CHANGES IN THE NUTRITIVE VALUES OF SOME NIGERIAN DIETS AFTER COOKING BY CERTAIN SOUTH EASTERN NIGERIAN TRADITIONAL METHODS

A THESIS PRESENTED

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October, 1972.

TO MY PARENTS

TABLE OF CONTENTS

Page

Page

A B S T R A C T

A nutritional study was carried out on six commonly used traditional diets of South-Eastern Nigeria.

In this study, the various recipes and the proportions of the foodstuffs used in the formulation of the peasant dietaries in South Eastern Nigeria were determined by survey. From these, the six diets were prepared using the ordinary traditional cooking methods.

Results on the nutrient composition of the raw and the cooked diets showed some losses of the vitamins, minerals and total fats as a result of cooking. Losses of total crude proteins and total ash were negligible.

The effects of cooking on the nutritive values of the diets were evaluated by a rat assay method. The procedure involved the measurement of the operative biological value (B.V.), true digestibility (TD), protein efficiency ratio (PER), net protein utilization (NPU), net protein ratio (NPR), weight gains and the net dietary protein calories percent (NDP Cals %) for male and female rats fed on the raw-mixed and cooked homogenised diets respectively. The studies on the rat also provided evidence of higher sensitivity of the male rats in dietary assays and of sex difference in response to the 'Ekpan Nkukwo' diet (diet D5).

The cooked diets showed higher TD, NPR, PER, NPU and NDP Cals % in the rats than the raw. Both forms of the six diets investigated showed lower BV, NPU, PER, NPR and NDP Cals % as compared with the control diet.

The determination of the serum protein levels, serum protein patterns and of the total serum cholesterol levels in the experimental rats gave no evidence of the effect of traditional cooking on them. Comparison of the NDP Cals % of these diets with the FAO recommended scale of allowance of NDP Cals % for man at various physiological ages and states shows that the peasant diets are inadequate especially for the most vulnerable groups - infants, growing children, pregnant women and lactating mothers.

The quantitative estimation of the various amino acids in the raw-mixed and cooked diets was also carried out. Some losses due to cooking were observed.

The implications of the various results are discussed.

CHAPTER ONE

1. INTRODUCTION

Food is essential to life. Throughout history the procuring of food has been the main preoccupation of all human beings. Many materials have been accepted as foods depending on such factors as geographic environment, climate, nearness to the sea, traditions, economic status, religious beliefs, and to some extent, individual tastes.

Fables and stories have been narrated in Africa and in other parts of the world about food. In Chapter 25 of the Book of Genesis we read about Esau and Jacob and a mess of pottage in exchange for a birth right. In the same book of Genesis we also read about Joseph the dreamer and the storage of maize in Egypt to avert starvation, while in the Lord's Prayer we make a strong request to Our Creator for the provision of the daily bread. The indication of all these is that hunger and starvation had been constant threats to mankind since the earliest time.

It is common knowledge now that to survive one has not only to eat, but must eat a nutritionally balanced diet. Attention has also shifted from the early concept of the dietary needs in terms of protein, carbohydrate, fat and minerals to the present formidable catalogue of nutrients required by man for a healthy life. It is also known that a suitable food supply and its rational utilization are of cardinal importance in maintaining the health and efficiency of the community.

In many parts of the world today people neither know what to eat nor have enough food to eat to satisfy their physiological requirements.

The assistant director-general of the FAO, Dr. Glosinger (1969) recently said that over 50% of the people living in some parts of Asia, Africa and Latin America suffer from hunger and malnutrition, whilst others in various parts of the world are either undernourished or malnourished. FAO studies showed that between 300 million and 500 million people suffer from actual lack of food while between one third and one half of the world population of 3,000 million suffer from varying degrees of malnutrition (FAO, 1960).

The latest population projections (Glosinger, 1969) indicate that between now and the end of the century we should anticipate a growth of about 2500 million people while between the year 2000 and 2030 the world will expand by 4,000 million people. About 75% today and 90% of mankind by the year 2030 live in the under-developed non-industrialized regions of Asia, Africa and Latin America where the balance between food, population and purchasing power is still precarious.

2. History of nutrition

From time immemorial people have known that they have to eat to survive. They always must have been conscious that certain foods were more desirable than others because of the taste, odour and sense of satisfaction derived when these were eaten. The palatability and wholesomeness of certain foods gave them preference over others. Certain foods were eaten for their medicinal

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properties while others were tabooed because of their magic influences.

Individual experiences taught many people the folly of over-eating. The notorious Roman banquets were as uncomfortable as are the over-indulgent in food and drink today. Socrates told his students to eat only when hungry and drink only when thirsty and never to leave the table with a feeling of satiety. The Latin proverb that over-eating destroys more than hunger, and the Scotch proverb that one should food sparingly and defy the physicians, are also known examples. The sickness following eating spoiled food probably led Herodotus to suggest to the early Persians that all diseases to which mankind is subject proceed from food.

Hippocrates made a broad classification of foods on the basis of his observations of the effects of individual foods upon the consumer. He advised the obese to labour much and drink little while those suffering from dysentry should take emesis with helleboro (McCollum, 1957). Athenaeus wrote the first cookery book "Sophists at Dinner" in which he recommended cabbage as a remedy against the intoxicating effects of wine.

Cornaro (1556), about thirteen years after the Sophists banquet, wrote "A easy "The Sure and Certain Method of attaining a Long and Healthful Life", in which he laid emphasis on abstemiousness in eating. He restricted his daily dietary allowance to 12 ozs. of wine and later on, to one oz a day. He lived for 95 years (McCollum, 1957).

3. The Discovery of the Vitamins and their Roles in Nutrition

Historically, the dietary requirements of man and animal were considered to be fats, proteins and carbohydrates, as well as various inorganic salts. Dumas (1871) was the first to question the adequacy of such diets. In his paper on "The Constitution of Blood and Milk" he commented on the increased mortality rate among infants fed on artificial milk constructed to substitute milk shortage in Paris following her siege by the Germans. The artificial milk consisted only of an albuminous substance, sugar and an emulsion of fat. Dumas concluded that in the natural milk there were still unknown chemical substances of nutritional significance.

Dumas' observations were supported by the experiments of Lunin (1885), who failed to maintain good health in mice fed only on purified casein, milk sugar, milk fat and some inorganic elements. The mice fed on this diet soon died, whilst the mice fed only on fresh milk remained healthy for about 60 days. Lunin concluded that milk must contain nutrients other than casein, lactose milk fat and minerals and that these other unknown essential nutrients were in whey constituents.

Socin (1891), Coppola (1890), Hall (1896), Pasqualis (1895) and many other investigators also failed to nourish mice or chicken on simplified diets of known chemical constituents.

Rohman (1908) reported highly instructive results by feeding small animals on purified diets with only small additions of natural foods.

Stepp (1909) experimented on the value of fats and related substances in animal nutrition. His alcohol and ether – extracted bread diets failed to support rats even for 30 days whilst the animals on the unextracted bread survived the six week - long experiments. He later extracted egg with cold alcohol and divided the extract into two portions. One portion was heated for two days in water bath with 95% alcohol and the other portion evaporated with mild heating. Each of these two proportions was added to extracted bread and fed to mice. The group of mice on the heated preparation died within thirty days and the other group on the evaporated one remained healthy. He concluded that unextracted bread contained some substance of physiological importance to the mice and that this substance could be easily destroyed by heating.

Eijkman (1896) showed that polyneuritis developed in chickens fed on scraps from hospital diets, on which patients had developed beri - beri, and concluded that it was due to nerve poison in rice. In 1908, Grijns postulated that beri - beri was the result of a nutritional deficiency. He concluded that rice contained an essential nutrient in outer layers of the grain which are removed in polishing.

Funk (1912) propounded the theory that beri - beri, scurvy, pellagra, and rickets were all caused by the absence in the diet of 'special substances which are of the nature of organic bases which we call VITAMINES'. The view of Funk according to McCollum (1957)

opened the eyes of physiologists and biochemists to new vistas of exploration techniques.

McCollum and Davis (1913) experimented with purified casein and milk sugar which was mixed with they constituents. They observed that young rats grew well when they ether-soluble extract of butter or egg-yolk was included in the diet. There was a growth failure when lard or olive oil was mixed with the basal diet and fed to rats. From these results it was evident that an unsuspected nutrient existed and that it was carried in certain fats. The nutrient was called 'fat-soluble A' and later named vitamin A. Further experiments by the same authors revealed that animals normally required two factors, the 'fat-soluble A' and the 'water-soluble B'.

Steenbock and Gross (1919) found that the yellow pigment in plants was a precursor of Vitamin A. McCollum and his associates (1922), following the observations of Mellanby (1918), demonstrated the requirement for two fat-soluble factors (factors A and D). They demonstrated also, the existence of antirachitic vitamin D, and noted that young rats kept indoors, away from direct sunlight, and deprived of this vitamin, developed abnormal bones and were stunted.

Kinnersley and Peters (1929) discovered that lactic acid accumulated in the brain of a pigeon maintained on a thiamine - deficient diet. They also observed reduction in the oxygen uptake by thiamine - deficient pigeon brain in vitro when the tissues were treated

with glucose, lactate, or pyruvate in Ringer - phosphate solution. This condition was corrected by the addition of thiamine.

Lohmann and Schuster (1937) discovered that in yeast a diphosphate ester of vitamin B_1 functions in the decarboxylation of the α - keto acids. Peters and associates (1939) presented evidence that vitamin B_1 in the pyrophosphate form, is indespensable for the removal of pyruvic acid, and indirectly lactic acid, in the normal metabolic scheme.

Abbott and Hokin (1947) provided evidence to show that extracts of rice polishings, tested on both pigeons and rats, contained two vitamins one of which cured polyneuritis in pigeons and the other stimulated growth in the rat. Both factors were shown to be sensitive to heat at varying degrees.

Salmon et al. (1928) separated the antineuritic substance from a crude vitamin B_2 extract by absorption on Fuller's earth at different hydrogen ion concentrations. Their work was supported by that of Smith and Hendrick (1926) who showed that there were at least two water-soluble vitamins. The antineuritic factor was subsequently named vitamin B_1 and the growth - promoting factor called vitamin B_2.

Ricker et al. (1946) isolated vitamin B_{12} and Smith (1948) showed that it relieved the symptoms of pernicious anaemia. Also, through observations on nutritional deficiency diseases, the other fat - soluble vitamins E and K, and the water - soluble B vitamins were discovered and their roles in cellular metabolism ascertained.

Holst and Fiocllich (1907) carried out extensive studies on the effect of diets in inducing or curing scurvy in guinea pigs. Their animals remained healthy on a diet of several cereal grains and cabbage, but, when restricted to grains alone, they developed scorbutic lesions and died after twenty to forty days. These workers further showed that supplements of fruits, fresh vegetables or their juices to a diet of grain protected the animals against scurvy. They noted that the daily allowances of thirty grams of fresh raw cabbage, cranberries, or carrot prevented the development of scorbutic symptoms in guinea pigs fed solely on a grain diet. These antiscorbutic foods were shown to lose their effectiveness when heated to 100°C for a half to one hour. Similarly, dried unheated carrot, dandelion leaves, or cabbage leaves, or dried potatoes were of no value as antiscorbutic foods.

The fact that fresh and raw vegetable food, raw milk, and numerous other substances contain an antiscorbutic substance, labile to heat, drying and exposure to oxidation, excited great interest among biochemists. Many investigators had begun to study specific problems relating to experimentally induced scurvy in animals. They studied the lability of the antiscorbutic factor under diverse conditions; pathological lesions in blood, bone, teeth, skin and other tissues.

Svirbely (1928) proposed to call the antiscorbutic substance vitamin C, a name which was widely adopted until in 1933 when Szent - Gyorgyi suggested that it be renamed ascorbic acid.

Progress in the study of experimentally - induced scurvy in guinea pigs and monkeys led naturally to the study of ascorbic acid by organic chemists, and its subsequent synthesis. Such developments opened the way for the study of the distribution, functions in metabolism of ascorbic acid, the pathology of its deficiency, minimum requirements and method of preservation.

4. INTERRELATIONSHIPS OF NUTRIENTS

Food may be defined as any solid or liquid which when swallowed, is capable of providing the human body with materials enabling it to perform its physiological functions. The components which give a substance its right to be regarded as food are known as 'nutrients'.

When the basic principles of nutrition were being established, nutrients were often studied independently in order to assess their significance and determine quantitative needs. This approach tended to overshadow the obvious fact that individual nutrients are not consumed in isolation but as a part of a diet providing a large number of variable components. For instance, the determination of the nutritive value of proteins under conditions ensuring their maximal utilization does not adequately represent the role of the protein content of a cereal diet, in which, absorbed amino acids are not only derived from several foods, but the nutritive value is affected by other factors such as calorie, mineral and vitamin intake. In view of this,

it has become necessary to explore the mechanisms underlying nutrient interactions; reasons for such interactions between nutrients can be varied and not confined to intermediary metabolism (Munro, 1964).

(i) Proteins, Carbohydrates and Fats

It is known that dietary supplements of either carbohydrates or fats given to rats grown on a diet containing an adequate amount of protein cause a conservation of the body nitrogen (Campbell, 1964). This phenomenon, often called the "nitrogen-sparing action", has led to many investigations to elucidate its mechanism on a physiological or biochemical basis or both, (Munro, 1951). Munro et al. (1959) presented experimental data to show that the effect of dietary carbohydrate might be attributable to its stimulative effect on protein synthesis in the muscle. Narloski et al. (1965) reported that dietary fat exhibits a protein - sparing effect in a manner similar to that caused by dietary carbohydrate. Nakano et al. (1970) have ascribed the protein - sparing action of carbohydrate and fat diets to their effect on the amino acid - degrading enzymes. These workers proposed that the inhibitory effect of dietary carbohydrate and fat is not due to suppression of enzyme activities but to the retardation of the inducible formation of the amino acid - degrading enzymes such as, threonine dehydratase, tryptophan pyrrolase and Arginase, and the resulting marked conservation of the body nitrogen.

Munro (1964) attributed the effect of dietary carbohydrate on the

utilization of food protein, to a number of interacting mechanisms. Lee and Hanna (1950) proposed a chemical reaction of the carbohydrates present in the foodstuffs with free amino groups, thereby removing amino acids, particularly lysine, unavailable to the body (Henry and Kon (1950). The type of carbohydrate in a meal has been shown to influence the efficiency of absorption of the protein consumed in the same food. Harper and Kotaysky (1955) observed that a low protein diet was better utilized by rats when sucrose was replaced by starch as the dietary carbohydrate, which was attributed to the retardation of the transit of food by the polysaccharide, so that the amino acids liberated from the protein were more efficiently utilized. But Cuthbertson and Munro (1939) and Munro (1949) showed that nitrogen balance underwent temporary deterioration when protein and carbohydrate in a diet were consumed in separate meals, whereas no such change occurred in a similar separation of dietary protein and fat.

Geiger and Mirsky (1956) did not observe any growth in rats fed on essential amino acids unless the diet contained some carbohydrate which was needed for the synthesis of non-essential amino acids. Dietary carbohydrate acts non-specifically as an energy source which like other energy sources, is a factor of utilization of dietary protein (Mayer, 1964).

(ii) Protein and Fat

(a) Protein and Fat in the Diet

The metabolism of proteins and their constituent amino acids

has been known to be grossly affected by many vitamins. For instance, in man and animals, tryptophane can substitute for nicotinic acid and will produce increased excretion of N^1- methylnicotinamide (Campbell, 1964). Also, Copelan and Srinivasin (1960) presented data to show that there is a leucine - nicotinic acid relationship. These workers suggested that in diets marginal in protein, amino acid imbalance caused by a relative excess of leucine may lead to depletion of nicotinic acid in the tissues. Belavady et al. (1963) indicated that the excretion of quinolic acid was increased to a greater extent than that of N^1- methylnicotinamide.

(b) Protein and Vitamin A

Experimental evidences are not consistent in the literature as to the role of protein deficiency on the absorption and utilization of vitamin A and carotene. For instance, Loh et al. (1960) reported reduced storage of vitamin A in a diet containing insufficient or poor quality protein. On the other hand, Murray (1961) showed that rats were able to store adequate amounts of vitamin A on diets of poor - quality protein or even on a protein - free diet. This report has been confirmed by Mathur and Sexton (1963) using diets containing 9, 4 and 20% casein. Rucheigh et al. (1962) assayed the livers and kidneys of rats fed on varying qualities and quantities of protein. The highest vitamin A content was observed in the livers and kidneys of rats fed on a protein - free diet. With increasing proportions of dietary protein,

there was a progressive decrease in the amount of vitamin A in the liver and an increase of the vitamin in the kidneys. The efficiency of vitamin A utilization was decreased by feeding a protein of inferior quality but was not affected by the level of dietary protein. Results reported by Mathews and Seaton (1963) suggest that protein is closely concerned with vitamin A transport.

In developing countries, the effect of protein deficiency on carotene absorption and utilization is probably more important, since carotene is the precursor of vitamin A activity and, the consumption of good quality protein is low in such countries. Friend et al. (1961) showed that pigs on low-protein diets had lower stores of vitamin A than animals on diets adequate in protein. This was considered to be due to both the impaired conversion of carotenoids, and to reduced absorption of vitamin A in protein - free animals. Berger et al. (1962) reported that a nitrogen - free diet decreased but did not prevent the conversion of carotene and the storage of vitamin A. Mathews and Seaton (1963) found that the liver and the blood of rats given carotene contained less vitamin A when the diets were low in protein. Their data in general suggest an interference connected with the absorption or conversion of carotene at low protein levels and may explain the low serum levels of vitamin A found in kwashiorkor. Arroyave et al. (1961) found that the treatment of kwashiorkor increased the serum levels of vitamin A although the diets did not contain vitamin A or carotene. Protein deficiency seems to interfere with the absorption

and utilization of carotene and to retard the utilization of vitamin A.

(c) Protein and Riboflavine

Bro-Rasmussen (1958), protonted some data which established clearly that animals and man on low protein intakes excrete relatively large amounts of riboflavine. Czaczkes and Gurgonheim (1946) showed experimentally that rats on low protein diet cannot retain and make use of riboflavine. The same authors obtained similar results for dietary fats and concluded that the effect of both dietary protein and fat was to reduce intestinal synthesis of riboflavine. Their theory was supported by the work of Bro - Rasmussen (1958). Lloyd (1964) suggested that the cause of suboptimal riboflavine retention during periods of protein inadequacy is the high lability of the flavo-proteins. That is, during negative nitrogen - balance the labile proteins are lost first, and when these are reduced, dietary riboflavine cannot be utilized because the proteins with which it would normally combine are not available.

(d) Protein and Vitamin B_{12}

In the last few years, many papers have linked vitamin B_{12} in a general way with the metabolism of proteins in different animals. Henry and Kon (1951) reported that the addition of vitamin B_{12} increased the biological value of casein for the vitamin B_{12} - deficient rat. Indian authors (Baliga and Rajagopalan, 1954; Baliga et al. 1954) obtained similar increases in biological value for

vegetable proteins with normal rats. There are then growing indication of a more specific connection between vitamin B_{12} and the biosynthesis of the essential amino acid, methionine (Schaffer et al.1949; Ogasky, 1950) through its effect on the formation of methyl groups (Arnstein, 1955). Further experiments by Henry and Kon (1956) with rats fed on casein, showed a similar increase in biological value when 1% methionine was added as by the addition of 1% homocystoine and vitamin B_{12} supplement.

(iii) Carbohydrates and the Vitamins.

It has been shown that carbohydrate and lipid metabolism are disturbed in ascorbic acid - deficient animals. For instance, lowered glucose tolerance was observed in scorbutic guinea pigs (Banerjee and Ghosh, 1947) and in scorbutic monkeys (Sarkar and Banerjee, 1957). A significant decrease in glucokinase activity was observed in the tissues of scorbutic guinea pigs (Banerjee and Ghosh, 1961). Banerjee and Kawishwar (1959) also showed that tissues from scorbutic guinea pigs had much higher contents of citric, lactic and oxalic acids than normal pair-fed controls, suggesting faulty operation of the tricarboxylic acid cycle in scurvy. Banerjee and Singh (1958) showed that total body cholesterol was greatly increased in scorbutic guinea pigs. Banerjee and Ghosh (1960) fed ascorbate to scorbutic guinea pigs and observed lowered total body lipids and increased total body cholesterol. These observations suggest

preferential channelling of the acetate 'pool' of the body towards cholesterol synthesis in vitamin C - depleted animals. The defects in the pathway of metabolism of carbohydrate and fats in scorbutic guinea pigs are probably due to diminished insulin production in the scorbutic condition.

Although not strictly a carbohydrate in the biochemical sense, D-sorbitol has been known to enhance the absorption of orally administered vitamin B_{12} in man and in animals and the absorption of iron by rats (Campbell, 1964). Morgan and Yudkin (1957) found that by adding D-sorbitol to a diet deficient in thiamine, normal growth in rats was brought about. Okuda et al. (1960) showed that D-sorbitol added to a diet deficient in vitamin B_6 increased the urinary excretion and the liver concentration of vitamin B_6 in adult rats and improved growth rate in weanling rats. These workers, however, obtained contradictory results, when D-sorbitol which was given at 10 - 20% level for 8 - 12 weeks, reduced intestinal absorption of orally administered radioactive vitamin B_{12} in rats. They explained this in terms of the difference in the physical state of vitamin B_{12} and sorbitol.

Popplor et al. (1960) were unable to confirm the thiamine - sparing action of sorbitol in man. Morgan and Yudkin (1962), reviewing the whole field of D-sorbitol and the B-vitamin interactions, proposed at least two mechanisms of sparing action involved. The first is represented by the limited sparing action on vitamin B_{12} and folic acid, and the second is illustrated in experiments with rats and mice in which they may be entirely independent on the dietary sources of a variety of B-vitamins.

(iv) Minerals and Vitamins

Minerals may influence the requirements for several vitamins and one of such interesting interrelationships is that of selenium and vitamin E which has been discussed by Diplock et al. (1961). An inverse relationship has been established by Gershoff and Ferngalin (1959) between the amount of pyridoxine in the diet and the endogenous oxalate excretion of rat, cats and rats. This is an interesting relationship involving a human health problem when the formation of urinary calculi is discussed. Gershoff and Andrus (1961) showed that high levels of dietary magnesium (400 mg/100 gm diet) provided protection against oxalate deposition in vitamin B_6 - deficient rats, given diets supplemented with glycine which is a precursor of endogenous oxalate.

Booth and Spray (1962) have reported iron and vitamin B_{12} interrelationship. Those workers treated gastrectomised rats with iron by intramuscular injection and found that the treated animals had less vitamin B_{12} in their livers and sera than the untreated operated animals. It was suggested that the artificial maintenance of high levels of tissue iron permitted increased haematopoiesis, with the resulting loss of vitamin B_{12} from the serum and liver. However, those data appear to suggest that a high level of a particular nutrient may not be best under all conditions.

(v) Vitamin and Vitamin

Apart from the interrelationship between groups of nutrients

in a diet, some interdependence of nutrients within groups has also been reported in the literature. For instance, the excess or absence of some vitamins has profound influence on the requirement for and the efficiency of utilization of others. Renke et al. (1960) found that vitamin B_6 - deficient animals on a diet low in vitamin B_6 became deficient in vitamin B_{12} although the diet contained unusually large amounts of vitamin B_{12} (all other nutrients being sufficient). Also, excessive amounts of vitamin B_6 caused a marked decrease in serum and liver levels of vitamin B_{12}. Morrison and Scott (1959 a), showed those effects to apply to other B-vitamins. They gave weanling rats diets, otherwise adequate, containing low, adequate or high levels of one B-vitamin in combination with low, adequate or high levels of a second B-vitamin. A deficiency of thiamine, riboflavine, pyridoxine or pantothenate was considered significant by reduced weight gain and efficiency of food utilization. Morrison and Scott (1959. b) further reported that excess thiamine or pyridoxine or both had no effect on the weight or reproductive performance of animals after parturition and lactation.

Some metabolic interrelationships between vitamin B_{12} and pantothenic acid have been reported by Aiyer and Srikantan (1961). Hsu (1963) had suggested that the effect of vitamin B_6 deficiency on vitamin B_{12} utilization is due probably to reduced secretion of gastric juice and the production of vitamin B_{12} - binding substance.

Diminution in the riboflavine content of the liver and decrease in activities of some liver dehydrogenases have been reported in vitamin B_{12} deficiency by Murthy et al. (1956), Williams et al. (1953) and many other workers.

Lloyd (1964) claimed that carotene can interfere with the antirachitic action of vitamin D, particularly when the levels of the latter are low. Though this evidence is not conclusive, with the chemical relationship between carotene and vitamin A, the possible deleterious effect of high levels of the latter on vitamin D metabolism cannot be discounted.

(vi) Mineral and Mineral

Certain minerals have been shown to interfere with or modify the requirement for others. For instance, Dick (1956) and Mills and Dell (1960) showed that the metabolism of copper was markedly effected by the dietary content of molybdenum. The magnitude of this effect of molybdenum is in turn influenced by the dietary level of sulphur - containing compounds in the diet. Dietary supplements of inorganic sulphate protected the urinary excretion of molybdenum in the rat and sheep and restricted or completely prevented its accumulation in the liver (Miller et al. 1956).

Adverse effects upon growth and upon the activity of the molybioflavoprotein enzyme, xanthine dehydrogenase, have been noted in rats (Higgins et al. 1956) given dietary supplements containing

tungstates. In a critical review of the interrelationships between Fe, Cu and Zn, Matrone (1960) concluded that contrary to opinions earlier held, there is little evidence to suggest that copper ion influences Fe absorption other than by diminishing the need for iron during copper - deficiency anaemia. There is, however, an interaction between Fe and Cu during haemoglobin synthesis in the rat (Matrone, 1960), and the chick (Hill and Matrone, 1961).

Studies in the rat showed that magnesium deficiency causes a decrease in the muscle potassium (Shaw and Holt, 1963; McIntyre and Davidson, 1950; Cotlove et al. 1951; Farber, 1966) and an increase in muscle sodium. Grace and Gibson (1969), observed that in magnesium - deficient guinea pigs there was an increased concentration of sodium in the skeletal and cardiac muscles. Ignatius and Epstein (1963) postulated that the change in the sodium concentration is due to the disruption of the mechanism which maintains the concentration gradient between the intracellular and extracellular fluids.

5. NUTRIENT CHANGES IN COOKING AND PROCESSING OF FOODS

In recent years much attention has been focussed on the nutrient changes which occur during the preparation of food. During this process, each individual has his plan of cooking and method of preparation even before cooking begins. He determines according to his own taste the rate at which cooking takes place, the point at which it stops. He

decides on the amount of cooking water used, the size of his food and many other details which are all points of importance in determining the extent of nutrient losses which may occur.

(i) Protein Changes

Heat processing has been shown to impair the nutritive value of some proteins. On such cases, the evidence indicates that lysine deficiency is the first limiting factor (Patton, 1950). Pader et al. (1948) showed that in heat processing, a new lysine linkage which is either not digestible by enzymes or is so slowly digested that part of the lysine enters the blood stream too late to participate in tissue formation with the other assimilated amino acids. The interaction of the free amino acids and amino ——— vitamins (B - complex vitamins) with reducing sugar resulting in a non - enzymic browning reaction (Maillard reaction) during food processing caused a decrease in nutritive value (Patton and Hill, 1948 a). Further studies by Patton and Hill (1948 b) on purified casein and soy globulin in 5% glucose solution maintained at 96.5°C for 24 hours showed significant losses of lysine, arginine, tryptophane and histidine. The losses were 27.4, 22.7, 15.3 and 14.3% respectively in case of soy globulin as measured by microbiologic assay after acid and alkaline digestion.

Sauberlich and Mitchell (1935) and Boas - Fixsen (1935) showed that the nutritive values of such materials as casein, meat, liver, kidney, heart, muscle, cereals and fish meal decreased when they were exposed

to high temperatures for a considerable time. Chick et al. (1935) found for rats a decrease in biological value from 64 to 44 and true digestibility from 93 to 73, when casein was heated at 150°C for 66 hours and fed at 9% protein level. Heating at 120°C for 72 hours also reduced the true digestibility of lactalbumin in the same experiments from 95 to 69%.

Heat treatment, on the other hand, has been shown to improve the nutritive value of various beans and peas probably by destroying the toxins and trypsin inhibitors these legumes usually contain. For instance, Osborne and Mendel (1917) found that ground raw soybeans when fed to rats as the sole source of protein in an otherwise complete ration did not support appreciable growth. However, normal growth resulted when they were fed on soybeans which were previously cooked.

Osborne and Mendel (1917) and other workers have shown that cooking ground soybeans increased the digestibility of the total protein by 10% for rats. There was also increased food consumption which together with increased nitrogen absorption might be responsible for the increased nutritive value of cooked soya beans. Finks and Johns (1921) reported better growth in rats when they were fed on cooked protein isolated from raw velvet beans. Johns and Finks (1920) have also reported better growth in rats on cooked phaseolin, an isolated protein of the navy beans. It therefore appears

that heat causes some essential protein fraction of these isolates which are unavailable in the raw form to become available for absorption and metabolic use when cooked.

The beneficial effects of mild heating used in ordinary domestic cooking have also been reported in the literature. For instance, Malik (1967) showed that in all the traditional diets he studied, domestic cooking increased the true digestibility of the dietary nitrogen. Hutchinson et al. (1964) showed similar beneficial effects of mild heating in the destruction of trypsin inhibitor, increased palatability for experimental animals, and improved digestion through the destruction of the capacity of such cereals as rice, oats, maize, barley, wheat and rye. Therefore, it seems that only when heating becomes excessive, that is, at temperatures greater than about 120°C does damage occur.

(ii) Carbohydrate Changes

Zamora (1966) reported insignificant losses of carbohydrates and minerals by leaching in wet processing of some foodstuffs. Sinclair et al. (1969) have, however, reported some transformations of carbohydrates during heat processing. The most affected by heat was starch which was converted into a soluble form and ultimately to dextrin. These workers also showed that oat starch gelatinized at 85°C, and potato starch at 65°C. In acid solutions, such as in stewed fruits and jams, heat causes 'inversion' of cane - sugar into

glucose and fructose. Its partial conversion to caramel is one of the means of developing flavours in foods by cooking (Sinclair et al. 1969).

(iii) Vitamin Changes

The vitamins are the most affected and the most sensitive of all the nutrients to heat processing. Several may suffer considerable losses during cooking (Bender, 1966). The greatest losses during processing are caused by leaching out of water-soluble vitamins in operations such as washing and blanching (Adam et al. 1942; Lee, 1958). However, some of the early work on the chemical nature and properties of vitamins showed that, while all are to some extent thermolabile, they differ widely from one another as regards the conditions which influence their destruction.

Both vitamin A and β- carotene are destroyed at high temperatures in the presence of oxygen (Harris and Von Loesecke, 1960). They are oxidized by fat peroxides and in the presence of traces of copper and, to a lesser extent, of iron (Bender, 1966). De (1936) reported a loss of carotene in vegetables after boiling for one hour. Malik (1967) reported losses of vitamin A (including carotene) which ranged from 25 to 50% from the cooking oil used in his studies. These results suggest that the loss of vitamin A activity may be expected when vegetables are incorporated in stews and cooked for long periods.

Isomerization of vitamin A and the provitamin can lead to losses as in canning. Falconer et al. (1964) reported unpleasant flavour due to degradation of carotene in dehydrated carrots. Haqsood et al. (1963) reported losses of vitamin A in enriched ghee and vanaspati. They observed that frying at 200°C caused a 40% loss in 5 minutes, 60% in 10 minutes and 70% in 15 minutes whilst boiling in water caused 16% loss in 30 minutes, 40% in one hour and 70% in two hours.

Vitamin B_1 often suffers considerable damage during cooking and processing of foods. Like all water soluble vitamins, vitamin B_1 is mainly lost from foods by leaching. Rosoce (1930) found that in 15 minutes boiling about 50% of the vitamin had gone into the cooking water. Langley et al. (1933) reported that the antineuritic potency of carrots was damaged to a high degree during cooking. Guerrant et al. (1936) and Nelson et al. (1932) have reported similar losses.

Vitamin B_1 is stable in acid but not in alkaline or neutral medium. Mild alkalinity of many natural waters has been shown to cause much destruction of vitamin B_1 in foods. For instance, Roy and Rao (1963) observed a loss of about 35 - 85% of thiamine in gruel cooked with waters of moderate to high alkalinity. Cooking in distilled water caused a loss of about 5%; with tap water, there was about 8 - 10% loss while well water caused a loss of up to 30%. Fish lost up to 50% of its thiamine on boiling and 7% on canning. Eggs lost about 9% of vitamin B_1 when scrambled and 29% when boiled (Line et al. 1942).

Losses of vitamin B_1 content of meat during cooking varied with size of the cut, fat content, and other factors, but the average was about 15 - 40% on boiling, 40 - 50% on frying, 30 - 60% on roasting and 50 - 70% on canning (Harris and van Loosecke, 1960).

Losses of riboflavine occurs mainly through leaching. The vitamin is stable to oxygen and to acid conditions but unstable to light and alkali. Harris and van Loosecke (1960) have reported 50% loss of riboflavine in two hours exposure to bright sunlight and 20% loss on a dull day. The riboflavine is converted into lumiflavine. Bone - Fixen (1938) has reported losses of vitamin B_2 into the cooking water. Loving and Remington (1937) found only a slight loss of riboflavine when cotton seed meal and soya beans were cooked in a pressure cooker. Malik (1967) reported losses of up to 50% for riboflavine in his diets and attributed these to leaching and open pan cooking.

Vitamin C is one of the most labile of the vitamins. Its retention in foods is often used as an index of severity of processing and storage conditions (Bender, 1966). Losses of the vitamin occur through oxidation aided by the presence of enzyme, ascorbic acid oxidase, heat, sunlight, oxygen and alkali, and by leaching into the processing water, this depending on the volume of the water (Bone - Fixen, 1938; Bender, 1966). Prolonged heating completely destroys the vitamin. Oxidation of the vitamin is also catalysed by traces of copper (Kellie and Silva, 1935; Szent - Gyorgy, 1928).

Trossler et al. (1936) observed that after some vegetables were boiled for 20 minutes, about 30% of the ascorbic acid was destroyed and about 30% was present in the cooking water. Fenton et al. (1937) observed that after two minutes boiling, swiss chard lost 10 - 14% of its original ascorbic acid content by total destruction and 18 - 24% had passed into the cooking water. During an examination of hospital diets, Platt et al. (1963) observed that peeled potatoes when soaked overnight in water, lost 45 - 60% of their ascorbic acid content. McHenry and Graham (1935) found that cooked peas had 42% less vitamin C than the raw peas. Fenton et al. (1936) found losses of 7 - 10% of vitamin C when peas were boiled in sodium chloride solution in an enamel pan. Fixen and Roscoe (1938) reported a loss of 5 - 10% of ascorbic acid in boiled carrots, peas and swisschard.

Could et al. (1936) have noted that the greatest loss of vitamin C occurs from the time the vegetable is plunged into the boiling water to that when the boiling point of the cooking medium is again reached. Mack (1936) showed that this was due to the activity of enzymes present in the raw vegetables which reacted to oxidise ascorbic acid whenever a cut surface of the vegetable was exposed to air. Thus two factors, solubility of the vitamin and the rapidity of its destruction by oxidising enzymes, both influenced by a third variable, the method of cutting the vegetables, operate simultaneously in unknown degrees.

Losses of vitamin C vary with the method of cooking. Kohman (1942) showed that losses became progressively greater with steaming, boiling and baking. Wellington and Trossler (1938) reported a loss of 10 - 30% of ascorbic acid on cooking cabbage by various methods, but the cooking water extracted as much as 66%.

It is not only during the actual cooking process that losses of vitamin C can occur. The handling of the material before and after cooking also provides opportunities of the vitamin loss. Many practices current in an average kitchen undoubtedly tend to increase the extent of such losses above the unavoidable minimum. Vegetables and fruits are often pealed, chopped up, or otherwise prepared for cooking sometime even before they are needed, and at times left in cold water. Green vegetables are nearly always soaked in salt and water for sometime to kill slugs and caterpillars (Boas - Fixen, 1938). It can then be stated categorically that in such circumstances losses of vitamin C through oxidation and leaching and of the other vitamins by leaching are bound to occur.

Vitamin D is stable and as such no losses have been reported for it in the literature. However, Bender (1966) reported 25 - 30% loss of the vitamin when vitamin D - enriched milk was dried.

Pyridoxine is not stable to heat and some losses are often incurred on cooking foods. Davies et al. (1959) have reported some losses of the vitamin on storage.

Nicotinic acid is unaffected by light, heat, oxygen, acid or alkali.

The only losses reported by Bender (1966) for nicotinic acid was by leaching into the cooking water. Similar losses have been observed by Russel et al. (1943) who also recorded 22% loss for leafy plants, 17% for flowery plants, 8% for legumes and 9% for tubers and roots.

(iv) Mineral Changes

Mineral changes during cooking of various materials have been reported. For instance, Bender (1966) reported an increase in sodium content of peas from 1.7 mg/100 gm in the raw vegetable to 12 mg/100 gm in the cooked form. This increase was probably due to the absorption of the mineral from the cooking water. Horner (1936) reported similar increases in calcium content of foods when cooked in hard water. Synder (1891) noted only a trifling loss of total mineral content of unpeeled potatoes on boiling. Griebel and Miermeister (1926) reported a loss of 6 - 7%. Pfund et al. (1942) observed an average of 10% loss for iron in potatoes soaked for six hours prior to boiling. Phosphorus retention in some vegetables ranged from 60 - 87% when cooked in open kettle, from 68 - 83% when cooked in waterless cookers and from 81 - 92% when cooked in pressure sauce pan (Brinkman et al. 1942).

6. Evolution and Concept of Animal Feeding Experiments

Feeding experiments with animals were designed based on previous experiences with good and bad foods and observations of the early nutritionists. From long experience with domestic animals, McCollum

had concluded that human diets could contribute to the preservation of the characteristics of youth if they were richer than average in the nutrients, calcium and vitamins A and C.'

However, the earliest record of feeding experiments with human subjects reported by McCollum (1957) was performed in 607 BC, in which two contrasting diets were compared. This 'experiment' is recorded in the Book of Daniel (1: 1 - 15) in the Bible.

Lawes and Gilbert (1884) showed the difference in the value of protein of cereal and legume seeds in what was the first animal experiment ever performed. Fordyce (1791) conducted an experiment with a large number of chicks to show the need of "calcareous substance" to the birds especially at the time of laying.

Magendie (1816) was the first to describe n symptoms of a dietary deficiency disorder in an experimental animal. He observed this in a series of experiments in which he fed some dogs on a diet of sugar or olive oil and water. The animals soon exhibited the symptoms of inanition, suffered from malnutrition and died. There was also the ulceration of the cornea, a condition which was later prevented by the administration of vitamin A.

Thaer (1804) observed that when farm animals such as cattle, horses, and sheep were given only well-cured hay to eat, they remained in good condition for a long time. From this observation he conceived the idea of determining by feeding experiments the amount of one food which was equivalent to a given amount of hay, in maintaining good nutritive condition in the animal. This later gave

rise to the popular Thaer's "Haye Equivalents."

Animal experiments since then opened new approaches to the solution of most nutritional problems. The knowledge of nutritional deficiency diseases with animal experiments led to the discovery of the vitamins, minerals, amino acids and the other nutrients and their roles in nutrition. It is now possible to study foods and diets for their quality, and drugs for their potency and pathological changes or interference with special functions such as fertility and foetal development by animal experiments. An important feature of many feeding trials which have been developed along with the use of laboratory animals is the use of purified diets as well as controls.

7. The Conventional Methods of Dietary Protein Evaluation

The primary function of dietary proteins is to furnish a mixture of amino acids of the proper pattern for the synthesis of tissue proteins and for maintenance (Allison, 1955, 1959). Any method for measuring the value of food proteins must directly or indirectly evaluate this function.

Many methods for determining the quality of dietary proteins have evolved since the recognition of the contrasting nutritive values of proteins from different sources by Lawes Gilbert (1854) and Thaer (1804). These methods have been reviewed by Allison (1949, 1955, 1959), Frost (1959) and Campbell (1963). Modifications have also been made of the original techniques.

The concept of biological value of proteins originally put forward

by Thomas in 1909 and later modified by Mitchell (1923 - 24), and Allison (1955) was based on the nitrogen gained or lost by an animal determined by the analyses of the food, the urine and faeces. Although the method is regarded by many workers as being reliable when properly carried out, it is difficult and time consuming so that many attempts have been made to simplify it. For instance, as differences in the biological values of proteins also produce differences in growth rate of the experimental animals, a number of growth procedures have been developed for protein evaluation. Other workers have approached the problem through the effect of dietary protein on the composition of the body tissues. Foods have also been screened in respect of their nutritive values by chemical and microbiological methods in an attempt to correlate biological value with differences in amino acid composition.

(i) The growth method

Osborne et al. (1919) introduced the concept of the protein efficiency ratio (PER) which is the grams gain in weight of the experimental animal per gram of protein consumed. In their original design, PER was determined for several levels of the test protein until the maximum value which was regarded as the best estimate for the protein was obtained. Barnes and Bosshardt (1946) however, supported this procedure though they obtained maximum PER value at 10% dietary protein level and at a higher level for poor quality proteins. Block

and Mitchell (1946 - 47) strongly criticized this procedure and stated that the insistence of the previous authors on the determination of maximum efficiency ratios seemed ill-advised. The actual practice of most workers has been to test proteins at a single level, usually about 10%. Also, the greater precision of the PER method over the use of the body weight gain only of Hogsted and Worcester (1947) and Sherwood and Holden (1953) has been demonstrated by the Rutger's Collaborative Study (1950). These workers observed that variation between laboratories was reduced by about 50%. Chapman et al. (1959) showed that there was much reduced variation within groups of animals when weight gains were related to food consumption.

Hoagland and Snider (1926) and Beveridge (1947) showed that PER varied with the sex of the rat. Morrison and Campbell (1960 a) observed that female rats tended to give maximal PER values at lower protein levels than males. In addition, the two sexes did not always show the same difference between proteins. Campbell (1963) stated that since female rats, at normal protein levels, did not gain as rapidly as males, and the variation within groups not consistently smaller, the precision and sensitivity of a test using males may be expected to be better than one using females. Jones (1951) observed that with diets which supplied adequate protein both in quality and quantity, male rats surpassed the females in weight gains.

In spite of its obvious advantages of simplicity, convenience and widespread use, the PER assay method has been subjected to various criticisms. For instance, Mitchell (1944) and Bender and Doell (1957) pointed out that (a) the results may vary with level of protein in the diet and food intake, (b) the assumption that body weight gain is constant in composition is not necessarily valid, and (c) PER makes no allowance for maintenance but assumes that all protein is used for growth. Bender and Doell (1957) also mentioned that the PER does not permit the evaluation of proteins which do not support growth and consequently that PER yields a much more variable result than NPU. Also, PER is non-specific to test proteins as other nutrients in the food may enhance weight increase, for example, fatty liver which may occur as a result of amino acid imbalance leads to increase in body weight. Though these criticisms have been refuted by various workers it is necessary and also desirable to use other parameters such as the biological value, true digestibility, net protein utilization and net dietary protein calories because of their precision and less dependence on any of the draw-backs of PER.

(ii) Nitrogen Balance and Biological Value

As Allison (1955) pointed out, amino acids may be considered as entering a dynamic 'pool' contributed to both by dietary and tissue proteins. Since nitrogen balance is the sum of the gains and losses of all tissue proteins of the body, it may be used as a measure of dietary protein to the animal concerned. The fraction of the absorbed

nitrogen retained in the body has been defined as the 'biological value' (Campbell, 1963).

Nitrogen balance was determined from the equation:

$$B = I - (U+F)$$

where B is the nitrogen balance, I is the nitrogen intake and U and F are the nitrogen excreted in the urine and faeces respectively. As all the faecal nitrogen does not originate entirely from the food, a correction was nooessary for the metabolic or endogenous nitrogen. To overcome this, Mitchell (1923 - 24) expressed biological value as:

$$BV = \frac{\text{N intake} - (\text{Faecal N} - \text{Metabolic N}) - (\text{Urinary N} - \text{Endogenous N})}{\text{N intake} - (\text{Faecal N} - \text{Metabolic N})} \times 100$$

'True digestibility' defined as the fraction of the ingested nitrogen absorbed, was similarly expressed as:

$$T.D. = \frac{\text{N intake} - \text{Faecal N} - \text{Metabolic N}}{\text{N intake}} \times 100$$

The figures for the metabolic faecal N and the endogenous N were obtained from the Kjeldahl analyses of faeces and urine collected for a control group of litter mate animals fed a nitrogen - free but isocaloric diet during the experimental periods.

The biological value is affected by many factors, including, the level of dietary protein (Forbes et al. 1956), amino acid imbalance

(Doshpando et al. 1956), caloric intake (Rosenthall and Allison, 1951), hormones (Guggenheim and Halevy, 1958) and season of the year (Rice and Flodin, 1960). Similarly, factors affecting the balance-sheet method for determining biological value have been discussed by Henry and Kon (1957) and by Forbes and Yoho (1955). Henry and Kon (1957) have expressed the importance of a standard protein for use in each assay. Forbes et al. (1956) and Rippon (1959) showed the linear decrease in the biological value of various proteins as the concentration in the diet or intake increased. Differences between the carcass analysis method and the balance - sheet method were discussed by Rippon (1959) and Henry and Foothill (1962) who found that the latter method yielded higher results.

(iii) The N P U method (by Carcass - Nitrogen)

The method was first described by Bender and Miller in 1953 for determining the net protein value (Bender and Miller, 1953 a). It gave results similar to the biological values determined by the Thomas - Mitchell method according to the procedure of Henry et al. (1937). The method has been described in detail by Miller and Bender (1955) who defined NPU, that is, BV X TD, as:

$$\text{NPU} = \frac{\text{Body N of test group} - \text{Body N of N - free group} + \text{N consumed by N - free group}}{\text{N consumed by test group.}}$$

Further simplification of the method of carcass nitrogen analysis by the use of body N: H_2O ratio has also been described and applied in

(Deshpande et al. 1955), calorie intake (Rosenthall and Allison, 1951), hormones (Guggenheim and Halevy, 1958) and season of the year (Rico and Flodin, 1960). Similarly, factors affecting the balance-sheet method for determining biological value have been discussed by Henry and Kon (1957) and by Forbes and Yoho (1955). Henry and Kon (1957) have expressed the importance of a standard protein for use in each assay. Forbes et al. (1956) and Rippon (1959) showed the linear decrease in the biological value of various proteins as the concentration in the diet or intake increased. Differences between the carcass analysis method and the balance - sheet method were discussed by Rippon (1959) and Henry and Foothill (1962) who found that the latter method yielded higher results.

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NPU estimation by Bender and Miller (1953 b) and many other workers.

Bender and Doell (1957) proposed the use of the net protein ratio (NPR) which is simply the weight loss of a negative control group added to the weight gain of the test group divided by the protein consumed by the latter group. This calculation was claimed to overcome the variations in food intake encountered in PER determinations. The NPR multiplied by an experimentally determined factor is called the 'Protein Retention Efficiency'. For Bender and Doell's (1957) data, the factor was 16. Forbes and Yohe (1955) found somewhat lower precision of NPU values particularly when calculated from water content of carcass than when determined by classical methods.

(iv) Microbiological Method

The need for a simple rapid and inexpensive method for protein evaluation had served to focus attention on the possibilities of using microbiological assays. A variety of methods have been proposed and used by Anderson and Williams (1951), Rosen and Fernell (1956) and many other workers, but each of the methods has always been found to distinguish between a limited number of proteins (Campbell, 1963). Commenting on the limited applicability of microbial methods of protein evaluation, Campbell (1963) said that their shortcomings limit, at present, their general application to merely screening procedures for predicting the nutritive quality of proteins in some foodstuffs.

(v) Chemical Scoring Method

Block and Mitchell (1946) devised a system of chemical scores based on the amount of the essential amino acid in greatest deficit in a protein compared to the level present in a reference protein (egg protein). However, as Mitchell observed, the chemical score is an index of the value of protein for growth only, since it assumes that the absence of an essential amino acid renders the protein completely unavailable even for tissue maintenance. Though available evidence suggests that the value of many foods may be predicted accurately from their amino acid content (Osor et al. 1960; Osor, 1951), Campbell (1963) has pointed out the possibility that the use of these methods may overestimate the true biological value.

(vi) Protein Regeneration Method

Frost (1959) and Allison (1955) have reviewed methods of evaluating protein quality based on regeneration of protein determined either by liver protein stores or by the body weight increase after depletion.

The protein content of the liver was shown to be a function of both the amount and value of dietary protein. Kesterlitz and Campbell (1945, 1945 - 46), and Campbell and Kesterlitz (1948) developed several methods for the estimation of labile liver protein after brief fasting in rats. The methods have, however, been criticized by Allison (1955) in that under certain circumstances, the liver may not reflect the state of the other labile protein stores in the body, and by Campbell (1963) as being laborious and offering

no advantage over the PER methods.

The repletion of adult depleted rats has been recommended by Campbell (1963) in that the same rats can be used as many as five times although Summers and Fisher (1960) have questioned the validity of this method with chicks.

The activities of liver enzymes are decreased by repletion of protein stores, and these have been used to study various functions of protein in the body (Allison, 1955). Levels of xanthine oxidase were found (Linack et al. 1953, 1954) to correlate fairly well with growth methods. Xanthine oxidase was found to be more dependent on protein content of the diet than the d - amino acid oxidase activity by Ramakrishnan et al. (1961).

(vii) Blood Amino Acid Pattern and Availability Methods

Several other methods have been developed for the evaluation of dietary protein quality. The blood amino acid methods have been demonstrated with adult dogs (Longenecker and Rouse, 1959) with rats (Guggenheim et al. 1960; Goldberg and Guggenheim, 1962) and humans (Albanese, 1959); Rootall et al. 1958; Frame, 1958). These methods are, however, cumbersome and often fail to give good correlation with growth methods (Campbell, 1963). Arroyave et al. (1962) found in kwashiorkor patients that fasting plasma levels of amino acids were reduced to about one - half of those in healthy children.

Availability of amino acids has been assessed by chemical, microbiological, enzymatic and animal techniques. The methods have all

been shown to correlate very well with biological assays. For instance, Clarke and Kennedy (1962) found that both carcass nitrogen gain and body weight gain were linearly related to the available lysine ingested as determined by animal assay.

(viii) Evaluation of Diets

It has long been known that protein in food should be evaluated in terms of quality and quantity. Mitchel (1922) proposed multiplying the protein content of a food with its biological value to obtain a measure of the 'net protein value' of the food. Hegsted (1957) and others have stated that both quality and quantity are necessary to express the true protein value of a food. Block and Mitchell (1946) suggested that the classification of individual protein foods as good, bad or indifferent in covering the requirements when fed alone, is of little significance in assessing the protein value of diets because of the supplementary relationships. This statement questions the validity of determining the quality of individual foods as applied to diets as a whole.

Platt and Miller (1958) called attention to the scarcity of measurements of quality and quantity of dietary protein. They listed some of the difficulties that may be encountered in such measurements. These include (a) the complexity of the mixtures eaten, (b) the nature and ticing of snacks and meals, (c) the effects of processing and cooking, the level of protein and other nutrients and (d) the balance of protein and other nutrients. These factors would influence NPU

values determined by the standardised procedures of Miller and Bender (1955). Therefore, NPU values so determined would not apply in diets where the utilisation of the protein may be influenced by such factors and others, for example, the protein to calorie ratio (Campbell, 1963).

To overcome these effects, Platt and Miller (1959) fed freeze - dried diets and meals without modification to rats. In this way, they felt that the value of the protein in the food would be influenced by whatever factors, or lack of them, existed in the diet and at a concentration of protein representative of the diet. The NPU so obtained would be designated NPU (operative) while that obtained under standardized conditions with semi-synthetic but otherwise complete diet would be NPU (standardized) (Miller and Bender, 1955).

When the NPU (operative) was multiplied by the crude protein content (N x 6.25), Miller and Bender (1955) derived the term, Net Dietary protein Value (NDpV), which represents the utilizable protein in the mixture and is a function of quality and quantity. Also, when NPU(op.) was multiplied by the total protein calories percent of the diet, the factor, the net dietary protein calories percent (NDp Cals %), was obtained.

Miller and Payne (1960) determined NPU (op) values for three different proteins, wheat gluten, casein and beef, fed at concentrations from maintenance to 45% protein calories. The relationship between NPU (op.) and the level of protein fed was linear. From these data, they obtained curves relating protein, calorie ratio,

NPU(at) and NDp Cals %. In further studies of the application of NDp Cals %, Miller and Payne (1961 a,b,c) found it possible to predict the protein values of diets. Such results were often in good agreement with those of bioassay. This method has been found particularly valuable for the estimation of the protein values of human diets (Campbell, 1963).

8. Use of the Laboratory rat in Feeding Experiments

The study of the nutritive quality of human diets by means of the laboratory rat is not a novel procedure. More than forty years ago, McCarrison (1927) and some years later, Cuha (1934) produced in rats symptoms of dietary deficiencies which they regarded as characteristic of human population groups in several parts of the world, by feeding the rats on diets commonly eaten in the respective regions. Orr et al. (1935) reported poor growth, non-thrifty appearance, and poor reproduction, rather than specific deficiencies, occurring in the same species on a diet approximately that of an average working class community in Scotland. The efficacy of whole milk in correcting the condition was shown by Gaunt et al. (1939) to be attributable to their calcium and phosphorus content.

The experiences of a number of investigators who have attempted to appraise human - type diets (including those of the U. S. wage earners in cities, army rations and war-time European and Chinese diets) by animal studies have been summarized by Williams (1947). Hosphill et al. (1943) and Williams (1947) reported satisfactory

growth in rats fed a natural cooked food. Skin sores about the head have been reported by Hewitt et al. (1949) in rats given a diet riboflavine - deficient for human adults. Saxton (1945) and Saxton et al. (1946) have likened bronchiectasis in the rat to arteriosclerosis in man, in that, these are chronic progressive diseases which limit the life span in their respective species.

Both Hegsted et al. (1947) and Mitchell (1954) reported independently that man resembles closely the young rat in his metabolic utilization of food protein. They indicated that the results of rat growth test were, on the whole applicable to the evaluation of human diets. Mitchell (1959) showed that the biological values of six proteins for the growing rat resembled closely those for adult man. Using literature data Flodin (1959) found fairly good correlation between PER values determined with the rat and biological values using adult man. While there are known differences in the amino acid requirements of man and rat, Hegsted (1957) pointed out that these are relatively minor. Since the requirements of children for amino acids are more critical than those of the adults, it would seem that the growth of rats should furnish a reliable indication of the value of food protein to children (Campbell, 1963).

Miller (1970) commented, in a symposium, that an 'appeal to the rat' is often a less laborious and more direct way of assessing diets consumed by man, in that apart from giving an idea of the nutritive value of the diet to the consumer, it has the added advantage that

the presence of toxic factors may also be detected.

9. Nutritional Studies in Nigeria

A number of nutritional studies has been carried out in Nigeria by a number of workers or by groups of workers. The procedures used range from nutritional surveys through a biochemical more to the direct assessment of some Nigerian diets and new food formulations with human beings and small laboratory animals.

Among the nutritional surveys conducted in Nigeria, the most extensive and informative are those of Nicol which were carried out over a period of some 12 years (1949, 1952, 1956 a and b, 1958 and 1959 a and b). The results of Nicol's studies showed that Nigeria could be divided into two main dietary zones, the southern zone and the northern zone, according to the pattern of food consumption (Oke,1967b). Nicol observed also that the main daily intakes of individual foods for adults (over 12 years of age) at the different locations reflected the pattern in the different zones. There was a general agreement between intakes and needs of adults who were not very active. Children, on the other hand, did not receive enough food to meet their calorie requirements as a result of a family poor food distribution pattern. This pattern allows a 'lion share' of the already insufficient family meal go to the father, then the women, so that the children are considered last. The UNICEF Follows (1967) report on the nutritive value of food taken by an average Nigerian family, indicated that the food would be comparable to

that in other parts of the world but for the poor distribution pattern already mentioned. However, the average diet is deficient in animal protein.

Broad generalizations of the nutritional status of the Nigerian community have been made from the food consumption surveys conducted in some isolated places in the country by McCulloch (1930), Thomson (1956 a, b; Calloti et al. (1956), Wilson (1954 a, b), Chartres (1951), Martin (1956), Ekpo (1965), UNICEF Fellows (1964, 1966). These studies have been re-enforced by the work of Collis and his associates at the Institute of Child Health, University of Ibadan (Collis et al. 1962 a and b; Dean 1962, 1963, 1964 a, b) and by the report of the Inter - Departmental Committee on Nutrition for National Development (ICNND) (1967). The information obtained from these studies is still inadequate especially as there has been no nation - wide food surveys and above all, in the isolated few reports already mentioned, the nutritive values of the diets assessed are almost invariably calculated from tables of food composition compiled in other countries rather than making use of values determined by analyses of foods produced and processed locally (Oyenuga, 1967) or of the diets prepared to be served at table.

These reports all indicate that the food consumed is largely derived from domestic peasant sources. The diets are ill-balanced with regard to protein, calories and certain other essential nutrients. On the medical side, evidence on the morbidity and mortality from

protein - calorie malnutrition is strong in many areas of the country. The high rate of mortality in infants and young children as a result of kwashiorkor and marasmus is a common feature in many peasant homes. There is undoubted scarcity of suitable foods containing protein of high biological value with which to prevent these conditions and treat established cases. The impaired health and growth rates which are associated with these conditions indicate the gravity of the problem of protein - calorie malnutrition in all Nigerian communities (Idusogie, 1972).

Nasir (1963 a) conducted experiments with rats and human beings using gari supplemented with full fat soya flour. He noted the effect on growth reproduction and lactation. In some experiments the soya - gari diets were supplemented with lysine and methionine separately and together. His results showed that soya flour alone, if heated to destroy the antitryptic factor, was well utilized as a supplement to indigenous local diets for lactating mothers, and that such diets enhanced milk production. The experiments also showed that milk proteins could be satisfactorily substituted with soya protein in gari - based diets, provided optimal amounts of methionine were added. Also, the addition of lysine together with methionine to such diets was shown to make them more valuable in their growth - promoting qualities than the best commercial stock diet.

Scattered through the literature are reports all pointing to the

various nutritional deficiencies and their impact on health in Nigeria. In a review of most of the nutritional studies carried out in Nigeria between 1933 and 1953, Bassir (1953) pointed out that a good deal of the general morbidity and inefficiency of the people of this country can be attributed to dietary insufficiency, especially with respect to animal protein, vitamins and certain inorganic salts.

Malik (1967) carried out a study of the peasant diets of the Yorubas of western Nigeria. His results showed that most of the diets have high biological values and that the protein contents of the diets were very highly utilised. Since the different parts of this country have different feeding habits and dietary patterns, similar studies on the nutritional status of the other areas of Nigeria are not only necessary but very desirable.

10. Factors Affecting Nutrient Intake By Peasant Nigerian Families

Nutrient intake by the Nigerian community varies greatly from one state to the other, and within the state, from one family to the other. This is influenced by a number of factors such as the sowing and harvesting season of the year. According to Dema (1964 b) the average food intake of children in the land hungry seasons provides just about 53% of their total energy requirements. He observed that the total nutritive value of the diets of families sampled in the rural areas of Ilesha in Western State of Nigeria is at its best at the beginning of the farming season, a period when the energy expenditure of the farmers is high. Bassir (1972) referred to the time in

many parts of Nigeria when millions of people do not have enough of any kind of food to eat and mild starvation ensues as the 'hungry season.' At this period, the foods are scarce and the prices are high. Ekpo (1970) attributed seasonal food shortages to low production, poor storage facilities and losses in the farms, lack of organised marketing of foodstuffs, poor distribution facilities and inadequate processing of food crops. As a result of this, the average food intake may fall to about 60% of the actual energy requirements. This discrepancy is most marked during the farming season when much energy is needed for farming and for preparation of fields for farming.

Idusogie (1971) mentioned the size and composition of the family unit as being a determinant factor in regulating nutrient intake in many Nigerian homes. In recent years, the traditional system of poly-nuclear family in many Nigerian societies makes it compulsory or obligatory for a slightly better placed relative to feed and cater for a host of unemployed or poorly-placed relatives. With limiting food and money resources, the practice has been for housewives, faced with the problem of having to feed a large number of people from the same cooking pot, to provide diets based on cheap, starchy and low quality staple foodstuffs such as cocoyams, cassava, and plantain, poorly supplemented with low-quality animal products such as the skin and foot of cows, pigs, goats, and sheep. The effect of family size on household food consumption has been shown by the survey data

of Deen (1967), Chon (1967) and UNICEF Follow-up Report (1967).

Dean (1965) mentioned certain other factors which affect nutrient intake by Nigerian communities. Among these are the resources of the family as measured by the extent and nature of cropping or money-earning capacity; losses of foods and nutrients through post infestation of man and his crops and livestock, and poor storage facilities. Others include the peasant farmer's ignorance of the nutritional needs of his family and the means of fulfilling them.

Other significant determinant factors of nutritional status are the changing food habits within the Nigerian community. Unfortunately, these food habits are governed by definite taboos which take different anthropological and sociological forms. For instance, the moslems in the Nigerian community do not eat pork, a habit which could be traced down to the earliest Jewish people who probably introduced this to get rid of the then prevalent pork tapeworm, which was thought to be sapping the strength of the Jewish people (Latham, 1965). Many other factors which contribute to the malnutrition and undernutrition problem in Nigeria have been extensively discussed in the FAO/UNICEF draft report on the Extension Seminar for the Federation of Nigeria (1962).

11. Nutrient Content of Some Locally Produced Foods and Foodstuffs

Nigerian community consists largely of peasant families whose diet is consumed is grown locally. Since a variety of food crops and livestock thrive in different parts of the country, nutrient consumption

varies appreciably in the different areas of the country.

Studies have been carried out on the nutrient content of some Nigerian foods and foodstuffs used in peasant dietaries with a view to assessing the nutritional status of sections of the community. For instance, improved strains of sorghum grown in Zaria have been shown by Mathieson (1964) to have up to 13% crude protein content. Though sorghum proteins are usually deficient in lysine and to a lesser extent in arginine, isoleucine and methionine, these improved strains of sorghum are known to have a fairly good essential amino acid pattern. Expressing the results on basis of 16 gm of nitrogen, Mathieson (1964) observed that one improved strain showed lysine content to be 2.4 against 1.8 for the indigenous cereal, arginine 3.5 against 3.2, isoleucine 5.1 against 5.0.

Oke (1965) carried out chemical studies on cassava (Manihot utilissima), one of the main staple food crops of West Africa. The changes in the food value, mineral content and poisonous substances during conversion of raw cassava to 'lafun' (flour from pooled fermented raw cassava) reported by him indicated that in the process about 70% of the protein is lost. There was a slight decrease in the total ash content so that the Ca : P ratio changed from 0.87 : 1 for cassava to 1 : 6 for 'lafun' compared with the optimum ratio of 1 : 2.

Pulses have been known to furnish the bulk of the protein in human diets in some parts of Nigeria. The most commonly used pulses include cowpea (Vigna unguiculata), groundnut (Arachis hypogaea),

lima bean (Phaseolus lunatus), pigeon pea (Cajanus cajan) and soya bean (Glycinu max). Analysis of those pulses by Oke (1967b) showed soya bean to be the richest in crude protein, 43% dry weight. Soya bean protein has been ranked next to animal protein, especially in its lysine content, and could therefore be an excellent protein supplement for the low income group who cannot afford sufficient animal protein. Unfortunately, soya bean is not eaten in Southern Nigeria. Bassir and Loebel (1968) have shown, however, that soya - ogi and soya - gari (enriched starchy staples with soya bean meal) are adequate for the treatment of kwashiorkor.

The protein contents of cowpea, groundnut, lima bean and pigeon pea are 29.63, 26.00, 20.75 and 22.38% dry weight respectively (Oke, 1967 b). Ground nut is more concentrated in calories (600 cal/ 100 gm dry matter) than the other pulses (350 cal/100 gm dry matter of each pulse). The Ca: P ratios are 1 : 6 for cowpea, 1 : 4 for groundnut, 1 : 7 for lima bean and 1 : 3 for soya bean (Oke, 1967 b)

Akinrele (1967) commented on the poor nutritive value of gari as being incompatible with its role as a staple food for millions of people in Nigeria and elsewhere. He raised the biological value of gari from 47 to 68 for rats by supplementing 5.4 gari with a protein - rich supplement consisting of 70 parts of full fat soya flour, 20 parts of whole fat sesame flour, 5 parts of defatted groundnut flour and 5 parts of dried yeast powder. Since an average Nigerian consumes about 300 gm gari (dry weight) per meal, the fortified

product would provide about 24 gm of protein which is more than a third of the minimum daily requirement.

Munro and Bassir (1969) carried out analyses of some fruits and vegetables commonly used in Nigeria for their ash, calcium and oxalate contents. They showed that there was more calcium than oxalate in tomato, sweet corn, onion, yam, cowpea, carrot, lime pulp and okro. On the other hand, garden egg, orange pulp, cucumber, cocoyam, plantain, spinach, tangarine pulp, groundnuts, red pepper, green beans and melon seeds were shown to be richer in oxalate than in calcium. Since the importance of calcium in animal and human nutrition is well known, the possible danger of calcium - oxalate interaction in the body cannot be overemphasized (Munro and Bassir, 1969).

Also scattered throughout literature has been the report on nutrient composition of some locally produced foodstuffs. Oyenuga (1968) has published a detailed analysis of the nutrient composition of some Nigerian foods and feedingstuffs. Further comprehensive information on the nutrient contents of some local and foreign foods has been given by Platt (1962). In all those publications, it is the composition of the raw food materials that is given.

Despite the available data above, information is still insufficient on the nutrient composition of an uncountable variety of the conventional and unconventional foodstuffs used in many parts of Nigeria. Also, cooking has been known to bring about highly complex reactions which

have a direct bearing on the nutritional value of food before it is ready to be served. Several methods of cooking and preparation of the materials prior to cooking are used in many parts of Nigeria. Members of the FAO/UNICEF study group for the Federation of Nigeria (1962) unanimously recognized the traditional cooking methods employed in various parts of Nigeria as one single factor contributing in no small measure to the malnutrition and undernutrition problems in the country.

Some studies of the apparent losses under controlled conditions of cooking and processing of individual foods have been reported in developed countries and have already been reviewed in this thesis. In Nigeria where the methods of cooking are different from the ones employed in those countries, this type of information is still required. However, Malik (1967) carried out an experimental study and obtained detailed results of some traditional peasant dietaries of the Yorubas of Western State of Nigeria. The changes which the traditional cooking methods bring about on the individual constituents as well as on the mixture as a whole were duly evaluated. The application of his results to other areas of Nigeria would however be misleading on account of the varying cultures, customs, feeding habits, religious beliefs, economic and nutritional status, tastes and the agricultural products of the people of this country.

In South - Eastern Nigeria information on the nutritional status of the peasants is seriously and significantly lacking despite the

already formidable catalogue of diseases of malnutrition and undernutrition among the peasant communities in the area. Therefore, the work described in this thesis was carried out to investigate the quality of six commonly used peasant diets of the area and the effects of the traditional cooking methods on their nutritive values. It is hoped that this information from the chemical, biochemical, and biophysical analyses of the formulations of these peasant dietaries described in the following pages will go a long way to portraying the nutritional needs of these people.

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CHAPTER TWO

MATERIALS

1. Sources and Nature of Foodstuffs

The foodstuffs used for the preparation of the various traditional dishes were bought in some local markets within eight miles of the University of Ibadan Campus. No reference was made to the geographical origin, storage conditions or treatment of the foodstuffs following harvest. They were quickly brought to the laboratory for cooking and analyses.

Studies were carried out on the following six diets commonly used in South-Eastern Nigeria.

1. Gari, 'afan' soup with smoked fish
2. 'Anyan ekpan' (grated cocoyam and wateryam) and 'abak' (pulmut) soup
3. Cassava foofoo (cooked fermented cassava tubers) and 'odikan ikon' (thick vegetable) soup
4. Pounded yam and 'afia efere' (plain soup)
5. 'Ekpan nkukuro' (grated cocoyam) and dried shrimps
6. 'Ewa ukot' (plantain porridge) and smoked fish, and for the purpose of comparison.
7. 'Reference' diet (containing cornstarch, casein, and optimum quantities of minerals and vitamins for the growth of the laboratory rat) was also studied.

Gari (fried cassava flour) and raw fermented cassava (for preparation of cassava foofoo) were bought as such in the market. All the diets were prepared in the laboratory. In preparing these diets, the ordinary traditional proceduros were used so that the diets would be identical with the food as actually consumed.

2. Recipe for the Preparation of the Various Diets

In order to work with the actual proportions of the condiments as would be used by a peasant farmer's wife for the preparation of the diet, a survey was conducted. Ten women, all from South Eastern Nigeria and mainly wives of junior workers in the University of Ibadan, were invited. The foodstuffs were given out to them in greater quantities than they would have used to prepare a single meal for five people. Each took as much as she could have used in the preparation of the meal. The quantity of each item taken by each woman was collected and weighed. The means of these weights for all the materials were computed. Each diet was then prepared with these predetermined proportions of the ingredients. The raw diet was similarly compounded.

The results of the survey and the actual proportions of the condiments used for the preparation of the diets are shown in the tables that follow.

Table I (a)

Survey on Recipe for Diet No. I (Gari, afan soup with smoked fish)

SUBJECT	CONDIMENT AND WEIGHT USED (G.)									
	Crayfish(dry) (Palaemonetes varians)	Dried, ground pepper (Capsicum frutescens)	Salt	Palm oil (Elaeis guineensis	Gari(fried cassave flour) (Manihot utilissima)	'Afan' (picked) (Gnetum africanum)	Waterleaf(picked) (Talinum africanum)	Periwinkle	Smoked fish	Egusi (Vivarium quadreta)
1	19.6	11.0	19.1	123.5	110.1	413.7	110.5	110.5	174.0	176.0
2	19.6	15.0	18.0	120.0	851.6	78.6	330.4	101.1	206.3	220.5
3	20.1	10.1	21.1	140.0	830.0	115.3	370.1	130.0	206.4	175.0
4.	30.5	21.0	16.5	133.0	820.1	109.1	360.5	131.0	180.9	180.5
5	25.0	20.3	19.0	90.3	790.9	130.5	351.0	79.8	171.3	300.1
6	19.5	18.6	19.3	120.1	821.8	111.3	600.3	108.0	174.0	201.5
7	24.8	6.6	20.3	150.8	835.0	126.6	313.5	100.0	163.1	180.0
8	20.3	13.1	19.6	183.5	624.3	100.6	290.7	104.1	145.5	208.1
9	31.5	9.8	18.7	194.9	1,003.5	65.8	340.3	106.0	174.3	154.0
10	19.0	10.1	21.3	100.4	938.2	132.2	344.1	90.3	150.1	176.5
Mean	22.9	13.5	19.3	123.4	841.9	108.0	351.7	106.1	174.6	197.3

Table 1 (b)

Recipe for Diet No. 1 (Gari, 'afan' soup with cooked fish)

CONDIMENT	WEIGHT USED (gm)
Gari	841.9
Crayfish	22.9
Pepper	13.5
Salt	19.3
Palm oil	123.4
'Afan' vegetables	108.0
Waterleaf	351.7
*Periwinkle	106.1
*Smoked fish	174.6
*Snails	197.3

*Edible portions only.

Table 2 (a)

Survey on Recipe for Diet No. 2 ('Anyan akoon' with 'abak' soup)

SUBJECT	COMPONENT AND WEIGHT USED (GM)								
	Water yam (Discorea alata Linn)	Cocoyam (Xanthosoma) (caffa Schott)	Palm fruit (Elaies guinoensis)	'Atama' (picked) (Heinsia crinita)	Smoked fish	Crayfish (Palaemoneteo varians)	Onions (Allium cepi)	Pepper(dried) (Capsicum frustescens)	Salt
1	2150	2250	1905	503	301	23.5	250.0	8.9	39.1
2	2010	1980	956	433	150	30.5	130.0	16.0	25.0
3	2500	2223	1875	550	209	31.1	130.8	14.3	23.3
4	1950	2513	1553	519	365	25.0	200.6	14.2	23.0
5	2005	2116	1894	431	150	40.5	250.0	16.5	21.9
6	2451	1870	777	511	196	33.7	190.5	10.1	25.4
7	2221	1871	1239	297	250	35.0	130.0	12.3	28.3
8	1693	2503	1050	444	511	21.9	178.3	14.0	19.4
9	1880	2019	1001	398	210	30.1	205.0	12.0	20.5
10	2047	1992	934	501	170	41.6	250.1	10.9	23.7
Mean	2090	2134	1258	459	251.0	31.3	191.3	12.9	25.8

Table 2 (b)

Recipe for Diet No. 2 ('Anyan okpan' with 'abak' soup)

CONDIMENT	WEIGHT USED (GM)
Wateryam (peeled)	2090.0
Cocoyam (peeled)	213.0
Palm fruit	1258.0
'Atama' vegetables (picked)	459.0
*Smoked fish	251.0
Crayfish	31.3
Onions	191.3
Dried pepper	12.9
Salt	23.8

*Edible portion only

Table 3 (a)

Survey on Recipe for Diet No. 3 (Cassava foofoo with 'ediwan ikon' soup

SUBJECT	COMBINENT AND WEIGHT USED (GM)							
	Crayfish(dried) (Palaemonetes varians)	Smoked fish	Waterleaf(pieced) (Talinum triangulare)	Palm oil (Elaeis guineensis)	Dried pepper (Capsicum frutescens)	Salt	Fermented cassava (Manihot utilissima)	'Ikon utoo' (Pieced) (Telferia spp.)
1	35.0	198.0	637	195.1	21.3	44.5	4,570	803
2	30.5	221.0	489	201.5	25.0	30.1	3,995	661
3	25.1	181.3	553	180.0	30.0	42.8	6,003	790
4	31.9	198.1	600	181.7	20.5	33.3	5,331	795
5	40.0	201.0	701	196.1	19.3	36.5	4,535	651
6	21.8	189.1	593	150.1	20.8	40.2	4,500	739
7	31.8	177.4	666	246.2	27.1	50.0	4,153	557
8	30.7	180.0	570	144.8	27.5	41.6	3,817	795
9	28.8	218.0	431	190.5	17.5	41.9	4,444	699
10	36.0	174.3	748	180.7	29.1	50.7	5,006	890
Mean	31.2	194.3	598.2	166.7	23.8	41.2	4,635	736

Table 3 (b)

Recipe for Diet No. 3 (Cassava foofoo with 'odikan ikong')

CONDIMENT	WEIGHT USED (GM)
Fermented Cassava	4,635
'Ikong ubong' vegetables (picked)	758
Crayfish (dried)	31.2
*Smoked fish	194.3
Waterleaf vegetables (picked)	578.2
Palm oil	186.7
Dried, ground pepper	23.8
Salt	41.2

*Edible portion only

Table 4 (a)

Survey on Recipe for Diet No. 4 (Pounded [illegible] with 'Ofe efore')

SUBJECT	CONDIMENT AND WEIGHT USED (GM)								
	Okro (Hibiscus esculentus)	Onions (Allium cepa)	Smoked fish	'Etinyan' (picked) (Amaranthus spp.)	Yam (fresh) (Dioscorea spp.)	Palm oil (Elaeis guineensis)	Pepper (ground) (Capsicum frutescens)	Salt	Crayfish (Palaemonetes varians)
1	364.5	385.6	278.3	333.1	4900	91.3	16.5	26.5	40.3
2	300.0	401.3	281.5	200.0	5015	100.3	18.9	14.9	21.9
3	431.2	288.8	211.1	161.0	5300	150.0	20.8	30.8	30.6
4	340.1	333.6	213.9	116.7	4398	83.0	15.3	31.8	37.1
5	398.5	401.7	185.3	301.1	5873	94.9	12.1	25.5	28.8
6	299.7	348.5	443.4	226.4	4443	130.3	11.9	31.8	35.7
7	303.0	383.1	186.5	201.0	5581	101.1	25.7	29.7	50.1
8	167.4	453.4	221.9	222.2	5005	105.0	21.8	15.8	20.8
9	275.9	225.1	300.0	130.7	4871	88.7	9.5	29.9	19.3
10	495.2	581.9	461.3	186.8	5190	200.1	10.1	20.6	25.5
Mean	360.6	380.3	280.3	209.7	5058	114.5	16.3	26.8	31.0

Table 4 (b)

Recipe for Diet No. 4 (Pounded yam with 'efia ofere')

CONSTITUENT	WEIGHT USED (GM)
Yam tubers (peeled)	5,058
Okro	360.6
Onions	380.3
*Smoked fish	280.3
'Efiryon' vegetables(picked)	209.7
Palm oil	134.5
Pepper (dried, ground)	16.3
Salt	26.8
Crayfish	31.0

*Edible portion only

Table 5 (a)

Survey on Recipe for Diet No. 5 ('Ekwa Nkukwo')

SUBJECT	CONDIMENT AND WEIGHT USED (GM)					
	Cocoyam (peeled) (*Xanthosoma mafaffa Schott*)	Crayfish (dried) (*Palaemonetes varians*)	Smoked fish	Dried, ground pepper (*Capsicum frutescens*)	SALT	Palm oil (*Elaeis guineensis*)
1	3205	40.1	301.1	19.0	50.0	355.0
2	4059	36.4	280.0	23.5	39.1	400.0
3	3111	30.1	185.3	12.8	36.3	369.9
4	3358	29.9	277.7	11.7	55.5	228.8
5	2981	36.5	280.9	10.5	58.1	483.1
6	5813	38.9	330.1	30.3	59.8	187.5
7	1896	37.7	401.9	31.5	44.4	291.4
8	2221	48.1	183.4	25.1	39.9	501.1
9	3887	40.1	299.1	15.9	58.1	333.8
10	3300	32.0	241.9	20.0	60.0	355.8
Mean	3383	37.0	278.2	20.0	48.1	350.6

Table 5 (b)

Recipe for Diet No. 5 (Edem Ikwo)

CONDIMENT	WEIGHT USED (GM)
Cocoyam (pooled)	3383
Dried crayfish	37.0
*Smoked fish	278.2
Dried ground pepper	20.0
Salt	48.1
Palm oil	350.6

*Edible portion only

Table 6 (a)

Survey on Recipe for Diet No. 6 ('Ibuk Ukom')

SUBJECT	CONDIMENT AND WEIGHT USED (G.)						
	Plantain (peeled) (*Musa paradisiaca*)	Crayfish (*Palaemonetes varians*)	Pepper (*Capsicum frutescens*)	Salt	Smoked fish	Palm oil (*Elaeis guineensis*)	Waterleaf (picked) (*Talinum triangulare*)
1	2,845	41.5	20.0	50.5	331.5	241.1	199.3
2	4,351	28.0	32.8	56.1	163.1	200.0	301.1
3	3,132	30.3	25.1	36.6	189.8	150.9	289.3
4	1,578	30.3	15.5	29.9	120.0	101.0	130.5
5	1,893	31.0	19.8	50.9	411.3	133.1	93.6
6	4,006	50.8	18.8	63.5	125.8	190.5	140.0
7	2,589	43.3	29.4	45.9	237.0	218.3	401.1
8	3,305	19.8	30.0	49.8	609.0	381.5	106.4
9	2,911	29.1	36.9	66.6	113.1	111.1	165.6
10	5,015	20.7	21.7	50.2	101.9	175.8	171.7
Mean	3,163	32.5	25.0	50.0	220.3	190.3	196.9

Table 6 (b)

Recipe for Diet No. 6 ('Inuk Ukon')

CONDIMENT	WEIGHT USED (GM)
Plantain (peeled)	3163
Crayfish (dried)	32.5
Dried ground pepper	25.0
Salt	50.0
*Smoked fish	220.3
Palm oil	190.3
Waterleaf (picked)	198.9

*Edible portion only.

3. Composition of the Reference (Casein) and Non-protein Diets

The basal protein - free diet used was that of Rippon (1959) slightly modified. It was made of the following on air-dried basis:

Sucrose	140	gm.
*Vitamin mixture	100	"
Butter (Danish)	200	"
Non-nutritive cellulose	100	"
+Salt mixture	80	"
Cod liver oil	20	"
Corn starch	1360	"

The corn-starch was added after all other ingredients had been added and thoroughly mixed together.

+ Salt Mixture

The composition of the salt mixture was that of Rippon (1959) and was made of the following:

NaCl	50	gm.
$Ca_3(PO_4)_2$	400	"
$FeC_6H_5O_7 \cdot 3H_2O$	35	"
$MgSO_4 \cdot 7H_2O$	80	"
NaH_2PO_4 (anhydrous)	105	"
KCl	250	"
KI	1.0	"
$MnSO_4 \cdot 4H_2O$	0.2	"
NaF	0.04	"

"Vita-dr" Mixture

The vitamin mixture used in Glaxo Laboratories reported by Cuthbertson (1957) was used. This consisted of the following vitamins per kilogramme weight of the semi-synthetic ration.

Vitamin A	4000 I.U.
Vitamin D (calciferol)	2000 I.U.
Vitamin E (- tocopherol acetate)	280 mg.
Vitamin K (menaphthone)	2 "
Thiamine	30 "
@ Riboflavine	30 "
Pyridoxine	8 "
Ca D- pantothenate	100 "
nicotinic acid	100 "
Vitamin B_{12}	50 ug.
Choline	1000 mg.
@Pteroylglutamic acid	1.0 "
Biotin	0.2 "
Inositol	220 "
p-aminobenzoic acid	75 "

(@ Riboflavine and Pteroylglutamic acid were added last.)

The reference (casein) diet was prepared by incorporating casein (B.D.H. Casein, non-defatted) at the expense of the corn-starch in the basal protein-free diet to give 10% protein (N X 6.25).

Both the basal protein-free diet and the casein reference diet were each made into a thick paste with a small quantity of water to reduce spillage by the animals.

4. Materials for the determination of Dry Matter (A.O.A.C., 1960)

Oven (Griffin over, Model 3/300PC)

Porcelain crucibles (100-ml capacity)

Analytical balance (Mettler H20, Gallonkamp)

A desiccator

5. Materials for Estimation of Crude Fat (Ether Extract) A.O.A.C, 1960

Soxhlet extraction apparatus (B & T, 251/2000)

Fat extraction thimbles

Beakers (100-ml capacity)

Analytical balance (Mettler H20, Gallonkamp)

Desiccator

Reagent:

Anhydrous ether: This was prepared by washing commercial ether with three portions of distilled water. Solid NaOH was added and the ether left to stand for 15 hours. This was then decanted into another dry bottle and small pieces of metallic sodium added. The bottle was loosely stoppered and left to stand until the evolution of hydrogen ceased.

6. Materials for Estimation of Total Ash (A.O.A.C., 1960)

As for section 4 above, and,

Muffle furnace (Gallenkamp, 220/240 V)

7. Materials for Estimation of Crude Protein (Total Food Nitrogen x 6.25)

(Markham, 1942)

(Plock, 1970)

Electric Heater box (BTL, 321/0010)

Distillation apparatus (Micro-Kjeldahl, Gallenkamp MR 650)

Kjeldahl digestion flasks (500-ml)

Concentrated sulphuric acid (C.P.R.)

Potassium sulphate (C.P.R.)

Mercuric oxide (C.P.R.)

Zinc dust (A.R.)

Sodium hydroxide solution: A 4% solution was prepared.

Methyl red Indicator: 1 gm was dissolved in 200 ml.
95% ethanol

0.1 N HCl solution: 8.9 ml conc HCl were diluted to 1000 ml and standardized against 0.1 N Na_2CO_3 (anhydrous) solution using methyl red as indicator.

Boric acid: A 4% solution was prepared.

8. Materials for Estimation of Tryptophane

(Miller, 1967)

Barium hydroxide, octahydrate $(Ba(OH)_2 .8H_2O)$ (A.R.)

K_2SO_4 (A.R.)

Hydrochloric acid (C.P.R.)

6N HCl : This was prepared by diluting 534 ml of conc HCl to 1000 ml.

0.4% (W/v) p-dimethylaminobenzaldehyde: 5 gm of the reagent were dissolved and made up to 1000 ml with conc HCl.

0.2% $NaNO_2$: 2 gm. A.R. $NaNO_2$ were dissolved in 100 cl distilled water and stored in refrigerator until required.

DL - Tryptophane

UNICAM Spectrophotometer (SP600).

9. Materials for Estimation of Calcium

(A.O.A.C., 1960)

Conc HCl (C.P.R.)

Conc HNO_3 (C.P.R.)

Conc Sulphuric acid (C.P.R.)

Ammonium oxalate solution: A saturated solution was prepared.

Methyl red indicator: Prepared as described on Page 72.

2% Ammonium hydroxide solution: 10 ml of conc 'analar' ammonia solution were diluted to 500 ml with distilled water.

Muffle furnace (Gallenkamp, 220/240 V)

Porcelain dish

$KMnO_4$ Solution: N/10 $KMnO_4$ solution was prepared and standardized against N/10 sodium oxalate solution.

10. Materials for Determination of Iron

(A.O.A.C., 1960)

As for section 4 above, and,

Conc HCl (B.D.H.)

α,α-dipyridyl solution: 0.4 gm α, α-dipyridyl reagent was dissolved and made up to 400 ml with distilled water. This was stored in the refrigerator until required.

10% Hydroxylamine - HCl Solution: 40 gm of the 'analar' reagent was dissolved in 400 ml water. This was stored in the refrigerator until required.

Acetate buffer Solution: 8.3 gm anhydrous Na acetate were dissolved in distilled water, 12 ml glacial acetic acid were added and volume made up to 100 ml.

Standard Fe Solution: This was prepared by dissolving 3.512 gm 'analar' $Fe(NH_4)_2SO_4 . 6H_2O$ in distilled water and adding 2 drops of conc HCl. The solution was made up to 500 ml with more distilled water. 10 ml of this solution were diluted to 1 litre (1 ml ≡ 0.01 mg Fe).

11. Materials for Estimation of Phosphorus

(Gomori, 1942)

As for section 4, and

Conc HCl

(C.P.R.)

10N H_2SO_4: Prepared by slowly adding 282 ml conc H_2SO_4 to 600 ml distilled and making volume up to 1000 ml with more water.

5% Sodium Molybdate Solution: 5 gm analar $Na_2MoO_4.2H_2O$ were dissolved and made up to 100 ml with distilled water.

Molybdate - Sulphuric acid reagent was prepared by mixing together 2 parts of 5% $Na_2MoO_4.2H_2O$, 1 part of 10N H_2SO_4 and 1 part of distilled water just before use.

Standard Phosphorus Solution: 3.592 gm. KH_2PO_4 were dissolved in water and made up to 1000 ml. A few drops of chloroform were added. (1000 ml solution = 0.5 gm P).

Metol (p-dimethylamino phenoloulphate): 2 gm were dissolved in 200 ml of a 5% solution of sodium bisulphite.

10% Trichloroacetic acid: 10 gm of C.P.R. trichloroacetic acid were dissolved and made up to 100 ml with distilled water.

12. Materials for Estimation of Vitamin A

(Carr - Price, 1926; Assoc. Vitamin Chemists, 1964).

100% KOH solution: 50 gm A.R. KOH were dissolved and made up to 50 ml with distilled water

Diethyl ether	A.R.
Ethanol	98%
Anhydrous Sodium sulphate	A.R.

Phenolphthalein: 1% solution was prepared in 100 ml
98% ethanol

Chloroform A.R.

Antimony trichloride: 250 gm (A.R.) $SbCl_3$ were dissolved in
1000 ml pure chloroform

Standard Vitamin A Solution: Crystalline vitamin A acetate in cotton seed oil, encapsulated in gelatin. Potency 30 ug vitamin A per gm of oil. (30 ug vitamin A = 100,000 I.U.).

Soxhlet extractor apparatus (B & T, 231/2000)

Fat extraction thimbles

Separating funnels (500 - ml)

Photo electric colorimeter (B & T, 307/3630)

13. Materials for estication of β-carotene
(Bassir, 1963 b)

~~100%~~ KOH Solution: Prepared as described for vit. A (page 75)

Diethyl ether A.R.

Ethanol 98%

Anhydrous Na_2SO_4 A.R.

Standard β-carotene Solution: 50 mg of pure β - carotene were dissolved in 'analar' chloroform and made up to 500 ml. (1 ml = 100 ug β - carotene)

Soxhlet extraction apparatus (B & T, 231/2000)

Fat extraction thimbles

Spectrophotometer SP500 (Unicam Instrument S.L - 50).

14. Materials for Determination of Vitamin B_1 (Assoc. Vitamin Chemists, 1966)

Anhydrous sodium sulphate (A.R.)

15% NaOH Solution: 15 gm were dissolved and made up 100 ml with distilled water.

1% $K_3Fe(CN)_6$ Solution: 1 gm of the reagent was dissolved in 100 ml water. It was stored in a dark brown bottle in the refrigerator until required.

Alkaline $K_3Fe(CN)_6$ Solution: 3 ml of the 1.0% $K_3Fe(CN)_6$ were diluted to 100 ml with 15% NaOH solution. This solution was usually prepared at the time of use.

0.1N HCl Solution: Prepared and standardized as described on page 72.

0.1N H_2SO_4 Solution: 2.8 ml of conc H_2SO_4 were diluted to 1000 ml with distilled water and standardised against 0.1N Na_2CO_3 (anhydrous) solution using methyl red as indicator.

Enzyme Solution: Fresh 1% aqueous solution containing Takadiastase, claraso, pancreatin, trypsin in 2.5 M sodium acetate solution was prepared.

25% KCl solution: This was prepared by dissolving 250 gm of KCl in distilled water and diluting to 1000 ml.

0.1N 25% KCl solution: 8.5 ml of conc HCl were diluted to 1000 ml with 25% KCl solution.

Activated Decalso: 'Special decalso for Thiochrome Determination' was activated by stirring continuously 500 gm of the material in 1 litre 3% boiling acetic acid for 15 minutes. This was decanted and the washing repeated thrice. It was then similarly washed 3 times with hot KCl solution and finally with hot distilled water so that the last washing gave no chlorine reaction. The product was dried in the oven at 100°C for 24 hours and stored in a dark brown bottle until required.

Stock Thiamine Solution: 100 mg of thiamine - hydrochloride dried over P_2O_5 in a desiccator for 24 hours were dissolved in 25% ethanol. 0.5 ml conc HCl was added and the solution diluted to 1000 ml. This was stored in a dark brown bottle in the refrigerator.

Intermediate thiamine solution: 5.0 ml of stock thiamine solution at room temperature were diluted to 100 ml with distilled water.

Working thiamine solution: 4.0 ml of the intermediate thiamine solution were transferred to a flask containing 75 ml 0.1N H_2SO_4, and 5 ml acetate solution were added and the volume adjusted to 100 ml with distilled water. This solution was prepared fresh when required. (1 ml = 0.2 ug thiamine).

Stock quinine solution: This was prepared by dissolving 100 mg (A.R.) quinine sulphate in 0.1N H_2SO_4 and diluting to 1 litre with the same solvent. The solution was stored in a dark brown bottle in the refrigerator until required.

Working quinine sulphate solution: 3 ml of the stock quinine sulphate solution were diluted to litre with 0.1N H_2SO_4.
(1 ml ≡ 0.3 µg)

Ethanol 95%.

Acetic acid: 30 ml glacial acetic acid were diluted to 1 litre with distilled water.

Hitachi Perkin - Elmer Fluorescence spectrophotometer (Model 203)

15. Materials for Estimation of Riboflavine
(Hodson and Norris, 1939)

0.1N H_2SO_4: Prepared and standardized as described on page 77.

2.5 M sodium acetate solution: 205 gm anhydrous sodium acetate were dissolved in water and diluted to 1000 ml.

4% $KMnO_4$ solution: 4.0 gm of 'analar' $KMnO_4$ were dissolved in water and made up to 100 ml.

3% hydrogen peroxide: This was prepared fresh by diluting 30% H_2O_2 (1:10) with distilled water.

Stock riboflavine solution A: 50 mg of USP reference standard riboflavine which had been dried in a vacuum desiccator over H_2SO_4 for 24 hours were dissolved in warm 1500 ml distilled water, 2.4 ml glacial acetic acid were added. After cooling to room temperature, this was made up to 2 litres with water and stored under toluene in a refrigerator (1 ml = 25 µg riboflavine).

Stock riboflavine solution B: 40 ml of A were diluted to 100 ml with distilled water and preserved under toluene in the refrigerator (1 ml = 10 ug of riboflavine).

Riboflavine working standard (1.0 ug/ml): 10 ml stock solution B were diluted to 100 ml with water. This was prepared fresh when required.

Stock solution of sodium Fluoresoein: 50 mg of sodium fluoresoein were dissolved in water and made up to 1000 ml.

Sodium Fluoresoein working solution: 1 ml of stock sodium fluoresoein was diluted to 1 litre with distilled water.

Sodium hydrosulphite ($Na_2S_2O_4$) (G.F.R.)

60 - 80 mesh Florisil

20% pyridine in 2% acetic acid: 200 ml of pyridine and 20 ml glacial acetic acid were diluted to 1000 ml with distilled water.

Hitachi Perkin - Elmer Fluoroscence spectrophotometer (Model 203).

16. Materials for Estimation of Vitamin C
(Assoc. Vitamin Chemists, 1966)

Meta Phosphoric acid,

6% HPO_3 Solution (0.005 M Ethylene diamine tetra acetic acid, EDTA).

60 gm of (A.R.) HPO_3 and 1.8 gm EDTA were dissolved and made up to 1000 ml with water. This was stored in the refrigerator until required and prepared weekly.

3% HPO_3 solution (0.0025 M EDTA): 500 ml of the above solution was diluted to 1 litre with distilled water.

Ascorbic acid, standard: 100 mg of ascorbic acid (USP Reference standard) were dissolved in 3% HPO_3 solution and diluted to 500 ml with the same solvent. This solution was immediately standardized with the dye.

0.025% 2, 6 - dichlorophenolindophenol solution: About 50 mg of sodium salt of 2, 6 - dichlorophenolindophenol dye were dissolved in 100 ml hot distilled water containing 42 mg of $NaHCO_3$, cooled and diluted to 200 ml with distilled water.

Standardization of the dye: A 5 - ml aliquot of the standard ascorbic acid solution (containing 1 mg ascorbic acid) was diluted to 5 ml of 3% HPO_3. This was titrated with the dye solution to a pink colour which persisted for 15 seconds. Since this volume of the dye represents 1 mg of ascorbic acid, the ascorbic acid equivalent (T) of 1 ml of dye solution is equal to 1 ml divided by the volume (in ml) of the dye solution used in this titration.

8% acetic acid: 80 ml glacial acetic acid were diluted to 1 litre with distilled water.

17. Materials for estimation of Pyridoxal (Vitamin B_6) (Sinclair et al. 1943)

Enzyme Mixture: A solution of papain and takadiastase in

3% HPO_3 solution (0.0025 M EDTA): 500 ml of the above solution was diluted to 1 litre with distilled water.

Ascorbic acid, standard: 100 mg of ascorbic acid (USP Reference standard) were dissolved in 3% HPO_3 solution and diluted to 500 ml with the same solvent. This solution was immediately standardized with the dye.

0.025% 2, 6 - dichlorophenolindophenol solution: About 50 mg of sodium salt of 2, 6 - dichlorophenolindophenol dye were dissolved in 100 ml hot distilled water containing 42 mg of $NaHCO_3$, cooled and diluted to 200 ml with distilled water.

Standardization of the dye: A 5 - ml oliquot of the standard ascorbic acid solution (containing 1 mg ascorbic acid) was diluted to 5 ml of 3% HPO_3. This was titrated with the dye solution to a pink colour which persisted for 15 seconds. Since this volume of the dye represents 1 mg of ascorbic acid, the ascorbic acid equivalent (T) of 1 ml of dye solution is equal to 1 ml divided by the volume (in ml) of the dye solution used in this titration.

8% acetic acid: 80 ml glacial acetic acid were diluted to 1 litre with distilled water.

17. Materials for Estimation of Pyridoxal (Vitamin B_6)
(Bina et al. 1943)

Enzyme Mixture: A solution of papain and takadiastase in

acetate buffer of pH4.5 made up so that 5 ml of the solution contained 0.2 gm of each enzyme. This was prepared just before use.

Acetate buffer: 54.4 ml of glacial acetic acid and 111 gm of hydrated sodium acetate crystals were dissolved and diluted to 1000 ml with distilled water.

Sodium tungstate: A 25% solution was prepared.

Alcoholic NaOH Solution (0.5%): 1 gm of A.R. NaOH was dissolved and diluted to 200 ml with 95% ethanol.

12% Acetic acid: 12 ml glacial acetic acid were diluted to 100 ml with H_2O.

5% Sodium acetate: 50 gm sodium acetate crystals were dissolved and made up to 100 ml with distilled water.

5.5% Na_2CO_3: A solution containing 5.5 gm C.P.R. anhydrous Na_2CO_3 in 100 ml distilled water was prepared.

Sulphanilic acid reagent: A solution of 1.6 gm sulphanilic acid in 350 ml water was prepared. 45 ml conc. HCl were added and the mixture diluted to 500 ml and stored in the refrigerator until required.

~~10% Sodium Nitrite solution~~: 10 gm A.R. sodium nitrite was dissolved in 100 ml water.

~~Diazotised reagent~~: 2.5 ml of sulphanilic acid reagent were pipetted into 10 ml brown glass-stoppered volumetric flask and iced in an ice bath. After 5 minutes, 0.4 ml of 10% $NaNO_2$ solution

was added, mixed and made up to the 10-ml mark with distilled water. This was prepared just before use.

Standard pyridoxine solution: 10 mg pyridoxine were dissolved in 50 ml distilled water to which 10 ml 1N HCl were added. This was made up to 100 ml with more distilled water. This was stored in a refrigerator in a brown bottle (1 ml = 100 ug B_6).

Spectrophotometer (SP500) (Unicam instrument).

18. Materials for the Determination of nicotinic acid

(Melnick and Field, 1940)

Spectrophotometer SP600 (Unicam SL - 505)

Cyanogen bromide: This was prepared under a hood by saturating water at 5 - 10°C with 80 gm cold bromine. This was immediately transferred to a glass - stoppered 1000 - ml volumetric flask which contained 500 ml cold water. The flask was kept in an ice - bath. A solution of 10% NaCN from a burette was then cautiously added to the bromine water until it just became colourless. 10 more drops of NaCN were added. The solution was diluted to the mark and stored in a glass-stoppered brown bottle.

Absolute alcohol 95%.

4% aniline solution: 4 gm re-distilled aniline were dissolved in absolute alcohol and made up to 100 ml.

Standard nicotinic acid solution: 100 mg of nicotinic acid were

dissolved in absolute alcohol and made up to 100 ml. 10 ml of this solution were diluted with more absolute alcohol to 100 ml (1 ml ≡ 100 ug).

Daroo: An activated animal charcoal

18 N NaOH solution: 72 gm NaOH pellets were dissolved and made up to 100 ml with water.

Alcoholic phosphate buffer: 10 ml of 85% H_3PO_4 were dissolved in 1960 ml distilled water. 30 ml of 18% NaOH and 333 ml of absolute ethanol were added and the volume made up to 2 litres.

19. Materials for determination of calorific value of diets (Miller and Payne, 1959).

Pellet press

Griffin oven (Model 3/300 PC)

Cotton thread (firing cotton, Gallenkamp)

Benzoic acid (Thermochemical standard)

Analytical balance (Mettler H20)

Ballistic Bomb Calorimeter (CH-385, Gallenkamp).

20. Materials for quantitative Estimation of the Amino Acids (Spackman et al. 1956).

40% NaOH solution: This solution was prepared by dissolving 800 gm 'analar' grade NaOH in distilled water with cooling under a running tap and then making up to 2000 ml with more distilled water.

Preparation of the buffers: All the reagents used for the preparation of the buffers were of analytical - reagent grade and

the solutions were prepared with double - distilled water. The quantities of the various reagents used in the preparation of the buffers of the different pH values were as shown in the table on page 86. The buffers were then adjusted to their correct pH with glass-electrode. They were boiled to exclude the air and 1 ml of caprylic acid added to each buffer to prevent fungal growth. While still hot, the buffers were covered with a layer of paraffin and stored in plastic bottles in an air - conditioned room.

Methyl Cellosolve, technical grade: The reagent was distilled to remove the peroxides after the addition to each litre of solvent of 10 ml of a solution prepared by dissolving 50 gm of $FeSO_4 \cdot 7H_2O$, in 100 ml of 2N H_2SO_4.

Ninhydrin reagent: 750 ml of methyl cellosolve were mixed with 250 ml of the buffer of pH 5.5. Nitrogen dried by passing the gas from the cylinder through concentrated H_2SO_4 was bubbled through the solution for 15 minutes to displace the air. Immediately after, a mixture of 20 gm of indanetriene hydrate (ninhydrin) and 0.4 gm of $Sn\,Cl_2 \cdot 2H_2O$ were added and dissolved. The resulting solution was covered with a layer of paraffin and then stored in a plastic bottle in an air-conditioned room.

Conc. HCl - reagent grade

Resins: The Hitachi spherical resin No. 3105, and Amberlite CC - 120 type 3 were used in the columns.

TABLE 7

Quantities of Various Chemicals Used for the Preparation of the Buffers

Buffer pH	Citric acid monohydrate (gm)	Sodium hydroxide (gm)	Sodium acetate (gm)	Sodium citrate (gm)	Glacial acetic acid (ml)	HCl 32% (ml)	Thiodiglycol (ml)	Polyethylene glycol mono-lauryl ether (ml)	Volume made up to (litre)
2.2 ±0.05	105	42	-	-	-	80.0	-	-	5
3.1 ±0.02	210	83	-	-	-	135.0	50	50	10
3.22	-	-	-	196	-	123.5	50	20	10
4.25	-	-	-	196	-	83.7	50	20	10
5.1 ±0.02	105	47	136	-	20.0	-	50	50	10
5.28	-	-	-	343.5	-	65.0	-	20	10
5.5	-	-	360	-	250.0	-	-	-	2.5
7.0 ±0.02	210	120	-	-	-	-	-	-	5

21. Materials for Estimation of Total Serum Proteins
(Malik, 1967).

Solution A: 45 gm of potassium sodium tartrate (Rochelle salt) were dissolved in 400 ml of 0.2% NaOH in a beaker. 15 gm of $CuSO_4$ were added and dissolved completely. 5 gm of KI were added to the mixture which was brought to 1 litre with more 0.2% NaOH.

Solution B: 0.5% KI was made up in 0.2 N. NaOH.

Working Biuret Reagent: 50 ml of solution A were diluted to 250 ml with solution B.

Versatol A - As standard protein.

22. Materials for Starch - gel Electrophoresis
(Smithies, 1955; 1959)

Trays - 25 cm x 11 cm x 0.5 cm deep with thicker ends.
All made of plastic.

Two plastic Buffer containers, each 6" x 6" x 4" deep with a partition 2" high in the middle.

Each container carries an electric terminal.

Power supply (Shandon, Type 25 23 MK II).

Staining trays (2 plastic trays each 6" x 12 x 3" deep).

Hydrolysed starch (Connaught Medical Res. laboratories, Toronto, Canada).

Boric acid - sodium hydroxide solution: This was prepared by dissolving 8.39 gm boric acid and 2 gm NaOH in 5 litre distilled water.

Buffer (pH 8.6): 92.76 gm boric acid and 12 gm NaOH were dissolved in 1000 ml distilled water and the pH checked.

23. Materials for Estimation of Total Serum Cholesterol (Abell et al. 1952)

Spectrophotometer SP500

Absolute alcohol, redistilled

Petroleum ether (B.P. 68°C), redistilled

Glacial acetic acid (A.R.)

Sulphuric acid (C.P.R.)

Acetic anhydride (A.R.)

33% KOH solution: 10 gm of (C.P.R.) KOH were dissolved in 20 ml distilled water.

Alcoholic KOH Solution: This was usually prepared fresh when required. 6 ml of the 33% KOH solution were diluted with 94 ml absolute alcohol.

Standard Cholesterol solution (0.4 mg/ml): 100 mg of recrystallised cholesterol were dissolved in absolute alcohol and the volume made up to 250 ml with the same solvent.

Modified Liebermann - Burchard Reagent: 20 ml of acetic anhydride were cooled in an ice - bath and 1 ml of conc H_2SO_4 added. The mixture was shaken and cooled for 9 minutes. 10 ml of glacial acetic acid were added, and the mixture shaken and warmed to room temperature. This was prepared just before use.

CHAPTER THREE

METHODS

1. Preparation of the diets

The diets were all cooked as for human consumption. The recipes and the cooking methods were those traditionally used in South - Eastern Nigeria. The cooking methods are described below.

Diet No. 1 - Gari, 'afan' soup with smoked fish.

Gari

Ingredients: Gari (fried cassava flour) (Manihot utilissima)

Preparation: Gari was sprinkled evenly on some quantity of boiling water in a basin. This was stopped before all the water was absorbed, and the chaff collected on top drained off. The guri was then stirred continuously to form a smooth paste.

'Afan' soup with Smoked Fish:

Ingredients: 'Afan' vegetables (Gnetum africanum), waterleaf (Talinum triangulare), palm oil (Elaeis guineensis), periwinkle, snails (Vivapara quadrata), smoked fish, salt, dried shrimps (Palaemonetus variens) and pepper (Capsicum frutescens).

Preparation: The 'afan' vegetables were picked and cut into very small pieces. The pieces were gathered up and pounded in a morter to form a paste. Waterleaf vegetables were picked, washed two times with tap water and cut into small pieces. The periwinkles were first boiled for 15 minutes, removed from the shells and washed three times with tap water. Snails were removed from the shells and washed with

lime juice to remove the slime. These were then cut to pieces. Some quantity of water was added to the cooking pot, followed by palm oil, the snails and pepper. After boiling for about 20 minutes, the vegetables were added and after another 10 minutes, the periwinkle, fish, crayfish and salt. The cooking was continued for another 30 minutes with occasional stirring.

DIET NO. 2. - 'Anyan okpan' and 'obek' (palm nut) soup.

Anyan okpan (Cocoyam/water yam mix)

Ingredients: Grated water yam and cocoyam

Preparation: Water yam tubers (Dioscorea alata Linn) and Cocoyam (Xanthosoma mafaffa Schott), were peeled, grated and mixed together to form a homogeneous pulp. Bits of the pulp were wrapped with pre-softened plantain leaves in cylindrical forms of length 6 - 8 inches. The materials were then steam-cooked. The native pot steamer was prepared by collecting and breaking sticks to lengths that would enter and fit the pot. These sticks were arranged crosswise to act as support for the wrapped food. A small quantity of water was put to cover the sticks. The pot was covered and heated to boiling. The wrapped bits of the pulp were then neatly arranged on the sticks and the pot covered tightly to keep the steam. The ~~cooking~~ was continued for about one and a half hours.

'Abok' (palm nut) Soup:

Ingredients: Palm fruits (Elaeis guineensis) 'atama' vegetables (Heinsia crinita), onions (Allium cepa), Pepper (Capsicum frutescens) crayfish (Palaemonetes varians), smoked fish and salt.

METHOD:

The ripe palm fruits were washed and cooked in an iron pot until the periocarp were succulent. The water was drained off and the palm fruits beaten up in a mortar until the kernels were free. Warm water was then added to the palm husks and mixed. The slurry was filtered, and the filtrate used for the preparation of the soup.

To this filtrate were added pepper, onions, smoked fish and salt and then boiled. Meanwhile the 'atama' vegetables were picked, cut into very small pieces and beaten up in a mortar to give a smooth paste. This vegetable was then added to the boiling soup, followed with crayfish and the cooking continued for about 45 - 65 minutes with occasional stirring.

DIET NO. 3: - Cassava foofoo and 'edikan ikon' (thick vegetable) soup.

Cassava-foofoo (cooked fermented cassava) (Manihot utilissimum)

Preparation: Some water was added to the raw cassava foofoo to make it into a paste. The paste was cooked in an iron pot and stirred continuously with a wooden turner until well cooked. This was shown by the transparency and change in colour of the foofoo. It was then removed, put into a mortar and pounded with a pestle to further smoothen it. The foofoo was then ready to be served.

'Edikan Ikon' Soup (Thick vegetable soup)

Ingredients: 'Ikon ubon' vegetables (Telferia spp.), waterleaf (Talinum triangulare), smoked fish, crayfish (Palaemonetes varians).

palm oil (Elaeis guineensis), pepper (Capsicum frutescens) and salt.

Preparation: The 'ikon ubon' vegetables were picked, cut to small pieces and washed in water with squeezing and rubbing to further reduce the pieces. The waterleaf was equally treated. Meanwhile, the soup pot was heated to boiling with some quantity of water to which palm oil and pepper were added. The vegetables were then added in bits to the boiling water to avoid overflowing. When every bit had been added, the pot was covered and cooked for about 50 minutes. The other ingredients, crayfish, fish and salt were added and the cooking continued for another 50 minutes.

DIET NO. 4 - Pounded yam and 'afia eforo' (plain) soup.

Pounded yam

Ingredients: Boiled white yam tubers (Dioscorea spp.)

Preparation: White yam tubers were peeled, washed and cut to suitable pieces ready for cooking. The tubers were then boiled until the pieces were soft. This took about one and a half hours. The pieces of yam while still hot were removed and put into a wooden mortar and pounded. A few pieces at a time were pounded adding more from the pot and pounding quickly and firmly until as much as could be comfortably managed was added. Pounding was continued until the yam became a solid mass. The mass was then molded into a smooth ball.

'Afia nfuro' (plain soup).

Ingredients: Okro (Hibiscus esculentus), onions (Allium cepa), smoked fish, 'utinyon' vegetables (Amaranthus spp.), palm oil (Elaeis guineensis), pepper (Capsicum fruticosum), crayfish (Palaemonetes varians) and salt.

Preparation: The water used for boiling the yam was used in the preparation of the soup. The okro was washed, topped and tailed and cut into small pieces. The onions were washed, the outer scale leaves were removed and discarded and the fleshy leaves sliced. The vegetables were picked, washed and cut into small pieces. The pepper and crayfish were separately ground up. The crayfish, pepper, palm oil, onions, okro and the vegetables were added to the cooking water and heated to boiling. Smoked fish was then added, the cooking pot was covered up and the soup left to cook for about one hour.

DIET NO. 5. - 'Ekpan Nkukwo' (pecled grated cocoyam and palm oil).

Ingredients: Cocoyam (Xanthosoma mafaffa Schott), Crayfish (Palaemonetes varians), dried fish, pepper (Capsicum frutescens), palm oil (Elaeis guineensis), cocoyam leaves (Xanthosoma mafaffa), and salt.

Cocoyam tubers were peeled, washed and grated using a local grater. The resulting pulp was thoroughly mixed with a large quantity of palm oil. Small bits of the pulp at a time were wrapped up in fresh cocoyam leaves ready for cooking. A small quantity of water was added to the cooking pot and heated to boiling. The wrapped bits of the cocoyam were put into the boiling water and cooked for 45 minutes. To this

were added the other ingredients - crayfish, dried fish, pepper and salt. Cooking was continued for another one hour and extra palm oil added. It was occasionally stirred to avoid localized heating.

Diet NO. 6. - 'Iwuk Ukom' (plantain porridge with smoked fish).

Ingredients: Mature unripe plantain (Musa paradisiaca), crayfish (Palaemonetus varians), pepper (Capsicum frutescens), water leaf (Talinum triangulare), palm oil (Elaeis guineensis), Salt and smoked fish.

Preparation: The plantains were peeled, washed and sliced across the length into pieces. These were boiled in a small quantity of water together with the waterleaf until well cooked. A large quantity of palm oil was then added and the food stirred. Then ground crayfish, pepper, salt and smoked fish were added. The cooking was continued with occasional stirring for about 40 minutes.

2. Formulation of the Diets

As described on pages 89 - 94, the diets consisted of two dishes, one made up of a staple which is often carbohydrate. The protein is supplied mainly by fish and crayfish from the soup. Diets 5 and 6 were single dishes. The two parts of diets 1 - 4 were mixed together and homogenized in bits. The bits were then pooled and finally mixed in a "Kenwood 'Chef' Domestic Food Mixer" (Model A 700D supplied by Kenwood MFG. Co. Ltd., Woking Surrey, England.). Diets 5 and 6 were similarly homogenized and mixed to give a homogeneous paste.

The raw forms of all the diets were similarly homogenized and mixed.

A 'conversion' was made to the feeding habits of the laboratory rat by drying the diets before feeding them to the rats. Each form of the diets was spread thinly on aluminium trays of size 24 x 12 x 3 inches and dried in a dehydrator with a forced draught at 65°C for 24 hours as described by Callinan et al. (1951) and Monphill et al. (1943). When drying was completed, the products were 'conditioned' by exposure to the air in thin layers with frequent mixing. It was stored in the refrigerator. Representative portions of the diets were taken for nutrient analyses.

3. Estimation of Dry Matter (A.O.A.C., 1960)

Method

A known weight of the homogenized, mixed diet in a weighed porcelain crucible was dried in an oven preheated to a temperature of 100 - 105°C. Weights of the crucible with the diet were taken at intervals of 12 hours until the last two observations remained constant. The crucible was further heated for one hour and then transferred to a desiccator to cool and later weighed. The weight of the crucible plus wet diet minus the weight of crucible plus dried diet (loss in weight) represented the moisture lost. The percentage dry matter for each form of the diet was calculated. The experiment was carried out in triplicate for each form of each diet.

4. Determination of Crude Fat (ether - extract) (A.O.A.C., 1960)

Method:

About 10 gm of the dried sample were placed in a porous fat extraction thimble inside the soxhlet extraction apparatus and extracted continuously with dry ether for eight hours. The solvent was evaporated in a water bath to about 20 ml. This was transferred into a weighed 100 ml beaker. The flask was rinsed thrice with 5 - ml portions of dry ether and each time transferred to the beaker. The solvent was evaporated off in a water bath and the extract dried for 30 minutes at 100°C, cooled in a desiccator and weighed. The percentage ether extract was calculated. The determination was carried out in triplicate for each form of the diets.

5. Estimation of Total Ash in the Diets (A.O.A.C., 1960)

About 5 gm dry weight of each form of the diets were weighed into a porcelain crucible of known weight. This was placed in a muffle furnace pre-heated to a temperature of 600°C. The material was held at this temperature for 48 hours. The crucible was then transferred to a desiccator to cool and was later weighed. The percentage ash was calculated. The estimation was carried in triplicate for each form of the diets.

6. Determination of Crude Protein (total food nitrogen x 6.25)

The semi-micro Kjeldahl method was used throughout in these determinations. In this, 4 gm dry weight, in triplicate, of each diet sample were placed in 500 - ml Kjeldahl digestion flask. 40 ml conc sulphuric acid, 17 gm potassium sulphate and 0.7 gm HgO (Fleck, 1970) were added. The flasks were placed in inclined position and heated gently until frothing ceased. The digestion was continued until the

solution cleared. The mixture was then maintained just below the boiling point for 2 hours. This was then cooled and quantitatively transferred to a 100 - ml volumetric flask and made up to volume with distilled water.

5 ml portions of this solution were transferred into a semi-micro Kjeldahl distillation apparatus and treated with 20 ml 40% NaOH solution and about 5 mg of zinc dust to precipitate the mercury. The ammonia was steam - distilled as described by Markham (1942) into 10 ml of 4% boric acid and 3 drops of methyl red indicator in 100 - ml conical flask. The tip of the condenser was immersed in the boric acid and the distillation was continued until about 10 ml had come over. The tip of the condenser was rinsed with a few millilitres of distilled water and the distillation stopped. The contents of the distillation apparatus were removed by automatic suction and the apparatus rinsed several times with distilled water before the next determination. The distillate was then titrated against the standard HCl solution. Distillation was carried out in triplicate for each digest. By calculation and multiplication with the appropriate factors, the nitrogen content (in gm) per 100 gm dry weight of the diet sample was determined. The crude protein was then calculated as food N x 6.25.

7. Estimation of Total Carbohydrate

The percentage of total carbohydrate in the diet samples was determined by difference. This was done by subtracting the percentages of ash, fat and crude protein from 100 as described by the A.O.A.C. (1960).

8. DETERMINATION OF MINERALS

Preparation of mineral Solution

The A.O.A.C. (1960) dry ashing method was used to get the minerals into solution. In this method, 10 gm dry weight of each diet sample were weighed into a porcelain dish of 50-ml capacity and 60 mm diameter. This was ashed in a muffle furnace preheated to 600°C. The sample was maintained at this temperature for 48 hours. It was cooled and 1 ml of conc HNO_3 was added. The acid was evaporated off on a steam-bath and the residue ignited in the muffle furnace for another 24 hours. The product was finally cooled and 5 ml conc HCl added.

The acid was evaporated to dryness on a steam bath. The residue was redissolved in 2.0 ml conc HCl and heated for 5 minutes on a steam bath with a watch glass on the dish. The watch glass was rinsed with distilled water. The solution was then filtered with a Whatman No. 540 ashless filter paper into 100-ml volumetric flask. The filtrate was cooled and made up to volume with distilled water.

(a) Estimation of Calcium (A.O.A.C., 1960)

METHOD:

10 ml aliquots of the above mineral solution, in triplicate, were pipetted into 250-ml conical flasks and 2 drops of methyl red indicator added. The solution was neutralized to a yellow colour with dropwise addition of 2% ammonium hydroxide solution from a burette. The solution was then diluted to 150 ml with distilled water.

This was heated to boiling and 10 ml of saturated ammonium oxalate solution added. The mixture was left overnight to fully precipitate the calcium oxalate. It was then filtered through a Whatman No.540 ashless filter paper and the precipitate washed thoroughly with 2% ammonia solution. The filter paper together with the precipitate was put back into the original flask and a mixture of 150 ml distilled water and 25 ml conc H_2SO_4 added. The contents of the flask were heated to 70°C and titrated hot against 0.05 N $KMnO_4$ solution to the first faint pink colour. A correction for the reagent blank was made.

Calcium was calculated as shown:

1 ml of 0.1N $KMnO_4$ = 0.002 gm Ca.

(b) Estimation of Iron (A.O.A.C., 1960)

Procedure:

10 ml of the mineral solution for each diet, prepared as described on page 98 were transferred, in triplicate, into 50 - ml volumetric flasks. In the same way, 2.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0 and 45.0 ml, respectively, of the final diluted stock standard iron solution were transferred to a number of 50-ml volumetric flasks. To each flask was added 1 ml of 10% hyroxylamine hydrochloride solution and after 5 minutes, 5 ml of the acetate buffer solution and 2 ml of α, α - dipyridyl and the mixture made up to mark with distilled water. The solution in each flask was mixed by inverting the flask several times.

An aliquot was transferred into a 2 cc cuvette and the optical density read at 510 mμ in an SP600 spectrophotometer against a blank containing only dilute HCl and the colour reagents. A standard graph of the concentration of iron against optical density was plotted from which the values for the test samples were obtained by interpolation. The iron content of the diets was then calculated and expressed in terms of mg/100 dry matter.

(o) Estimation of Phosphorus (Gomori, 1942)

Preparation of Standard Graph : 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 ml, respectively, of the standard phosphorus solution were pipetted into seven 25-ml volumetric flasks containing 2.5 ml of the molybdate - sulphuric acid reagent. A reagent blank was prepared containing 1 ml of distilled water in the eighth 25 - ml volumetric flask. 1 ml of the metol reagent was added to each of the flasks. The solutions were then made up to volume and mixed by inverting the flasks several times. The blue colour developed was read at 600 mμ in an SP600 Unicam spectrophotometer against the reagent blank when the solution was just 15 minutes old.

Determination of Phosphorus in the Diets: 5 ml aliquots of the mineral solution of each diet, in triplicate, were pipetted into 25 - ml volumetric flasks and treated as described for the standard phosphorus solution. Their optical density values were similarly read at 600 mμ in an SP600 spectrophotometer against the reagent blank. The concentration of phosphorus in the original sample was determined using the standard

graph and was expressed in terms of ug V per 100 gm dry matter.

3. Determination of the Vitamins

(a) Vitamin A

(Carr - Price, 1926; Assoc. Vitamin Chemists, 1946)

Preparation of Vitamin A extract: 10 gm dry weight of each sample were extracted continuously in a soxhlet extraction apparatus with ether for six hours. The ether was evaporated to dryness in a water bath. 30 ml of ethanol and 3 ml of a 100% KOH solution were then added to the flask containing the oily residue. The flask was connected to a reflux condenser and the contents gently refluxed until saponification was complete (about 30 minutes). The flask was cooled and the contents diluted with 100 ml of distilled water. The contents were then quantitatively transferred into a separating funnel. The separating funnel was gently shaken to avoid the formation of emulsions and the contents allowed to separate into 2 layers. The aqueous lower layer was drawn off into another separating funnel, whilst 50 ml of ether which had been used to rinse the saponification flask were added to it. This second separating funnel was then shaken gently and the liquid allowed to separate into two layers. The lower layer was again drawn off and the extraction process repeated twice. The ether layers were combined in the first separating funnel. The bulked ether solution was then washed by pouring through 100 ml of distilled water without shaking. The aqueous layer was drawn off and discarded.

About 100 ml of 0.5N NaOH solution were then added to the separating funnel was gently swirled and allowed to settle. The contents separated into two layers and the lower layer was again drawn off and discarded.

The ether layer was then repeatedly washed with distilled water until the aqueous layer when tested was colourless with phenolphthalein. The alkali - free ether layer was then dried by filtering through anhydrous Na_2SO_4 placed inside a filter paper in a funnel into a dry 1000-ml volumetric flask. The separating funnel was rinsed twice with 25 - ml portions of ether and poured through the Na_2SO_4 in the glass funnel into the volumetric flask. A glass bead was placed in the flask and the solution was then slowly evaporated to dryness in a water bath. The oily residue was taken up in dry chloroform and quantitatively transferred into a dry 100-ml volumetric flask and later made up to volume with pure chloroform.

Preparation of the Standard Graph: 0.50 gm of vitamin A acetate containing 3.0 mg of vitamin A per gram was weighed into a saponification flask and treated as described for the test sample. The final residue was taken up in chloroform and made up to 1000 ml (1 ml = 1.5 ug vit. A). Then, 1.0, 2.0, 3.0, 4.0 and 50 ml aliquots of the standard solution were pipetted into 10-ml volumetric flasks and made up to volume with chloroform. Similarly 1 ml of the sample extract, in triplicate, was diluted to 10 ml. 2 ml chloroform were introduced into the colorimeter tube placed in the instrument. 10 ml of $SbCl_3$ were then added from a rapid delivery pipette. This was the blank and was used to set the

instrument at zero absorbance. To a series of colorimeter tubes, 1 ml of each of the diluted standards and of the sample extract and 1 ml of chloroform were added. Each tube was placed in turn in the instrument and 10 ml $SbCl_3$ reagent added from a rapid delivery pipette. The optical density was taken at maximum deflection of the instrument. A graph of concentration of vitamin A was plotted against absorbance. The reading for the sample extract was converted into concentration by interpolation from the standard vitamin A graph.

(b) β - carotene

(Bassir, 1963 b)

Preparation of β - carotene extract: 10 gm of the diet, on dry weight basis, were placed in an extraction thimble and continuously extracted in a soxhlet apparatus for six hours and the extract treated as already described for vitamin A estimation. The final oily residue was taken up in chloroform and made up to 100 ml.

A series of β - carotene standards were set up as shown on the following table:

Tubes	(1)	(2)	(3)	(4)	(5)	(6)
ml standard solution (0.01 mg/ml)	0	0.25	0.5	1.0	2.0	3.0
ml chloroform	10	9.75	9.5	9.0	8.0	9.0

Three other test tubes were set up each containing 1 ml of the sample extract and 9 ml of chloroform. For the purpose of comparison,

the spectrophotometer SP500 was set at zero absorbance with the contents of tube I. The reading for each of the standards was taken at 440 m μ and also for the sample extract. A standard graph of absorbance against the concentration of the various standard solutions of β - carotene was plotted. The concentration of β - carotene in the test sample extract was then determined by interpolation on the standard graph. Estimation was in triplicate for each form of diet.

(c) Estimation of Vitamin B_1 (Thiamine)

(Assoc. Vitamin Chemists, 1966).

Preparation of the extract: 10 gm of the dry diet, in triplicate, were weighed and placed in 250 - ml conical flask. About 100 ml of 0.1N HCl were added and the sample gently mixed so as to be evenly dispersed in the liquid. The sides of the flask were then washed down with more of the 0.1N HCl. The flask with its contents was then heated for 30 minutes in a boiling water bath with occasional shaking. This was then cooled under a running tap to 40°C.

Enzyme hydrolysis: The pH of the extract was adjusted to 4.0 - 4.5 with a few millilitres of 2.5 M sodium acetate solution, using bromocresol green as an external indicator on a spot plate. About 10 ml of the freshly prepared enzyme suspension were added to the extract, followed by 0.5 ml of benzene and the sample incubated overnight at 40°C in an incubator with a shaking device. The mixture was cooled to room temperature and diluted to 200 ml with distilled water. It was then

thoroughly mixed and filtered through a Whatman No. 540 filter paper into a dry flask, discarding the first few millilitres of the filtrate.

Purification: A chromatographic column was prepared by adding to an absorption tube already plugged with glass wool a distilled water suspension of 5.0 g of the activated decalso. Care was taken to wash down all the decalso from the walls of the tube and a layer of liquid was always left above the surface of the decalso during the absorption process to keep out air. The column was again cleaned by passing through two 5 - ml portions of 3% acetic acid.

Then 25 ml of the sample filtrate were pipetted and slowly discharged along the side of the column onto the decalso. This was allowed to pass through the decalso and the filtrate discarded. The column was then washed with three successive portions (about 10 ml each) of hot water, discarding the washings.

The thiamine was eluted from the column by passing through it five 4.0 - 4.5 - ml portions of almost boiling acid potassium chloride solution, taking care to prevent the surface of the liquid from falling below the surface of the activated decalso until the final portion of acid - KCl solution had been added. The eluate was collected in 25-ml volumetric flask and the volume made up with the acid-KCl solution. This was the 'assay' solution.

Oxidation of the Thiamine to Thiochrome: 5 ml aliquots, in triplicate, of the working standard thiamine solution were pipetted into 50-ml separating funnels. 1.5 g of sodium chloride were added to each

separating funnel which was then gently swirlled to produce a rotary motion in the liquid. By means of a fast delivery pipette 3 ml of the freshly prepared alkaline ferricyanide solution were added. The pipette was immediately removed and the separating funnel swirlled again to ensure adequate mixing. Immediately after, 15 ml of redistilled iso-butyl alcohol were added, the separating funnel was stoppered with a glass stopper and shaken vigorously for 90 seconds. The solution at this stage was protected from light to which thiochrome is sensitive. The flask was allowed to settle so that the contents separated into two layers. The lower aqueous layer was run off and discarded while the iso-butyl alcohol layer was then dried by addition of 2 gm anhydrous Na_2SO_4. This was shaken for 30 seconds and allowed to stand and settle. This was run off into a clean brown bottle ready for thiochrome fluorescence measurement. A standard blank was similarly treated except that the oxidizing agent was replaced with 3 ml of 15% NaOH solution.

Into three other separating funnels, 5 ml aliquots of the 'assay' solution were pipetted. These were treated as described for the thiamine standards. The 'assay' blank was prepared as for the standard blank. The clear, dried isobutyl alcohol extracts of the 'assay' blank and 'assay' solutions were also run off into labelled brown bottles for thiochrome fluorescence measurement.

Measurement of Thiochrome: The fluorescence of the iso-butyl alcohol solutions were determined at 365 m/u in terms of galvanometer deflections in the Hitachi Perkin-Elmer fluorescence spectrophotometer (Model 203).

The reproducibility of the instrument was governed with the working standard quinine sulphate solution.

The fluorescence of the isobutyl alcohol extract from the oxidized 'assay' solution was measured and designated 'A'. The fluorescence of the 'assay' blank was measured and designated 'b'. Then the fluorescence of the extract from the oxidized standard solution was measured and called 'S'. Finally, the fluorescence of the extract from the standard solution which was treated with 3 ml of the 15% NaOH solution was measured and this reading (standard blank) 'd'.

Calculation: The thiamine content of 5 ml of the 'assay' solution was given as:

$\frac{A - b}{S - d}$ ug. This value multiplied by the appropriate factors of dilution gave the thiamine content in mg/100 gm dry diet sample.

(d) Estimation of Vitamin B_2 (riboflavine)
(Hodson and Norris, 1939)

Preparation of samples: 10 gm dry weight of sample, were placed in 250-ml conical flask. 75 ml of 0.1N H_2SO_4 were added and the mixture autoclaved for 15 minutes at 15 lbs/sq. inch pressure. The flask was left to cool to room temperature. The pH was adjusted to 4.5 by the addition of about 5 ml of 2.5 N sodium acetate solution.

Enzyme hydrolysis: The flask was then incubated at 38°C overnight with 10 ml of enzyme suspension containing 0.1 gm of papain and 0.1 gm of taka-diastase in distilled water and to which 0.5 ml of glycerin had been added.

Oxidation with $KMnO_4$ solution: When the mixture had cooled to room temperature, 3 ml of the 4% $KMnO_4$ solution were added and shaken to ensure mixing. After two minutes, 3 ml of 3% H_2O_2 solution were added and the contents of the flask thoroughly mixed. Excessive foaming of the mixture was prevented by addition of one drop of octyl alcohol. The mixture was then finally made up to 100 ml with distilled water. This was then filtered into a dry flask after discarding the first 5 ml of the filtrate.

Absorption and elution: An absorption column (glass chromatographic tube, 275 mm in length and reservoir capacity of 60 ml) was prepared by carefully pouring down the absorption tube 5 gm florisil suspended in 5 ml of distilled water. The florisil was washed down the column and flow rate maintained at 1 ml per minute. The column was then washed with about 25 ml of water. Care was always taken to leave a layer of liquid above the florisil in the chromatographic column.

25 ml of the sample extract were then pipetted and carefully discharged onto the florisil. The filtrate from this was collected and discarded. The column was then eluted by the addition of about three 15 - ml portions of 20% pyridine in 2% acetic acid and the eluate collected in a 50 - ml volumetric flask. This was then made up to volume with more pyridine - acetic acid solution when about 35 - 45 ml of it had collected in the 50 - ml flask.

Fluorometry: The reproducibility of the fluorometer was governed with the working standard solution of sodium fluorescein. The

fluorescence of the eluate was then measured as follows: To cuvette A, 9 ml of the sample eluate when added. To cuvette B, 1 ml of freshly prepared working standard solution of riboflavine and 9 ml of the sample eluate. The contents of cuvette B were then mixed by agitating cautiously to ensure homogeniety. Their fluorescence was seperately measured at 440 m/u in the Hitachi Perkin-Elmer fluorescence spectrophotometer. The fluorescence of A was designated 'A' and that of cuvette B was called 'B'. About 5 mg of sodium hydrosulphite were added to cuvette A. This was immediately mixed and the remaining fluorescence measured and called 'C'. Since riboflavine is light - sensitive, care was taken to avoid exposure of the extract to bright light. Determinations were in triplicate for each diet.

Calculation: The riboflavine content of the eluate was calculated as:

$$\frac{A - C}{B - 0.9A} \times 0.1 \text{ ug riboflavine/ml eluate.}$$

(e) **Estimation of Vitamin C**

(Assoc. Vitamin Chemists, 1966).

Procedure:

100 gm of each dry diet were blended with an equal weight of 8% acetic acid to give a homogeneous slurry. 50 gm of this slurry were weighed into a beaker and then quantitatively transferred into 250-ml volumetric flask and made up to volume with more 8% acetic acid. The solution was then thoroughly mixed and centrifuged. The supernatant was decanted and filtered through a Whatman No. 540 filter paper into a dry flask. 20 ml aliquots of the filtrate were taken in 100 - ml

conical flasks and titrated immediately against the otandardized solution of the 2, 6 - dichlorophonol indophonol dye to a faint pink end point which persisted for 15 seconds.

Calculation:

The ascorbic acid content of the sample assayed is given by the following formula:

$$\frac{V \times T \times 100}{W} = \text{mg ascorbic acid/100 gm sample}$$

where V = ml dye used for titration of aliquot of diluted sample
T = ascorbic acid equivalent of dye solution expressed as mg/ml of dye
W = gm of sample in aliquot titrated.

Each form of each diet was assayed for vitamin C in triplicate.

(f) Pyridoxal (Vitamin B_6)
(Bino et al., 1947)

Preparation of the Sample:

10 gm dry weight of each form of the diet were weighed into a 250 - ml Erlenmeyer flask and 100 ml of 0.04 N H_2SO_4 were added and mixed. The suspension was autoclaved for 30 minutes at 15 lbs/sq. inch pressure. The flask was cooled and the pH adjusted to 4.5 with acetate buffer solution. 5 ml of the enzyme solution were added and the mixture incubated at 40°C for 2 hours.

The material was then transferred to 250-ml centrifuge tube and centrifuged for 5 minutes. The extract was decanted into a 200-ml

volumetric flask and the residue washed with 15 to 20 ml of distilled water and again separated in the centrifuge. The combined extracts and the washings were adjusted to pH of 7 with sodium hydroxide solution and then diluted to 200 ml.

A 35-ml aliquot of the extract were pipetted into a 50 ml centrifuge stoppered-tube and 2 ml of 25% sodium tungstate solution added, followed by 0.5 ml of concentrated sulphuric acid. The contents were mixed by inverting the tube a few times and then centrifuging for 2 to 3 minutes. The supernatant was decanted into 50 ml glass centrifuge tube and precipitate was washed with 5 ml of distilled water. This was further centrifuged for 5 minutes and the washing added to the first extract. The pH of the bulk solution was adjusted to 3 with strong NaOH solution using glass electrode. To this clear solution, 0.5 gm superfiltrol was added and the mixture shaken frequently to suspend the superfiltrol for a period of 30 minutes. The mixture was again centrifuged and the extract discarded. The superfiltrol layer was washed twice by suspension and centrifugation with 15 ml portions of the acetate buffer (pH = 3). The residue was well drained after the last washing.

Elution of the Vitamin:

To the superfiltrol, 20 ml of the 0.5% alcoholic NaOH solution was added. The tube was well shaken to mix the contents and latter placed in water bath at 60 - 65°C for 30 minutes. The material was mixed several times within this time. This was cooled, centrifuged and the supernatant

decanted into a 50 ml beaker. The superfiltrol was washed twice with 5 ml portions of 0.5% alcoholic NaOH and centrifuged so that all the washings were decanted into the 50 ml beaker. The pH of the bulked eluates was adjusted to 7.3 with 12% acetic acid using glass electrode. The mixture was transferred quantitatively into a 50-ml volumetric flask and made up to volume.

Colour determination with Diazotized sulphanilic acid:

To 10 ml of the above solution, 4 ml of 50% sodium acetate solution were added and then 2 ml of distilled water. This was followed by 1 ml of diazotized reagent and 2 ml of 5.5% sodium carbonate solution. The solution was mixed gently after the addition of each reagent. An extract blank was prepared by using 10 ml of the extract, 3 ml of water, 4 ml of 50% sodium acetate and 2 ml of 5.5% sodium carbonate solution. A reagent blank was also prepared using 12 ml distilled water, 4 ml of 50% sodium acetate solution, 1 ml of diazotized reagent and 2 ml of 5.5% sodium carbonate solution. The colour of the extracts was read at 326 mμ in an SP500 spectrophotometer. The optical density obtained was converted to concentration by interpolation from a standard graph prepared as described below after allowing for the sample blank reading.

Preparation of a standard graph for pyridoxine:

1 ml of the standard pyridoxine solution was diluted to 100 ml with distilled water (1 ml of this final solution = 1 ug of vitamin B_6). Then 1.0, 2.0, 3.0, 5.0, 8.0 and 10.0 ml of this solution were pipetted into boiling tubes. To each tube was added with mixing after each addition,

4 ml of 50% sodium acetate, 2 ml of distilled water, 1 ml of diazotised sulphanilic acid reagent, and 2 ml of 5.5% Na_2CO_3 solution. Each tube was then diluted to 19 ml with distilled water. The colour was allowed to develope in an ice bath and was read at 420 mμ in the SP500 spectrophotometer against the reagent blank. A graph of optical density was plotted against the concentrations of the vitamin. Estimation was carried out in triplicate for each diet sample.

(g) Determination of Niacin

(Melnick and Field, 1940)

Preparation of the sample:

5.0 gm dry weight of each diet sample were weighed into 100-ml Erlenmeyer flask and 5 ml of concentrated HCl (S.G. 1.18) and 15 ml of water were added. The flask was immersed with occasional shaking into a boiling water bath for 35 minutes. This was then cooled to room temperature and further diluted with 15 ml of distilled water. This was followed with 10 ml of absolute alcohol and 200 mg of activated charcoal. The mixture was well shaken and filtered through Whatman No. 540 filter paper at room temperature.

8.33 ml of this filtrate were transferred into a dry 10 - ml volumetric flask. A drop of phenolphthalein solution was added and the mixture cautiously neutralised with 18N NaOH and finally made up to mark with water.

3 ml aliquots of this solution were pipetted into three boiling tubes. To the first tube, 7 ml of the alcoholic phosphate buffer were added and this was the sample blank. To each of the remaining two tubes, 6 ml of cyanogen bromide solution, and 1 ml of the 4% aniline solution

were added with stirring to mix after each addition. A reagent blank was prepared by mixing together 2 ml of distilled water, 1 ml of absolute alcohol, 6 ml of cyanogen bromide and 1 ml of 4% aniline solution.

The colour produced was read 7 minutes after mixing at 385 mμ in an SP500 spectrophotometer. The concentration of niacin in the sample was determined from the standard graph on which the absorbance of standard solutions of niacin were plotted against concentration after allowing for the sample blank reading. The determination was carried out in triplicate for each form of the diet.

Preparation of Standard Graph: 1.0, 1.5, 2.0, 2.5 and 3.0 ml of the working standard solution of niacin (1 ml = 100 ug) were pipetted into 5 boiling tubes. To each were added 6 ml cyanogen bromide solution and 1 ml 4% aniline solution with mixing after each addition. The solution in each tube was diluted to 10 ml and mixed again. The colour produced was read 7 minutes after mixing at 385 mμ in the SP500 against the reagent blank. A graph of absorbance against concentration was then plotted.

10. Determination of Calorific Value of the Feed

The method described by Miller and Payne (1959) was used. A known weight of the dried sample was completely burnt in a ballistic bomb calorimeter and the heat of combustion recorded in terms of the maximum deflection of a spot of light in the galvanometer. This was compared with the deflection caused by the heat of combustion of a known weight a benzoic acid after allowing for the blank estimation (empty crucible and a known length of the cotton thread).

Method:

About 1 gm of the dried, ground homogenous diet was weighed into a pellet press and squeezed in a vice. The pellet (about 10 mm in diameter) was removed and dried for 24 hours at 105°C in an oven. This was later transferred into a desiccator, cooled and weighed. The sample was then ready for the calorific value determination.

The weighed pellet of food was put into the bomb crucible and placed on the crucible stand in the bomb. One end of a piece of cotton thread (5 cm long) was tied to the firing wire and the other end allowed to make contact with the pellet in the crucible. The top of the bomb was screwed on to the base and oxygen passed gently into the system for about 2 minutes to expel the atmospheric gases. The exit valve was then closed and the bomb filled with oxygen till 15 atmospheres pressure was obtained. The oxygen cylinder was closed and the system isolated by closing the oxygen valve on the bomb. The galvanometer was set at zero on the scale making sure that (1) the thermocouple wire was fixed tightly onto the top of the bomb and (2) the galvanometer needle was moving freely.

The firing button was pressed and left to release itself automatically. The movement of the galvanometer needle was observed on the scale and the maximum deflection of the needle was taken as the final reading. The air-pressure in the bomb was released gently through the gas release valve. The top of the bomb was removed, cooled and cleaned. The crucible was allowed to cool and equally cleaned ready for next estimation. Determination was performed 6 times for each of the dried diets.

Standard estications were made with 1 gm of benzoic acid instead of the food sample. Six estimations were also carried out with the standard and the mean deflection determined. A blank determination was performed using 5 cm of the cotton thread and empty crucible. The maximum deflection for this was usually subtracted from the maximum reading for the test and the benzoic acid samples.

Calculation:

For benzoic acid, the heat of combustion is obtained as follows:

$$2C_6H_5COOH + 16O_2 \longrightarrow 14\ CO_2 + 6H_2O + \text{heat}$$

i.e., 244 gm + 16 x 22.4 litres ——→ 1542.4 Kcal.

Hence, 1 gm ——→ $\frac{1542.4}{244}$ K cals

= 6.32 K cals

The gross energy (GE/gm) of the diet was calculated as:

$$\frac{(\text{Cal. Reading for diet} - \text{Blank}) \div \text{Wt of diet}}{(\text{Galvanometer Reading for Benzoic acid} - \text{Blank}) \div \text{Wt of benzoic acid}} \times 6.32 .$$

The gross energy was then converted to the metabolisable energy by the equation :

$$ME/gm = (GE/gm \times 0.95) - (N\% \times 0.075)$$

where ME - metabolizable energy and GE - gross energy, both in kilocalories as described by Miller and Payne (1959), and N% - nitrogen content of the diet per 100 gm dry weight.

11. Quantitative Estimation of the Amino Acids

The quantitative estimation of amino acids was carried out by a specially trained technician in the department. The procedure followed was based on the report of Spackman, Stein and Moore (1956). The Hitachi Amino acid Analyser Model KLA - 3B was used. The operation of this instrument was as directed by the manufacturers in the manual which accompanied the instrument.

The operation consists of using ion-exchange resin columns of suitable length and internal diameter packed with synthtic sulphonated cation exchange resins of fine particle size. A small quantity of the protein hydrolysate (corresponding to 1 - 2 mg of protein) is applied to the column and the individual amino acids are eluted by sodium citrate buffers at a gradient pH 3 to 7. The whole operation takes place under highly standardized conditions of flow rate, temperature and pressure on the column. The eluate at constant flow rate is mixed with specially prepared ninhydrin reagent and is passed through a reaction chamber (a capillary tubing of about 20 metres long in a bath of boiling water). The blue colour of the ninhydrin complex develops within 20 minutes. It is then passed through an automatic recording colorimeter by which the photometric data are recorded by a 12 - dot recorder which gives three different colours, green, blue and red, representing respectively the absorption at wavelengths of 570, 440 and 640 mμ. The unknown components are measured quantitatively by comparing the area defined by the curve for each component against that given by the standard amino

acid sample. The results obtained are expressed on the basis of mg/100 gm dry diet sample.

Cystine was determined as cysteic acid on the column after performic acid oxidation as described by Schram et al. (1954) (see below).

Tryptophane was determined by the chemical method of Miller (1967) described below.

12. Oxidation of Food Samples for Cystine determination (Schram et al., 1954)

About 25 ml of the performic acid reagent (1vol. of 30% (w/w) H_2O_2 to 9 vol. of 88% w/w formic acid) previously cooled to 0°C were added to 5.0 gm of the dry diet sample in a 100-ml quickfit volumetric flask. The mixture was then gently swirled and placed in the refrigerator for 16 hours.

At the end of the reaction time, most of the solvent was removed under pressure at bath temperature (30 - 40°C) on rotary evaporator. The concentration was stopped as soon as the bulk of the reagent was removed, yielding a syrupy ribbon on the wall of the rotary flask. The sample was then ready for acid hydrolysis using 6 N HCl.

13. Estimation of Tryptophane

The method of Miller (1967) was used in this estimation. In this, 2 gm dry weight of each diet sample, in triplicate were placed in a 100-ml conical flask and 15.4 gm of powdered $Ba(OH)_2.8H_2O$ were added. 15 ml distilled water were pipetted into the flask which was

capped with tinfoil and autoclaved for 7 hours at 15 lbs/sq in pressure. This was left to cool overnight.

Preparation of the hydrolysate: The cold hydrolysate was neutralized by titrating with 6 N HCl until colourless to phenolphthalein. The small white precipitate of barium carbonate formed was dissolved by further addition of 6 drops (0.3 ml) of the acid. The solution was then quantitatively transferred into a 100-ml centrifuge tube and 40 ml of a solution of sodium sulphate containing 175 gm anhydrous Na_2SO_4/litre added. This was mixed and centrifuged at 1500 rpm for 20 minutes. The supernatant was decanted into a 250 - ml volumetric flask. The residue was re-suspended in 10 ml distilled water and further centrifuged. This was repeated thrice and all the washings added to the flask. The hydrolysate was then made up to the mark with distilled water.

Colorimetric determination of tryptophane in the hydrolysate:

2 ml aliquots of the hydrolysate were transferred into four stoppered tubes. To three of these tubes, 5 ml of 0.5% (W/V) p-dimethylamino-benzaldehyde (DM AB), after 20 minutes 0.2 ml of 0.2% (W/V) sodium nitrite solution were added. The 4th tube served as the blank. To it was added 5 ml conc HCl followed by 0.2 ml of 0.2% (W/V) sodium nitrite solution after 20 minutes. The mixture in each tube was filtered after 23 minutes using 9 cm Whatman No. 540 filter Paper. The optical density was determined in a Unicam SP600 spectrophotometer at 590 mμ against the sample blank. The tryptophane content of the sample was read from a standard graph and was expressed on mg/100 gm food basis. Estimation was in

triplicate for each diet sample.

Preparation of the standard graph: 50 mg dl-tryptophan was dissolved and made up to 100 ml with distilled water (1 ml = 0.5 mg). 2 gm hydrolysed potato starch were weighed into eleven 100-ml conical flasks. 1 ml, 2 ml, 3 ml, 4 ml and 5 ml aliquots of the standard tryptophane solution, in duplicate, were pipetted into 10 of the 11 flasks. The 11th flask served as the blank. Then 15 ml distilled water and 15.4 gm of powdered $Ba(OH)_2.8H_2O$ were added and these flasks capped and treated as described for the test samples. The optical density was determined against the blank at 590 mμ in the Unicam SP600 spectrophotometer. A standard graph of optical density against concentration was plotted.

14. ANIMAL FEEDING EXPERIMENT

(i) Animals: Male and female albino rats of the Wistar strain from our own colony were used in all the experiments to estimate the weight gains, net protein ratio, protein efficiency ratio, net protein utilization, biological value, true digestibility of dietary nitrogen and the net dietary protein calories present of the cooked and uncooked diets. The animals were weaned at 23 - 24 days of age and fed on stock diet for a week so that they were always 30 - 31 days old and weighed between 50 - 60 gm at the commencement of the experiments.

(ii) Selection and Grouping of Animals: Five litters of rats known to contain more than 10 offsprings per litter were selected. The rats were then screened so that those which were unusually large or small were discarded and only eight rats (four males and four females) of each litter used

for the experiments. The five litters were then divided into eight groups (4 groups of males and 4 of females) of five in such a way that each contained one rat from each litter and the mean weight of each group did not exceed others by more than 2 gm. The rats were then numbered and housed individually in a battery of wire screen - bottomed metabolic cages each of size, 30 x 24 x 20 cm. (See Plates 1a, 1b).

(iii) Management and Feeding: Two groups of the rats (1 of males and 1 of females) were fed on the cooked diet. The next two groups (1 of males and 1 of females) were assigned to the raw diet. The third batch of 2 groups (1 of males and 1 of females) was placed on the reference casein diet while the remaining two groups (1 of males and 1 of females) were fed on the basal protein-free diet for a period of 10 days.

The food was placed in pots specially designed to minimize spillage and scattering. Finer wire screens were placed below the wire screen - bottomed cages to collect faeces and spilled food. Water and food were given ad libitum to each rat. The weights of the animals were recorded every other day. The first three days were regarded as acclimatization period during which time no records were kept of food consumption, and no collection of faeces was made. Collection of faeces was made daily for the last 7 days of the feeding experiment. The faeces of individual rats were pooled, dried at 105°C for 24 hours and ground into powder for faecal nitrogen determination. Daily

Plate I(a). A block of metabolic cages for housing the individual rats during the feeding experiments

Plate I(b). The perspex trays used for the separate collection of urine and faeces of the rats.

PLATE I a

PLATE I b

records of food consumption were kept for the last 7 days by weighing the food given and what remains together with that spilled after 24 hours.

(iv) Collection of Urine and faeces: Each rat was placed in a single cage with a wire screen-bottom. Under each cage was placed a plastic tray (about 2 cm larger on all sides than the cage) bent at the middle along the whole length so that the long sides sloped towards the middle. The plastic tray carried a finer wire screen with holes about 0.1 cm square. This wire screen allowed only urine to pass through while collecting the faeces and the spilled food. The urine was finally collected in 100 ml beakers which contained 10 ml of $MgSO_4$ as a preservative. The faeces were carefully separated from any hair or particles of the spilled food. All spilled particles of food were put back into the food pot, dried and weighed. The faeces were dried at 105°C overnight and stored in bottles till the end of the experiments.

At the end of the feeding period the animals were weighed and killed with chloroform. Incisions were made into the skull, thorax and body cavities of the animals. The animals of each group were laid in aluminium trays (24" x 12" x 3") covered with filter paper and dried to a constant weight in a hot circulating air in an oven at 105°C for 48 hours. The contents of the alimentary canal were not removed from these animals. The dried carcasses were ready for carcass nitrogen determination. The experiment was repeated with each diet. Since the different batches of rats from the same stock show varying response

to treatment (Henry and Kon, 1957), each diet (raw-mixed and cooked) was run with its own control group and a basal protein - free group fed on the reference casein and the basal protein-free diets,respectively.

(v) Determination of Carcass and faecal Nitrogen: Carcass - N: The carcass nitrogen was determined by a modified method of Rippon (1959). In this procedure, the dried carcass of each rat including the contents of the alimentary tract as well as the small pieces of nitrogen - free filter paper used to absorb spilled blood during the dissection was first pounded in a mortar and transferred to a 1 - litre conical flask. 100 ml concentrated H_2SO_4 were poured over it and after 10 minutes 100 ml of distilled water were cautiously added. Disintegration of the carcass began immediately on addition of the water and digestion was well advanced in one hour without the application of any external heat. After this had cooled down to room temperature, the sample in the flask was autoclaved for 3 hours at 16 lbs/sq. inch pressure. Hydrolysates were cooled, stirred and made up to 250 ml. 25 ml portions of the slurry were taken in triplicate for carcass nitrogen determination by the Kjeldahl - Markham (1942) method previously described.

Faecal - N: The dried pooled faeces for each rat throughout the experimental period were weighed and ground into powder. 2 gm aliquots of each powder, in duplicate, were taken and digested according to the Kjeldahl procedure and ammonia determined by the micro-method of Markham (1942).

15. Criteria for Assessment of the Nutritional Quality of the Diets

The following criteria of nitrogen utilization were worked out on the basis of analyses of the diets, faeces and the dried carcasses for nitrogen content.

(i) True digestibility (T.D.) of the Dietary Nitrogen

This was determined by the original 'balance - sheet method' of Mitchell (1923 - 24) and is defined as the percentage of the food nitrogen consumed that is absorbed. It is calculated as:

$$\text{True Digestibility (T.D.)} = \frac{\text{N intake} - (\text{Faecal N} - \text{Metabolic N})}{\text{N intake}}$$

Metabolic N is the total faecal nitrogen excreted by the animals on basal protein-free during the experimental period.

(ii) Net Protein Utilization (N.P.U.or.): This was estimated by the carcass nitrogen method of Miller and Bender (1955) and is defined as:

$$\text{N.P.U.} = \frac{\text{Body N content with test diet} - \text{Body N content with N - free diet} + \text{N consumed by N - free diet group}}{\text{N intake by Test diet group.}}$$

That is,

$$N\ P\ U = \frac{B_T - (B_K - I_K)}{I}$$

where I is the nitrogen intake by animal on test diet.

(iii) Biological Value (B.V.): This was originally defined by Mitchell (1923 - 24) as the fraction of the absorbed nitrogen that is retained. In these investigations, the biological value was calculated from N.P.U.

by dividing it by T. D. according to the procedure of Miller and Bender (1955), Bender and Haizelden (1957) and Campbell (1963).

That is,

$$BV = \frac{N.P.U.}{T.D.}$$

(iv) Protein Efficiency Ratio (P.E.R.) : This was first used by Osborn et al. (1919) and this method expresses the biological value of the proteins in the diet as the gain in body weight per gram of protein eaten over the experimental period. It was calculated as

$$P.E.R. = \frac{\text{Weight Gain}}{\text{Protein consumed.}}$$

(v) Net Protein Ratio (N.P.R.) : This was first introduced by Bender and Doell (1957) as is assumed to overcome certain of the drawbacks of PER (Mitchell, 1944).

It is defined as:

$$N.P.R. = \frac{\text{Gain in weight with test protein + Loss in weight with basal protein - free diet}}{\text{Protein intake by Test group}}$$

(iv) Net Dietary Protein Calories Percent (NDP Cal%)

This was obtained by the method of Platt and Miller (1959) and Platt et al. (1961) using the procedure of Idusogie (1970) outlined below.

The caloric value of the protein was taken as 4 K Cal/gm for the determination of percentage of calories from protein. The caloric value of the dried diets was determined with the ballistic bomb calorimeter

as described on page 114, the gross energy so obtained was converted into metabolizable energy by the equation:

$$ME/gm = (CE/gm - 0.95) - (N\% \times 0.075)$$

where ME = metabolizable energy and CE = gross energy both in Kilocalories as described by Miller and Payne (1959). Hence the Protein Calories as a percentage of total calories (P Cals %) and the Net Dietary Protein Calories Percent (NDP cals %) were calculated:

$$\text{Protein Calories \%} = \frac{N\% \times 6.25 \times 4}{ME/gm}$$

where N is the nitrogen content of the diet.

$$NDp\ Cals\ \% = NPU(op) \times P\ Cals\ \%$$

where NPU(op) is the NPU obtained when no adjustment is made in the level of protein in the food mixture assayed (Platt and Miller, 1959; Platt et al., 1961).

16. Preparation of sera for analyses: A third feeding run was carried out with each diet and its control as described on page 120. There was, however, no collection of urine and faeces and no record was kept of food consumption. All animals were fed ad libitum for 10 days. At the end of the 10th day, the animals were killed by decapitation and the sera from all the animals in each group were pooled. This was allowed to clot at room temperature and then centrifuged for 5 minutes in the refrigerated centrifuge at 1000 r.p.m. The clear sera were stored in screw - cap bottles in the refrigerator until required for analyses.

17. Determination of Total Serum Proteins

The biuret method of King reported by Malik (1967) was followed in these investigations.

Preparation of Standard Curve: Versatol A was used as the standard protein and calculated quantities of the protein solution (5 - 500 mg/100 ml) were taken in test tubes. The volume was made up to 3 ml with distilled water in each case. 3 ml of biuret reagent were then added to each test tube. All the tubes were incubated at 37°C in a water bath for 10 minutes. A reagent blank was also prepared containing 3 ml of distilled water and 3 ml of biuret reagent. The optical density of the colour developed was read at 540 mμ in an SP500 spectrophotometer against the reagent blank. The optical density was plotted against the known protein concentration and the standard curve obtained.

Analysis of Test Sample: 0.1 ml of the pooled serum from the animals in each group fed on the test and control diets were taken in test tubes. The volume was made up to 3 ml in each case with distilled water and 3 ml of the working biuret reagent added to each tube. The optical density was determined in the same manner as for the standard protein. The protein concentrations were read from the calibration curve. The determinations were made in triplicate for each pooled serum.

18. Separation of the serum Protein fractions by Starch - gel electrophoresis.

The method of Smithies (1955; 1959) was used for the separation of the serum proteins. Hydrolysed starch made into gel by heating 72 g

in 400 ml of boric acid - sodium hydroxide solution was allowed to set for 12 hours. The samples were applied in incisions across the depth of the gel block and the block was fixed in a vertical position. Current was passed through at 15 m A for 15 hours. At the end of this period the gel blocks were removed and carefully sliced across the thickness. These slices were then stained using amido Black 10B dye. The excess dye was drained off in successive washings with methanol - acetic acid - water solution in the proportion of 5:1 : 5. The gel plates photographed the protein fractions as discrete bands.

<u>Preparation of the gel:</u> 72 gm of the hyrolysed starch (supplied by the Connaught Medical Research Laboratories, Toronto, Canada) were placed in a one - litre conical flask containing 400 ml of boric acid - NaOH solution. The mixture was gently heated to boiling with shaking until the starch was cooked as indicated by the change in colour and the transparency of the solution. The material was de-aerated by mild suction using a water pump. It was then poured into 25 x 11 x 0.5 cm plastic trays and covered with a plastic sheet cover, care being taken to exclude all air bubbles. The gel was allowed to set for 12 hours.

<u>Application of the Samples and Separation:</u> At the end of the 12 hours, the plastic cover sheet was removed and at a distance of about 9 cm from one end of the gel, 3 uniform incisions were made. About 0.07 cl of the serum sample was applied to each incision. Three pieces of polythene

sheets each a little larger than the incision were used to cover the incisions and the entire gel surface covered with a piece of clean polythene sheet to prevent evaporation. The gel was then placed in a vertical position in one of the buffer containers into which 100 ml of the buffer had been placed in each compartment. The buffer in the outer compartments of the two containers were connected to the inner ones and to the ends of the starch gel by means of filter paper wicks. The terminals were connected to the power pack and the current passed through for 15 hours.

Slicing and Staining: At the end of the operation time, the gel was gently removed from the tray, sliced and stained as described above.

19. Determination of Total Serum Cholesterol

Procedure:

The total cholesterol content of the pooled sera of the different groups of rats fed on the various test and control diets was determined by the method of Abell et al. (1952).

Treatment of Serum Samples: 0.5 ml samples of the test serum were measured into 25-ml glass stoppered centrifuge tubes, and 5 ml of alcoholic KOH were added to each tube. The tubes were stoppered, well shaken, and incubated in a water bath at 37°C for 55 minutes. When the samples had cooled to room temperature, 10 ml of petroleum ether were added and mixed well with the contents of each tube. 5 ml of water were then added and the tubes shaken vigorously for 1 minute. These were then centrifuged at slow speed for 5 minutes. 5 ml aliquots of the petroleum ether layers were transferred to a small dry bottle. The petroleum ether was

evaporated by placing the bottles in a water bath at 60°C and blowing a gentle stream of air into them. The bottles were then allowed to cool to room temperature and stoppered with clean dry corks ready for colour development with the Liebermann - Buchard reagent.

Preparation of Standard : Standards were prepared for the inclusion in each series of determinations. The standards were run along with the test samples. Duplicate 5 ml samples of the standard cholesterol solution and 0.3 ml of 33% KOH solution were mixed in 25 ml glass-stoppered centrifuge tubes and incubated for 55 minutes at 37°C. 10 ml petroleum ether and 5 ml of water were added and the tubes shaken vigorously for 1 minute. After centrifugation, 1.0, 2.0 and 3.0 ml samples of the petroleum ether layer were measured out into 10-ml volumetric flasks and evaporated to dryness to provide standards equivalent to 0.2, 0.4, and 0.6 mg of cholesterol.

Development of Colour: The bottles containing the dry residues from the samples and the standards were arranged in a wire basket so that a set of standards containing 0.2, 0.4, and 0.6 mg of cholesterol came at the end of the series. A clean empty bottle was placed at the beginning to receive the blank. The samples were set in a water bath at 25°C. A stop-watch was started, and 6 ml of the modified Liebermann - Burchard reagent were added first to the empty bottle and at regular intervals thereafter to the other samples. The bottles were corked and shaken and returned to the bath. The optical density of each sample was read against the blank in an SP500 spectrophotometer at 620 mμ, 33 minutes after the

reagent was added.

Calculation of Results:

The optical density equivalent to 1 mg of cholesterol was calculated from the readings of the standards:

$$\frac{\text{O. D. of std}}{\text{mg. cholesterol in std}} = S.$$

The average of all such values for the standard was used for calculating the total cholesterol content of the test samples:

$$\frac{\text{O. D. of unknown}}{S} \times \frac{10}{\text{vol of petroleum ether aliquot}} \times \frac{100}{\text{vol. of serum sample}} = \text{mg cholesterol/100 ml serum}$$

Estimation was carried out in triplicate for each serum sample for each group of rats.

20. Statistical Analysis of Results:

The results for the bioassay with rats were compared statistically to establish the differences between sexes and between forms of the diets fed, using the student's t - test (Bailey, 1966). The differences between the means were considered 'significant' if the corresponding probability values, P, were less than or equal to 0.05 (or $P \leq 0.05$). Where the values of P were greater than 0.05 ($P > 0.05$), the differences between the means of such parameters were regarded as 'not - significant'.

CHAPTER FOUR

EXPERIMENTS AND RESULTS

1. The determination of nutrients in the raw-mixed and cooked diets.

The foodstuffs for the preparation of the various diets were collected as described on page 55. The diets were prepared as described on pages 89 - 94 using the recipes and the traditional cooking methods of the peasants in South Eastern Nigeria. To assess the effect of the traditional cooking on the nutritive quality of the six diets representative portions of the raw-mixed and cooked diets were analysed for the various nutrients.

(a) Determination of the major nutrients in the raw-mixed and cooked diets.

Procedure: Representative samples of the raw-mixed, traditionally cooked and control diets were analysed for crude fat and total ash by the method of the A.O.A.C. (1960). The nitrogen content of the diets was estimated by the Kjeldahl - Markham procedure using the selenium - digestion catalyst of Plack (1970). The crude protein content of the food was calculated as 6.25 times the nitrogen content. The total carbohydrate content was obtained by difference. That is, the percentages of fat, ash and crude protein were subtracted from 100.

Results: Table 8 shows the main nutrients in the diets. Negligible losses of the protein in all the diets were observed. In diets D1 and D2 these losses were slightly greater than for the other diets. This might be due to the losses by leaching of the soluble proteins of the periwinkle which was cooked and washed several times before being incorporated into the

TABLE 8

NUTRIENT COMPOSITION OF THE DIETS

DIET	GM/100 GM DRY MATTER			
	CRUDE PROTEIN	ETHER EXTRACT	TOTAL ASH	TOTAL CARBOHYDRATES
RAW	17.39 ± 0.05	13.34 ± 0.31	4.52 ± 0.09	64.75 ± 1.11
D1 COOKED	16.82 ± 0.32	11.63 ± 0.13	4.34 ± 0.15	67.30 ± 0.50
RAW	10.30 ± 0.03	7.86 ± 0.01	4.90 ± 0.11	76.91 ± 0.39
D2 COOKED	9.01 ± 0.05	6.71 ± 0.07	4.92 ± 0.13	79.37 ± 1.51
RAW	6.92 ± 0.38	8.76 ± 0.02	3.06 ± 0.12	81.47 ± 3.00
D3 COOKED	6.89 ± 0.24	6.67 ± 0.05	3.24 ± 0.07	83.20 ± 2.51
RAW	11.84 ± 0.07	9.68 ± 0.11	4.95 ± 0.06	73.53 ± 2.10
D4 COOKED	11.30 ± 0.10	6.56 ± 0.31	4.98 ± 0.13	77.15 ± 3.01
RAW	15.87 ± 0.28	19.51 ± 0.57	7.35 ± 0.38	59.57 ± 3.21
D5 COOKED	13.69 ± 0.54	13.20 ± 0.15	7.45 ± 0.15	65.66 ± 4.33
RAW	10.50 ± 0.00	20.39 ± 0.13	5.74 ± 0.02	73.37 ± 1.03
D6 COOKED	10.09 ± 0.14	10.11 ± 0.12	5.61 ± 0.09	74.19 ± 3.21
CONTROL (CASEIN)	10.20 ± 0.10	11.00 ± 0.40	4.00 ± 0.11	74.86 ± 0.90

soup in D_1, and in D_2 similar losses might result from the separate cooking of the Anyan okpan diet as described on page 90. In both cases, the cooking and washing water was usually discarded. Losses of total fats varied between 3% in diet D6 and 32% in each of the diets D4 and D5. These losses could be attributed to the open-pan cooking and to the high temperature reached in the cooking processes. Slight increases were observed in the value of total ash in the cooked diets D2, D3, D4 and D5.

(b) Determination of Minerals in the raw-mixed and cooked diets.

Procedure: Analyses for minerals in the various diets were carried out using the mineral solution prepared by the dry ashing method of the A.O.A.C. (1960). Calcium was determined by the permanganate titration method of the A.O.A.C. (1960). Phosphorus was estimated spectrophotometrically as a phospho-molybdate complex by the method of Gomori (1942). Iron was determined with α,α- dipyridyl by the method of Ramsay reported by the A.O.A.C. (1960). The dry matter content of the diets was obtained by the method of the A.O.A.C. (1960).

Results: The mineral contents of the test diets are shown in Table 9. Losses of the minerals investigated were least in diets D5 and D6. This is because these two diets were single dishes and as described on pages 93 - 94 their preparation involved mixing all the condiments in the same cooking pot. These were unlike diets D1, D2, D3 and D4, one part of which was usually cooked separately and then eaten with the other also separately cooked. The highest losses observed for

MINERAL COMPOSI

DIET	DRY MATTER (%)	P R Calcium (m
D1	32.88 ± 0.31 24.60 ± 0.68	1220.10 ± 2 1079.10 ± 3
D2	26.66 ± 1.11 21.46 ± 2.01	863.49 ± 745.52 ±
D3	45.14 ± 0.95 30.23 ± 0.24	326. 5 ± 2 315. 0 ± 4
D4	31.85 ± 0.22 25.25 ± 0.22	934. 2 ± 9 871. 2 ± 26
D5	48.26 ± 0.41 34.45 ± 0.33	1104. 0 ± 25 1096. 0 ± 51
D6	44.91 ± 0.71 34.71 ± 0.57	55.43 ± 4. 53.50 ± 7.

TABLE 9

MINERAL COMPOSITION OF [illegible]

Diet	DRY MATTER (%)	PER 100 GM DRY MATTER		
		Calcium (mg)	Phosphorus (mg)	Iron (mg)
D1 RAW	32.88 ± 0.31	1220.10 ± 21.1	247.40 ± 1.30	15.34 ± 0.90
D1 COOKED	24.60 ± 0.68	1079.10 ± 35.8	207.80 ± 2.22	14.33 ± 0.15
D2 RAW	26.66 ± 1.11	863.49 ± 1.11	367.20 ±11.30	13.03 ± 0.31
D2 COOKED	21.46 ± 2.01	745.53 ± 0.93	316.60 ± 9.31	9.33 ± 0.40
D3 RAW	45.14 ± 0.95	326. 5 ± 21. 5	184. 6 ±12. 4	19.57 ± 1.83
D3 COOKED	30.23 ± 0.24	315. 0 ± 45. 0	173. 3 ±11. 5	16.57 ± 2.15
D4 RAW	31.85 ± 0.22	934. 2 ± 93. 0	321. 4 ±12. 3	7.58 ± 0.28
D4 COOKED	25.25 ± 0.22	871. 2 ± 26. 6	280. 8 ±14. 4	6.79 ± 0.30
D5 RAW	48.26 ± 0.41	1104. 0 ± 25. 0	443. 8 ±25. 5	8.55 ± 0.61
D5 COOKED	34.45 ± 0.33	1096. 0 ± 51. 0	435. 3 ±26. 6	8.10 ± 0.30
D6 RAW	44.91 ± 0.71	55.43 ± 4. 00	326. 9 ±22. 5	8.67 ± 0.63
D6 COOKED	34.71 ± 0.57	53.50 ± 7. 00	320. 9 ±12. 3	8.34 ± 0.38

diets D_1 and D_2 were probably due to leaching of some soluble salts of these minerals into the cooking water which was usually discarded.

(c) Determination of Vitamins in the raw-mixed and cooked diets

Procedure: In the representative portions of the diets, thiamine (vitamin B_1) was determined by thiochrome fluorescence method of the Association of Vitamin Chemists (1966), and riboflavine, by the fluorometric method of Hodson and Norris (1939). Niacin was estimated spectrophotometrically by the procedure of Melnick and Field (1942). Pyridoxine was determined by the chemical method of Bano et al. (1943), vitamin C by the dye - reduction method of the Association of Vitamin Chemists (1966) and vitamin A by the Carr-Price (1938) method. Beta-carotene was estimated by the colorimetric method of Bassir (1963 b).

Results: The various vitamins showed varying degrees of destruction during the traditional preparation of the diets (See Table 10). The most affected were vitamin C and the B - vitamins.

Table 10

VITAMINS IN THE RAW AND COOKED DIETS

DIET	PER 100 GM DRY WEIGHT OF DIET						
	Vit. A (I.U.)	β-Carotene (I.U)	Vit.C (mg)	Vit. B_1 (mg)	Vitamin B_2 (mg)	Niacin (mg)	Pyridoxal (mg)
B1 RAW	104800 ± 125.1	22110 ± 111.3	24.63 ± 0.41	1.35 ± 0.11	1.11 ± 0.10	14.41 ± 0.50	15.52 ± 0.73
B1 COOKED	92955.0 ± 331	21125.0 ± 281.6	14.14 ± 0.01	0.95 ± 0.20	0.99 ± 0.06	10.05 ± 0.15	11.28 ± 0.08
B2 RAW	71650.0 ± 231.1	3099.0 ± 131.6	8.09 ± 0.01	0.39 ± 0.01	0.09 ± 0.00	3.40 ± 0.01	17.56 ± 0.83
B2 COOKED	40990.0 ± 583.9	2483.1 ± 81. 5	Trace	0.27 ± 0.03	0.08 ± 0.00	3.20 ± 0.11	9.00 ± 0.04
B3 RAW	71387.0 ± 650.1	3904.3 ± 256.4	29.20 ± 1.17	0.50 ± 0.07	0.24 ± 0.02	6.87 ± 0.20	11.20 ± 0.98
B3 COOKED	55663.1 ± 128.0	3723.7 ± 135.1	17.53 ± 2.90	0.35 ± 0.04	0.18 ± 0.01	5.13 ± 0.30	9.20 ± 0.87
B4 RAW	31385.8 ± 721.3	5111.4 ± 69. 3	15.73 ± 0.052	0.68 ± 0.01	0.39 ± 0.04	10.23 ± 0.24	20.20 ± 0.09
B4 COOKED	23393.2 ± 430	4244.7 ± 83. 0	5.12 ± 0.00	0.45 ± 0.01	0.27 ± 0.00	8.25 ± 0.14	19.51 ± 0.13
B5 RAW	132930.0 ±115.4	24823.5 ± 110.0	37.75± 0.43	2.35 ± 0.15	0.42 ± 0.01	9.34 ± 0.23	12.08 ± 0.43
B5 COOKED	109241.4 ±246.7	22934.5 ± 222.2	12.50 ± 2.02	1.23 ± 0.07	0.33 ± 0.02	8.13 ± 0.11	29.46 ± 0.10
B6 RAW	90230.1 ± 762.1	9555.5 ± 386.0	14.90 ± 0.29	0.19 ± 0.01	0.19 ± 0.01	27.03 ± 0.43	29.88± 0.33
B6 COOKED	75127.0 ± 231.0	8868.7 ± 161.8	7.54 ± 1.02	0.12 ± 0.01	0.14 ± 0.01	23.27 ± 1.01	27.48 ±0.27

(d) Quantitative estimation of the Amino Acids in the diets.

Procedure: The total amino acid analysis of the raw-mixed traditionally cooked and control diets was carried out by the modified method of ion-exchange chromatography of Spackman et al. (1956). Hydrolysis of the diets for the estimation of the amino acids, except cystine and tryptophane was carried out as described by Schram et al. (1953).

Cystine was estimated, as cysteic acid after oxidation with performic acid and hydrolysis with 6N HCl by the procedure of Schram et al. (1954).

Tryptophane was estimated spectrophotometrically at 540 mμ by the modified method of Miller (1967), using p-dimethylaminobenzaldehyde.

Results: The total amino acids of the raw and control diets are shown in Table 11. Lysine was the most affected by the different cooking processes of the different diets. Losses of this amino acid were highest (6% and 6%, respectively) in diets D6 and D3. The highest loss of about 6% of methionine was observed in the cooked diet, D1. Oesterhout et al. (1959) reported 5% and 6% losses of methionine and methionine + cystine respectively after heating tunny - fish meal.

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2. Determination of Protein Calories Percent and Metabolizable Energy of the raw-mixed, cooked and control diets.

Procedure: The gross energy of the dried diets was determined by the method of Miller and Payne (1959). The metabolizable energy (in Kcals/gm of dry diet) and the protein calories percent were calculated using the procedure of Idusogie (1970) described on pages 126 - 127..

Results: Table 12 shows the PCal% and the metabolizable energy values of the test and control diets. The PCal% of 6.28 obtained for diet D3 based on cassava and fish is close to the value of 6.1 obtained by Miller and Payne (1961 b) for a Canbian diet based also on cassava and fish. The same workers obtained PCal% values of 12.5 and 14.0, respectively, for some Nigerian diets based on sorghum, pulses and fish; and on, sorghum, fish and milk. The PCal% and metabolizable energy values of the control diet were the means of six separate estimations. The metabolizable energy of the test diets varied between 3.90 and 5.05.

3. Determination of the Effect of Feeding The Raw-Mixed and The Traditionally Cooked Diets To Rats.

Procedure: The weanling male and female albino rats of the Wistar strain from our own colony were selected and grouped as described on pages 120 - 121. Food and water were given ad libitum. The animals were fed on the test and control diets for 10 days. The first 3 days of the feeding trial were regarded as the acclimatization period during which no collection of faeces was made and no record of food consumption was kept. Observations were made for the last 7 days on:

(1) Food intake every 24 hours.

Table 12

Protein Calorion % and Metabolizable Energy of Raw and Cooked and Control Diets.

DIET		Metabolizable Energy (Kcal/gm)	Protein Cal%
D1	RAW	4.64	15.83
	COOKED	4.39	15.33
D2	RAW	4.76	10.64
	COOKED	4.97	9.07
D3	RAW	3.99	6.75
	COOKED	4.30	6.28
D4	RAW	4.08	11.62
	COOKED	3.90	11.58
D5	RAW	4.71	11.53
	COOKED	4.05	13.51
D6	RAW	4.86	8.46
	COOKED	5.05	9.46
CONTROL		4.37*	9.96*

* Mean of six separate determinations.

(ii) Weight gain/loss during the feeding period. This was the difference between the initial and the final weights of each rat.

(iii) Faeces were collected every 24 hours, dried and stored,

(iv) The nitrogen content of the diets was determined and nitrogen intake of the animals calculated from the food consumption data.

(v) Faecal nitrogen was determined in the dried pooled faeces for each rat at the end of the experiment.

From these observations, the effect of cooking on the nutritional value of the peasant diets were assessed using the following parameters:

(a) Protein efficiency ratio (PER),

(b) Net Protein Ratio (NPR),

(c) The true digestibility of dietary nitrogen (T.D.)

(d) Biological value (B.V.)

(e) Net Protein Utilization (NPU)

(f) Net dietary protein Calories percent (NDpCal%), and

(g) Weight gain/loss by the animals. These parameters have already been defined on pages 125 - 127

The results were compared statistically using the Student's t - test for sex differences on cooked diets, and for differences between the raw-fed and cooked diets in the rats. The results were also compared with those for a casein (reference) diet fed at 10% protein level in an otherwise complete diet to littermate rats. The various

results are shown in Tables 13 - 36.

Results: Tables 13 - 20 show the variations in responses of the male and littermate female rats to the different traditionally cooked peasant diets. On diet D3 the female rats consumed significantly greater nitrogen (Table 13) and throve better (Table 20) than the males. The highest body weight gains and PER values were observed in both sexes when fed on diet D1. This might be attributed to the higher crude protein content (Table 9) of this diet than of the others. All the animals on diet D6 lost weight during the experimental period. This might be explained in terms of the very low NPU (Table 17) value of this diet. This observation would support the previous findings of Bender (1956) that negative PER values are usually obtained when NPU is about or less than 40%. In all cases, however, the two sexes did not always show the same differences between diets. Similar results were obtained by Morrison and Campbell (1960 a) with male and female rats fed respectively on different levels of the same and of different proteins.

Effect of Traditional Cooking on the Utilization of the Test Diets:

Tables 21 - 28 show the responses of the weanling littermate rats (ten in each case, 5 males and 5 females) to the raw-mixed and cooked peasant diets. The animals on raw-mixed diets D2 and D5 consumed more nitrogen ($P<0.001$) than those on the cooked diets (Table 28). The lowest

nitrogen intake was observed in raw-mixed and cooked diet D3. The rats on all the cooked diets except diet D1 had higher PER values (Table 25). The slightly higher PER value with the raw-mixed diet D1 could be due to similar slightly higher biological value (Table 23) for this form of the diet. Mendor (1956), Block and Mitchell (1946 - 47) and Munry et al. (1947) reported high correlation between NPU and PER for rats on different proteins.

Cooking in all cases improved the coefficient of true digestibility considerably (Table 24). Except in diets D1 and D3 cooking also improved the biological value of the diets (Table 23). Higher NPCal% values were obtained for all the cooked diets except diet D1 (Table 21). The slightly higher NPCal% value for raw-mixed diet D1 could be attributed to the slightly higher P Cal% (Table 12) and NPU values of this form of the diet.

Comparison of the test and control diets as shown in Tables 29 - 36 indicated better utilisation of the control diet than either the raw-mixed cooked test diets in almost all the cases.

Table 13

Comparison of the Nitrogen Intake by the Male and Female Rats on the Cooked Diet.

DIET	N INTAKE (Mean of 5 rats)	Standard Deviation	't'	Level of Significance
Male D1	3.07	0.339	0.47	P > 0.05
Female	3.20	0.542		(Not significant)
Male D2	0.983	0.113	1.82	P > 0.05
Female	0.685	0.347		(Not significant)
Male D3	0.617	0.076	4.22	P < 0.01
Female	0.828	0.083		(Significant)
Male D4	1.301	0.396	0.98	P > 0.05
Female	1.519	0.209		(Not significant)
Male D5	1.538	0.099	1.65	P > 0.05
Female	1.023	0.757		(Not Significant)
Male D6	1.141	0.187	1.11	P > 0.05
Female	1.007	0.196		(Not Significant)

Table 14

Comparison of the Protein Efficiency Ratio in the Male and Female Rats on Cooked Diet.

DIET	PER (Mean for 5 rats)	Standard Deviation	't'	Level of Significance
Male D1	2.190	0.091	0.50	P > 0.05
Female	2.132	0.244		(Not significant
Male D2	1.977	0.096	2.03	P > 0.05
Female	1.347	0.688		(Not significant)
Male D3	1.403	0.179	1.07	P > 0.05
Female	1.566	0.288		(Not significant)
Male D4	1.453	0.341	1.05	P > 0.05
Female	1.202	0.233		(Not significant)
Male D5	1.622	0.181	3.77	P < 0.01
Female	0.748	0.537		(Significant)
Male D6	-0.196	0.250	1.15	P > 0.05
Female	-0.439	0.405		(Not significant)

Table 15

Comparison of the Biological value (B.V.) of the Protein in the Cooked Diet for Male and Female RATS

DIET	B V (Mean for 5 rats)	Standard Deviation	't'	Level of Significance
D1 Male	58.17	3.180	0.84	P > 0.05
D1 Female	55.99	4.860		(not significant)
D2 Male	70.72	0.823	1.71	P > 0.05
D2 Female	74.26	5.572		(not significant)
D3 Male	73.32	4.550	1.84	P > 0.05
D3 Female	68.30	4.050		(not significant)
D4 Male	51.23	1.690	0.45	P > 0.05
D4 Female	51.76	2.050		(not significant)
D5 Male	66.34	1.811	5.74	P < 0.001
D5 Female	60.18	1.561		(significant)
D6 Male	50.56	3.530	0.19	P > 0.05
D6 Female	50.01	5.201		(not significant)

Table 16

Comparison of the NDp Cals % of the Control Diet for the Male and Female Rats

DIET	NDp Cals % (Mean for 5 rats	Standard Deviation	't'	Level of Significance
D1 Male	7.72	0.471	1.06	P > 0.05
D1 Female	7.37	0.591		(not significant)
D2 Male	5.25	0.090	1.29	P > 0.05
D2 Female	4.07	0.201		(not significant)
D3 Male	3.97	0.180	4.24	P < 0.01
D3 Female	3.51	0.161		(significant)
D4 Male	5.37	0.151	0.60	P > 0.05
D4 Female	5.22	0.191		(not significant)
D5 Male	7.79	0.260	5.18	P < 0.001
D5 Female	6.82	0.290		(significant)
D6 Male	3.34	0.220	0.71	P > 0.05
D6 Female	3.23	0.270		(not significant)

Table 17

Comparison of the Net Protein Utilization of the Cooked Diet in the Male and Female Rats.

DIET	NPU (Mean for 5 rats)	Standard Deviation	't'	Level of Significance
Male	50.36	3.040	1.06	P > 0.05
D1 Female	48.05	3.820		(not significant)
Male	57.81	1.001	2.08	P > 0.05
D2 Female	56.05	1.610		(not significant)
Male	63.19	2.920	4.24	P < 0.01
D3 Female	55.85	2.551		(significant)
Male	46.95	1.330	0.60	P > 0.05
D4 Female	46.82	1.630		(not significant)
Male	57.03	1.910	6.61	P < 0.001
D5 Female	50.45	1.510		(significant)
Male	41.83	2.700	0.71	P > 0.05
D6 Female	40.50	3.391		(not significant)

Table 18

Comparison of the True Digestibility (T.D.) of the Nitrogen in the Cooked Diet in the Male and Female Rats

Diet	T. D. (Mean for 5 rats)	Standard Deviation	't'	level of Significance
Male D1	86.61	2.300	0.48	P > 0.05 (not significant)
Female	85.90	2.320		
Male D2	81.77	1.950	4.59	P < 0.002 (significant)
Female	75.63	2.291		
Male D3	86.29	2.900	2.84	P < 0.05 (significant)
Female	81.87	2.430		
Male D4	90.81	1.210	0.24	P > 0.05 (not significant)
Female	90.68	1.090		
Male D5	86.88	1.971	3.03	P < 0.02 (significant)
Female	83.82	1.111		
Male D6	82.47	1.380	1.19	P > 0.05 (not significant)
Female	81.20	2.820		

Table 19

Comparison of the Net Protein Ratio. (NPR) of the Cooked Diet for the Male and Female Rats

Diet	NPR (Mean for 5 rats)	Standard Deviation	't'	Level of Significance
Male	2.72	0.071	0.48	P > 0.05
M Female	2.69	0.121		(not significant)
Male	3.39	0.120	0.38	P > 0.05
M Female	3.36	0.111		(not significant)
Male	3.76	0.101	1.44	P > 0.05
M Female	3.56	0.251		(not significant)
Male	2.74	0.111	1.02	P > 0.05
M Female	2.63	0.051		(not significant)
Male	3.61	0.120	2.61	P < 0.05
M Female	3.18	0.320		(significant)
Male	1.05	0.250	3.71	P < 0.01
M Female	1.70	0.315		(significant)

Table 20

Comparison of Weight Gains/Losses by Male and Female Rats on Cooked Diet

DIET	Weight gains/losses (Mean for 5 rats	Standard Deviation	't'	Level of Significance
Male D1	40.66	5.990	0.74	P>0.05
Female	43.20	4.760		(not significant)
Male D2	12.70	1.410	3.67	P<0.01
Female	9.07	1.301		(significant)
Male D3	5.46	1.190	2.55	P<0.05
Female	7.76	1.651		(significant)
Male D4	12.62	7.151	0.19	P>0.05 (not significant)
Female	13.36	4.771		
Male D5	15.67	2.430	2.34	P<0.05
Female	10.95	3.510		(significant)
Male D6	-1.14	1.640	1.00	P>0.05
Female	-2.44	2.400		(not significant)

Table 21

Comparison of the Net Dietary Protein Calories Percent of the Raw-Mixed and Cooked Diets for Rats.

DIET	NDPCAL% (Mean for 10 rats)	Standard Deviation	't'	Level of Significance
RAW D1	7.91	0.550	0.76	P > 0.05 (not significant)
COOKED	7.72	0.560		
RAW D2	4.64	0.331	4.65	P < 0.001 (significant)
COOKED	5.17	0.135		
RAW D3	3.73	0.219	0.00	P > 0.05 (not significant)
COOKED	3.74	0.288		
RAW D4	4.46	0.275	8.97	P < 0.001 (Highly significant)
COOKED	5.39	0.175		
RAW D5	5.25	0.602	8.01	P < 0.001 (Highly significant)
COOKED	7.30	0.515		
RAW D6	3.00	0.206	2.82	P < 0.02 (significant)
COOKED	3.29	0.251		

Table 22

Comparison of the Net Protein Utilization of the Cooked and Raw Mixed for the Rats

DIET	NPU (Mean for 10 rats)	Standard Deviation	't'	Level of Significance
M1 RAW	49.92	3.480	0.45	P>0.05
M1 COOKED	49.21	3.641		(not significant)
M2 RAW	44.88	3.200	10.78	P<0.001
M2 COOKED	56.93	2.493		(Highly significant)
M3 RAW	54.90	3.256	2.55	P<0.02
M3 COOKED	59.52	4.583		(significant)
M4 RAW	38.41	2.367	9.14	P<0.001
M4 COOKED	46.52	1.372		(Highly significant)
M5 RAW	45.55	5.217	4.02	P<0.001
M5 COOKED	54.84	3.804		(significant)
M6 RAW	34.77	2.386	5.14	P<0.001
M6 COOKED	43.19	3.144		(significant)

Table 23

Comparison of the Biological Value (B.V.) of the Raw-Mixed and Cooked Diets for the Rats

DIET	B. V. (Mean for 10 rats)	Standard Deviation	't'	Level of Significance
RAW D1	65.06	4.751	3.98	P<0.001 (significant)
COOKED D1	57.08	4.248		
RAW D2	65.31	2.214	4.72	P<0.001 (significant)
COOKED D2	72.49	3.500		
RAW D3	75.77	5.882	2.22	P<0.05 (significant)
COOKED D3	70.81	4.990		
RAW D4	46.33	3.378	4.20	P<0.001 (significant)
COOKED D4	51.50	1.898		
RAW D5	59.53	4.896	1.95	P>0.05 (not significant)
COOKED D5	63.88	3.476		
RAW D6	48.42	3.392	1.06	P>0.05 (not significant)
COOKED D6	52.43	3.412		

Table 24

Comparison of the True Digestibility of Nitrogen in the Raw-fixed and Cooked Diets in the Rats.

DIET	T. D. (Mean for 10 rats)	Standard Deviation	't'	Level of Significance
RAW D1	76.80	2.178	9.36	P < 0.001
COOKED	86.26	2.339		(Highly significant)
RAW D2	67.65	3.772	7.26	P < 0.001
COOKED	78.70	3.599		(Highly significant)
RAW D3	72.60	2.948	8.30	P < 0.001
COOKED	84.06	3.310		(Highly significant)
RAW D4	83.62	4.857	4.74	P < 0.001
COOKED	91.49	1.109		(Significant)
RAW D5	77.28	2.816	7.05	P < 0.001
COOKED	85.65	2.272		(Highly significant)
RAW D6	73.77	1.399	10.18	P < 0.001
COOKED	84.99	2.376		(Highly significant)

Table 25

Comparison of the Protein Efficiency Ratio (PER) of the Raw-Irad and Cooked Diets in the Rat

DIET	P. E. R. (Mean for 10 rats)	Standard Deviation	't'	Level of Significance
D1 RAW	2.30	0.633	0.57	P > 0.05
D1 COOKED	2.16	0.187		(not significant)
D2 RAW	1.39	0.276	2.97	P < 0.02
D2 COOKED	1.83	0.183		(significant)
D3 RAW	0.81	0.335	3.69	P < 0.01
D3 COOKED	1.48	0.239		(significant)
D4 RAW	0.73	0.425	2.72	P < 0.05
D4 COOKED	1.40	0.350		(significant)
D5 RAW	1.30	0.300	0.11	P > 0.05
D5 COOKED	1.37	0.294		(not significant)
D6 RAW	-0.72	0.280	2.31	P < 0.05
D6 COOKED	-0.32	0.266		(significant

Table 26

Comparison of the Net Protein Ratio. (NPR) of the Rats on Raw-mixed and Cooked Diets

DIET	N. P. R. (Mean for 10 rats)	Standard Deviation	't'	Level of Significance
RAW D1	2.75	0.230	0.62	P > 0.05
COOKED	2.70	0.105		(not significant)
RAW D2	2.75	0.408	4.22	P < 0.001
COOKED	3.41	0.167		(significant)
RAW D3	3.19	0.362	3.14	P < 0.01
COOKED	3.66	0.244		(significant)
RAW D4	2.26	0.117	8.67	P < 0.001
COOKED	2.68	0.100		(significant)
RAW D5	2.77	0.277	5.19	P < 0.001
COOKED	3.43	0.291		(significant)
RAW D6	0.95	0.227	3.71	P < 0.002
COOKED	1.53	0.429		(significant)

Table 27

Comparison of the Weight Gain/Loss by the Rats on Raw-mixed and Cooked Diets

DIET	Weight GAIN/LOSS (Mean for 10 rats)	Standard Deviation	't'	Level of Significance
RAW D1	36.12	3.553	2.79	P < 0.02 (significant)
COOKED	41.93	5.555		
RAW D2	4.98	4.025	2.93	P < 0.01 (significant)
COOKED	10.62	2.315		
RAW D3	4.20	2.193	3.08	P < 0.01 (significant
COOKED	7.22	2.060		
RAW D4	5.83	3.902	3.13	P < 0.01 (significant)
COOKED	12.99	6.416		
RAW D5	17.11	5.135	1.64	P > 0.05 (not significant)
COOKED	12.99	6.087		
RAW D6	-5.16	1.977	3.69	P < 0.002 (significant)
COOKED	-1.79	2.155		

Table 28

Comparison of the Nitrogen Intake by the Rats on Raw-mixed and Cooked Diets

DIET	N Intake (Mean for 10 rats)	Standard Deviation	't'	Level of Significance
RAW	2.77	0.401	1.92	P > 0.05 (not significant)
COOKED	3.13	0.457		
RAW	1.73	0.345	6.20	P < 0.001 (significant)
COOKED	0.83	0.238		
RAW	0.79	0.193	0.91	P > 0.05 (not significant)
COOKED	0.72	0.132		
RAW	1.19	0.190	1.68	P > 0.05 (not significant)
COOKED	1.41	0.367		
RAW	2.08	0.072	8.00	P < 0.001 (significant)
COOKED	1.52	0.209		
RAW	1.18	0.183	1.18	P > 0.05 (not significant)
COOKED	1.07	0.213		

Table 29

Comparison of the Nitrogen Intake of the Rats on the Test and Control Diets

	DIET	N Intake (Mean for 10 rats)	Standard Deviation	't'	Level of significance
31	COOKED	3.13	0.457	8.80	P < 0.001 (Highly significant)
	CONTROL	1.68	0.257		
	RAW	2.77	0.401	7.23	P < 0.001 (Highly significant)
	CONTROL	1.68	0.257		
32	COOKED	0.83	0.298	4.59	P < 0.001 (significant)
	CONTROL	1.32	0.147		
	RAW	1.73	0.345	3.48	P < 0.01 (significant)
	CONTROL	1.32	0.147		
33	COOKED	0.72	0.132	9.53	P < 0.001 (Highly significant)
	CONTROL	1.53	0.224		
	RAW	0.79	0.193	7.19	P < 0.001 (Highly significant)
	CONTROL	1.53	0.224		
34	COOKED	1.41	0.367	2.53	P < 0.02 (significant)
	CONTROL	1.77	0.261		
	RAW	1.13	0.190	5.69	P<0.001 (significant)
	CONTROL	1.77	0.261		
35	COOKED	1.52	0.209	3.45	P<0.01 (significant)
	CONTROL	1.23	0.153		
	RAW	2.08	0.072	16.0;	P<0.001 (Highly significant)
	CONTROL	1.23	0.153		
36	COOKED	1.07	0.213	0.88	P>0.05 (not significant)
	CONTROL	1.14	0.116		
	RAW	1.13	0.103	0.58	P > 0.05 (not significant)
	CONTROL	1.14	0.116		

Table 30

Comparison of the Weight Gain/Loss in the Rats on Test and Control Diets

DIET	Weight Gain/Loss (Mean of 10 rats)	Standard Deviation	't'	Level of Significance
COOKED	41.93	5.555		
CONTROL	29.43	5.831	4.91	P <0.001 (significant)
RAW	36.12	3.553		
CONTROL	29.43	5.831	3.10	P <0.01 (significant)
COOKED	10.62	2.315		
CONTROL	22.64	3.869	8.31	P <0.001 (Highly significant)
RAW	14.98	1.025		
CONTROL	22.64	3.869	4.34	P< 0 .001 (significant)
COOKED	7.22	2.060		
CONTROL	27.32	4.922	10.82	P <0.001 (Highly significant)
RAW	4.20	2.193		
CONTROL	27.32	4.922	12.25	P <0.001 (Highly significant)
COOKED	12.93	6.416		
CONTROL	34.18	6.555	7.30	P <0.001 (Highly significant)
RAW	5.83	3.902		
CONTROL	34.18	6.555	11.75	P <0.001 (Highly significant)
COOKED	12.99	6.937		
CONTROL	21.18	3.754	3.63	P <0.002 (significant)
RAW	17.11	5.135		
CONTROL	21.18	3.754	2.02	P >0.05 (not significant)
COOKED	21.79	2.155		
CONTROL	19.91	2.732	19.72	P <0.001 (Highly significant)
RAW	-5.16	1.977		
CONTROL	19.91	2.732	23.51	P <0.001 (Highly significant)

Table 31

Comparison of the Protein Efficiency Ratio (PER) in the Rats on Test and Control Diets

	P. E. R. (Mean for 10 rats)	Standard Deviation	't'	Level of Significance
[illegible]	2.16	0.187	5.60	P < 0.001 (significant)
[illegible]	2.81	0.316		
[illegible]	2.30	0.633	2.27	P < 0.05 (significant)
[illegible]	2.81	0.316		
[illegible]	1.83	0.183	10.83	P < 0.001 (Highly significant)
[illegible]	2.74	0.190		
[illegible]	1.39	0.276	12.74	P < 0.001 (Highly significant)
[illegible]	2.74	0.190		
[illegible]	1.48	0.239	13.43	P < 0.001 (Highly significant)
[illegible]	2.85	0.216		
[illegible]	0.81	0.335	16.18	P < 0.001 (Highly significant)
[illegible]	2.85	0.216		
[illegible]	1.40	0.350	12.95	P < 0.001 (Highly significant)
[illegible]	2.98	0.163		
[illegible]	0.73	0.425	15.62	P < 0.001 (Highly significant)
[illegible]	2.98	0.163		
[illegible]	1.37	0.294	11.17	P < 0.001 (Highly significant)
[illegible]	2.74	0.219		
[illegible]	1.30	0.300	12.27	P < 0.001 (Highly significant)
[illegible]	2.74	0.219		
[illegible]	-0.32	0.266	27.15	P < 0.001 (Highly significant)
[illegible]	2.80	0.247		
[illegible]	-0.72	0.240	29.81	P < 0.001 (Highly significant)
[illegible]	2.80	0.247		

Table 32

Comparison of the Net Protein Ratio (N.P.R.) in the Rats on Test and Control Diets

Diet	N. P. R. (Mean of 10 rats)	Standard Deviation	't'	Level of Significance
1 COOKED	2.70	0.105	11.76	P < 0.001 (Highly significant)
1 CONTROL	4.19	0.385		
1 RAW	2.75	0.230	10.12	P < 0.001 (Highly significant)
1 CONTROL	4.19	0.385		
2 COOKED	3.41	0.167	14.06	P < 0.001 (Highly significant)
2 CONTROL	4.48	0.144		
2 RAW	2.75	0.408	12.62	P < 0.001 (Highly significant)
2 CONTROL	4.48	0.144		
23 COOKED	3.66	0.24	5.43	P < 0.001 (significant)
23 CONTROL	4.36	0.293		
23 RAW	3.19	0.362	7.08	P < 0.001 (Highly significant)
23 CONTROL	4.36	0.293		
24 COOKED	2.68	0.100	19.60	P < 0.001 (Highly significant)
24 CONTROL	4.28	0.252		
24 RAW	2.26	0.117	22.95	P < 0.001 (Highly significant)
24 CONTROL	4.28	0.252		
25 COOKED	3.43	0.291	10.47	P < 0.001 (Highly significant)
25 CONTROL	4.61	0. 204		
25 RAW	2.77	0.277	16.94	P < 0.001 (Highly significant)
25 CONTROL	4.61	0.204		
26 COOKED	4.53	0.429	15.23	P < 0.001 (Highly significant)
26 CONTROL	4.30	0.303		
26 RAW	0.95	0.227	23.77	P < 0.001 (Highly significant)
26 CONTROL	4.30	0.303		

Table 33

Comparison of the True Digestibility of the Nitrogen in the Rats on Test and Control Diets

Test	True Digestibility (Mean of 10 Rats	Standard Deviation	't'	Level of Significance
[illegible]	86.26	2.339	0.33	P > 0.05
[illegible]ROL	85.86	2.932		(not significant
[illegible]	76.80	2.178	7.85	P < 0.001
[illegible]ROL	85.86	2.932		(Highly significant)
[illegible]	78.70	3.599	3.01	P < 0.01
[illegible]ROL	83.61	3.689		(significent)
[illegible]	67.65	3.772	9.57	P < 0.001
[illegible]ROL	83.61	3.689		(Highly significant)
[illegible]	84.08	3.310	0.62	P > 0.05
[illegible]ROL	84.85	2.133		(not significant)
[illegible]	72.80	2.948	10.97	P < 0.001
[illegible]ROL	84.85	2.133		(Highly significant)
[illegible]	90.40	1.109	3.51	P < 0.002
[illegible]ROL	86.75	3.038		(significant)
[illegible]	83.82	4.657	1.66	P > 0.05
[illegible]ROL	86.75	3.038		(not significant
[illegible]	85.35	2.272	0.77	P > 0.05
[illegible]ROL	86.39	3.591		(not significant)
[illegible]	77.28	2.816	6.31	P < 0.001
[illegible]ROL	86.39	3.591		(significant
[illegible]	81.99	2.376	4.14	P < 0.001
[illegible]ROL	86.66	2.667		(significant)
[illegible]	72.87	1.393	12.11	P < 0.001
[illegible]ROL	86.66	2.094		(Highly significant)

Table 33

Comparison of the True Digestibility of the Nitrogen in the Rats on Test and Control Diets

Diet	True Digestibility (Mean of 10 rats	Standard Deviation	't'	Level of Significance
[illegible]	86.26	2.339	0.33	P > 0.05
[illegible]ROL	85.86	2.932		(not significant
[illegible]	76.80	2.178	7.85	P < 0.001
[illegible]ROL	85.86	2.932		(Highly significant)
[illegible]	78.70	3.599	3.01	P < 0.01
[illegible]ROL	83.61	3.689		(significant)
[illegible]	67.65	3.772	9.57	P < 0.001
[illegible]ROL	83.61	3.689		(Highly significant)
[illegible]	84.08	3.310	0.62	P > 0.05
[illegible]ROL	84.85	2.133		(not significant)
[illegible]	72.80	2.948	10.97	P < 0.001
[illegible]ROL	84.85	2.133		(Highly significant)
[illegible]	90.40	1.109	3.51	P < 0.002
[illegible]ROL	86.75	3.098		(significant)
[illegible]	83.82	4.657	1.66	P > 0.05
[illegible]ROL	86.75	3.098		(not significant
[illegible]	85.35	2.272	0.77	P > 0.05
[illegible]ROL	86.39	3.591		(not significant)
[illegible]	77.28	2.816	6.31	P < 0.001
[illegible]ROL	86.39	3.591		(significant
[illegible]	81.99	2.376	4.14	P < 0.001
[illegible]ROL	86.66	2.667		(significant)
[illegible]	72.87	1.393	12.11	P < 0.001
[illegible]ROL	86.66	2.094		(Highly significant)

Table V:

Comparison of the Biological Value (B.V.) of the Test and Control Diets for the Rats

Diet	Biological Value (Mean of 10 rats)	Standard Deviation	't'	Level of Significance
SOAKED	57.00	4.248	15.90	P<0.001 (Highly significant)
CONTROL	85.68	3.784		
RAW	65.06	1.751	10.74	P<0.001 (Highly significant)
CONTROL	85.68	3.784		
SOAKED	72.49	3.500	8.33	P<0.001 (Highly significant)
CONTROL	90.50	5.882		
RAW	66.31	2.214	12.17	P<0.001 (Highly significant)
CONTROL	90.50	5.882		
SOAKED	70.81	4.990	6.16	P<0.001 (significant)
CONTROL	84.40	4.885		
RAW	75.77	5.025	3.89	P<0.001 (significant)
CONTROL	84.40	4.885		
SOAKED	51.50	1.898	26.33	P<0.001 (Highly significant)
CONTROL	84.41	3.471		
RAW	46.33	3.378	24.90	P<0.001 (Highly significant)
CONTROL	84.41	3.474		
SOAKED	63.88	3.476	9.76	P<0.001 (Highly significant)
CONTROL	83.87	5.153		
RAW	59.53	4.896	10.56	P<0.001 (Highly significant)
CONTROL	83.87	5.153		
SOAKED	52.42	3.413	11.10	P<0.001 (Highly significant)
CONTROL	82.26	3.166		
RAW	48.42	3.392	22.40	P<0.001 (Highly significant)
CONTROL	84.26	3.754		

Table 35

Comparison of the Net Protein Utilization (N.P.U.) in the Rats on Test and Control Diets

DIET	NPU (Mean of 10 rats)	Standard Deviation	't'	Level of Significance
COOKED	49.21	3.641	16.22	P < 0.001
CONTROL	73.51	3.033		(Highly significant)
RAW	49.92	3.480	16.16	P < 0.001
CONTROL	73.51	3.033		(Highly significant)
COOKED	56.93	1.493	12.80	P < 0.001
CONTROL	75.52	3.470		(Highly significant)
RAW	44.88	3.200	20.51	P < 0.001
CONTROL	75.52	3.470		(Highly significant)
COOKED	59.52	4.584	6.83	P < 0.001
CONTROL	72.38	3.407		(significant)
RAW	54.90	3.256	10.78	P < 0.001
CONTROL	72.38	3.407		(Highly significant)
COOKED	46.52	1.372	31.31	P < 0.001
CONTROL	73.18	2.314		(Highly significant
RAW	38.41	2.367	33.21	P < 0.001
CONTROL	73.18	2.314		(Highly significant)
COOKED	54.04	3.804	10.00	P < 0.001
CONTROL	71.87	3.967		(Highly significant)
RAW	45.55	5.717	13.31	P < 0.001
CONTROL	72.53	3.720		(Highly significant)
COOKED	41.19	3.141	22.86	P < 0.001
CONTROL	72.11	3.033		(Highly significant)
RAW	34.77	2.336	31.14	P < 0.001
CONTROL	72.77	3.033		(Highly significant)

Table 36

Comparison of the Diet Dietary Protein Calories Percent of the Standard Control Diets in the Rats

Diet	KILOCALS% (Mean of 10 rats	Standard Deviation	't'	Level of Significance
SOYBEAN	7.72	0.560	2.86	P<0.01 (significant
CONTROL	6.98	0.287		
RAW	7.91	0.550	4.74	P<0.01 (significant)
CONTROL	6.98	0.287		
SOYBEAN	5.17	0.135	19.74	P<0.001 (Highly significant
CONTROL	7.49	0.345		
RAW	4.64	0.331	18.87	P<0.001 (Highly significant)
CONTROL	7.49	0.344		
SOYBEAN	3.74	0.288	25.92	P<0.001 (Highly significant)
CONTROL	7.88	0.370		
RAW	3.73	0.219	27.72	P<0.001 (Highly significant)
CONTROL	7.88	0.370		
SOYBEAN	5.39	0.175	23.48	P<0.001 (Highly significant)
CONTROL	7.60	0.240		
RAW	4.46	0.393	21.57	P<0.001 (Highly significant)
CONTROL	7.60	0.240		
SOYBEAN	7.30	0.515	3.01	P<0.01 (significant)
CONTROL	6.69	0.343		
RAW	5.25	0.602	6.56	P<0.001 (significant)
CONTROL	6.69	0.343		
SOYBEAN	5.29	0.251	31.65	P<0.001 (Highly significant)
CONTROL	7.20	0.300		
RAW	2.00	0.206	34.10	P<0.001 (Highly significant)
CONTROL	7.20	0.300		

4. Determination of the Total serum proteins of the animals on raw-mixed, cooked and control diets.

Procedure: The total serum proteins of the male and female rats were determined as described on page 128 using the biuret method of King reported by Kalu (1967). The results are shown in Table 37.

Results: Higher values (6.09 - 6.60 g/100 ml serum) were obtained in the animals on the control diet. Slightly higher values were obtained in the male rats than in the females. There did not appear to have been much variation between the serum protein levels of the animals on the raw-mixed and cooked diets. There was also no noticeable variation in the serum protein levels of the animals on the various diets.

Table 37

Total Serum Proteins of the male and female rats on the 5 diets and Control diets (gm/100 ml serum)

(Each value is the mean of 3 determinations)

SEX OF ANIMAL	D1			D2			D3			D4			D5			D6		
	RAW	COOKED	CONTROL	RAW	COOKED	CONTROL	RAW	COOKED	CONTROL	RAW	COOKED	CONTROL	RAW	COOKED	CONTROL	RAW	COOKED	CONTROL
MALE	5.93	5.93	6.60	6.30	5.90	6.49	5.75	5.65	6.65	5.80	5.90	6.45	5.97	5.80	6.30	5.99	5.69	6.35
FEMALE	5.73	5.78	6.42	5.10	5.81	6.51	6.60	5.24	6.60	5.55	5.70	6.35	5.83	5.70	6.31	5.49	5.62	6.02

D1 = Gari, afiin, smoked fish

D2 = Amyan ekpan and pork soup

D3 = Cassava foofoo with edikan ikong soup

D4 = Fermented yam and fish stew

D5 = Akpu (fufu)

D6 = Plantain porridge with smoked fish

5. Separation of the serum Protein patterns of the animals on test and control diets.

Procedure: The effect of traditional cooking on the nutritional quality of the peasant diets was investigated with the starch - gel electrophoresis of the serum proteins of the rats fed on these diets. The procedure used was that of Smithies (1955, 1959) described on pages 128 - 130. The pooled serum of the male and female rats on the raw-mixed and cooked diets was run simultaneously with that of the control. The aim here was to show any variations in the bands which might be caused by the differences in the nutritional quality of the diets. The results are shown on plates 2a - 7b.

Results: The various protein bands (albumin, post albumin, α_1-, α_2-, β- and γ- globulins) were represented in the separations regardless of the diet or sex of the animal.

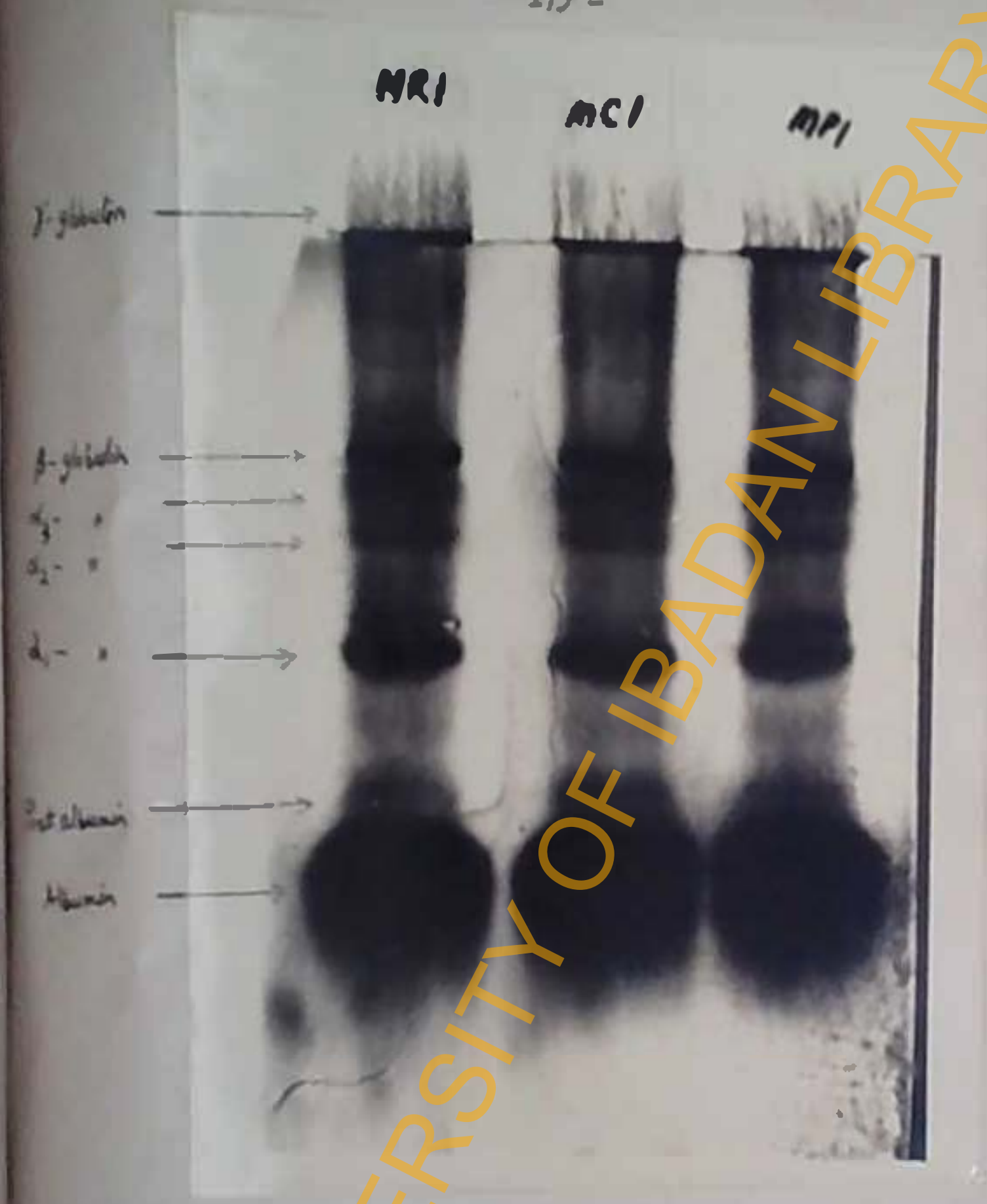


Plate 2(a) Starch - gel electrophoretic separation of the serum proteins of the male rats fed for 10 days on the test and control diets.

NR1 = Male rats on raw-mixed diet D1

MC1 = " " " cooked " D1

MP1 = " " " control diet

Plate 2(b). Starch - gel electrophoretic separation of the serum proteins of the female rats fed for 10 days on the test and control diets.

FRI = Female rats on raw-dried diet

FCI = " " " cooked " "

FFI = " " " control diet

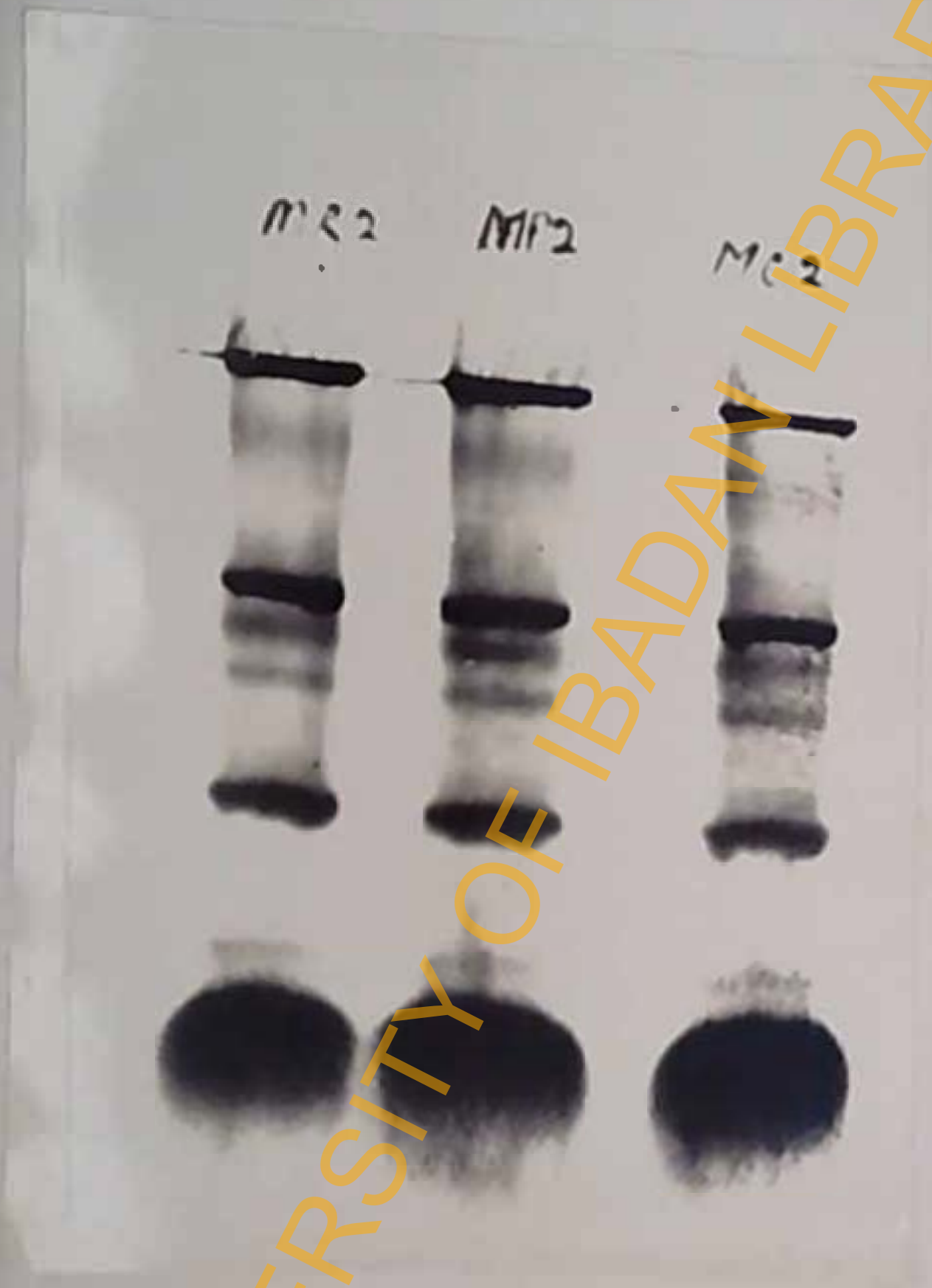


Plate 3(a). Starch - gel electrophoretic separation of the serum proteins of the male rats fed for 10 days on the test and control diets.

MR2 = Male rats on raw-mixed diet D2

MC2 = " " " cooked " "

MF2 = " " " CONTROL diet.

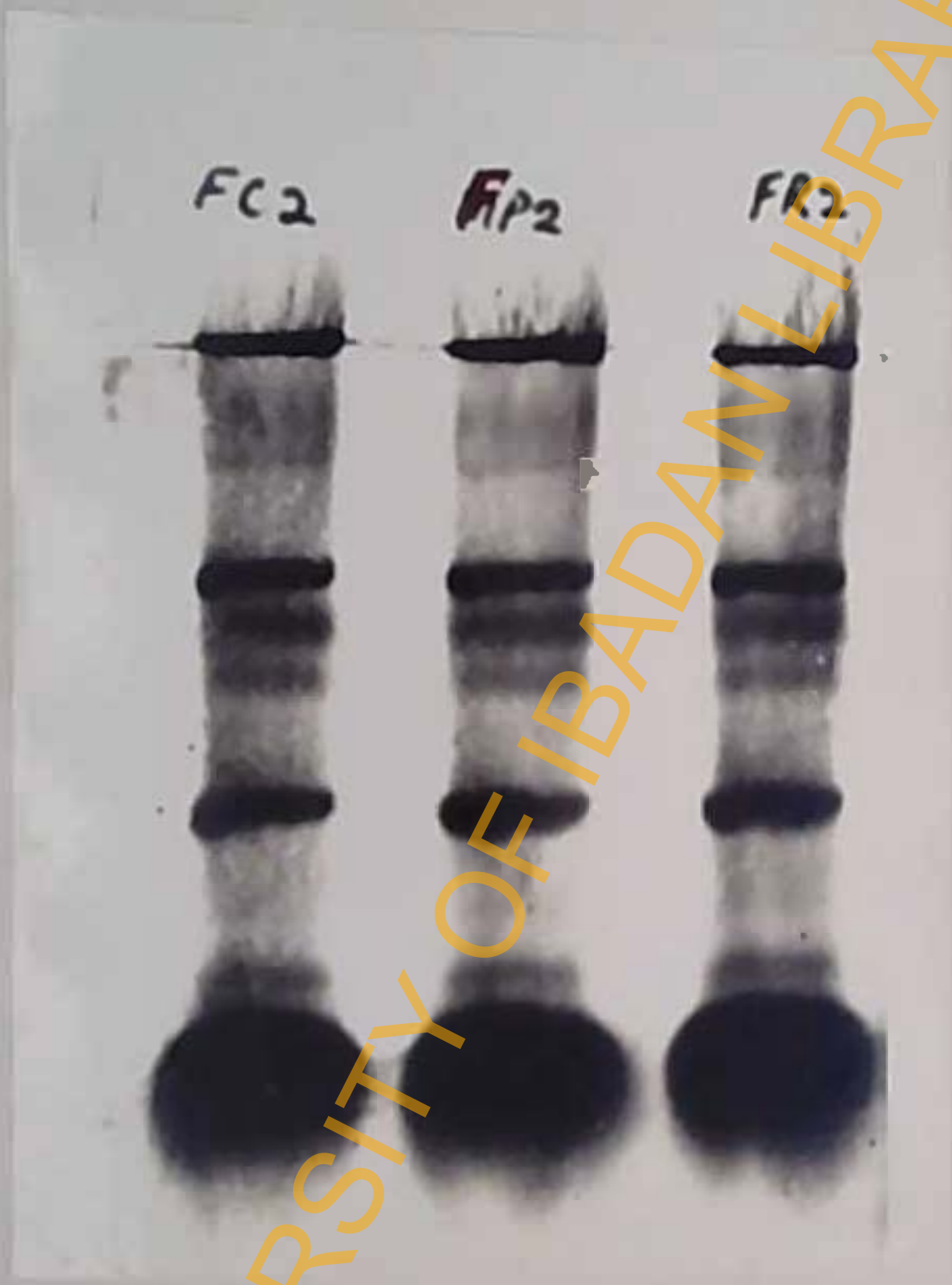


Plate 3(b). Starch - gel electrophoretic separation of the serum proteins of the female rats fed for 10 days on the test and control diets.

FR2 = Female rats on raw-dried diet D2

FC2 = " " " cooked " "

FP2 = " " " control diet.

Plate 4(a). Starch - gel electrophoretic separation of the serum proteins of the male rats fed for 10 days on the test and control diets.

MC3 = Male rats on cooked diet D3

MR3 = " " " raw-mixed diet D3

MP3 = " " " control diet.

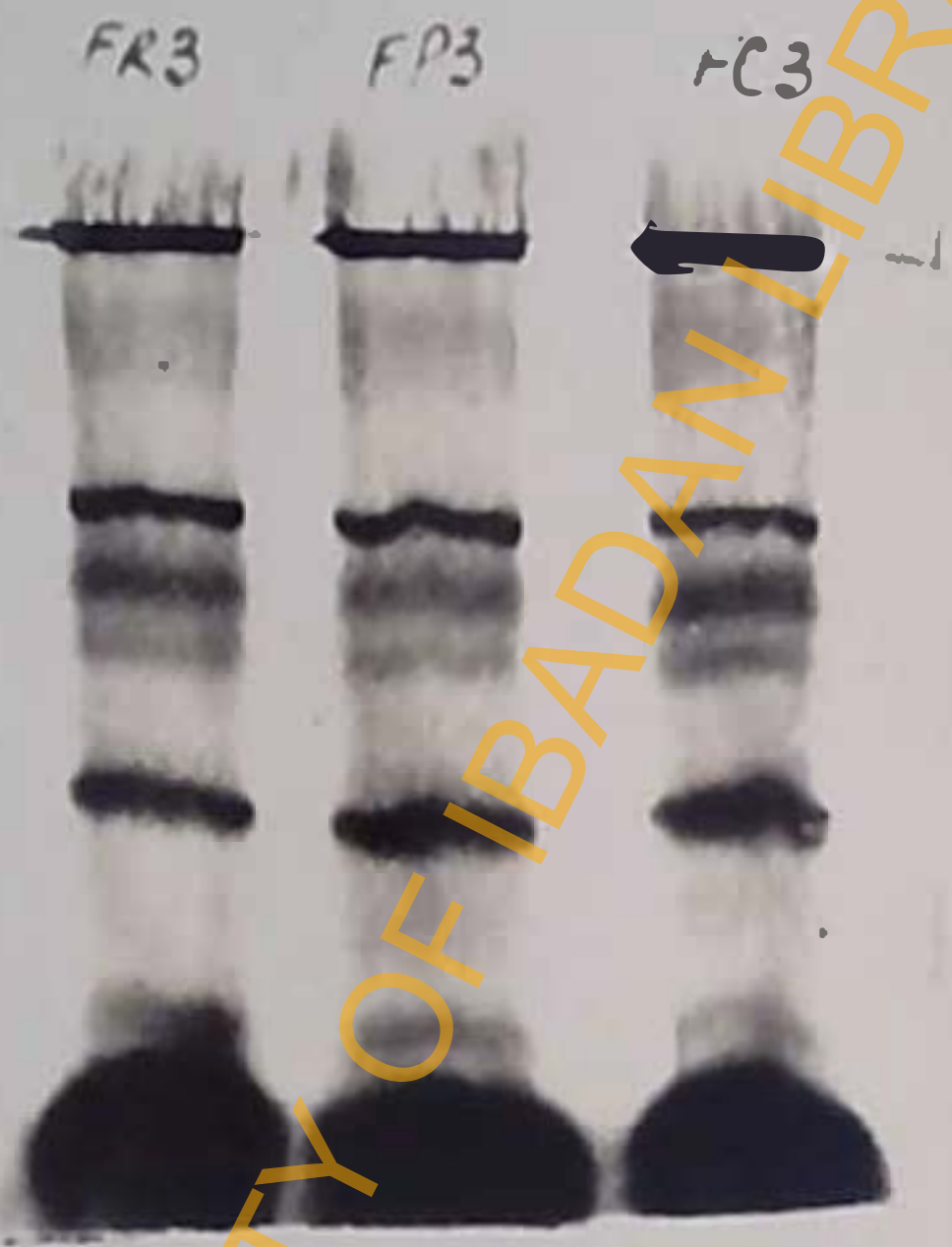


Plate 4(b). Starch - gel electrophorotic separation of the serum proteins of the female rats fed for 10 days on the test and control diets.

FR3 = Females rats on raw-mixed diet D3

FC3 = " " " cooked " "

FP3 = " " " control diet.

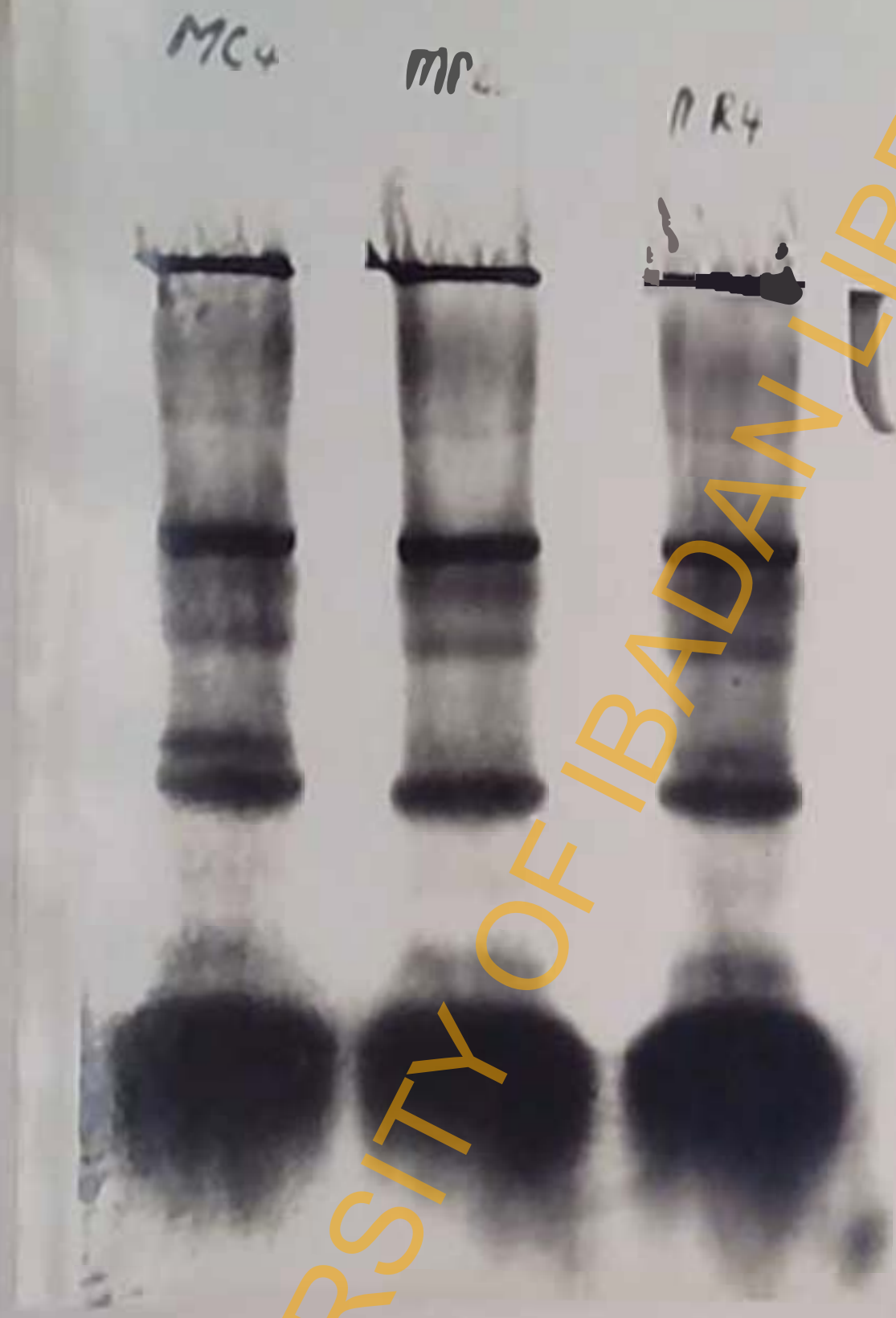
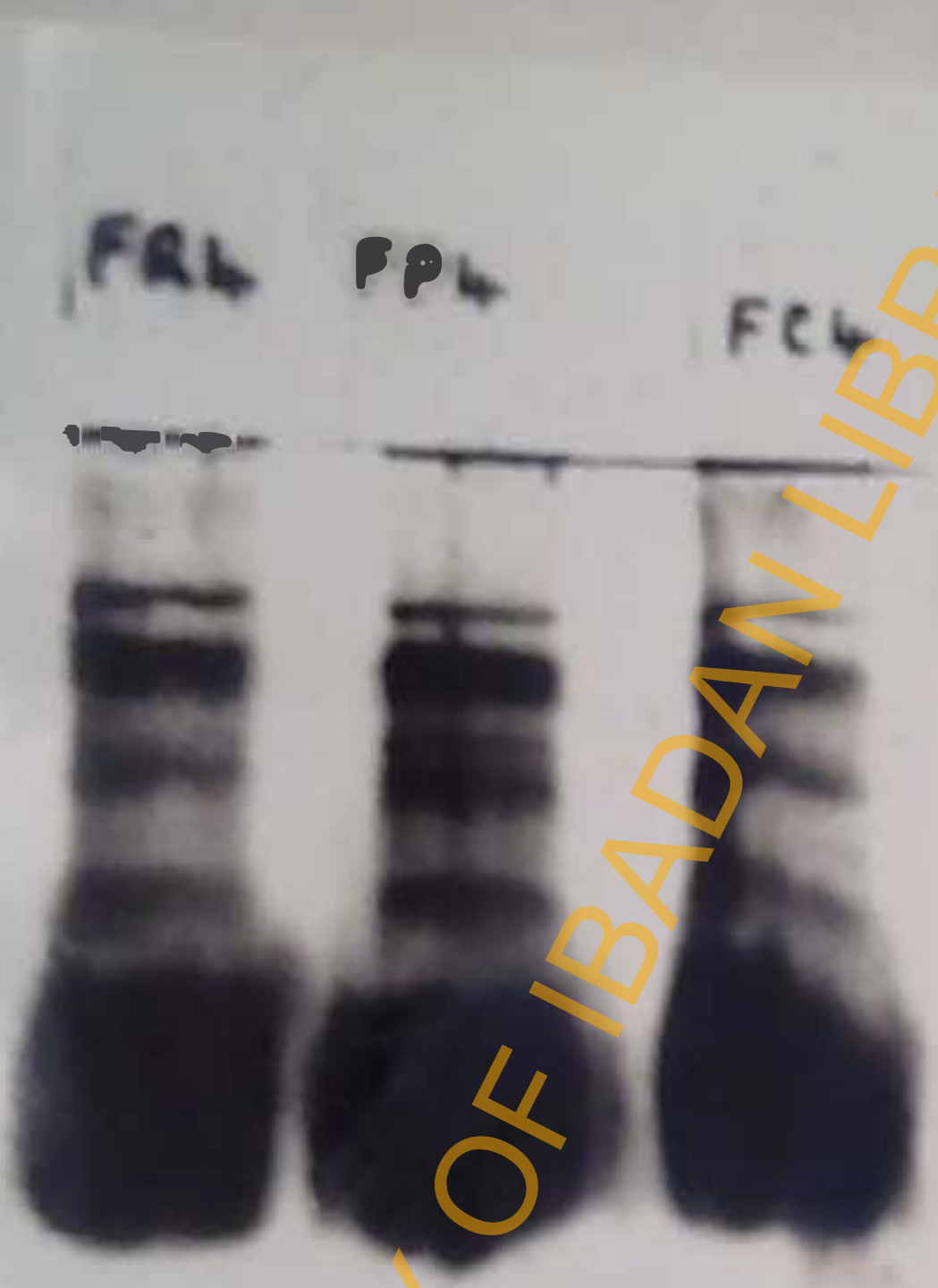


Plate 5(a). Starch - gel electrophoretic separation of the serum protoins of the male rats fed for 10 days on the test and control diets.

MC4 = Male rats on cooked diet D4

MR4 = " " " " raw-mixed diet D4

MD4 = " " " " control diet.



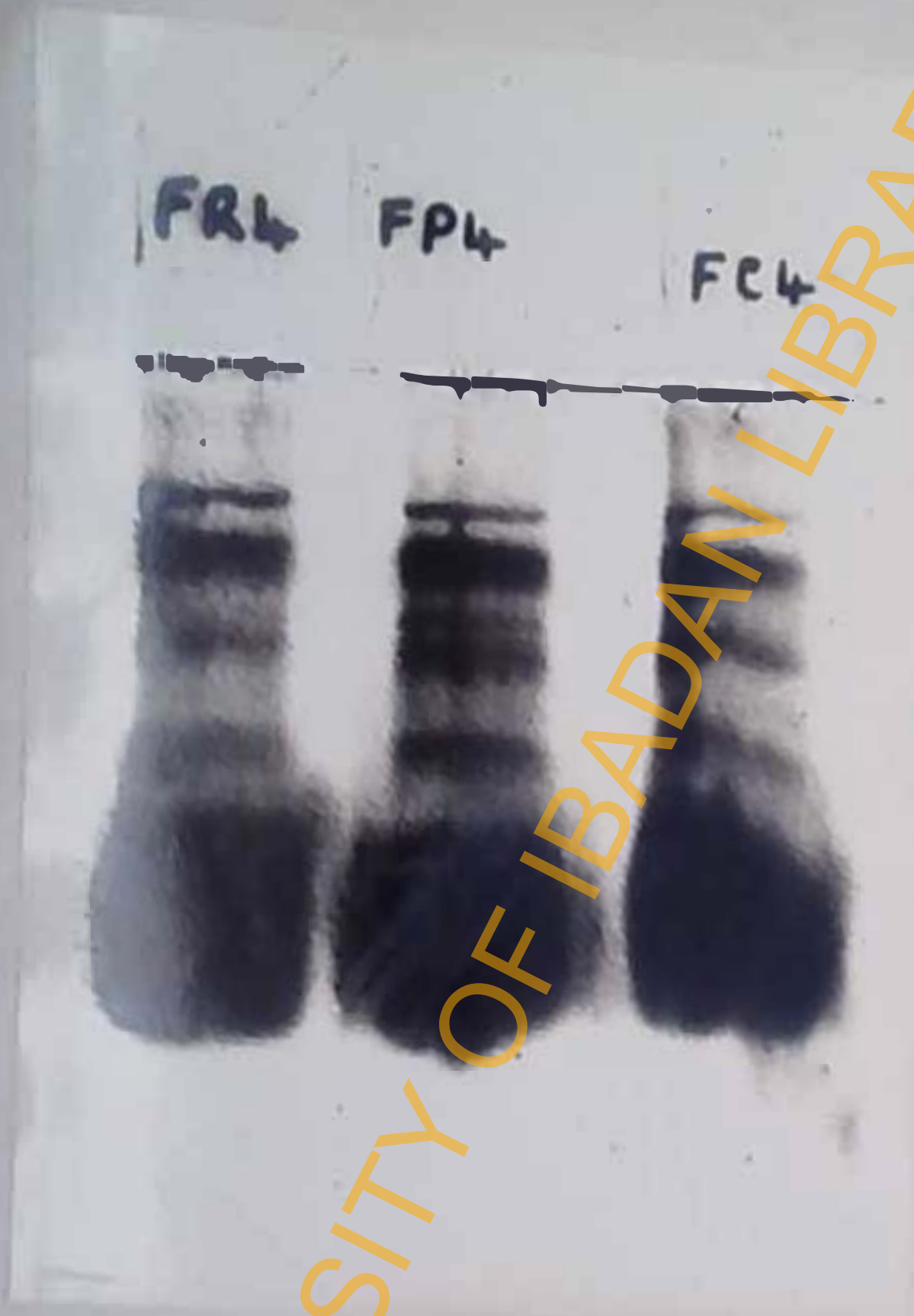


Plate 5(b). Starch - gel electrophoretic separation serum proteins of the female rats fed for 10 days on the test and control diets.

FR4 = Female rats on raw-mixed diet D4

FC4 = " " " cooked " "

FP4 = " " " control diet.

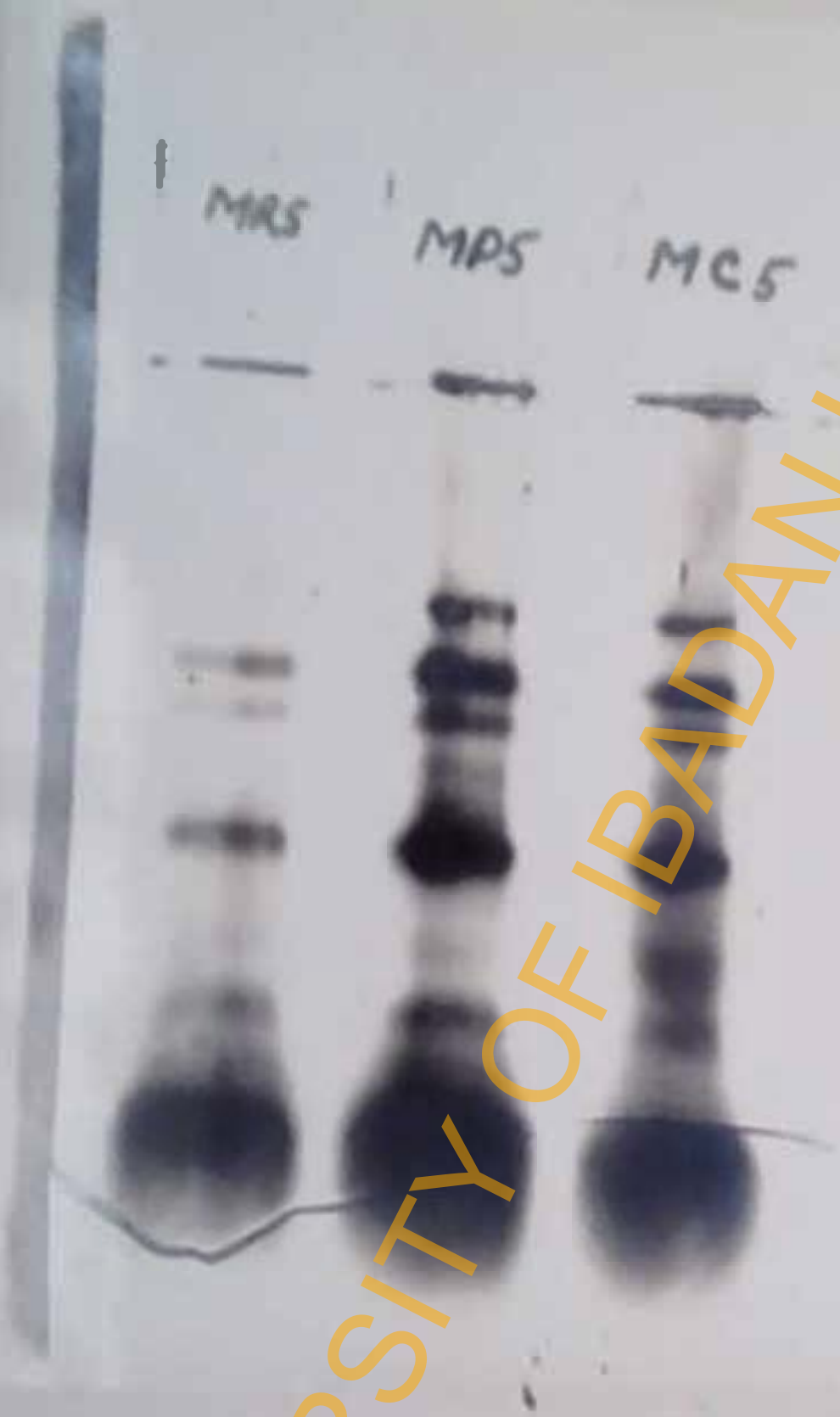


Plate 6(a). Starch - gel electrophoretic separation of the serum proteins of the male rats fed for 10 days on the test and control diets.

MRS = Males on raw - mixed diet RS

MPS = " " cooked " "

MCS = " " control diet.

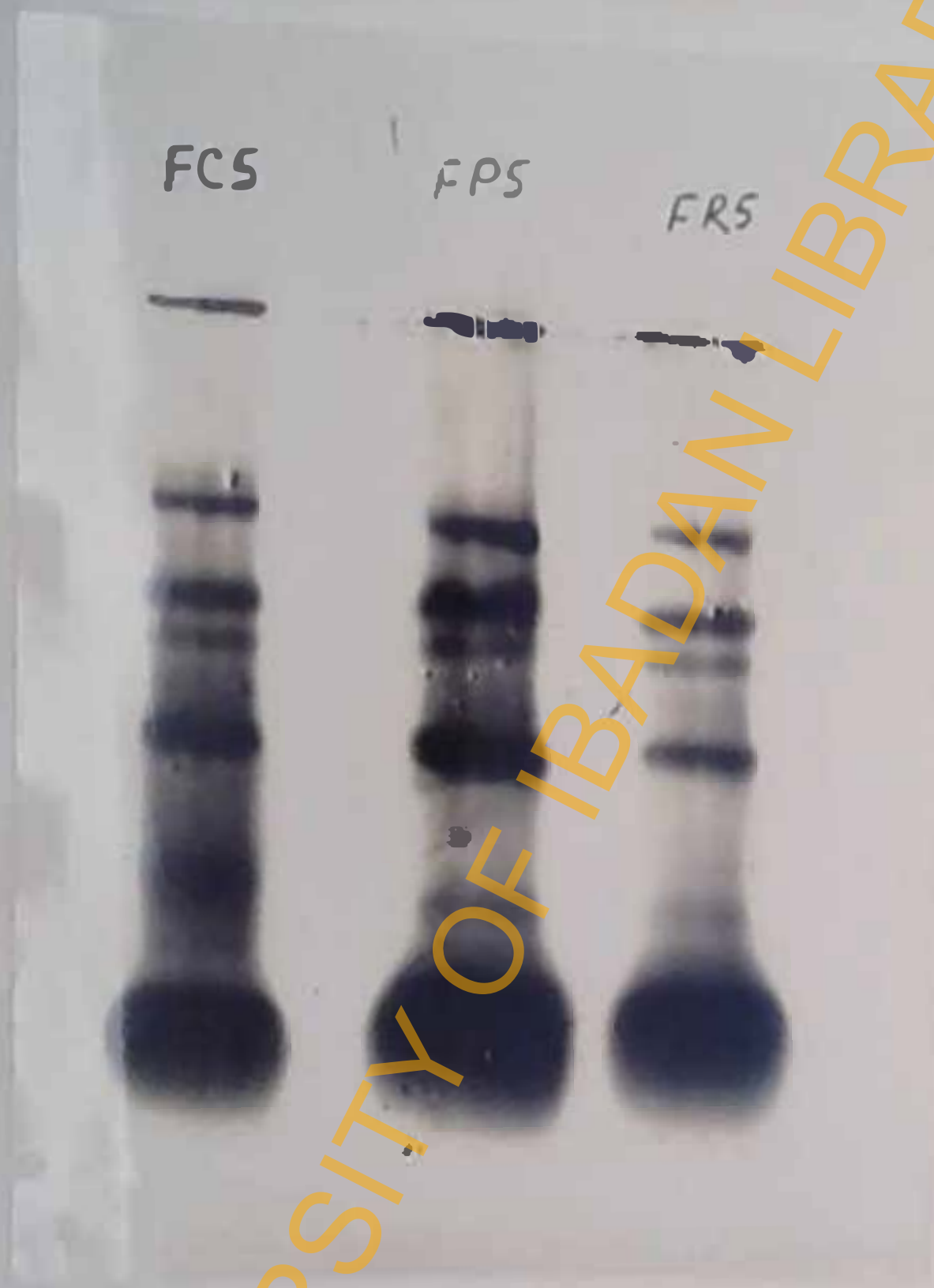


Plate 6(b). Starch - gel electrophoretic separation of the serum proteins of the female rats fed for 10 days on the test and control diets.

FCS - Female rats on cooked diet D5

FRS - " " " raw-mixed diet D5

FrS - " " " control diet.

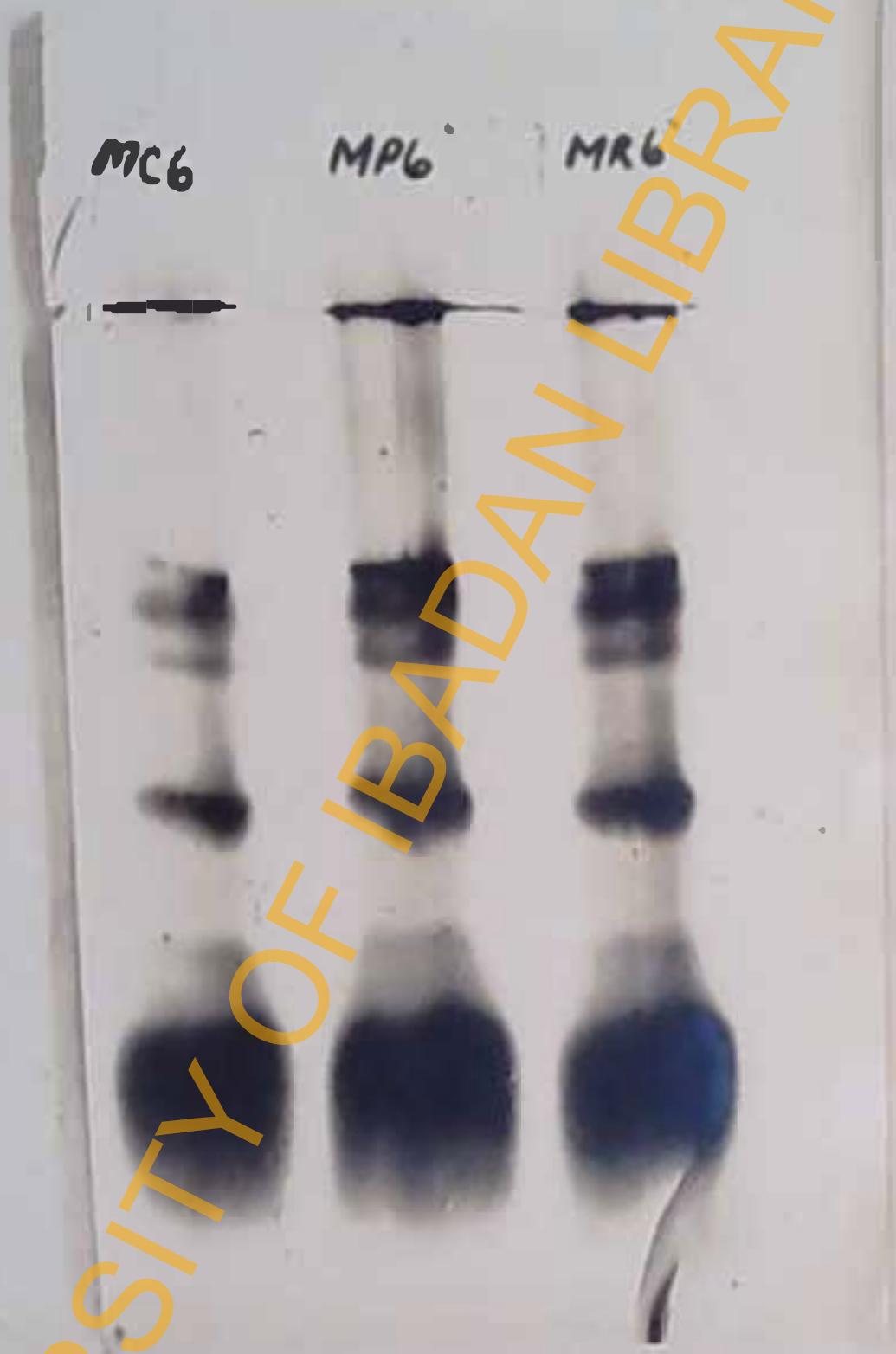


Plate 7(a). Starch - gel electrophoretic separation of the serum proteins of the male rats fed for 10 days on the test and control diets.

MR6 = Male rats on raw-mixed diet D6

MC6 = " " " cooked " "

MP6 = " " " control diet.

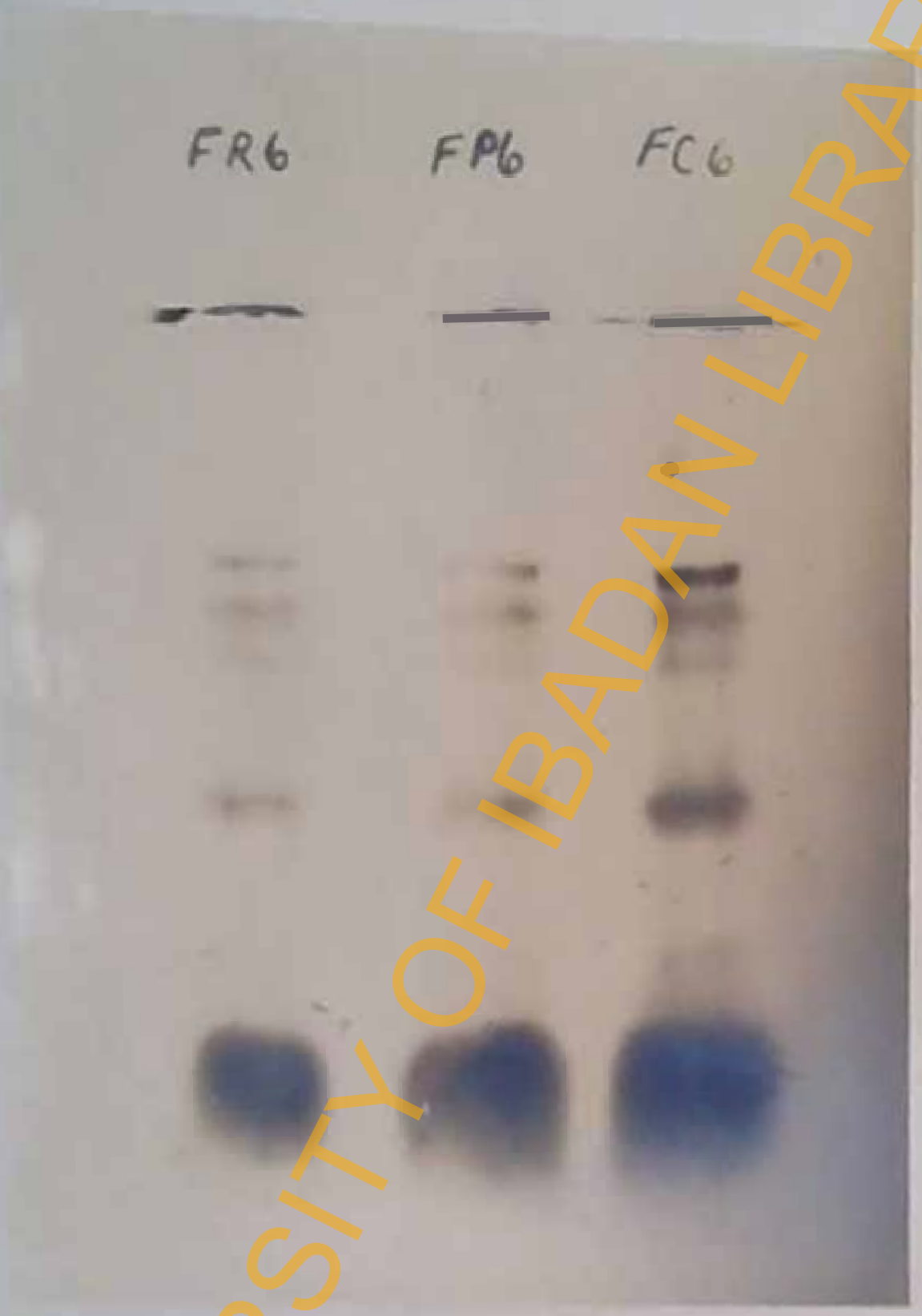


Plate 7(b). Starch - gel electrophoretic separation of the serum proteins of the female rats fed for 10 days on the test and control diets.

FR6 = female rats on raw-rice diet D6

FP6 = " " " cooked " D6

FC6 = " " " control diet.

6. Determination of Total serum cholesterol of the animals on the raw-mixed, cooked and control diets.

Procedure: The total serum cholesterol levels in the animals on the test and control diets were determined in the pooled serum as described on pages 130 - 132 using the method of Abell et al. (1952). The results were expressed as mg cholesterol per 100 ml of serum.

Results: The variations in the total serum cholesterol levels in the male and female rats are shown in Table 38. The values (120 - 140 mg/100 ml serum) for both sexes on the control diet were higher than the values in the littermate rats of either sex on any of the test diets. Rats on test diets D1 and D5 showed slightly higher serum cholesterol values (100.0 - 125. 9 mg/100 ml serum) than in the animals on any of the other test diets.

Table 38

Total serum cholesterol in the male and female rats on the test and Control diets (mg/100 ml serum)

(Each value is the mean of 3 determinations)

DIET OF ANIMAL	D1			D2			D3			D4			D5			D6		
	RAW	COOKED	CONTROL	RAW	COOKED	CONTROL	RAW	COOKED	CONTROL	RAW	COOKED	CONTROL	RAW	COOKED	CONTROL	RAW	COOKED	CONTROL
MALE	100.0	99.1	138.0	85.1	92.0	126.5	94.5	80.0	125.0	88.1	85.3	127.8	110.5	102.3	133.1	70.5	78.5	129.0
FEMALE	120.2	108.0	140.3	91.5	103.5	130.1	96.2	90.0	130.0	102.0	92.1	131.0	125.9	110.0	136.4	80.1	92.2	140.0

D1 = Garri, afan and smoked fish

D2 = Anyen ekpan, and ebak soup

D3 = Cassava fooloo and edikan ikon soup

D4 = Powded yam and afia efere

D5 = Ekpin ikuhwo

D6 = Plantain porridge with smoked fish.

CHAPTER FIVE

DISCUSSION

Several factors, environmental, social or economic, have contributed to the problems of malnutrition and undernutrition prevalent in the Nigerian peasant homes. The results of these are the predominant poor health and the much reduced physique in this section of the population (Nicol, 1959 a,b; Collis et al.1962 a; Expo, 1964). Since food is the most important environmental factor affecting health (Hamm and Stare, 1951), the studies reported in this thesis were carried out in an attempt to evaluate the nutritional quality of the peasant diets of South Eastern Nigeria. The effect of the traditional methods of preparation and cooking on the quality of these diets was also studied on the following six diets commonly used in the area :

(1) Gari, afan and smoked fish,

(2) Anyan okpan and abak soup,

(3) Cassava foofoo and edikan ikon,

(4) Pounded yam and efia efere,

(5) Anyan Ekwuru, and,

(6) Iwuk ukom, with smoked fish and palm oil.

The various parameters used in the assessment of the nutritional quality of these diets were :

(a) The recipes and methods of preparation of the various diets,

(b) The amino acid composition of the raw - mixed and cooked diets,

(c) The losses of the nutrients during the traditional preparation of the foods,

(d) The effects of feeding the raw - mixed and cooked diets to rats,

(e) The total serum cholesterol levels in the experimental animals,

(f) The total serum protein levels and the serum protein patterns of the experimental animals.

Though the results of all these assessments have been presented and explained at the appropriate places in this thesis, the following is a general discussion on all of them.

(a) Sampling and preparation of the diets : Tables 1a - 6 show the results of the survey conducted among the peasant families on the composition and the proportions of the foodstuffs used in the preparation of the peasant diets studied. Several important conclusions are apparent from these tables.

(1) The foodstuffs needed in the formulation of these peasant dietaries are few in number and almost without variety. It is possible that the monotony of these foods may impede the satisfaction of the mineral and vitamin requirements, as revealed in my later investigations, which are more likely to be satisfied in a more varied diet.

(2) The soups used by the peasants have little variety, consisting almost invariably of pepper, palm oil, fish and salt.

(3) The chief sources of protein in the diets are relatively few in number. Smoked fish seems to be the only source of protein of significance. Minor contributions are also, however, made by crayfish, snails and periwinkle;but,as shown in the survey tables, the relative proportions of these items in the diets are too small.

(4) Though variation occurred with regards to the proportions of the condiments, required by each woman for the preparation of the meal, this was not large enough to reflect anything else apart from the slight variations in individual tastes. Since the families sampled were all of the low income group, it is possible that the generally low proportions of the more nutritious but costly food items such as fish, crayfish, and the complete absence of meat of any kind from the diets, were due to economic status and not to taste. People in the high income group from the area, including the author, have been known to consume such foods as milk, meat, eggs and a wide variety of imported foods of high nutritional value.

(5) There is a very high proportion of the starchy staple, about 75% dry weight, in all the diets. This also appears to relate to the economic status and the purchasing power of these peasant families. With their limiting food and money resources, it is a

common practice within the rural homes, for house-wives, faced with the problem of having to feed a large number of people (characteristic of the peasant families) from the same cooking pot, to provide diets based on cheap, starchy and low quality staple foodstuffs such as cocoyam, cassava and plantain, poorly supplemented with low quality or inadequate animal products (Idusogie, 1971).

The results of the survey, however, supported the reports of McFie (1967) and the Inter-Departmental Committee on Nutrition for National Development (ICNND) (1967) that rural Nigerians depend only on traditional diets prepared from what is grown locally with little or no supplementation. The dietary supplements, when these are used, completely depend on the income and the purchasing power of the family. This therefore limits the use of the more costly but nutritious foods to the higher wage - earners (Idusogie, 1971). Unconsciously, however, this section of the population raises the level of the daily protein intake by consuming large quantities of such unconventional items of foods as bush meat, crabs, insects, snakes and similar articles when these are not tabooed (Nicol, 1959 a,b; Idusogie, 1971).

In general, the average African peasant family is very poor and has no money to buy sufficient food for adequate nutrition and also pay for other family necessities. Nutritional and dietary

surveys in parts of Nigeria by Dema (1967) show that poverty is one of the major factors contributing to the existence of Protein-calorie deficiency in the country, especially in the 'hungry' season when the foods are scarce and prices are high.

(b) The amino acid composition of the raw-mixed and cooked diets : Table 11 gives the amino acid composition of the raw-mixed and cooked diets, expressed as mg/100 gm dry diet, obtained by the ion-exchange method of Spackmann et al. (1958). Cystine and tryptophane were obtained by other methods. Some losses were observed in the individual amino acids and the degree varied from diet to diet. The losses incurred might be due to the slight losses by leaching of the soluble proteins observed in the cooked diets. As described on pages 87 - 92, the preparation of the South - Eastern Nigerian traditional diets often involves a number of processes which are likely to affect the constituent amino acids of the dietary proteins. The methods of the preparation of the materials prior to cooking, the presence of metallic ions and the reaction of the cooking medium are also highly complex processes which can exert some adverse influence on the amino acid patterns of the cooked diets. Miller et al. (1965 a, b) reported that the presence of moisture and the changes in the pH of the material during cooking can influence the availability of the sulphur - containing amino acids. The same authors found considerable losses of Cystine when heated in the presence of glucose. Such reactions are likely in

the dietary preparation of foods during which the carbohydrate and protein materials come in contact.

The low values of the total amino acids in the diets might be due to the low crude protein content of the dishes and also due to the slight hydrolytic losses of some of the labile amino acids during the preparation of the materials for analysis. Such hydrolytic losses have been reported by Smith et al. (1954), Rees (1946) and Schram et al. (1954). Hughes (1958) reported hydrolytic losses of 9.4% for serine; for threonine, 2.5%, for arginine, 1.4% and for methionine 8.8% after 24 hours hydrolysis of food mixtures. This discrepancy on the results of acid - hydrolysed materials not withstanding, a more serious objection has been raised by workers like Bender (1966) who contend that these results are different in animals where hydrolysis of the protein materials is by enzymes. Under these circumstances, these workers argued validly that the amino acids may not be so available as in acid hydrolysis.

In all cases, higher values were obtained for glutamic and and aspartic acids. This might be due to the additional quantities derived from asparagine and glutamine, which would yield ammonia and free acids during acid hydrolysis (Hughes, 1958).

It is evident from these results that almost all the essential amino acids are present in inadequate quantities in the diets. Cooking losses of the amino acids, and especially of the essential amino acids are therefore bound to be nutritionally

significant.

(c) Losses of the nutrients during the traditional preparation and cooking of the diets. The figures for the nutrient contents of the raw-mixed and cooked diets are given in Table 8. The losses of the proteins and crude fats had been explained in the appropriate sections and are not likely to be of nutritional significance. The slight increases observed in the total ash in diets D2, D3, D4 and D5 might be due to absorption from the cooking medium. This result agreed in principle with the reports of Bender (1966), Horner (1936), McCance et al. (1936) and Stewart (1946). The apparent increases observed in the percentages of total carbohydrates in the cooked diets might be due to the losses in cooking reported for the other nutrients in the diets.

Ekpan Nkukwo (D5) seems to be exceptionally high in the total ash content, this being about 6% dry weight of this diet. This might be due to the high proportion of cocoyam in this diet. This foodstuff has been reported to be rich in total ash by Anonymous (1957) and Oyenuga (1968). Similarly, the low ash content (about 3%) observed in diet D3 could be due to the proportion of cassava in the diet. Cassava is low in ash content and very poor in calcium (Oyenuga, 1968; Lathar, 1965).

The losses of minerals (Table 9) observed in the traditional preparation and cooking of the various peasant diets have been

explained on pages 135 - 136. Similar losses of iron and calcium in domestic cooking have been reported by various workers. Saito (1969a,b) reported losses of 17% and 32% iron respectively while cooking daily foods by the Japanese and the European methods. The losses of calcium reported by the same author in the same foods were 5% and 9% respectively using the same two cooking methods. McCance et al. (1938) reported 16% loss of iron in cooked potatoes whilst Stewart (1946) observed that in cooking 150gm potatoes the losses were 1.0 mg of iron, 0.27 mg of calcium and 4.5 mg of Phosphorus. Pfund (1942) observed 10% loss of iron on boiling Potatoes. Diets D1 and D5 were rich in calcium, probably as a result of the snails and periwinkle (Wood et al., 1967) in diet D1, and cocoyam (Oyenuga, 1968) in diet D5. Losses of phosphorus were highest in diets D1 and D4, being about 16.2% and 12.6% respectively. Noble and Halliday (1937) reported losses between 13% and 40% of phosphorus during the cooking of some vegetables in an open pan. Also, in keeping with the household practice, the cooking utensils were not rinsed when the food was removed. It is possible too that some quantities of these minerals might escape estimation in this way.

The vitamin contents of the various diets are shown in Table 10. The vitamins were the most affected of the nutrients by the traditional methods of cooking used in these investigations. The losses incurred might be due to some of these vitamins being thermolabile (Rose - Fidler, 1939) and others being both thermolabile

and water-soluble (Bondor, 1966; Loo, 1958; [illegible], [illegible], — 1942).

Losses of vitamin A and β - carotene varied between 11% and 43% during the cooking of the diets. Similar losses of the vitamin and the pro-vitamin have been reported by De (1936), Lanning and Van Veen (1937), Maqsood [illegible] (1963), DellaMonica and McDowell (1965) and Falconer [illegible] (1964). The loss might be due to the high temperatures reached and the presence of oxygen (Harris and Von Loesecke, 1960), and to the presence of traces of copper and iron (Bondor, 1966) in the diets. The highest loss of vitamin A and β - carotene was in diet D2 and the least in diet D1. This could be the result of the interplay of the factors mentioned previously which could cause isomerization of the vitamin and its subsequent unavailability for estimation.

The loss of vitamin B_1 varied in the diets between 29% and 48%. This vitamin incurred significant losses in all the diets. The losses could in part be due to leaching into the washing water during the preparation of the vegetables prior to cooking. These results agreed in principle with the results reported by Roscoe (1930), Munsell and Kifer (1932) and Langley [illegible] (1933). Actual destruction of the vitamin might occur during cooking as a result of the possible change in the pH of the cooking medium coupled with the high temperature reached. Losses due to these

factors were reported for vitamin B_1 by Newton (1931), Roy & Rao (1963) and Harris and Von Loesecke (1960).

Vitamin B_2 was more stable to the cooking processes than vitamin B_1. The losses observed varied between 1.2% and 30%. The highest loss incurred was in diet D4. These losses might be due to leaching and open - pan cooking. Losses, by this means, of vitamin B_2 have been reported by Bone - Pixson (1938) and Levine and Remington (1937). Malik (1967) reported losses of up to 50% for riboflavine in his investigations.

Cooking losses of niacin and pyridoxine varied between 5.9% and 30.3% and 3.4 and 48.7% respectively. Though niacin is reported to be heat-stable (Bender, 1966), pyridoxine is thermolabile (Woodring and Storvick, 1960). It is possible that niacin might be lost by leaching during the preparation of the foodstuffs, while losses of pyridoxine could be due to both leaching and the high temperature of the cooking medium. This could therefore be responsible for the higher losses of pyridoxine than niacin. These results agreed in principle with the results obtained by Bender (1966), Woodring and Storvick (1960), Russel et al. (1943) and Davies et al. (1959).

Losses of vitamin C varied between 40% and 100%. These losses might be due to leaching, heat and oxygen (Bender, 1966; Bone - Pixson, 1938). Losses might also have been occasioned by the presence of traces of copper and other metallic ions (Szent - Gyorgyi,

1928; Kellie and Zilva, 1935) in the diets. The highest loss of 100% of the vitamins in diet D2 might as well have been caused by the processing method and the longer period required for cooking this diet as described on page 90. As a result of these, the vitamins and the other water soluble nutrients in the vegetables and ooooyum must have been lost to the cooking water which was discarded. The results agreed in principle with the reported losses of the vitamin as a result of leaching by several workers including Treasler et al., (1936) and Fenton (1937). Platt et al. (1963) reported a loss of 45%- 60% vitamin in pealed soaked potatoes used in hospital diets. McHenry and Graham (1935), Fenton et al. (1936). Fixsen and Roscoe (1938), Eddy and Kohman (1924) and Leapitt et al. (1943) also reported very considerable losses of vitamin C by leaching in cooking foodstuffs.

Losses might also be due to the traditional method of preparing the vegetables. This, in all cases, involved preparation of the vegetables sometime before cooking began by picking, washing, cutting into very small pieces, and revashing often with squeezing or pounding in a mortar to further reduce the sizes of the pieces. These processes often expose the bruised surfaces of the vegetables to oxygen and the enzyme, ascorbic acid oxidase, so, it would not be surprising if much of the vitamin were lost even before cooking began. Losses of vitamin C due to the oxidizing

essays were reported by Gould et al. (1936), Hunt (1936) and Hess - Pixson (1936).

From the foregoing, the following conclusions and comments seem justified :

(1) The cooking losses of the total crude proteins were negligible; but, the level of protein in the diets was regretably low.

(2) There was considerable loss of total fats in the cooking processes.

(3) The total fat content of the peasant diets even though palm oil is plentiful is less than [redacted] the amount found in good European diets. However, it is likely that no adverse effects on health due to low consumption of essential fatty acids would be noticed since there is often a high supplementary consumption of palm oil with roasted yam, plantain or cocoyam outside the normal meal times of the peasants.

(4) There is a significantly low quantity of the B - vitamins in all diets and this situation was further worsened by the severe traditional processing and cooking methods which induced such losses.

(5) Cooking hardly affected the minerals. The calcium content of most of these diets is high, but it is doubtful if the iron present in some of these diets would allow for the heavy intestinal parasitic infestations and endemic malaria encountered in these peasant populations.

(6) Ascorbic acid is seriously and significantly destroyed in the diets during cooking.

(7) Vitamin A (including beta - carotene) content of the various diets was high despite the cooking losses.

(d) The effects of feeding the test diets to rats: It has been shown by various workers that the nutritive value of a diet prepared for human consumption is influenced by a number of inherent factors including the way the food is prepared, the quantity and quality of the protein, the amount of food consumed (Miller, 1970), the level of fats and carbohydrates (because of their protein-sparing action) (Platt and Miller, 1958). If such a diet is assayed with rats, its nutritive value would depend on whether the rats were normal or infected with malaria (Dema et al., 1959) or hookworm (Orraca - Tottah and Platt, 1967).

Tables 13 - 20 show the responses of the male and the littermate female rats to the various diets. The nitrogen intake (Table 13) between the sexes did not vary significantly except in diet D3 (Cassava foofoo with odikan ikon) on which the females consumed more than the males ($P < 0.01$). Slightly higher nitrogen intake values were also observed for the females on diets D1 (Cari with afan and smoked fish) and D4 (Pounded yam with plain soup). It is likely that these diets were more palatable to this sex than the other diets. The males, on the other hand, consumed more of

diets D2 (Anyan akpan with abak soup), D5 (Akpan Okoho) and D6(Iwuk ukon) than the females. The influence of taste in limiting the acceptance of food has been demonstrated in rats by Light (1955). Light and Tornabon (1953a, b) have shown that sex is an important factor in regulating food intake in male and female weanling rats.

The data on PER (Table 14) indicated no significant difference between the sexes except in diet D5. The male rats on diet D5 showed significantly higher PER than the females ($P < 0.01$). Slightly higher PER values were also observed in the males on the other diets except diet D3. These observations might be due to both the higher nitrogen consumption by the male rats on these diets and to the fact that animals of this sex utilized these diets better than the females as shown by the higher true digestibility (Table 18) and the net protein utilisation figures (Table 19). The female rats on diet D3 showed both higher weight gains ($P < 0.05$) (Table 20) and higher PER than the males. This could be attributed to both the higher nitrogen intake by the female rats and the lower protein content of this diet than in others. Morrison and Campbell (1960 a) presented evidence to show that female rats tend to give maximal PER at lower dietary protein levels than the males. This would suggest a possible sex difference in protein requirements. Jones (1951) obtained evidence indicating that

female rats had lower protein requirements than males, and Shelton et al. (1951) presented data to show that lysine requirement was higher in male weanling pigs than in female. Weight gains by the two sexes related highly to nitrogen intake (Table 13). These results supported in principle those obtained by Hegsted and Worcester (1947), Thorwood and Weldon (1953) and Ijaa (1959a,b).

The coefficient of true digestibility of the dietary nitrogen was always higher in the male rats than in the females. It is possible from these observations to conclude that protein digestibility may not only depend on the chemical nature of the protein component of the diet but also on other factors. For instance, Mitchell et al. (1950) suggested that the other components of the diet may modify the action of the digestible enzymes, by interposing indigestible barriers between protease and substrate, by disturbing the proper relation between the secretory and motor activities of the gastro-intestinal tract, or by other means. Any of these factors may be influenced by the sex of the animal. The significantly higher T.D. in male rats than in the females on some of the diets might be the result of the extent to which these factors influence each other in the diets.

The biological values of the diets (Table 15) did not differ significantly between the sexes except in diet D5 on which the males showed higher biological value ($P < 0.001$). This might be

due to the significantly higher ($P < 0.001$) NPU (Table 17) in the male rats. The female rats on diets D2 and D4 showed higher B.V. than the males. This might be due to the lower true digestibility of these diets in this sex. This could result in an economy of utilization of the less available nitrogen from these diets by the female rats. The net protein utilization (Table 17) was higher in all cases in the male rats. This might be explained by the high true digestibility of the dietary nitrogen in this sex. More nutrients might be made available to this sex in this way. This result supports the proposition of Henry and Kon (1958) that digestibility and amino acid pattern determine the value of food protein to the animal.

The male rats on all the diets showed higher NDpCals % values (Table 16) than the females on all the diets. These differences were significant ($P < 0.01$; $P < 0.001$, respectively) for the sexes on diets D3 and D5. The NPR values of the rats are shown in Table 19. The male rats showed higher NPR values than the females on all the diets. For diets D5 and D6 these differences were significant at 5% and 1% levels respectively. The higher NPR values for the males could indicate a possible higher maintenance requirement by this sex. This would support the previous findings of Morrison and Campbell (1960a) which suggested some sex differences in requirements of nutrients by rats.

The results for PER and weight gains for the male and female rats on diet D6 (Iwuk ukom, smoked fish and palm oil) showed that the animals lost weight during this period. The data for the total amino acid contents of this diet did not reveal such differences from the other diets as shown by the feeding experiment. This discrepancy can be explained by assuming the presence in the diet of non - protein growth inhibitors (Lopkovsky, 1955). The NPU data for this diet indicated positive values despite the weight loss. This might mean that the animals still retained some of the nitrogen ingested during the experiment, so that the loss in body weight could be explained only in terms of the loss of body fat and body water. These observations agreed in principle with the results of Morrison et al. (1962).

From these results, it could be seen that in almost all the cases higher values for all the variables and greater variations between the diets were obtained with the male rats. This would therefore indicate a greater sensitivity and precision with this sex in dietary evaluation than with the females. Significant differences between the sexes were always observed in the animals on diet D5 (Ekpan Nkukwo) despite a non - significant difference in their nitrogen consumption data.

Table 28 shows the nitrogen intake of the rats on raw-mixed and cooked diets. The rats on raw diets D2 (Anyan okpon with abak soup), D3 (Cassava foofoo with odikan ikon), D5 (Ekpan Nkukwo) and

D6 (Iwuk ukax with smoked fish and Palm oil) consumed more nitrogen (P 0.001 for each of diets D2 and D5) than their littermates on the cooked diets. Slightly higher nitrogen intake values were obtained for the rats on cooked diets D1 (Gari, afee with smoked fish) and D4 (Pounded yam with plain soup). The comparison of the nitrogen intake of the raw-dried and cooked diets against that of the animals on the control diet is shown in Table 29. The rats on raw and cooked Gari, afan with smoked fish (D1) consumed more nitrogen than the corresponding littermates on control diet ($P < 0.001$). On diets D2, D3, D4 and D6 the dietary nitrogen intake was often less than the control. These differences in the dietary nitrogen intake could be attributed to the varying degrees of palatability of these forms of diets to the rats (Light, 1955). The weight gains of the animals on the raw and cooked diets are shown in Table 27. It is possible from these results and especially in diets D3 and D6 that nitrogen intake was not the only factor controlling weight gains of the animals. The weight gains varied with the type of diet. This was further supported by greater body weight gains of the control animal (Table 30) which were in all cases significantly higher than the gains in body weight of the test animals except in diet D1. These results supported the previous findings of (Kik (1938), Hamilton (1939), Mitchell and Carman (1926), Fraps and Carlyle (1939).

In Table 25 the PER values of the animals on the raw and cooked diets showed that the cooked diets were better utilised in all cases. This was shown by the higher PER values for the rats on the cooked diets except in the case of diet D1 on which the animals fed the raw diet faired better. Since similar slight increases were observed in the NPR (Table 26), SV (Table 28), NPU (Table 22) and NDpCals % (Table 21), it would appear that the effect of traditional cooking on this diet was slightly deleterious. Significantly higher PER values ($P < 0.001$) were observed in all cases for the control animals than for the test.

As shown in Table 31 slightly different PER values were obtained for the control animals from experiment to experiment. The PER obtained in the six separate experiments were 2.81, 2.74, 2.85, 2.98, 2.74 and 2.80. This variation in the PER values could be attributed to the varying responses of the different batches of rats of the same breed to the same diet. Similar observations were made by Henry and Kon (1957) in their experiments. These workers then suggested that a standard protein should always be examined together with the proteins to be tested and that comparisons should always be made with reference to the particular standard protein. This procedure would have much to commend it, and implied that the values determined possess relative rather than an absolute significance (Mitchell et al., 1936). Chapman et al. (1959) obtained PER values of 2.41, 2.53, 2.55, 2.50 and 2.58 for casein fed at 10% protein level in five separate tests. Bender (1956) found

the PER values of dried skimmed milk to be 2.47, 2.68, 2.77, 1.70, 1.29 and 2.76 under his experimental conditions. The slightly higher PER values obtained in these investigations with the control animals might be explained from the observations of Keane et al. (1962, 1963) that higher PER values are obtained when the diets are given moistened than when given dry. Though the latter appears a general practice, in our laboratory, the control diets were mixed with water to a thick paste to minimize scattering. With this method of feeding, Henry (1965) obtained even higher PER values of 3.63, 3.52, 3.58, 3.54 in four separate determinations. The difference between my values and here might be due to the percentage of moisture in the diet (Keane et al, 1962, 1963) and to the strain difference of the rats (Janson, 1962).

In Table 26, except in diet D1, significantly higher NPR values were often obtained for the animals on the cooked diets than for their littermates on raw - mixed diets. This result could be attributed to higher maintenance requirement of the faster growing rats on the cooked diets as indicated by their higher PER values (Table 25). Similar significantly higher values ($P < 0.001$) were obtained for the control rats when compared with their littermates on raw or cooked diets. Hegsted and Worcester (1947) observed that the maintenance requirements of the faster growing rats tends to be higher because of the increase in body size.

In Table 24, significantly higher coefficient of true digestibility ($P < 0.001$) of the dietary protein was observed in the cooked than the raw diets. For instance, the true digestibility increased from 78.6% to 86.3% in diet D1 as a result of cooking. In diets D2, D3, D4, D5 and D6, the increases were 11.0, 11.3, 6.6, 8.1 and 9.1%, respectively. The lower coefficient of true digestibility of the protein in the raw diet could be the result of an unavailable protein fraction in the raw diet, or due to the conversion of a part of dietary and endogenous protein in the intestine into an unavailable protein fraction by the action of the raw diet. Both possibilities could operate in reducing the true digestibility of the raw diet. Cooking might therefore have increased the availability of the entire protein fraction and the other nutrients in the diet. Comparison of the true digestibility of the raw diets with the controls indicated better digestibility of the control diet in all cases (Table 33). With the improvement of the coefficient of true digestibility as a result of cooking, there were smaller differences between the T.D. values of the cooked and the control diets in the rats. The coefficient of true digestibility was high for all the cooked diets, being between 78% - 90%. This could be attributed to the quality of the dietary protein. As shown in Tables 1(b) - 6(b). Fish was the major source of protein in all the diets. This foodstuff has been shown by several workers (Bender and Heisoldon,

1957; McLaughlan and Morrison, 1960; Beveridge, 1947; Sure, 1957a,b; Morrison and Campbell, 1960b) to have high true digestibility and other excellent nutritive qualities as a source of dietary protein. The nearly constant T.D. for all the cooked diets despite the varying levels of the crude protein (Table 8) in the diets could be explained by the view expressed by Henry and Kon (1957) that for good quality proteins, the changes in the dietary levels do not always affect the true digestibility, and that such changes when they do occur are usually small. Ijaz (1959b) showed that true digestibility was constant for one and the same protein irrespective of dietary level, in agreement with the findings of Allison et al. (1946) and Forbes et al. (1958).

As shown in Table 23 cooking considerably improved the biological values of diets D2, D4, D5 and D6. The slight decrease in biological value of diet D1 has already been explained on pages 202-203. In case of diet D3, the lower digestibility of the raw diet coupled with the very low protein level of the diet (Table 8) could induce a considerable economy in the utilization of the less available dietary nitrogen which might be reflected in the higher biological value of this diet for the animals. Comparison of the biological value of the test diets with the control (Table 34) showed in all cases higher values for the casein diet. This could be the result of better utilisation of this diet than

any of the test diets. It is possible that this diet has a more proper nutrient balance in addition to the higher proportions of the essential amino acids (Table 11) to meet the requirements of the growing rats.

Further advantage due to cooking has been shown in the significantly higher NPU of the cooked diets in the rats ($P < 0.001$ for D2, D4, D5 and D6, and $P < 0.02$ for diet D3). This might be due to the higher true digestibility of the cooked diets which might have compensated for the losses of nutrients incurred during the traditional preparation and cooking of the diets. In all cases, however, a significantly higher NPU (Table 35) was obtained when the littermate rats were fed on the casein diet. The lower NPU of the peasant diets could be the result of the low and improper balance in the mineral and vitamin contents of these diets. Morrison et al. (1962) observed lower values of NPU in rats fed on casein diets with low mineral and vitamin contents. The values obtained for the NPU in rats on the cooked diets were in close agreement with the figures, obtained by Miller and Dean (1958) with the peasant diets assessed by Nicol (1949, 1952, 1956) during a nutritional survey in the drier northern parts of Nigeria.

The NDpCal% of values (Table 21) in all cases except in diet D1 showed increases as a result of cooking. The NDpCal % values obtained for the rats on cooked diets were in close

agreement with the previous results of Miller and Dema (1958). The generally low NDpCals % of these diets could be due to the low PCals % (Table 12) and the low NPU values previously explained. Since the generally poor nutritional status and the concomitant poor health of the peasant families are always attributed to the low PCals % and NDpCals % of their diets together with the inadequate consumption of these diets (Idusogie, 1970; Jelliff, 1955; Ekpo, 1970; Dumo, 1965 and many other workers), the NDpCals % of these diets as assayed with healthy growing rats were compared with the recommended allowances of Miller (1963) and FAO (1965) as shown in Table 39. From the table, the following conclusions could be drawn that:

(1) Gari, afan with smoked fish and ükpan ukukwo would under the conditions of adequate caloric intake (Miller and Payne, 1961) satisfy the needs of the child, the adult and possibly the mixed household.

(2) Anyan ukpan and Pounded yam diets would only satisfy a healthy adult when consumed in adequate quantities.

(3) Cassava foofoo and Ivwk ukom are too poor as routine foods for any member of the family.

(4) The folly of trying any of the diets on infants as a substitute for breast milk is seen in the NDpCals % figure for breast milk compared with the peasant diets studied.

TABLE 39

Comparison of the DpCals % of the various raw mixed and cooked diets as determined by Rat assay with the Recommended Scale of Allowances of Miller (1963), F. A. O. (1965)

SUBJECT	AGE	Recommended Allowances of NDpCals % (Miller, 1963; F.A.O., 1965)	DIETS													
			Gari, afan with smoked fish		Anyan akpan with abak soup		Cassava foo-foo with edikan ikon		Pounded yam with plain soup		Ekpan Nkukwo		Iwuk Ukom with smoked-fish		CONTROL DIET	Human Breast milk (Platt & Miller, 1961)
			RAW	COOKED	RAW	COOKED	RAW	COOKED	RAW	COOKED	RAW	COOKED	RAW	COOKED		
			7.91	7.72	4.64	5.17	3.73	3.78	4.46	5.39	5.25	7.30	3.00	3.29	7.31*	8.7
INFANTS	0 - 3 months	8.3														
	3 - 9 "	8.0														
Toddler	9 months-3 years	7.8														
Child	3 - 9 years	5.9														
Adolescent	-	8.4														
Adult	-	4.8														
Pregnancy (2nd & 3rd Trimester)	-	7.0														
Lactating mother	-	9.5														
Mixed Household Community Group	-	6.5														

* Mean of six separate estimations

(5) The inadequacy of any of the common diets as routine foods for the more vulnerable groups (infants, toddlers, adolescents, the pregnant and the lactating women) is also shown in the table since the requirements of this group is very great.

(f) Total Serum Cholesterol levels in the animals

The determination of total cholesterol in serum has assumed an added significance in recent years because of the possible implication of cholesterol in the etiology of arteriosclerosis. In these investigations, serum cholesterol levels were determined in male and female rats fed for 10 days on raw - mixed, traditionally cooked peasant diets of South Eastern Nigeria, and on casein diet for comparison purposes (Table 38).

As shown in the table, the rats on the casein diet consistently exhibited higher cholesterol levels than either the littermates on raw-mixed or traditionally cooked diets. Female rats on all the diets exhibited slightly higher total serum cholesterol levels than the corresponding males. On both forms of the test diets the total cholesterol levels were within the range of 80 - 120 mg/100 ml serum in both sexes. The range of 120 - 140 mg/100 ml serum was obtained for both sexes on the control diet.

Several dietary components have been shown to affect the concentration of serum cholesterol, e.g. the type and amount of

carbohydrate and fat (Fortson et al., 1955; Hegsted et al., 1957), the amount of protein and magnesium (Meyer et al., 1956). In the adult rat the concentration of serum cholesterol appears to determine the degree of atherosclerosis produced (Hegsted et al., 1957) although other factors may be concerned in the young rat (Vitale et al., 1957). The range of 120 - 140 mg/100 ml obtained for the control animals is in close agreement with the results obtained by Meyer et al. (1957) in young rats fed on 10% casein. Cuthbertson et al. (1959) obtained mean values of 117 mg and 130 mg/100 ml serum respectively for male and female rats fed for 14 days after weaning on stock diet. The higher serum cholesterol levels in the control rats on a diet with butter as the source of fat support the findings of Aftergood et al. (1956) who observed higher values for lard than for cotton seed oil as the source of fat in the diet for rats. Jones and Huffman (1956) observed that long - term feeding of high levels of dietary casein tend to increase serum cholesterol levels in the normal rat. Dragdon et al. (1957) observed that the addition of butter to the diet of swine caused a significant elevation in total serum cholesterol but corn oil was without effect. In all the test diets, the source of fat was palm oil, a vegetable oil. Except for the raw form of diet D5, the levels of total fats in the test diets have been below 15% and much lower in the cooked

diets. Analysis of palm oil by Oyenuga (1967) shows that it contains more than 50% unsaturated fatty acid (oleic and linoleic). It is possible that the consistently low levels of the serum cholesterol in the test animals are the results of the low dietary levels of fats, and the high unsaturation of the dietary fats. Aftergood et al. (1957) observed that the substitution of unsaturated fats for saturated fats in the diets tends to lower the serum cholesterol in experimental animals. Also, the analyses of the diets showed that they were rich in vitamin A (including beta - carotene). Ward (1960) observed the hypocholesterolemic effects of high dietary level of vitamin A in chicks and Weitzel et al. (1956) had reported that high dietary levels of Vitamin A reduced the atherosatous lesions in old hens. It is therefore possible that all these dietary constituents together with the high unsaturation of the dietary fats act synergistically to depress the serum cholesterol levels in the experimental animals. The mild variations within the groups of animals and between the raw and cooked diets might occur as a result of the different levels of fats and carbohydrates (Portman et al. 1955; Nagstad et al., 1957), and proteins (Meyer et al., 1956) in these diets. Nagstad et al. (1960) observed that by varying the amount of dietary fat, a complicated situation is created since such fat does not affect the serum cholesterol level proportionately

at different dietary levels. Hath et al. (1959) found little effect on total serum cholesterol level when the level of fat was varied.

In these experiments the females rats in all cases exhibited higher but statistically non-significant values of serum cholesterol than the males. A similar result was obtained by Cuthbertson et al. (1959) who observed no significant differences in the serum cholesterol levels in young male and female rats given stock diets for 14 days from weaning. Though significant sex differences in the serum cholesterol values sometimes occur at this age but they were unusual (Cuthbertson et al., 1959). The absence of significant differences between the sexes as observed in adult male and female rats by Cuthbertson et al. (1959) and Aftergood et al. (1956), apart from age effects, might as well be attributed to the short feeding period in these experiments. This was 10 days compared with 24 weeks used by Aftergood et al. (1956). Cuthbertson et al. (1959) explained their observations for adult male and female rats to be a result of sex hormones. For young rats some other factor regulating the serum cholesterol would seem necessary to explain the different rates of increase in the total serum cholesterol in the two sexes.

(g) Total Serum Proteins and Serum protein patterns in the experimental rats. The total serum protein levels were higher in the control animals than in the animals of either sex fed on the

test diets (Table 37). This might indicate a more efficient incorporation of the proteins in the control diet into the body proteins of the animals. This would be supported by the higher NPU of the control diets. The same explanation could be offered for the slightly higher values in the males than in the females. No differences were observed in the serum protein bands regardless of sex or diet. The absence of significant differences in the total serum protein values and in the protein bands between forms of diets and between diets could be due to the experimental period which might be too short to produce any such effects. It might also be that the traditional cooking of the diets had no effect, adverse or otherwise, on the serum proteins in the rats. The results support the previous observations of Vera Cabak et al. (1963) and Malik (1967).

General Summary

Nutritional studies on six commonly used South Eastern Nigerian peasant diets showed that Gari, afan and smoked fish, and Ukpan Nkukwo were the best diets. These were followed by Pounded yam and afia efere (plain soup), Ukpan okpan with abak soup, and Cassava foofoo with edikan ikon in that order. Ikpa ukor, smoked fish with palm oil (Diet D6) was the worst diet, in that the rats on this diet lost weight. In all cases, however, all the other diets maintained suppressed growth rates in the rats. Cassava foofoo with edikan ikon soup was the poorest in protein content. All the diets were deficient in the B - vitamins.

The following recommendations can therefore be made :

(1) The existing recipe can be improved by increasing the proportions of fish, periwinkle, snails and crayfish in the diets.

(2) More vegetables and fruits be consumed to make up for the losses of vitamin C incurred in the preparation and cooking of the diets.

(3) The existing traditional methods of handling, preparation and cooking can be improved in order to prevent the destruction of the more labile nutrients.

(4) The peasants can be encouraged to supplement their protein intake with materials from cheap sources such as bush meat, insects, snakes, crabs and similar

unconventional foods.

(5) They should also consume some cereal foods so as to increase the intake of both proteins and the B - vitamins which are conspicuously low in their diets based only on starchy roots.

CONTRIBUTIONS TO KNOWLEDGE

The investigations reported in this thesis have thrown new light into the understanding of the problems of malnutrition in peasant Nigerian homes. The major contributions to knowledge include :

(1) The various recipes and proportions of the foodstuffs for the preparation of six South Eastern peasant diets have been elucidated.

(2) The levels of the various nutrients in the raw - mixed and cooked peasant diets have been determined and the losses due to the traditional preparation and cooking procedures were assessed.

(3) The true digestibility, biological value, protein efficiency ratio, net protein ratio, net protein utilization, weight gains and losses and the net dietary protein calories percent have been determined using male and female rats fed on the traditionally cooked diets.

(4) The nutritive adequacy of the peasant Nigerian diets has been elucidated.

(5) The metabolizable energy and the protein calories percent values of the traditional South - Eastern Nigerian diets have been determined.

(6) The amino acid patterns of the raw - mixed and cooked peasant diets have been determined.

(7) The serum protein levels and the serum protein patterns of the rats fed on six traditional South - Eastern Nigerian diets have been determined.

(8) The total serum cholesterol levels in the rats fed on the raw - mixed and traditionally cooked diets have also been elucidated.

ACKNOWLEDGEMENTS

I am particularly grateful to my Head of Department and Supervisor, Professor Olumbe Bassir, for the keen interest and invaluable guidance throughout the course of these investigations.

I also thank my friends and colleagues in the Department and outside for their encouragement, and the University of Ibadan, Ibadan, Nigeria for the Scholarship awarded for the course.

The author acknowledges the significant and invaluable services of the entire technical staff of the Department of Biochemistry. Messrs. J. Ogunsuoi and H. Chimbo deserve special thanks for the management of the animals and the production of the photographs.

Lastly, my thanks go to Mrs. E.J. Ogunsuoi and others for their help in designing the recipes for the diets studied and Messrs. E.N. Ekoriamma and A.O. Ogwo for typing this thesis.

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