NUTRITIONAL EVALUATION OF CEREAL AND LEGUME-BASED COMPLEMENTARY DIETS USED IN JOS, PLATEAU STATE

BY

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DECLARATION

I **MARIAM SOLOMON**, do hereby declare that:

(a) This thesis has been writing by me and that it is a record of my original research work.

(b) No part of the thesis has, to the best of my knowledge, been presented anywhere and at anytime for the award of any higher degree.

(c) All quotations and references have been duly acknowledged.

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Mariam Solomon
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CERTIFICATION

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I dedicate this work to:

(1) God Almighty for his Grace, Wisdom and Favour more than ever before.

(2) My late mother, Mrs Rifkatu S. Monde

(3) All Boi Women

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LIST OF ACRONYMS

AHRTAG	Appropriate Health Resources and Technologies Action Group.
FBDG	Food Based Dietary Guidelines
FMOH	Federal Ministry of Health
IAEA	International Atomic Energy Agency
ICN	International Conference on Nutrition
NACMD	National Advisory Committee on Micronutrient Deficiency
NNN	Nigeria Nutrition Network
NPAN	National Plan of Action on Nutrition
IITA	International Institute of Tropical Agriculture

ABSTRACT

Three multimix complementary diets were prepared from local foodstuffs i.e. maize *(Zea mays),* acha grain *(Digitaria exilis)* rice *(Sativa oryza),* sorghum *(Sorgum bicolar),* millet (*Pennisatum typhoides*), groundnut *(Arachis hypogea),* bambaranut *(V-oadzeia subterranea),* sesame *(Sesamun indicum),* carrots *(Daucas carota),* crayfish *(Macrobrachuim Sp),* garden egg *(Solanum incanum),* palm oil *(Elaeis guinensis)* and table salt (NaCl). The formulated diets were subjected to biochemical analysis-along with a commonly used proprietary infant cereal (Nestle Cerelac) as control. Standard chemical methods were used to determine the proximate nutrient composition, some vitamins and antinutritional factors. Atomic Absorption Spectrophotometric and Ion Chromatograhic techniques were used to determine mineral composition while Automated Amino Acid Analyzer was used to identify and quantify amino acids. The formulations and the control were fed to rats under the same conditions. Physical, biochemical and haematological parameters of the rats fed were used to asses the suitability of these formulated diets as a possible substitute for the proprietary infant foods. Analysis of variance (ANOVA) was used to establish any significant difference in the analytical data for formulated and control diets. The assessment results show that the formulated diets are comparable nutritionally to Nestle Cerelac-supporting animal growth without any significant organ impairment as indicated in the liver and kidney function tests. The diets were well accepted as shown by the amounts consumed by the rats. Results of molar ratios of some minerals and antinutritional factors in the compounded diets suggest that the

antinutrients will not pose any serious problem in the usage of the complementary diets. The cost of producing the formulated diets is about N50-N100 cheaper than Cerelac. The study has therefore, revealed that with proper selection of local foodstuff, it is possible to prepare nutritious complementary diets that would be acceptable, readily available, affordable and nutritionally adequate. Dissemination of the findings at scientific and community levels is very desirable.

CHAPTER ONE

INTRODUCTION

Despite abundant global food supplies, widespread malnutrition persists in many developing countries. The World Health Organization (WHO) and UNICEF have been concerned about this trend, particularly of Protein Energy Malnutrition (PEM) and micronutrient deficiencies (hidden hunger) among infants, children and pregnant women. The united Nations system Standing Committee on Nutrition (SCN) pointed out that malnutrition is directly and indirectly associated with more than 50% of all children mortality, and is the contributor to disease in developing world (SCN,2004).

In Nigeria, and indeed most developing countries, the underlying problems have been identified to include poverty, inadequate nutrient intake particularly during pregnancy, period of rapid growth and complementary feeding in infants, ignorance about nutrient values of foodstuff and parasitic infections (NPAN, 2002). Results of the 2001-2003 food consumption and nutrition survey showed a steep increase in the incidence of child wasting between 6 and 12 months, which is the period of complementary feeding for most children (IITA, 2004).

Major international and national efforts towards addressing these problems include nutritional supplementation, fortification of staple foods and modification of traditional diets to meet specific requirements. The promotion and support of exclusive breast-feeding, access to and the initiation of nutritious complementary foods between ages 6-24 months remain essential components of achieving optimal nutrition and malnutrition control programmes for infants and children (WHO, 1998;

2001b) Failure to achieve these components predisposes the infant to malnutrition, growth retardation, infection and increased risk of mortality.

Complementary feeding is instituted according to a country-specific infant feeding guidelines, which also takes into cognizance the availability and affordability of infant instant cereal formulas. Proprietary formulas are usually considered nutritious, acceptable and safe to the infant but their high cost has put them beyond the reach of most families, especially those in the low income "bracket." Most families depend on locally formulated diets to feed infant and young children. The locally formulated foods (pap and porridges) are low in protein and high in anti-nutritional factors that reduce the bioavailability of some micronutrients. Poor processing and cooking methods also contribute substantially to loss of micronutrients, leading to micronutrient deficiency disorders in infants fed these foods.

Different approaches have been adopted to combat the problem particularly of "hidden hunger" in Nigeria and most developing countries. One of such immediate approach is oral supplementation of micronutrients especially the global focal micronutrients Fe, Zn, I_2 and Vitamin A. Various organizations and individuals (ICN, 1992; FAO/WHO, 1998; Kennedy et al 2003) have upheld that, the most sustainable solutions are those that are likely to be maintained in the long-term. These would include food-based approaches like diet diversity, food fortification and biofortification. Kennedy et al (2003) further suggested that food fortification and biofortification could be the most cost effective of all public health interventions and thus within the economic reach of even the world's poorest. The 1992 International

Conference on Nutrition (ICN) suggested other approaches to include improved food availability, food preservation, research nutrition education and hygiene.

Based on these recommendations, a joint FAO/WHO consultative forum in 1998, established the scientific basis for the development and evaluation of Food-Based Dietary Guidelines (FBDG) for various regions of the world. Sequel to this, the Federal Ministry of Health (FMOH) in Nigeria published the Food-Based Dietary Guidelines (FMOH, 1999). The guidelines recommended some sustainable food-based approaches that encourage dietary diversification through the production and consumption by all population groups, of both macro- and micro-nutrient-rich foods, including traditional foods found in different parts of the country. The report recommended the use of staple starchy roots, tubers and cereals in combination with legumes, vegetables, fruits and if possible animal sourced foods in preparing weaning foods for infants and children. Based on these recommendations, different variables affecting micronutrient intake and ways to combat deficiency have been considered in several nutrition fora (NNN, 2000; NACMD, 2003).

Besides, the reports by many researchers on the nutritive potentials of cereals, legumes, vegetables and fruits in the formulation of complementary foods have been promising (Badamosi et al, 1995; Owolabi, 1996; FAO, 1997; Okoh, 1998; Ladeji et al, 2000;). It is imperative that blends of such foodstuffs found in various communities be formulated and biochemical studies be carried out on their composites for possible use as complementary foods. This study, which is part of an exploratory effort on the improvement of the nutritional quality of traditional complementary foods, was designed to use staple foodstuffs indigenous to Plateau State to formulate composite blends that can be nutritious, readily available and

affordable to both rural and poor urban mothers. Findings from the study would offer answers to the questions (a) whether such local blends can meet the various dietary recommendations for infants and children and (b) whether they can substitute the more expensive proprietary formulas sold in the markets.

1.1 SPECIFIC OBJECTIVES

The research was undertaken principally to

(i) formulate composite blends using some selected cereals, legumes and vegetables commonly cultivated and consumed in Plateau State.\

(ii) determine the macro- and micro-nutrient composition of the composite blends, and compare their nutrient profiles with a reference proprietary formula, Nestle Cerelac and Recommended Dietary Allowances (RDAs).

(iii) carry out a bioassay of the nutritive quality of each blend in a rat model system, using biochemical indices of nutritional status.

(iv) to further fortify the composite blends that hold greater promise using other available foodstuffs.

(v) reevaluate the fortified blends as in (i) – (iii)

(vi) cost the local blends and compare to the current cost of the proprietary formula in the market.

(vii) make informed recommendations on the use and nutritive adequacy of the local composites.

1.2 REALIZATION OF THE SPECIFIC OBJECTIVES

The objectives outlined above will be achieved through

(i) the use of standard chemical methods to evaluate the proximate nutrients, vitamins and anti-nutritional factors of the local formulations

(ii) the use of Atomic Absorption Spectrophotometer and Ion Chromatographic Analyzer to determine the mineral components (cationic and anionic levels).

(iii) the use of Technicon Amino Acid Analyzer to determine the amino acid profiles.

(iv) animal studies to assess the effect of consumption of the local diets on biochemical indices of nutritional status and growth rate of weanling rats as compared to those fed proprietary formulas.

(v) data analysis to warrant recommendations on the use of the local diets.

CHAPTER TWO

LITERATURE REVIEW

2.1 INFANCY, EXCLUSIVE BREASTFEEDING AND COMPLEMENTARY FEEDING PERIOD

Infancy is a period of rapid physical growth as well as physiological, immunological and mental development when nutritional requirements are at their highest. For instance most brain development, for which protein, cholesterol, zinc and some essential fatty acids are crucial requirements, occur in the first two years after birth (Guthrie, 1989). Most tooth development, requiring calcium, phosphorus and vitamins A, D and C occurs in the first one to five years of life (Uphoff, 1993). Deficiency of these and other nutrients can therefore have dire consequences, some of which can be long lasting or even lead to death. It is estimated that of nearly 13 million young children who died in developing countries in 1993, half died of malnutrition and its effect in potentiating infectious diseases (Uphoff, 1993). WHO/UNICEF (1997) also reported that millions of children are physically and mentally disabled due to PEM and micronutrient deficiencies. These shortfalls have been attributed to shortened period of exclusive breastfeeding, low dietary intake and poor bioavailability of nutrients in complementary foods (Lutter and Rivera ,2003)

Nutritious dietary intake during this critical period therefore becomes very vital and priority must be accorded to its improvement. WHO/UNICEF (1993) have recommended that, after 6 months, nutritious and safe foods (also known as complementary foods) be gradually introduced and continued up to 2 years of age, thereafter the normal family diet be introduced (Yeung, 1998). The use of complementary foods does not only prime the infant’s system to an adult diet, but also

serves as a source of additional nutrients required for optimal growth and development (Fernandez et al, 2002). Proper weaning practices coupled with lengthened breastfeeding period of at least two years can eliminate or at least reduce the high incidence of infant mortality and morbidity experienced in developing countries (Walker, 1990). It is believed that gradual introduction of complementary foods after six (6) months of age to the infant diet would have no adverse or shock effect on the digestive system of the child (Okungbowa, 1986).

2.2 COMPLEMENTARY FOODS

In most developed societies, nutrient-fortified cereals are the first complementary foods introduced to the infant, followed by fruits, vegetable and meat products. In developing countries, although a number of convenient fortified proprietary formulas are available, they are often too expensive and out of the reach of most families.

The use of home-based complementary foods that can be readily prepared, available, affordable, is one feeding option that has been recommended. (WHO, 1981; Fashakin and Ogunsola, 1982; Temple et al, 1996; FMOH, 1999; Nnam, 2002) to stem the deleterious effects of malnutrition in infants and young children. A number of research efforts aimed at improving the nutrient quality of existing local complementary foods, and the formulation of new ones have are being quite promising. Tables 2.1and 2.2 show some of the blends in Nigeria (Fashakin and Ogunsola, 1982) and some West African countries (Onofiok and Nnanyelugo, 1998).

The composition of local complementary foods varies from place to place and from country to country. In West Africa generally, the common family diets and staple foods on which infants are weaned include dishes from cereals, starchy tubers,

legumes and vegetables. Some of the diets are single, while some are mixed diets. In Ghana for instance, the main traditional weaning food is Koko-a fermented maize porridge (Armer-Klemesu and Wheeler, 1991), while Ogi prepared from maize or sorghum is used in Sierra Leone (Jonsyu, 1985). In Nigeria, a fermented maize or millet (akamu) and fermented gari (foo-foo) are commonly used in the Northern and Southern parts respectively. The weaning foods and practices in other African countries are similar to those indicated in Table 2.1.

Table 2.1 Some Traditional Weaning Foods in West Africa

Country	Food	Age of Introduction (months)	Description
Nigeria	Pap (English), Ogi (Yoruba) Akamu (Hausa)	3 - 6	Fermented cereals e.g. maize, millet or sorghum
Ghana	Koko, Kankey	3 - 6	Fermented corn porridge
Sierra Leone	Ogi, Couscous ogi	4 - 6	Cereal gruel from fermented maize or sorghum
Benin Republic	Ogi	3 - 6	Cereal gruel from fermented maize, sorghum or millet

Source: Onofiok and Nnanyelugo (1998).

Table 2.2 Nutritive Value of Some Single and Mixed Weaning Foods Compared with Commercial Products in Nigeria.

Food	Energy (Kcal)	Ash (g/100g dm)	Protein (g/100g dm)	Carbohydrate (g/100g dm)
Guinea corn pap	415	0.5	4	92
Maize pap	417	0.2	6	91
Millet pap	419	0.5	7	88
Millet-soya-milk pap	420	1.8	19	74
Guinea corn porridge	412	1.0	5	91
Millet-soya -porridge	413	2.2	23	70
Lactogen®	463	4.8	22	52
Similac®	517	3.0	11	56
Cerelac®	412	3.3	16	67

Source: Fashakin and Ogunsola (1982).

2.3 NUTRITIONAL PROBLEMS OF TRADITIONAL COMPLEMENTARY FOODS

Traditionally, the diets of most human societies consist of a starchy plant staple, such as cereal, root or tuber, combined with herbs, vegetables and fruits (mainly gathered or self-grown). Sometimes legumes serve as additional staple food. Only people with a good income or those who hunt, fish or keep livestock themselves consume significant amounts of animal protein.

A common feature of plant foods is their high content of water, fibre, low energy and micronutrient densities (Solomon, 2000). This characteristic becomes particularly worrisome during the complementary feeding period in infants and children. Hence, traditional weaning foods from plant staples often fail to meet the nutritional needs of the infants, due to stiff consistency and high volume which combine to offer a low-cost filling meal that often lacks adequate nutrients (Fernandez et al, 2002). They are therefore known to poorly support growth and development. For instance, Naismith (1973) and Fashakin and Ogunsola, (1982) have associated the aetiology of protein energy malnutrition in children to the frequent use of maize pap (Koko) and millet gruel during the weaning period.

It is possible to achieve an adequate nutrient intake by increasing the daily intake of such low nutrient-dense foods, but the volume of the food to be consumed may be too large to allow the child to ingest all the food necessary to cover nutrient needs. For instance, an infant aged 4 – 6 months would need about 62g of corn gruel to meet daily need of energy of 740 (kcal), and protein need of 13g (Eka and Edijala, 1972). This is an impossible target considering the size of an infant's stomach.

Poor combination and formulation has partly contributed to the poor performance of traditional complementary foods. A number of researches (Fashakin and Ogunsola, 1982; Marcos et al, 1983; Oyeleke et al, 1985; Abbey and Nkanga, 1988; Abbey and Mark-Balm, 1988; Omoruyi et al, 1994; Badamosi, et al, 1995), in Nigeria have shown that a combination of cereals and legumes or tubers with vegetables and animal sourced food rather than the single diets, better supported growth and development.

Poor processing methods and hygiene have also been identified as other factors responsible for low nutrient density in local complementary foods. Processes such as milling, fermentation, and parboiling are intended to achieve specific purposes but they however tend to affect the nutrient content of the food negatively. Okoye (1992) reported a 90% loss of free folic acid content of cereals and certain vegetables, 50% of yam thiamine and ascorbic acid and up to 20% of milk vitamin E content during boiling.

The presence of non-nutrient constituents (antinutritional factors) in plant-based foods has been shown to also negatively influence the bioavailability of nutrients. The best documented being oxalic acid which forms oxalate precipitates with dietary calcium, while phytic acid forms insoluble phytates with Ca, Fe, Zn and possibly other metals. For instance the relatively poor availability of the fairly high Fe content of cereals is mainly due to their correspondingly high phytic acid levels (Okoye, 1992).

2.4 FORMULATION OF LOCAL COMPLEMENTARY FOODS OF HIGH NUTRITIVE VALUES

The use of high nutrient dense foodstuffs such as cereals, legumes, vegetables and animal food products to prepare complementary foods for infants and children has been suggested by a number of researchers (Temple et al, 1996; Onofiok and Nnanyelugo, 1998; Nnam, 2002). Cereals generally are known to be relatively low in lysine and tryptophan, but fair in sulphur-containing amino acids ie methionine and cysteine (FAO, 1997; Okoh, 1998). On the other hand, legumes are relatively rich in proteins (19-26%) and fat (40–46%), and contain moderate quantities of tryptophan and threonine. This class of foodstuff can therefore form a good supplement to cereals. It is however evident that cereals and legumes are low in trace minerals and vitamins (Osagie and Eka, 1998). Fruits and vegetables are valuable sources of these micronutrients. They could therefore provide significant quantities of the nutrients if properly processed and blended with the staple foods. Animal source foods like crayfish, egg and milk have further been suggested as source of enrichment (Badamosi et al, 1995; Temple et al, 1996; Ladeji et al 2000).

In Nigeria a number of cereal–legume combinations have been formulated, but conclusions have it that double mixes are deficient in many micronutrients as rightly observed by Osagie and Eka (1998). A triple mix consisting of a staple grain ‘acha’, sesame and garden egg, formulated by Temple et al (1996) was found to be superior in mineral content. Germination, sprouting and fermentation processes of plant foods have been suggested as other ways of improving the digestibility, nutrient densities and bioavailability of micronutrients (Mlingi, 1988; Guthrie, 1989; Davidson et al 1994;). Dietary diversification, supplementation, and fortification of locally available

foods could also result in improved micronutrient intake by Nigerian infants during complementary feeding period (NNN, 2000; Nnam, 2002; NACMD, 2003).

2.5 HIV AND INFANT FEEDING

It is a well-known fact that breast feeding significantly improves child survival by protecting against diarrheal diseases, pneumonia and other potentially fatal infections. It enhances the quality of life of the infant through its nutritional, psychosocial and many other benefits. As a result, lack of it presents substantial disadvantages and risks to both children and mothers (UNAIDS/WHO/UNICEF, 1997; UNICEF, 2004). The risk of not breastfeeding, hence increases child morbidity and mortality.

The finding that HIV is transmitted through breastmilk presents a serious dilema particularly for mothers in poorer countries. The finding also poses a greater dilema to policy makers and health workers striving to strike some sort of compromise between breastfeeding children or not breastfeeding (Latham and Preble, 2000). For such poor women, the choice not to breastfeed is much more problematic because of inadequate access to resources necessary to obtain sufficient breast milk substitutes, as well as equipment, fuel and potable water to prepare it safely.

The new guidelines on HIV and infant feeding outlined by UNAIDS/WHO/ UNICEF (1997) calls for urgent action to educate, counsel and support HIV-positive women in making decision about how to nourish their infants safely. The guidelines stress that, in order for a mother to take such a decision, she should among other things, have access to information about other feeding options and the risk and benefits associated with them. One of the suggested options is shortened duration of breast-feeding. It therefore follows that nutritious complementary foods must be

introduced to enhance the infant's immune system. Such foods should be micronutrient-dense and safe-feeding practices must be used in order to prevent infectious diseases. This will give the child the benefits of safe and healthy feeding while avoiding the risk of HIV transmission through breastmilk.

2.6 NUTRITIVE NEEDS OF INFANTS

According to Okoye (1992) age is one of the factors affecting individual nutrient requirements, and these requirements are met only from a restricted selection of foodstuff. Consequently, special attention is given to infant nutrition. Okoye (1992) further stated that the unique nature of infant nutrition resides in factors such as the immaturity of the digestive system and related biochemical machineries e.g. detoxication mechanism. Hence the need for a special starting diet to compensate for inadequacies of any insufficient maternal endowments and the continuously changing nutrient requirements of a growing child.

Breast-milk satisfies the nutrient and energy requirements of the infant for the first 6 months. As the child grows, the nutrient composition of milk increasingly becomes inadequate to meet the infants' requirements. The nutrients most affected are iron, zinc, vitamins A and C. Therefore, to be able to meet the changing requirements of the infants, there is the need to supplement the breastmilk with a nutritious diet, which could be a proprietary formula or locally prepared at home, while breastfeeding continues for at least two years (Okoye, 1992). This gradual shift from breast-milk to solid or semisolid foods is called complementary feeding (WHO/UNICEF, 1993).

2.6.1 Energy Needs

For a newborn infant exclusively breast-fed, breast-milk provides all the needed calories for the first 6 months. An intake of 95–145 Kcal/Kg (150 ml) has been considered adequate. By 6 months, energy needs increases for a very placid infant by 32Kcal, and a fretful infant by 60 Kcal (Guthrie, 1989). An excessive intake of calories, leading to a rapid gain in weight, is as undesirable in infants as in adults which supports the concept that maximal growth is not synonymous with optimal growth.

Most traditional weaning foods are usually bulky (meaning, food of high viscosity but low energy density), and have been found to provide low energy for the growing child (Akinyele and Omotola, 1986). The factors limiting energy intake of an infant weaned on such low energy foods are the volume the child can consume at a time and the frequency of feeding (Walker, 1990). Low energy density weaning foods caused by high bulk gruels have long been implicated in protein-energy-malnutrition (Guthrie, 1989; Okoye, 1992; Devlin, 1997). Protein energy malnutrition (PEM) refers to all disease conditions attributable to inadequate dietary protein and energy, comprising mainly of kwashiorkor and marasmus (Gurney et al, 1985; Okoye, 1992). Children with mild or moderate PEM adapt to their inadequate diet by growing slowly and often become lethargic. They are usually susceptible to infectious diseases such as diarrhea and may further develop other micronutrient deficiency disorders (Gurney et al, 1985).

2.6.2 Protein Needs

The need for protein during the period of skeletal and muscle growth of early infancy is high. An intake of 2.1g of high biological-value protein per kg of body weight permits nitrogen retention of about 45%, as long as energy intake is adequate (Guthrie, 1989). By 5 to 6 month the protein needs drop slightly to 2g/kg. If the protein is of low biological value the amount needed increases proportionately. Protein with high biological value of at least 70 to 85%, e.g. eggs, milk, meat, with almost half of amino acids being essential amino acids, have been recommended to be used for infants (Picciano, 1987).

There has been no evidence of advantages from protein intakes above recommended levels. Protein in excess of the body's need for growth and repair of tissue must be deaminated in the liver so that the carbon skeleton of the amino acids can be used as a source of energy, and the amino portion is excreted as urea. Since the infant has a limited capacity to concentrate waste metabolites, such as urea in the urine, the excretion of more waste requires a large volume of water. If the necessary water is not available, urea will accumulate, and ironically, the infant will suffer from protein edema (Guthrie, 1989). It is therefore suggested that protein intake by 6 to 12 months old infant should not exceed 6g/kg body weight (13 – 15g/day) (Guthrie, 1989). Infants' amino acid requirements are proportionately higher than those of adults. In addition, histidine is essential for infants at a level surpassed in both breastfeeding and bottle-feeding.

Some of the weaning formulations, which have been advocated for developing countries, have high levels of protein. Fashakin and Ogunsola (1982) have however reported poor protein quality of some traditional weaning foods. It was concluded that

the amino acid patterns of the mixes were not sufficiently complementary, and suggested other protein sources (of animal source) be added to raise the quality.

2.6.3 Micronutrients

Micronutrients are vitamins and mineral elements, which are needed in minute quantities for the normal functioning of the body. They are normal chemical components of foods in their active forms or as precursors of the active forms. They form components of enzymes or co-factors needed for metabolic reactions in the body (Devlin, 1997).

There has been reports of high prevalence of micronutrient deficiency among children and women especially in developing countries (WHO/UNICEF, 1995; Collaway, 1995; FAO/WHO, 1998). This according to the reports is due to increase requirements for growth and development, poor bioavailability in plant-based diets, and inadequate attention given to the problem by the various communities and governments. Other identified immediate causes of micronutrient deficiency in children include, inadequate dietary intake, diseases and poor breast-feeding practices, while remote causes include inadequate household food security, basic health services, sanitation and hygiene (Harrison 1996; UNICEF, 1998; NNN, 2000). Other causes noted by Campen (1996) are nutrient-nutrient interaction and nutrient-drug interaction. Campen (1996) further stated that absorption and assimilation of iron and zinc are influenced by heavy metals such as lead, cadmium and mercury, and that anti-nutrients such as phytates and oxalates antagonize the uptake of calcium and iron from grains, legumes and vegetables.

The common practice of milling and polishing of grains, parboiling of vegetables and legumes used during the processing and preparation of foods for

infants and children, have been found to reduce the levels of micronutrients (La Roche, 1990), and could cause micronutrient and other nutritional deficiency disorders in infants and children (Lorri, 1996). Depending on the nutrient and severity of deficiency, the consequences of micronutrient malnutrition in infants and children may include growth retardation, anorexia, susceptibility to infections, behavioural changes and learning disability. The high mortality and morbidity rate among infants and children has led to the view that the modern world should not tolerate the persistence of these deficiencies. UNICEF (2000) has put the global mortality rate of children due to "hidden hunger" at 114 per 1000 (11.4%) live births.

2.6.4 Micronutrients of International and National Concern

The World Health Organization (WHO) and UNICEF have identified iron deficiency anemia (IDA), vitamin A deficiency (VAD), iodine deficiency disorders (IDD) and zinc deficiency as the four micronutrient deficiencies of greatest significance. These two International Organizations have also linked the deficiencies with the high prevalence of childhood diseases and mortality. The deficiencies are also known to have negative impact on general growth and development, reproduction, maturity and immunity responses.

International fora including the World Summit for Children and International Conference on Nutrition (ICN) among others, called on national governments and the entire world community to draw up plans of action aimed at limiting and possibly eliminating these deficiencies by ensuring and legislating for the fortification of children's foods with the deficient micronutrients.

In Nigeria, reliable data on micronutrient nutrition are hard to come by. However, the national micronutrient survey that became available about 1993,

suggested that VAD, IDA, IDD and zinc deficiency are prevalent. Consequently, interventional strategies have been suggested, discussed and programme implementation recommended at several fora (NNN, 2000; NACMD, 2003).

So far, some programmes directed at alleviating micronutrient deficiencies in Nigeria, particularly among children and women include salt iodization, iron and vitamin A supplementation, fortification and bio-fortification of staple foods. The 2001 National Nutrition Survey was also aimed at identifying and eradicating vitamin A, zinc and iron deficiencies (IITA, 2004).

2.6.5 Vitamins

Vitamins are organic substances that can be found in plants or chemically synthesized in animals, which are required in trace amounts for health, growth and reproduction. When ingested by animals, most of them as the active vitamin or provitamins, are modified into co-enzymes that act in concert with enzymes to catalyze biochemical reactions (Zubay, 1993). The scientific evidence supporting the important role of vitamins in promoting health and preventing non-communicable diseases, independent of other nutritional constituents, has been stressed (Blunberg, 1995). Vitamins essential for human are classified into fat-soluble (Vitamins A, D, E and K), and water-soluble (Vitamins of the B group, vitamin C, niacin folic acid and pantothenic acid). In recent times vitamin A has received global attention due to high prevalence of its deficiency particularly in infants and children.

In many parts of the world, efforts to ensure adequate vitamin status for the primary prevention of corresponding deficiencies still continue to receive serious attention. Recommendations offered are focused on the consumption of appropriate (or fortified) foods that provide adequate vitamin A to promote growth, prevent night

blindness and strengthen corneal structure and immune function in children; folic acid to help reduce the risk of neural-tube defects as well as anaemia and prenatal mortality in women of childbearing age; Vitamin D to promote bone health in children, Vitamin C, E and certain B vitamins (B_6, B_{12}) and folate to reduce the risk of cardiovascular and cerebrovascular diseases (Key, 1994). The putative protective role of anti-oxidant vitamins (Vitamin A, C, E) against vascular diseases, appears to be based in part on their ability to inhibit the oxidative modification of LDL cholesterol, a critical early step in the atherogenic process (Steinberg, 1991). Increased intake of vitamins from fruits and vegetables by children may provide a wide spectrum of benefit in respect of these physiological functions and many diseases.

Vitamin A

Vitamin A (a fat-soluble vitamin) occurs in many forms; as retinol (alcohol), retinal (aldehyde) retinyl acetate or palmitate (esters) and vitamin A carotenoids (β-carotene, α-carotene). Vitamin A is relatively unstable, particularly in harsh environments. The instability is mostly due to its chemical nature, which contains many double bonds that are susceptible to degradation (Wirakartakusumah and Hariyadi, 1998).

In animals, Vitamin A exists largely in the preformed state as retinol or as its related compounds. In plants, it occurs in the precursor, or provitamin form as carotenoids, which animals convert into vitamin A after ingestion in the diet (Bloem and Darnton-Hill, 1999). Dietary sources of vitamin A include meat, diary products carrot, green leafy vegetables, deep-yellow fruits and red palm oil (UNICEF, 2004). Preformed vitamin A can be obtained from foods of animal origin. However, low consumption of these foods in poor countries and families has limited this important dietary source of the vitamin especially in children. Infants who are exclusively

breastfed receive vitamin A from breast milk for the first 4-6 months, and thereafter in addition, from complementary foods.

Functions of Vitamin A.

Vitamin A has been identified as essential for vision, growth, cell differentiation, immune system and reproduction (Guthrie, 1989; Okoye, 1992; UNICEF, 2004). Clinical physical deficiency can manifest in children in any one of the following ways:

(i). Irreversible blindness (Xerophthalmia) which is one of the most visible dramatic consequences of vitamin A deficiency and a leading factor in the prevalence of childhood mortality and disability (Sommer and West, 1996).

(ii) Increased severity of measles in children due to damage of epithelial tissues throughout the body, and also plays a role in corneal blindness (Bloem and Darnton-Hill, 1999) It has also been shown that improved vitamin A status of children before the onset of measles or after markedly reduced the risk of complication and death.

(iii) Vitamin A and its derivatives are important for the normal differentiation of the haematological cell systems (Bloem, and Darton-Hill,1999). Epidemiological studies have also indicated that Vitamin A deficiency and anaemia often coexists, and that there are significant associations between serum retinol and biochemical indicators of iron status (Mohanram et al, 1977; Mejia and Arroyave, 1988). In another study in Indonesia involving pregnant women, Suharno (1992) showed that 100% of aneamic pregnant women were cured by a combined therapy of Vitamin A with iron, whereas only 40% by vitamin A alone, and 60% by iron alone. This clearly indicated that vitamin A supplementation has greater impact on reducing the prevalence of anaemia than the usual iron therapy alone.

(iv) Vitamin A plays an important role in stimulating the immune system and in the maintenance of mucosal surfaces. Deficiency may lead to reduced T-cell and B-cell functions, which may in turn contribute to high viral loads or lower levels of maternal antibodies crossing the placenta. Deficiency may also compromise the integrity of the placenta, or makes the birth canal more susceptible to trauma and exposure of infants to maternal blood. High viraemia in breast milk has also been associated with vitamin A deficiency (Semba et al, 1994; SCN, 1995). Vitamin A has been shown to reduce infant and children mortality from diarrhea and other gastrointestinal disorders (AHRTAG, 1993).

Causes of Vitamin A Deficiency and Control Measures

Vitamin A deficiency (VAD) occurs when body (liver) stores are depleted to the extent that physiological functions are impaired. In recent times VAD is considered as one of the most frequent nutritional disorders in children all over the world. Globally extrapolations from the best available data suggest that 140 million preschool children and more than 7 million pregnant women suffer from Vitamin A deficiency every year (SCN, 2004). In countries where immunization programs are not widespread and vitamin A deficiency is common, millions of children die each year from complications of infectious diseases such as measles with 2.8 million showing frank signs of xerophthalmia (Kennedy et al, 2003).

The situation in Nigeria reviewed at the meeting of the National Advisory Committee on Micronutrient Deficiencies (NACMD, 2003) revealed that the 1993 survey on the nutritional status of Nigerian women and children showed a VAD prevalence of 9.2% and 7.2% for under five children and mothers respectively, with wide spatial variations (2% in the Southwest and as high as 16.9% in the Northeast)

attributable to food consumption patterns across the country (green leafy vegetables, fruits and palm oil, are much more prominent in the diets in southern Nigeria). In terms of specific figure the review indicted that more than 9 million children are vitamin A deficient in Nigeria, and over 300, 000 child deaths could occur from vitamin A deficiency over the next ten years if nothing is done, and warned that when combined with other nutritional deficiencies and ill-health, VAD would pose a serious threat to child survival. Over the years, strategies to control VAD in Nigeria rely on vitamin A supplementation, food fortification and dietary diversification. NACMD observed that supplementation through the National Immunization Days (NIDS) have been successful, but the costly option is unlikely to be sustainable beyond the short term. They therefore saw the need for alternative mechanisms like food fortification that would ensure wider coverage of high-risk populations at low cost.

Following the benchmark survey in 1995, three staple foods (Wheat/Corn flour, sugar and vegetable oil) have been identified and chosen as vehicles for fortification with vitamin A. This is because of their relative affordability, accessibility and technical feasibility. Fortification of these foods at the household, community and industrial levels has been identified to be the most cost-effective, sustainable and of high coverage in the medium term. In view of this, effective implementation of fortification of flour has started, while oil and sugar is expected to follow (NACMD, 2003). Other intermediate and long term strategies that have been suggested include breastfeeding, appropriate feeding and weaning practices, production and consumption of micronutrient-dense foods, bio-fortification of staple grains like maize, nutrition education and poverty alleviation (NACMD, 2003).

Recommended Daily Allowance for Vitamin A

The biological vitamin A activities of diets are computed in retinol equivalent (RE). Recommended daily allowance (RDA) for vitamin A set for some developed countries show that adult male would require 1000 RE daily, while adult female would need 800 RE. The RDA for infants based on the content and volume of human milk is 375 – 420 RE until the age of six months and 375 – 400 RE thereafter until the first year (Guthrie, 1989). These recommendations are expected to vary from country to country. There is paucity of data on the RDAs of vitamin A for the Nigerian population. Recommendations are usually based on foreign literature values and the Cordex Alimentarius Commission for food standards.

2.6.6 Mineral Elements

Mineral elements are generally classified as either microelements or macroelements. The macroelements are those present in relatively higher amounts (750ppm) in animal tissues. They include Ca, K, Na, Mg, S., PO_4^{3-}, Cl. The microelements, also called trace elements, are present at less than 50ppm. Trace elements essential for human nutrition include among others Fe, Zn. Mn, Se, F, I_2. Mineral elements unlike proteins and vitamins, cannot be synthesized in the body, and so must be obtained through dietary means. Hence the amount of a mineral element in an animal tissue reflects the amount present in the food consumed by the animal, which is in turn a function of the element present in the soil, and the extent to which the plant concentrates it during growth (Mertz, 1980).

Functions and Bioavailability of Mineral Elements.

Minerals serve one or more functions in the body. They are constituents of skeletal tissues, cofactors to enzymes, carrier proteins, protein hormones and electrolytes in body fluids and cells (Okoye, 1992). Because of the rapid rate of growth and development in infants and children, mineral element nutriture has become very essential.. For instance, calcification of bone is needed in early infancy to support the weight of the body by the time the baby walks. Also the availability and utilization of calcium in the postnatal period is a crucial factor in adequate tooth formation. Iron is needed especially in the first year of life to prevent iron-deficiency anaemia. Zinc is critical during the developmental period because of its role in normal brain development and immune system. Iodine deficiency have been implicated in various Iodine deficiency disorders (IDDs). Flourine and phosphorus are important in the development of teeth (Guthrie, 1989).

Bioavailability of mineral elements is affected by a number of factors. Foods and diets of animal origin have been shown to contain mineral elements in forms that are more readily absorbed (Pennington et al, 1988), while those of plant origin are less available due to the presence of some antinutritional factors (FAO/WHO, 1998). For instance, Okoye (1992) observed that iron deficiency anaemia might result from poor iron content of staple diet, poor absorption from the gut lumen and excessive concentration of phytates and tannins in the diet or the form of iron present in the staple diet. Zinc absorption is also impaired by phytates and fibre (FAO/WHO, 1998). Dietary calcium deficiency in Nigeria is attributed to the consumption of diets with high phytate and phosphate levels (Okonofua, 2002). Children often have an inadequately low calcium intake thereby giving rise to high prevalence of nutritional ricket (Oginni et at,

1996; Thacher et at, 2000). Other factors include mineral – mineral interaction, processing methods that result into loss of minerals, and ignorance of mineral–rich foodstuff found in the communities. In developing countries, the diets of most people or families, which are basically plant-based, are marginal for micronutritional adequacy. These and other anti-nutritional effects may be of significance especially when formulating infant diets.

In addressing these problems, and that of micronutrients in general, a combination of strategies involving the promotion of breast feeding, dietary modification/diversification (to include increased consumption of animal – source foods), food fortification and supplementation, have been emphasized and implemented in some countries (Kennedy et al, 2003).

Assessment of Mineral Element Status in Children

The most effective way of determining whether an elemental deficiency is present or absent is really that of monitoring the response of biochemical and functional indices, and of plasma and tissue concentrations to supplementation. (Jacob, 1987). The most popular of currently employed analytical techniques for biological materials include Atomic Absorption Spectrophotometer (AAS), Electron Spin Resonance (ESR), Mass Spectroscopy (MS) and Colorimetry. However, certain drawbacks such as complexity, expense of equipment and materials, high level of operator skill, matrix sensitivity etc., have placed some constraints to the use of most of the techniques leaving AAS and Colorimetry as the most suitable methods of choice (Jacob, 1987). There are however specific static indices of deficient mineral intake that can be used to assess status (Okoye, 1992).

Iron

It is highly unlikely that life in any form can exist without iron because of its diverse functions. It is essential for the production of haemoglobin, which helps deliver oxygen from the lungs to body tissues, transport electrons in cells, and synthesis of iron-containing enzymes that are required to utilize oxygen (O_2) for the production of cellular energy (Cook, 1982). The body's iron stores, iron absorption and iron loss determine iron balance. At least two-third of body iron is functional iron, mostly haemoglobin within circulating red blood cells, with some myoglobin in muscle cells and parts of iron-containing enzymes. Most of the remaining body iron is storage iron (existing as ferritin and haemosiderin), which serves as a deposit to be mobilized when needed (ACC/SCN, 2000). The reduction of body iron has three main stages;

i. Iron depletion, which refers to a decrease of iron stores measured by reduction in serum ferritin concentration.

ii. Iron deficient erythropoeisis, when storage iron is depleted and there is insufficient iron absorption to counteract normal body losses.

iii. Iron deficiency anemia, which is the most severe degree of iron deficiency that ensues if the haemoglobin concentration falls below normal (Gillespie and Johnson, 1998).

While the biochemical liabilities of deficiencies are evident, the efficacy of body iron conservation and iron's ability to generate reactive species should caution against supplying excess iron to those with adequate iron reserves. If body iron reserves are present at birth, the infant will need virtually no dietary iron for at least three months after birth. Intake from six to twelve months of age can be obtained mainly through diet,

such as iron-fortified formulas to meet the cut-off point for blood hemoglobin concentration of 110g/liter for children ages 6-59 months (Gillespie and Johnson, 1998).

Prevalence of Iron Deficiency

Iron deficiency and its anaemia (IDA) is the most common micronutrient malnutrition problem in the world as it affects more than 3.5 billion people globally, of which about 2 billion are children and women (UNICEF, 1998; UNICEF/UNU/WHO/MI, 1999). It is associated with an estimated 111,000 maternal deaths each year (SCN, 2004). The reports indicated that prevalence in developing countries is 3 to 4 times higher than in industrialized countries. In Nigeria, the 1993 micronutrient survey data showed that more than 50% of women and young children in the southeast suffered from IDA.

Iron deficiency has continued to weaken children's learning abilities through compromised mental development, lowers resistance to infections, fetal growth retardation, maternal death and increased morbidity in the developing world. (WHO, 1997).

Anaemia in Children

Anaemia is when the level of haemoglobin in the blood is below normal either because there are too few red blood cells, or there is too little haemoglobin in each cell or both (Draper, 1997). There are different types of anaemia, but iron deficiency anaemia is the most common. Deficiency of other micronutrients (Folate and B_{12}) causes a different kind of anaemia.

Until recently, most efforts to reduce anaemia was focused on women, but there is now increased efforts to prevent anaemia in children aged from six months to two

years. This has become necessary because severe anaemia among this age group has been found to cause slow mental development, reduced learning capacity, weakness and increased susceptibility to infections (Draper 1997). Major causes of iron deficiency in children after six months of age have been identified to include inadequate dietary intake, poor absorption and parasitic infections. The iron (non-haem iron) present in many traditional cereal – based complementary foods in Nigeria and developing countries in general, are not well absorbed by the body due to inhibitors (Draper, 1997; NACMD, 2003). Haem iron is found in foods containing blood and muscle, and is relatively well absorbed, but the economic situation in Nigeria does not allow for the consumption of such food in most families.

Prevention and Control of Iron Deficiency in Children

One of the acceptable recommendations for infant feeding to ensure good iron status and prevent deficiency is breastfeeding for at least four to six months. This is followed by iron-rich foods while breastfeeding continues for at least two years. The presence of lactoferrin in breastmilk has been shown to enhance iron bioavailability to approximately 50% to 80% (ACC/SCN, 1997; UNICEF, 1998). Thus, the prevalence of iron deficiency anaemia in early infancy has been inversely correlated with the incidence of breastfeeding (Yeung, 1998).

Because of the limited food choices of infants, fortification of complementary foods has been one of the approaches used to prevent and control iron deficiency in children. For instance in many developed countries, commercially prepared infant foods are fortified with iron and other deficient micronutrients, and their wide acceptability/consumption has been shown to lower incidences of iron deficiency (UNICEF, 1998). In developing countries, where cereals and legume –based single or

double mixes are usually the first semi-solid foods introduced to infants, incidences of iron deficiency has been high (UNICEF, 1998). Fortification of staple foods through plant breeding or their products at processing level, has been advocated as low-cost and sustainable approach (ACC/SCN, 2000). In Nigeria however, salt fortification with iron (as well as iodine), supplementation with iron-folate, and dietary diversification are some of the intervention measures taken. Others are prevention of worm infestation and malaria control through use of insecticide treated nets (NACMD, 2003).

To effectively prevent IDA, intake of vitamins A, C, E, B_2, B_{12} and folate, as well as zinc and copper must be promoted. Vitamin. C and copper improve iron absorption while vitamin A and zinc mobilize iron stores. Folate and B_{12} are important for the production of red blood cells. Vitamin E protects against oxidative damage (Nutriview, 2002/1).

Food Sources of Iron

Liver is the only rich and readily available source of iron, but it has never been a popular diet item in developing countries. Most people however depend on a variety of alternative sources. No one-food group is responsible for a large share of iron in the diet, but meat, cereals, and vegetable group make significant contributions. Because of wide difference in iron absorption from various sources, knowledge of the iron content of foods does not always give a true picture of its availability (Nutriview, 2002/1).

Effect of Food Preparation on Iron Content.

The major cause of loss of iron in the preparation of food are the discarding of iron–rich cooking water and the removal of iron-concentrated vegetable peelings. So any cooking method that minimizes the possibility of iron dissolving in the cooking water, such as using relatively large pieces of food, cooking foods with skin or peels on,

and simmering rather than boiling, will increase the amount of iron available in the diet. Steamed vegetables have more iron than boiled vegetables as do those cooked for a short time in a small amount of water, so also those cooked in their skins than peeled ones. The use of vegetable stock in soups or foods prepared for children also helps minimize iron losses. The use of iron cooking pots increases the iron in food products (Guthrie, 1989). These and other fortification options should be taken into consideration when complementary foods are prepared and fed to infants.

Test of Iron Status of an Individual.

Although there are many indicators of iron status in the human body, the commonly used are serum ferritin, red cell protoporphyrin, transferrin saturation, mean corpuscular volume and haemoglobin/haematocrit content. Serum ferritin is the most sensitive indicator. Transferrin saturation also known as total iron-binding capacity (TIBC), which occurs with a depletion of iron stores, can be used. TIBC of less than 16% indicates that iron reserves are inadequate to meet the needs for iron (Cook, 1982).

The determination and use of haemoglobin content of the blood is insensitive to the early stages of iron deficiency. It is however useful in assessing the severity of anaemia (Cook and Skikne, 1989).

Zinc

Of the forty or so micronutrients essential in the human diet, deficiencies of only three namely vitamin A, Iron and Iodine are generally thought to be of public health significance and targeted for prevention in development programmes (Guthrie, 1989). There is now growing knowledge about the essentiality of zinc in human nutrition where it has been shown to play a number of roles in biochemical reactions.

Essentiality of Zinc in Human Nutrition

Zinc is one of the essential mineral elements that is found in almost every cell, where it stimulates the activity of over 100 enzymes needed for various biochemical reactions (NIH, 2002). In fact, it has been established that the six categories of the international nomenclature are zinc metalloenzymes (Goldez and Vallee, 1983). Important enzymes stimulated by zinc, support metabolic processes such as the immune system, wound healing, organoleptic abilities of taste and smell, brain development, synthesis of DNA and RNA, normal growth and development during pregnancy, cell division, sexual maturation, storage and release of insulin among others (Atinmo, 1980; Guthrie, 1989; Shrimpton, 1993; Bahijri, 2001; NIH, 2002).

Because of its roles in the biochemical processes of growth and development, zinc is considered as one of the most essential mineral elements in foetal, infant and early childhood development. For instance, the essentiality of zinc in normal brain development suggests a clinical function for the element during the prenatal period (Prasad, 1982; Guthrie, 1989). The role of zinc in the immune system has also been associated with mother- to –child transmission of HIV (ACC/SCN, 2000).

Zinc Metabolism

Zinc seems to have an interrelated metabolic pathway with vitamin A, iron and copper. Zinc has been shown to be an essential component or cofactor in the enzyme that converts provitamin A into retinol, and its deficiency seems to interfere with vitamin A metabolism (Ross, 1999). This seems to support the notion that any vitamin A supplementation will require an increase of zinc.

Food Sources and Bioavailability of Zinc.

During the early feeding period of infants, the high level of zinc in colostrum (4mg/L) covers the requirement of the period. This level however declines to 1.2mg/L at one year (Sandstead, 1985). Lack of supplementation or introduction of zinc-fortified complementary foods at this stage results in deficiency. Total dietary zinc intake may be adequate, but bioavailability particularly from plant-based foods may be reduced due to high phytate contents (SCN, 1995). Populations that largely depend on plant-based foods would require supplementation and fortification of foods meant for infant and children. Beneficial effects of zinc supplementation and fortification of foods have been demonstrated (Shrinpton, 1993).

To evaluate whether the amount of zinc provided by the food is adequate and appropriately selected, zinc reference values are often used to compare the level of zinc in the food consumed and its bioavailability in animal models with the RDAs. Allowances for infants based on the zinc concentration of human milk are set at 3 – 5mg/day (Guthrie, 1989) and 2 – 3mg/day for ages 0 – 1 year (NIH, 2002).

Zinc Deficiency and Control Measures

Zinc deficiency is widespread but under-recognized.. (Shrimpton, 1993). Developing countries are the worst hit, and is often closely associated with iron deficiency (SCN, 1995). The most vulnerable groups are infants, children, pregnant, and lactating women. In children, deficiency is an important but often an overlooked factor in the aetiology of poor child growth, increased incidence and severity of infection, and in impaired cognitive development. Zinc deficiency impairs the mobilization of vitamin A from the liver and may also decrease its absorption thereby contributing to vitamin A deficiency. Deficiency has also been shown to contribute to

complications during pregnancy and poor pregnancy outcome (SCN, 1995; UNICEF, 1997).

There are very scanty data available on the causes, distribution or magnitude of zinc deficiency in Nigeria. This was the view expressed by Chibuzo (2003) in a paper presented at the meeting of the National Advisory Committee on Micronutrient Deficiency Control. In the presentation, two published data or studies seem inadequate. Although an overview of the National Survey on zinc in ten states in Nigeria seems to indicate that pregnant women had serum zinc level above the cut-off point, the study does not include all subjects. This lack of appropriate research work on zinc status in Nigeria highlights the urgent need for epidemiological studies to determine if indeed there is zinc deficiency in Nigeria, and the dose to recommend for supplementation of the nutrient if a deficiency actually occurs.

Besides supplementation, other two important determinants of good zinc nutriture in infants and children are food supply and the bioavailability of the zinc in food. Dietary diversification and fortification can therefore be used to improve the content and bioavailability of zinc, and this would involve the use of zinc–dense foods in complementary food productions, as well as using food processing and preparation techniques to reduce the phytate content of unrefined cereals, legumes and tubers.

There is no fortification medium/vehicle recommended for zinc in Nigeria yet. But Zambia and South Africa have identified maize meal and wheat flour as vehicles for zinc fortification. Since vitamin A and zinc metabolism are interrelated and since vitamin A fortification has been initiated in Nigeria, Chibuzo (2003) is of the view that the vehicles used for vitamin A could also be used for zinc fortification.

Iodine

Iodine is an inorganic mineral, and one of the many mineral elements that are essential to humans. It is required by the thyroid gland for the synthesis of the thyroid hormones (tyrosine and triiodotyronine), which mediate a number of processes such as energy and heat production, synthesis of body tissues as well as proper growth and brain development. It is therefore required throughout life. It is however estimated that nearly two billion people (35.2%) worldwide have inadequate intake of this important mineral element (SCN, 2004). In Nigeria, a 20% prevalence rate of iodine deficiency disorders (IDD), with an estimated 25 to 35 million at risk, have been reported (NACMD, 2003)

Food Sources and Utilization of Iodine

While the soil is a repository of iodine, seafoods are the richest and only reliable sources. The iodine content of non-seafoods depends to a large extent, on content in soil on which food crops are grown or animals are raised (Okoye, 2003). This assertion has also been demonstrated in a study to examine the goiter-soil-water-diet relationship in some communities in Plateau State by Ubom (1991), in which it was shown that low iodine content in drinking and cooking water, soil and diets were partly responsible for endemic goiter in the goitrous areas. Apart from the poor iodine in soil and water, many of our staple foodstuffs and domestic water sources have been shown to contain cyanogenic glycosides (goitrogens), which are anti-thyriod agents that impair the body's ability to utilize iodine (Ubom, 1991; Okoye, 2003).

The human body has very efficient machinery for utilization of dietary iodine. Iodine ion, which is the form present in food and drinking water, is also the form in which iodine is absorbed from the gut lumen into the blood. The uptake of iodide by the

thyroid gland from the circulating blood is energy-dependent and very efficient. Once inside the gland, iodine ion is converted (oxidized) to the molecular form of iodine (I_2). It is the molecular iodine that serves as a building block for the manufacture of the thyroid hormones. Many of the naturally occurring non-nutrient compounds in staple foodstuffs and domestic water sources interfere with the various stages of utilization of iodine by the body, the ultimate effect of which is condition of iodine deficiency (Okoye, 2003).

Iodine Deficiency and Control Measures

According to Okoye (2003), failure to derive sufficient iodine from the diet either as a result of lack of iodine in diet or presence of goitrogens in food or water, has a negative effect on the production of thyroid hormones by the thyroid gland. The ultimate consequence depends on the extent of deficiency. Where deficiency is marginal, the thyroid gland responds by increasing its size (cell number and cell size) to create additional surface area for uptake of iodine from the blood. By so doing, the gland is able to mop up the iodine in the blood circulation to sustain a normal hormone production level. Where the deficiency persists for a long period, the adaptive changes in the thyroid gland may lead to a pronounced increase in size of the gland. This manifests outwardly as a swelling in front of the neck, a condition termed simple goitre.

Simple goiter is a painless condition but has some undesirable effects on physical appearance. If the thyroid gland continues to grow, it exerts pressure on either side of the trachea leading to difficulty in breathing (Guthrie, 1989). Goitre is a good indicator of iodine deficiency in a community, and epidemiologists use the number of goiter cases (expressed as total goiter rate, TGR), especially among children of school age as a measure of incidence of IDD (Okoye, 2003)

In a situation where iodine deficiency is significant, the thyroid gland is unable to manufacture sufficient amount of thyroid hormones (hypothyroidism), which is most telling at the developmental and growing stages of human life (foetal and infant stages) particularly the brain and the central nervous system. Even mild deficiency may lead to decrease of Intelligence Quotient (IQ). The good news is that IDD is one of the most preventable disorders if appropriate control majors are taken. (Okoye, 2003) to meet the body's needs for energy metabolism, development of the brain and central nervous system, and growth. As a result, these functions are impaired and may, if untreated, culminate in definite disease conditions or disorders, which include low mental acuity, partial paralysis, deafmutism, dwarfism, facial and physical deformity, lassitude, neurological damage and cretinism. An individual may present with two or more of the above manifestations (Okoye, 2003).

As a result of the unreliability of non-seafoods as sources of nutrient iodine on account of the ubiquity of iodine poor soils and the presence of goitrogens, iodine supplementation has been suggested (Ubom,1991; Okoye, 2003) as a means of providing human iodine needs. In this regard, universal salt iodization has proved the most efficient method of providing iodine in the human diet since most humans consume salt on a daily basis (Okoye, 2003). Worldwide iodization of salt has been reported to produce major reduction in iodine deficiency disorders (Kennedy et al, 2003).

The 2003 report of the National Advisory Committee on Micronutrient Deficiencies indicated that over 98% of Nigerian households consume iodized salt, and impact evaluation of some previously IDD endemic locations indicated drastic reductions in total goiter rate (TGR), and more significantly, urinary iodine excretion

rate. This success of the IDD control program is attributed to the willingness, political support and commitment to salt iodization of the salt industries in the country.

Good food processing practices to neutralize the naturally occurring goitrogens in foodstuffs (Okoye, 2003), and water purification to eliminate pollutants have been suggested as part of an effective IDD eradication program (Ubom, 1991). Adequate intake of iodine by pregnant and lactating women from various iodine sources in the maternal food, would increase maternal and child endowment and increased iodine in milk. Foodstuffs and water used in the preparation of complementary foods must be properly processed and treated respectively in order to reduce the level of goitrogens, and water pollutants, thereafter further fortified with iodine if necessary.

Recommended Daily Allowances

Man requires only about a teaspoonful of iodine throughout his life-time (Okoye, 2003). The Food and Nutrition Board found that an intake of 1μg of iodine/kg of body weight is adequate for most adults. A daily intake of 150μg for both adult male and female is recommended. For pregnant and lactating women, the requirement is 50μg higher. The need of growing children, especially girls, may exceed the suggested level of 1μg/kg of body weight. 40 to 50μg has been recommended for infants and children up to 6 months of age (Guthrie, 1989). There are no recommended intake for Nigerians.

Evaluation of Iodine Status

Creatinine urinary excretion is directly proportional to iodine availability. Hence excretion of iodine in a single sample of urine relative to the amount of excreted creatinine is used to evaluate iodine status. Ubom (1991) showed a strong correlation between iodine excreted in the urine with goiter endemia. The uptake of a stable isotope of iodine by people with a normally, functioning thyroid is also useful in assessing

iodine status. Others are measurement of T3, T4 and thyroid stimulating hormone levels and the assessment of protein-bound iodine (PBI) in the blood. Amount parallels both the level of thyroxine in the blood and the basal metabolic rate. Since basal metabolic rate reflects thyroxine levels, it is an indirect measure of iodine status (Guthrie, 1989).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Experimental Food Samples

The study was conducted in two parts. The first part involved the formulation and analysis of seven different cereals and legume based complementary diets using staple foodstuff readily available in Plateau State. This was to allow for the use of a wide range of foodstuff available to mothers that could be used to prepare diets inexpensively, and nutritious enough to meet the nutritional requirement of infants and children. Analysis of the formulated diets was carried out alongside a commonly used proprietary infant cereal (Nestle Cerelac) as control. This was to identify the diets that closely mimic the control, hence hold promise.

The foodstuffs used were–maize (*Zea mays*), acha grains (*Digitaria exilis*), rice *(Oryza sativa),* millet *(Pennisatum typhoides),* Sorghum *(Sorghum bicolar),* soya beans (*Glycine max*), groundnut (A*rachis hypogea*), bambaranut (*V-oandzeia sub terranea*), sesame (*Sesamun indicum*), carots (*Daucus carota*), crayfish (*Macrobrachium sp*), garden egg (*Solanum incanum*), palm oil (*Elaeis guineensis),* table salt (NaCl). The foodstuffs and the proprietary formula were purchased from Jos main markets.

3.1.2 Experimental Animals

Male Winster strain weanling rats weighing between 40 – 55g, purchased from the Animal House of the University of Jos, were used in the animal experimentation aspects of the study.

3.1.3 Chemicals and Reagents

Vitamin A palmitate, riboflavin, thiamine hydrochloride, ascorbic acid and pyridoxine hydrochloride were products of Roche Switzerland, Deutschland, and were kind donations from Europharm Laboratories, Jos, Plateau State.

Citric acid, copper sulphate, selenium dioxide, anhydrous glucose, sodium potassium tartrate, trisodium citrate, sodium pyruvate, sodium tungstate dehydrate, sodium bisulphate, magnesium carbonate, ammonium molybdate, sodium EDTA, 1-amino-1-naphthol-4- sulfonic acid, potassium iodide, silver nitrate, sodium hydrogen phosphate, L-aspartic acid, α-ketoglutaric acid, crystalline phenol detached and NADH were purchased from Sigma Chemical Company, St. Louis, USA.

Disodium phenyl phosphate, thiourea, chloroform, acetone, methanol, petroleum ether, nitric acid, perchloric acid, calcium oxide, hydrogen peroxide, 2, 6–dichlorophenol indophenol, bromocresol green, sodium hydroxide pellets, tungstophosphoric acid, anhydrous picric acid, creatinine, oxalic acid, urea, diacetyl monoxide were products of May and Baker (M&B) Laboratory Chemicals, Dagenham, England.

Sulphuric acid, sodium sulphate, boric acid, methyl red, hydrochloric acid, sodium carbonate, sodium hydrogen carbonate, glacial acetic acid, potassium ferricyanide, potassium permanganate, absolute ethanol, antimony trichloride, trichloro acetic acid, acetic anhydride, potassium hydroxide, ammonia solution, sodium nitrate, o-toluidine, sodium azide, dl-alanine, magnesium chloride, sodium indigotin disulfonate and thiosemi carbazine were products of British Drug House (BDH) Chemical Limited, Poole, England.

Diethyl ether was purchased from Thomas Baker (Chemicals) Ltd., Bombay. 1, 4–dinitrophenyl hydrazine was of East Anglia Chemicals, Hadleigh, Ipwichs, Suffolk, UK. Anion exchange resin AG-1-x4 was obtained from Bio-Rad Laboratories. Reagent kits containing buffer solutions (vial R1), enzymes (vial R2) and standards (vial R3), used for cholesterol and triglycerides determination, were obtained from Biolabo Place Albert Camus, France. Serum iron kit (Cat. No.4/257) containing chromagen, reductant, buffer and standard, used for the determination of serum iron and total-iron-binding capacity, were of Biotech Laboratories Ltd, Ipswich, and Suffolk, United Kingdom.

3.1.4 Equipments

A Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer (Model 180-80) was used in all elemental analysis. Ion Chromatographic Analyzer (ICA) Model IC 100-25 was used to detect and quantitate anions, and peaks printed on a YEW Kokusai Chart. MSC Mistral 2L refrigerated Ultra centrifuge and MSE bench-top centrifuge were employed in centrifugation procedures. Cecil 373 and CE 505 double beam ultraviolet spectrophotometer were used in absorbency determinations. MSE orbital shaker was employed in procedures that require vigorous shaking. Technicon Amino Acid Analyzer (TSM-1) was used in amino acid analysis. Gallenkamp Soxhlet extraction apparatus unit (model 300–10R) was used in fat extraction processes. Gallenkamp drying oven (model OVL 270-0900) was used for drying purposes while Barkmeyer Muffle Furnace (Model Ney-525) was used for ashing purposes.

3.2 METHODS

3.2.1 Preparation of Specialized Reagents and Buffers

0.1M Carbonate-Bicarbonate Buffer (pH 10.0). Sodium bicarbonate (8.4g) and Sodium carbonate (28.6g) were separately dissolved in small amount of distilled water before mixing the two in one litre volumetric flask and made up to the mark. The pH was measured, adjusted and stored at 4^oC until required.

0.1M Phosphate Buffer (pH 7.4) Anhydrous Na_2HPO_4 (11.3g) and anhydrous KH_2PO_4 (2.7g) were separately dissolved in distilled water and the two poured into a one litre volumetric flask and more distilled water added to the mark. pH was adjusted and solution stored at 4^oC until required.

0.1M Citrate Buffer (pH 4.2). This was prepared by dissolving 29.4mg trisodium citrate powder with distilled water in a one litre volumetric flask. Similarly, 21.0g citric acid monohydrate was dissolved with distilled water in a separate one litre volumetric flask. Exactly 300ml of the trisodium citrate and 400ml of the citric acid solutions were mixed and pH adjusted to 4.2. The buffer was also stored at 4^oC until required.

Acetate Buffer (pH4.5). Exactly 2.5g anhydrous $CaCl_2$ was weighed and dissolved in 50ml acetic acid (1:1v/v) and added to solution of 33g sodium acetate diluted to 50ml with distilled water. pH was adjusted to 4.5 and stored at 4^oC until required.

Stock and Working Phenol Standard. Stock phenol solution (1mg/ml) was prepared by dissolving 100mg of crystalline phenol in 100ml 0.1M HCl. Exactly 1.0ml of the stock solution was diluted to 100ml with distilled water to give a concentration of 0.01mg/ml

Stock and Working Pyruvate Standard. Stock pyruvate standard was prepared by dissolving 220mg of sodium pyruvate in 100ml phosphate buffer pH7.4), and stored frozen in 1 ml aliquots. Just before use, 1ml aliquot of the stock solution was diluted 1 in 5 with more phosphate buffer to give a working pyruvate standard solution (20mM).

Stock and Working Bromocresol Green (BCG) Dye Solution. Stock BCG (0.6mM) was prepared by dissolving 419.0mg Bromocresol Green in 10ml 0.1M NaOH solution and diluted to one litre with distilled water. Sodium azide (100mg) was added as preservative. 30% Brij 35 was prepared by heating 30g of pure Brij 35 in a beaker on a hot plate to melt. The melted brij was diluted to 100ml with distilled water. 4 ml of the 30% brij and 100mg sodium azide were then added to diluted BCG stock solution (250ml made up to 1litre with citrate buffer pH4.2) and mixed thoroughly. The resultant solution was stored in a brown reagent bottle at 4^{o}C.

Stock and Working Urea Standard. 50mg/ml stock urea solution was prepared by dissolving 50g urea in 1 litre distilled water; 10ml of the stock was diluted to 250ml with more distilled water to give a solution of 2mg/ml.

Creatinine Standard Solution. A 1mg/100ml creatinine standard solution was obtained by dissolving 10mg creatinine in 1 litre 0.1M HCl in a volumetric flask and kept at room temperature until needed.

Phosphate Standard Solution. This solution was prepared by dissolving 0.35g KH_2PO_4 in a 1 litre volumetric flask with 500ml distilled water. 10ml 10N H_2SO_4 was added and mixture made up to 1 litre with more distilled water.

Stock and Working Ascorbic Acid Standard. Stock ascorbic acid solution was prepared by dissolving 25mg ascorbic acid in 100ml 0.5% oxalic acid to give a

solution of 25mg/100ml. 1ml of the stock solution was then diluted to 50ml with 4% TCA just before use to give the working standard solution.

Disodium Phenyl Phosphate Substrate. Disodium phenyl phosphate (436mg) was accurately weighed and dissolved in distilled water. 1% $MgCl_2$ solution (0.4ml) was added and mixture made up to 100ml mark in a 100ml standard volumetric flask with more distilled water. Three drops of chloroform were added and solution stored at 4^oC until required.

Aspartate Transaminase Substrate Solution. This substrate was prepared by dissolving 13.3g of DL-aspartic acid in 90ml 1M NaOH solution. Exactly 0.146g α-ketoglutaric acid was added and dissolved by the addition of more NaOH solution. The mixture was checked and adjusted to pH 7.4. The Solution was then made up to 500ml with phosphate buffer and stored frozen.

Alanine Transaminase Substrate Solution. Nine grams of L-alanine was dissolved in 90ml of distilled water, 0.146g α-ketoglutaric acid was added followed by 2.4ml NaOH solution to adjust the pH to 7.4. The Solution was then made up to 500ml with phosphate buffer, dispensed into 5ml aliquots and stored frozen.

Nicotinamide Adenine Dinucleotide (NADH) Reduced Solution. Reduced NADH solution was prepared by dissolving 20mg accurately weighed NADH in 2 ml phosphate buffer just before use.

2, 4-Dinitrophenyl Hydrazine (2, 4-DNPH) Reagent. This reagent was prepared by dissolving 200mg 2, 4-DNPH powder in 85ml concentrated HCl in a 1 litre volumetric flask and made up to mark with distilled water. The reagent was stored in brown reagent bottle at 4^oC until required.

Diazo Reagent. Sulfanilic acid was diluted 1 in 10 with water and then 0.3ml was mixed with 10ml sodium nitrate solution just before use.

Biuret Reagent. $CuS0_4.5\ H_2O$ (3.0g) and NaK tartrate (9.0g) were dissolved in 500ml 0.2M NaOH solution. Five grams of KI was added and mixed thoroughly. Volume was made up to 1 litre with more of the NaOH solution.

O-Toluidine Reagent. Five grams of thiourea and 90ml o-toluidine solution were mixed to dissolve and made up to 1 litre with concentrated glacial acetic acid in a volumetric flask. Solution was stored in an amber bottle at 4^oC until required.

Carr-Price Reagent. Carr–Price reagent was prepared by dissolving 12.50g of crystalline antimony trichloride in a litre of chloroform and kept in a tightly stopperred brown reagent bottle at room temperature just before use.

Colour Reagent for Urea Determination. Exactly 67ml of diacetyl monoxime solution (20g/L) was mixed with 67ml thiosemicarbazine solution (5g/L) and made up to 1 litre with distilled water.

Picric Acid Solution. A 0.04M Picric acid solution was prepared by dissolving 8.25g anhydrous picric acid with water and made up to 1 litre. Solution was stored in an amber bottle until required.

Tungstic Acid Solution. One gram polyvinyl alcohol was dissolved in 100ml distilled water with heating. Solution was allowed to cool to room temperature, and then transferred to a 1 litre volumetric flask containing sodium tungstate dihydrate solution (11.1g in 300ml distilled water); and mixed thoroughly. Dilute H_2S0_4 (2.1ml conc. in 200 ml H_2O) was added and made up to 1 litre with distilled water.

Molybdate Solution. Molybdate solution was prepared by dissolving 12.5g-ammonium molybdate in 200ml distilled H_2O in a 500ml volumetric flask, and 50ml 10N H_2S0_4 added. This was then made up to volume with more distilled water.

Sulfonic Acid Reagent. Accurately weighed 0.16g 1-amino-2 – naphthol- 4 – sulfonic acid, 1.92g Na_2CO_3 and 9.6g $NaHSO_4$ were dissolved in 90ml distilled water and then transferred quantitatively into a 100ml volumetric flask. Thereafter the mixture was heated slightly to dissolve, and made up to volume with distilled water. The solution was stored in amber bottle at 4^oC until required.

Indigo Solution. The solution was prepared by dissolving 6.0g sodium indigotin disulfonate in 500ml distilled water by heating. The solution was allowed to cool and 50ml conc. H_2S0_4 added. The resulting mixture was then made up to 1 litre in a volumetric flask with distilled water, and filtered just before use.

3.2.2 Collection and Preparation of Experimental Foodstuffs

All the foodstuff used in formulating the complementary diets were purchased from local markets in Jos, Plateau State, in adequate quantities and processed as follows:

Soyabeans was cleaned by removing unwanted particles and then soaked in clean water overnight. Testa was removed by rubbing in-between the palms and washed several times with more water. The washed beans was then boiled in water for 20 minutes, strained of water and air-dried for two days. The dried beans sample was then slightly roasted in an oven at 70^oC for 30 minutes.

Groundnut and bambaranut were separately cleaned and slightly roasted at 70^oC for 30 minutes.

Sesame was washed with clean water to remove unwanted particles, rubbed in between the palms to remove testa, and then washed again several times before air-drying for two days. It was then roasted at 70^{o}C for 10 minutes

Paddy rice, sorghum, millet, maize and acha grains were separately washed with clean water and air-dried for 24 hours.

Fresh carrots and ripped garden egg were separately washed, grated and air-dried for two days.

The dried foodstuffs were milled to smooth homogenous powder and packaged into airtight containers until required.

3.2.3 Formulation of the Experimental Diets (Composite Blends)

The protein content of each processed foodstuff was determined by the Micro Kjeldahl method, and the composite diets formulated on the protein basis. The first set of seven diets were formulated as follows:

Diet 1 – Yellow maize: Soyabeans: Groundnut (60:30:10% w/w)

Diet 2 - Sorghum: Soyabeans: Groundnuts (60:30:10% w/w)

Diet 3 – Millet: Soyabeans: Groundnut (60:30:10% w/w)

Diet 4 – Rice: Groundnut: Bambranut: Carrot (60:20:10:10% w/w)

Diet 5 – Soyabeans Alone (100%)

Diet 6 – Acha: Sesame: Crayfish: Garden egg (60:25:10:5% w/w)

Diet 7 - White maize: Sesame, Groundnut: Crayfish (60:20:10:10% w/w)

Diet 8 – Nestle Cerelac (Served as standard or control diet)

The eight diets were analyzed for their proximate nutrient composition and then fed to laboratory rats. Their nutritional potential in terms of feed intake, growth rate, efficiency ratios, and mortality rate were assessed. Diets that were found to be

promising were enriched and modified by the addition of red palm oil, salt and crayfish. These modified diets formed the set of samples used in the second part of the study. The modified diets were re-designated as follows:

Diet 1 – Rice: Groundnut: Bambaranut: Crayfish: Carrot: Salt* (50:20:10:10:10% w/w)

Diet 2 – Acha: Sesame: Crayfish: Garden egg: Palm oil: Salt*(60:20:10:5:5% w/w)

Diet 3 – Yellow maize: Soyabeans: Groundnut: Crayfish: Palm oil: Salt* (60:10:10:15:5% w/w)

Diet 4 – Nestle Cerelac (Served as the standard control diet).

* 0.5g of salt was added to every 100g composite blend just before feeding.

The modified diets were again subjected to various nutritional evaluations, and results compared with the standard control diet to warrant recommendations.

3.2.4 Chemical Analysis of the Diets

Moisture Content Determination

The standard method of AOAC (1990) was used to determine the moisture contents of the experimental diets.

Procedure

Clean petri dishes with lids were labeled and dried in an oven at 100^{o}C for 30 minutes, cooled in a dessicator containing reignited CaO as desiccant, and weighed to a constant weight. Two grams each of the powdered samples was accurately weighed into the labeled petri dishes. The dishes and samples were weighed again before drying in the oven at 60^{o}C for 5 hours in the first instance, and then quickly transferred into a dessicator containing CaO as dessicant to cool. After cooling, the

dishes containing the samples were then quickly weighed with minimum exposure to atmosphere. The procedure was repeated, but dried for 3 hours for each subsequent drying to constant weight. Triplicate determinations were carried out on each sample.

Calculation

The moisture content of each sample was calculated as the difference in weights before and after drying to constant weights. Values were expressed as percentage moisture. Each dried sample was transferred into clean dry labeled airtight sample container and kept until required for other analytical procedures.

Ash Content Determination

The ash content was determined by the method of AOAC (1990)

Procedure

Porcelain crucibles with lids were ignited for 5 minutes in a muffle furnace (M-525) at 550^{o}C, cooled in a dessicator and weighed. Two grams of each sample was separately weighed into the appropriately labeled crucible and weighed again. Crucibles and contents were ignited in the muffle furnace (Model M-525) at 550^{o}C for 18 hours to light gray ash. Thereafter, they were removed and placed immediately in a dessicator to cool and weighed.

Calculation

The difference in weight or loss in weight of the crucible and samples before ashing gave the organic matter content of each diet sample, while the difference between the weight of the crucibles alone and crucible plus ash, gave the weight of ash of each sample. Values for ash were calculated and expressed in percentages.

Crude Lipid Estimation

Crude lipid, also known as ether extract, is a combination of fatty acids, esters, sterols, simple and complex fats, vitamins (A, D, E, K.) and carotenes. Crude fat can be estimated by repeated extraction of the samples with petroleum ether (preferably 60 – 80^{o}C). The method of Pearson (1973) was employed. The method is based on the principle that non-polar components of the samples are easily extracted into organic solvents.

Procedure

Three grams, (moisture – free) of each sample, was placed into labeled fat-free thimbles. These were then weighed, plugged with glass wool and introduced into the soxlet extractors containing 160ml petroleum ether (b.p 60 – 80^{o}C). Clean dry receiver flasks were also weighed and fitted to the extractors. The extraction units were then assembled, and cold water was allowed to circulate, while the temperature of the water bath was maintained at 60^{o}C. Extraction was carried out for eight hours. At the end of this time, the thimbles containing the samples were removed and placed in an oven at 70^{o}C for three hours and dried to constant weight.

Calculation

The crude lipid was obtained as the difference in weight before and after the exhaustive extraction. The de-fatted residues were kept in airtight sample containers for use in other analysis.

Crude Protein Determination

A modified method of micro-Kjeldahl as described by Oyeleke (1984) was used for crude protein determination. The nitrogen content of proteins varies from 15

– 18%. Assuming an average value of 16%, then crude protein is estimated as the nitrogen content multiplied by the appropriate factor (AOAC, 1990).

Equations

The digestion involves oxidation of the organic matter with sulphuric acid:

Organic N $\xrightarrow{\text{conc. } H_2SO_4}$ $(NH_4)_2SO_4$

Carbon $\xrightarrow{\text{conc. } H_2SO_4}$ CO_2

Distillation is carried out on Markham's steam distillation apparatus (Markham, 1942). This involves liberation of ammonia by caustic soda, which is trapped in an (excess) acidic buffer and the excess acid titrated with standardized 0.1M NaOH solution.

Procedure for Digestion

Three grams each of the de-fatted samples were separately weighed on pre-weighed Whatman ashless filter paper No. 4 and placed in micro-Kjeldahl digestion flask together with few anti-bumping granules. Two grams of catalyst mixture ($CuSO_4$: Na_2SO_4: SeO_2, 5:1:02 w/w) was added to each flask, and then 10ml nitrogen free concentrated H_2SO_4 also added to each flask. The flasks were placed in inclined position on a heating mantle in a fume cupboard. Digestion was started at temperature of 30^oC until frothing ceased, and then heating was increased to 50^oC for another 30 minutes, and finally at full heating (100^oC) until a clear solution was obtained. Simmering was continued below boiling point for another 30 minutes to ensure complete digestion and conversion of nitrogen to ammonium sulphate.

After digestion was completed, samples were allowed to cool, then transferred quantitatively to 100ml volumetric flasks with washing and cooling to room temperature. Volumes were made up to mark with distilled water.

Distillation

Ten milliliters of each digested sample was pippetted separately into the Markham's steam distillation apparatus, followed by the addition of 20mls 40% NaOH solution. Distillation was started and the liberated ammonia was trapped into 2% boric acid in a 100ml conical flask containing 4 drops of mixed indicator (0.1% BCG and 0.1% methyl red in 95% alcohol) to a volume of 50ml.

Titration

The ammonia trapped in the boric acid was titrated against 0.01M HCl to an end point of light gray colour. Titre values were obtained in triplicate for each sample and blanks.

Calculation

Percentage nitrogen in the food samples were calculated according to Markham (1942 procedure as follows:

$$\%\text{Nitrogen} = \frac{(a-b)x\ 0.01x14.005xCx100}{D\ x\ Ex\ 1000}$$

Where:

a = average titre value for samples

b = titre value for blank

C = volume to which the digest was made up to.

D = aliquot taken for distillation

E = weight of dried samples taken for digestion.

% Crude Protein = %Nitrogen x 6.25 (AOAC, 1975).

Estimation of Total Carbohydrate

The total carbohydrate content of the diet samples was obtained by subtracting the sum of percentage crude protein, crude fat and ash from 100 (AOAC, 1980).

Calculation of Caloric Value of the Diets

When samples of carbohydrate, fat and protein are burnt in a bomb calorimeter, the amount of heat produced (heat of combustion) is always the same for each of these nutrients. It is the maximum amount of energy that the sample is capable of yielding when it is completely burnt or oxidized. Values of 4.1 Kcal/g for carbohydrate, 9.45 Kcal/g for fat and 5.65 Kcal/g for protein have been obtained. However, the net heat of combustion in the human body is slightly different from that in the bomb calorimeter. In the calorimeter, the heat of combustion comes from the energy produced by oxidation of C to CO_2, hydrogen to water and nitrogen (from protein) to nitrous oxide. The body is capable of releasing the energy potential of carbon and hydrogen, but cannot use the energy of nitrogen. Therefore the heat from the oxidation of nitrogen cannot be considered available to the body. This amounts to 1.3Kcal. A potential of only 4.3Kcal/g of protein is therefore available to the body (Guthrie, 1989).

Calculation

Based on these values, the factors 4, 9 and 4 representing the approximate amount of energy available to the body per gram of carbohydrate, fat and protein respectively (physiological fuel value) were used in arriving at the caloric values of the diets analyzed as follows:

Total caloric value = Sum (gram of each nutrient in diet x factor)

Crude Fibre Determination

This was carried out according to the procedure of AOAC (1980). Four grams of each moisture-free sample was weighed into a 250ml beaker, and 50ml 4% H_2SO_4 added followed by distilled water to a volume of 200ml. This was then heated to boiling and kept boiling for exactly 30 minutes, with constant stirring using a rubber-tipped glass rod to remove all particles from sides of beaker.

The volume was kept constant by addition of hot distilled water. After 30 minutes of boiling, the content was poured into a buchner funnel fitted with an ashless Whatman filter paper No. 40 and connected to a vacuum pump. Beaker was washed several times with hot distilled water and then transferred quantitatively with a jet of hot water. Washing continued on the funnel until the filtrate was acid-free as indicated by litmus paper.

The acid-free residue was transferred quantitatively from the filter paper into the same beaker removing the last traces with 5% NaOH solution and hot water to a volume of 200ml. Again the mixture was brought to boil and kept boiling for 30 minutes with constant stirring as earlier described, keeping the volume constant with hot water. The mixture was then filtered and washed as earlier described until alkaline free.

Finally, the resultant residue was washed with two portions of 2ml 95% alcohol. Residues on filter paper were transferred to a pre-weighed porcelain crucible. The content of the crucible was then dried in an oven maintained at 110^{o}C to a constant weight after cooling in a desiccator. Crucible content was then ignited in a muffle furnace at 550^{o}C for 8 hours, cooled and weighed. Triplicate determinations

were carried out on each diet sample. Crude fibre was calculated as loss in weight after ignition.

Determination of Amino Acid Profile

The amino acid content of the formula and control diets was determined using the method described by Spackman et al (1958). Due to technical limitations only the second set of diets and the control were analyzed.

Procedure

The moisture-free samples were defatted, hydrolyzed, evaporated before loading into a Technicon Analyzer (TSM-1).

Defatting of Samples

Two grams of the moisture free samples were separately weighed into labeled extraction thimbles and fat extracted with chloroform/methanol mixture (1:1 v/v) using a soxhlet extraction apparatus as earlier described. The extraction lasted for 15 hours.

Hydrolysis of the samples

40mg of each defatted sample was separately weighed into glass ampoules. 7 ml of 6N HCl, was added and oxygen expelled by passing nitrogen into the ampoules (This was to avoid possible oxidation of some amino acids during hydrolysis). The glass ampoules were then sealed with flame and put into an oven preset at 105^{o}C and left for 22 hours to hydrolyze. The ampoules were allowed to cool and the tips broken. Contents were then filtered. The filtrates were evaporated to dryness at 40^{o}C under

vacuum in a rotary evaporator. Residues were dissolved in 5 ml acetate buffer (pH2.0) and stored in plastic sample bottles at $-4^{o}C$ until required.

Loading of the hydrolysate into the TSM-1 analyzer

Ten microliter of each hydrolysate was dispersed into the cartridge of the analyzer. The analyzer then separated and analyzed free acidic, neutral and basic amineswhich lasted for 76 hours. Norleucine was employed as the internal standard. 10µl of the standard solution mixture of amino acids was also loaded into the analyzer. Values of both the standard and samples were recorded and printed out as chromatogram peaks by the chart recorder.

Calculation from the Peaks

The net height of each peak produced on the chromatogram (each representing an amino acid) was measured. The half-height of each peak was located and the width of the peak at half-height accurately measured. Approximate area of each peak was then obtained by multiplying the height with the width of half height. All measurements were in millimeters (mm). The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated as:

$$NE = \frac{\text{Area of norleucine peak}}{\text{Area of each amino acid in the standard mixture.}}$$

A constant (S_{std}) for each amino acid in the standard mixture was then calculated.

$$S_{std} = NE_{std} \text{ x mol.weighed of amino acid x } \mu\text{mole } AA_{std}.$$

The amount of each amino acid (in g/100g protein) in each diet sample was calculated as follows:

$$\text{Concentration of amino acid (g/100g protein)} = NH \times \frac{NH}{2} \times S_{std} \times C$$

Where C = $\frac{\text{Dilution x 160}}{\text{Sample wt x \%N x 10 x volume loaded x NH x W(nleu)}}$

NH = net height

W = width

nleu = norleucine

Assay of Antinutritional Factors in the Diet Samples.

Determination of Phytate

This was achieved using the method of AOAC (1990).

Phytate is extracted using dilute HCl and then extract mixed with Na_2 EDTA – NaOH solution, and placed in an ion-exchange column. The extracted phytate is diluted with 0.7ml NaCl solution and wet-digested with H_2SO_4/HNO_3 mixture to release phosphate, which is measured colorimetrically after reacting with ammonium molybdate solution. The amount of phytate in original sample is obtained as hexaphosphate equivalent.

Procedure

Two gram of each diet sample was separately weighed into labeled 150ml Erlenmeyer flasks. Exactly 40 ml of 2.4% HCl was added to each sample, covered and shaken vigorously on an MSE orbital shaker for 3 hours at room temperature. Meanwhile, a column was prepared by adding 3 ml of distilled H_2O to the slurry of 0.5g-anion exchanger resin AG 1-x 4 chlorides obtained from BIO-Rad laboratories. This was allowed to settle and then washed with 15ml of 0.7M NaCl solutions, followed by 15ml of distilled water.

Samples were removed from the shakers and filtered through Whatman filter paper No. 42. One millilitre of each filtrate was separately mixed with 1.0ml Na_2 EDTA – NaOH reagent in a 25ml volumetric flask. Mixtures were diluted to the mark with distilled water, mixed and transferred quantitatively to the prepared column. The first eluent from the column was discarded. The Column was washed with 15ml 0.1M NaCl, which was also discarded. The column was then washed with 15ml 0.7M NaCl and fraction collected into a digestion flask. Concentrated H_2SO_4 (0.5ml) and 3.0ml concentrated HNO_3 were added to the flask. Before the next sample was added to the column, 15ml of distilled water was passed through it. Mixtures in flasks were digested on micro-Kjeldahl rack at 50^oC until active boiling ceased and thick yellow vapour was given out. Heating continued for another 10 minutes before burner was turned off. Flasks were allowed to cool to room temperature. Exactly 10ml-distilled water was added to flask and swirled to dissolve the digests. All solutions were separately transferred quantitatively to labeled 50ml volumetric flasks. 2.0ml molybdate solution was added to each sample, mixed thoroughly, then followed by 1.0ml sulfonic acid reagent and mixed again. The solutions were then diluted to volume with more distilled water. Mixtures were allowed to stand for 15 minutes and absorbance read at 640nm. A blank solution was prepared by mixing 1ml 2.4% HCl with 1.0ml Na_2EDTA – NaOH reagent, and diluted to 25ml with distilled water before pouring into column and treated as samples

Preparation of Standard Curve

A standard curve was prepared by pipetting 1.0, 3.0, 5.0, 7.0ml phosphate standard solution containing 80, 240, 400 and 560µg phosphorus respectively, into labeled 50ml volumetric flasks. 20ml distilled water was added to each flask, mixed

thoroughly, followed by 2.0ml Molybdate solution with continuous mixing. 1.0ml sulfonic acid solution was added mixed well, diluted to volume with distilled water and mixed again. Absorbances of the solutions were read at 640nm. A standard curve was generated (Appendix 1). Triplicate determinations were carried out on all the samples and standards.

Calculation

Phytate concentration in the diet samples was extrapolated from the generated standard curve, and expressed as mg/100g sample.

Determination of Total Oxalate

Total oxalate in the diet samples was assayed using the method of AOAC (1990). It was however slightly modified where powdered sample were used instead of canned vegetable juice.

Oxalate is precipitated as insoluble calcium oxalate, which is collected by centrifuging. The precipitate is dissolved in an excess of hot dilute H_2SO_4 and the oxalate titrated (in hot) with standardized $KMnO_4$.

Procedure

Two gram of the powdered diet samples were separately weighed into labeled 250ml beaker, and 150ml distilled water and 55ml 6M HCl added. Two drops of alcohol were added and mixture boiled for 15 minutes, cooled, and transferred quantitatively into 500ml volumetric flask, diluted to volume with distilled water and mixed again. Mixture was allowed to stand over night, mixed thoroughly, and then filtered through No. 42 Whatman filter paper.

Exactly 25ml of the filtrates were separately pipetted into labeled 50ml flasks and then 5ml tungstophosphoric acid added, mixed and let to stand for 5 hours. Mixtures were filtered through Whatman filter paper, and 20ml of filtrates were pipetted again into centrifuge tubes followed by ammonium hydroxide solution dropwise until a pH of 4.5 was achieved using indicator paper. 5ml acetate buffer (pH 4.5) was then added to maintain a constant pH.

Mixtures were allowed to stand again overnight at room temperature, after which they were centrifuged for 15 minutes at 1700rpm to compact the precipitate. Supernatants were carefully decanted and calcium oxalate precipitates washed three times with centrifugation and decantation using cold washing liquid (12.5ml HoAc, diluted to 250ml with distilled water). Precipitates were re-dissolved in 5ml dilute H_2SO_4 (1:9v/v). The dilute H_2SO_4 also served as the blank solution. All mixtures were then heated in a boiling water bath for 15 minutes and the hot solutions titrated with 0.01N $KMnO_4$ until a persistent pink colour was obtained. Triplicate titrations were carried out on each sample.

Calculation

The volume of $KMnO_4$ used to titrate the hot solution of each sample was used to calculate the oxalate content of each sample as follows:

$$\text{Mg oxalate/100g sample} = \frac{\text{ml of 0.01N } KMnO_4 \times 1350}{\text{Weight of sample taken}}$$

Where 1350 = 0.45 (mg oxalic acid equivalent to 1ml 0.01N $KMnO_4$) x [(30/20) x (50/25) dilution factors] x 100 (to convert to 100g sample)

Determination of Hydrocyanic Acid.

The alkaline titration method of AOAC (1990) was employed.

Procedure

Ten gram of each powdered sample was weighed separately into Kjedahl digestion flask and 200ml distilled water added. Flasks were connected to the distillation unit, and let stand for 3 hours before steam distillation commenced. 150ml of distillate was collected into 250ml volumetric flask containing NaOH solution (0.5g in 20ml distilled water). Distillates were made up to volume with more NaOH solution. 100ml of the mixtures were measured out into another set of flask and 8ml 6N NH_4OH and 2ml 5% KI solutions added to each flask. These were then titrated with 0.02N $AgNO_3$ to faint but permanent turbidity end point. Triplicate titrations were carried out on each sample.

Calculation

The amount of HCN in each sample was calculated using the relationship:

1ml 0.02N $AgNO_3$ ≡ 1.08mg HCN (AOAC, 1990).

Determination of Tannins.

The method of AOAC (1980) was also used in this determination.

Procedure

Two grams residues of petroleum ether extracts as earlier described were boiled with 300ml distilled water for 2 hours, cooled and diluted to 500ml with more water and then filtered. 25ml of the filtrates were transferred separately into 2L porcelain dish. 20ml indigo solution and 750ml distilled water added, followed by 1ml of $KMnO_4$ (earlier standardized with 0.1N oxalic acid) at a time until the blue solution turned green; then few drops until solution became golden yellow. Triplicate analyses

were carried out on all the samples. Mixture of 20ml indigo solution and 750ml distilled water was titrated with $KMnO_4$ as blank.

Calculation

The difference between the sample and the blank titre values was multiplied by the factor of 0.006235* to obtain the concentration of tannin in the samples.

*1ml oxalic acid solution = 0.006235 querci tannic acid.

Vitamin Assay

Vitamin A

Vitamin A concentration in the samples was determined using the method of Carr and Price (1926). The method is based on measuring the blue colour obtained when saturated antimony trichloride is added to a solution containing vitamin A in chloroform at 620nm. Dry materials containing fat or oil are first of all extracted with fat solvent before saponifying with alcoholic alkali. Vitamin A is present and assayed in the unsaponifiable fraction.

Extraction

Five grams of the powdered samples were each weighed into extraction thimbles and extracted with petroleum ether (60 – 80^{o}C) on a soxlet extractor as earlier described. The petroleum ether was evaporated and the oil residues saponified with 10ml of alcoholic KOH under reflux for 45 minutes at 58^{o}C.

The alcoholic hydrolysate was diluted with 50ml of distilled water and the vitamin A extracted with 20ml of diethyl ether. The process was repeated twice, and the combined organic extracts washed free from alkali with more water, and then dried over anhydrous sodium sulphate overnight under reduced light. Solvent was

evaporated on a water bath at 40^{o}C. The resultant residue was re-dissolved in chloroform and made up to 25ml with more chloroform.

Assay

One milliliter each of the chloroform solution was pippetted into l cm-glass cuvette. Saturated antimony trichloride solution (3mls) was added to each sample extract and absorbance read immediately at 620nm against chloroform blank. Triplicate analysis were carried out on each sample.

Calculation

The vitamin A content of each sample in microgram were read off from a calibration curve prepared from different concentrations of oily vitamin A palmitate. All samples were shielded from light during the analytical procedures.

Vitamin B_1 (Thiamine) Determination

Measurement of vitamin B_1 (thiamine) was done using the method of Stroebecker and Henning (1965).

In this method, B_1 is readily decomposed in neutral or alkaline solution, and extracted in acid medium. It is best extracted in 0.001 – 0.02M HCl. B_1 is stable in acid medium even on prolonged heating.

Extraction

Five grams of each powdered sample was weighed and ground in a porcelain mortar with 5ml 0.02M HCl. The mixture was rinsed quantitatively into a 100ml volumetric flask and more 0.02M HCl poured to the 70ml mark. The mixture was warmed for 1 hour at 50^{o}C with occasional shaking on water bath. At the end of the heating, the content of each flask was allowed to cool to room temperature before

making up to 100ml with distilled water. It was vigorously shaken several times and allowed to stand for 15 minutes each time. Suspensions were filtered through No. 1 Whatman filter paper.

Spectrophotometric Measurement

Five milliliter of each sample extract was pippetted into labeled test tubes, and 5ml of oxidation solution (potassium ferricyanide: NaOH mixture 1:9v/v) added. Each mixture was shaken and then allowed to stand for 1 minute. Three drops of hydrogen peroxide solution was added to each test tube and shaken again. Absorbance of each preparation was determined at 369nm against a blank prepared in the same manner, but 5ml of water added instead of the sample extract. Triplicate determinations were carried out on each sample extract.

Calculation

The thiamine content in mg per 100g samples was obtained as follows:

$$\text{Mg Vit. } B_1 = \frac{\text{Abs.x100*x110x1000}}{5}$$

Where 100* is the volume to which extract was made up to. 110 is a conversion factor. 5 is weight of sample taken for extraction.

Vitamin B_2 (Riboflavin) Determination.

This assay was based on the method of Stroebecker and Henning (1965). Vitamin B_2 occurs in natural products almost entirely in combined form as riboflavin-5- phosphoric acid ester (flavine mononucleotide), and linked to protein as a constituent of "yellow enzymes". Extraction with hot dilute acid (steam bath or water bath) splits vitamin B_2 - protein complex. Protein is precipitated with excess acetone or methanol, while riboflavin stays in solution without being absorbed by the

precipitated protein and other impurity. Dry samples such as cereal products and feeding stuffs, are first of all powdered and then defatted by extraction with ether or light petroleum.

Extraction

Powdered moisture free samples were defatted using light petroleum ether (40 – 60^{o}C) for 8 hours. One gram each of the defatted samples was weighed into a 50ml conical flask and shaken with 120ml of 0.1N HCl on a water bath set at 70^{o}C for 90 minutes. Fifteen (15) milliliters of acetone was added and shaken for 5 minutes. Mixtures were filtered through No.1 Whatman filter paper. Excess acetone in the filtrate was evaporated on water bath until odour-free. Each extract was diluted to 10ml with distilled water, 1ml of glacial acetic acid added, and then shaken.

Assay

Standard solutions of riboflavin were prepared by accurately weighing out 50, 100, 200, 400 and 600mg riboflavin and dissolving in 10ml distilled water to give concentration of 5, 10, 20, 40, 60mg/ml respectively. To 10ml of both sample extracts and standard solutions of riboflavin, 0.5ml of 4% $KMnO_4$ solution was added with shaking and allowed to stand for exactly two minutes. To this was added 0.5ml of 3% H_2O_2 solution and shaken vigorously to expel excess oxygen. Solutions that were turbid or had precipitates of MnO, were centrifuged before absorbance of the resultant yellow colour solutions was measured at 444nm. Triplicate determinations were carried out on each extract.

Calculation

Absorbance obtained for the different concentrations of standard riboflavin were used to plot a calibration curve (Appendix 3) while the vitamin B_2 concentration of each sample extract was read from the linear curve.

Vitamin B_6 (Pyridoxine) Determination.

Vitamin B_6 was determined by the modified method of Stroebecker and Henning (1965). The method is based on the principle that pyridoxine molecule possesses a phenolic hydroxyl group that gives a brown colour on interaction with ferric chloride, whose intensity is proportional to the concentration of the phenolic hydroxyl in the pyridoxine.

Extraction Procedure

Exactly 1g of each powdered samples was weighed separately into a 100ml conical flask and extracted with 10ml 0.1M HCl with vigorous shaking for 10 minutes. Samples were filtered through No. 1 Whatman filter paper. Filtrates were then made up to 10ml with distilled water. Five milliliters of the slightly acidic filtrates were each treated with 1ml ferric chloride solution that had been diluted 1 in 50 with distilled water. The optical density of the resultant brown colour was measured in a 1cm cell cuvette against a blank solution consisting of 1ml dilute ferric chloride solution at 450nm. Triplicate determinations were carried out on each sample.

Calculation

The absorbencies obtained from the various sample extracts were converted into pyridoxine concentration by means of a calibration curve generated. (Appendix 4).

Vitamin C (ascorbic acid) Determination.

This was achieved by the method of Roe and Kuether (1943). Ascorbic acid is oxidized to dehydroascorbic acid, which is coupled with 2, 4 – DNPH. The oxazone so formed reacts with conc. H_2SO_4 to yield a red coloured complex, which absorbs at 520nm.

Extraction and Assay

Five gram of each powdered diet sample was weighed into labeled porcelain dishes and grounded with 25ml of 0.5% oxalic acid for 10 minutes, mixed thoroughly and allowed to settle. Supernatants were decanted and 0.5ml pipetted into clean dry test tubes, followed by the addition of 1.5ml of 4% TCA. Into two separate test tubes, 2.0ml standard vitamin C solution (0.5mg/100ml) was pipetted into two separate test tubes followed by 2.0ml 2, 4 – DNPH, mixed and incubated at 50^oC for 1 hour. All tubes were then transferred to an ice bath for 5 minutes. With tubes still in ice, 2.5ml of 85% H_2SO_4 was added to each tube drop wise with constant mixing. Tubes containing mixtures were removed from the ice bath and allowed to stabilize to room temperature for about 30 minutes. Absorbance was read at 520nm using the blank solution to zero the spectrophotometer. Triplicate determinations were carried out on each sample extract.

Calculation

Milligram of vitamin C in 100g of samples was calculated using the formula:

$$\text{mg vit. C} = \frac{\text{Absorbance of sample extract x conc. of standard x 25 x 100}}{\text{Absorbance of standard} \qquad 0.5 \times 5}$$

Where 25 is the volume of extract for each sample

0.5 is the volume of extract taken for assay

5 is the weight of samples taken

100 is to convert to 100g sample.

Elemental Analysis

Determination of Cations

All atomic absorption spectrophotometric measurements were carried out with a Hitachi 180-80 spectrophotometer which is equipped with a data processing unit and a strip chart automatic recorder. This model of the atomic absorption spectrophotometer (AAS) has provisions for flame and flameless procedures of elemental concentration determinations. The flame technique was used for the determination of Na, Mg, Zn, Ca and Fe which were the cations of interest. The flameless method can be used for cations that are usually found in very low concentrations in biological fluids eg Cd, Pb, Cu and Co.

Table 3.1 Analytical Conditions by Flame Atomic Absorption Spectrophotometry

Element	Na	Ca	Mg	Zn	Fe
Lamp current (MA)	10	7.5	7.5	10	10
Wavelenght (nm)	589	422.7	285.2	213.8	248.3
Oxidant (Air) L./min	9.4	9.4	9.5	9.4	9.4
Slitwidth	0.4	2.5	2.5	1.3	0.2
Fuel (C_2H_4)	2.2	2.6	2.0	2.0	2.3

Source: Wilson et al (1995)

In the two techniques, the flame and the graphite atomizer are subjected to a strong magnetic field during atomization of the element of interest. This produces a zeeman effect on the atomic vapour of the element. The energy emitted from a hollow cathode lamp is thus split into two arrays-one parallel (P//) and the other perpendicular (P⊥) to the magnetic field. The two beams are affected by the light scattering and broad band molecular absorption while the beam parallel is affected additionally by sample absorption. Electronic subtraction of P⊥ from P// gives the true absorption of the sample.

A more detailed treatment of the principles of atomatic absorption spectroscopy is given by Price (1972), Whiteside (1979), Wilson et al (1995) and Williams et al (1979). The instrumental flame conditions for the elements analyzed are given in Table 3.1

Procedure

Three grams each of the moisture free samples was accurately weighed into 100ml labeled dry pyrex beakers. Ten (10) milliliters of digestion mixture (conc. HNO_3: $HCLO_3$, 6:1v/v) was added to each sample and allowed to soak for three minutes. The resulting mixture was placed on a heating mantle in a fume cupboard and heated slowly at first until frothing ceased. Heating continued with the addition of more of the digestion mixture until white residue was obtained. The resultant white residue was allowed to cool, then re-dissolved in 5ml 1M HCl and transferred quantitatively into dry labeled tubes and stored as stock solution of each digested

sample. Further dilutions of the stock solutions were made where necessary before elemental analyses were effected.

With the Hitachi model the concentration of each element was calculated directly in parts per million (PPM) using a 180-0205 data processing unit. Results obtained in ppm were converted to mg/ 100g samples.

Determination of Anions

The Ion Chromatographic Analyzer (Model IC 100-25), is a product of Yokogawa equipped with a conductivity and a U.V detector. This equipment has a dual column system comprising a suppressor and a separator column (SAX-1, YEW, 250 x 4.6mm in diameter), which uses a strong base anion exchange resin and a concentrator (SAX –2, 100x 4.6mm in diameter).

Procedure

One gram each of the food samples was weighed into labeled 50ml volumetric flask and 10ml distilled water added. Flasks and contents were shaken continuously for 3 hours after which samples were centrifuged at 3000rpm for 1 hour. The resultant supernatants were filtered using a 0.22mm teflon filter after which it was injected into the Ion Chromatographic Analyzer (ICA) which was used to determine anions in the experimental diets as described by Ubom and Tsuchiya (1988). Anions were detected under the following operating conditions:

Eluent = 30mM $NaHCO_3/Na_2CO_3$ (flow rate 2ml min^{-1})

Scavenger = 0.05M H_2SO_4 (flow rate 4ml min^{-1})

Injector volume = 100µs cm^{-1}

UV detector = WL. 226nm, Absorbance 0.002au

Temperature = 40^oC

Ten ml aliquot of standard solution containing various anions [NO_2^- (15 ppm), NO_3^- (30ppm), Br^- (10ppm), Cl^- (10ppm), F^- (5ppm), $S0_4^{2-}$(40ppm), PO_4^{3-}(30ppm)], was separately injected into the ICA. Anions were then identified using retention times of the standards against those of sample filtrates and quantified by peak areas. Triplicate determinations were carried out.

3.2.5 Animal Experimentation

Animals

Male Winter-strain weanling rats weighing between 40 – 55g purchased from the Animal House of the University of Jos, were used in these studies. The rats were randomly distributed into eight (8) groups of 10 rats each during the first feeding and four (4) groups during the second feeding experimentation (10 rats per group). They were kept in metabolic cages made of Perspex sheets. The rats were allowed to stabilize on the normal laboratory feed for 3 days and starved for one day before feeding with the experimental diets commenced.

Animal grouping

During the first feeding experimentation, rats were allotted the diets as follows:

Group 1: Yellow maize: Soybeans: Groundnut blend. (diet 1)

Group 2: Sorghum: Soyabeans: Groundnut blend (diet 2)

Group 3: Millet: Soyabeans: Groundnut blend (diet 3)

Group 4: Rice: Groundnut: Bambaranut: Carrot blend (diet 4)

Group 5: Soyabeans alone (diet 5)

Group 6: Acha: Sesame: Cray fish: Garden egg blend (diet 6)

Group 7: White maize: Sesame: Groundnut: Crayfish blend (diet 7)

Group 8: Nestle Cerelac as control group (diet 8)

In the second set of experiment, the modified diets were allotted to rat as follows:

Group 1: Rice: Groundnut: Bambaranut: Crayfish: Carrot: Salt blend (diet 1)

Group 2: Acha: Sesame: Crayfish: Garden egg: Palm oil: Salt blend (diet 2)

Group 3: Yellow maize: Soyabeans: Groundnut: Crayfish: Palm oil: Salt blend (diet3)

Group 4: Nestle Cerelac as control (diet 4)

Animal feeding

Just before every feeding, known quantity of each diet was mixed thoroughly with enough boiling water to a thick paste and allowed to cool before feeding to the animals. Rats were given feed and water ad libitum for 28 days. Feaces and urine were collected separately to avoid mixing with the feed. Daily records of feed, water and weights of rats were kept.

Collection of Blood

At the end of the feeding period, rats were anaesthetized with diethyl ether and blood collected by cardiac puncture into light-shielded centrifuge tubes. A portion of whole blood was collected from each rat into sample bottles containing EDTA (1mg/ml) for parameters that required the use of whole blood. The remaining blood samples were allowed to clot for 20 minutes, before centrifuging at 3000rpm for 15 minutes in a refrigerated centrifuge, (to obtain serum for parameters determined in sera). Serum was carefully transferred with pasteur pipettes into clean, dry labeled light-shielded sample bottles and stored frozen until required.

Haematological Parameters

Packed Corpuscular Volume (PCV) and Haemoglobin (Hb) concentration were determined from a portion of the whole blood collected. Triplicate determinations were carried out.

Packed Cell Volume (PCV)

This was determined by the method of Green and Ezeilo (1978). In this method, clotting is prevented in blood, which then separates into layers under the influence of gravity when spun in a centrifuge. The cells settle down and pack themselves because of centrifugal force.

Procedure

Blood-containing anticoagulant was drawn up into a special micro-capillary tube leaving at least 15mm unfilled. One of the blank ends of the tube was then carefully sealed with plastacine, and centrifuged for 5 minutes in a microhaematocrit centrifuge at 12000 rpm. The PCV was read in percentage directly on the microhaematocrit reader by sliding the tubes along the chart until the meniscus of the plasma intersected the 100% mark.

Calculation

PCV value was calculated as the ratio of the height of the cells to the total height of fluid in the tube.

Haemoglobin Estimation

This was achieved using the method of Green and Ezeilo (1978). This method involves haemolysing the red cells and then comparing the colour intensity of the haemolysed solution obtained with prepared commercial standards.

Procedure

Whole blood obtained from each rat was sucked up to the $20cm^3$ mark of a micropipette and outer surface wiped clean. The blood was then carefully discharged into 4ml ammonia solution (0.04%) in a calibrated tube. This was allowed to stand for 5 minutes, and the absorbance read at 520nm. The haemoglobin content of each blood sample was read from a standard curve obtained by treating different concentrations of standard haemoglobin in the same manner. Triplicate results were obtained for each sample.

Assay of Some Biochemical Parameters

Triplicate assay of the biochemical indices were carried out on the sera of rats fed the experimental diets.

Serum Bilirubin

This was determined using the colorimetric method of Malloy and Evelyn (1937). The method is based on the diazo reaction commonly called the Vander-Bergh's reaction. In the process, dizotized sulphanilic acid converts bilirubin in serum or urine sample to its azo-dye derivative. The intensity of the red colour of azo-bilirubin is directly proportional to the concentration of the yellow coloured parent bilirubin. Only conjugated bilirubin (the water-soluble) interacts with the diazo regent to give the red colour (direct Vander-Bergh's reaction). However, in the presence of solubilizers like methanol or catalysts like caffeine, unconjugated bilirubin undergoes diazotisation (indirect Vander Bergh's reaction).

It therefore follows that total (conjugated and unconjugated) bilirubin concentration can only be obtained in the presence of a solubilizer or catalyst. The concentration of unconjugated bilirubin is obtained by subtracting that of conjugated bilirubin from total bilirubin.

Procedure

Exactly 0.4ml of the light-shielded serum samples from the different diet fed rats were pippetted into each of two test tubes labeled A and B containing 3.6ml distilled water. One milliliter of freshly prepared diazo reagent was added to tube labeled A and blank solution (dilute HCl) to tube labeled B. Tube contents were mixed immediately and allowed to stand at room temperature for 5 minutes. The absorbance of mixture in tube A was read against mixture in tube B as blank at 450nm. Absolute methanol (5ml) was added to all the mixtures. After a further 30 minutes, absorbance was again read at 540nm of mixture A using B as blank. An

absorbance of 0.3 for methyl red was used as the standard and assumed to be of 0.016mg/100ml concentration of bilirubin. (Malloy and Evelyn 1937).

Calculation

The concentration of both conjugated and total bilirubin (in μmol/100ml of serum) in the serum of rats fed the different diets were calculated as follows:

$$\frac{A_{sample} \times 0.016 \times 100}{A_{std} \times 0.4}$$

Where: A_{sample} is the absorbance of serum sample

A_{Std} is the absorbance of standard methyl red (0.3)

0.016μmoles is the concentration of the standard.

Serum Glucose

The o-toludine method of Hultman (1959) was employed in the estimation of glucose levels in the serum of rats fed the various diets. In this method, glucose reacted with o-toludine in glacial acetic acid to yield N-glycosylamine, which is blue-green in colour. The intensity of the colour, which is proportional to glucose concentration, was measured at 625nm against a blank solution.

Procedure

Exactly 0.1ml of serum was added to labeled tubes containing 5.0ml of o-toludine reagent. The mixtures were then placed in a boiling water bath ($100^{o}C$) for 10 minutes, after which the mixtures were removed and placed in cold water to cool. The blue-green colour that developed was read at 625nm.

Calculation

The glucose concentration was determined from a generated standard curve (Appendix 5) and expressed as mMol/L.

Serum Total Protein

Total protein in serum of rats was determined using the Biuret method of Plummer (1978). Copper ions in the alkaline solution of biuret reagent react with protein to form purple colour complex. Colour intensity which is proportional to the number of peptide bonds, hence the concentration of proteins is read at 540nm.

Procedure

Serum (0.1ml) from rats was mixed with 5.0ml of biuret reagent in test tubes. The mixtures were incubated in water bath at 37^{o}C for 10 minutes. The mixtures were removed and allowed to cool for one hour. Absorbance was read at 540nm against blank solution containing 0.1ml distilled water and 5.0ml biuret reagent. Bovine serum albumin (BSA) was used to prepare a standard curve as indicated in appendix 6.

Calculation

The concentration of total protein in the serum of the different rats was evaluated from the standard curve generated from the absorbencies and corresponding concentrations of bovine serum albumin.

Serum Albumin

Serum albumin was determined by the dye binding method of Doumas et al (1972). When a solution-containing albumin is added to a buffered solution of bromocresol green (BCG) at pH. 4.2, the BCG solution undergoes a change in colour

as if there has been a shift in pH (to the alkaline range) when in fact there has been none. The green colour produced is proportional to the albumin concentration.

Procedure

Into labeled test tubes, 8.0ml of BCG solution was delivered followed by 0.1ml serum samples and mixed. Then mixtures were incubated in a water bath for 15minutes at 37^{o}C for colour development. Absorbance was read at 640nm against a blank containing 8.0ml of BCG and 0.1ml distilled water. Albumin concentrations (g/100ml) in the different serum samples were determined from a generated standard curve as shown in appendix 7.

Serum Alkaline Phosphatase

The phosphatases catalyze the hydrolysis of most orthophosphomonoesters and related compounds. Alkaline phosphatase (ALP) catalyzes the hydrolysis of the substrate disodium phenyl phosphate, releasing phenol and inorganic phosphate at pH. 10.0. The amount of either phenol or inorganic phosphate so liberated under standard conditions can be estimated and used to evaluate the activity of phosphatase present.

In this assay, the method of Wright et al (1972) was used to determine the amount of phenol liberated. This is achieved by using 4-amino phenazone, which reacts with phenol to form a pink quinine, that absorbs maximally at 510nm.

Procedure

Into labeled test tubes, containing 1.1ml carbonate-bicarbonate buffer, 1.0ml of freshly prepared 0.02M disodium phenyl phosphate was added. 1.0ml serum was added to tube labeled ‘test.’ This was followed by 1.0 ml distilled water to tube labeled ‘standard blank.’ Exactly 1.0ml of working phenol standard was added to tube

labeled ‘standard’, then 0.8ml of 0.5M NaOH solution to all tubes labeled ‘test blank.’ Mixtures were then incubated at 37^{o}C for 15 minutes. Reaction in the ‘test’ and ‘standard’ tubes was stopped by the addition of 0.8ml of 0.5M NaOH solutions. To all tubes was added 1.1ml each of 0.5M $NaHCO_3$ solution followed by 1.0 ml of 0.6% 4-aminophenazone, 1.0 ml 4% potassium ferricyanide and mixed thoroughly. The pink colour that developed was immediately read at 510nm using the appropriate blank to set the colorimeter to zero.

Calculation

The alkaline phosphatase activity in each serum sample was calculated in King Amstrong Unit/100ml then converted to 1U/L. The King Amstrong unit (K.A) is the amount of enzyme that will liberate 1mg of phenol in 15 minutes at pH. 10.0.

$$KA = \frac{Abs_{test} \times \text{conc. of std}}{Abs_{std}} \times \frac{100}{0.1}$$

Where: Abs_{test} is the absorbance of the test serum

Abs_{std} is the absorbance of the standard

1.0 is the volume of serum used.

Serum Alanine Aminotransferase (ALT) (E.C 2.6.1.2)

The assay is based on the method of Reitman and Frankel (1957), in which the enzyme catalyzes the transfer of the amino group between alanine and ά-ketoglutarate to yield the product pyruvate.

L-alanine + ά-ketoglutarate $\xrightarrow{ALT}$ pyruvate + glutarate.

Pyruvate is coupled to 2, 4-dinitrophenylhydrazine (2, 4 DNPH) in an alkaline medium to yield phenyl hydrazine. The activity of the enzyme is thus estimated by quantifying the amount of pyruvate produced.

Procedure

In labeled test tubes, was added 0.5ml each of ALT buffered substrate solution and incubated at 37^{o}C for 3 minutes. 0.1ml of the different serum samples was added to the tubes labeled 'test,' 0.1ml working pyruvate standard into tube labeled 'standard' and 0.1ml distilled water into tube labeled 'standard blank.' Tube contents were mixed and incubated at 37^{o}C for 60 minutes, after which 0.5ml of 2, 4-DNPH was added to each tube and mixed. All mixtures were allowed to stand at room temperature for exactly 5 minutes. Absorbance of the resultant coloured complex of each mixture was read at 510nm.

Calculation

The pyruvate formed (mMoles) per minute per litre of serum is the unit of enzyme activity at 37^{o}C given by:

$$\frac{T - TB \times 0.4 \times 1/60 \times 1000}{S - SB \qquad\qquad 0.1}$$

Where: T is the absorbance of test serum

TB is the absorbance of the test blank

S is the absorbance of the standard pyruvate

SB is the absorbance of standard blank

0.4 is the concentration of standard.

60 is the time of incubation in minutes

0.1 is the volume of serum used

1000 is to allow for the expression of enzyme activity per litre of serum.

Serum Aspartate Aminotransferase (AST) (EC: 2.6.1.1)

The method of Reitman and Frankel (1957) was used in this assay. 2, 4-DNPH was used to produce a coloured phenylhydrazine of oxaloacetate in the aspartate – ά-KG transamination reaction catalyzed by the AST.

L – aspartate + ά-KG $\xrightarrow{AST}$ oxaloacetate + glutarate.

The activity of the enzyme is estimated by measuring the amount of oxaloacetate – phenylhydrazone complex produced colorimatrically.

Procedure

The procedure and assay conditions were the same as for ALT assay except that aspartate was used as substrate and the period of incubation was 30 minutes instead of 60 minutes.

Calculation

The oxaloacetate formed (mMoles) per minute per litre of serum is the unit of enzyme activity calculated as follows:

$$\frac{T - TB \times 0.4 \times 1/30 \times 1000}{S - SB \qquad\qquad 0.1}$$

Where all terms are as explained earlier, except that the incubation time in this assay was 30 minutes.

Serum Lactate Dehydrogenase (LDH)

LDH activity in the serum of rats was assayed using the method of Vassault et al (1983). Lactate dehydrogenase catalyzes the reversible reaction of the oxidation of lactic acid to pyruvate.

$$\text{L-Lactic acid} + NAD^{+} \overset{LDH}{\longleftrightarrow} \text{Pyruvic acid} + NADH + H^{+}$$

The activity of LDH can therefore be assayed by following the changes in the concentration of NADH in the forward reaction, or the backward reaction of reduction of pyruvate. When the backward reaction is used, pyruvate is used as substrate, in which case, the amount of pyruvate left unreduced after a definite time interval, is estimated by reacting with DNPH.

Procedure

Into labeled test tubes (test and control), 0.1ml of buffered substrate was pipettes, followed by 0.1ml of serum sample, and allowed to equilibrate in water bath at 25^{o}C for 3 minutes. To the tubes labeled test, 0.1ml of NADH solution was added, while 0.2ml of buffer to tube labeled ‘control’. 1.2ml of phosphate buffer was added to tube labeled ‘blank’. All contents were incubated for exactly 15 minutes at 25^{o}C, removed from bath and reaction stopped by addition of 1.0ml of 2, 4-DNPH. All the reaction mixtures were allowed to stand at room temperature for 20 minutes, thereafter, 1.0ml 0.4M NaOH solution was added to each tube. After a further 10 minutes, absorbance was read at 510nm.

Calculation

LDH activity is the µmole pyruvate reduced per minute per litre of serum. Control tube contained 0.75 µmole pyruvate. Therefore the mount of unreduced pyruvate in µmole is given by:

$$\frac{A_t \times 0.75}{A_c} = \text{amount of unreduced pyruvate}$$

Where: A_t is the absorbance of test serum

A_c is the absorbance of control.

It then follows that the pyruvate reduced is obtained by subtracting the mount of unreduced pyruvate from 0.75 µmoles.

i.e. 0.75 – amount of unreduced pyruvate $\times \frac{1000}{0.1} \times 1/15$ = LDH activity in µmole/L

Where: 1000 is to allow expression per litre

0.1 is the volume of serum used

15 is the time allowed for incubation.

Determination of Creatinine in Serum of Rats Fed the Diets.

This assay was achieved by the method described by Varley et al (1976). Creatinine reacts with alkaline picrate to form an amber yellow solution that is measured spectrophotometrically at 540nm (Jaffe reaction).

Procedure

This involved two stages. In the first stage 2ml of each serum was pipetted into labeled test tubes followed by 2 ml distilled water. These were then left to stand for 5 minutes, 3ml 5% sodium tungstate was added followed by 2ml 2/3N H_2SO_4. The mixtures were centrifuged for 15 minutes at 3000rpm.

In the second stage, 3.0ml of the supernatants were pipetted into another set of clean dry test tubes one of which contained 3.0ml standard creatinine (1mg/100ml) and 3.0ml distilled water as blank. To all the mixtures, 1.0ml 0.04M picric acid solution and 1.0ml 0.75M NaOH solution were added and mixed after each addition. These were left at room temperature for 15 minutes and absorbance read at 540nm against the blank.

Calculation

Creatinine in serum of rats fed the experimental diets were calculated as follows:

$$\frac{\text{Absorbance of serum}}{\text{Absorbance of standard}} \times \text{conc. of standard} \times 100 \times 2$$

Determination of Blood Urea Nitrogen

Urea nitrogen was assayed by the Diacetyl Monoxine method of Wybeng et al (1971). In this method, diacetyl monoxine reacts with water under acidic condition to form diacetyl, which in turn reacts with urea to give a yellow diazine derivative, read at 520nm.

Procedure

Exactly 0.1ml of each serum sample and 0.1ml of working urea standard solution (2mg/ml) were separately pipetted into labeled test tubes and diluted to 10ml with distilled water and mixed thoroughly. 0.1ml of the diluted samples were then pipetted into another set of labeled test tubes and 1.0ml of distilled water added followed by 2.0ml colour reagent. Mixtures were mixed thoroughly and placed in boiling water bath for 20 minutes. The incubated mixtures were removed from bath, allowed to cool and absorbance read at 520nm against a blank solution containing water and colour reagents. Triplicate determinations were carried out for each serum sample.

Calculation

The concentration of urea in the serum was calculated using the relationship

$$\frac{\text{Absorbance of serum}}{\text{Absorbance of standard}} \times \text{conc. of standard} = \text{Conc. of urea in serum}$$

Determination of Total Cholesterol

Cholesterol concentration in serum of rats fed the experimental diets was determined using the CHOD-PAP method of Allain et al (1974).

The method involves the enzymatic hydrolysis and oxidation of cholesterol esters and cholesterol respectively.

Cholesterol ester $\xrightarrow{\textit{Chol. esterase}}$ Cholesterol + free fatty acid.

Cholesterol + O_2 $\xrightarrow{\textit{Chol. Oxidase}}$ cholesten-4-one + H_2O_2.

H_2O_2 + Chlorophenol + 4-amino-antipyrine $\xrightarrow{\textit{peroxidase}}$ Quinoneimine (pink) + $4H_2O$

The intensity of the pink colour formed is proportional to the cholesterol concentration.

Procedure

The determination was achieved using a reagent kit (code 80106) purchased from Biolab Diagnostic, France. The reagent vials contained after reconstitution, phosphate buffer and chlorophenol (vial R 1), cholesterol oxidase, cholesterol estarase, peroxidase, 4-amino-antipyrine (vial R 2), and cholesterol standard (200mg/dl) (vial R 3)

Just before use, the reagents were reconstituted by transferring the entire contents of Vial R2 (enzymes) into Vial R1 (buffer) mixed gently and allowed to stand for 5 minutes. This served as the working reagent. Into four sets of 3 test tubes labeled 'samples,' 'standard', and 'blank', 10ml of sample (serum) standard (Vial R3) and distilled water were separately pipetted respectively. 1ml each of the reconstituted reagent was added to all test tubes, mixed, and incubated for 5 minutes at 37^{O}C. Absorbencies of the sample and standard were measured against reagent blank at 500nm. Triplicate determinations were carried out on each serum sample.

Calculation

The cholesterol concentration in the serum of rats fed the different diets was calculated as follows:

$$\text{Cholesterol conc.} = \frac{A_{\text{sample}} \times \text{conc. of standard}}{A_{\text{standard}}}$$

Determination of HDL – Cholesterol

This was determined, by the combined method of Lopes–Virella et al (1977) and Allain et al (1974).

Law density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicron fractions are first of all precipitated by the addition of phosphotungstic acid in the presence of Mg^{2+} ions. After centrifugation, HDL-cholesterol which remains in the supernatant is quantitated. The precipitating reagent, which was a product of RANDOX Laboratories, contained 50ml phosphotungstic acid and MgCl.

Procedure

Into labeled centrifuge tubes, 200µl of serum samples were pipetted, followed by 200µl of precipitating reagent. These were mixed and allowed to stand for 10 minutes at room temperature. The resulting mixtures were centrifuged for 10 minutes at 4000rpm. The clear supernatants were separated, and cholesterol contents determined by the CHOD-PAP method as earlier outlined. Triplicate analyses were carried out on each serum sample.

Calculation

$$\text{HDL-cholesterol} = \frac{A_{sample} \times \text{conc. of standard} \times 2}{A_{standard}}$$

Where 2 is to correct for dilution effect of precipitate.

Determination of Triglycerides

The concentration of total triglycerides in serum of rats was assayed by the method of Esders and Michrina (1979).

The method involves the enzymatic cleavage, phosphorylation, oxidation and peroxidation of triglycerides, glycerol, glycerol-3-phosphate and 4-chlorophenol respectively. A reagent kit was purchased from Biolab, France consisting of buffer (vial R1), enzymes (Vial R2) and standard solution (Vial R3). The reactions involved in the determination are as follows:

$$\text{Triglycerides} \xrightarrow{\textit{lipase}} \text{glycerol + free fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow[\textit{Kinase}]{\textit{Glycerol}} \text{glycerol –3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow[\textit{Oxidase}]{\textit{G-3- P}} \text{DHAP + } H_2O_2$$

$$H_2O_2 \text{ + 4-chlorophenol + 4-amino antipyrine} \xrightarrow{\textit{peroxidase}} \text{Quinoneimine (pink) + } H_2O$$

The intensity of the pink colour that develops is directly proportional to the concentration of triglyceride.

Procedure

Vial R2 containing the enzymes lipase (1500µ/L), peroxidase (1800µ/L), glycerol-3-P oxidase (4000µ/L), glycerol kinase (1000µ/L), as well as 4-amino antipyrine (0-30mM/L) and ATP – Na (1.72mM/L), was reconstituted by quantitatively transferrin its content into Vial R1 (buffer). This was mixed gently and

allowed to stand for 5 minutes then followed by the CHOD-PAP method as earlier described. Triplicate determinations were also performed for all the serum samples.

Calculation

$$\text{Triglyceride conc.} = \frac{A_{sample} \times \text{conc. of standard}}{A_{standard}}$$

Determination of Total Iron Binding Capacity (TIBC)

TIBC was determined using the combined colorimetric methods of Ramsey (1958) and Ferruccio and Giovanni (1980).

An excess of iron is added to the serum to saturate the transferrin. The unbound iron is precipitated with basic $MgCO_3$. After centrifugation the iron in supernatant is quantified. All plastics and glasswares were soaked in 0.1M HCl and rinsed three times with iron-free de-ionized water to avoid contamination with iron.

Procedure

Into labeled centrifuge tubes, 0.5ml of serum samples were separately pipetted followed by 1.0ml iron solution (500µg iron/100ml), and mixed. These were let to stand for 30 minutes at room temperature. $MgCO_3$ (180mg) was separately weighed and added to each mixture and allowed to stand again for 30 minutes at room temperature with frequent mixing. Mixtures were then centrifuged for 10 minutes at 3000rpm. 5ml of each supernatant was pipetted into labeled cuvettes and 2.0ml acetate buffer (pH 4.5) added, followed by 0.1ml ascorbic acid (1.3M) as reductant.

Standard tube was also set up by pipetting 0.5ml iron standard (35.8µM) into a tube containing 2.0ml acetate buffer and 0.1ml reductant. A reagent blank containing 2.0ml buffer, 0.1ml reductant and 0.5ml deionized water (iron free) was also set up. Absorbance of the samples and standard were read against reagent blank at 610nm.

After this, 0.1ml of iron chromogen was added to all the tubes, mixed and incubated for 5 minutes at 20^{o}C. Final absorbance was read at 610nm. Triplicate analyses were carried out on all the samples.

Calculation

The initial absorbance was subtracted from the final absorbance to give absorbance for the samples and standard.

$$\text{TIBC (µM)} = \frac{\text{A sample x 107.4}}{\text{A standard}}$$

3.2.6 Statistical Analysis

All data with statistical analysis were expressed as means ± SD. Analysis of variance (ANOVA) was used to establish any significant difference between the formulated and control diets (Kelly and Onyeka, 1992).

CHAPTER FOUR

RESULTS

4.1 RESULTS OF THE COMPOUNDED DIETS

4.1.1 Proximate Nutrient Composition

Results of the proximate nutrient composition of the compounded diets (diet 1-8) are presented in Table 4.1. It was observed that values of the moisture content of diets 3, 4 and 6 of 4.5, 4.9 and 5.15% dm respectively were significantly higher ($P<0.05$) than the control value (3.25%), while the rest of the diets had comparable value to the control ($P>0.05$). All the values were however, either within or below the 5% level recommended for dry foods for enhanced keeping quality (Temple et al 1996).

Values for crude protein were significantly higher ($P<0.05$) in diets 4 and 5 (16.1 and 20.6% respectively, lower in diets 2(11.1%) and 7 (11.3%) than control (13.4%). Crude fat was observed to be significantly higher ($P<0.05$) in all the compounded local diets (10.7 – 28.1%) than in the control (3.72%). Diet 1 showed the highest value of 28.1%. Nitrogen Free Extract (NFE), which represents the total carbohydrate value was significantly higher ($P<0.05$) in the control (79.5%), than in all the locally formulated diets (50.2 – 67.9%). The high NFE value observed in the control was expected because NFE was calculated by the "difference" method.

Table 4.1: Proximate Nutrient Composition of the Compounded Diets

Nutrient (%dm)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8 (control)
Moisture	3.70[a]±0.14	2.95[a]±0.07	4.50 ±0.21	4.90 ±0.14	3.90 [a]±0.14	5.15 ±0.08	4.09 [a]±0.20	3.25 [a]±0.21
Crude Protein	13.8 [a]±0.22	11.1±0.28	13.4[a]±0.14	16.1±0.12	20.6±1.28	14.4[a]±0.08	11.3±0.60	13.4[a]±0.52
Crude fat	28.1±0.57	11.7±0.78	10.7±0.27	15.6±0.21	19.7±0.55	17.7±0.07	18.2±0.36	3.72[a]±0.06
NFE	50.2±1.80	67.4±1.58	67.9±1.54	59.0±0.26	52.6±1.33	57.1±2.40	60.7±2.01	79.5[a]±2.10
Crude fibre	9.07±0.26	10.4±0.38	7.96±0.06	9.29±0.83	6.05[a]±0.99	10.8±0.35	9.79±0.41	3.41[a]±0.48
Ash	2.05±0.07	1.40±0.14	0.99±0.01	2.06±0.08	5.75±0.35	2.60[a]±0.14	3.05[a]±0.07	3.48[a]±0.48
Energy (Kcal)	508.1±14.24	419.3[a]±13.13	421.5[a]±18.61	440.8[a]±16.90	470.1±16.90	445.3[a]±17.29	451.8[a]±17.74	452.6[a]±10.08

Results are means ± SD for three determinations

Figures in the same horizontal row that share the same superscript are not significantly different from control at P>0.05

Crude fibre was however found to be lower in the control (3.41%) than the local diets (6.05 – 10.8% dm). Ash was significantly higher ($P<0.05$) in diet 5 (5.75%), lower in diets 1, 2, 3 and 4 (0.99 – 2.06%) than the control (3.48% dm). Energy values in all the local diets and the control were however comparable ($P>0.05$). On the whole, the control diet cannot be said to be superior in terms of proximate composition. Based on protein content, diets 1, 3 and 6 were found to be much more comparable to the control. Rats fed diet 5 recorded the highest mortality rate.

Table 4.2 shows the calculated amount of each nutrient (in gram) that can be derived from 65g of the various diets [dry weight estimate of the daily intake of local weaning food by a six-month old infant in Jos, Nigeria (Fernandez, et al 2002)]. The calculation allowed comparison of the amount of nutrients to some Recommended Daily Allowances (RDAs to 1 year). Results indicated that only diet 5 (13.4g) could meet the RDA for protein of 13 – 14 g, followed by diets 4, 6 and 1 (10.5, 9.36 and 9.04g respectively). Diets 1, 4, 5, 6 and 7 would meet the RDA of 10 –25g for fat, while diets 2 and 6 fell below the recommended values. Fat in the control diet (2.42g) fell far below the RDA. All the diets (Local and control) fell below RDA for crude fibre (15 –20g) and energy (650Kcal).

Table 4.2: Amount of Nutrients Provided by a Daily Intake of 65g of Diets compared with some RDAs.

Nutrient	**Amounts in 65g**								
	Diet1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8 (Control)	RDA* to 1yr (g/day)
Moisture	2.41	1.92	2.93	3.18	2.54	3.35	2.66	2.11	
Crude protein	9.04	7.22	8.71	10.5[a]	13.4[a]	9.36	7.35	8.71	13-14g[a]
Crude fat	18.3[a]	7.61	6.96	10.1[a]	12.8[a]	11.5[a]	11.8[a]	2.42	10-25g[a]
NFE	32.6	43.8	44.1	38.4	34.2	37.1	39.6	51.7	-
Crude fibre	5.89	6.76	5.17	6.04	2.63	7.02	6.36	2.22	<15 – 20g
Ash	1.33	0.91	0.64	1.34	3.74	1.69	1.98	2.26	-
Energy(Kcal)	330.3	272.5	274.0	286.5	305.6	289.4	293.7	294.2	650Kcal[a]

*Recommended dietary allowances from Guthrie (1989)

Figures in the same horizontal row that share the same superscript are not significantly different from the RDA (P>0.05).

4.1.2 Mineral Element Composition

The amounts of mineral elements in the diets as well as in 65g (estimated daily intake by infant) are presented in Tables 4.3 and 4.4 respectively. It was observed that K, Ca, Zn, Fe, F, Cl and PO_4 were significantly higher ($P<0.05$) in the control diet than in the local formulations. Elements such as Na in diet 6 (47.4mg), Mn in diet 5 (2.38mg) and SO_4 in diet 6 (66.7mg) were statistically higher than in control for these elements. However, in general terms, it can be said that mineral elements are higher in the control diet than in the local formulations. Recommended daily allowances for the elements indicated that they can be met by the daily intake of 65g of the local diets for Mg, Mn and F, while values for all the other elements fell short of their respective RDAs. Diets 4 and 6 would make significant contribution to Zn intake, while diets 1, 6 and 7 to Fe intake.

4.1.3 Vitamin Content

Results are as presented in Tables 4.5 and 4.6. Ascorbic acid and thiamine were significantly lower in all the local diets than in the control. Amounts of thiamine would however meet the RDA of 0.3 – 0.4mg. Ascorbic acid fell short of the recommendation (25 –35mg). Riboflavin content of the local diets was also significantly lower than the control, but would meet the RDA of 0.4 – 0.5mg. Pyridoxine in diet 4 was significantly higher ($P<0.05$) than control while values in diets 1, 2 and 6 were comparable ($P>0.05$) to the control. Only diet 4 would meet the

Table 4.3: Mineral Element Composition of the Compounded Diets

Mineral element (mg/ 100g sample)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8 (control)
Na	21.1[a]±0.08	0.19±0.05	0.89±0.00	17.2±0.03	1.43±0.11	47.4±0.45	1.12±0.00	29.2[a]±0.03
K.	129.7±33.83	69.6±0.50	59.3±2.50	99.7±0.18	78.6±5.33	85.1±1.17	128.8±15.17	284.4[a]±10.09
Ca	36.7±0.18	23.6±0.24	25.7±1.43	185.6±31.67	46.4±5.00	172.5±10.83	31.0±0.58	298.0[a]±41.02
Mg	38.5±1.33	73.4±0.67	84.4±10.7	110.9±20.50	222.2±20.67	158.5±10.67	84.8±1.72	54.6[a]±0.30
Zn	2.01±0.01	1.65±0.02	2.28±0.03	3.08±0.02	3.22±0.01	4.77±0.09	2.75±0.01	14.1[a]±0.12
Fe	2.86±0.60	2.41±0.12	1.69±0.01	2.08±0.02	0.98±0.08	3.84±0.60	2.65±0.64	15.4[a]±0.13
Mn	0.37±0.02	0.65±0.03	0.98±0.01	0.82±0.01	2.38[a]±0.01	0.91±0.04	0.71±0.03	1.47[a]±0.05
F	14.6±1.52	14.6±2.33	4.59±0.22	33.3±3.02	5.00±0.66	16.7±1.22	17.9±2.33	42.0[a]±5.43
Cl	46.9±5.21	16.7±2.14	6.25±0.67	114.5±12.2	2.71±0.14	31.3±4.11	28.3±1.92	178.1[a]±10.7
PO_4	45.8±5.01	40.8±3.42	2.50±0.23	24.2±2.44	35.0±4.01	18.4±2.78	26.3±3.20	76.2[a]±7.21
SO_4	43.3[a]±4.88	9.33±1.76	4.00±0.17	46.7[a]±6.10	13.3±0.76	66.7±6.50	26.7±3.21	47.1[a]±4.11
NO_3	9.08[a]±0.56	0.65±0.01	0.65±0.11	6.52[a]±0.88	0.65±0.03	6.52[a]±0.52	0.65±0.01	8.00[a]±1.23

Values with statistical analysis are means ± SD of two determinations

Figures in the same horizontal row that share the same superscript are not statistically different from the control values at P>0.05

Table 4.4: Amount (mg) of Mineral that can be provided in 65g of the Diets.

	Amount (mg) in 65g samples								
Mineral	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8 (control)	RDA*(mg)
Na	13.7	0.12	0.58	11.2	0.93	30.8	0.73	18.3	120
K	84.3	45.2	38.5	64.5	51.3	55.3	83.7	184.9	500
Ca	23.9	15.3	16.7	120.6	30.2	112.1	20.2	193.7	400
Mg	25.0	47.7	54.9	72.1	144.4	103.0	55.1	35.5	40
Zn	1.31	1.07	1.48	2.05	2.09	3.50	1.79	9.17	5.0
Fe	1.86	1.57	1.10	1.35	0.64	2.50	1.72	10.0	6.0
Mn	0.24	0.42	0.64	0.53	1.55	0.59	0.46	0.96	0.3 – 0.6
F	9.49	9.49	2.98	21.6	3.25	10.8	11.6	17.3	0.1 – 0.5
Cl	30.5	10.9	4.06	74.4	1.76	20.4	18.4	115.8	180
PO_4	29.8	26.5	1.63	15.7	22.8	12.0	17.1	49.5	300
SO_4	28.2	6.06	2.60	30.4	8.65	43.4	17.4	30.6	
NO_3	5.90	0.42	0.42	4.24	0.42	4.24	0.42	5.20	

*Recommended Daily Allowance (RDA) from Guthrie (1989).

Table 4.5: Vitamin Content of the Compounded Diets

Vitamin (mg/100gdm)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8 (control)
Ascorbic acid (C)	0.21±0.13	0.48±0.03	0.36±0.09	0.30±0.09	0.62±0.04	0.53±0.11	0.64±0.05	2.91^{a}±1.30
Thiamine (B_1)	0.33±0.02	0.63±0.01	0.71^{a}±0.06	0.32±0.05	0.69^{a}±0.12	0.23±0.04	0.36±0.09	0.88^{a}±0.02
Riboflavin (B_2)	0.50±0.14	3.20±0.28	1.75±0.07	0.20±0.04	1.80±0.14	3.85±0.07	0.20±0.01	6.50^{a}±0.14
Pyridoxine (B_6)	0.11^{a}±0.05	0.10±0.07	0.03±0.01	0.85±0.09	0.05±0.01	0.09±0.02	0.03±0.00	0.14^{a}±0.06
Vitamin A (µg)	850.0±28.28	250.0±28.28	75.0±4.24	407.5±10.61	392.1±18.39	487.5^{a}±18.39	347.5±13.55	559.0^{a}±14.14

Values are means ± SD for three determinations

Figures in the same horizontal row that share the same superscript are not statistically different from the control diet (P>0.05).

Table 4.6: Amount of Vitamins Provided by 65g of the Diets.

Vitamin (mg)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8 (control)	RDA* up to 1 year
Ascorbic acid (C)	0.14	0.31	0.23	0.20	0.40	0.34	0.42	1.89	25 – 35mg
Thiamine (B_1)	0.22	0.41	0.46	0.21	0.45	0.15	0.23	0.57	0.3-0.4mg
Riboflavin (B_2)	0.33	2.08	1.14	0.13	1.17	2.50	0.13	4.22	0.4-0.5mg
Pyridoxine (B_6)	0.07	0.06	0.02	0.55	0.03	0.06	0.02	0.09	0.3-0.6mg
Vitamin A (µg)	552.5	162.5	48.8	265.6	254.9	316.9	225.9	357.5	375-420µg

*RDA from Guthrie (1989).

RDA of 0.3 – 0.6mg. Vitamin A (as RE) was significantly higher in diet 1 (850 µg) and would meet the RDA of 375-420 µg, followed by the control diet (357.5µg). The rest of the diets fell short of the recommendation. The inclusion of carrot in diet 1 could be responsible for the high vitamin A content observed.

4.1.4 Food and Water Intakes

Results of the cumulated water and food intake of rats fed with the experimental diets are shown in Figures 1 and 2 respectively. In both cases, rats fed with the control diet consumed significantly higher quantities of water (1585ml) and food (1536g) probably because of its palatability. Quantities of food consumed by rats fed diets 1, 4 and 6-ranked next (857, 945, 792g respectively).

4.1.5 Weight Gain and Growth Rate

Figure 3 shows the weight change and growth rate of rats recorded during the feeding period of 28 days. Again rats fed the control diet recorded the highest weight gain/growth rate, followed by rats fed diets 1, 4 and 6. There was no mortality recorded in these groups of rats. Diet 5 fed rats weighed the least, and had the highest mortality rate (3 rats) followed by rats fed diets 2 and 3 (1 rat each). These observations therefore suggest that diets 1, 4 and 6 were better tolerated and supported higher growth rate.

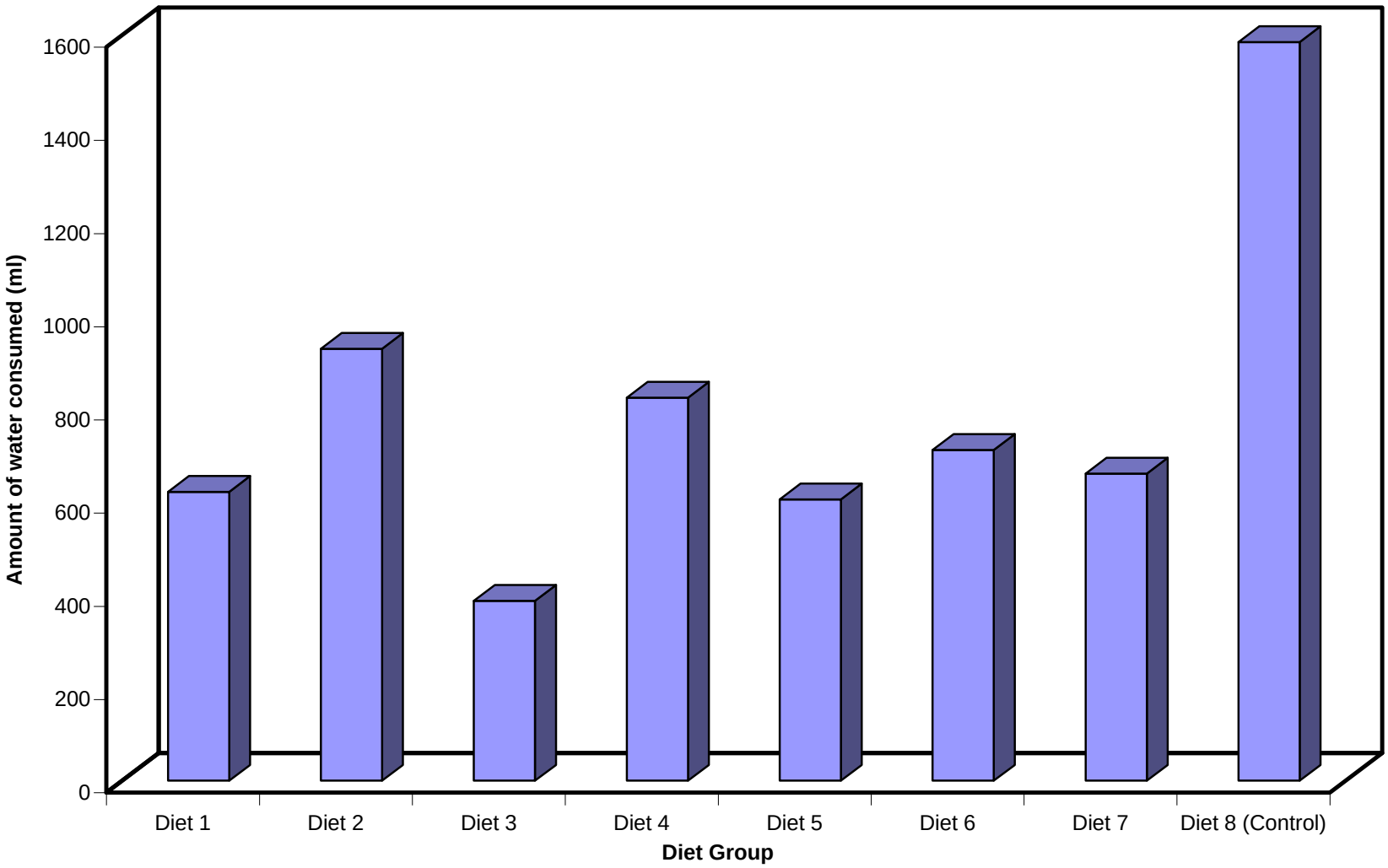


Figure 1: Water Consumed by Rats over the Experimental Period of 28 Days

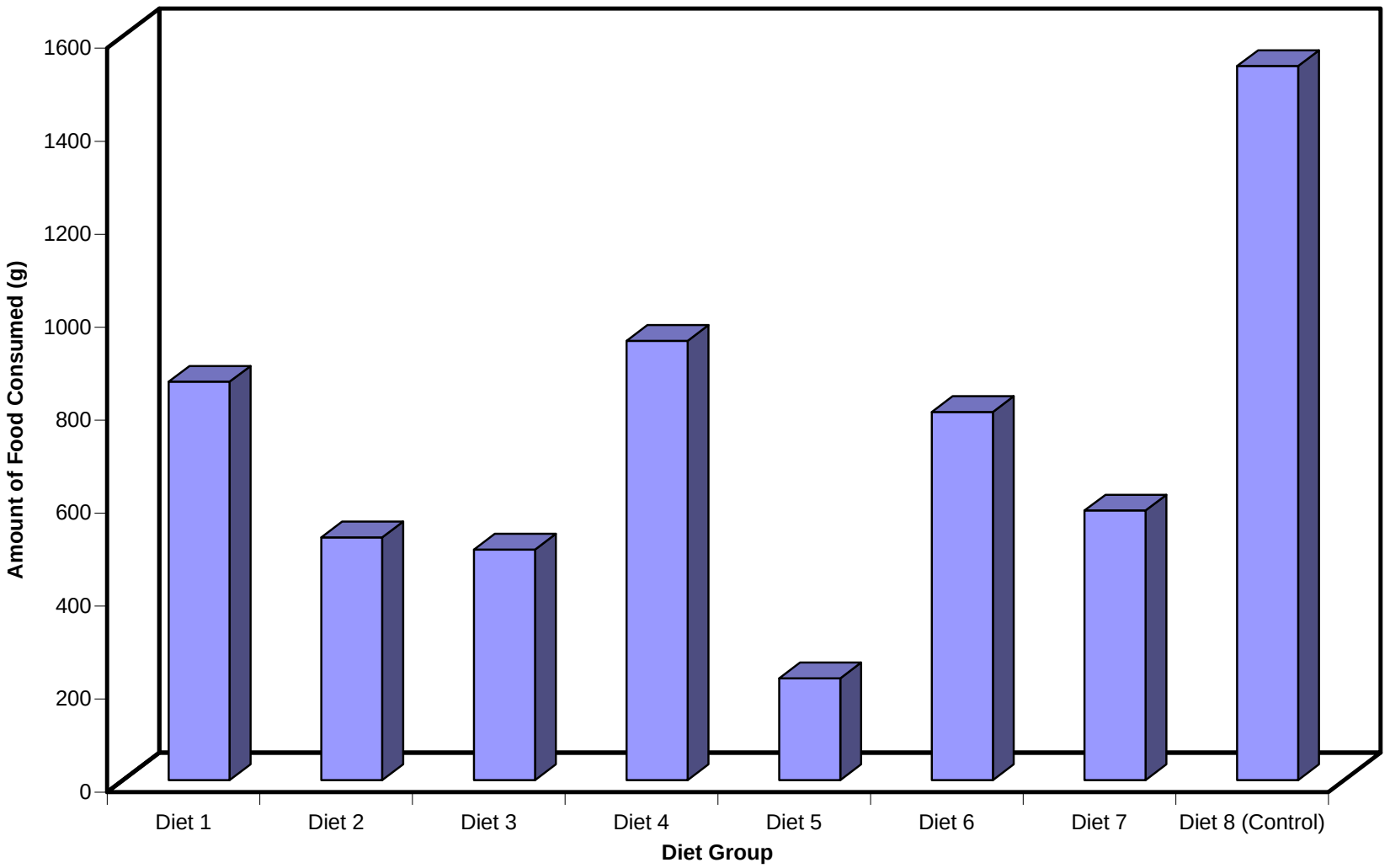


Figure 2: Food Consumed by Rats over the Experimental Peroid of 28 Days

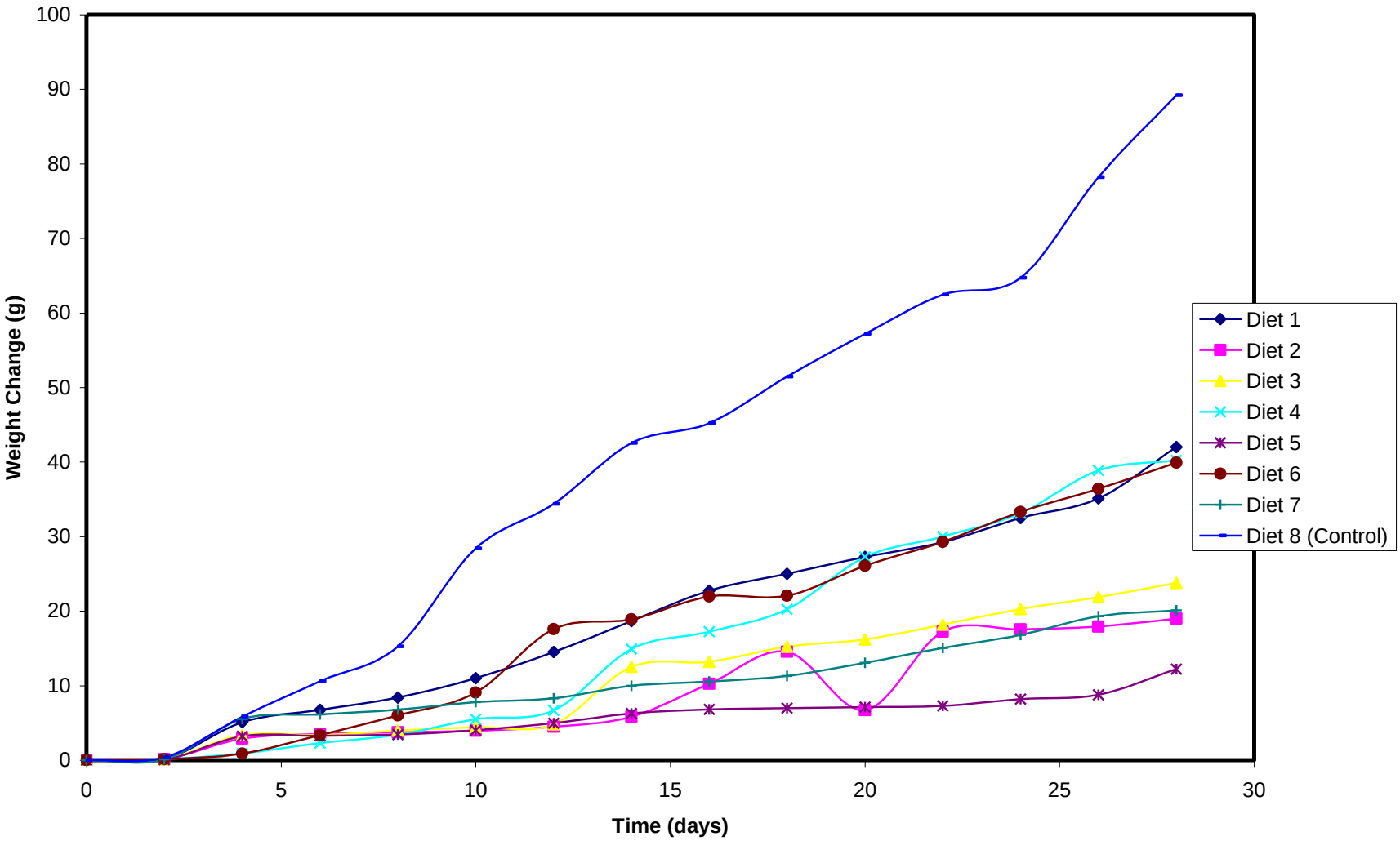


Figure 3: Change in Weight (Growth Rate) of Rats Fed with the Diets for 28 Days

4.1.6 Efficiency Ratios

The higher food intake, protein intake and weight gain observed in rats fed with the control diet (Table 4.7) also resulted in higher protein efficiency ratio (3.8) followed by diets 1, 4 and 6 (2.3, 1.9 and 2.4 respectively) Diet 5 showed the lowest value of 0.6.

4.1.7 Diet Selection

The preliminary investigation of the compounded diets revealed some potentials in them, for instance diets 1, 4 and 6 were found to hold greater promise in terms of protein content, food intake, growth rate, protein efficiency ratio and survival rate of rats fed with the diets. These and other strength observed informed their choice for further evaluation. Diets 1, 4 and 6 were therefore modified by changing the ratio of foodstuff used, inclusion of red palm oil, salt and crayfish where necessary to improve nutrient content, palatability, food (and nutrient) intakes and growth rate. The modified diets (redesignated diets 1, 2 and 3) were analyzed chemically as well as in the rat model to ascertain their new status along side the control (diet 4).

Table 4.7: Food intake, Weight Gain and Efficiency Ratios of Compounded Diets

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diets 5	Diet 6	Diet 7	Diet 8 (control)
Food intake (g/rat)	857	522	496	945	219	792	580	1536[a]
Protein intake (g/rat)	13.6	11.1	13.4	16.1	20.6[a]	14.4	13.3	23.4[a]
Weight gain (g/rat)	32	19	23	30	12	35	19	89[a]
FER	26.8	27.5	21.6	31.5	18.0[a]	22.6	30.5	17.3[a]
PER	2.3	1.7	1.7	1.9	0.6	2.4	1.7	3.8[a]

$$\text{Food Efficiency Ratio (FER)} = \frac{\text{Food intake}}{\text{Weight gain}}$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Weight gain}}{\text{Protein intake}}$$

Figures in the same horizontal row that share the same superscript are not statistically different from the control ($P>0.05$).

4.2 RESULTS OF THE MODIFIED DIETS

4.2.1 Proximate Nutrient Composition of Modified Diets

Results of the proximate nutrient composition of the modified diets are shown in Table 4.8, while amounts of nutrient derivable from 65g of the diets are shown in Table 4.9. The modified diets were significantly higher ($P<0.05$) in moisture, fat and crude fibre, than the control. Moisture content was found to be generally higher in the modified than the first set of diets, while crude fat was observed to be lower. The increased moisture in the modified diets could be attributed to climatic changes (atmospheric humidity) since the modified diets were prepared during the humid season.

Crude protein was observed to be statistically higher ($P<0.05$) in the modified diets (16.7 – 19.8%) than the control (14.4%). Also values were higher than the preliminary diet, possibly due to modification of ratios of the foodstuff used. Ash, NFE and energy values of both sets of formulations did not differ significantly ($P>0.05$).

Comparison between derivable nutrients from 65g of the modified diets and the RDAs, showed that only diet 3 came close to meeting the recommended allowances for crude protein (12.9g) and crude fat (10.7g) None of the diets met the recommended values for crude fibre and energy. Similar observation was made about the first set of diets.

Table 4.8: Proximate Nutrient Composition of the Modified Diets

Nutrient (%dm)	Diet 1	Diet 2	Diet 3	Diet 4 (Control)
Moisture	6.27±1.12	7.13±0.67	6.27±0.27	4.12^{a}±0.47
Crude Protein	16.7±2.47	16.8±1.56	19.8±0.75	14.4^{a}±1.34
Crude fat	11.4±0.94	12.7±0.23	16.5±0.31	3.00^{a}±0.83
NFE	63.8±4.13	62.9±2.67	55.4±1.60	79.0±2.63
Crude Fibre	8.15±1.65	7.56±0.65	8.30±1.12	3.58^{a}±0.63
Ash	3.13^{a}±0.25	3.0±0.44	2.68^{a}±0.16	2.89^{a}±0.41
Energy (Kcal)	430.0^{a}±17.37	533.1^{a}±13.71	549.3^{a}±14.05	400.6^{a}±18.03

Values are means ± SD for three separate determinations

Figures in the same horizontal row that share the same superscript are not significantly different from control (P>0.05).

Table 4.9: Amount of Nutrient in 65g of the modified diets

Nutrient (%dm)	Diet 1	Diet 2	Diet 3	Diet 4 (Control)	RDA* up to 1 year
Moisture	4.08	4.63	4.08	2.68	
Crude Protein	11.0	10.9	12.9[a]	9.36	13–14[a]
Crude fat	7.41	8.26	10.7 [a]	1.95	10 [a]-25
NFE	41.5	40.9	36.0	51.4	
Crude Fibre	5.30	4.91	5.40	2.33	<15-20g
Ash	2.03	1.95	1.74	1.88	
Energy (Kcal)	279.5	346.5	357.0	263.9	650 [a]

Values in the same horizontal row that share the same superscript are not significantly different from the RDA
* Recommended Daily Allowance (RDA) from Guthrie (1989).

4.2.2 Mineral Content of the Modified diets

The mineral element composition of the modified diets is as presented in Table 4.10, while amounts of nutrients derivable from 65g of the diets are shown in Table 4.11. Values showed that while Ca, Zn and Fe remained higher in the control. Na (38.1 – 51.8mg), Mg (125.4 – 158.8mg), Mn (1.76 – 3.24mg) and the anions were significantly higher ($P<0.05$) in the modified diets than the control (29.7, 60.6, 0.72 respectively). Values for Na, Mg, Zn, Fe, Mn and all the anions were found to be higher in the modified diets than the first set of formulations, which suggests that there was improvement in the mineral content as a result of the modification carried out. Consequently, amounts that can be provided by 65g of the modified diets also increased for these elements. However, Na, Ca, Fe and PO_4 still fell short of the recommended daily allowances for children up to 1 year, while Mg, Zn, Mn, F and Cl would meet the recommendations for infants. On the whole, the modified diets would provide higher amounts of all mineral elements compared to the preliminary formulations.

4.2.3 Vitamins of the Modified Diets

Values obtained and calculated amounts in 65g of vitamins in the modified diets are shown in Tables 4.12 and 4.13 respectively. It was observed that only ascorbic acid was significantly higher in control than all the modified diets while vitamin A, which is a global focal vitamin, was observed to be higher in the modified

Table 4.10: Mineral Element Composition of the Modified Diets

Mineral element (mg/100g dm)	Diet 1	Diet 2	Diet 3	Diet 4 (Control)
Na	49.8±0.18	38.1±0.87	51.8±2.90	29.7±0.31[a]
Ca	22.1±0.33	38.6±0.95	15.6±0.04	72.4±0.76[a]
Mg	38.8±0.00	158.8±0.26	125.4±0.21	60.6±2.60
Zn	10.8±0.11	11.3±0.15	7.27±0.03	19.8±0.11[a]
Fe	5.17±0.09	3.49±0.22[a]	2.90±0.33[a]	13.48±0.09[a]
Mn	1.76±0.04	2.52±0.03	3.24±0.06	0.72±0.06[a]
F	17.6±1.28	18.0±0.68	70.0±4.25	48.0±7.42
Cl	260.0±20.5	290.0±11.5	176.0[a]±19.4	189.0[a]±12.9
PO_4	113.0±10.8	63.0±9.42	41.0±6.82	95.0±8.42
SO_4	86.0±6.40	41.0[a]±4.52	64.0±8.94	41.0[a]±6.92

Values with statistical expression are means ± SD of two determinations

Figures in the same horizontal row that share the same superscripts do not differ significantly from the control ($P>0.05$).

Table 4.11: Amount of Mineral Element (mg) that can be provided in 65g of the Modified Diets

Mineral	Diet 1	Diet 2	Diet 3	Diet 4 (Control)	RDA* up to 1 year
Na	32.4	24.8	33.7	19.3	120
Ca	14.4	25.1	10.1	47.1	400
Mg	25.2	103.2	81.5	39.4	40
Zn	7.02	7.40	4.73	12.9	5.0
Fe	3.36	2.27	1.89	2.26	6.0
Mn	1.14	1.64	2.11	0.47	0.3–0.6
F	11.4	11.7	45.5	31.2	0.1-0.5
Cl	169.0	188.5	114.4	122.8	180
PO_4	73.5	41.0	26.6	61.8	300
SO_4	55.9	26.6	41.6	26.6	-

*RDA from Guthrie (1989).

Table 4.12: Vitamin Contents of the Modified Diets.

Vitamin (mg/100g dm)	Diet 1	Diet 2	Diet 3	Diet 4 (control)
Ascorbic acid (C)	0.12±0.03	0.37±0.05	0.54±0.02	2.01[a]±0.17
Thiamine (B_1)	1.21±0.14	0.42±0.15	0.61±0.14	0.79[a]±0.21
Riboflavin (B_2)	1.00[a]±0.34	0.34±0.04	0.35±0.04	0.80[a]±0.22
Pyridoxine (B_6)	0.33±0.03	0.50[a]±0.10	0.31±0.02	0.57[a]±0.11
Vitamin A (µg)	875.1±45.11	625.3[a]±16.72	702.0±30.22	600.2[a]±22.31

Values are means ± SD for two determinations

Figures in same horizontal rows that share the same superscript are not significantly different from control diet (P>0.05).

Table 4.13: Amount of Vitamins that can be provided in 65g of Modified Diets

Vitamin (mg)	Diet 1	Diet 2	Diet 3	Diet 4 (control)	RDA* up to 4yrs
Ascorbic acid (C)	0.08	0.24	0.35	1.31	25-35mg
Thiamine (B_1)	0.79	0.27	0.40	0.51	0.3-0.4mg
Riboflavin (B_2)	0.65	0.22	0.23	0.52	0.4-0.5mg
Pyridoxine (B_6)	0.22	0.32	0.20	0.37	0.3-0.6mg
Vitamin A (µg)	568.8	406.4	456.3	390.0	375-420µg

RDA from Guthrie (1989)

diets (625.3 – 875.1µg) than the control (600.2 µg). Results were also higher than the first set of diets. This could be due to the beneficial effect of red palm oil included during the modification. There were no variations in the values of thiamine, pyridoxine and riboflavin between the first and modified formulations. Amounts in 65g of the modified diets indicated that ascorbic acid fell short of the recommended value of 25 – 35g, while vitamins B_1, B_2, B_6 and A would meet the recommended amounts. However the effect of cooking on these vitamins in the diets remains to be established.

4.2.4 Amino acid Composition and % Chemical Scores of the Modified Diets

The amino acid profile and the chemical scores of the Essential Amino Acids (EAA) in the modified diets and control are shown in Tables 4.14 and 4.15 respectively. Seventeen amino acids were detected, out of which nine were essential amino acids, namely, Iso, leu, lys, met, thr, val, tyr, phe and his. Tryptophan was not detected. On the whole, there was no significant difference ($P>0.05$) in amino acid composition between the control and the modified diets.

The calculated chemical scores of the essential amino acids indicated that diet 1 contained the lowest amounts of EAAs, while diets 2 and 3 compared well with the control. It was also clear that EAAs such as threonine and cysteine found to be limiting in the local diets, were also limiting in the control.

Table 4.14: Amino Acid Profile of the Modified Diets

Amino acid (g//100g protein)	Diet 1	Diet 2	Diet 3	Diet 4 (control)
Lysine	3.23[a]	3.60[a]	5.12[a]	4.41[a]
Histidine	1.66	2.02[a]	2.04[a]	2.51[a]
Arginine	4.00 [a]	4.08[a]	4.95	3.02[a]
Aspartic acid	5.07 [a]	5.23[a]	5.38[a]	5.30[a]
Threonine	1.53 [a]	1.69[a]	1.56[a]	1.71[a]
Serine	2.72 [a]	3.40[a]	3.26[a]	2.27[a]
Glutamic acid	9.48	9.30	12.20[a]	12.25[a]
Proline	2.35 [a]	2.34[a]	2.11[a]	1.80[a]
Glycine	2.83 [a]	3.14[a]	2.48	3.00 [a]
Alanine	3.09 [a]	2.99[a]	4.04	3.27 [a]
Cystine	1.35 [a]	1.25[a]	0.97[a]	0.98 [a]
Valine	3.06 [a]	3.47[a]	3.84[a]	3.90 [a]
Methionine	2.07 [a]	2.21[a]	2.24[a]	2.36 [a]
Isoleucine	3.26	4.27	5.21	7.16 [a]
Leucine	3.43	4.51[a]	4.20	5.67 [a]
Tyrosine	2.80[a]	5.44	2.68[a]	3.57 [a]
Phenylalanine	2.81[a]	3.36[a]	3.35[a]	3.47 [a]
Tryptophan	ND	ND	ND	ND

Figures in the same row that share the same superscript are notsignificant different from control at P>0.05.

ND = not determined.

Table 4.15: Chemical Scores (%) of Essential Amino acids in the Modified Diets

Amino acid	FAO Ref. Value	% Chemical Score*			
		Diet 1	Diet 2	Diet 3	Diet 4
Isoleucine	4.2	77.7	101.7	124.0	170.5
Leucine	4.2	82.78	107.4	100.0	135.0
Lysine	4.2	76.9	85.7	121.9	105.0
Methionine	2.2	94.1	100.5	101.8	107.3
Threonine	2.8	54.6[a]	60.4 [a]	55.7[b]	61.1 [a]
Phenylalanine	2.8	100.4	120.0	119.6	123.9
Valine	4.2	72.9	82.6	91.4	92.9
Tyrosine	2.8	100.0	194.3	95.7	127.5
Cystine	2.0	67.5[b]	62.5[b]	48.5[a]	89.0 [b]
Tryptophan	1.4	ND	ND	ND	ND

FAO (1970).

*% Chemical score = $\frac{\text{Value of amino acid in diet (g/100 protein)}}{\text{FAO Ref. Value}} \times 100$

a= first limiting amino acid

b=second limiting amino acid

ND = Not determined.

4.2.5 Antinutritional Factors (ANF)

Antinutrients assayed in this study are phytate, total oxalate, tannins and hydrocyanic acid, and values are as shown in Table 4.16. All the antinutrients were found to be lower ($P<0.05$) in the control than the modified diets. Total oxalate was observed to be higher (6.66 –11.4mg), while hydrocyanic acids the least (0.04–0.1mg). The calculated ANF: mineral molar ratios are shown in Table 4.17. Values for the control diet were significantly lower ($P<0.05$) than those for the formulated diets. Values for Phytate: Fe and Oxalate: Ca were significantly higher compared to the other parameters.

4.3 ANIMAL EXPERIMENTATION WITH THE MODIFIED DIETS

4.3.1 Water and Food Intake

These are depicted in Figures 4 and 5 respectively. There was no significant difference ($P>0.05$) in the amount of water consumed by rats fed with the modified and the control diets. There was however difference between the control (1527g) and local diet groups (1357 – 1429g) in terms of food consumption. There was also marked increase in the amount of food consumed when rats were fed with the modified diets (1357-1429g) as compared to the amounts consumed when fed with the first set of diets (219 – 945g) which suggests that the modified diet were more accepted to the rats.

Table 4.16: Antinutritional Factors (ANF) in the Modified Diets and Control

Antinutrient (mg/100g dm)	Diet 1	Diet 2	Diet 3	Diet 4 (Control)
Phytate	0.56±0.02	0.61±0.14	0.40±0.10	0.07[a]±0.02
Total oxalate	8.56±1.01	11.4±3.11	6.66±1.22	0.11[a]±0.01
Hydrocyanic acid	0.10±0.01	0.04±0.01	0.10±0.02	0.01[a]±0.01
Tannins	0.86±0.20	1.93±0.42	2.11±0.13	0.03[a]±0.00

Results are means ± SD for three determinations.

Values in the same horizontal row that share the same superscript are not significantly different from the control diet at P>0.05.

Table 4.17: Annutrient: Mineral Molar Ratios in the Modified Diets.

ANF: Mineral	Diet 1	Diet 2	Diet 3	Diet 4 (control)
Phytate: Calcium	2.5	1.6	2.6	0.1
Phytate: Magnisium	1.4	0.4	0.3	0.1
Phytate: Zinc	5.1	5.4	5.5	0.4
Phytate: Iron	10.7	17.6	13.8	2.2
Phytate: Calcium	28.6	19.5	24.2	0.2
Tannins: Iron	6.7	5.1	7.5	0.9

$$\text{Molar Ratio} = \frac{\text{Millimoles of ANF intake per day}}{\text{Millimoles of mineral intake per day}}$$

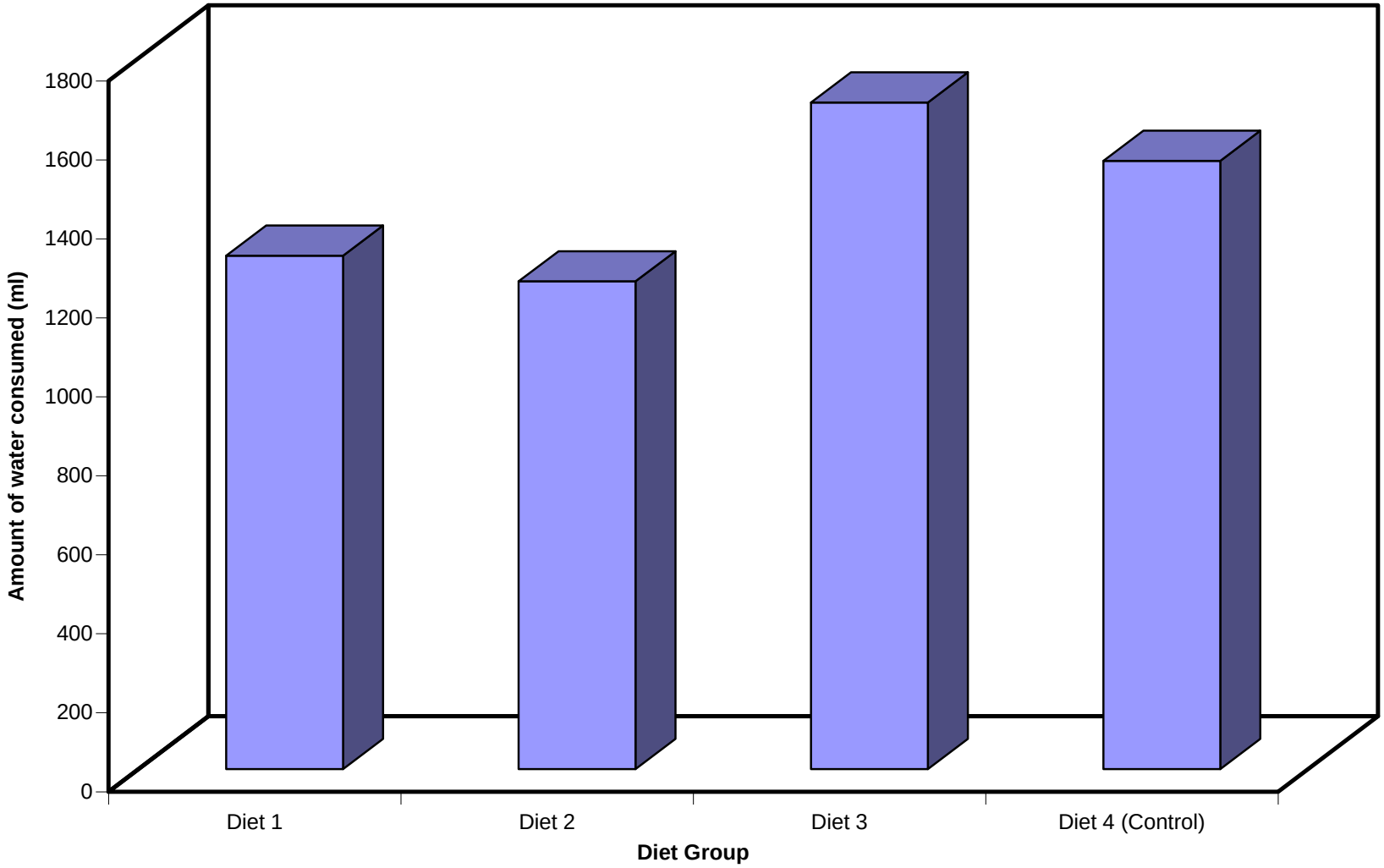


Figure 4: Water Consumed by Rats Fed with the Modified Diets for 28 Days

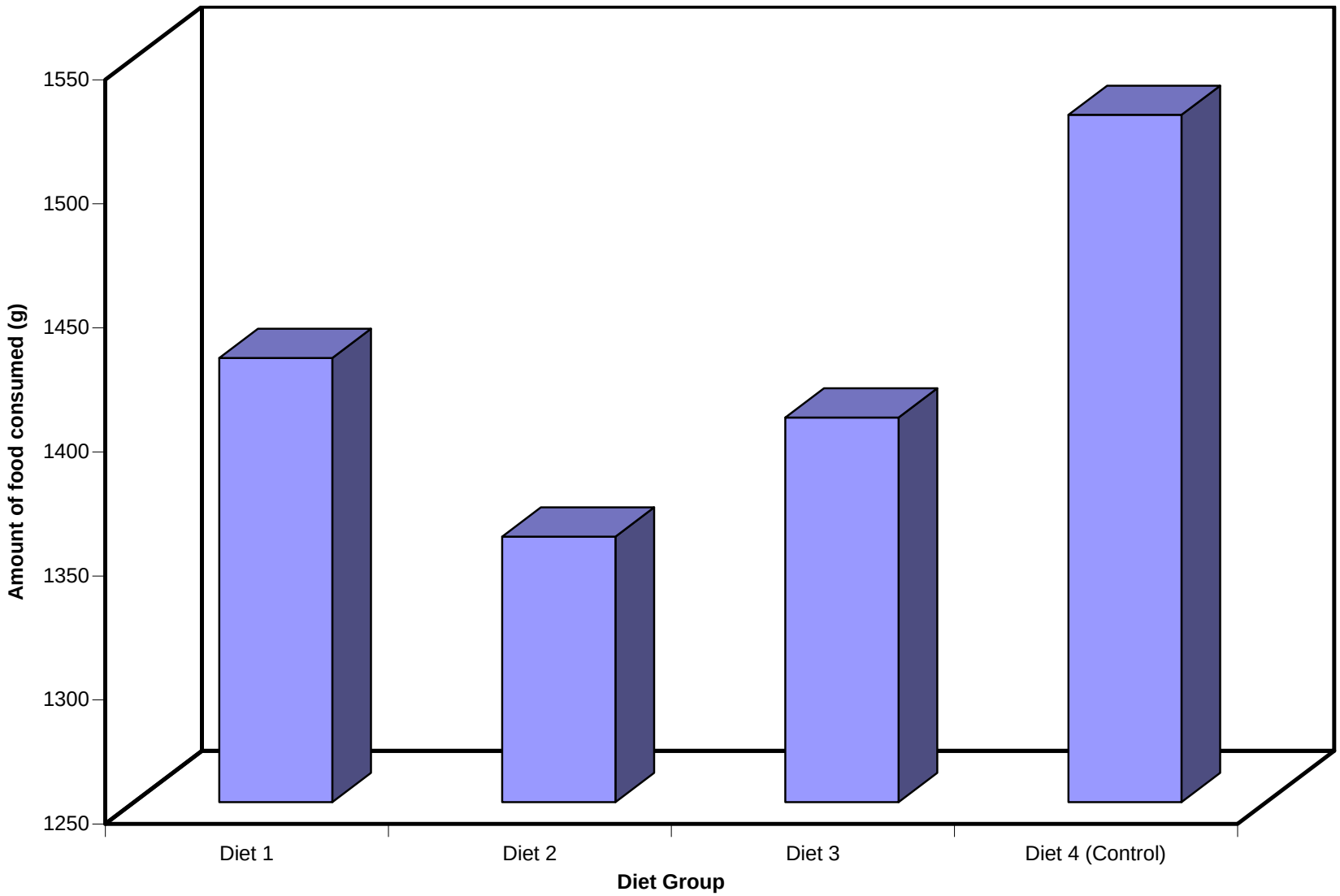


fig. 5: Food Consumed by Rats Fed with the Modified Diets for 28 Days

4.3.2 Growth Rate of Rats

The growth rate of rats fed with the modified diets is depicted in Figure 6, which shows that rats fed with diets 1 and 3 had comparable growth rate to the control group. Diet 2 group showed significantly lower rate. However, the modified diets supported higher growth rate than the first set of formulated diets (Figure 3).

4.3.3 Weight Gain and Efficiency ratios

Table 4.18 shows the protein intake, weight gain, protein and food efficiency ratios of the modified diets fed to rats. Again diets 1 and 3 showed comparable values to the control, while diet 2 showed lower values. When values were compared with those of the first set of formulated diets, the modified diets showed higher weight gain, protein intake as well as better food and protein efficiency ratios. Again this suggests that the modified diets were superior to the first formulated diets in terms of acceptability and ability to support growth in rats.

4.3.4 Haematological Indices

The haemoglobin (Hb) and Packed Cell Volume (PCV) of rats fed with the modified diets re presented in Table 4.19. There was no statistical difference ($P>0.05$) in both Hb and PCV between the modified and control fed rats. Rats fed the modified diets had Hb value of 10.7 – 12.3g/dl, and PCV value of 35.3 – 41.0% while the control group had 11.0g/dl for Hb and 36.7% for PCV. These comparable values suggest that the modified diets supported physiological functions as the control.

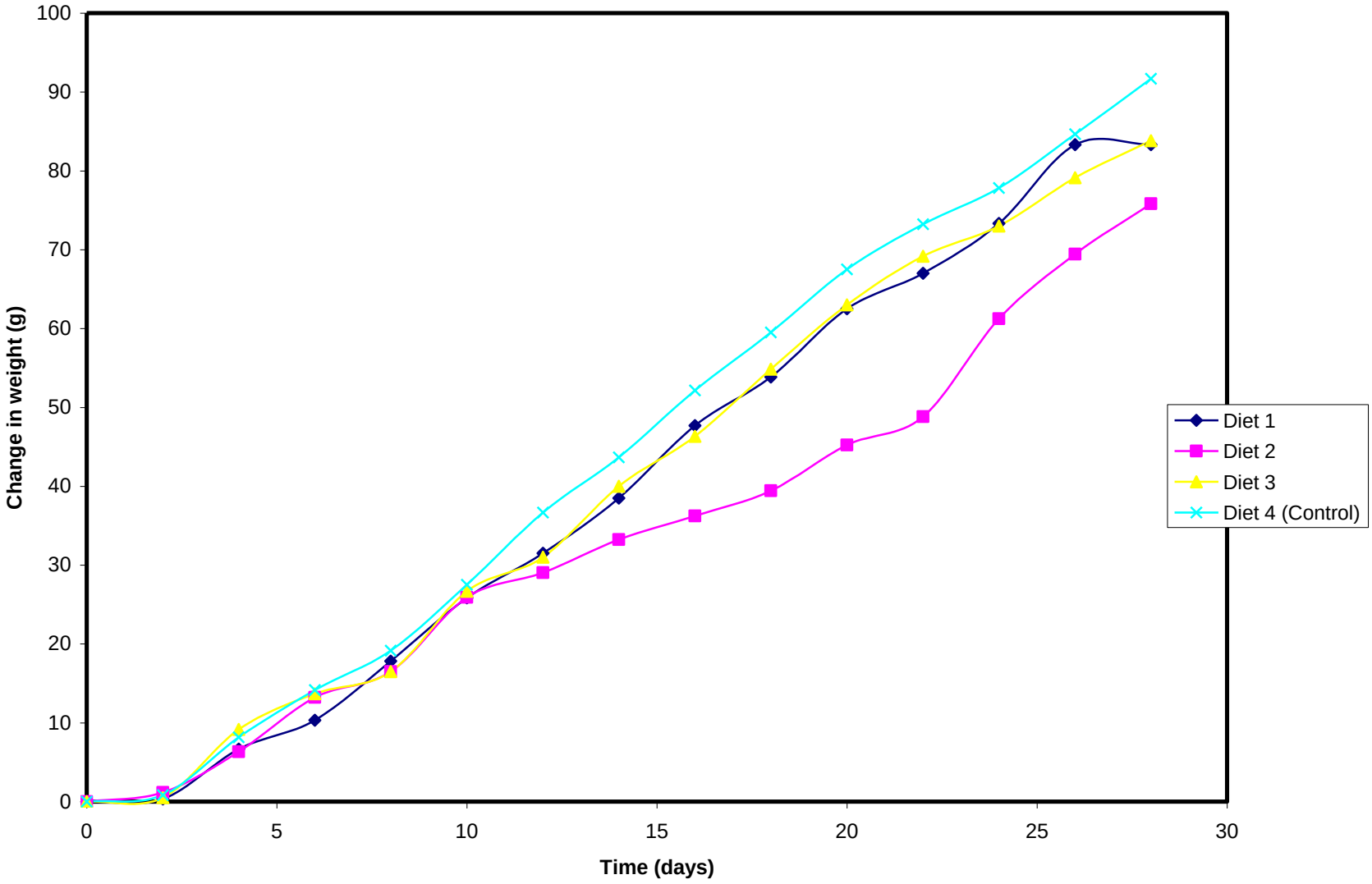


Figure 6: Change in Weight (Growth Rate) of Rats Fed with Modified Diets for 28 Days.

Table 4.18: Food intake, Protein intake, Weight gain and Efficiency Ratios of the Modified Diets

Parameter	Diet 1	Diet 2	Diet 3	Diet 4 (control)
Food Intake (g/rat)	1420[a]	1348 [a]	1390[a]	1543 [a]
Protein Intake (g/rat)	45.3	38.0 [a]	53.0	36.6 [a]
Weight gain (g/rat)	147 [a]	105	139	153 [a]
FER	9.7	12.8	10.0	10.1
PER	3.3	2.8	2.6	4.2 [a]

Food Efficiency Ratio (FER) = $\frac{\text{Food Intake}}{\text{Weight gain}}$

Protein Efficiency Ratio (PER) = $\frac{\text{Weight gain}}{\text{Protein Intake}}$

Figures in the same row that share the same superscript are not statistically different from the control at P>0.05.

Table 4.19: Some Haematological Indices of Rats Fed with the Modified Diets.

Diets	Hb (g/100ml)	PCV (%)
Diet 1	10.7 [a]±0.64	35.3 [a]±1.53
Diet 2	10.4 [a]±0.84	35.3 [a]±2.52
Diet 3	10.3 [a]±0.42	41.0±1.41
Diet 4 (control)	11.0 [a]±0.61	36.7 [a]±1.53

Values are means ± SD for three determinations

Figures in the same vertical column that share the same superscript are not significantly different from the control P>0.05.

4.3.5 Serum Biochemical Parameters

Results of serum glucose, protein, albumin, urea, creatinine and bilirubin in rats fed the modified diets are shown in Table 4.20. Results indicated that diets 1 and 2 had comparable value of serum glucose (8.35 and 7.80 mM) with the control (8.70mM), while diet 3 group rats had lower value of (5.95mM). There was no statistical difference ($P>0.05$) in urea concentration between the control and local diet groups. Diets 1 and 2 differed significantly from the control for creatinine, while rats fed diet 3 had comparable value to the control. Total protein, albumin, globulin and bilirubin (total and conjugated) in rats fed the modified and control had comparable values. On the whole, it can be said that the modified diets also supported normal biochemical functions since values of biochemical indices compared well with the control.

4.3.6 Serum Enzymes

Activities of some enzymes assayed in serum of rats fed with the modified diets are outlined in Table 4.21. It shows that alkaline phosphatase, lactate dehydrogenase and the transaminase activities in serum of rats fed with diets 1 and 3 were significantly higher than in the control group, while diet 2 group showed comparable values to the control ($P>0.05$).

Table 4.20: Serum Biochemical Parameters of Rats Fed with the Modified and Control Diets

Serum parameter	Diet 1	Diet 2	Diet 3	Diet 4 (Control)
Glucose (mM)	8.35[a]±1.48	7.80[a]±0.99	5.95±0.78	8.70[a]±0.98
Urea (mM)	4.00±0.50	3.27[a]±0.31	4.43[a]±0.21	3.13[a]±0.31
Creatinine (µM)	45.3±3.11	46.7±3.43	38.0±2.65	28.3[a]±2.03
Total protein (g/100ml)	6.35[a]±0.50	5.65[a]±0.45	6.20[a]±0.28	6.80[a]±0.42
Albumin (g/100ml)	3.63[a]±0.35	4.10[a]±0.42	3.88[a]±0.50	4.22[a]±0.38
Globulin (g/100ml)	2.72[a]±0.15	1.55[a]±0.03	2.32[a]±0.22	2.58[a]±0.04
Total bilirubin (µM)	10.4[a]±0.97	10.2[a]±0.86	10.2[a]±0.95	10.5[a]±0.89
Conj. bilirubin (µM)	5.71[a]±0.86	5.41[a]±0.81	5.22[a]±0.77	5.32[a]±0.42

Values are means ± SD for three determinations.

Figures in the same horizontal row that share the same superscript do not differ significantly from the control at $P>0.05$.

Table 4.21: Activities of Some Serum Enzymes in Rats Fed with the Modified Diets

Enzyme (IU/L)	Diet 1	Diet 2	Diet 3	Diet 4 (Control)
Alkaline Phosphatase	63.7±6.70	81.5[a]±14.1	65.7±9.50	87.0[a]±10.4
Alanine Transaminase	16.2±2.25	13.7[a]±2.52	17.7±3.31	11.65[a]±2.42
Aspartate Transaminase	21.2±1.57	15.5[a]±2.29	21.8±3.27	14.4[a]±1.64
Lactate Dehydrogenase	166.7±13.13	153.1±10.23	161.1±9.11	152.2±12.73

Values are means ± SD for three determinations.

Figures in the same horizontal row that share the same superscript do not differ significantly from the control values $P>0.05$.

4.3.7 Blood Lipid Profile

Table 4.22 shows the lipid profile in blood of rats fed with the experimental diets. Total cholesterol was observed to be statistically higher ($P<0.05$) in rats fed with the control diet (3.3mM) than in rats fed with diets 1 and 3 (1.73 and 1.56mM) respectively. HDL-cholesterol differed significantly between rats fed the control (0.01mM) and diet 1 (0.36mM), while diets 2 and 3 did not differ significantly from the control. Triglyceride concentrations were also comparable in rats fed the control and all the modified diets. When total cholesterol: HDL-cholesterol ratios were calculated, significant difference was observed between the control (33:1) and the three modified diets (5:1, 6:1 and 21:1).

4.3.8 Serum Retinol, Iron and Total Iron Binding Capacity (TIBC)

Values are as shown in Table 4.23. There was no significant difference in TIBC and retinol between rats fed the control and modified diets. There was however difference in serum iron between the control (19.7µM) and diet groups 1 and 2 (33.0µM and 31.3µM respectively).

4.4 ESTIMATED COST OF THE MODIFIED DIETS

Table 4.24 shows the estimated cost of approximately 450g of each modified diet based on the current prices of foodstuff used in the market, and the cost of a 450g tin of control diet (Nestle Cerelac). The estimates indicate that the local diets would cost between N100.00 to N180.00, while Cerelac costs N280.00. The local formulations cost less by 25 to 64% and therefore more cost effective and affordable.

Table 4.22: Some Lipid Profile in the Blood of Rats Fed with the Modified and Control Diets

Type of lipid (mM)	Diet 1	Diet 2	Diet 3	Diet 4 (Control)
Total cholesterol	1.73±0.87	2.37[a]±0.90	1.56±0.29	3.30[a]±0.72
HDL-cholesterol	0.36±0.03	0.11[a]±0.04	0.25[a]±0.07	0.10[a]±0.02
Triglyceride	1.15[a]±0.32	1.13[a]±0.40	0.76[a]±0.19	1.47[a]±0.35
Total chol.: HDL chol. Ratio	5:1	21:1	6:1	33:1[a]

Values with statistical analysis are means ± SD for three separate determinations. Figures in the same horizontal row that share the same superscript are not statistically different from the control values (P>0.05).

Table 4.23: Serum Retinol, Iron and Total Iron Binding Capacity (TIBC) of rats fed with the Modified and Control Diets.

Parameter	Diet 1	Diet 2	Diet 3	Diet 4 (Control)
Serum Fe (µM)	23.0±2.25	21.3±0.64	21.3±1.44	39.7[a]±2.41
TIBC (µM)	81.7[a]±1.54	74.8±1.27	83.2[a]±3.00	88.0[a]±1.22
Serum Retinol (µM)	57.3[a]±2.40	42.5±1.66	52.4[a]±1.35	53.8[a]±1.08

Values are means ± SD for three determinations

Figures in the same row that share the same superscript are not significantly different (P>0.05) from the control value.

Table 4.24: Estimated Cost of the Modified Diets at the Time of Study.

Diets	Estimated cost per 450g
Diet 1	N180.00
Diet 2	N150.00
Diet 3	N100.00
Diet 4 (control)	N250.00

CHAPTER FIVE

DISCUSSION

The use of locally available foodstuff to formulate the diet (blends) agrees with the guideline and criteria set out by the federal Ministry of Health in Nigeria (FMOH, 1999), calling for the use of available foodstuff in various communities in the country to prepare food for infants and children, so as to ensure availability and affordability. Such guideline are particularly important in a developing country like Nigeria, where gross malnutrition has largely been attributed to inadequate intake of food materials due to inability of parents or families to afford the proper diets (especially animal-source foods), and disease states that could prevent adequate utilization of the food materials as well as loss of appetite associated with these diseases (NNN, 2000). In some cases, ignorance about the essential food materials that could provide a balance diet has resulted in poor dieting.

Carbohydrate, fat, protein, vitamins and minerals elements are particularly important both in quantity and quality when formulating or preparing diets for infants, where requirements must be met for rapid growth, development and maintenance of good health. The first set of diets were assessed in both qualitative and quantitative terms, while the second set (Modified diets) were further evaluated in the rat model. Nutrient contents in one gram of each diet were multiplied by 65g in accordance to the estimated amount of weaning food consumed by 6-month old infant in Jos (Fernandez et al, 2002), to allow for comparison with the recommended daily allowances (RDAs) of some nutrients.

Proximate Nutrient Composition

The law moisture content observed in the first set of diets, and which were comparable to the control values, is a valuable attribute. Values of 2.95-5.15% reported in the current study fall within the 5% moisture content recommended for dry foods reported separately by Atinmo, (1986) and Badamosi et al, (1995). Law moisture content would tend to increase the shelf life of the diets, more so that the diets may be prepared in quantities that would require storage. Hence, the lower the moisture content, the better the keeping quality. Moisture values were however observed to be higher in the modified diets probably due to climatic difference during the experiments. The high moisture content would encourage the growth of microorganisms (mould), hence render the diets unsuitable for consumption. It is therefore recommended that small quantities of the diets be prepared at a time especially during humid seasons. Proper and hygienic cooking is also recommended to avoid diarrhea and other infections as observed by AHRTAG (1993).

Crude protein content in both the first set of diets (11.1-20.6%) and the modified diet (16.7-19.8%) fall within the protein Advisory council recommendations of 11.9-23.3% for infant diet. The calculated amount in 65g of the diets showed that only diet 5 in the first set of formulations would meet the RDA of 13-14g/day. Although the modified diets would not meet this recommendation it would however supply more protein per 65g (9.36-12.9g) than the first set of diets. The improved protein content in the modified diets could be attributed to the inclusion of crayfish, which is a good source of protein recommended by other researchers (Badamosi et al, 1995; Temple et al, 1996; Ladeji et al, 2000), in the formulation of infant's food. Protein is particularly important both in quantity and quality for rapid growth and

development of a child. Infant's amino acid requirements are proportionately higher than those of adults. In addition histidine is essential for infant at a level surpassed in both breastfeeding and bottle-feeding. The protein efficiency ratios (PER) calculated for the modified diets after the feeding experimentation were also higher than values observed for the first set of diets. This could be due to improved palatability, higher food consumption, and the positive contribution of crayfish, which is an animal source protein of higher biological value. Other researches (Temple et al, 1996; Ladeji et al, 2000; Nnam, 2002) have also suggested the inclusion of milk and egg in infant diets to improve the protein quality. This is based on the fact that proteins of plant origin have low biological value. The comparable food intake, weight gain and growth rate between the modified and control diet groups could also be partly responsible for the higher PER values observed for these diets.

The consistently high crude fat content observed in both the first sets of diets (10.7-28.1%) and the modified diets (11.4-16.5%) could be due to the inclusion of some oil-dense legumes such as sesame, groundnut and soyabeans, as well as red palm oil in the modified diets. Values in the control (3.0-3.7%) were significantly lower. Fats contribute substantially to the energy value of foods as well as provide essential fatty acids for optimal neurological, immunological and functional developments in children (Guthrie, 1989). Furthermore, lipid matrix in fat-based foods is inhospitable to bacteria (food spoilage) hence the spread of food-borne infection is avoided. This is particularly important in countries where household electricity and facilities for refrigeration are a luxury for most people. A product or food with a long shelf life at ambient tropical temperature is a valuable innovation. On the other hand, the high fat content in the formulated diets may lead to and excessive

intake of calorie, more so that carbohydrate content of the diet are sufficiently high. This may therefore lead to a rapid gain in weight, which is as undesirable in infants as in adults.

Furthermore, the high fat content of the diet may affect the keeping quality of foods on prolonged storage and frequent exposure, due to peroxidation of polyausaturated fatty acid (rancidity) that would impact unpleasant odour and reduced intake of food and nutrient. It is hoped that red palm oil in the modified diets would not only improve the palatability of the foods, but is believed to also provide antioxidant vitamins such as vitamins A and E, which will go a long way in reducing the degree of peroxidation in the foods.

Carbohydrates most of which come from vegetable foods notably grain products, tubers, roots and fruits, are the main source of energy in the diet. The cereal-legume based diets were found contain carbohydrate in the range of 55.4-67.9%. According to Gibney (1995), when formulating diets for infants, the ratio of carbohydrate to fat and protein must be taken into consideration. This is because a high protein and low carbohydrate diet would mean that the amino acids of the protein will be diverted to glucose synthesis and the body is deprived of protein meant for body building with resultant wasting and stunting. Values obtained in this study seem to suggest that carbohydrate will be sufficient to meet requirements.

Although there is no fixed RDA for carbohydrate intake, FAO/WHO (1998) has recommended an intake of about 50g per day as being sufficient to meet energy need. In this regard it would seem that only the control diet could meet this recommendation, while all the local diets fall short based on the estimated intake of 65g of weaning food by an infant per day (Fernandez, et al, 2002). It is however

believed that since breastfeeding is expected to continue, frequent feeding with local diets would provide sufficient carbohydrate. The high growth rate observed in rats fed the modified diets seems to suggest that protein was spared for normal metabolic processes, hence energy needs were met by the diets.

Dietary fibre consists primarily of the indigestible complex carbohydrate of cell wall in plants. Crude fibre values were found to be higher in the two sets of formulated diets (6.05-10.8%) than in the control (3.58%). The observed high values in the formulated diets could be due to the inclusion of whole grains and legumes with little or no refinement. The calculated amount of fibre in 65g of the formulated diets (2.33-5.40g) shows that there could be no risk of high intake by infants. FAO/WHO (1998) has recommended 10-15g/day. It is however, important to note that high fiber content or excessive intake may reduce the availability of nutrients such as Ca, Zn especially in food that marginal in these elements. High fibre content is also known to increase viscosity of food. This is particularly worth considering in infant feeding because highly viscous foods would reduce intake.

On the other hand, high dietary fibre observed in the diets can have some beneficial biological effects such as laxative effect on GIT, increased feacal bulk and help reduce plasma cholesterol level (Okoye, 1992). The higher food intake, weight gain and growth rate in rats fed these diets partly suggest that there was no significant negative effect of fibre.

Energy values in the two sets of formulated diets (452-508 Kcal/100g) and the control (401 kcal/100g) were comparable and agreed with some literature reported values for locally prepared weaning foods (Fashakin and Ogunsola, 1982; Ketiku and Olusanya, 1987; Temple et al, 1996; Ladeji et al. 2000; Islam et al, 2004). However,

none of the estimated daily intake values (263-330 Kcal) of the formulated diets met the RDA (650 Kcal) set for infants. Low calories as a result of inadequate intake of energy–giving nutrients notably carbohydrates and fats, will deprive the body of needed basal metabolic processes and strength for physical activities. It is believed that frequent consumption of the diets along with breast-milk, would satisfy the daily energy requirements of infants.

Micronutrients

Analysis of the first set of formulated diets revealed that mineral and vitamin contents were significantly lower than in the control. When some global focal micronutrients were considered, it was observed that Diets 1, 4 and 6 were relatively better in vitamin A (RE) contents. Zinc was also present in appreciable amounts in diets 4 and 6, while iron in diets 1 and 6. On the whole diet 1 would meet the RDA for vitamin A while all other diets fell short of the RDAs for ascorbic acid and most of the mineral elements except F^- and Mn. The higher micronutrient values observed in the control could be due to fortification.

The appreciable amounts of global focal micronutrients found in diets 1, 4 and 6 in the first sets of diets partly informed the selection of these diets for reevaluation. When crayfish, red palm oil and salt were included in these diets, it was found that all the diets would now meet the RDA for vitamin A possibly due to the positive contribution of red palm oil. The B vitamins would also meet the recommended daily intakes. Vitamin C still fell short of the RDA. Vitamins and minerals are very vital for growth and development in infants. Fruits and vegetables are therefore recommended to be included in infant’s diets to increase micronutrient intakes.

Similarly, the modified diets fell short of the RDA for Ca and PO_4 up to 1 year. Due to the importance of these minerals in the skeletal development and growth of infants, (Nduka, 1999; Affi, 2000), it would be necessary to increase intake through fortification and or supplementation. Since complementary foods are intended to complement breast milk, it is believed that continuing breastfeeding along with the consumption of these local formulations, would go a long way in meeting most of the requirement for infants. This practice is highly recommended by WHO/UNICEF (1995) and FMOH (1999).

Weight Gain and Growth Rate

The rate and pattern of growth as well as absence of illness and mortality can also assess the adequacy of a diet (Guthrie, 1989) during the first feeding experimentation, it was observed that rats fed diets 2, 5 and 7 performed relatively poorly and recorded varying mortality rates. Rats fed diets 1, 4 and 6 showed higher weight gain and no mortality was recorded in these groups of rats. This could be attributed to higher food intake and PER (ability of protein to support growth) also recorded for these diets.

Having shown that diets 1, 4 and 6 had greater nutrients in several respects, the diets were modified as earlier mentioned and then fed unto rats again for reassessment. At the end of the feeding period, the modified diets were found to compare well with the control in terms of food intake, weight gain, growth rate and PER, and no mortality was recorded. Only diet 2 performed relatively lower. There was no clear understanding and explanation for the low performance of rats fed with diet 2, but one could speculate that the inclusion of garden egg which has a bitter taste, might have imparted an unpleasant taste to the diet hence lowered food

consumption and nutrient intake. The PER of the modified diets (2.8-3.6) compared well with the literature reported values for casein (2.5), whole egg (3.8) and cow's milk (2.0) as reported by Okoye (1992). These observations clearly demonstrated the positive contribution of crayfish, red palm oil and salt to the diets which improved the palatability and acceptability, hence increased food and nutrient intake.

Having observed that the modified diets are capable of supporting growth as well as closely mimicked the control, further analysis was carried out to assay for the amino acid composition, the levels of some antinutritional factors and indices of nutritional status in rats fed with the diets.

Amino Acid content

One of the known procedures for assessing the biological value (quality) of proteins is the determination of the amino acid profile in general and the essential amino acids (EAA) in particular. The amino acids as well as the EAA profile of the modified and control diets revealed that, those amino acids present in the control were also present in the formulated diets. When the chemical scores of the EAAs were calculated relative to the FAO (1970) reference values, it was observed that threonine and cystine were the most limiting amino acids in all the diets. Guthrie (1989) has reported that methionine is limiting in legumes, lysine in cereal, while soyabeans and nuts do not have enough of one or more of the EAAs. However, Lysine and methionine were not found to be limiting in the local diets. This is expected since the formulated diets were cereal-legume based blends hence they were mixed rather than single diets.

Essential amino acids are critical for the maintenance of nitrogen balance as well as other body-building functions, so much that if any one of them is not present in sufficient amounts when the body proteins are being synthesized, the amino acid in short supply becomes the limiting factor. The detection of histidine in the diets was also an added advantage because histidine is considered an essential amino acids in children since it cannot be synthesized in their bodies due to underdeveloped mechanism for that.

Antinutrients

Antinutritional factors (ANF) such as phytate, tannins, oxalate, sapanins, etc. can negatively affect the bioavailability of mineral elements and digestibility of protein and carbohydrate in the diet of infants. According to Friedman (1985), this effect may be of significance when diets are marginal for nutritional adequacy.

Some research observations recorded in literature suggest that the absolute effect of high levels of ANF on mineral bioavailability are dependent on the relative concentration of minerals in such foods, as well as the ANF: mineral molar rations of the food. The higher the molar ratio, the less available the mineral, with particular reference to zinc bioavailability, phytate: Zn molar ratio of 5.5 has been shown t=not to affect zinc bioavailability. Values of 9.1 and above can induce marginal Zn deficiency (Davies and Olpin, 1979; Greger and Snecker, 1980; Sandstrom, 1997).

With the exception of total oxalate, values of the ANF obtained are comparable to values reported for most Nigerian cereal-legume based foodstuff and diets (Mbofung et al, 1984; Atinmo, 1986; Badamosi et al, 1995).

The calculated molar ratios of some of the ANF were observed to be within the tolerable levels. Consequently, phytate and tannins may not affect the bioavailability of Ca, Mg, Zn, Fe. High values obtained for phytate: Fe and Oxalate: Ca may however impede absorption and utilization of Fe and Ca in the formulated diets but not in the control.

It has been recognized that simple processing techniques such as soaking, cooking, germination (sprouting) and fermentation can significantly reduce the content of ANF (Osagie, 1998). Soaking, cooking, roasting were some of the processes used in this study. This may partly be responsible for the low values of ANF observed, and the possible beneficial effect of increased bioavailability of some mineral elements.

Biochemical Indices

The state of intake of a nutrient can also be measured by assaying some biochemical indices such as the level of nutrient or its metabolic derivative in body fluids, and accessible storage tissues. Values significantly below the normal range are usually taken as suggestive of inadequate dietary intake.

In this study, some iron-dependent physiological parameters such as heamoglobin (Hb); packed cell volume (PCV) as well as serum iron and total iron binding capacity (TIBC) were used to assess iron intake, metabolism and deficiency. Although the calculated iron content in 65g of the control diet was found to be significantly higher than in all the modified diets, values obtained for Hb, PCV, serum iron and TIBC in rats revealed that there was no marked difference in physiological response to iron intake. For instance, Hb, PCV serum iron and TIBC in the control

serum (11.0g/100ml, 36.7%, 39.7µM and 88.0µM, respectively) and in serum of rats fed the formulated diet (10.3-10.7g/100m, 35.3-41.0%, 21.3-23.0µM, 74.8 - 81.7µM respectively) did not differ significantly. This suggests that the local diets would support normal iron metabolism. This could be partly due to the positive contribution of crayfish and the low level of phytate earlier observed in the local diets. However, continued breastfeeding would ensure adequacy, absorption and utilization of iron and other micronutrients.

Biochemical tests that relate to various functions of the liver and the assessment of hepatocellular injury were assessed in this study. The activities of the serum enzymes (transaminases, alkaline phosphatase and lactate dehydrogenase) in both the control and local formular fed rats were comparable. This suggests that the local diets did not cause impairment of functions due to nutritional stress or leaking of enzymes into the blood by hepatocelluar injuries. These observations attest to the safety of the local formulations and to support normal biochemical functions.

The measurement of individual plasma proteins provided definite information about changes that could arise due to nutritional, physiological and disease condition. The comparable values in albumin concentration reflect the transport function of the protein while globulin reflects immunoglobulin hence, good immune response. This suggests that there was at least normal synthesis and functional levels of the proteins. The absence of mortality in all the groups of rats could be as a result of the good immune response earlier speculated.

Values of plasma urea in rats fed with the modified diets obtained in this study were again comparable to the control. When results were extrapolated to humans, values obtained were within the paediatric reference range of 1.5-4.0 mM given by

Nduka (1999), supporting earlier observation that the modified diets are adequate in protein and amino acid content and would support normal liver and metabolic functions. Plasma urea is a useful test of renal excretory function because it correlates well with the clinical consequence of retained metabolic products in renal dysfunction.

Creatinine is derived from the turnover of creatine phosphate in muscle (Devlin, 1997). Serum creatinine was observed to be higher in rats fed the modified diets (38.0-46.7µM) than in the control group (28.3µM). This suggests that there were greater changes in the daily turnover of the total muscles mass in rats fed the modified diets which was not the case in the control group.

Total cholesterol and cholesterol: HDL cholesterol ratios in serum of rats fed the modified diets were lower than the control group this was expected because plant source foods are known to contain little or no cholesterol, and high in unsaturated fatty acids (Guthrie, 1989). Total plasma concentration of cholesterol and triglycerides can give a clear insight into hyperlipidaemia arising either from excessive dietary intake or genetic disorders. High dietary intake of saturated fat for instance has been shown to initiate LDL-Cholesterol synthesis by the liver. Values of triglyerides in both the control and modified groups were comparable all of which suggest that consumption of the local formulations would not pose any risk of hyperlipidemia or even be a cardio vascular risk factor.

Cost Implication

An estimated cost of the modified diets revealed that an average quantity of 450g would cost between N100-180, while the same quantity of Nestle Cerelac costs

N250. It therefore follows that locally formulated complementary foods can be prepared inexpensively. The home based formulation would also ensure availability at all times.

Conclusion

In conclusion, with proper selection and combination, it is possible to use local household foodstuff to formulate multimixes that can be used as home-based complementary foods. The blends formulated in this study are strongly recommended for used particularly by rural and poor urban mothers to feed their infants and children during the complementary feeding period. This will ensure availability and affordability as well as help in alleviating some economic and time-related constraints faced in child feeding practices.

Summary of findings

The research work has clearly demonstrated that:

(i) Nutritious, acceptable and affordable complementary foods can be formulated using our locally available or household food items, and which can be comparable to the conventional proprietary infant formulations.

(ii) In terms of health needs, the local formulations met in some aspects and in other instances surpassed the health needs met by the proprietary formula.

(iii) The formulated blends were found to be marginal in some micronutrients notably vitamin C, Fe, Zn, Ca. Fortification and or

supplementation may be necessary to meet the recommended dietary allowances.

(iv) The antinutritional factors assayed in the blends were within the tolerable levels, and may not therefore pose serious problem to nutrient availability and utilization.

(v) The formulated blends are obtained at prices 50 – 60% cheaper than the proprietary formula, hence are more cost effective.

Suggestions on Areas of Further Work

(i) Processing techniques other than soaking and roasting should be used to further reduce the levels of antinutrients such as oxalate.

(ii) Fortification and or supplementation of the formulated blends with nutrients that are marginal should be explored.

(iii) Digestibility and bioavailability studies of nutrients in the home-based formulations should be undertaken.

(iv) Effect of cooking on the nutrients and the shelf-life studies of the formulations should be undertaken.

(v) Studies should be expanded to other adjacent states to establish nutrient-dense foodstuff that are low in antinutritional factors, and which can be used to formulate complementary diets.

(vi) Information adduced from this study should be disseminated at scientific and community levels.

(vii) Comprehensive food composition tables and recommended nutrient intakes for all population groups in Nigeria should be established to facilitate meaningful nutrition studies and dietary recommendations.

Contribution to Knowledge

(i) This study has contributed to the understanding that same staple foodstuff cultivated in Plateau State have great nutrient potentials, and which can be used to formulate nutritious and more cost effective complementary foods as part of effort to improve child nutrition, and reduce morbidity and mortality rates.

(ii) The study has also led to the observation that the levels of some antinutritional factors may not affect the availability and utilization of nutrients in the formulated diets.

(iii) The qualitative and quantitative data from this study can be used to establish and or update food composition tables and dietary guidelines for achieving the Millennium Development Goals (MDGs) in child feeding.

REFERENCES

Abbey, B.W. and Mark – Balm, T. (1988) Weaning foods prepared from composite ungerminated and germinated cowpea. Nutri Report International. 38 (3): 519-528

Abbey, B.W. and Nkanga, U.B. (1988) Production of high quality weaning products from maize – cowpea – crayfish mixtures. Nutri Report International. 37 (5): 951-957.

ACC/SCN (1997) Third Report on the World Nutrition Situation. Geneva: ACC/SCN.

ACC/SCN (2000) Fourth Report on the World Nutrition Situation: Nutrition throughout life cycle. January 2000 pp 33-41.

Affi, A.T. (2000) Preventive Nutrition: A practical handbook. Malthouse Press Limited Lagos.

AHRTAG (1993) Dialogue on Diarrhoea. The InternationalNewsletter on the control of diarrhoeal disease. Issue No. 53.

Akinyele, I.O. and Omotola, B.D. (1986) Energy and Protein Intake of infants and children from the low income group of Ibadan. Nutrition Research 6:129-137.

Allain, C.C; Poon. L.S; Chan, C.S.G; Richmond, W. and Fu, P.C. (1974) Quantitative determination of Serum Cholesterol. Clin. Chem. 20:4470.

AOAC (1975) Official methods of Analysis. 12th edn. Association of Official Analytical Chemists. Washington DC.

AOAC (1980) Official methods of Analysis 13th edn. Association of Official Analytical Chemists. Washington DC.

AOAC (1990) Official methods of Analysis. 15th edn. Association of Official Analytical Chemists. Washington DC.

Atinmo, T. (1986) The cultural significance of food and the nutritional values of Nigerian foods with respect to trace element nutriture. A seminar paper on Nigerian food culture. University of Ibadan, Nov. 1986.

Armar-Klemesu, M.A. and Wheeler E.F. (1991) Weaning practices and their outcome; a critical look with reference to Ghana. Bulletin of Noguchi Memorial Institute for Medical Research 4:3-2

Bahijri, S.M. (2001) Effects of diet and Diarrhea in relation to Growth. Annals of Saudi Medicine 21 (5-6): 323-328.

Badamosi, E.J; Ibrahim, L.M. and Temple, V.J. (1995) Nutritional Evaluation of a locally formulated weaning food, JUTH-PAP. West Afri. J. Biol. Sci 3:85-93.

Bloem, M. and Darnton – Hill, T. (1999)Micronutrient Deficiencies: The First Link in a Chain of Nutritional and Health events in economic crisis. HKI, Jakarta (draft)

Blumnberg, J. (1995) Role of Vitamins in Health Promotion and the prevention of non communicable diseases (Background paper prepared for the joint WHO/FAO Consultation on the preparation and use of Food-based Dietary Guidelines, Nicosia, Cyprus. March, 1995.

Briend, A., Lacsala, R and Prudhon, C. (1999) Ready-to-use therapeutic food for treatment of marasmus (letter). Lancet 353:1767-168.

Calloway, D.H. (1995) Human Nutrition: Food and micronutrient relationships. Working paper on Agricultural Strategies for Micronutrients. Internal food policy. Res. Inst. Washington DC pp. 23.

Campen, V.R.D. (1996) Role of food and meal composition in determining bioavailabilities of micronutrients In: food-based Approaches to preventing micronutrient malnutrition workshop. Salt Lake City, USA Nov. 1995.

Carr, F.H. and Price, E.A. (1926) Photometric assay of Vitamin A. Biochem. J. 20:497.

Carvil, J; Jacob, A. and Worwood, M. (1986) "Dignostic methods for Iron Status" Ann. Clin. Biochem. 23:168-171.

Cook, J.D. (1982) Assessing Iron status of a population. Am. J. Clin. Nutr.32:2115

Cook, J.D. and Skikne, B.S. (1989) Iron Fortification: An Update. Am. J. Clin. Nutr 38:648-659.

Chibuzo, E.C. (2003) Update on Zinc deficiency control and zinc nutrition in Nigeria. A paper presented at the meeting of the National Advisory Committee on Micronutrient Deficiency control Abuja. 27th March, 2003.

Davidson, L; Galan, P. and Kaolenmayer, P. (1994) Iron Absorption in infants: The influence of phytic acid and ascorbic acid in formulas based on soy isolate. Paediatr. Res. 36:816-822.

Devlin, M.T. (1997) Textbook of Biochemistry with Clinical Correlations. 4th ed. Wiley – Liss Inc. New York/Chichester/Toronto pp 540-548.

Doumas, B.I. and Biggs, H.G. (1972) Determination of Serum albumin. In: G.A. Cooper (ed). Standard methods of Clinical Chemistry. Academic press Inc. New York. Pp 175.

Draper, A. (1997) Child development and iron deficiency. Early action is critical for healthy mental, physical and social development. INACG.

Eka, O.U. and Edijala, J.K. (1972) Chemical composition of some traditionally prepared Nigerian foods. Nig. J. Sci 6:157-62.

Esders, T.W. and Michrina, C.A. (1979) Triglyceride assay J. Biol. Chem. 254:2710.

Fashakin, J.B. and Ogunshola,F. (1982) The Utilization of local Foods in Fermentation of weaning foods. Trop. Paed. 28:93-6

Federal Ministry of Health (FMOH) (1999). Food-based Dietary Guidelines for Nigeria. Nutrition Division, Abuja.

Fernandez, D.P; Vanderjagt, D.J; Williams, M; Huang, Y.S; Chuang, L; Millson, M; Andrews, R; Pastuszyn, A. and Glew, R.H. (2002), Fatty acid, amino acid and trace mineral analyses of five weaning foods from Jos, Nigeria. Plant Foods for Human Nutrition 57:257-274.

Ferruccio, C. and Giovanni, C. (1980) Improved Direct specific determination of serum iron and total iron-binding capacity. Clin. Chem. 26:327-331

FAO (1970) Amino Acid content of foods and Biological Data on Proteins. FAO Nutritional studies 24, FAO, Rome.

FAO (1997) Preventing Micronutrient malnutrition: A guide to food-based approaches. A manual for policy makers and programme planners. Food and Agricultural Organization of the United Nations and International Life Science Institute. Washington DC. Pp. 2-6.

FAO/WHO (1998) Preparation and use of Food-Based Dietary Guidelines. A report of a Joint FAO/WHO Consultation. WHO Technical Report Series 880 Geneva.

Friedman, M. (1985) Nutritional and Toxicological aspects of food safety. Plenum Press, London. Pp. 112.

Gibney, M. (1995) Consumption of sugars. Workshop on the Evaluation of the Nutritional and Health Aspects of Sugar. Am J. Clin Nutr. 62: (suppl.) 1985-1945.

Gillespie, S. and Johnson, J.L. (1998) Expert consultation on Anaemia. Determinants and Interventions. Micronutrient Initiative: Ottawa.

Goldez, A. and Vallee, B.L. (1983) Metal ions and Biological Systems. John Wiley Inc. New, York pp 112-118.

Green, J.H. and Ezeilo, G.C. (1978) An Introduction to human physiology. Fourth Edn. Oxford University press. Ibadan, Nairobi. Pp7-17.

Greger, J. L. and Snecker, S. M. (1980) Effect of dietary protein and phosphorus levels in utilization of zinc, copper and manganese by adult males. J. Nutr. 110:2243-2253.

Gurney, M., Jelliffe, D.B. and Harland, E. (1985) Protein-Energy malnutrition In: D.B. Jelliffe (ed). Child Health in the Tropics. 5th edn. English Language Book Society/Edward Arnold Publishers. London. Pp. 65-82.

Guthrie, A.H. (1989) Introductory Nutrition. 7th edn. Times Mirror/Mosby College Publishing. Pp. 485-576.

Harrison, G.G (1996) Potential for changing food consumption patterns. In: G.F.Jr. Combs, R.M. Welch, J.M. Duxbury, N.T. Uphoff, M.C. Nesheim (eds). Food –Base Approaches to preventing Micronutrient Malnutrition: An International Research Agenda. Cornell University Ithaca NY. Pp 102-108.

Hultman, E. (1959) Nature: In: J. Richard, C.C. Donald, W.J. Winkelman eds. Clinical Chemistry, Principles and techniques. 2nd edn. Harper and Row Publishers, New York, Evanston, San Francisco. London pp. 215-314.

IAEA (2003) Improving Nutrition through Nuclear Science. International Atomic Energy Agency, Bulletin Austria, June 2003.

ICN (1992) Caring for the socio-financially Deprived and Nutritionally Vulnerable. International Conference on Nutrition. Theme paper No. 3 FAO/WHO, Rome.

Islam, M.M; Peerson, M.J; Ahmed, T; Dewey, G.K and Brown, H.K. (2004)Effect of Energy Density of Complementary Foods on total Energy intakes. Centre for Health and Population Research Publication Dhaka, Bangladesh.

IITA (2004) Summary proceedings of the stakeholder meeting for the project on Micronutrient Enrichment of Maize and Plantain in Nigeria: A

sustainable Approach to Mitigate Hidden Hunger. International Institute for Tropical Agriculture 7-8th September, 2004.

Jacob, A.B. (1987) Trace Elements: In W.N. Tietz (ed). Fundamentals of clinical Chemistry. W.B. Saunders Company, New York. Pp 517-531

Jonsyn, F.E. (1985) Fungi associated with selected fermented foodstuffs in Sierra-Leone. In: IFS/UNU Workshop book of proceeding. Development of indigenous fermented food and food technology in Africa. Duala, October, 1985.

Kelly, M. G. and Onyeka, J. O. A. (1992) Introduction to Statistics and Experimental Design for the Life Sciences. ABC Publishers, Enugu. Pp. 44-85.

Kennedy, E., Mannar, V. and Iyengar, V. (2003) Alleviating Hidden Hunger: Approaches that work. IAEA Bulletin No. 45/1, June 2003.

Ketiku A.O. and Olusanya, J.O (1987). The Nutritient composition of traditional weaning foods in Nigeria. Paper presented ad the 1st National Workshop on food Composition. University of Ibadan, 31st March-5th April 1987.

Key, T. (1994) Micronutrients and Cancer aetiology: the epidemiological evidence. Proceedings of the nutrition society. 53:605-6114.

Ladeji, O; Temple, V.J and Solomon, M. (2000) Chemical evaluation of the nutritional potential of two locally formulated Complementary foods. West Afr. J. Biol. Sci: 25-32

La Roche, F. (1990) Vitamins and Carotenoids in Pasta.Basel Switzerland.

Latham, M. and Preble, E. (2000) Appropriate Feeding Methods for infants of HIV-Infected mothers in sub-Saharan Africa. British Medical Journal . 320:1656-1660.

Lewis, B. (1987) "Plasma Lipid concentration: The concept of normality and its implications for detection of High Risk". J.Clin. Pathol. 40:1118-1127.

Lopes – Virella, M.F. (1977) HD2-Cholesterol precipitant. Clin. Chem. 23:883.

Lorri, W. (1996) Potential for Developing new Home-Based Food processing and preparation techniques to improve Micronutrient Nutrition. In: G.F.Jr. Cambs, R.M. Welch, J.M. Duxbury, N.t. Uphoff and M.C. Nesheim (eds) Food-Based Approaches to preventing micronutrient malnutrition: An International Research Agenda. Cornell University Ithaca, NY.

Lutter, K.C. and Rivera, A.J (2003) Nutritional Status of infant and young children and characteristic of their diets. J. Nutr. 133:2941S-2949S.

Malloy, H.T. and Evelyn, K.A. (1937) Determination of direct and total bilirubin by the Diazo reaction. J.Biol. Chem. 119: 481-484

Markham, R. (1942) Protein analysis in Foods. Biochem. J. 36:790-800.

Mbofung, C. M. F., Atinmo, T. and Omololu A. (1984) Zinc and phyltate concentrations, phytate-zinc molar ratio and metallocaloric ratio of zinc and protein contents of some selected Nigerian dietary foods. Nutr. Res. 4:567-576.

Mejia, L.A. andArroyave, G. (1988) The effect of Vitamin fortification of sugar on Iron metabolism in Pre-School Children in Guatemala. Am. J. of Clin. Nutr. 3:87-93.

Mertz, W. (1980) The Essential Trace Elements. Science. 213:1332.

Mlingi, N.V.L. (1988) Reducing dietary bulk in cassava-based weaning foods by fermentation. In: proceedings of a Workshop on Improving Child Feeding in Eastern and Southern Africa: Household Level Food Technology 1987, Nairobi, Kenya.

Mohanram, M; Kulkarni, K.A. and Peddy, V. (1977) Haematological studies in vitamin A Deficient Children. Int. J.Vit. Res. 47:389-393.

Marcos, S. R; Siad, A.K; Gabriel, G.N and Hady, N.E.A (1983) Supplementary and Weaning Foods for the Egyptian Child. In: A.F Walker (ed) The contribution of weaning foods to Protein-energy malnutrion Nutr. Res. Review 3(37): 199.

Naismith, D.J (1973) Kwashiorkor in Western Nigeria: a Study of Traditional Weaning Foods with particular respect to energy and linoleic acid. Br. J. Nutri. 80:567-76.

National Advisory Committee on Micronutrient Deficiency (NACMD) (2003). Papers Distributed/Presented at the meeting of the NACMD. 27-28 March, 2003 Abuja.

National Institute of Health (NIH) (2002) Facts about Dietary supplements. Clinical Nutrition services, warren Grant Magnuson Clinical center. Office of Dietary supplement, National Institute of Health.

NPAN (2002) National Plan of Action on Food and Nutrition (NPAN) in Nigeria. National Planning Commission, Abuja.

Nduka, N. (1999) Clinical Biochemistry for students of Pathology. Longman Nigeria PLC pp 57-73.

Nestle, P. (1993) Nestle cenelac Infant Milk cereal. Information for the medical Profession only printed in Nigeria for nestle foods Nigeria PLC.

Nigeria Nutrition Network (2000) Communiqués and Papers presented/distributed at the 1st Annual Nigeria Nutrition Network (NNN) meeting. 12-13 Dec. 2002, Abeokuta.

Nnam, N.M (2002) Evaluation of complementary foods based on maize, groundnut, paw paw and mango flour blends. Nig J. Nutri. Sci 23 (1&2). 8-18

Nutriview (2001/2) A quarterly Newsletter on the role of Micronutrients in Nutrition and Health. Roche Vitamins, Europe Ltd. Bassel, Switzerland.

Oginni, L.M; Worsfold, M; Oyelami, O.A; Sharp, C.A; Powell, D.E. and Davie M.W.J. (1996) Etiology of Rickets in Nigerian children. J. Pediatr. 128:692-694.

Okoh, P.N. (1998) Cereal grains. In: A.U. Osagie and O.U Eka (eds). Nutritional Quality of plant foods. Post harvest Research unit. Unversity of Benin, Benin City PP 32-52.

Okonofua, E. (2002) Calcium and Vit.D nutrition in Nigerian women and Children. A paper presented at the Workshop on Nutritional Rickets in Nigerian Children: The Way Forward. 13-15 March 2002, Abuja.

Okoye Z.S.C (1992) Biochemical Aspects of Nutrition Prentice-Hall of India, New Delhi pp 147-195

Okoye (2003) Iodine Deficiency Disorders. Paper Distributed/presented at the meeting ofthe National Advisory Committee on Micronutrient Deficiencies 27-28 March, 2003 Abuja.

Okungbowa, P. (1986) Nigerian weaning diets for use by community health workers and mothers. Federal Nutrition Division. FMOH, Lagos.

Osagie, A. U. (1998) Antinutrional Factors. In: A. U. Osagie and O. U. Eka (eds). Nutritional Quality of Plant Foods. Post Harvest Research Unit. University of Benin, Benin City. Pp. 221-244.

Osagie, A.U and Eka, O.U. (1998) Nutritional quality of Plant Foods. Post Harvest Research Unit. University of Benin, Benin City Nigeria.

Omoruyi, F, Osagie, U.A and Adamson, I, (1994) Blood Proteins and Tissue enzymes in Malnourished rats rehabilitated with corn-crayfish-protein diet. Bioscience Research Communications 6:1-8.

Onofiok, N.O and Nnanyelugo, D.O (1998) Weaning foods in West African. Food and Nutrition Bulletin. The UN University.

Owolabi, A.O; Mac-Inegite, J.O; Olowoniyan, F.O. and Chindo, H.O (1996). A Comparative Study of the Nutritional Status of Children in Villages in Northern Nigeria using and not using Soya beans. Food Nutr. Bull. 17:42-48.

Oyeleke, A.O (1984) Outlines of food analysis. Department of Biochemistry. Ahamadu Bello University, Zaria.

Oyeleke, A.O Morton I.D and Pender, A.E (1985) The use of cowpea (Vigna unguiculata) in improving a popular Nigeria weaning food. Brit. J Nutr. 54:343.

Pearson, D. (1973) Laboratory techniques in food analysis. Butterworths, London

Pennington, J.A.T., Wilson, D.B, Newell, R.F Harland, B.F, Johnson, R.D and Vanderveen, J.E. (1988) Selected Minerals in food survey. J. Am-Diet Assoc. 84:771-780.

Peter, J.W. (1979) An Introduction to Atomic Absoption Spectrophotometry. Pye Unicam Ltd, Cambrige, England.

Picciano, M.F(1987) Nutrient needs of infants. Nutrition Today 22:8-10.

Plummer, D.T. (1978) An introduction to Practical Biochemistry 2nd edn. Mc Graw-Hill Book co.Uk. pp 144.

Prasad, S.A. (1982) Clinical, Biochemical and Nutritional Aspects of Trace Elements. Alan-Liss Inc. New York. Pp112-118.

Price, W.J (1972) Analytical Atomic Absorption Spectrometry. Heyden and son limited, England pp. 118.

Ramsey, W. N.M (1958) Determination of the Total Iron binding capacity of serum. Adv. Clin Chem 1:1.

Reitman, S. and Frankel, 5 (1957) Colorimentric method for the determination of serum transaminases. Am J. Clin. Path 28:56-61.

Roe, J.H and Kuether, C.A (1943) Photometric assay of Vitamin C. J. Biol. Chem 147:399.

Ross, A.C (1999) Vitamin A and retinoids. In: M.Shils (ed). Nutrition in Health and diseases 9th edn. Baltimore. Williams and Wilkins.

Sandstead H.H (1985) Zinc: Essentially for Brain development and functions. Nutrition Reviews 43:129-137 343:1595-1597

Sandstorm, B. (1997) Bioavailability of Zinc. Eur. J. Clin. Nutr. 51:S17-S19.

Schneeman, B.C and Tieiven, I. (1994) Dietary Fibre. In: Modern Nutrition in Health and Disease (Shils, M.E; Ohlson, I.A Shike, eds) 8th ed. Philadelphia, Lea Febiger. 89-100

Semba, R.D; Miotti, P.G and Chiphangwi, J.D. (1994) Maternal Vitamin A Deficiency and mother-to-child transmissionof HIV-1. The Lancet. 343:1593-1597.

Shrimpton, R. (1993) Zinc Deficiency – Is it widespread but under-recognize? SCN news 9:24-27

Solomon, N.W. (2000) plant-based diets are traditional in developing countries. 21st century challenges for better nutrition and health. Asia Pac. J Clin. Nutri (Suppl) 541-5-54

Sommer, A. and West, K.P (1996) Vitamin A deficiency: Health, Survival and vision. Oxford University Press NY.

Sparkman, D.H, Stein, E.H and Moore, S (1958) Automatic recording apparatus for use in the chromatography of amino acids Analytical Chemistry 30:1190-1991.

Steinberg, D. (1991) Antioxidants and atherosclerosis A current assessment. Circulation 84: 1420-1425.

Stroebecker, R. and Henning M.H (1965) Vitamin Assay-Tested Methods. Verlag Chemic GMBH Welnheim/ Bergstr.

SCN (1995) Behavioural changes and nutrition Programmes. Standing Committee on Nutrition (SCN) News # 13: Geneva.

SCN (2004) Nutrition for Improved Development Outcomes. 5th Report on the World Nutrition Situation Standing Committee on Nutrition (SCN). Geneva

Suharno, D.M. (1992) Vitamin A and Nutritional Anaemia. Food and Nutrition Bulletin 17:7-10.

Temple, V.J; Badamosi, E.J; Ladeji, O and Solomon, M. (1996) Proximate chemical composition of three locally formulated complementary foods. West Afr. J. Biol Sci. 5:134-143.

Thacher, T.D; Fischer, P.R; Pettifor, J.M; Lawson, J.O; Isichei, C.O and Chan, G.M. (2000) Case-Control Study of Factors Associated with Nutritional Rickets in Nigerian children. J Pediatr. 137:367-373.

Ubom, G.A (1991). The goiter-soil-water-diet relationship: case study of plateau State, Nigeria. The Science of total Environment. 107:1-11

Ubom, G.A and Tsuchiya, Y. (1988). Determination of Iodine in natural water by Ion Chromatography. Wat. Res. 22 (11); 1455-1458.

UNAIDS/WHO/UNICEF (1997) HIV and Infant feeding: A policy statement developed collaboratively by UNAIDS WHO and UNICEF. UNAIDS: Geneva.

UNICEF (1997) The care initiative: Assessment Analysis and Action to improve care for Nutrition. New York : UNICEF

UNICEF (1998) The state of the word's children. New York: UNICEF

UNICEF (2000) State of the World's children. New York: UNICEF

UNICEF (2004) Eliminating Micronurient Deficiencies: Expert Opinion. New York: UNICEF.

UNICEF/UNU/WHO/MI (1999) Preventing Iron Deficiency in women and children. Technical consensus on key issues. Technical workshop, October 7-9, 1998. Boston and Ohawa. International Nutrition and MI.

Uphoff, N T. (1993) Monitoring and evaluating population participation in world Bank –assisted project. In B. Bhatangar and A. Williams (eds) Participatory Development and the World Bank: Potential directions for change. World bank Disc. Paper No 183. World Bank, Washington DC.

Vans, S., Kusin J.A, Voorhoeve, A.M and James A.A.J (1978) Agents affecting health of Mothers and child in rural area of Kenya : Food intake, feeding habits and nutritional state of the Akamba infant and toddler. Tropical and Geographical Medicine 30:505-522.

Varley, H., Gowenlock H.A and Bell M. (1976) Practical clinical Biochemistry (Vol 1). William Heinemann Medical Books Ltd. London Pp 35-37.

Vassault A., Wahlefeld, A. W and Deneke U. (1983) Lactate Dehydrogenase In: H.U Bergmeyer, J.Bergmeyer and M.Grassl (eds) Methods of Enzymatic Analysis Vol 3, 3rd edn. Weinheim, Verlag-Chemie pp 118-138

Walker A.F (1990) The contribution of Weaning foods to Protein energy Malnutrition. Nutr. Res. Rev 3:25-47

WHO (1981) International Code of Marketing of Breastmilk Substitutes, 1981. World Health Organization. Geneva.

WHO (1997) Global Database on Child Growth and Malnutrition Geneva: WHO

WHO (1998) Complementary Feeding of Young Children in Developing Countries: A Review of Current Scientific Knowledge. WHO/NUT/98.1 1998 World Health Organization. Geneva.

WHO (2000) Working group on the growth reference protocol and WHO task force on methods for the natural regulation of fertility growth patterns of breastfed infants in seven countries. Acta Paediatric 89:215-222

WHO (2001a) New Data on the Prevention of Mother –to-Child Transmission of HIV and their policy implications. Conclusion and Recommendations. WHO Technical Consultations on behalf of the UNINFP/UNICEF/WHO/UNAIDS. WHO: Geneva

WHO (2001b). Infant and Young Child Feeding. World Health Assembly 2001. Document 54.2 world Health Organization. Geneva.

WHO /UNICEF, (1995) Global Prevalence of Vitamin A Deficiency: Micronutrient Deficiency Information System. Working Paper 2 Geneva: WHO

WHO/UNICEF (1997) Integrated Management of Childhood Illness: a training course for first-level health workers. WHO/CHD/97.3 A-L. Geneva: WHO.

Williams, B.L and Wilson, K. (1979). Principles and Techniques of Practical Biochemistry pp. 153-158 Publishers Edward Arnold. New York.

Wilson, K and Walker, P (1995). Practical Biochemistry-Principles and Techniques. Pp. 375-377. Publishers- Cambridge. University Press, England.

Wirakartakusumah, A.M and Hariyadi, P. (1998) Technical Aspects of food fortification. Food and Nutrition Bulletin 9(2): 101-108

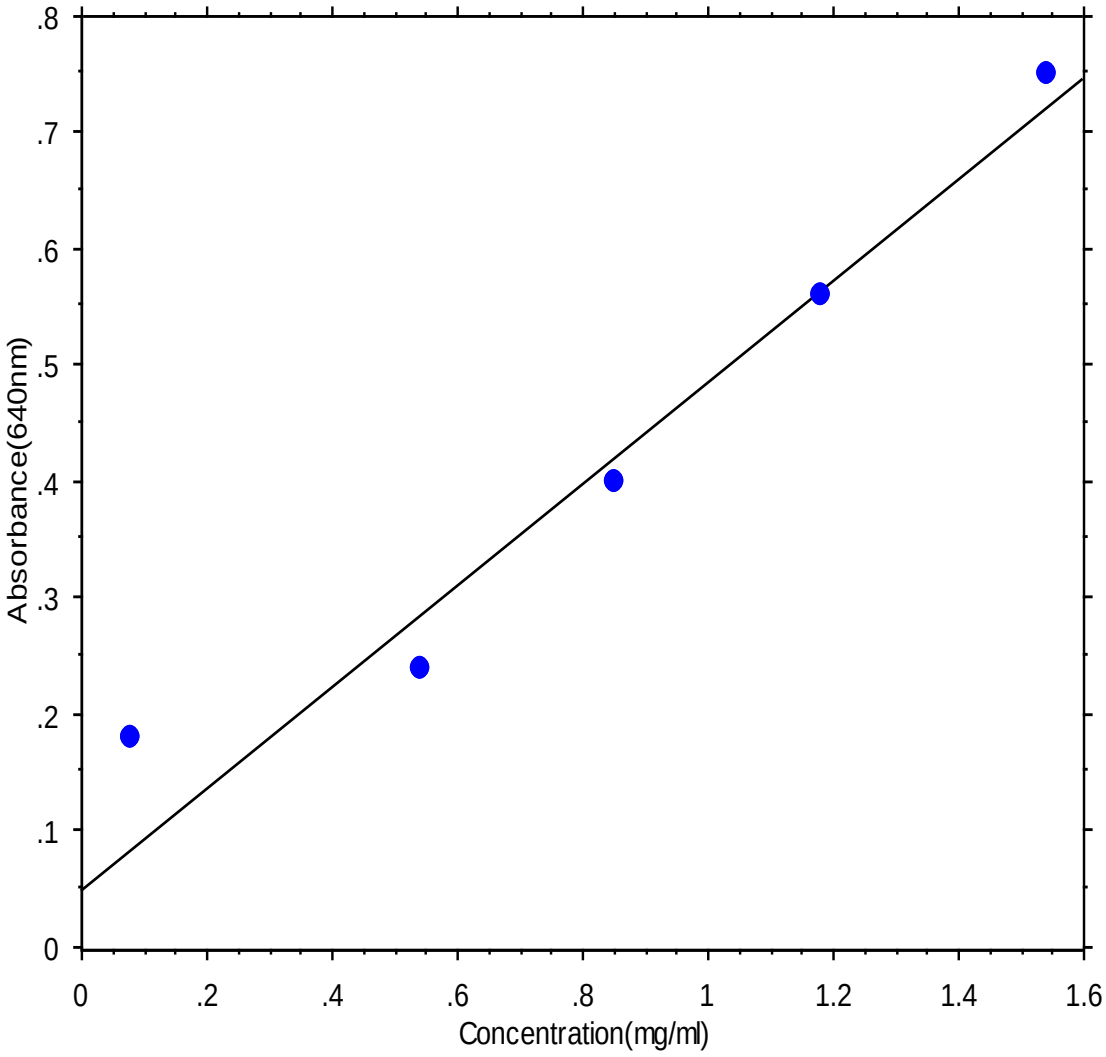
Wright, P.J Leathhood, P.D and Plummer, D. T. (1972) Enzymes in Rat urine: Alkaline Phosphatase. Enzymol 42:317-327

Wyberg, D.R; Digiorgio, J and Pileggi, V.J (1971) Direct Manual determination of Urea Nitrogen. Clin Chem. 17:891.

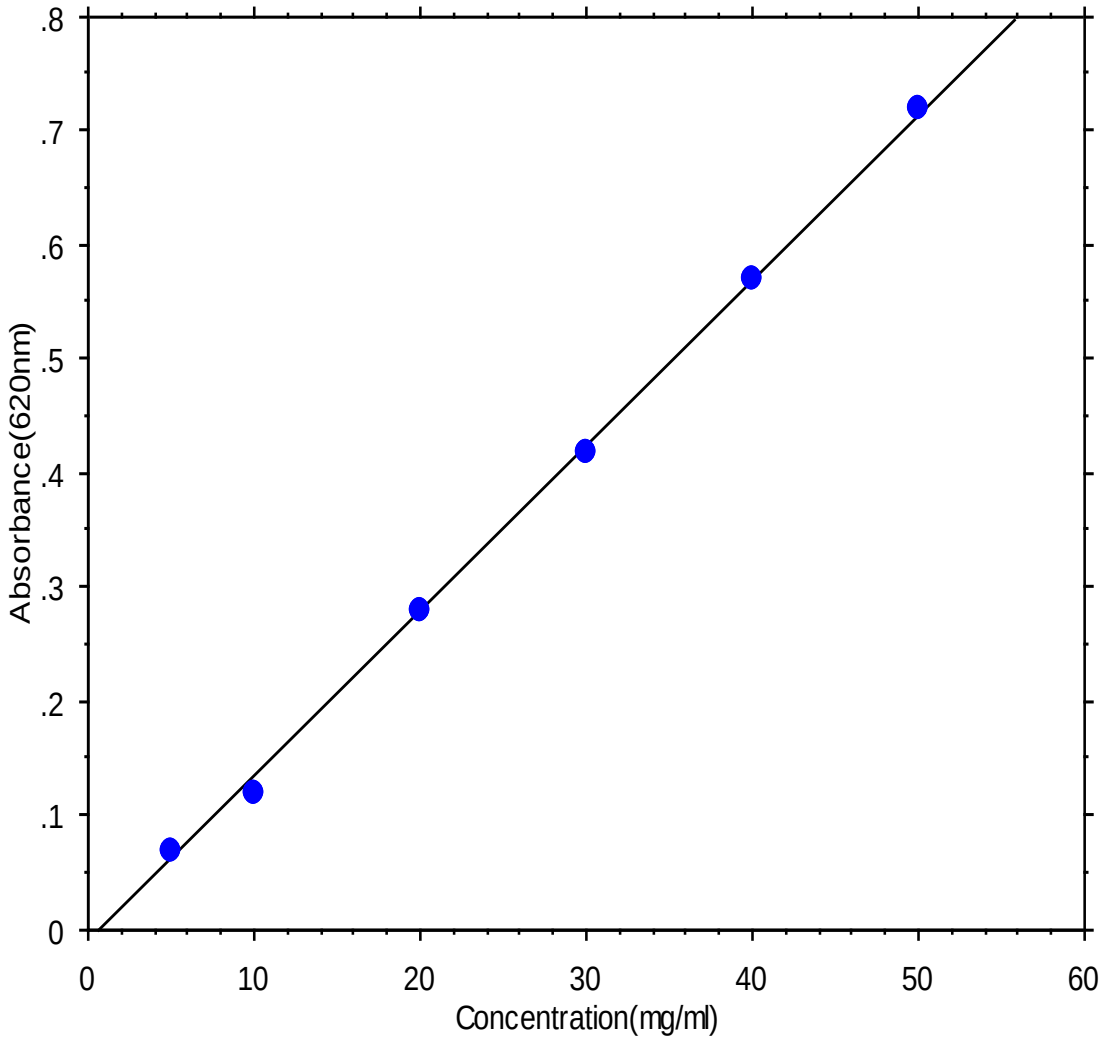
Yeung, D.L (1998) Iron and Micronutrient: Complementary food fortification. Food and Nutrition Bulletin 9(2): 159-163

Zubay, G. (1993) Biochemistry. 3rd ed. EMC Brown Publishers. Dubuque. Iowa. Melbourn. Australia. Oxford English PP. 223-229.

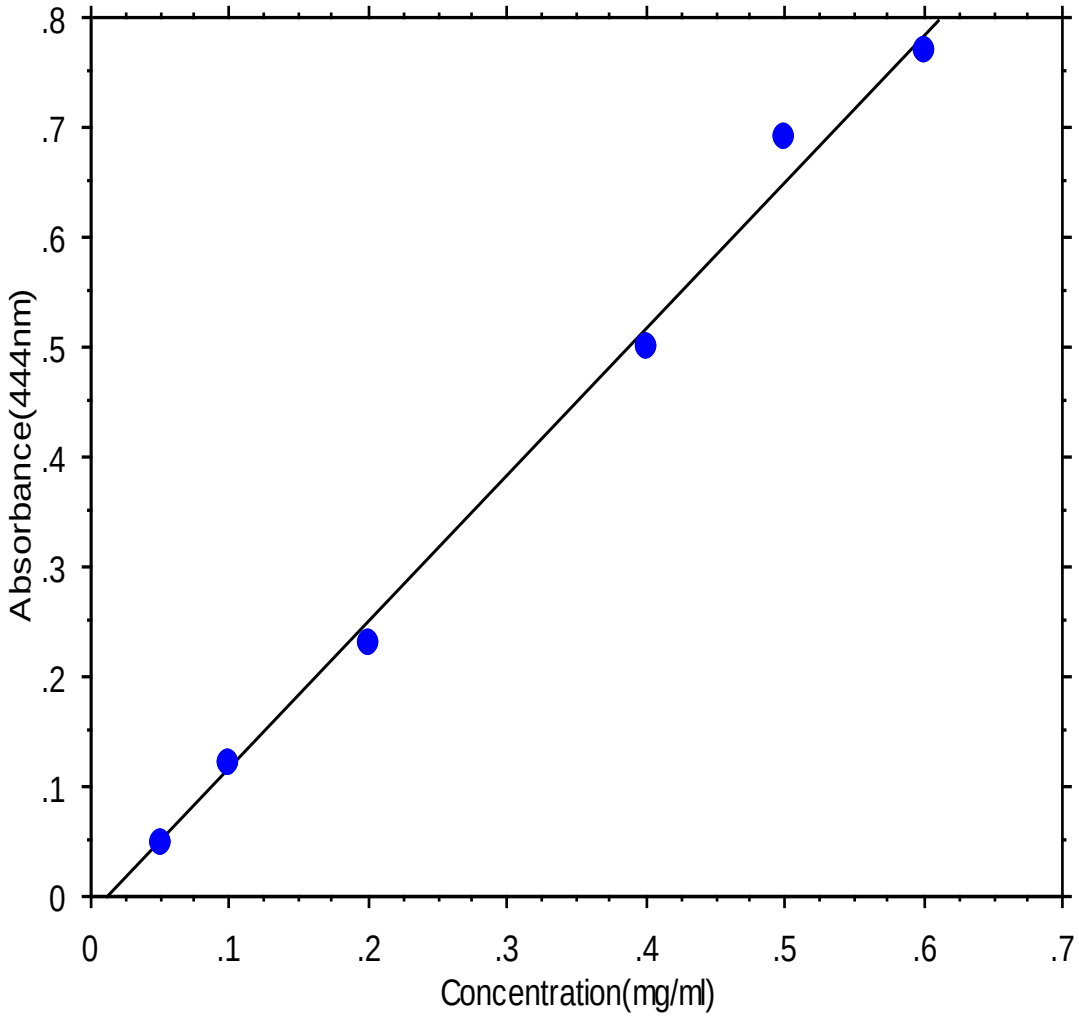
APPENDICES



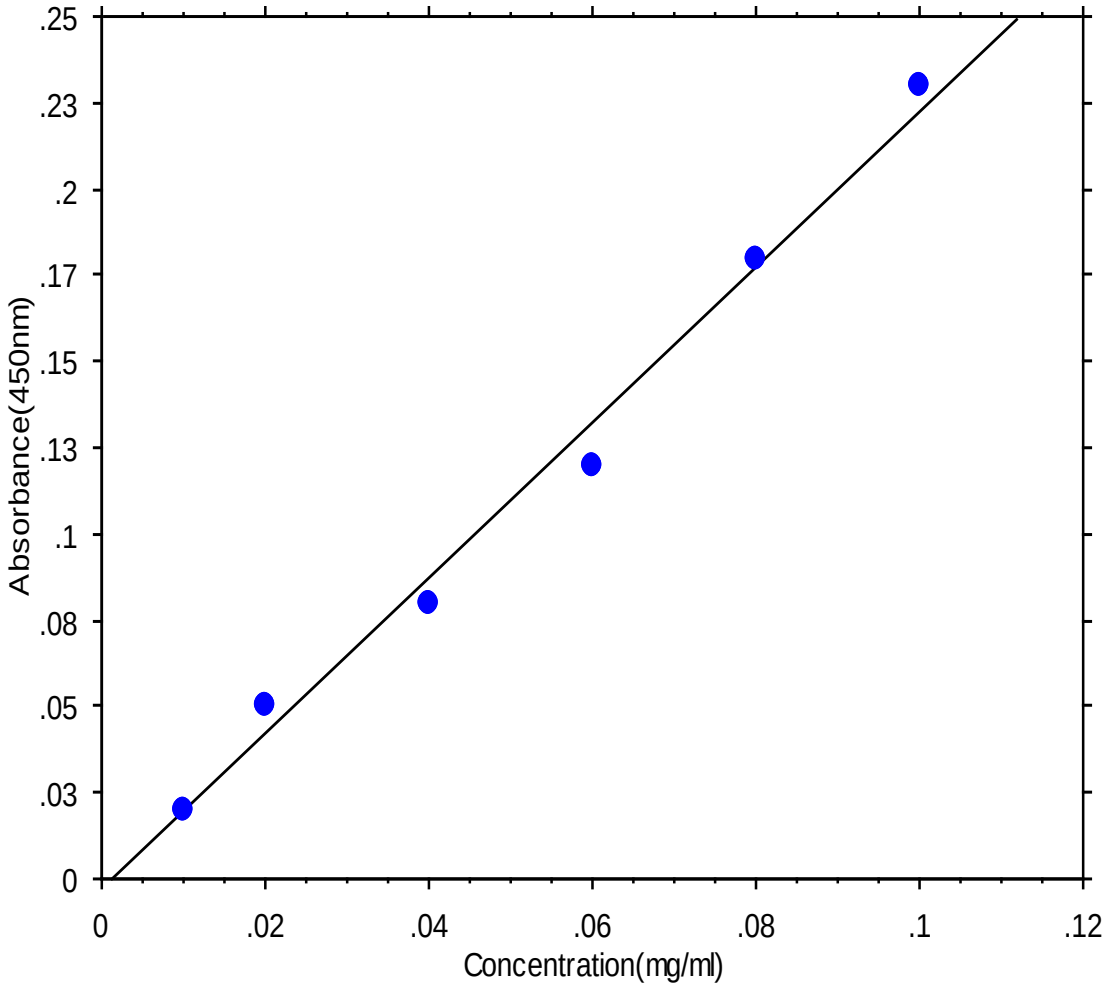
Appendix 1: Standard Curve for Phytate Determination



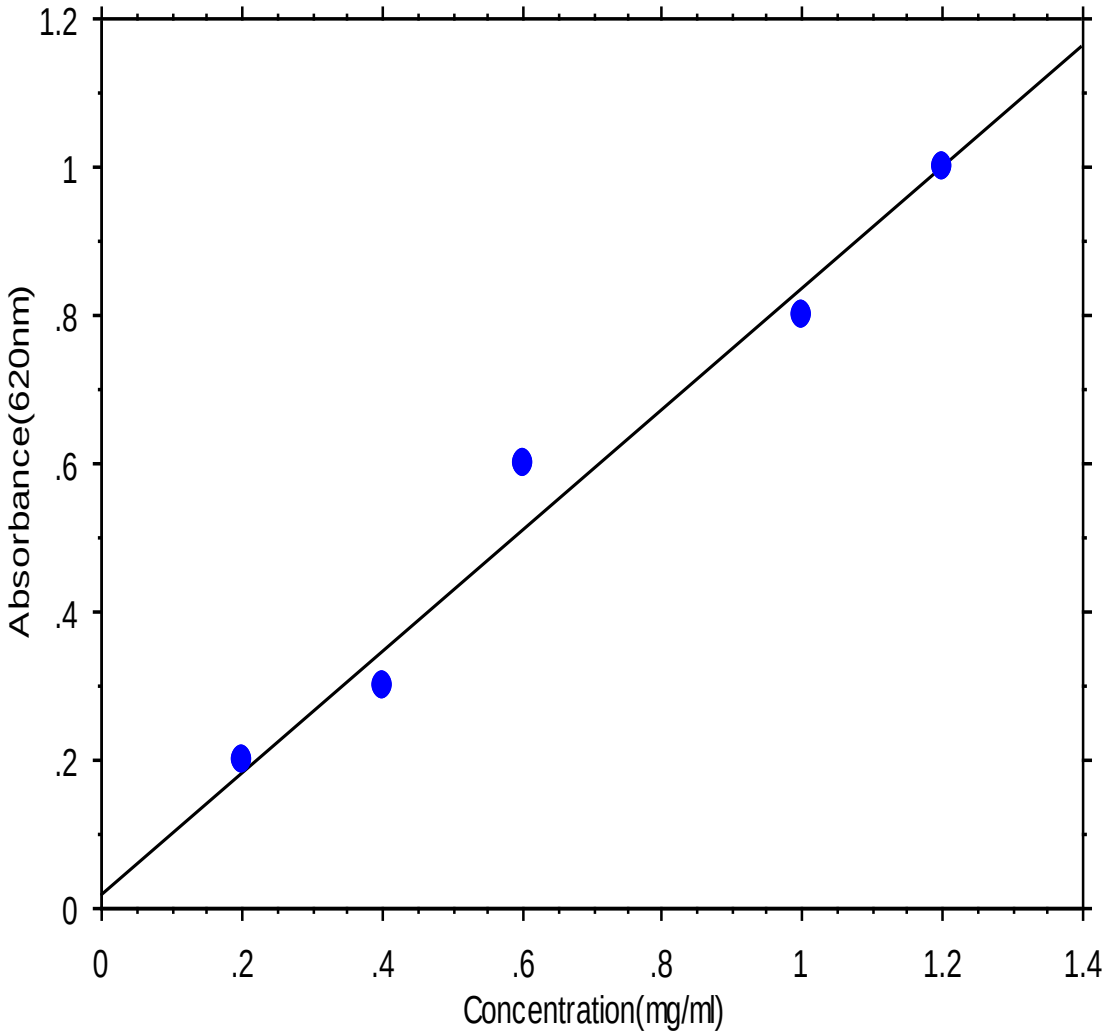
Appendix 2: Standard Curve for Vitamin A Determination



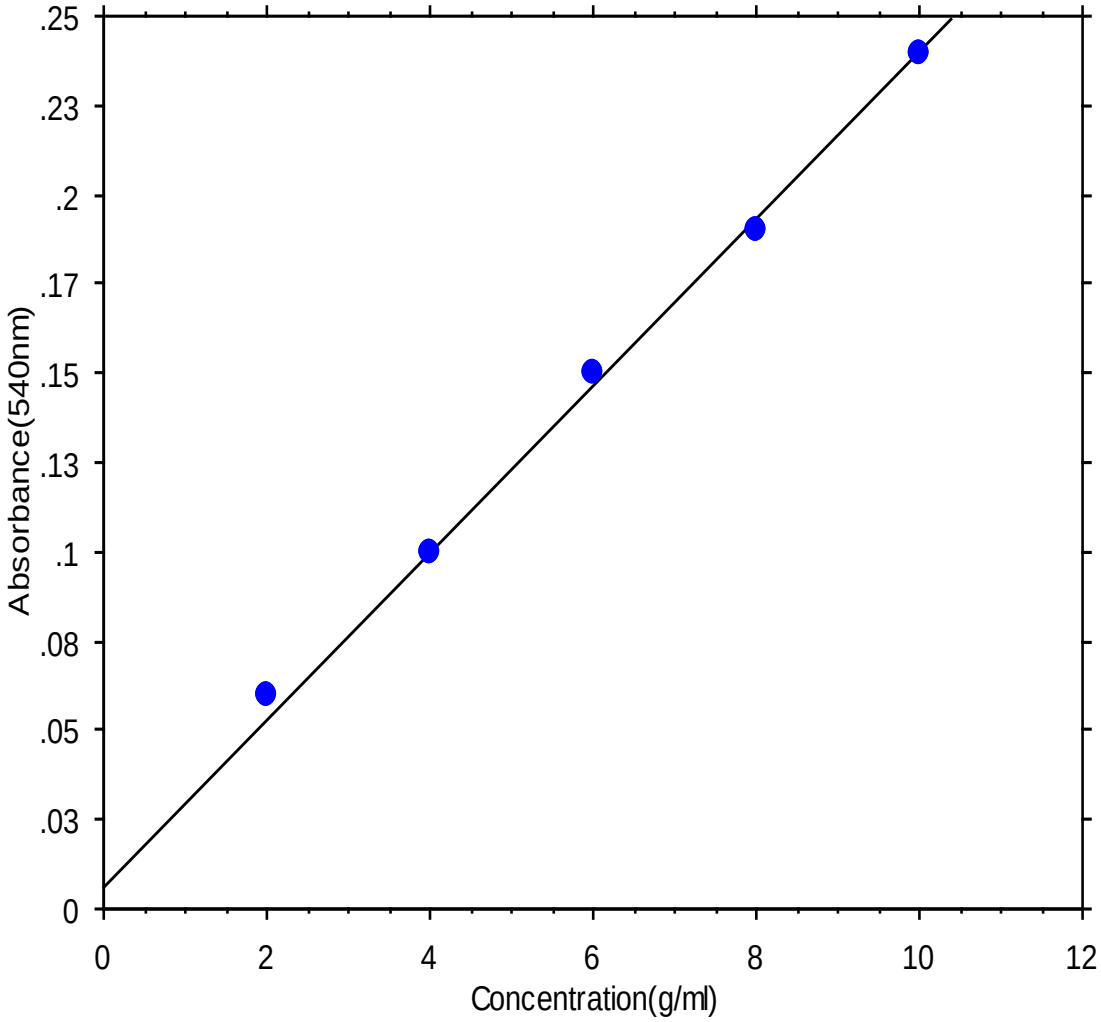
Appendix 3: Standard Curve for the Determination of Riboflavin



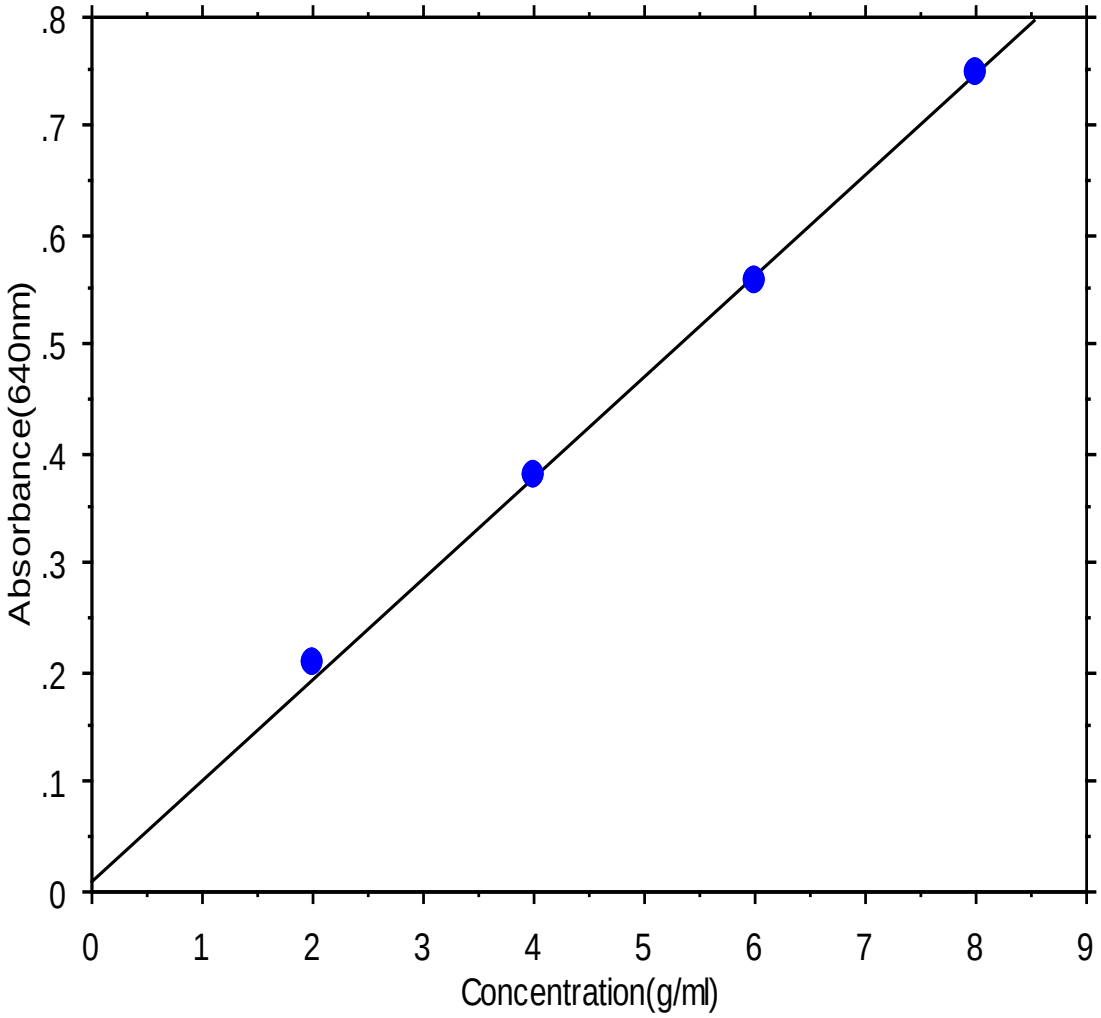
Appendix 4: Standard Curve for Pyridoxine Determination



Appendix 5: Standard Curve for Glucose Determination



Appendix 6: Standard Curve for Total Protein Determination



Appendix 7: Standard Curve for Albumin Determination