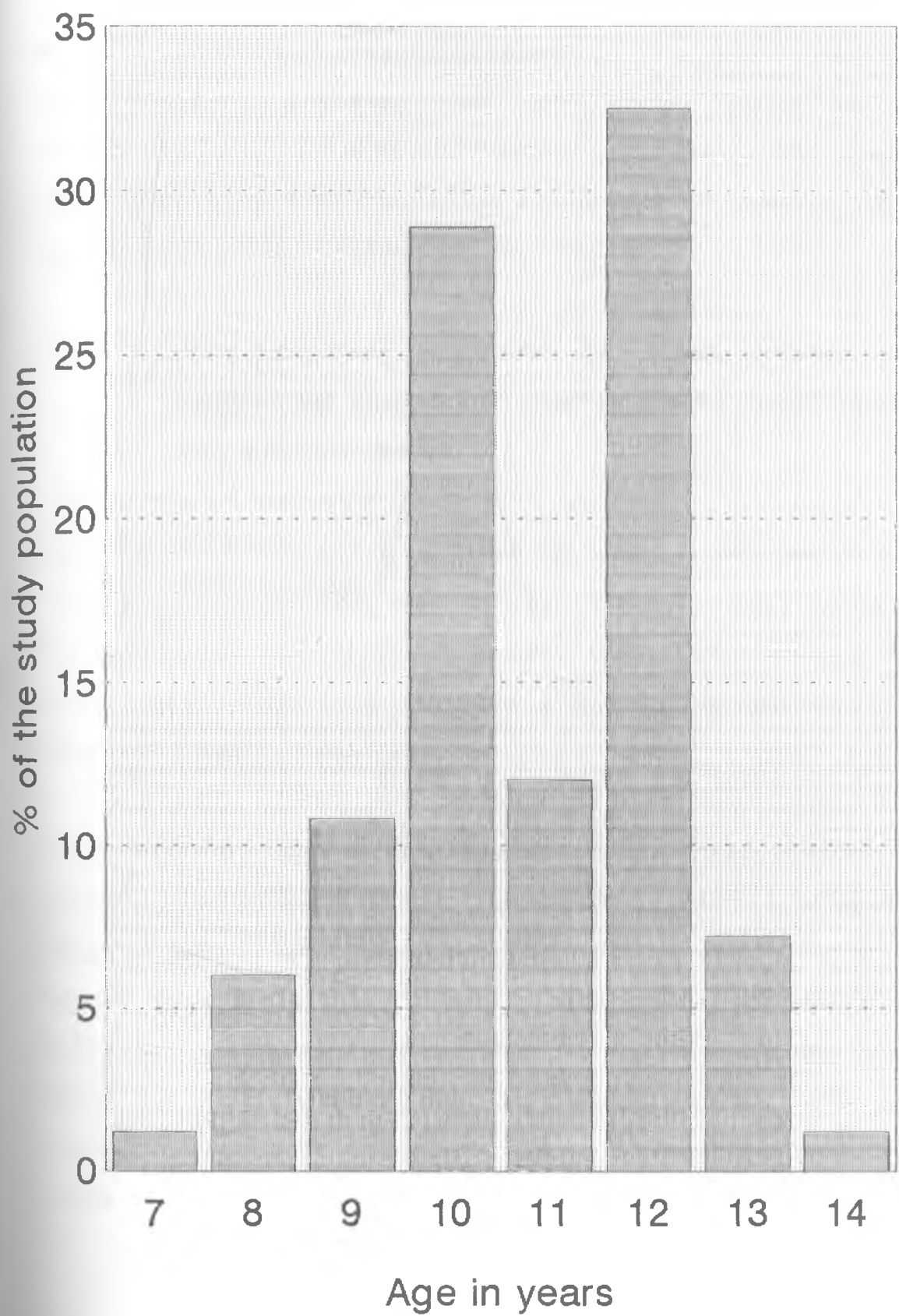
4.5 *The proximate composition of comfrey*

4.5.1 *Water and moisture content*

Water content for green fresh leaves grown during the wet season was 81.8 ± 3.5 % on fresh weight basis. The powder contained 7.1 ± 0.4 % moisture content on dry matter basis (Dm) this was done during the wet period.

4.5.2 *Beta carotene contents of fresh comfrey and unblanched oven dried comfrey powder grown during wet and dry season*

The content of ß-carotene of fresh comfrey leaves grown during the dry season (December-March) was 30.6 ± 0.4 mg/100 gms Dm and lower than that of fresh comfrey leaves grown during the rainy season April-June was 43.4 ± 0.6 mg/100 gms Dm (i.e. about 30% higher) Table:5.

The ß-carotene content of unblanched-oven dried powders of vegetables grown during the rainy season (49.03 ± 0.14 mg/100 gms on Dm basis) was higher than those of the comfrey grown in the dry season (35.4 ± 0.2 mg/100 gms) on Dm. It should be noted that the ß-carotene content of unblanched-oven dried powder in both seasons was not significantly different ($P > 0.05$). However it was higher than for the fresh comfrey leaves (Table 5:).

Table 5: ß-carotene levels of comfrey grown during different seasons

ß-carotene (mg/100g)	Dec.-March (Dry period)	April-june (wet season)	P-Value
Unblanched oven dried powder	35.4±.2	49.0±.14	0.15
Fresh leaves	30.6±.4	43.4±.6	

4.5.3 *ß-carotene content of blanched-oven dried comfrey powder, solar dried and cooked Comfrey Powder.*

The ß-carotene content of unblanched comfrey powder (49.03 ± 0.14 mg/100 g) was practically similar to that of whole leaf which was steam blanched-oven dried comfrey powder with slight but negligible loss of ß-carotene. The ß-carotene content of whole leaf steam blanched for one minute and three minutes were 48.9±.20 and 47.9±1.0 mg/100 g on dm basis respectively. These represented losses of 0.26% and 2.3% respectively (Table 6:). For the chopped vegetables the time of steam blanching was critical. The percentage loss were higher, that is about 2.7% and 16.9% after 1 and 3 minutes respectively.

Powder from unblanched solar dried comfrey had ß-carotene content (34.3 ± 1.9 mg/100 g) which was 30% lower than that of the unblanched oven dried comfrey powder (49.03 mg/100 g). This shows a significant difference with $P < 0.05$.

The ß-carotene content for comfrey powder cooked for 5 minutes was 46.8 ± 0.1 mg/100 g which represents only 4.5% loss over that of comfrey powder grown in the wet season. After 7 minutes of cooking there was a further loss of about 21.68% to 38.4 ± 0.5 mg/100g.

4.5.4 *ß-carotene content of comfrey powder stored at 25°c*

The data on ß-carotene content during storage at 25°c under carbon dioxide CO_2 in black polythene bags is presented in Figure:5. There was relatively rapid loss of ß-carotene during the first 2 weeks, from about 49.03 ± 0.14 mg/100 gms to about 35.4 ± 0.24 mg/100 gms. The loss was gradual for the rest of the period such that after 5 weeks of storage the ß-carotene content was about 22.04 ± 0.3 mg/100g) representing 55.03% loss.

4.5.5   *Sieve analysis and adsorption capacity of comfrey Powder*

The adsorption capacity of various particle sizes of comfrey vegetable powder show that decreasing the particle size increased the surface area distribution that led to high adsorption capacity. Adsorption capacity was above 35 mg/g of powder when particle size was between 0.075 to 0.125 mm and it was less than 30 mg/g of powder when particle size was 1.7 to 2.0 mm (Figure 6). The curve showed that there would be practically no increase in adsorption capacity with further reduction of particle size.

4.5.6   *Serum ß-carotene and retinol levels*

There was no significant difference between the serum ß-carotene levels of the control and experimental group at the beginning of the study (which were $0.50 \pm .49$ μm/l and $0.40 \pm .46$ μm/l respectively) (Table 7). Post-intervention serum ß-carotene levels of the experimental group (4.39 ± 1.28 μm/l) were, however, significantly higher than that of the control group which were 0.39 ± 0.34 μm/l ($p < 0.0001$).

Similarly the serum retinol levels in both groups (CCD and CFD) were not significantly different both at baseline and post-intervention ($P > 0.05$) (Table 7).

Table 6. ß-carotene content of dry Comfrey powder processed in various ways (mg/100g DM basis)

Comfrey Product (wet season)	Unblanched (mg/100g)	Blanched (mg/100g)			
		Chopped		Whole	
		1min	3min	1min	3min
Oven dried comfrey powder	49.0±.1	47.7±.2	40.7±.2	48.9±.2	47.9±.1
Sun dried comfrey powder	34.3±1.9	-**	-	-	-
Cooked comfrey powder (5 min)	46.8±.1	-	-	-	-
Cooked comfrey powder (7 min)	38.4±.5	-	-	-	-

** Not determined

Table 7: Serum ß-carotene and retinol levels before and after supplementation

Treatment type	n	Baseline levels		Post-intervention levels	
		ß-carotene μmol/l	Retinol μmol/l	ß-carotene μmol/l	Retinol μmol/l
Control grp (unsupp.)	41	0.50±.49	0.48±.25	0.39±.34	0.37±.17
Experimental grp (comfrey supp.)	44	0.40±.46	0.46±.20	4.39±1.28	0.29±.18
P-value		0.50	0.37	0.000	0.70

grp	- group
unsupp	- unsupplemented
supp	- supplemented

Figure 3: The β-carotene content of unblanched dry comfrey powder stored at 25°C
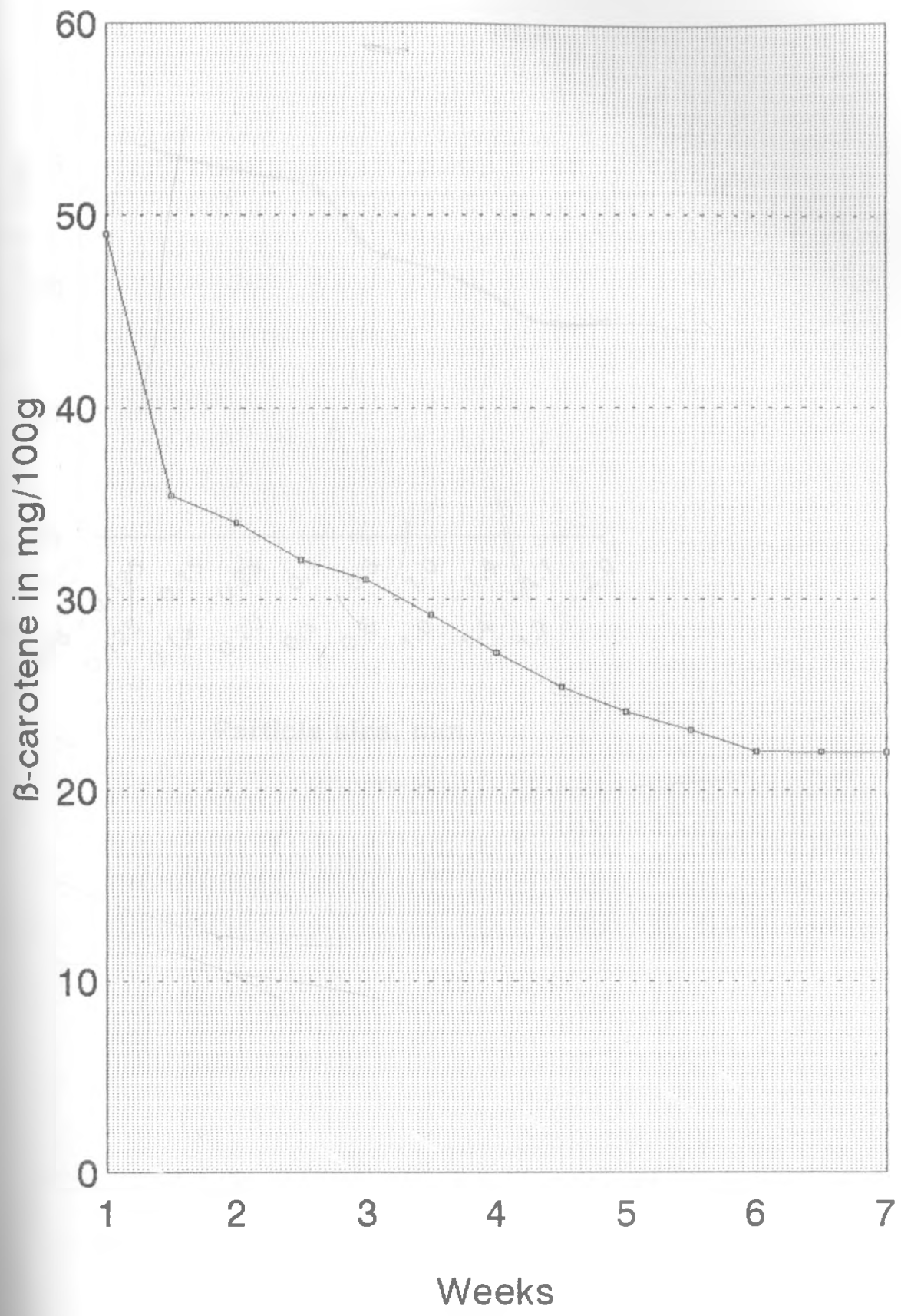
Methylene blue sorption capacity of various particle sizes of Russian cormfrey vegetable powder
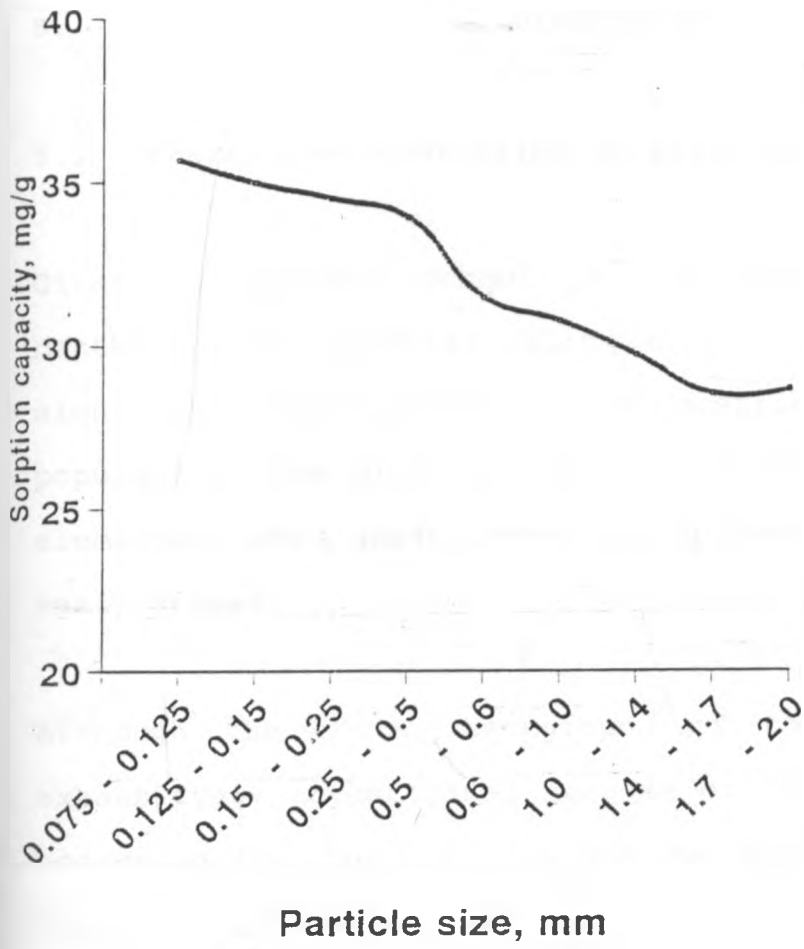
CHAPTER FIVE

5.   DISCUSSION

5.1 *General characteristics of study subjects*

Gitathuru Approved School is the only national reception center for all juvenile delinquents in Kenya. There were no significant differences in age distribution of the study population. The Education level of most of the children was elementary and a great number had dropped out of school at the early stages.

Although the social background of the children was not exhaustively investigated because of logistical problems of accessing the families, it can be safely assumed that the family background of these children in both the experimental and the control group would not be significantly different. The distribution of children by social characteristics would also not be significantly different because, the study though serially conducted took a short time within which, factors such as migration flows and seasonal changes which could lead to different rates of admission of children and diverse areas and environment would not have affected it. This observation is partially supported by the fact that the children were of similar age distribution, and educational levels.

Further, the observation that the Hb status and the prevalence of parasitic infestations which can affect serum retinol levels were not significantly different at baseline suggests that the children in both groups were subject to similar environmental conditions. Thus the serum ß-carotene and retinol levels, protein levels and nutritional status would be expected to be the same.

5.2 *Dietary intake*

The feeding patterns and food intake, were similar for the two groups since there was laid down menu in the institution. The observation that the study subjects had an inadequate intake of protein and energy is expected in such institutions. However, such low deficiency levels of caloric intake could be expected to cause clinical presentations of malnutrition which would result in mortality. This low intake is confirmed by the unbelievably low body mass index. Fortunately the children are in transition to other juvenile approved schools where the living conditions and nutrition status are better.

Vitamin A is one of the four most important nutrition deficiency problems in the developing countries as aforementioned.

It is one of the nutrients which even in the developed countries has not been found to be adequate for an appreciable proportion of the population regardless of the socio-economic status (UNICEF, 1994). Thus, subclinical state of vitamin A deficiency are common. An appreciably high proportion of the study subjects would therefore be expected to have poor vitamin A status. The inadequacy of Vitamin A is aggravated by inadequate protein and energy intake that lead to low serum Retinol-Binding-Protein (RBP) which is essential in transport of absorbed carotenoids for conversion into active form of vitamin A. This among other factors discussed below could account for low serum retinol level.

The observation of no significant difference in serum retinol levels between the two groups may be explained by either or a combination of the following:

(i) Poor serum protein level arising from inadequate intake. This would result in low retinol binding protein (RBP) which is required for transport of absorbed carotenoids for conversion into active form of vitamin A

(ii) Poor conversion (metabolism) of ß-carotene into retinol which can be associated with nutritional status or factors in the physiological status of the individual.

(iii) Low reserves of retinol in the liver may mean that generated retinol initially goes to saturate the liver.

(iv) Lipid malabsorption which is common among children of poor nutritional status could markedly increase vitamin A needs (Ionnides, 1993)

(v) Excessively high levels of retinol in the liver above $300\mu g/g$ of liver or severely low levels would be expected to show little or practically no increase in serum retinol levels (Gibson, 1990).

5.3 *Potential of comfrey as a source of Vitamin A*

The case of comfrey as a source of vitamin A is strengthened by the fact that it has high ß-carotene content compared with other vegetables which are high in this provitamin such as amaranths, pumpkin leaves and the fact that it can be processed without significant loss of ß-carotene (section 3.5.1).

The fact that comfrey is best blanched whole and not chopped (in order to retain more ß-carotene) decreases labour as well as nutrient loss. The general practice of blanching of chopped vegetables which, in any case increase losses of other nutrients particularly water soluble ones should be discouraged.

Further, that an organoleptically acceptable comfrey cake can be obtained by cooking comfrey powder briefly for 5-7 minutes with little loss of ß-carotene content makes comfrey powder a convenient ingredient for institutional meals. A lot of institutions, particularly in marginal areas would be interested in using comfrey powder.

It is also evident that such simple method as solar drying may be applicable despite the fact that there was some loss of ß-carotene. The comfrey powder would still have higher contents of ß-carotene than other vegetables referred to above.

The relatively high ß-carotene content of comfrey powder stored simply under CO_2 for a period of 2 weeks ($35.4\pm0.24mg/100g$) to 5 weeks ($22.04\pm0.3mg/100g$) is encouraging. This further supports consideration of comfrey powder for processing and use in institutional settings because it can be bought and stored in bulk.

Absorptive studies show that increasing adsorptive surface area of comfrey powder by decreasing the particle size would enhance the absorption of the ß-carotene, which would also increase the availability of the ß-carotene due to the fact that the protein-carbohydrate matrix in which the protein is embedded has been broken down.

Since the serum ß-carotene levels of the intervention group was higher than normal as aforementioned; use of comfrey or other vegetables should be encouraged. Efforts should however, be made to establish the real factors which prevented the serum retinol levels from increasing and whether this applies for other vegetables.

The fact that the retinol levels were not significantly different between control and experimental group should not discourage consideration of comfrey as a means to the improvement of vitamin A intake in diets particularly in institutions since serum ß-carotene levels were increased considerably in the experimental group $(4.39 \pm 1.28 \mu m/l)$.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 *CONCLUSIONS*

The ß-carotene levels of comfrey are among the highest compared to most vegetables. The season of growth of vegetables as shown by this study is critical in determining ß-carotene content. High ß-carotene levels in comfrey are obtained during wet season.

Blanching whole comfrey leads comparatively to lower losses of ß-carotene than blanching chopped leaves. Oven drying (at 60°c) and solar drying of whole vegetables results in levels of ß-carotene which are comparable to those of fresh vegetables.

An acceptable product with little loss of ß-carotene can be obtained by cooking powders from whole processed comfrey/vegetables for brief periods of 5-7 minutes.

Storing vegetables under CO_2 reduce the rapid destruction of ß-carotene allowing a longer period of storage (2 weeks). With a wide range of particle size between (0.075-0.2 mm) there is little difference in the increase in sorption capacity of and therefore there would be no need to grind the powder further (<0.075mm).

The increase in serum retinol levels arising from increased ß-carotene or possibly from readily available sources is not automatic and may be dependent on other factors which have been suggested to be degree of embedment in leaf matrix, fat intake, nutritional status or the liver vitamin A reserves.

RECOMMENDATIONS

Communities should be encouraged to grow comfrey inorder to incorporate it in their diet. They should be encouraged to process it for it is easy to preserve and keep for longer periods.

Blanching of whole other than chopped vegetables should be encouraged to decrease nutrient losses in the process.

Controlled intervention studies with green leafy vegetables powders should be carried out.

Identifying the factors leading to low serum retinol levels after administration with a powder relatively high in ß-carotene should be a priority in curbing vitamin A deficiency. In vitro Studies should be carried out to determine the effect of particle size distribution and surface area of different vegetables on ß-carotene availability and its conversion into retinol.

The nutritional status of the subjects should be improved by increasing the caloric, protein and vitamin A intake.

REFERENCES

Abdilla, D.C. 1976. Comfrey: Natures's Health Food. Capital Publishing House, Quezon City.

ACCN/SCN, 1992. Vitamin A deficiency. In: Micronutrient Malnutrition in Developing Countries: A Periodic Review of Developments In International Nutrition Publication No.1, WHO, Geneva.

Annon, Vit A⁺ Sieve, 1994.

Antia, F.P. 1973. Clinical Dietetics and Nutrition. Second edition. Oxford University Press, London, pp 121-132.

Arroyave, G., Aguilar, J.R., Guzman, M.A. and Flores, M. 1979. Evaluation of sugar fortification with vitamin A at the national level. Pan American Health Organization Scientific Publication No.384, Washington DC.

Bahl, R. 1995. Vitamin A immunity and infection. Indian Journal of Paediatrics 62(2):195-199.

Bender, D. 1993. Introduction to Nutrition and Metabolism. UCL Press Ltd, University College London, PP. 236-241.

Cromption, D.W.T. and Nesheim, M.C. 1984. Malnutrition insidious partner. WHO Magazine, March 1984, PP. 18-21.

Daniel, W.W. 1991. Biostatistics: A Foundation for Analysis in the Health of Science. Fifth edition, John Wiley and Sons Inc., Toronto, PP. 592-595.

Drabkin, D.L., Austin, J.H. 1932. Spectrophotometric studies: Spectrophotometric constants for common haemoglobin derivatives in human, dog and rabbit blood. Journal of Biological Chemistry 98: 719-733.

Dekker, M. 1994. Vitamin A in Health and Disease. Blomhoff, R. **(Ed).** Marcel Dekker, Inc., New York.

Dennet, G. 1984. Retinoids and the immune system: Immunostimulation by vitamin A. **In:** The Retinoids, Vol.2. **Sporn, M.B. Roberts, A.B and Goodman, D.S. (Eds.).** Academic Press, New York-San Diego, PP. 373-375.

Erdman, J. W., Fahey, G. C., and White, C.B. 1986. Effects of purified dietary fiber sources on ß-carotene utilization by the chick. Journal of Nutrition. **116:2415.**

Erdman, J. 1988. The physiologic chemistry of carotenes in man. Clinical Nutrition **7:101**

Food & Agricurture Organization/World Health Organization. 1989. Requirements of Vitamin A, Iron, Folate & Vitamin B_{12}. Report of Joint FAO/WHO Expert Committee. FAO Food & Nutrtion Series 23, FAO, Rome, PP. 1-107.

Food & Agricurture Organization/World Health Organization. 1992. International Conference on Nutrition. World Declaration and Plan of Action FAO/Rome.

Gibney, M. J. 1986. Nutrition, Diet and Health. Cambridge University Press, Cambridge-Sydney.

Gibson, R.S. 1990. Principles of Nutritional Assessment. Oxford University Press, New York, PP. 378-388.

GoK/UNICEF, 1992. Children and Women in Kenya: A situation analysis, UNICEF-Kenya Country Office, Nairobi, Kenya PP. 77.

Goodman, D.S., and Blaner, W. S. 1984. Biosynthesis absorption and hepatic metabolism of retinol. **In:** The Retinoids, Vol. 2, **Sporn, M.B. Roberts, A.B and Goodman, D.S. (Eds.)**, Academic Press, San Diego, PP. 1-39.

Goodman, D.S. and Huang, H.S. 1965. Biosynthesis of vitamin A with rat intestinal enzymes. Science **149:879-880.**

Gregg, S.J. and King, S.W. 1967. Adsorption, Surface Area and Porosity. Academic Press, London, PP. 290-295.

Guthrie, H.A. 1989. Introductory to Nutrition. Seventh edition. Times Mirror/Morsby College Publishers. St. Louis-Toronto, PP. 394-359.

Guthrie, H. and Mary, F.P.,1995. Human Nutrition. Seventh edition. Times Mirror/Morsby College Publishers. St. Louis-Toronto, PP. 394-411.

Hills, L.D. 1976. Comfrey: Fodder, Food and Remedy, Universe Books, New York.

Imungi, J. K. 1996. Changes in vitamin and mineral contents during preparation and processing of Kenyan traditional GLV: A review. The Journal of Food Technology in Africa 1:17-19.

IVACG 1982. Biochemical Methodology for the Assessment of Vitamin A Status. International Vitamin A Consultative Group, Washington DC.

Ionnides, C., Walker, R. and Parke, D. 1993. Food and Nutrition and Chemical Toxicity. Smith-Gordon Nishimura Publishers, London PP. 57-59.

Keith, P. West, Jr. 1991. Dietary vitamin A deficiency: effects on growth, infection and mortality. Food and Nutrition Bulletin **13:119-131.**

Keith, P. West, Jr. 1993. Vitamin A Deficiency and Childhood Mortality. Hellen Keller International, New York, PP. 73-79.

Kjolhede, C. 1992: Child Health Priority for the 1990s. Report of a Seminar held June 20-22, 1991 at the Johns Hopkins University School of Hygiene and Public Health, PP. 221-239.

Koesler, J. E. 1926. Vitamin A-iron interactions. In: Nutrient interactions. **In:** Nutrient Interactions. Revised edition. Bodwell, C.E. and Erdmann, J.W.**(Eds.),** Marcel Dekker Inc., New York. 1988, PP. 175-205.

Kuhnlein, H.V., 1992. Food sources of vitamin A and provitamin A, Food and Nutrition Bulletin **14:3-9.**

Machlin, L.J., 1991. Handbook of Vitamins. Second edition, Marcel Dekker, Inc., New York, PP. 11-57.

Mackigney, 1983. In Micronutrient Survey of Kenya. UNICEF, Nairobi, Kenya, 1994.

Mahalanabis, D., Simpson, T. W., Chakrabarty, M.L., Chameli, G., Bhattacharjee, A.K. and Mukherjee, K.L. 1979. Malabsorption of water miscible vitamin A in children with giardiasis and ascariasis. Am J. Clin. Nutr. **32:313-318.**

Mason, J.B., Susan, J. and Mahshid, L. 1993. Introduction and policy implications in delivery of oral doses of vitamin A to prevent vitamin A deficiency and nutritional blindness. A state-of-the-art review. **Keith, P.W. Jr. and Somer, A (Eds.).** Nutrition Policy Paper No. 2. Administrative Committee on Cordination/Subcommittee on Nutrition (ACC/SCN) of the United Nations.

Mejia, L.A. 1985. Vitamin A as a factor in nutritional anaemia. Proceeding of Nutrition Society **54(2): 501-508.**

Mejia, L.A. 1986. Vitamin A nutrient interrelationships. **In:** Vitamin A Deficiency and its Control. **Banernfeind, J.C. (Ed.).** Academic Press, Orlando Florida, PP. 69-100.

Mejia, L.A. 1977. Vitamin A-iron interactions. **In:** Nutrient Interactions. Revised edition. **Bodwell, C.E. and Erdmann, J.W.(Eds.),** Marcel Dekker Inc., New York, 1988, PP.221-224.

Morris, S.S., Kirkwood, B.R., Tomkins, A.M.,Arthur, P., Ross, D. A., Gyapong, J.O. and Abbott, R. A. 1993. Determinants of vitamin A deficiency in Northern Ghana. XV International Vitamin A Consutative Group meeting **(Abstract).**

Muroki,N. 1995. Personal Communication, Department of Food Technology and Nutrition, University of Nairobi. P.O Box 442, Uthiru, Nairobi, Kenya.

Neeld, J.B. Jr. and Pearson, W.N. 1963. Macro and micro methods for the determination of serum vitamin A using trifluoroacetic acid. Journal of Nutrition **79:454-462.**

Noel, W.S., Jesus Bulux,1993. Plant sources of provitamin A and human nutriture. International Life Sciences Nutrition Reviews **51(7):199-204.**

Olson, J.A. 1996. Vitamin A. In: Present Knowledge in Nutrition. Seventh edition. **Ziegler, E.E. and Filer, L.J. Jr. (Eds.).** ILSI Press, Washington DC. PP. 109-119

Olson, J.A. 1987. Recommended dietary intake (RDI) of vitamin A in humans. Am J. Clin. Nutr. **45:705-716.**

Olson, J.A., and Hayaishi, O. 1965. The enzymatic cleavage of ß-carotene into vitamin A by soluble enzymes of rat liver and intestine. Proc. Natl. Acad. Sci. USA **54:1364-1370**

Olson, J.A. 1994. Absorption, transport & metabolism of carotenoids in humans. Pure Appl. Chem **66:1011-1016.**

Olum, I.O., Mirza, N. M. and Muhudhia, S. O. 1990. Vitamin A and child health: A review of the effects of deficiency and the benefits of supplementation. UNICEF KCO/Kenya - 85 - Paediatric Association, 1990, PP.56.

Passmore, R. Eastwood, M.A. and Davidson, L.P. 1986. Human Nutrition and Dietetics. Eigth edition. Churchill Livingstone Press, Longmans Group of Companies, Edingburgh-London, PP.133-137.

Rankins, J., Green, N.R., Tremper, W., Slacewitcz-Sapuntzakis, M., Bowen, P. and Ndiaye, M. 1993. Undernutrition and VAD in the department of Linguere Louga region of Senegal. Am J. Clin. Nutr. **58:91-97.**

Rao, C.N. 1967. The vitamin A value of some vegetables. Journal of Nutrition and Dietitics **4:10-15.**

Reddy, V. and Sivakumar, B. 1972. Studies on vitamin A absorption. Indian Paediatrics. **9:307-310.**

Saskia De Pee, 1996. Food based approaches for controlling vitamin A deficiency: Studies in breastfeeding women in Indonesia. PhD. thesis. Department of Human nutrition, Wageningen Agricultural University, the Netherlands.

Savage, K. and Burgess, A. 1993. Nutrition For Developing Countries. Second edition. Oxford University Press, New York, PP. 255-266.

Scrimshaw, N.S., Taylor, C.S. and Gordon, J.E. 1968. Interactions of Nutrition and Infections, World Health Organization, Geneva cited from Vitamin A in Health and Disease. **Rune Blomhoff, (Eds.)** Marcel Dekker, Inc. New York, PP.585-614.

Sehmi,J.K. 1993. National Food composition Tables and The Planning of Satisfactory Diets in Kenya. Government Printer, Nairobi, Kenya.

Simpson, K.L. and Chichester, C.O. 1981. Metabolism and Nutritional Significance of Carotenoids. Ann. Rev. Nutr. **1:351-375.**

Sivakumar, B. and Reddy, V. 1975. Absorption of vitamin A in children with ascaris. J. Trop. Med. Hyg. **78:114-115.**

Solon F. S., Latham, M.C., Guirriel, R., Florentino, R., Williamson, D.F. and Aguitar, J. 1985. Fortification of MSG, with Vitamin A: The Philipine experience. Food Technology **39(11):71-77.**

Sommer, A. and Tarwotjo I. 1983. Increased mortility in children with mild VAD. Lancet **2:585.**

Sommer, A., Tarwtojo, I. Djunaedi, E., West, K.P. Jr., Loeden, A.A., Tilden, R., Mele, L. and Aceh Study Group. 1986. Impact of vitamin A supplementation on childhood mortality. A randomised controlled community trial, Lancet **1:1169-1173.**

Sommer, A.I. Tarwotjo, I. and Katz, J. 1987. Increased risk of xerophthalmia following diarrhea and respiratory disease, Am J. Clin. Nutr. **45:977.**

Steel,R.G. and Torrie, J.H. 1980. Principles and Procedures of Statistics: A Biometrical Approach. Second edition. McGraw-Hill Book Company, Singapore.

Stephenson L.S. and Cecilia H., 1987. The Impact of Helminth Infection on Human Nutrition. Taylor and Francis, London-Philadelphia.

Stevenson and Earl, 1996. Zinc-vitamin A interactions. **In:** Nutrient Interactions, Revised edition. **Bodwell, C.E. and Erdmann, J.W.(Eds.),** Marcel Dekker Inc., New York. 1988, PP. 1P.173

Suharno, D., West, C.E., Muhilal, Longman. M.M.H.G., de Waart, F.G., Karyadi D, Hautvast, J.G.A.J. (1993). Cross-sectional study on the iron and vitamin A, iodine and iron status in school children in Shoa region, central Ethiopia **In:** Toward Comprehensive Programs to Reduce Vitamin A Deficiency. XV International Vitamin A Consultstive Group Meeting, 8-12 March 1993, Arusha Tanzania, PP. 49 (**Abstract**).

Temalilwa, C.R. and Mulokozi G. 1993. An overview of the National program for prevention and control of VAD and Xerophthalmia in Tanzania. **In:** Toward Comprehensive Programs to Reduce Vitamin A Deficiency. XV International Vitamin A Consultstive Group Meeting, 8-12 March 1993, Arusha Tanzania, PP. 28 (**Abstract**).

UNICEF-KCO, 1994. Vitamin A deficiency in Kenya: A Report on the National Micronutrient Survey, UNICEF Kenya Country Office, Nairobi, Kenya.

UNICEF, 1998. Zinc and vitamin A: Taking the sting out of malaria. The State of the Worlds children. Published for United Nations Children Children's Education Funds by Oxford University Press, New York, PP. 74-79.

Wadhwa A., A. Sighn, A. Mittal, S. Sharma. 1994. Dietary intervention to control vitamin A deficiency in 7-12 year old. Food and Nutrition Bulletin **15(1):53-57.**

Waterlow, J.C. 1992. Protein-Energy-Malnutrition. Edward Arnold Press, London.

Wolde-Gebriel Zewdie, 1993. Interrelationship between vitamin A, iodine and iron status in school children in Shoa region, central Ethiopia. Br J. Nutr **70 (2):593-607.**

WHO 1982. Control of vitamin A deficiency and xerophthalmia. Report of a joint WHO/UNICEF/USAID/HellenKeller International/IVACG meeting. World Health Organization Technical Report Series 672. WHO, Geneva.

WHO 1995. Physical status: The use and interpretation of anthropometry. PP. 270-305.

Yadar, S.K.,and Sengal, S. 1995. Effects of home processing on ascorbic acid and ß-carotene content of spinach and amaranths leaves. Plant Foods for Human Nutrition **47(2):125-131.**

Ziegler, E.E., Filler, L.J. 1997. Present Knowledge in Nutrition. Seventh edition. International Life Sciences Institute Press, Washington DC., PP. 109-119.

APPENDIX 1:

QUESTIONNAIRE

1.0 Demographic and background information:

Date of interview ___/___/19___

Name of interviewer _____

Name of the father _____

Name of the mother _____

Name of the respondent _____

Ethnicity_____

Age of the respondent _____ years

Date of admission to Gitathuru Approved School

Day _____ Month _____ Year _____

Education level of the respondent _____ years

	Name	Age	Relation to respondent	Level of education	Occupation	Religion
1						
2						
3						
4						
5						
6						
7						
8						

2. CLINICAL EXAMINATION

(2.A) Medical history

1. Have you had any disease in the last 7 days?

        1 - Yes   2 - No

2. If yes which ones :

        (i)   (iii)

        (ii)   (iv)

3. Have you had any treatment?

        1 - Yes   2 - No

(2.B) Physical examination

1. Dental status: 2nd Molars Upper/Lower
                   Canines Upper/Lower
2. Oral mucosa: Lesions present 1 - Yes   2 - No
                     Pain 1 - Yes   2 - No   Part_____
                     Bleeding gums   1 - Yes   2 - No
                     Abscess   1 - Yes   2 - No
3. Physical abnormality   1 - Yes   2 - No
4. Pallor   1 - Yes   2 - No
5. Jaundice   1 - Yes   2 - No

6. Do you have any problems seeing in dim light?
            1- Yes   2 - No
7. Bitots spots   1 - Yes   2 - No
8. Allergic conjuctivitis   1 - Yes   2 - No
9. ENT_____RS_____CVS_____
10.   PA_____CNS_____
11.   Skin abnormalities_____.

(2.C) Genitalia

1.   Pubic hair   1 - Yes   2 - No   Stage_____

3.   Social background

    i. Where is your home _____.
    ii.Where were you staying before you came to Gitathuru
       approved school _____.
    iii.For how long were you there?_____
    iv. Where were you before then _____.
    v. Whom were you staying with at that time _____.

4.   *Dietary history*

i.   What foods did you eat at home? mention them
      1. Maize and beans   2. Maize, beans and
                                        vegetables
      3. Ugali and green vegetables 4. Ugali and cabbage
      5. Ugali and beans   6. Ugali and fish
      7. Rice and beans   8.   Rice   and   green
                                       vegetables
      9.0thers
ii.   What foods did you eat at the police remand ? mention
         them :
      1. maize and beans   2. Rice and beans
      3. Ugali and beans   4. Ugali and cabbage
        5. Rice and cabbage   6.   Rice,   beans   and
                                       cabbage
      7. Maize, beans and cabbage   8.   Ugali,   bean   and
                                       cabbage

iii. Which vegetables have you consumed in the last 7 days

	Vegetables	Amount	Source	Frequency/ Week
(i)				
(ii)				
(iii)				
(iv)				
(v)				
(vi)				

iv. Do you consume any fruits?

           1 - Yes   2 - No

v. If yes which fruits have consumed in the last 7 days

	Fruits	Amount	Source	Frequency/ Week
(i)				
(ii)				
(iii)				
(iv)				
(v)				
(vi)				

5. *Information of dietary intake*

Gross meal composition for the pupils.

Meal	Ingredients	Amounts in (kgs)	Number of persons per taking	Average consumption in kgs
Supper	Beans Maize Rice Ugali Cabbage Comfrey Potatoes Fat Others			
Lunch	Beans Maize Rice Ugali Cabbage Comfrey Potatoes Fat Others			
Breakfast	**Porridge:** Flour Sugar **Tea:** Milk Sugar			
Snack				

6.0 *INFORMATION SHEET FOR SERUM ß-CAROTENE AND RETINOL ESTIMATES*

Information sheet for serum ß-carotene and retinol estimates

Serial No._____

School _____ Class _____

Childs name_____ Sex _____ Age _____

Village _____ District _____

Parents name _____ Address _____

Date of specimen collection_____

Date of serum vitamin A estimation_____

Serum levels

	Serum level in μm/l	Normal	Low	Acceptable	High VA status
Baseline levels					
Second test					

7.   *STOOL SPECIMEN SHEET*

Name of child_____ Class_____ Age_____

Container dispenced_____ Y/No Date _____

Stool specimen received_____ Y/No Date _____

Stool specimen examined_____ Y/No Date _____

Results (n=85)

Number of parasites_____

Parasites present_____

Parasite prevalence based on microscopic stool examinations

Parasite type	Egg count per gm of stool
Ascaris	
Hookworm	
Trichuris	
s.mansoni	
Others	