EFFECTS OF *CITRULLUS LANATUS* SEED (EGUSI) PROTEIN ISOLATE ON LIPID PEROXIDATION IN MALNOURISHED RATS FED HIGH FAT DIET.

OGUNYINKA BOLAJOKO IDIAT

201001567

February 2012

EFFECTS OF *CITRULLUS LANATUS* SEED (EGUSI) PROTEIN ISOLATE ON LIPID PEROXIDATION IN MALNOURISHED RATS FED HIGH FAT DIET.

OGUNYINKA BOLAJOKO IDIAT

201001567

Dissertation submitted to the Departments of Biochemistry and Microbiology, Faculty of Science and Agriculture, University of Zululand in Partial Fulfillment of requirements for the Masters (MSc) degree in Biochemistry.

Supervisor: Prof. A.R. Opoku

February 2012

Dedication

To my Family

Declaration

This is to certify that the work reported in the dissertation entitled "Effects of *Citrullus lanatus* seed (Egusi) protein isolate on lipid peroxidation in malnourished rat fed on high fat diets" is the original work of Mrs. Bolajoko Idiat Ogunyinka, carried out under the supervision and direction of Prof AR Opoku. The dissertation has been submitted to fulfill the requirements for the degree Master of Science (MSc) with the approval of the undersigned.

I, B. I. Ogunyinka, declared that the dissertation has not previously been submitted by me for a degree at this or any other university, that this is my own work in design and in execution and that all the material contained therein had been duly acknowledged.

B. I. Ogunyinka

Prof A. R. Opoku

ACKNOWLEDGEMENTS

My first thanks and profound gratitude go to Almighty God who through His grace and love granted me strength, wisdom and knowledge to make this research a reality.

I would like to use this opportunity to express my profound gratitude to my supervisor Prof. A. R. Opoku for his guidance, invaluable suggestion, constructive criticism, encouragement and patience throughout the research.

I would also like to express my special gratitude to Prof and Dr (Mrs) Kolawole, Pastor and Pastor (Mrs) Foli, Dr and Mrs Nejo, Mr Gbenga Soyingbe, Mrs Joyce Wajuiham and Mrs Esien Udoh, for their constant guidance, support and tremendous encouragement.

Special thanks to my friends and colleagues Mosa, Nthoko and Flexy and the entire staff of Biochemistry Department, for their moral support.

To the love of my life and my dear children my heart felt gratitude goes to you all for your patience, understanding, care, financial support and unwavering love throughout the years of study.

I would also like to appreciate the contribution of the following people who had contributed in one way or another for success of this research.

- Mr Nkwanazi (UZ).
- Mr Lukas (UZ).
- Mr Sibusiso (UZ, Chem Dept).

- Lancet lab (Richards Bay).
- Dr J. H. Voster (Vet, Pietermaritzburg).
- The Research Office (UZ).

ABSTRACT

Proteins play vital role in the normal physiology of an individual and the deficiency of it in the diet leads to many conditions often referred to as PEM. Due to high mortality rate among PEM children around the world, programs are aimed at improving the health and wellbeing of such vulnerable children. Animal proteins still remain the best source of good quality protein; however, those that are highly susceptible to PEM cannot afford protein of animal origin. There is need, therefore, to look at readily available proteins of plant origin. The potential of *Citrullus lanatus* (Egusi) seeds protein isolate, as an alternate protein source, on malnourished rats was investigated. De-hulled, deffated egusi seeds were extracted with water (pH 10). The extracted proteins were precipitated at pH 5, centrifuged and freeze-dried to obtain the protein isolate. Proximate composition shows that egusi seed isolate is rich in protein (63.4 %), and minerals. Functional properties indicate that the protein isolate has good oil absorbability and water binding capacity. The amino acid composition revealed the presence of a wide spectrum of essential amino acids.

The administration of the protein isolate (20% incorporated into low diet) to malnourished rats resulted in the alleviation of the detrimental effects associated with protein malnutrition. The variables investigated were the serum enzymes (ALP, AST, ALT, GGT, LDH), serum lipid (cholesterol, TAG), haematological parameter (WBC, RBC, Hb, PCV, MCV, MCH, MCHC, RDW, Platelets, Neutrophils, Monocytes, Lymphocytes, LUC, Eosinophils, Basophils), and the histology of the liver and kidney.

It is concluded that egusi protein isolate could be a candidate in the search for solution to PEM.

TABLE OF CONTENTS

LIST OF TABLES

Tables

LIST OF FIGURES

List of figures

ABBREVATIONS

ALP – Alkaline Phosphatase

ALT – Alanine amino tranferase

AST – Aspartate amino tranferase

ATP – Adenosine tri phosphate

B - Basophils

C - Casein

E – Eosinophils

EFA – Essential Fatty Acid

FA – Fatty Acid

GGT – Gamma-glutamyl transpeptidase

Hb – Hemoglobin content

HCT – Hematocrit

HDL – High density lipoprotein

HFD – High Fat Diet

LCC – Leucocyte cell

LDH – Lactate dehydrogenase

LPC – Lymphocyte cells

LPD – Low Protein Diets

LPD + C – Low Protein Diet and Casein

MCH- Mean Corpuscular Hemoglobin

MCHC – Mean Corpuscular Hemoglobin Concentration

MCV- Mean Corpuscular Volume

NTP - Neutrophils

PC – Platelet Count

PI – Protein Isolate

RBC – Red blood cell

WBC – White blood cell

CONTRIBUTION TO KNOWLEDGE

Ogunyinka IB, Opoku AR. Effect of *Citrullus lanastus* seeds (Egusi) protein isolate on lipid peroxidation in malnourished rats fed on high-fat-diet. 6th Annual Faculty of Science and Agriculture Research Symposium, University Of Zululand, October 2011.

CHAPTER ONE

INTRODUCTION

Nutrition is the science of food in relation to the nutrients and the health of the organism. It is the study of the quality and quantity of nutrients derived from the food we eat in relation to proper functioning of the body and the overall growth. Food is needed for three basic things-

(a) Energy to power all activities.

(b) Structural materials for growth, repair and maintenance.

(c) Chemical agents that regulates the vital processes within the body.

The nutrients in food must be taken in right quality and quantity for optimum health, too little or too much quality and quantity of nutrients result in malnutrition. Poor nutrition is a powerful constraint to realizing ones potentials; children who grow up in environment of poverty and malnutrition in developing countries have diminished capacity for learning and are not able to take full advantages of even the limited educational opportunities to which they have access to (Pollitt, 1990). The Wold Health Organization (WHO, 1973) defines malnutrition as the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance and specific function.

Malnutrition is a life threatening condition caused by diets lacking essential protein, fats, vitamins and minerals. Malnutrition can be due to over nutrition, undernutrition and inability to use the nutrient ingested. The most important form of malnutrition is protein energy malnutrition (PEM). Malnutrition among children in developing

countries is mainly due to consumption of cereal based porridge which is bulky, low in energy and density, and high in anti-nutrients (Michealsen and Henrick, 1998). Its clinical symptoms are classified into three main classes, namely Kwashiorkor, Marasmus and Marasmic-kwashiorkor according to their nutritional deprivation (Touyz, 2000; Prata, 2007). Kwashiorkor is principally protein deficiency; Marasmus is predominantly energy deficiency while Marasmic-kwashiorkor is a combination of chronic energy deficient and chronic or acute protein deficiency. Studies indicated that about 60% of deaths in children under the age of 5 years in developing countries are related to malnutrition (UNICEF, 1989).

Protein energy malnutrition is characterized by low weight for age, edema, dermatitis, hair changes, mental changes, hepatomegaly and diarrhoea (Etukudo *et al* 1999). According to Akinola *et al* (2010) protein malnutrition which is a common problem in children of the developed world occurs due to socio-economic reasons. Plant proteins play a significant role in human nutrition, particularly in developing countries where average protein intake is less than required. Onweluzo *et al* (1994) indicated that because of inadequate supplies of animal proteins, there had been a constant search for new protein sources for use as both functional food ingredient and nutritional supplement. Singh *et al* (1993) and Lohlum *et al* (2010) suggested that one of the least expensive ways of increasing protein level in the diet of low income group is by encouraging the consumption of local indigenous edible seeds especially the legumes and oil seeds.

Cucurbitaceae consists of various squashes, melons, and guords, including crops such as cucumber, pumpkins, luffas and water melons. The melons includes members of the plants family *Citrullus lanatus* (Egusi melon). They are among the

economically most important vegetable crops worldwide and grown in both temperate and tropical region (Pitrat *et al* 1999; Fokou et al (2004). It is grows anywhere from humid gullies to dry savannahs to tropical highlands making it possible source of food for farmers in even the worst conditions. It has about 120 genus and about 735 species (Mabberley, 1987). The industrial utilization of these oil seeds as source of protein food supplementation is non-existent outside West Africa due to lack of information on chemical composition and functional properties of these oil seeds.

The present study aims at isolating protein from defatted meal of *Citrullus lanatus* (Egusi) seeds and to examine its biological value by feeding the isolate to malnourished rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 MALNUTRITION

Malnutrition is a serious and often life-threatening condition caused by a diet lacking essential nutrients. It is often manifested as over nutrition (diabetes, dental decay, obesity) and under nutrition (digestive problems, improper absorption and unbalanced diet etc.).

People suffering from malnutrition have extremely compromised immune system which makes them to be susceptible to illness and diseases which often claim their lives. Young children and babies are the most vulnerable, if left untreated, their physical growth and mental development can be permanently impaired or loss of life in severe cases. The most common manifestation of under nutrition in children is PEM.

2.1.1 PROTEIN ENERGY MALNUTRITION

PEM also known as Calorie Malnutrition occurs in children whose consumption of protein and energy is not sufficient to meet the nutritional need. In most cases deficiency will exist in both total calorie and protein intake. However, PEM occurs when a diet provides enough energy but lacks adequate amount of protein. It may also occur in children with illness that leaves them unable to absorb vital nutrients or convert them to energy essential for healthy tissues.

PEM has long been recognized as a common problem especially in children in the developing world, who lack of adequate nutrition due to some socio-economic reasons (Manary *et al,* 2000). It is the leading cause of death for children totally or partially among the children under the age of 5yrs (WHO, 2000).

Based on their nutritional deprivation, the clinical manifestation or symptoms of Protein Energy Malnutrition can be divided into 3 main classes:

i. Kwashiorkor.
ii. Marasmus.
iii. Marasmic-Kwashiorkor (Touyz; Prata *et al,* 2007).

Literature indicates that Marasmus represents adaptive response to starvation, while Kwashiorkor represents a maladaptive response to starvation and Marasmic-Kwashiorkor is having combined features (Berkow and Roberto 1999;, Jimoh *et al,* 2005).

2.1.1.1 KWASHIORKOR

Kwashiorkor is a disease that occurs when the diet is lacking protein and is more of energy. It is mostly common in areas with limited food supply such as famine areas, war zone and also as a result of nutrition ignorance due to lack of knowledge in diet requirement and feeding methods.

Kwashiorkor comes from a Ghanaian word which means 1^{st}, 2^{nd} i.e. the diseases that visit the 1^{st} child after the arrival of the 2^{nd} child. This abrupt withdrawer or switch of the child from the mother- nutrient-dense-protein -rich breast milk to being weaned with watery starch gruel with low protein reduce their immunity, and exposes or predisposes the child to infection and diseases.

It usually occurs between ages one and three years old, as a result of an increased metabolic activity, increase nutrient loses and inadequate diet (William *et al.*1935)**.** Kwashiorkor is characterized by growth retardation. The height and weight of these children fall below the curves of other children of their age. Growth retardation, reduced

skeletal muscle are due to lack of protein, the body is converting the muscular protein to meet up with the protein deficiency.

Presence of edema in both legs and abdomen are due to lack of plasma protein, albumin, which maintains the osmotic pressure of the blood and hence the fluids leaks out of the blood and accumulates in the legs and abdomen.

With the lack of tyrosine to make melanin, hair loses its color and strength. Inadequate production of Beta-lipoprotein results into an impaired transport of fat in and out of the liver hence there is an accumulation of fats in the liver leading to fatty liver.

The lack of protein to carry or store iron leave iron free. This unbound iron can act as pro- oxidant, thereby initiating a free radical reaction, which contributes to many illness and death if unattended to (Halliwell *et al.,* 1992).

2.1.1.2 MARASMUS

Marasmus is a Greek word which means waste or drying-away. Marasmus is a chronic condition resulting from insufficient or inadequate protein and energy intake to match the body requirements. As a result the body draws on its own stores resulting in emaciation (Ibukunolu - Alade, 2001). It usually occurs in the 1-2 years of life as a result of starvation; it is common in an impoverished and over populated area. Marasmus is characterized by growth retardation, gross weight loss and always hungered. Due to loss of subcutaneous fat and muscle, vital muscle functions are impaired. The child is wrinkled and has a low resistance owing to infection and diseases (Scherbaum and Furst 2000).

Recent advances in the in the understanding of the disease seem to suggest that PEM may be more complex than just the availability of rich protein diet, and adaptation

failure (Gopalan1993; 2001), genetic deficiency of transaminases (Phadke *et al*1995) and a buildup of free radical (Golden and Ramdath, 1987) have been implicated as causative agents of PEM. A buildup of free radicals damage tissues and explain many of the features of kwashiorkor—bleached or reddish hair. In addition, the concentrations of several molecules (glutathione, vitamin E, Zn, and selenium-containing enzymes glutathione peroxidase) that protect against free radical damage are reduced in PEM.

The deficiency of essential fatty acid also contribute to many of the clinical problems associated with PEM such as scaly dermatitis, loss of hair, edema, fatty liver, impaired wound healing, susceptibility to infection and growth retardation, etc. (Golden and Ramdath, 1987). UNICEF (1989), therefore, recommends the addition of oil in rehabilitating diets of PEM children to boost their energy intake. Addition of fats and oils increases the energy content of the food, but not the bulk. This is suitable for PEM children with small appetite and stomachs. Furthermore, vegetable oil contains essential fatty acids (EFA) that are important for growth and development and visual acuity (FAO 1994). EFA are important precursor of prostaglandin, thomboxane and prostacyclins (Achu *et al* 2008).

However, adding oil to rehabilitating diets has its own consequences. A diet rich in fatty acids (FA) can lead to change in oxidative stress system, obesity, diabetes, etc. (De La Cruz *et al* 1999) evidence suggests that the risk of circulatory diseases is closely related to the amount of total cholesterol (TC) and high density lipoproteins (HDL) and cholesterol (C) (Oluba *et al* 2008).

If the build-up of free radical is indeed a cause of PEM, then addition of oil to the diet of PEM children in other to boost their energy level, as recommended by UNICEF (1980), could be inappropriate.

It is apparent that the solution to Kwashiorkor and Maramus is still in protein rich diet. Animal protein still remains the best source of good quality protein because all the ten essential amino acids are present in appropriate ratio. However, those that are highly susceptible to PEM cannot afford to have protein of animal origin due to some or all of the following factors.

1. Poverty/ low purchasing power: Parents of low income groups cannot afford to buy food (animal protein) that contain all the essential amino acids for their children because such are expensive.
2. Lack of knowledge: Ignorance of the requirement of a growing child dietary need and the improper use of the available resources.
3. Lack of availability: This is due to scarcity of protein of animal origin which could be as a result of famine, drought, natural disasters, and war.
4. Traditional or cultural practices: The general believe that father and the older siblings should have the larger portion of the food, often deprive the children their need for proper growth.

There is need, therefore, to look at readily available protein of plant origin which will be of high biological value and has excellent digestibility and relatively cheap, which in turn will provide appropriate energy, protein, minerals and vitamins to meet all the nutritional need of a child requiring a well balance diet. Lohlum *et al* (2010) recommended that one of the least expensive way of increasing protein level in the diet of low income group is by encouraging the consumption of local indigenous edible seeds especially oilseeds and legumes.

2.2. OIL-SEED PROTEINS

For a variety of reasons more, individuals nowadays prefer to control their health with the help of natural products (Kincheloe, 1997; Thaipong *et al,* 2006). Hasler (1998) concluded that diet rich in fruits and vegetable reduces incident of heart diseases and certain cancer in human.

Plant protein always remains an important source of protein and oil for nutritional, industrial and pharmaceutical applications (Eromosele, 1997). Many of the oil-producing plants contain appreciable level (60-70%) of protein, which has great potential for use in human diet.

Typical oil seeds proteinsare those from soybean, sun flower, peanut and rape seeds(Ash and Dohlman, 2006; Onweluzo *et al,* 1994). According to Young (1991), Soy protein is considered a complete protein because of ample amount of all essential amino acids, and several other macronutrients that it contains; soy protein has a with a nutritional values equivalent to that animal proteins.Isolates are practically free from tanning acids, trypsin inhibitors and flatulent factor. They are rich in thiamine, niacin, trace element and are good source of Mg, K.

In alleviating protein energy malnutrition, it is necessary to make maximum use of available vegetables; one way of doing that is to encourage the consumption of local edible seeds especially the legumes and oil seeds (Singh *et al,* 1993; Lohum *et al,* 2010). A study of the *in vivo* antioxidant properties of pumpkin seed protein isolate in our laboratory indicated that the administration of protein isolate was effective in alleviating the detrimental effects associated with carbon tetra chloride intoxication (Nkosi *et al* 2005, 2006a, b). Several studies reveal that Cucurbitaceae (Egusi) seeds

are rich in proteins and oils(Murkovic *et al,* 1996; Silou *et al,* 1999; Achu *et al,* 2005, and 2006).

2.2.1*CITRULLUS LANATUS* (EGUSI MELON SEED)

Citrullus lanatus (Egusi melon seed), a biological ancestor of water melon, originated from West Africa.Egusi seed is a member of cucurbitaceous family comprising of 53% oil and 33% proteins (Ogbonna *et al*, 2009). From here onwards *Citrullus lanatus* will be refer to as Egusi.

The seed has both nutritional and cosmetic importance. A study reveals that Egusi is rich in essential amino acids like arginine, glutamine, aspartic and minerals such as zinc and essential fatty acids which may be of great importance to prostrate health (Ojeih *et al*, 2008).The fatty acids composition of its oil was reported to contain mostly linoleic and oleic acids, and to resemble that of safflower (Yaniv *et al,*1999), corn, cotton seed, sunflower, soy bean and sesame oil (Oluba *et al,* 2008). Melon seed, rich in unsaturated fatty acids (Achu *et al,* 2005; Achu, 2006) could possibly have a hypocholesterolemia effect (Oluba *et al,* 2008).

In Nigeria, where important meals of the day comprise of soup and stew, Egusi seed in paste form is consumed as Egusi soup, dried seeds are often fried into snack (Robo) and a fermented condiment (Ogiri) (Uruakpa *et al,*2004) sometimes the roasted seeds are used to make paste for spreading on bread. The seed are used to make healthy cholesterol free oil. In some rural part of South Eastern Nigeria, ground egusi, mixed with *Pleutorus* tuber *negium,*is shaped into balls to substitute for meat in the diets (Bankole *et al,*2005). They are also use as raw material in the production of margarine, salads, baby foods, and live stock feeds. The oil is used in the production of local pomade, soaps and its shell is used as poultry litter. The plants blankets the area where its grows, it stops the growth of weeds and it is also resilient to pests.

According to Frankel (1995) Egusi is a rich source of flavonoids and phenol and will thus have the ability to scavenge free radical and inhibit hydrolytic and inflammatory actions. Study also reveals that methanolic extracts of Egusi possessed high *in vitro* antioxidant activity (Gill *et al,*2010). Because of its rich source of phytochemical, minerals and vitamins it can also be a potential source of useful drug (Adesanya*et al,*2011).

Egusi is a relatively new crop despite the fact that many of these crops have ancient history of contributing to food security (Willis *et al* 1989). Wide water melon (*Citrullus lanatus*) is not a major crop in South Africa agriculture; it is a minor, underutilized crop whose economic potential is yet to be fully exploited.

2.3 SCOPE

This study aims to investigate the biological value of egusi protein isolates.

To explore the potential of *Citrullus lanatus* (Egusi melon seed) protein isolate in providing a high protein sources to supplement the diet of protein malnourished children.

The study will involve the:

1 Extraction of Protein Isolate from de-hulled and defatted seeds.
2 Proximate analysis of the protein isolate.
3 Determination of the functional properties of the isolate.
4 Determination of the amino acid composition of the isolate.
5 Incorporation of protein isolate into a high fat diet to rehabilitate malnourished rats.
6 Analysis of blood samples for hematological parameter, liver and kidney histology.

Chapter Three

MATERIAL AND METHODS

This chapter describes the materials and methods used in this study. The details of the preparation of reagents and the detailed methodology are presented in Appendix A and B, respectively.

3.1 Material

3.1.1 Raw material.

Dehalled sun-dried Egusi (*Citrulluslanatus var lanatus*) seed were obtained fromDurban local market in March 2010; they were confirmed and identified as *Citrullus lanatus* by a taxonomist in the Department of Botany, Faculty of Science, University of Zululand, Kwadlangeswa, South Africa. The seeds were sorted to remove dirt and decomposed ones, then washed, and dried in oven at 50^{o}Covernight. The seeds were stored in an air-tight container in a desiccator for further analysis.

Rat feed (Ideal Adult food nutrition) was obtained from AVI Product PTY LTD, Empangeni

Maize meal (samp-- Iwisa) was obtained from the local supermarket, Empangeni.

3.2 Methodology (See Appendix B for details)

3.2.1. Preparation of Egusi seed Protein isolate

Dried and de-hulled Egusi (*Citrulluslanatus var lanatus*) seeds were ground into 2mm mesh size powder. The powder was defatted twice with hexane. The residue

was air dried and then extracted (1: 20 w/v) with acidic butanol to remove possible antinutrients(phytate). Proteins in the butanol residue were extracted (1:5w/v; stirred continually overnight in the cold room) twice with distilled water (pH10; pH adjusted with 30% NaOH). The residue (after centrifugation at 4500rpm, 30min) was discarded and the pH of the combined filtrate was adjusted to pH 5 with 18% HCl. This was also allowed to stand in the cold room overnight. The sample was centrifuged at 10000 rpm for 15 minutes and the residue was freeze-dried(Nkosi *et al* 2005).

3.2.2 Proximate Composition

3.2.2.1 Moisture Determination

Sample of the protein isolate was put in a previously weighed glass crucible, placed in an oven (105°C) and dried to a constant weight. The change in weight was recorded and the percentage moisture content calculated.

3.2.2.2 Ash determination

The sample (2g) was put in a crucible and placed in furnace maintained at 600^0C for five hours; the resultant weight was used to calculate % ash content.

3.2.2.3. Crude fat determination

The moisture free samples were extracted into hexane on a Soxhlet extractor for 16hrs at low heat. The solvent was then evaporated and the residue was used to calculate the amount of crude fat.

3.2.2.4 Determination of total protein

Total protein was determined as Kjeldahl nitrogen. The sample was digested with concentrated sulphuric acid and the Kjedahl catalyst. The digest was then reacted with Nesslers reagent to produce color for colorimetric estimation at 520nM (William 1964). Ammonium sulphate solution was used as standard. Amount of protein is expressed as Kjeldahl N x 6.25.

Total soluble protein of the samples was determined by the method of Lowry et al (1951). Bovine serum albumin (B.S.A) was used as standard.

3.2.2.5 Carbohydrate Determination

Carbohydrate was determined by Hassid and Neufeld (1964) method. The samples were first percolated with 80% ethanol to obtain an extracts of soluble carbohydrate and then with 35% of perchloric acid to obtain the non-soluble carbohydrate (starch) extract. The percolates were reacted with anthrone reagent and the absorbance read at 630nM. Glucose and maize (starch) were used as standards for the soluble carbohydrate and starch respectively.

3.2.2.6 Mineral analysis

The ash of the sample (3.2.2.2) was dissolved in HCl and the mineral content analyzed using Carl Zeiss Jena AAS3 atomic absorption spectrophotometer (AOAC 1990).

3.2.3. Functional properties

These are protein properties which determine the processing behavior in food system in terms of quality of the food product. They reflect complex interactions

between composition, structure and conformation of protein and other food components as well as the nature of the environment in which these are associated (Kinsella 1976).

3.2.3.1 Solubility

The nitrogen solubility of the protein isolate was determined by the method of Bera *et al* (1989). The sample (2g) was dissolved separately in 100ml of distilled water whose pH has been variously adjusted to 2,3,4,5,6,7,8,9,10,11,12 with 2M HCl or 2M NaOH solution. The mixture was shaken for 1 hour and then centrifuged (4500rpm, 20 minutes). The Lowry *et al* (1951) method of protein determination was used to determine the amount of protein in the supernatant.

3.2.3.2. pH determination

The pH of the protein isolate in solution (2g in 50ml of distilled water) was determined with pH meter.

3.2.3.3 Foam capacity

Foam capacity was determined according to the method of Narayama and Marasinga-Rao (1982). The sample (2g) was homogenized for 5mins in an electric homogenizer at high speed. The homogenate was quickly poured into 50ml measuring cylinder and the total volume at zero time and after 30 sec were recorded. Foam capacity was expressed as percentage volume decrease per minute.

3.2.3.4 Foam stability

The sample (2g) was homogenized in 50ml of distilled water at high speed for 5mins. The homogenate was quickly poured into 50ml measuring cylinder and the

total volume was taken at interval of 30mins for 2hrs. The foam stability was expressed as percentage volume decreased per minute (Lawhon *et al*1972).

3.2.3.5 Water Absorption capacity

The water absorption capacity was estimated by the method of Cegla *et al* (1977). The sample (1g) was homogenized in 50ml of distilled water for 5mins in an electric homogenizer. The solution was left to stand for 30mins at room temperature and then centrifuged at 4500rmp for 15mins. The volume of the supernatant was measured and decrease in volume was used to calculate the percentage of water absorbed.

3.2.3.6. Oil absorption capacity

The fat absorption capacity was determined according to the method of Lin *et al* (1974). The method is similar to that reported for the water absorption capacity, except that 50ml of olive oil was used in place of water. The oil absorption capacity was expressed as percentage of oil absorbed. Specific gravity of olive oil is 0.91g/cm^3

3.2.3.7. Bulk density

A previously tarred 10ml graduated cylinder was filled with the sample. The bottom of the cylinder was gently tapped on the laboratory bench several times until there was no further diminishing of the level after filling to 10ml mark. The bulk density was calculated as weight of sample per unit volume of sample (g/ml).This was based on the procedure of Akpapunam and Markakis (1981).

3.2.4. Determination of amino acid composition

The sample was refluxed with 6M HCl and the resulting solution filtered. The amino acid composition of the hydrolysate was determined using a Beckman 121MB Amino Acid analyzer (Fasuyi and Aletor, 2005).

3.2.5. Preparation of rat feeds

Samp (maize meal) was milled to 2mm size and mixed with little water to make dough. The dough was then molded into finger like sticks and dried in the oven at 50^{o}C. This formed the basic (Low-protein-diet) into which different nutrients were added to formulate the various diets (see Table 3.1)

Table 3.1 Feed Formulation (g/100g)

Diet components (g/100g) diet	MM	Casein	PI	Cholesterol	Thimecil	Bile salt	Sunflower Oil	HFD
LPD	100							
LPD+C	80	20						
LPD+ PI	80		20					
LPD+ HFD	80							20
HFD	79.3			5	0.2	0.5	15	
HFD + PI			20					80
HFD+ C		20						80

MM = Maize meal, P.I = Protein Isolate, HFD = High fat diet, LPD = Low Protein diet, LPD+C = Low Protein diet and Casein, LPD+PI = Low Protein diet and Protein Isolate, LPD+HFD = Low Protein diet and High fat diet, HFD+PI =High fat diet and Protein Isolate and HFD+C = High fat diet and Casein.

3.2.6. Experimental animals

Ethical clearance for the use of animals in this study was obtained from the Research Animal Ethic Committee of the University of Zululand (see Appendix C)

Sprague-Dowley rats were obtained from the animal house, Department of Biochemistry and Microbiology. The fifteen week old rats (150-180g) were weighed and house in cages (4 animals per cage). They were fed with commercial rat feed

and had free access to water and feed. They were acclimatized to standard room conditions (24 – 27^{o}C; 12hour day/night; 60%RH).

After establishing the baseline the rats were divided into 2 main groups:

Group 1 comprised of 8 animals and they continued on the normal rat feed for the 28 days and

Group 2 animals were further divided into 2 subgroups (A and B).

Group 2A started on low protein diet for the next 10 days

Group 2B were kept on high fat diet for the next 10 days

After 10days 4 animals were sacrificed from each group i.e. 1, 2A and 2B

Group 2A were then divided into 4 subgroups while group 2B were divided into 3 subgroups (see the experimental design in Fig 3.1)

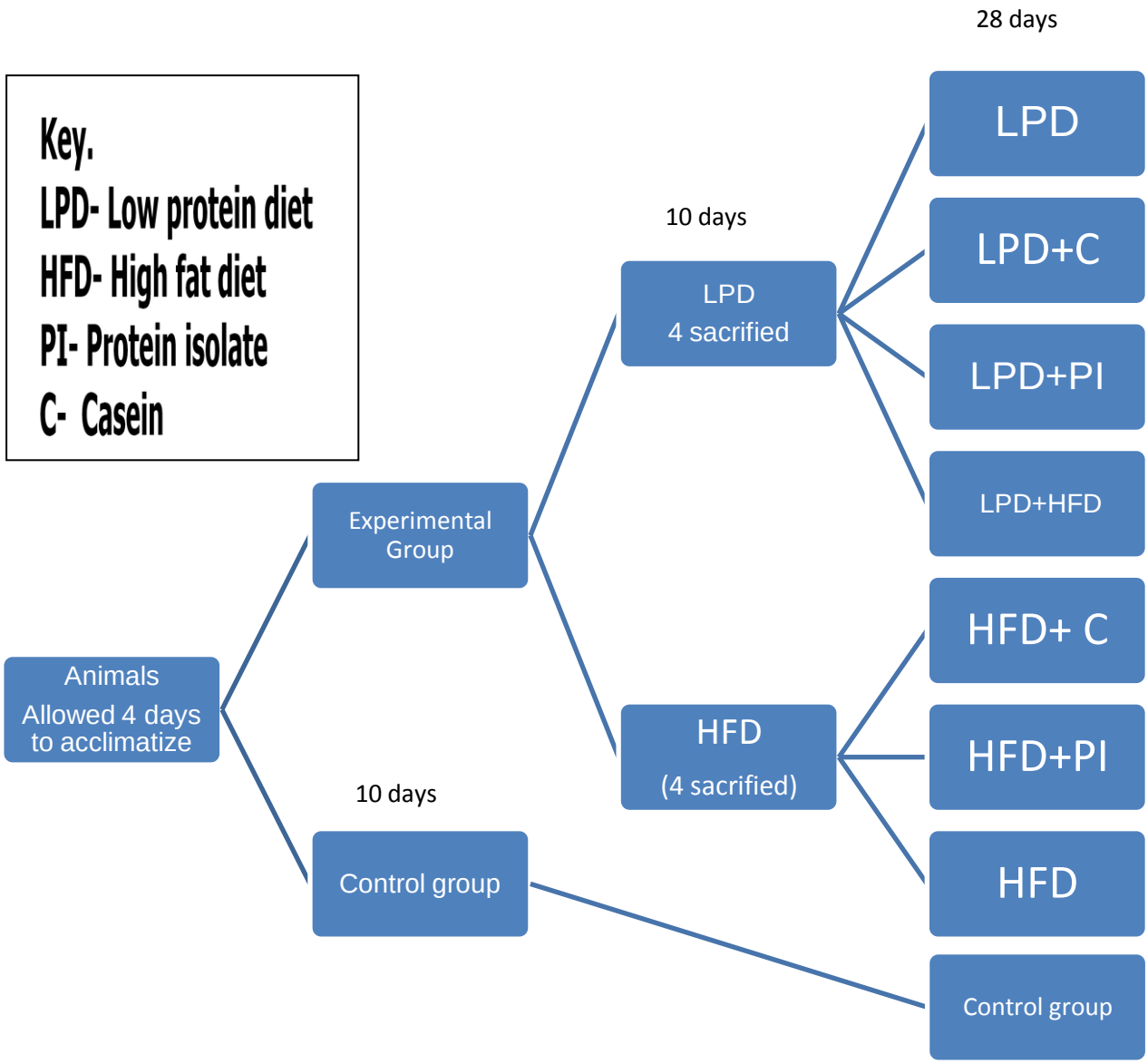

Fig 3.1 Experimental design

3.2.6.1. Blood collection for enzyme assay, lipid analysis and liver and kidney histology

After 4 days of acclimatization 4 animals were anaesthetized with diethyl ether and then sacrificed. Blood was drawn from the vein cava into anticoagulant tubes supplied by Lancet laboratory, this form the baseline result.

Similar procedures were carried out after day 10 and 28, 4 animals from each group i.e group 1, 2A and 2B were sacrificed. The animals were not fasted prior to the sacrifice; they have access to water and food.

3.6.1.2. Blood analysis

The analysis of the blood was carried out at the Lancet Laboratories (Richards Bay Hospital), using standard pathology procedures and equipment

The following tests were conducted:

Sodium, potassium, Chloride, Inorganic phosphate, Urea, Creatine, Calcium, Magnesium, Uric acid, Total bilirubin, Albumin, Globulin, Total protein, Alkaline phosphatase, γ-Glutamyl transferase, Alanine aminotransferase, Aspartate aminotransferase.Cholesterol, TAG..

WBC, RBC, PCV, MCV, MCH, MCHC, RDW, Platelets, Neutrophils, Monocytes, Lymphocytes, LUC, Eosinophils, Basophils were all determined.

3.6.1.3. Histology of liver and kidney

Liver and kidney were harvested and preserved in formalin. Histology studies were carried out at the Vet diagnostics Laboratories (Pietermaritzburg) by qualified pathologist having no prior knowledge to which group they belonged. This method

allowed for unbiased description of the histological lesions which were present or absent in the samples.

3.2.7 Statistical analysis

The mean and standard error of the mean of the three experiments were determined. Statistical analysis of the differences between mean values obtained for experimental groups were calculated using Microsoft excel program 2003. Data were subject to one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. In all cases, p values ≤ 0.05 were regarded as statistical significant.

CHAPTER FOUR

RESULTS

Protein isolate obtained from Egusi was analyzed for proximate composition, functional properties, and amino acid composition. The biological value of the protein isolate was examined by incorporating 20% (w/w) of the isolate into maize meal and feeding it to malnourished rat's over 28 days. The body weight, amount of food eaten and water consumed were monitored. After the 28 days feeding, the rats were sacrificed and the blood, liver and kidney were analyzed for biological parameters.

4.1 Proximate composition

The result of proximate analysis of the protein isolate is shown in Table 4.1. It is apparent that the isolate obtained contain an appreciable (63.4%) amount of protein.

Table 4.1 The approximate composition of Egusi protein isolate

	EPI
Moisture (%)	**5.11**
Ash (%)	**3.9**
Crude fat (%)	**3.4**
Protein (%)	**63.37**
Carbohydrate (%)	**Soluble – 2.4%** **Non-Soluble-20.18%**

(EPI = Egusi protein isolate)

4.2 Minerals composition.

The minerals content of the isolate is presented in table 4.2. The main minerals found in the isolate are Na, K, and Ca. Trace amounts of Mg and other minerals also existed in the isolate.

Table 4.2 The mineral composition of the isolate.

Elements	**Protein Isolate (mg %)**
Mn	0.04
Fe	0.45
Mg	0.57
Zn	0.19
Cu	0.12
Ca	1.38
Co	0.01
K	5.30
Na	19.5

4.3 The functional properties

The functional properties of the protein isolate (Table 4.3) indicates that the isolate possesses good water absorption capacity (WAC), oil absorption capacity (OAC) and low bulk density, foam capacity and stability.

Table 4.3 Functional properties of Egusi protein isolate

Properties	**Egusi Protein isolate**
pH (in solution)	5
Oil Absorption (g of oil/ g of protein)	5.5
Water Absorption (g of water/ g of protein)	10.5
Foam capacity (%)	1.02
Foam stability (%)	0.05
Bulk Density (g/ml)	0.05g/ml

4.4 The Protein Solubility

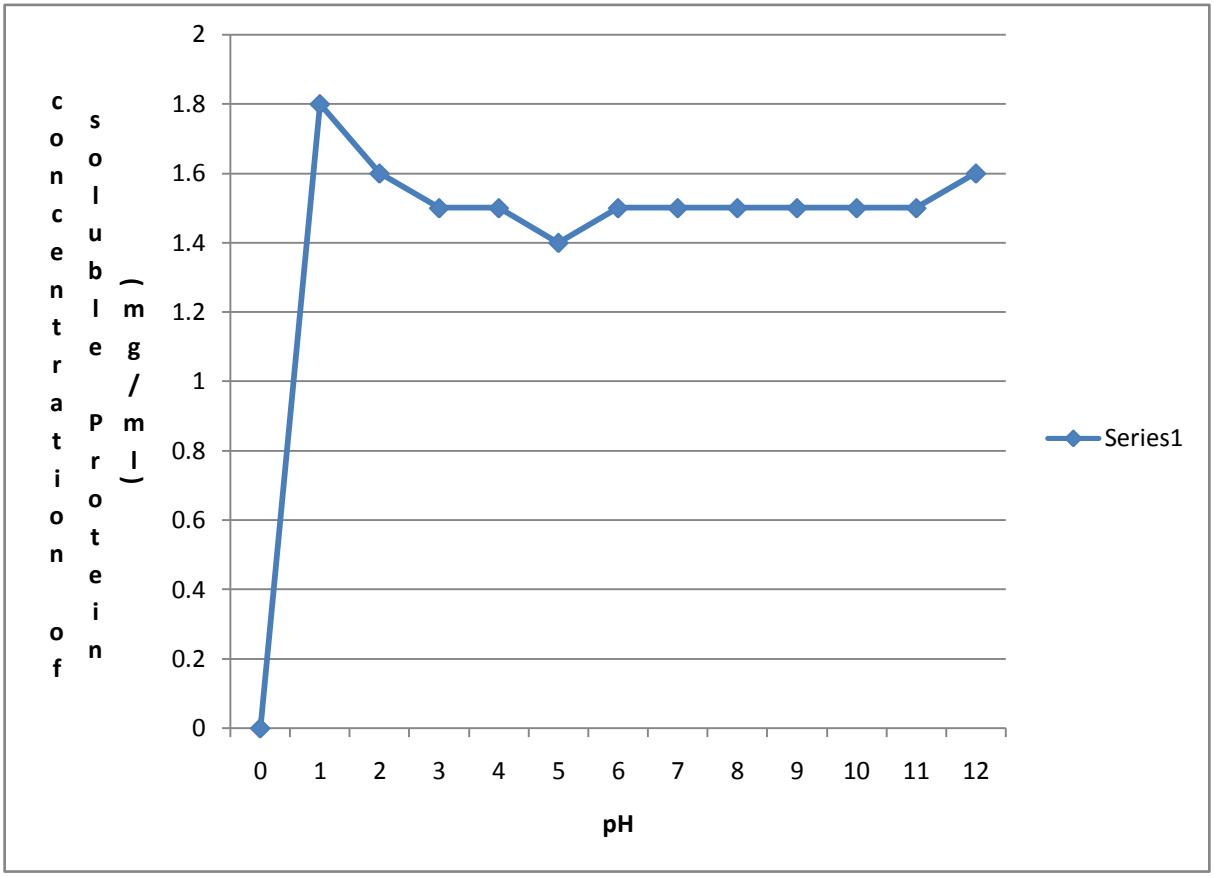

Figure 4.1 Protein solubility profiles of Egusi Protein isolate.

The protein solubility profile of the isolate, shown in fig 4.1, indicated that like any other protein, the solubility is pH dependent; the isoelectric point for the isolate is 5 which show that the isolate is slightly acidic.

4.5 Amino acid composition

Table 4.5 shows the Amino acid composition of the Egusi protein isolate. It is apparent that the protein isolate contains appreciable quantities of the essential amino acids like, arginine, valine, threonine and histidine, with methionine as the limiting amino acids.

Table 4.5 Amino acid composition of Egusi protein isolates (mg/g)

Amino acid	Egusi isolate (mg/g)
Asp	104.61
Thr	41.31
Ser	46.45
Glu	209.94
Gly	66.44
Ala	47.04
Cys	2.5
Val	71.31
Met	4.0
Ile	23.09
Leu	39.62
Tyr	34.97
Phe	32.38
Lys	33.04
His	29.33
Arg	122.81

Table 4.7 the proximate composition of the animal feeds (%)

Component g/ 100g	**LPD**	**LPD +C**	**LPD +PI**	**LPD +HFD**	**HFD + PI**	**HFD +C**	**HFD**
Moisture	14.4	6.5	9.4	9.1	12.5	17.5	7.7
Ash	0.4	1.1	0.8	0.3	0.9	0.8	8.0
Crude fat	5.3	4.8	4.2	8.5	3.5	20.7	19.6
Protein	10.71	17.28	15.72	11.53	13.69	13.73	9.5
CHO soluble	10.2	8.2	19.6	11.3	3.8	20.8	8.1
Non Soluble CHO	10.3	11.9	17.9	15.1	16.5	9.1	9.1

CHO soluble = soluble carbohydrate Non soluble CHO = non soluble carbohydrate.

4.6 The proximate composition of the animal feeds

The proximate composition of the animal feeds shown in table 4.6 indicates that the incorporation of the isolate into the various feeds increased the protein contents of the feeds to appreciable levels.

4.8 Effect of feed on animal weight

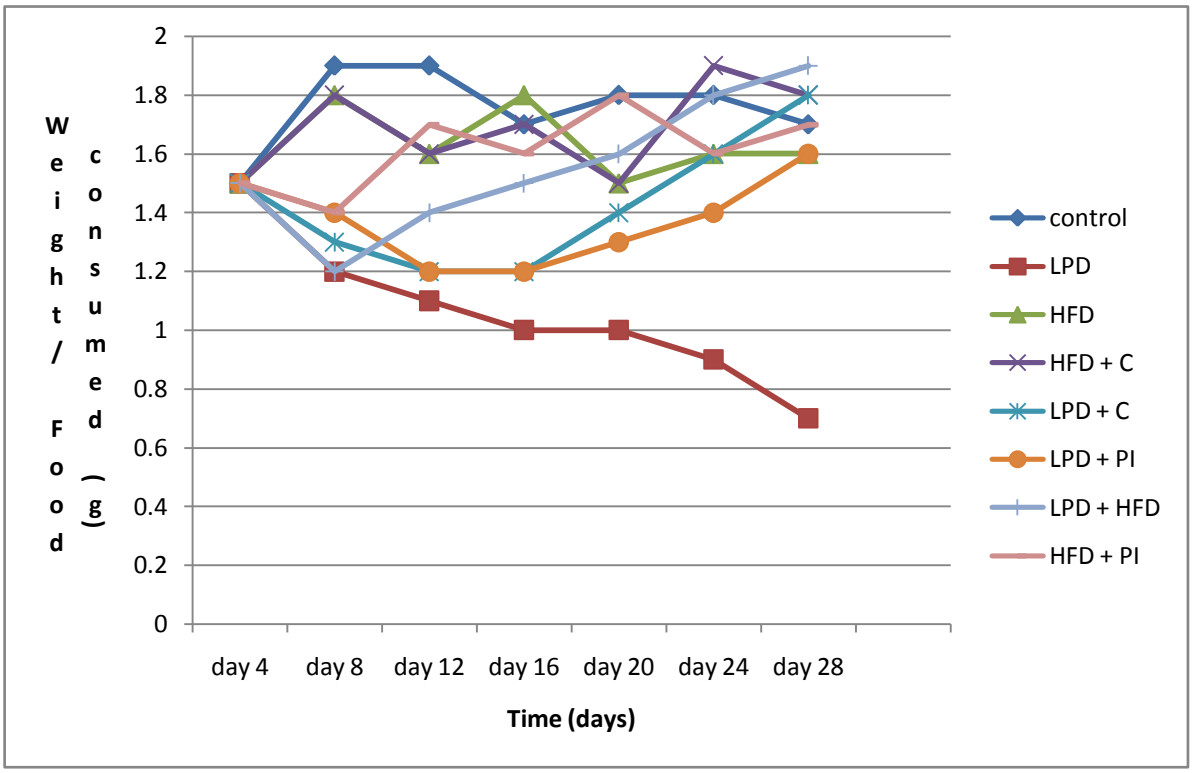

Figure 4.2 Changes in body weight of rats fed various feeds (diets)

The changes in body weights of rats fed with various diets are shown in figure 4.1. There is an indication that the rats fed the LPD were losing weight and that there was a general improvement (increase) in body weight when they were switched onto the protein enriched diets.

4.9 Effect of protein isolate on blood parameters

The results obtained when the blood of the rats fed on the various diets was analyzed are presented in table 4.9.1.to 4.14.3

Table 4.9.1Serum Protein (g/l), Albumin (g/l), Congugated bilirubin (umol/l) and total bilirubin (umol/l) after 10 days feed

10 days	T bilirubin	C bilirubin	Albumin	T Protein
Base line	1.97 ± 0.57 NS	1.92±0.56 NS	47.00±13.62 NS	80.25±23.25 NS
LPD	3.50 ±1.09* S*	1.80±0.51 NS	41.25±11.94 NS	62.25±17.97 NS
HFD	3.97±1.00** S**	1.80±0.53 NS	43.00±12.44 NS	72.75±21.19 NS

(n =4, mean ± SEM) S* = Significant ($p<0.05$), S** = Significant ($p<0.01$), NS = Not Significant.

Table 4.9.1 shows the parameters compared to the base-line (before the start of the feeding experiment) after 10 feeding on the LPD and HFD. The results indicate a significant difference in Total bilirubin both in LPD and HFD, but no significant deference was observed in the remaining parameters; the total protein in HFD was slightly higher.

Table 4.9.2 presents the blood parameters as they are compared across the groups after 10 days feed. The control group consisted of the rats that remained on the normal rat feed, while the LPD and the HFD were the experimental groups.

Table 4.9.2 Serum Protein (g/l), Albumin (g/l), Congugated bilirubin (umol/l) and total bilirubin (umol/l) after 10 days

10 days	T bilirubin	C bilirubin	Albumin	T Protein
CONTROL	1.95±0.56 NS	2.10±0.56 NS	48.25±13.97 NS	82.50±23.85 NS
LPD	3.50±1.09 NS	1.80±0.51 NS	41.25±11.94 NS	62.25±17.97 NS
HFD	3.97±1.15 NS	1.85±0.53 NS	43.00±12.44 NS	72.75±21.19 NS

(n = 4, mean ± SEM) NS = Not Significant

There was no significant difference observed in the parameters (Serum Protein, Albumin, Congugated bilirubin and total bilirubin). It is likely that the time frame was too short for the malnourishment to be established.

Even though there were observable differences between the groups after 28 days feeding (Table 4.9.3), there was still no significant difference in the data obtained.

Table 4.9.3-The Serum Protein (g/l), Albumin (g/l), Congugated bilirubin (umol/l) and total bilirubin (umol/l) after 28 days

28 days	T. Bilirubin	C.Bilirubin	Albumin	total protein
Control	1.80± 0.51 NS	2.20 ± 0.75 NS	43.25 ± 12.61 NS	70.75 ± 20.63 NS
LPD	2.77 ± 0.89 NS	3.32 ± 1.51 NS	39.75 ± 11.62 NS	65.50 ± 19.45 NS
LPD+C	2.25 ± 0.65 NS	1.82 ± 0.52 NS	42.75 ± 12.37 NS	71.00 ± 20.50 NS
LPD+PI	1.80 ± 0.51 NS	1.85 ± 0.53 NS	40.25 ± 11.63 NS	67.00 ± 17.97 NS
LPD+HFD	2.77 ± 0.99 NS	2.95 ± 1.01 NS	44.75 ± 12.99 NS	82.00 ± 23.78 NS
HFD+PI	2.10 ± 0.61 NS	2.87 ± 2.1 NS	48.75 ± 14.26 NS	86.75 ± 25.26 NS
HFD+C	2.70 ± 0.82 NS	2.50 ± 0.80 NS	48.75 ± 14.12 NS	86.25 ± 25.23 NS
HFD	3.77 ± 1.19 NS	2.57 ± 0.84 NS	29.00 ± 11.85 NS	ND

(n = 4, mean ±SEM), ND = not determined, NS = Not Significant.

Table 4.10.1 Serum lipids (mmol/l), creatinine (umol/l), urea and total carbohydrate (mmol/l)

10 Days	Urea	Creatinine	Triglyceride	Cholesterol	T Carbohydrate
CONTROL	6.05 ± 1.78 NS	66.25 ± 19.24 NS	1.79 ± 0.64 NS	2.20 ± 0.66 NS	12.25 ± 3.70 NS
LPD	4.67 ± 1.65 NS	59.75 ± 17.41 NS	0.94 ± 0.28* S*	1.88 ± 0.57 NS	11.50 ± 3.43 NS
HFD	5.22 ± 1.59 NS	73.00 ± 21.61 NS	1.84± 0.25** S**	4.83± 2.06* S*	11.75 ± 3.25 NS

(n = 4, mean ± SEM), S = Significant, ($p<0.05$) S = Significant ($p<0.01$), NS = Not Significant.

Table 4.10.1 shows serum concentration of Cholesterol, Triglyceride, Urea, Creatinine and Total Carbohydrate after 10 days feed. The results indicated a significant difference in triglyceride of LPD with decrease in concentration and cholesterol and triglyceride in HFD with increase in concentration. While the other parameters showed no significant difference when compared to control.

Table 4.10.2 Serum lipids (mmol/l), creatinine (umol), urea (mmol/l) and total carbohydrate (mmol)

28 days	Urea	Creatinine	Triglyceride	Cholesterol	T Carbohydrate
Control	5.92± 1.74 NS	53.00 ± 15.85 NS	1.02 ± 0.39 NS	2.07 ± 0.61 NS	17.25 ± 6.00 NS
LPD	3.87 ± 1.25 NS	56.75 ± 16.98 NS	0.95 ± 0.34 NS	2.36 ± 0.69 NS	17.00 ± 5.53 NS
LPD+C	11.80 ± 3.29 NS	68.25 ± 20.25 NS	0.50 ± 0.15 NS	1.65 ± 0.47 NS	14.75 ± 4.34 NS
LPD+PI	8.62 ±2.53 NS	67.25 ± 19.92 NS	0.99 ± 0.37 NS	2.11 ± 0.79 NS	11.00 ± 3.35 NS
LPD+HFD	6.52 ± 2.21 NS	77.50 ± 23.28 NS	0.64 ± 0.20 NS	13.7 ± 4.50 NS	10.25 ± 3.00 NS
HFD+PI	8.42 ± 2.55 NS	69.75 ± 20.28 NS	1.34 ± 0.79 NS	25.18 ± 11.34 NS	10.75 ± 3.80 NS
HFD+C	8.57 ± 2.50 NS	91.00 ± 31.53 NS	0.68 ± 0.27 NS	7.51 ± 3.97 NS	ND
HFD	4.92 ± 1.42 NS	115.50 ± 42.80 NS	1.95 ± 0.34 NS	6.25 ± 2.62 NS	7.25 ± 2.47 NS

(n = 4, mean ± SEM), NS = Not Significant.

Table 4.10.2 shows serum levels of Cholesterol, Triglyceride, Urea, Creatinine and Total Carbohydrate after 28 days feed. There was no significant difference observed among the various groups. Although the LPD group shows a slight increase in

concentration of cholesterol and creatinine, and slight decrease in concentration of triglyceride and urea, the HFD group shows high concentration of cholesterol, triglyceride and creatinine and low concentration of urea. LPD + HFD group shows low concentration of triglyceride and urea and high concentration of creatine when compared with control group. Although there was no significant difference recorded, upon administration of the protein (casein and EPI), there were observable decreases in triglyceride and cholesterol; urea levels however, increased with both casein and EPI.

The effect of the various diets on some serum enzymes are presented in table 4.11. Table 4.11.1 shows serum enzyme levels of day 10 compared to the base-line. It is apparent that the general levels of the enzymes were not significantly affected by the feeding experiments.

Table 4.11.1 Serum enzyme (IU/L) levels of day 10 compared to the base-line

4 DAYS	AST	ALT	ALP	GGT
Base line	148.75±67.55 NS	65.00±21.89 NS	261.25±78.79 NS	4.00±1.15 NS
LPD	133.50±38.77 NS	37.25±10.87 NS	241.00±78.47 NS	4.00±1.15 NS
HFD	254.50±79.85 NS	53.50±15.75 NS	393.25±129.72 NS	4.00±1.15 NS

(n = 4, mean ± SEM), NS = Not Significant.

It is however noted that after 10 days feeding, there was a significant decrease in ALT of the LPD rats (Table 4.11.2) as compared to the rats that remained on the normal rat feed. The levels of the other serum enzymes were not significantly affected.

Table 4.11.2 Serum Enzymes (IU/L) level after 10 days feed

10 DAYS	AST	ALT	ALP	GGT
CONTROL	233.00±79.49 NS	81.25±27.12 NS	271.50±84.65 NS	4.00±1.15 NS
LPD	134.25±38.95 NS	37.75±10.99 NS	241.00±78.47 NS	4.00±1.15 NS
HFD	252.00±78.28 NS	53.50±15.75 NS	393.25±129.72 NS	4.00±1.15 NS

(n = 4, mean ± SEM), NS = Not Significant.

A similar pattern was observed after the 28 days feeding experiment (Table 4.11.3) However, all the enzymes activity was observe to increase in concentration when compared to the control, but upon administration of the proteins (casein and EPI) the concentration of ALT, AST, ALP and GGT decreased, while LDH concentration remain the increased.

Table 4.11.3 Serum Enzymes (IU/L) after 28 days feed

28 days	G.Glutamyl	AST	ALT	ALP	LDH
Control	9.50 ± 4.50 NS	371.75 ± 196.95 NS	62.75 ± 25.38 NS	293.00 ± 97.86 NS	1993.75 ± 1040.85 NS
LPD	14.75 ± 7.06 NS	452.00 ± 178.12 NS	128.00 ± 73.44 NS	323.00± 93.81 NS	3023.00 ± 1448.64 NS
LPD+ C	4.00 ± 1.15 NS	184.00 ± 74.07 NS	22.00 ± 9.88 NS	193.00 ± 58.66 NS	3742.00 ± 1.31 NS
LPD + PI	7.75 ± 2.65 NS	255.75 ± 148.64 NS	60.50 ± 31.87 NS	250.25 ± 92.35 NS	3925.75 ± 1.16 NS
LPD + HFD	6.75 ± 2.71 NS	210.50 ± 96.93 NS	63.25 ± 23.77 NS	304.00 ± 87.98 NS	3104.50 ± 981.23 NS
HFD + PI	4.50 ± 1.80 NS	365.50 ± 174.39 NS	65.50 ± 27.45 NS	619.75 ± 187.64 NS	3042.00 ± 6.43 NS
HFD + C	6.32 ± 2.01 NS	342.00 ± 155.26 NS	75.50 ± 31.47 NS	495.50 ± 166.63 NS	2651.75 ± 1.01 NS
HFD	10.75 ± 4.60 NS	355.75 ± 117.37 NS	85.00± 17.69 NS	486.50 ± 180.87 NS	4029.50 ± 298.6 NS

(n = 4, mean ± SEM), NS = Not Significant.

The results of the mineral analysis are presented in tables 4.12.1to 4.12.3. There was no significant difference observed between the groups on the various days of

feeding; however the malnourished rats showed low concentration of sodium, slight increase in potassium and chloride. On rehabilitation with casein and EPI, no changes were observed.

Table 4.12.1 Serum minerals (mmol/l) of day 10 compare with base line

10days	Sodium	Potassium	Chlorine
BASE LINE	145.25 ± 41.93 NS	6.90±1.99 NS	47.00±13.67 NS
LPD	156.00 ± 45.92 NS	4.60±1.35 NS	41.25±11.94 NS
HFD	147.00±42.45 NS	4.85±1.42 NS	43.00±12.44 NS

(n = 4, mean ± SEM), NS = Not Significant.

Table 4.12.2 Serum minerals (mmol/) after 10 days feed

10 days	Sodium	Potassium	Chlorine
CONTROL	145.25 ± 41.94 NS	5.50 ±1.58 NS	104.50 ± 30.17 NS
LPD	154.50 ± 45.63 NS	4.60 ± 1.35 NS	114.00 ± 33.71 NS
HFD	147.00 ± 42.45 NS	4.85 ± 1.42 NS	106.75 ± 30.84 NS

(n= 4, mean ± SEM), NS = Not Significant.

Table 4.12.3 Serum Minerals (mmol/l) after 28 days feed

28 Days	Sodium	Potassium	Chlorine
Control	151.75 ±43.83 NS	6.57 ± 2.18 NS	113.00 ± 32.66 NS
LPD	150.00 ± 43.35 NS	7.50 ± 2.16 NS	114.25 ± 32.98 NS
LPD + C	155.25 ± 44.90 NS	3.88 ± 1.19 NS	106.75 ± 30.89 NS
LPD + PI	148.00 ± 48.83 NS	9.17 ± 2.69 NS	109.00 ± 3168 NS
LPD + HFD	145.50 ± 42.20 NS	8.65 ± 2.54 NS	109.50 ± 31.84 NS
HFD + PI	145.50 ± 42.20 NS	6.95 ± 2.06 NS	107.00 ±31.02 NS
HFD + C	152.00 ± 43.88 NS	4.82 ± 1.67 NS	103.75 ± 29.95 NS
HFD	143.50 ± 41.44 NS	5.67 ± 1.80 NS	108.75 ± 31.42 NS

(n= 4, mean ± SEM), NS = Not Significant.

The cells counts of the rats' blood are presented in table 4.13. There was no significant difference that was observed when compared with baseline, except for slight decrease in MCHC with HFD, and PC with LPD (Table 4.13.1)

Table 4.13.1 Serum blood counts after 10 days feed compared with baseline

10Days	Erythrocytes	Haemoglobin	Hct	Mcv	Mch	Mchc	RDW	Platelet count
BASELINE	6.77 ± 1.96 NS	14.37± 4.19 NS	0.42 ± 0.12 NS	61.95 ± 17.89 NS	21.25 ± 6.13 NS	34.10 ± 9.85 NS	12.77 ± 3.69 NS	205.25 ± 90.75 NS
LPD	6.14 ± 1.79 NS	12.97 ± 3.97 NS	0.39 ± 0.11 NS	63.80 ± 18.43 NS	20.20 ± 6.13 NS	33.20 ± 9.64 NS	13.80 ± 3.98 NS	186.50 ± 55.32 NS
HFD	6.19 ± 2.06 NS	13.60 ± 4.06 NS	0.44 ± 0.13 NS	64.05 ± 18.52 NS	19.70 ± 5.72 NS	30.87 ± 9.01 NS	13.02 ± 3.79 NS	265.25 ± 96.49 NS

(n =4, mean ± SEM), NS = Not Significant.

Hct-hamatocrit; Mcv-Mean corpuscular volume; Mch-Mean corpuscular hemoglobin; Mchc; RDW-Red cell distribution width

Table 4.13.2 Serum blood counts after 10 days feed

10 DAYS	Erythrocytes	Haemoglobin	Hct	Mcv	Mch	Mchc	RDW	Platelet count
CONTROL	6.92 ± 2.01 NS	14.40 ± 4.15 NS	0.44 ± 0.12 NS	63.02 ±18.19 NS	21.52 ± 6.21 NS	34.22 ± 9.88 NS	12.77 ± 3.69 NS	271.50 ± 101.59 NS
LPD	6.14 ± 1.79 NS	14.92 ± 4.35 NS	0.39 ± 0.11 NS	63.87 ±18.45 NS	21.20 ± 6.13 NS	33.20 ± 9.64 NS	13.80 ± 3.98 NS	198.75 ± 60.07 NS
HFD	6.91± 2.06 NS	13.60 ± 4.06 NS	0.44 ± 0.13 NS	64.05 ±18.52 NS	19.70 ± 5.72 NS	30.87 ± 9.01 NS	13.02 ± 3.79 NS	283.75 ± 100.07 NS

(n= 4, mean ± SEM), NS = Not Significant.

Hct-hamatocrit; Mcv-Mean corpuscular volume; Mch-Mean corpuscular hemoglobin; Mchc; RDW-Red cell distribution width

The results obtained when compared with the control (table 4.13.2) were similar in pattern to those obtained for the baseline.

Table 4.13.3 Serum blood counts after 28 days feed

28 days	Erythrocytes	Haemoglobin	Hct	Mcv	Mch	Mchc	RDW	Platelet count
Control	5.19 ± 1.51 NS	13.85 ± 4.00 NS	0.26 ± 0.08 NS	48.22 ± 19.79 NS	23.45 ± 6.86 NS	39.05 ± 11.27 NS	12.15 ± 3.50 NS	200.50 ± 57.80 NS
LPD	7.21 ± 2.10 NS	14.60 ± 4.24 NS	0.47 ± 0.13* S*	65.12 ± 18.80 NS	20.27 ± 5.85 NS	20.27 ± 5.85 NS	12.52 ± 3.61 NS	400.25 ± 129.00** S**
LPD+C	6.90 ± 2.02 NS	14.90 ± 4.32 NS	0.46 ± 0.13* S*	51.62 ± 21.31 NS	24.37 ± 7.20 NS	24.37 ±7.20 NS	14.50 ± 4.70 NS	489.50 ± 144.98** S**
LPD+PI	5.59 ± 1.66 NS	12.72 ± 3.70 NS	0.37 ± 0.11 NS	64.02 ± 18.49 NS	21.20 ± 6.13 NS	21.20 ± 6.13 NS	12.25 ± 3.56 NS	631.00 ± 214.50 NS
LPD+HFD	7.02 ± 2.06 NS	13.05 ± 3.79 NS	0.43 ± 0.12* S*	61.10 ± 17.66 NS	18.42 ± 5.37 NS	18.42 ± 5.37 NS	12.06 ± 3.64 NS	663.50 ± 204.13** S**
HFD+PI	2.95 ± 0.92* S*	7.50 ± 2.1 NS	0.20 ± 0.06 NS	57.88 ± 16.70 NS	20.50 ± 5.92 NS	20.50 ± 5.92 NS	11.22 ± 3.24 NS	115.75 ± 33.41* S*
HFD+C	ND	ND	ND	ND	ND	ND	ND	ND
HFD	6.84 ± 1.98 NS	13.22 ± 3.8 NS	0.42 ± 0.12 NS	62.60 ± 18.11 NS	19.45 ± 5.61 NS	19.45 ± 5.61 NS	11.85 ± 3.43 NS	521.00 ± 173.78** S**

(n = 4, mean ± SEM), ND = not determined, NS =Not Significant, S* = Significant, ($p<0.05$), S** = Significant ($p<0.01$). Hct-hematocrit; Mcv-Mean corpuscular volume; Mch-Mean corpuscular hemoglobin; Mchc; RDW-Red cell distribution width.

Table 4.13.3 shows blood count of day 28. Serum concentration of MCV, Hb, EC and MCH decreased in malnourished rat when compared with the control group. HFD fed shows low concentration in MCH, MHCH and RDW while EC and MCV increased in concentration. After administration of casein and EPI the platelet count increased significantly.

Table 4.14.1 Serum white blood cell after 10 days feed

10 DAYS	LCC	NTP	LPC	MC	ESP	BP
CONTROL	3.80 ± 1.40 NS	1.82 ± 0.79 NS	1.158 ± 0.52 NS	0.19 ± 0.06 NS	0.15 ± 0.05 NS	0.04 ± 0.01 NS
LPD	3.23 ± 0.98 NS	0.59 ± 0.20** S**	2.28 ± 0.72 NS	0.36 ± 0.11* S*	0.00 ± 0.00** S**	0.00 ± 0.00** S**
HFD	5.50 ± 2.01 NS	1.11 ± 0.46 NS	4.01 ± 1.48** S**	0.33 ± 0.11* S*	0.01 ± 0.01** S**	0.01 ± 0.01** S**

(n = 4, mean ± SEM) S* = Significant ($p<0.05$) S** = Significant ($p<0.01$), NS Not Significant. LCC-leucocyte cells; NTP-neutrophils; LPC-lymphocytes; MC-monocytes; ESP-eosinophils; BP-basophils

Table 4.14.1 shows serum White blood cells of day 10. A significant difference was observed in LPD fed rat with decrease in concentration in neutrophils (NTP), monocytes (MC), eosinophils(ESP) and basophils(BP) while, HFD fed rats also

showed a significant difference with decrease in concentration in eosinophils and basoplis and increase in concentration in lymphocytes (LPC), and monocyte.

Table 4.14.2 Serum White blood cells after 28 days feed

28 days	Leucocyte C	Neutrophils	Lymphocytes	Monocytes	Eosinophil	Basophils
Control	5.1 ± 1.47 NS	1.16 ± 0.35 NS	2.94 ± 0.91 NS	0.10 ± 0.03 NS	0.17 ± 0.05 NS	0.02 ± 0.01 NS
LPD	4.47 ± 1.32 NS	0.18 ± 0.06** S**	3.11 ± 0.92 NS	1.12 ± 0.42** S**	0.03 ± 0.01** S**	0.01 ± 0.01* S*
LPD+C	4.07 ± 1.18 NS	1.08 ± 0.48 NS	2.49 ± 0.74 NS	2.67 ± 2.06* S*	0.38 ± 0.38 NS	0.02 ± 0.01 NS
LPD+PI	3.22 ± 0.94* S*	1.18 ± 0.64 NS	1.63 ± 0.66* S*	0.18 ± 0.08 NS	0.04 ± 0.01** S**	0.01 ± 0.00** S**
LPD+ HFD	5.87 ± 1.75 NS	0.85 ± 0.35 NS	3.48 ± 1.26 NS	0.30 ± 0.11** S**	0.01 ± 0.01** S**	0.04 ± 0.02 NS
HFD+C	ND	ND	ND	ND	ND	ND
HFD	6.55 ± 1.96 NS	0.55 ± 0.35* S*	5.10 ± 1.61* S*	1.63 ± 1.11* S*	0.03 ± 0.01** S**	0.05 ± 0.02* S*

(n = 4, mean ± SEM), ND = Not Determined, S* = Significant, ($p<0.05$) S** = Significant ($p<0.01$) NS = Not Significant.

Table 4.14.2 shows activity of serum White blood cells of day 28, significant difference was observed in both LPD and HFD fed rats with neutrophils, monocytes, eosinophils and basophils with slight decrease in concentration when compared with control, on administration of casein an EPI, a significant difference was observed in LPD +PI with decrease in all except for neutrophil and monocyte.

4.15 The Microscopic examination of the liver and kidney

The result obtained from the histological parameter of kidney and liver of rats fed on various diets are presented in fig 4.3.1 to 4.4.4.

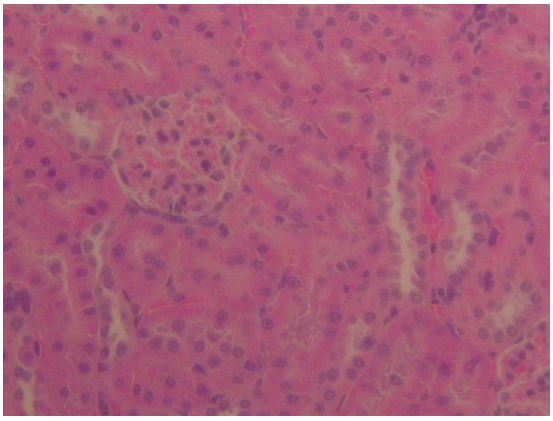

Figure 4.3.1
Kidney Control
(640 × 480 pixels)
(n = 4, mean ± SEM)

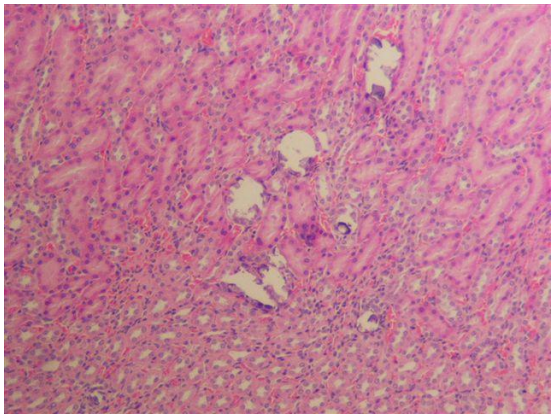

Figure 4.3.2
Kidney LPD
(640 × 480 pixels)
(n =4, mean ± SEM)

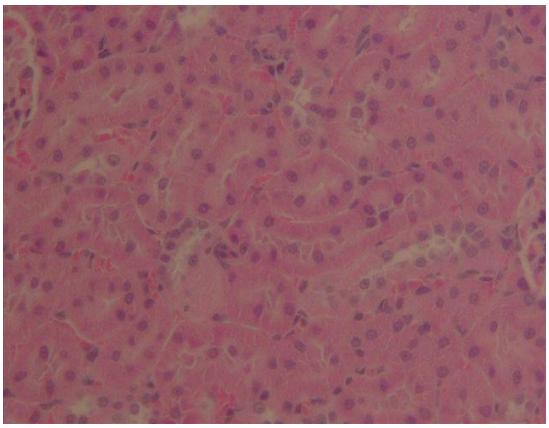

Figure 4.3.3
LPD +PI Kidney
(640 × 480 pixels)
(n = 4, mean ± SEM)

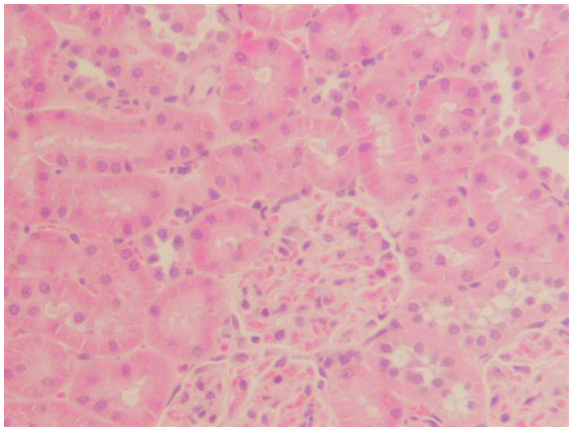

Figure 4.3.4
HFD + PI Kidney
(640 × 480 pixels)
(n = 4, mean ± SEM)

Fig 4.3.1 shows the microscopic examination of control kidney with no significant histological lesion was observed.

Fig 4.3.2 shows the microscopic examination of LPD kidney with very mild multifocal intratubular renal mineral deposition / calcification of some proximal convoluted tubules.

Fig 4.3.3 and Fig 4.3.4 show that upon administration of EPI, LPD + PI was able to reverse the change with no significant lesion observed while HFD + PI could not, but shows very mild multifocal intratubular renal deposit / calcification of some proximal convoluted tubules –mostly at the corticomedullary junction

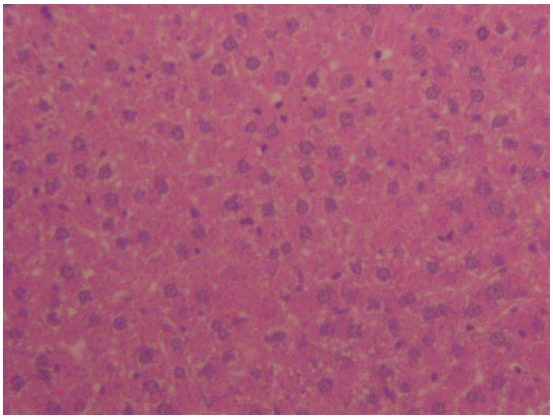

Figure 4.4.1
Liver Control
(640 × 480 pixels)
(n= 4, mean ± SEM)

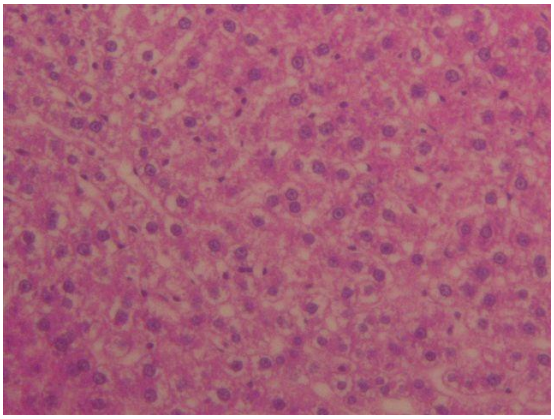

Figure 4.4.2
Liver LPD
(640 × 480 pixels)
(n = 4, mean ± SEM)

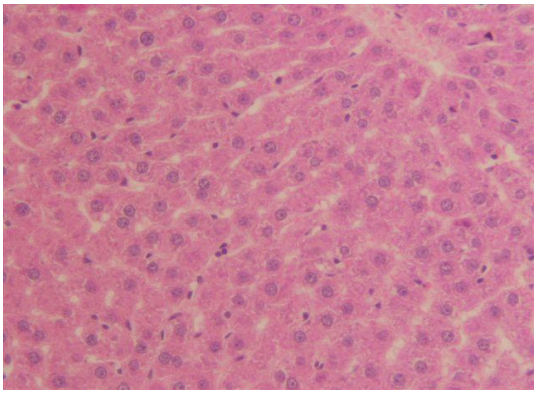

Figure 4.4.3
LPD +PI Liver
(640 × 480 pixels)
(n = 4, mean ± SEM)

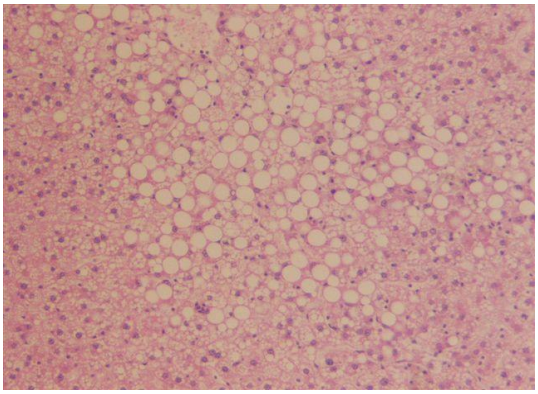

Figure 4.4.4
HFD + PI Liver
(640 × 480 pixels)
(n = 4, mean ± SEM)

Fig 4.4.1 shows the microscopic examination of control liver with no significant histological lesion was observed.

Fig 4.4.2 shows the microscopic examination of LPD liver with mild sinusoidal and portal congestion, mild diffuse hydropic degeneration and very mild suspect fatty changes in hepatocytes.

Figure 4.4.3 and Fig 4.4.4 shows that upon administration of EPI, LPD + PI was able to reverse the change with no histological lesion observed while HFD + PI could not, but showed variables but mostly mild hydropic degeneration and fatty changes of hepatocytes were observed.

CHAPTER FIVE

DISCUSSIONS

Proteins are the most important constituents of human diets because of their ability to provide the amino acids needed by the body to synthesize, repair and maintain its cells (FAO 1990).However, the cost and availability of conventional proteins have directed the attention of both governmental and non-governmental organizations to the search for new sources of protein. It is apparent that the protein isolate obtained from Egusi has the potential to be used as protein supplier to endemic protein undernourished groups: The proximate composition of the isolate is similar to those reported for other oil-seed isolates (Ige *et al*. 1984; Aremu *et al,*2006;Olaofe *et al*1994;Badifu and Ogunsua 1991). Oyenuga (1998) suggested that seed flour with low moisture content will have a long shelf life since the isolate would be free from microbial attack. The ash contents of 3.9% is low compared to the value recorded by Aremu *et al*(2006) for fluted pumpkin and Omosuli *et al*(2009) for defatted cashew nut flour. The value is closed to the report of Aletor and Aladetimi (1989) 3.0 to 3.87% for some cowpea variety, but in close agreement with Ekeayanwu (2010) 3.9% for Africa nutmeg and Essien et al (2010) for Egusi seeds. Pomeranz and Clifton (1981) establish that ash content of nuts, seeds and tuber should fall inthe range of 1.5—2.5% for it to be suitable for animal feeds. The carbohydrate content of EPI is similar to that of *C.melo* (Loukou *et al., 2007),* and Africa nutmeg (Ekeanyanwu *et al,*2010). The protein content of the egusi isolate(63.4%) is relatively higher, in comparison to 33.8% crude protein of the melon seeds (Ogbonna *et al 2009)* and*L. Siceraria* seeds (Ogundele and Oshodi 2010). The protein content of

EPI was however lower than soy protein isolate (Okezie and Bello, 1988),*Kargo* seed (Akin-Osanaiye 2009) and *M. Oleifera* seed (Govardhan *et al* 2010).

Minerals play important role in homeostasis (Abulude, 2001). The analysis of the elements of the isolate shows appreciable amount of the elements; the low levels of Co, Cu, Zn, Mn and Fe, could be attributed to the loss in the extraction process.

The broad spectrum of amino acids (table 4.5) and the functional properties (table 4.3) observed are indication of a good quality protein. According to Osundahunsi *et al.,* (2003)the ability of the isolate to absorb water and swell is desirable in food systems to improve the yield and consistency and give body to the food. The Egusi isolate showed good water absorption capacity; a value that compared with soy protein isolates (Okezie and Bello 1988). It is apparent that EPI could possess a great ability to swell, dissociate and unfold exposing additional binding site since carbohydrate, and most other non-protein component that could impair it has been removed (Kinsella 1979). This then suggests that any formulation with the isolate will have body and texture that would be desirable because of the good water biding capacity. Moure *et al* (2006) stated that protein solubility is influences the hydroplilicity / hydrophobicity balance, which depends on the amino composition, particularly at the protein surface. Higher solubility is related with the presence of a low number of hydrophobic residues, the elevated charge and the electrostatic repulsion and ionic hydration occurring at pH above and below isoelectric pH.

Kinsella (1979) also explained that the ability of protein to bind fat is very important for such applications as meat replacement and extender mainly because it enhances flavor and improves mouth feels. Also isolates with high 0AC can be a good candidate in cold meat industry especially sausages, where protein can bind the fat

and water in these products (Ogunwolu *et al*., 2009).This then suggests that the Egusi isolate can be used in the formulation of feed formula.

According to Damodaran (1997) foam properties are greatly enhanced by protein concentration; the higher the concentration, the greater the volume. Proteins increase the viscosity and facilitate the formulation of a multilayer, cohesive protein film at the interface.The poor-foaming properties of EPI implies that EPI would not be good for application in foods like beverages, candies, confections, cakes and coffee because they require proteins with relatively high foaming properties (Paul and Southgate 1980). However, low-foaming capacity and stability properties prevent any gastrointestinal troubles like flatulence associated with feed formula; hence the isolate can be suggested for weaning formula.

Fagbemi (1991) indicated that, low bulk density isa greater package advantage, as a greater quantity may be packed within a constant volume. Kruse *et al.,* (2001) also attributed this to the fact that since protein isolate is rich in protein because most of its carbohydrate and other non-protein that usually increases the bulk density have been removed, this will thus facilitate the transportation of the isolates. The Egusi isolate have low bulk density this thus suggest that the cost of package and transportation will reduce and this enhances the assessability of the targeted group.

Solubility profile of the Egusi protein isolate is pH dependent, the isoelectric point of the isolate is between pH 5--7, above or below which the protein will precipitated out. Basha and Pancholy (1982) stated at pHvalue above and below the isoelectic pH protein carry a net charge; electrostatic repulsion and ionic hydration promote solubilisation of protein, for most protein, minimum solubility occurs at their pI region,

where electrostatic repulsion and ionic hydration are minimum and hydrophobic interaction between surface nonpolar patches is maximum.

In meeting the dietary requirement of man protein is not important, if it cannot supply the essential or indispensable amino acids (the building blocks of protein), Behrman and Vaughan (1993) documented that infants have very critical nutritional requirements because of their rapid growth and immaturity of gastrointestinal function. According to Behrman and Vaughan (1993) infants needed nine essential amino acids for growth and maintenance; histidine is limiting amino acids in the standard reference casein. It is noteworthy that EPI is rich in histidine. Glutamic was the most prominent amino acid in the EPI. Similar results have been reported by Olaofe *et al.* (1994) and Adeyeye (1997). The Egusi protein isolate is high in other essential amino acids like arginine, threonine, valine. However, EPI is low in leucine, isoleucine, lysine, phenylalanine and methionine.

The performance of the rats fed the EPI is an indication of the biological quality of the protein. The rats fed with rat-feed were steady in growth while those fed with low protein diets decreased in body weight, this was expected since the rat -feed was a balanced meal and low protein diet was poor in protein. However, those fed with LPD + PI and LPD + C, gained weight, LPD + C was better because it was animal protein.The HFD fed rat gained more weight than any other group, when compared with the control group. Similar observations havebeen reported by Prasad (2005) and Park *et al.* (2009).

There were no significant changes (except bilirubin, triglycerides and cholesterol) in the blood parameters measured in the various groups over the 28 days feeding period. It is possible that the feeding time was very short for such changes to occur.

There were, however, decreases in such parameters as neutrophils, monocytes, eosinophils, basophils with LPD and eosinopils, and basophils eosinophils with HFD and increases in lymphocytes and monocytes with HFD indicating damage to the internal organs (liver and kidney). For example, the elevated bilirubin shows billiary or hepatocellular obstruction which indicated that the excretory function of the liver had been impaired; the elevated enzymes in the malnourished rats indicated that the liver had been injured or damaged resulting in enzyme leakage from the liver into serum. The liver cells damaged during malnutrition, are usually confirmed by the AST and ALT analysis of the liver. The increase in concentration observed in serum ALT and AST thus confirmed the hepatocellular damage. ALP and LDH are found in several tissues throughout the body, they are also important for diagnosis of liver diseases. The serum enzyme concentration in LPD was expected to be higher than that of HFD and control due to low protein in LPD hence there is a degradation of organ protein for vital need Gupta *et al* (1994). This shows that the levels of AST and ALT on liver and serum are greatly influenced by dietary proteins.

LDH is an enzyme that is found in almost all the body cells, use as a general marker of cell injury, elevated LDH could not be lowered with both proteins probably because of short time frame, 28 days. This research was designed for this period of time because similar research in literature uses the same time frame. However, upon the administration of EPI the serum concentration of protein and albumin increased, and the concentration of ALT, AST, ALP and GGT decreased. It is apparent that the protein isolate was able to reverse the damage done by low protein diets. The histology of these organs (figure 4.3.1 – 4.4.4) revealed the slight damage done by malnutrition, according to the pathologist this could be due to time frame.

Blood electrolytes are important in the maintenance of the functional integrity of cells. Cell membranes depletion of these ions, either singly or in combination, is associated with many pathological conditions, which in some cases may be fatal (Tiez, 1986). The lack of protein in the diets of malnourished children results in electrolyte abnormalities that lead to edema. This classical feature of kwashiorkor is a result of increase in permeability of a membrane to sodium, this also lead to low level of antioxidants which are likely to allow membrane alteration to occur, hence lipidperoxidation is enhanced. In this study, it was observed that the serum of malnourished rat show low concentration of sodium, slight increase in potassium and chloride. On rehabilitation with Egusi protein isolate and casein as standard, no significant differences were observed.

Although there were no significant changes in the cholesterol, triglyceride, urea, creatinine and the blood cells count levels in the rehabilitated rats, it is apparent that the EPI is a good protein and that the isolate could be used to fortify low protein diets to combat malnutrition most especially in developing countries where protein of animal source are not readily available. It might, however, be necessary to supplement the protein isolate with minerals, since some elements were not detected or possibly lost during extraction process.

CHAPTER SIX

CONCLUSION

In the exploration of Egusi seed protein isolate as a high protein sources to supplements the diet of protein malnourished children, the following conclusion were drawn:

---

1. The protein isolate has a high (63.4%) protein content.
2. The Egusi protein isolate possess valuable functional properties like

   (a) Low foaming capacity and stability which could prevent gastro-intestinal troubles like flatulence in food formula.

   (b) Good water binding capacities suggest that any formulation with the isolate will have body and texture that would be desirable.

   (c) Good oil absorbability enables it to be used as meat extender which enhances flavour and improve mouth feels.

   (d) Low bulk density reduces cost of package and transportation thus increases the accessibility to the targeted group.
3. The amino acids composition of the isolate shows that it comprises of 16 amino acids of which 9 are essentials and 7 non essential amino acids and also the isolate is rich in Histidine which is limiting amino acids in children.
4. The malnourished animals fed with Egusi protein isolate gained an appreciable weight which compares favourably well with casein an animal protein and commercial rat feed.
5. The malnourished animal's serum analysis shows high levels of serum enzymes most especially AST and ALT, after rehabilitation with protein isolate, this high concentration was normalized.

It is concluded that the Egusi protein isolate administration was effective in alleviating the detrimental effects associated with protein malnutrition.

Bibliography

Abulude, F.O. (2001). Mineral and Phytate content of vegetables grown in Nigeria and calculation of their phytate Zn and Ca: Phytate molar ratio *Adv Food Sci*, 23: 36—39.

Achu, M. B., Fokou E., Tchieang C., Fotso M., Tchouanguep F. M., Nutritive value of some cucurbitaceae oil seeds from different regions in Cameroun. *Afri. J. Biotechnol*. (2005). 4 (10): 1329—1334. Sativus oils from Cameroon. Afri.*J. of Food Sci*. Vol. (2) pp 021—025.

Achu M. B., (2006). A study of some Physciochemical characteristics and Nutritional Properties of five cucurbitaceae oil seeds from Cameroun. Thesis presented and defended for the award of Doctorate PhD in Biochemistry. Faculty of science, University of Yaounde 1.P. 180.

Achu, M. B., Fokou, E., Tcieang, C., Fotso, M., Tchouanguep F. M., (2008). Antherogenicity Cucumeropsis manii and Cucumins

Adeyeye, E. I. (1997).Amino acid composition of six variety of dehulled African yam bean (Spjenostylis stenocarpa) flour, *int J. Food.Sci. Nutri.*48: 345—351.

Akinola, F. F., Oguntibeju, O. O. and Alabi, O. O. Effect of severe malnutrition on oxidative stress in Wistar rats. (2010)*Sci. Res. and Ess* Vol 5 (10), pp 1145—1149, 18 May.

Akpapunam, M.A., and Markakis, P., (1981).Physciochemical and Nutritional aspects of cowpea flour.*Journal of Food Science* 46: 972—973.

Akpata, M. I. and P. I. Akubo, (1999). Chemical composition and selected functional properties of sweet orange (Citrus sinensis) seed flour. *Plant Food Hum.Nutr.,* 54: 353—362.

Aletor, V. A. And Aladetimi O. O. (1989) Compositional evaluation of some cowpea varieties and some underutilized legumes in Nigeria Nahrug. 10:999—1007.

AOAC, (1990).Method of analysis of the Association of Official Analytical Chemists 15th editon.Washington D. C.

Aremu, M. O., Olaofe, O. and Akintayo, E. T. (2006). Compositional evaluation of cowpea *(Vigria unguiculata)* and scarlet runner bean *(Phaseolus coccineou)* varieties grown in Nigeria. *J. Food Agric.* Env. 4(2):39-- 43.

Ash, M. and Dohlman, E. (2006). Oil crops situation and outlook year book Electronic out look report from the economic research service. United State Dept. of Agriculture.

Bankole, S. A., A. A. Osho, A. O. Joda, and O. A. Enikuomehin, (2005). Effects of drying method on storability of egusi melon seeds (Colocynthis citrullus L.).

Basha, S. M. and S. K. Pancholy, (1982).Composition and Characteristics of basic protein from peanut (Arachis hypogaea L.)*J. Agric. Food Chem*, 30: 1176—1179.

Basu, S., (2003) Carbon tetrachloride-induced lipid peroxidation: Eicosanoid formation and their regulation by antioxidants nutriends.*Toxicolog,* 189: 113—127.y

Barons, D. N. (1987)., A short Textbook of chemical pathology Ed. London, Hodder and Stoughon Richard Clay.

Bender, A., (1992). Meat and meat products in Human Nutrition paper 53, FAO /UN, Rome.

Bendich, A., (1993). Physiological role of antioxidants in the immune system. *J. Dairy Sci*., 76: 2789—2794.

Behman, R. Vaughan, V. Nelson and Nutritional Disorders In*NelsonTextbook of Pediatrics:* 12th edition; Nelson, W. Ed; W. Saunders Co; Philadelphia, P.A., 1983; pp 138—139.

Bera, M. B. and Mukherjee, R. K. (1989).v Solubility, emulsifying and foaming properties of rice bran protein concentrates *J. Food sci.* 54, 142—145.

Berkow, and Robert (1999). The Merck Manual of Medical Information.House station .*NJ Merck Research* pp 104—108.

Beuchat, L. R. (1977). Functional and Electrophoretic characteristics of succinylated peanut flour protein *J. Agric. Food Chem* 25: 258.

Birch, G. G., S. E. Kemp.(1989). Apparent Specific volumes and tastes of amino acids chemical Sense, 14(2), pp249—258.

Bourdon, Emmanuel and Blache, Dennis, Antioxidant and Redox signalling No 2, (2001) Mary Ann Lieberts incl. Importance of Protein in Defence Against Oxidation.

Cegla, G. F., Meinke, W. W. and Matitil, K. F., (1977). Composition and Characreristics of Aquous Extracted Textured Vegetable Protein Flours: Soy and Cottonseed. *J Food Sci*. 42: 807—813.

Damodaran, S. (1997) Food Proteins: An overview In S. Damodaran and A. Paraf (Eds). Food proteins and their application (pp 1—21) New York: Marcel Dekker.

De La Cruz J. P., Antioxidant potential of evening primrose oil administration in hyperlipemic rabbit's life science vol 65 Pp 543—55, (1999)

Ekeanyanwu, R. C., Njoku, O. and Ononogbu, I. K.(2010), The Phytochemical composition and some Biochemical Effects of Nigerian Tigernut (*Cyperus esculentus L)* Tuber. *Pakistan Journal of Nutrition***9** (7): 707—715.

Eromosele, C. O. (1997) *Biochemical and nutritionalcharacteristics of seed oil from wild plant.* Proceeding of 2nd international workshop and African Pear improvement and other New sources of veg oil Ngaoundere Cameroun pp 203—208.

Essien, E. B., Amaefule, O. I. and Ihenacho, E. (2010) Proximate Analysis and Physico-chemical properties of water melon *(Citrullus lanatus)* seeds Nigeria *Journal ofBiochemistry and Molecular Biology***24**(2): 6—10.

Etukudo, M. H., E. O. Agbedana, O. O. Akinyinka, and B. O. Osifo (1999). Plasma electrolyte, total cholesterol, liver enzymes and selected antioxidant status in Protein Energy Malnutrition. *Af. J. Med. Sci.,***28**: 81—85

Fagbemi, T. N. and A. Oshodi, A. A. (1991) Chemical composition and functional properties of full fat fluted Pumpkin seed flour (Telfairia occidentalis). *Nigerian Food Journal*, pp: 9.

F.A.O. / W.H.O. /U.N.U. (1985)Energy and Protein Requirements W.H.O. Technical Report series No 724, W.H.O., Geneva.

F.A.O. /W.H.O (1990) Protein Quality Evaluation Rome

F.A.O. / W.H.O (1991) Protein Quality Evaluation F.A.O. Food and Nutrition page 51, F.A.O. / U.N. Rome.

Fokou, E. Achu, M.B. Tchouanguep, (2004) M.P. Preliminary nutritional evaluation of five species of egusi seeds in Cameroun. *African Journal of food, Agriculture Nutrition and Development*.**4** (1): 11 (s).

Fasuyi, A.O., Aletor, V., (2005): Varietal composition and functional properties of cassava (*Manihot esculenta*, Cranzt) leaf meal and leaf protein concentrates. *Pakistan Journal of Nutrition***4** (1): 43-49

Frankel, E., (1995): Nutritional Benefits of Flavonoids, International Conference on Food Factors Chemistry and Cancer Prevention, Hamatsu Japan, Abstract C6—2.

Gill, N., Bansal, M., Grag, S., Sood, A., Muthuraman and Bali, M., (2010): Evaluation of antioxidant, anti-inflammatory and analgesic potential of *Citrullus lanatus* seed extract in rodent model. *Theinternet Journal of Nutrition and Wellness.***9**(2).

Golden, M.H.N. and Ramdath, D. (1987) Free radical in the pathogenesis of Kwashiorkor *Proc Nutr. Soc*. **46**: 53—68

Gopalan, C (1993). Child care in India: emerging challenges *Yoyana***37**: 6—8.

Gopalan, C (2001).Achieving household nutrition security in societies in transition an overview.*Asian Pac J Clin Nutr***10**: S4—S12.

Govardhan Singh R.S., Ogunsina, B. S., and Radhac, C., (2010). Protein extractability from deffated Moringa Oleifera Lam seeds flour *Ife Journal of Science*13 :1.

Graham, D.E and M.C. Philip (1976).The conformation of Protein at the Air – water interphase and their role. Akers, R. I (Ed), Stabilizing foams New York Academies Press, pp 237---255.

Hays, N.P., R.D. Starling, X. Liu, D. H. Sullivan, T. A. Trappe, J. D. Fluckey and Evan, W. J. (2004). Effects of an Ad Libitum low fat, high carbohydrate diet on body weight, body composition and fat distribution in older men and women. *Arch Intern Med*. **164**: 210—217.

Halliwell, B and Chirico S (1993), S. Lipid, peroxidation: Its mechanism, measurement and significance. *Am, J. Clin.Nutr*.**57**: 715S—724.

Halliwell B., (1996). Oxidative stress, nutrition and health experimental strategies for optimization of nutritional antioxidant intake in human.

Free Radical Resolution. **25**: 57—74.

Halliwell B., J.M. Gautheridge and C.E., Cross (1992). Free radical and antioxidants and human diseases; Where are we now? *J.lab clin.med.,* **119**; 598- 620.

Hanseh J. D. L. (1993). Protein Energy Manutrition in perspective *S. Afr J. Food Sxi Nutr*.(1993). Vol 5 no 1 p2—4.

Hansler, C. M. (1998). Functional foods: their role in diseases prevention and health promotion food *Technol* 52: 63—69.

Ibukun-Olu-Alade (2001).Public Health Nutrition 2nd ed. Tosco Press, Nigeria, pp 107—112.

Ige, M. M., Ogunsua, A. O. and Oke, O. L. (1994), Functional properties of the proteins of some Nigeria oil seeds Conophor seeds; and three varieties of Melon seeds.*Journal of Agricultural and Food Chemistry*.**32**: 822—825.

Jimoh, K. O., Oladunjoye, O.P. (2009). Evaluation of physicochemical and rheological characteristics of Soybean fortified yam flour *J. Appl. Biosci*, **13**: 703—706.

Kerr, J. F. R., Wyllie, A. H. and Currie, A. R., (1972).Apoptosis?a basic biological phenomenom with wide ranging implication in tissue kinectics. *British Journal of Cancer*; **26**: 239—257.

Kincheloe, L (1997). Herbal medicine can reduce cost in HMO *Herbalgralm*: 41: 49.

Kinsella, J. E. (1976).Functional properties of protein foods.Critical Reviews' in *Food Science and Nutrition* 1: 219—229.

Kinsella, J. E. (1979). Functional properties of Soy proteins *J. Amer. Oil Chem. Soc.,* 56, 242—58.

Kinsella J. E., S. Damodaran and German, B. (1985). Physicochemical and Functional properties of oil seed Proteins with Emphasis on Soy proteins. In: New Protein Foods, Alschul, A. M. And H. L. Wilke [Eds] vol 5, New York, Academic press, pp 107—179.

Kruse, J. P., Bagger and K. D., Schwenke, (2001). Reheological properties of modified lupin proteins.Naung / Food, 45: 412—415.

Lawhon, J. T. C., M. Carter, and Maltil, K. F. (1972). A comparative study of whipping potential of an extract from several oil seed flours cereal *science today* 17: 240.

Lin, M. J. Y., Humbert, E. S. and Sosulki F. W. (1974).certain functional properties of sunflower meal products. *Journal of Food Science* 39: 368—370.

Lohlum, S.A., Maikid,i G. H. and Solomon, M. (2010). Proximate composition, amino acid profile and phytochemical screening of Laphira 1Lanceloata seeds vol. 10 (1) *AFJAND 61*: 395—400.

Lowry, O. H., Rosenbrouh, N. J., Farr, A., Randall, R. J. (1951). Protein measurement and Folin phenol reagent.*J. Biol. Chem*. 193: 265—275.

Mabberley, D. I., (1987).The Plant Book. Cambridge: Cambridge Univ. Press, New York.

Manary, M. J., Leeunwenburghh, C, Heinecke, J. W. (2000). Increased stress in Kwashiorkor. *J. Paed*, 137: 421—424.

Majno G., and Jorris I., (1995) Apptosis, oncosis and necrosis.An overview of cell death.*Am. J. Panthol*; 146: 3—15.

Michealsen, K. F. and Hendrick, F. (1998). Complementary feeding: A global perspective. *Nutr*. 14 (10): 763—766. Products: A review Food International Research International 39: 945—963.

Morr, C. V., (1989). Whey Proteins: Manufacture, In: Fox, P. F., (Eds). Developments in Dairy chemistry 4: Proteins. Elsevier science Publishers, London p 245—248.

Moure, A., Sineiro, J., Dominguez, and Parajo, J. C., (2006). Functionality of oil seed protein

Murkovic, M., Hillebrand A., Winkler, J., Leitne,r E., Pfannhauser, W., (1996). Variability o fatty Acid content in Pumpkin seeds.(Cucurbita.Pepo. L.

Zeitschrift- fuer –Lebensmittel – Untersuchung –und – Forschum 203 (3): 216—219.

Nayarama, M. Narasinga, S. (1984). Effect of partial proteolysis on the functional properties of winged bean (Phorsphocarpus tetragonolobus) flour. *J. Food Sci*. 49: 944—947.

Nkosi, C. Z. Opoku, A. R. Terblanche, S. E. (2005). Effect of pumpkin seed (cucurbita pepo) protein isolate on the activity levels of certain plasma enzymes in CCI_4 induced liver injury in low protein fed rats *Phytother Res* 19: 341—345.

Ogbonna, P. E. (2009). Yeild response of Egusi Melon (*Colocynthis citrullus L*.) to rate of NPK 15:15: 15 Fertilizer *Am. Eurasia J. Sustain Agrc* 3 (4): 764—770.

Ogunwolu, S. O., Henshaw, F. O., Hans, – Peter N., Andrea Santos. (2009) Functional properties of protein concentrate and isolates produced from cashew (Anacardium occidentale L.) nut. *Food chemistry* 115 852—858.

Oguntayo, O. D., Otulana, J. O., Adesanya, A. O., Olaseinde O. O. and Adefule, A. K., (2011) Effect of Methanolic Extract of *Citrullus lanatus* Seed of on Experimentally Induced Prostatic Hyperplasia. *European Journal Medicinal Plants* 1(4): 171—179.

Ojieh, G. C., Oluba, O. M., Ogunlana, Y. R., Adebisi, K. E., Eidangbe, G. O, and Orole, R. T., (2008), Compositional studies of C. lana (Egusi) melon seed. *The internent Journal of Wellness* 6 (1).

Oluba, M. O., Adeyeye, O, Ojieh G. C. Isiosio I. O., (2008). Fatty acid composition of Citrullus lanatus (egusi) oil and its effect on serum lipids and some serum enzymes.*The internet J. Cardiovascular Res*. (5): 2.

Okezie, B. O. and Bello, A. B., (1988). Physicochemical and functional Cardiovascular Res., properties of winged bean flour and isolate compare with soy isolate. *J.Food Sci*. 53 (1): 450—454.

Okonkwo, J. N. Tochukwu, and Okonkwo, J. O. Chinedu, (2009). Antioxidant Properties of Diospyros Preussi (Ebenaceae Gurke) Seed oil. Tropical *Journal of Pharm. Research*, 8(6): 551—555.

Olaofe, O., Adeyem,i F. O. and Adediran, G. O. (1994), Amino acid, mineral composition and functional properties of some oil seeds *J. Agric, Food chemistry*, 42 (4), 879—881.

Osundahunsi, O. F., Fagbem,i T. N. Kesselman, E., Shirmoni, E. (2003). Comparison of the physicochemical properties and pasting characteristics of flour and starch from red and while sweet potato cultivar. *J. Agric Food Chem*., 51: 2232—2236.

Onweluzo, J. C., Obanu, Z. A. and Onuoha, K. C., (1994), Functional properties of some lesser known tropical legume.*Journal Food Sci Tech*. 31: 302—306.

Park, C. H., Cho, E. J. and T. Yokozwana, (2009). Protection against hypercholesterolemia by cornifructus extract and its related protective mechanism *J. Med. Food*, 12: 973—981

Paul A.A. and South gate D. A. T., (1978).McCance and Widdowsons.The composition of foods 4th edition. In: Food composition data production,

management and use H. Greenfield and D. A. T Southgate (eds) Elsevier Applied Science, London, pp: 227—228.

Peter T. J. (1996) All about Albumin, San Diego Academics Press.

Phadke, M. A., Khedkar, V. A., Pashankar, D., Kate, S. L., Mokashi, G. D. And Gambhir, P. S., Serum amino acids and genesis of protein energy malnutrition *Indian Pediatr* (1995); 32—301—6.

Pollitt, E. (1990).Malnutrition and infection in the classroom.United Nations Educational, Scientific and cultural Organization (UNESCO), Belgium.

Pomeranz, Y. and Clifton, D. (1981). In Mellon, E. E. Ed. Food analysis theory and practice West port C. T: AVI Publishing company.

Prasad, K (2005). Hypocholesterolemic and antiatherosclerotic effect of lignan complex isolated from flax seed. Antherosclerosis 179, 269—275.

Prata, F. J. A., Masedo, D. V., Rostrom, Mello, M. A. (2007).Oxidative stress during rehabilitation from protein malnutrition associated with aerobic exercise in rats *Braz. Arch. Biol. Technol*. 50: 1—12.

Pitrat, M., Chauvet, M. and Foury, C. (1999). Diversity, History and production of cultivated cucurbits *Acta Hort* 492: 21—28.

Ruano – Ravina A. Figueiras A, Freire – Garabal M.And Barros – Dios J. (2006), Antioxidant vitamin and risk of lung cancer, *Pharm Res* 12 (5); 599—613.

Scherbaum and Furst (2000). Protein – energy malnutrition. Curr Opin *Clin. Nutr.Metab. Care* 3; 31—38.

Scheraga, H. A., G. Nemethy, and Steinberg, I. Z. (1962).The contribution of hydrophobic bonds to the stability of protein conformation *J. Biol. Chem,* 237: 2506—2508.

Singh, U. V. P. Rao, N. Subrahmanuam and Sexena (1993). Cooking, chemical composition and protein quality of newly developed genotypes of pigeon pea (Cajanus cajan L.) *J. Sci. Food Agric* 61; 395—400.

Sindayikengera Severin, XIA Wen – Shui. (2006) Nutritional evaluation of casein s and whey protein, *Journal of Zhejiang University science* 7 (2): 90 – 98.

Sohal, R. (2002). Role of oxidative stress; and protein oxidation in the aging process, Free Radic :*Biol Med* 33 (1); 37—44.

Soriani, M., Pietrafore, D. and Minett, M (1994). Antioxidant potential of anaerobic human plasma: role of serum albumin and thiols as scavengers of carbon radicals. *Arch Biochem Biophys* 312: 180—188.

Silou, T., Mampoua, D., Loka, Lonyange, W. D. and Saadou, M. (1999). Composition globale et caracteristiques des huiles extraits de 5 especes de Cucurbitacees du Niger La Rivista Italiana Delle Sostance Grasse. LXXVI: 141—144.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros, L. and Byrne, D. H., (2006) Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruits extracts. *J Food Compos Anal*: 19: 669—675. (s)

Thompson, W. A. B. Infant Formulas and the use of Vegetables Protein.*J. Am oil Chem*. Soc 56: 386 (1979).

Tietz, N. W. (1986). *Textbook of Clinical Chemistry*, 3rd edn Philadelphia; W. B. Saunders.945—974.

Touyz, R. M. (2000).Oxidative stress and vascular damage in hypertension. Curr.*Hypertention* 2; 98-- 105.

U. N. I. C. E. F. (United Nation Children Funds) (1989), The UN convention on the Right of the child Adopted and opened for signature, ratification and accession by General Assembly resolution 44/ 25 of 20 Nov 1989.

Uruakpa, F. O. and Aluko, R.E. (2004). Heat induced gelation of white egusi (colocynth citrullus L.) seeds *Food chem*. , 87, 349—354.

Valko M., Morris H., Cronin M. (2005).Metal, toxicity and oxidative stress. *Curr. Med. Chem*. 12(10): 1161—1208.

Vertuani, S., August,i A., Man-Freclin,i S. (2004). The antioxidants and pro antioxidants network: an overview. *Curr. Pharm. Res*. 10 (14): 1677—1694.

WHO/FAO, (1973).Food and nutrition terminology, definition of selected terms and expressions in current use. WHO, Geneval (NUTR 173,2).

World Health Organization (2000).Turning the tide of malnutrition, responding to the challenge of the 21st century.Geneva Switzerland W.H.O. (WHO/ NHD/ 00. 7.

William, C. D. Kwashiokor: a nutritional diseases of children associated with a maize diet *Lancet* 1935; 2: 1151—2.

William, P. C. (1964). The colorimetric determination of total nitrogen in feedstuff.Analyst (London) 89, 276—281.

Young, V. R., (1991). Soy protein in relation of human protein and amino acid nutrition.*J. Am. Diet Assoc*. 91: 828—835.

Yaniv, Z., Shabelsky, E. and Schafferman, D., (1999). Colocynth: Potential arid land oil seed from an ancient curubit. In Perspective on New uses 1st Edition: Janicks J, Eds : ASHS Press: Alexandria, V. A., U.S.A., pp 257—261.

APPENDIX A

REAGENT DETAILS

A.1. Reagent

These chemicals were purchased from Sigma-Aldrich Co. Ltd (Steinheim, Germany) _Butanol, Casein, Cholesterol, Copper sulphate, Diethyl ether, Hexane, Sodium carbonate and Sodium tartarate.

Chemical from other suppliers include:

Ammonium sulphate (Saarchem)

Anthrone

Bovine serum albumin

Di –sodium hydrogen orthophosphate (Associated Chemical Enterprises Pty Ltd)

Ethanol (Saarchem)

Folin Ciocalteau.

Fomaldehyde (Saarchem)

Glucose (Associated Chemical Enterprises Pty Ltd).

Hydrochloric acid (Saarchem)

Nessler reagent

Olive oil

Perchloric acid (Merck)

Pig bile salt (SS by Capital lab).

Sodium hydrogen orthophosphate (Associated chemical enterprises pty ltd).

Sodium hydroxide (Saarchem)

Starch (Lab consumable and chemical supply)

Sulphuric acid (Radchem)

Thimecil (Saarchem)

A.2 Equipment

Drying oven – Gallenham

Homogenizer – IKA TIO basic ultra turrax

Atomic absorption spectrophotometer VR200-RS – United scientific PTY

Atomic absorption spectrophotometer AA50 Varian

Laboratory mill – MF 10 basic IKA polychem supplies Gallenham

Soxhlet extractor –JR selecta

Kjeldahl flask

Furnace

Blender – WARRING United scientific pty.

Electric stirrer- IKA RW 20 digital

Rotary evaporator Heidolph Laborota 4000 Efficient

Ultra centrifuge- Eppendof

pH meter -720- WTW series Inolab

Freeze dryer- benchtop K – Vitrus

Scale – Adventurer – OHAUS

A.3.1. Anthrone Reagent

Anthrone reagent was prepared by dissolving 1.0g of anthrone in 500ml of 72% sulphuric acid.

A.3.2. Nessler's reagent

An improved Nessler's reagent was prepared by dissolving 5.5g of red mercuric iodide ($H_g I_2$) and 4.125g potassium iodide in 25ml of distilled water. To this mixture was added a cooled solution of 14.50g sodium hydroxide in 50ml of distilled water. This was then made up to 100ml and kept in the dark reagent bottle.

A.3.3. Standard Nitrogen solution

Standard nitrogen solution was prepared by dissolving 4.7162g of Ammonium sulphate in 100ml of distilled water. 1mg of the solution is equivalent to 1mg of nitrogen.

A.3.4. Preparation of standard solution of glucose and starch.

The standard solution of starch was prepare by dissolving 0.1, 0.2…..0.6mg of maize starch in 20ml of perchloric acid each and shaken thoroughly after which anthrone reagent was added and used to plot the standard starch curve. This was

also prepared by dissolving 1, 2, 3…..10mg of glucose in 50ml of 80% ethanol each and shaken vigorously. Anthrone was added and used to plot the standard starch curve.

A.3.5 Ninhydrin preparation (for spraying)

This was prepared by dissolving 0.2g of ninhydrin in 100ml acetone.

A.3.6 Ninhydrin reagent

The reagent was prepared by dissolving 0.3g of hydrindantin in 2.0g of ninhydrin in 75ml of 2-methoxy-ethanol. About 4N sodium acetate buffer (pH 5.5) was added to make the solution up to 100ml.

A.3.7 Standard Amino acid solution

The standard amino solution were prepared by dissolving their respective weight in 10% isopropanol solution to obtained a concentration of of 0.02M each of 0.02M each of the amino acid.

A.3.8. Follin Ciocalteau reagent

This was prepared by dissolving 25g of sodium molybdate ($Na_2MoO_4.2H_2O$), 100ml of sodium tungstate (NaWO4.$2H_2O$), 700ml of distilled water, 50ml of 85% H_3PO_4 and 100ml of concentrated hydrochloric acid were introduced into a 1500ml flask and refluxed for 10hrs. Then 150g of lithium sulphate, 50ml distilled water and a few drops of bromine were added after cooling. The mixture was then boiled for about 15mins without a condenser, to remove excess bromine. The resulting mixture was cooled and diluted to 1 litre and filtered.

A.3.9. Solution for Lowry's Method of protein determination

A.3.9.1 Preparation of Solution A

This was prepared by dissolving 4.0g of sodium hydroxide crystals in 1000ml of distilled water to give a 0.1M sodium hydroxide solution. Then 20g of Na_2CO_3 was then dissolved in the 0.1M sodium hydroxide solution and stirred continuously to dissolve the sodium carbonate crystal. The solution was labeled A.

A.3.9.2 Preparation of solution B

Solution B was prepared by dissolving 2g of sodium tartate (HOOC (CHOH) COONa in 200ml of distilled water to give 1% sodium tartate solution. Then 1g of copper crystal was then dissolved in sodium tartate solution and the solution was labeled B.

A.3.9.3 Preparation of solution C

50ml of Lowry's solution A (A61) and 1ml of Lowry's solution B (A62) were mixed thoroughly to give solution C. this was prepared daily before use.

A.3.10. Formalin10 % buffered formalin was prepared by measuring 1.75g of sodium hydrogen orthophosphate ($Na_2HPO_4.2H_2O$) AND 3.25g of di - sodium hydrogen orthophosphate (Na_2HPO_4) was dissolved in 25ml of boiling water 50 ml of 40% formalin was added and the resulting mixture was made up to 400ml with distilled water.

APPENDIX B

DETAILS OF METHODOLOGY

B.2. Preparation of protein feeds

Different types of feeds were prepared.

I. Low protein diet: this comprises of 100% of maize meal mixed with little water to make dough. The dough was then molded to fingerlike sticks dried in the oven at 50oC

II. High fat diet: comprised of 15% of sunflower oil 0.5% of bile salt, 0.2% of thimecil. All together 20.7% of fat was incorporated into 79.3% of maize meal to give high fat diet. Mixed with water and molded dried in the oven into fingerlike sticks.

III. The extracted protein isolate was incorporated with maize meal in 20: 80(w/w) ratio mixed with water and molded into fingerlike sticks and dried in the oven at 50oC i.e. low protein diet and casein

IV. Casein was also incorporated into high fat diet in ratio 20:80(w/w) ratio to give high fat diet and casein

V. The extracted protein isolate was also incorporated into high fat diet in 20:80 (w/w) ratio to give high fat diet and protein isolate

VI. The normal rats fed were obtained from supplier.

B.3. Feed procedure

The total numbers of 48 sprague-Dowley rats were caged 4 animals per cage. The rates were allowed to acclimatize to the new environment for 4 days. They were all fed on normal rat feed and daily supplied with water. After the adaptation 4 rats were sacrificed for baseline. The remaining 44 rats were divided into 2 groups

Group 1 consisted of 8 animals = control group

Group 2 consisted of 36 animals =Experimental group

Group 1 continued on the normal rat fee for the next 10days. After which 4 animals were sacrificed.

Group 2 were further divided into 2 group A and B group 2A 16 were also malnourished with high fat diet. 4 animals were sacrificed from each group.

Group 2A was further divided into 4 groups

Group 2A (I) continued with low protein diet for the next 14 days

Group 2A (II) was rehabilitated with casein

Group 2A (III) was rehabilitated with protein isolate

Group 2A (IV) continued with high fat diet for the next 14days

Group 2B was further divided into 3 groups

Group 2B (I) was rehabilitated with casein

Group 2B (II) was rehabilitated with protein isolate

Group 2B (III) continued with high fat diet

After 28 days all the remaing animals from group 1, 2A and 2B were sacrificed. All the animals were given free access to water and food and their weight were taken every other day

B.4.Proximate Analysis

B.4.1.Moisture Determination

The sample (2g) was transferred to a previously dried and weighed crucible and placed in an oven thermostatically controlled at 105^0c for 5 hours. It was then removed placed in a dessicator to cool and weighed .This procedure was repeated until a constant weight was recorded. The loss in weight was expressed as percentage moisture content.

B.4.2.Ash Determination

The sample (2g) was transferred into a previously dried and weighed crucible and ignited in a furnace pre – heated at 600^0c for 2 hours. It was cooled and weighed. The recorded weight gave the ash content of the sample expressed as the percentage of the sample of the original eight.

B.4.3.Total Protein Determination

The sample (1g) was digested in a 500ml Kjeldahl flask with 20ml of concentrated sulphuric acid (specific gravity 1.82) and 2 Kjeldahl tablets (3.5g K_2SO_4 +0.0035 Selenium). The digestion was continued until the dark mixture became very clear. The digestion was continued for 30 extra minutes. The flask was allowed to cool and

the content transferred into a 200 ml volumetric flask and diluted to the mark with distilled water.

A 1ml portion was pipette into a 50 ml calibrated flask and diluted to 30 ml with distilled water. About 4 ml of Nesslers reagent was added and the mixture diluted to the mark with distilled water. The absorbance was read at 530nm (Table B1) and amount of nitrogen present calculated by reading off from standard calibration curve (Fig B.1). Such values were multiplied by 6.25 to obtain the total protein concentration.

The standard curve was prepared by dissolving 4.7262g of $(NH_4)_2SO_4$ in 1 litre of distilled water. Aliqiots containing 0.1- 0.6mg of nitrogen was diluted to approximately 30ml. About 4 ml of Nesslers reagent was added, allowed standing for 20 minutes for color development. The absorbance was read at 530nm. A plot of absorbance versus concentration gave the standard nitrogen curve (Table.B.1).

Protocol for the standard curve for Nitrogen

Test tube No.	1	2	3	4	5	6	7
$(NH_4)_2SO_4$	0.0	0.1	0.2	0.3	0.4	0.5	0.6
Nessler reagent (ml)	4	4	4	4	4	4	4
Distilled water (ml)	50.0	49.9	49.8	49.7	49.6	49.5	49.4
Total (ml)	54	54	54	54	54	54	54

Table B.1

B.4.4.Crude Fat Determination

The dried sample from moisture determination (B.5.1) was transferred to a 22 x 88 nm paper thimble using a power funnel. A glass wool plug was placed at the mouth of the thimble and placed in a Soxhlet extractor. About 80 ml petroleum ether (B.P 60^0C -80) was poured into a previously weighed round – bottom flask. The flask was then attached to the extractor and the extraction carried out for 16 hours on low heat. The round –bottom flask was removed after excess petroleum had been recovered It was then finally dried in an oven, cooled in a desiccator and weighed. The increase in weight of the flask was recorded as the amount of crude fat extracted

B.4.5.Mineral Analysis

The mineral composition of the protein isolates was determined by the method of AOAC (1980), 0.05g of the ash of the protein isolate was dissolved in 50ml of 20% HCI.

The mineral concentration of potassium and sodium were determined using a Corning EEL flame photometer that had been calibrated (Table B.2 and fig. B.2 and B.3)

The other (Mn, Fe, Mg, Zn, Cu, Ca, and Co) were determined with a CARL JENA AAS3 Atomic Absorption Spectrophotometer. The instrument had been calibrated with standard mineral solution (Table B.2).

Standard solutions of concentration ranging from 1ppm to 100ppm were prepared for each of the minerals and fed to instrument for the calibration. The Atomic Absorption

Spectrophotometer had an integrator incorporated and so it plotted all the standard curves and provided the corresponding concentration of the different metals in the ash solution of the protein isolates.

The Corning EEL flame photometer used gave only the absorbance readings for the standard curves were plotted (fig B.2 and B.3). The amount of sodium or potassium in the ash solution of the protein isolates were read off from the standard curves.

B.4.6.Total Soluble Protein Determination – Lowry's Method

Lowry *et-al* (1951) method was used for the estimation of soluble protein in the supernatant for the protein solubility determination at the various pHs (table B.4).

Bovine Serum Albumin (B.S.A) was used as standard. The calibration curve was plotted and the concentration of protein in 1ml of supernatant at a particular pH was read from the standard curve (Table B.2).

The absorbance at 660nm was read after standing for 30 minutes on Spekol II Spectrophotometer.

Protocol for the standard Bovine Serum Albumin curve

Test tube No.	1	2	3	4	5	6	7
Std Albumin solution (mg/ml)	0	0.1	0.2	0.3	0.4	0.5	0.6
Distilled	1	0.9	0.8	0.7	0.6	0.5	0.4

water (ml)							
Solution C (ml)	5	5	5	5	5	5	5
Left to stand at room temperature for 10mins							
Folin Ciocalteau reagent	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Left to stand at room temperature for 30mins							

Table B.2

B.4.7.Carbohydrate Determination

The sample (1g) of sample was quantitatively transferred into burette previously stuffed with glass wool. The sample was then wetted with 2ml of 80% ethanol and stirred to remove air bubbles to avoid channel formation. An air space was created by inserting a stopper of glass wool as shown in Fig B.1.

This was done to prevent the carbohydrate diffusing into the solvent. About 25ml of 80% ethanol was used to percolate the soluble carbohydrates. The percolation rate was adjusted to approximately 1.5m/hr.

After the ethanol percolation, the residue was then percolated with 25ml of 35% perchloric acid. Before then the residue had been thoroughly mixed with 2ml perchloric acid.

From test solution containing the starch or the glucose 2.0ml was pipette into a Pyrex 0^{o}c glass tube and kept at. About 10ml of Anthrone reagent which had been cooled to 0^{0}c was added to the 2ml test solution. The reaction mixture was shaken thoroughly and heated for exactly 10 minutes at 100^{0} c in the water bath. After this treatment, the tube was rapidly cooled at 0^{0}c and the absorbance read 630nm, against distilled water blank within an hour.

The amount of starch and soluble carbohydrate was then estimated from standard solution prepared (as shown in Appendix A.8) to give the standard graphs (Fig B.3 and B.4).

Protocol for standard Glucose curve

Test tube No.	1	2	3	4	5	6	7	8	9
Glucose solution (mg/50ml)	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Anthrone reagent (ml)	10	10	10	10	10	10	10	10	10

Table B.3

Protocol for standard starch curve

Test tube No.	1	2	3	4	5	6	7	8	9
Starch solution (mg/20ml)	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Anthron reagent	10	10	10	10	10	10	10	10	10

Table B.4

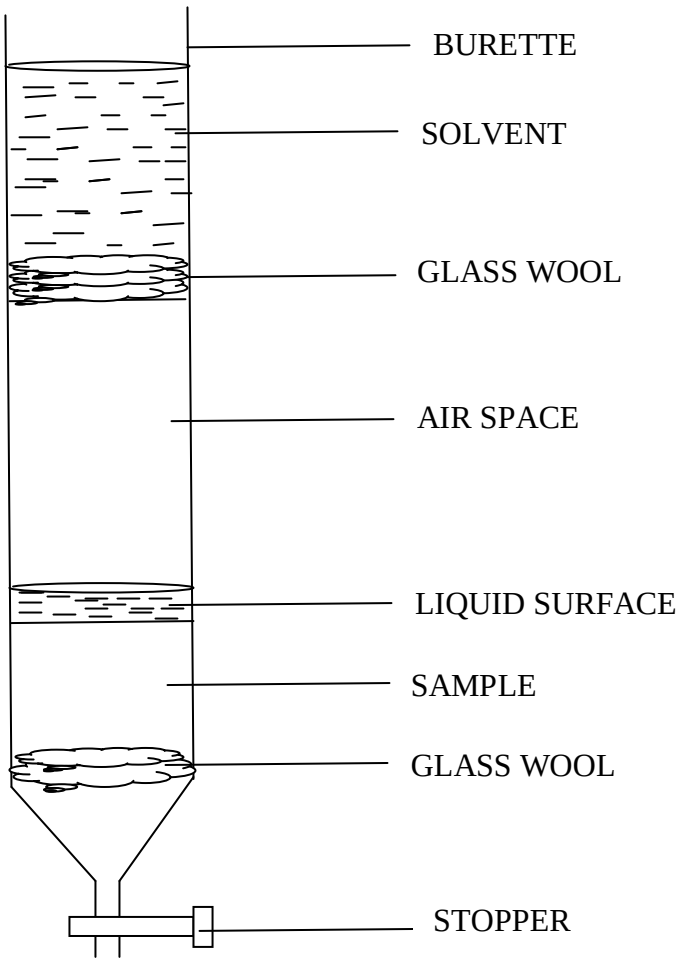

Fig B.1 Perculator

Table B. 5. Weight / Food consumed (g)

Days	4	8	12	16	20	24	28
control	1.5	1.9	1.9	1.7	1.8	1.8	1.7
LDP	1.5	1.2	1.1	1	1	0.9	0.7
LDP+C	1.5	1.8	1.6	1.8	1.5	1.6	1.6
LDP+PI	1.5	1.8	1.6	1.7	1.5	1.9	1.8
LDP+H	1.5	1.3	1.2	1.2	1.4	1.6	1.8
HFD+C	1.5	1.4	1.2	1.2	1.3	1.4	1.6
HFD+PI	1.5	1.2	1.4	1.5	1.6	1.8	1.9
HFD	1.5	1.4	1.7	1.6	1.8	1.6	1.7