EVALUATION OF THREE LOW COST RICE BASED VEGETABLE FOOD MIXTURES CONTAINING DIFFERENT QUANTITIES OF PROTEIN FOODS, TUBERS AND GREEN LEAFY VEGETABLES ON ALBINO RATS

By

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TABLE OF CONTENTS

			Page
	LIST OF TABLES	....	
	LIST OF FIGURES	....	
	LIST OF APPENDICES	....	
I	INTRODUCTION	....	1
II	REVIEW OF LITERATURE	....	4
	A. Nutritional deficiencies in poor Indian diets	....	4
	B. Role of indigenous low cost foods in combating malnutrtion	....	6
	C. Biological evaluation of Protein quality	....	10
III	EXPERIMENTAL PROCEDURE	....	18
	A. Selection of the diets	....	18
	B. Preparation and analysis of diets	....	20
	C. Evaluation of Protein quality of the diets by	....	22
	1. Protein Efficiency Ratio	....	22
	2. Hepatic Nitrogen and fat contents	....	23
	3. Nitrogen balance	....	24
	4. Determination of NPU	....	27
IV	RESULTS AND DISCUSSION	....	29
	A. Protein Efficiency Ratio	....	29
	1. Food and protein intake	....	29
	2. Body weight gains	....	31
	3. Protein Efficiency Ratio	....	34

			Page
	B.	Hepatic nitrogen and fat contents	36
		1. Hepatic nitrogen	36
		2. Hepatic fat	38
	C.	Nitrogen Balance	40
		1. Nitrogen retention	40
		2. Digestibility Coefficient and Biological Value	42
	D.	Net Protein Utilisation	45
V		SUMMARY AND CONCLUSION	47
VI		BIBLIOGRAPHY	50
VII		APPENDICES	60

LIST OF TABLES

TABLES		Page.
I	PERCENTAGE COMPOSITION OF THE BASAL DIET . . .	19
II	COMPOSITION OF THE EXPERIMENTAL DIETS . . .	20
III	PROTEIN CONTENT OF THE SELECTED DIETS . . .	21
IV	THE MEAN TOTAL FOOD AND PROTEIN INTAKE OF DIFFERENT DIETS . . .	30
V	MEAN INCREASE IN WEIGHT OF RATS FED DIFFERENT DIETS . . .	32
VI	PER OF BASAL AND THREE EXPERIMENTAL DIETS . . .	35
VII	MEAN INCREASE IN HEPATIC NITROGEN CONTENT OF RATS . . .	37
VIII	MEAN INCREASE IN HEPATIC FAT CONTENT OF RATS . . .	39
IX	MEAN DAILY NITROGEN RETENTION IN RATS FED DIFFERENT DIETS . . .	41
X	THE BIOLOGICAL VALUE AND DIGESTIBILITY COEFFICIENT OF THE TEST DIETS . . .	43
XI	NPU AND NDP CALORIES PERCENTAGE OF THE DIETS . . .	45

LIST OF FIGURES

Figure No.			Page
1.	MEAN DAILY NITROGEN RETENTION IN RATS FED DIFFERENT DIETS	...	47 (a)

LIST OF APPENDICES

Number			Page
I	A.	COMPOSITION OF MINERAL MIXTURE	60
	B.	COMPOSITION OF VITAMIN MIXTURE	61
II		ANALYSIS OF FOODSTUFFS FOR PROTEIN	62
III		WEIGHT GAINS, PROTEIN INTAKE AND PER OF RATS FED DIFFERENT DIETS	65
IV		HEPATIC NITROGEN OF RATS FED DIFFERENT DIETS	68
V		HEPATIC FAT CONTENT OF RATS FED DIFFERENT DIETS	69
VI		DATA ON NITROGEN BALANCE BY THE RATS FED DIFFERENT DIETS	70
VII		BIOLOGICAL VALUE AND DIGESTIBILITY COEFFICIENT OF THE DIETS	72
VIII		NET PROTEIN UTILISATION OF THE DIFFERENT DIETS	74
IX		NET DIETARY PROTEIN CALORIES PER CENT OF THE DIETS	75

I. INTRODUCTION

In a nation's march towards prosperity, extension of health facilities to all her people is of primary importance (Murthy, 1971). The World Health Organisation (WHO, 1956) has defined health as a state of complete physical, mental and social well being. Good nutrition paves the way for an optimal level of health and well being.

Malnutrition arises from faulty diets (Rama Sastri, 1968). It is a major cause of ill health in many developing countries. Malnutrition is invariably prevalent wherever the diet is composed largely of one of the five calorie yielding staples of the world namely, rice, wheat, maize, cassava or millets (Lowenberg, 1968).

The Indian diet is marked by the preponderance of cereals. According to Swaminathan (1972) cereals provide 70 to 80 per cent of the protein in the low income groups. The surveys carried out by Devadas et al (1967 and 1971) illustrate that the home diets of rural people are deficient in almost all the nutrients.

India lives in her half a million villages where 80 per cent of the population lives. Therefore, while considering

the improvement of health of the nation, rural areas need greater attention than they are receiving at present (Devadas, 1961 and Reddy, 1967).

In South India, the prevalence of kwashiorkor among toddlers in the poorest sections of the population is in the order of one to two per cent (Gopalan, 1970). Gopalan and Vijayaraghavan (1971) express that about 10 to 30 per cent of the Indian population suffer from iron deficiency anaemia and 52 per cent of toddlers among the poor socio-economic groups have haemoglobin levels below 10.8 per cent.

Protein-calorie malnutrition can be remedied by a number of alternative patterns of diet supplements (Joy, 1971). Animal protein is delicious and adequate but the present total animal protein deficit is about five million metric tons, or about the quarter of the existing supply (Altschul, 1966). Although 70 per cent of the population in India are non-vegetarians, because of the high cost of animal foods and limited purchasing power, people consume more starchy foods than animal foods (Lowenberg, 1968 and Whyte, 1968). Hence income influences not only the total quantity of protein in the diets, but also its quality (FAO, 1964).

Increases in the quantity and improvements in quality of proteins in the diets, can be brought about by increased

consumption of the protein foods commonly used, as well as, by the addition of protein concentrates and newly developed protein rich foods to the normal diets (FAO, 1964 and FAO, 1970). In the recent years, attempts have been made to develop novel protein foods such as the leaf protein, propagation of chlorella, and vegetable protein mixtures such as Incaparina and several others. However, the use of such foods has been restricted by lack of attractive products in certain cases, and by the problems encountered in their acceptability in other cases (Devadas 1967). Prevailing prejudices among people and taboos also limit the use of newer foods (Whyte, 1968). Hence attempts should be made to supplement the customary diets by locally produced low cost nutritious and acceptable foods (Stiebeling, 1964). As Devadas (1967) states, the nutritional superiority of a new food mixture should be established in the laboratories before advertising or educating the people regarding their use.

The present study is an attempt in this direction. It was undertaken to evaluate three low cost indigenous vegetable protein food mixtures containing tubers and leafy vegetables at different levels of incorporation in a basal diet as a basis for improving the normally existing rural diets around Coimbatore City. The three newly formulated diet mixtures with indigenous supplements were evaluated along with the basal diet for their Protein Efficiency Ratio, Biological Value and Net Protein Utilisation on albino rats.

II. REVIEW OF LITERATURE

The literature pertaining to this study is reviewed under the following headings:

A. Nutritional deficiencies in poor Indian diets.

B. Role of indigenous low cost foods in combating malnutrition.

and C. Biological evaluation of protein quality.

A. Nutritional Deficiencies in poor Indian diets:

The diets consumed by a vast majority of the people belonging to the low income groups in India are based mainly on cereals and millets. They contain only small quantities of pulses, vegetables, oils and fats and negligible amounts of milk and other animal foods, Murthy (1955) and Rao (1967). Sukhatme (1965) states that one third of the households in India can be considered to be undernourished or calorie deficient. The National Sample Survey Reports (1966) reveal that in the rural areas, 68 per cent of the total income is spent on food alone (40 per cent on cereals, 8 per cent on milk, 2 per cent on flesh foods and 18 per cent on other foods). According to the Indian Council of Medical Research (ICMR 1969), the average daily per capita consumption of calories is about 1890 as against the average per capita requirement of 2000 calories. Gopalan and Rao (1971) report that the intake of

calories is only 70 to 75 per kg. body weight as against the requirement of 100. They estimate the incidence of calorie deficiency to be 92 per cent among pre-school children in India.

Patwardhan (1961) warns that poor vegetarian diets based on rice and jowar are deficient in protein, B vitamins and minerals. Hariharan et al (1967) observe that the Indian diets based on rice and wheat are deficient in calcium and certain vitamins.

Vitamin A deficiency is very common in India, accounting for a quarter of the nutritional deficiencies in the country, Aykroyd et al (1966). Reddy (1969) states that eight per cent of the children between six months and six years around Hyderabad have vitamin A deficiency signs and that the incidence of vitamin A deficiency is particularly high among the preschool children whose dietary intake is about 100 μg/ day, most of which is derived from βcarotene, Reddy (1971).

There is also widespread prevalance of riboflavin deficiency in India as rice is a poor source of the vitamin, (Ramasastri, 1969). Swaminathan et al (1970) report that 28.5 per cent of children between four and five years of age in the rural areas are suffering from riboflavin deficiency as characterised by angular stomatitis.

Pellagra is a common nutritional deficiency condition around Hyderabad accounting for nearly one per cent of the general hospital admissions and nearly eight to ten per cent of the admissions to the mental hospital, Gopalan (1969).

B. Role of indigenous low cost foods in combating malnutrition:

In India, pulses are next in importance to major cereals, Patwardhan (1961). Parpia et al (1964) have experimented with several low cost protein rich food combinations which include legumes and oilseed meals. FAO (1964), Aykroyd (1966) and Yadav and Bharadwaj (1971) indicate that pulses are rich in protein and some of the B vitamins. An intake of 70g. pulses per day will improve the nutritive value of a diet which is largely composed of cereals. Pulses furnish not only protein but inevitably lead to an increase in the carbohydrate content of the diet, since all the pulses contain more than twice as much carbohydrate as protein, Butler (1969).

Gopalan et al (1969) emphasise the need for reduced consumption of cereals by both urban and rural groups and increased consumption of pulses and other protective foods in order to improve the Indian diets. Therefore, Rao (1969) stresses the consumption of pulses as an important way of ensuring adequate carbohydrate and protein intake in many developing countries.

Cereals and pulses continue to remain as the major source of nutrients providing 85 to 90 per cent of total protein, 30 to 52 per cent of total fat and 84 per cent of total calories in the Indian diets, Rao (1967). Pant (1971) indicates that in human dietaries addition of pulse to wheat can replace lysine fully. Use of wheat plus pulse in the ratio of 8:2 protein was proved to be as good as lysine supplemented wheat. This practice has the added advantage of providing extra calories, protein and other nutrients in the diet.

Rama Sastri and Mohan (1969) report that pulses and green leafy vegetables are relatively rich sources of folic acid. Green leafy vegetables are also good sources of riboflavin. Since they are relatively inexpensive, their inclusion in adequate amounts in the diet can improve considerably the riboflavin content. Greenleafy vegetables are very good sources of iron also, (ICMR, 1966). They are available throughout the year. They supply considerable quantities of vitamin A, folic acid, ascorbic acid and calcium (Iyengar, 1967 and Jelliffe, 1968).

Anandam et al (1966) found green leafy vegetables to be a good supplement in the school lunch programme in improving the nutritional status of children. Supplementation of 40g. of amaranthus providing 1200µg. carotene for 15 days brought about a significant increase in the Serum vitamin A levels of children in Hyderabad (Lala and Reddy, 1970).

To many nutritionists the simplest way of improving the nutritional status of our children is to increase the consumption of what they are eating presently, namely the cereal based diets with the addition of inexpensive and easily available protein foods in suitable forms in the cereal based diets, Reddy (1968).

The WHO (1963) suggests that vegetable protein foods in suitable combinations can be effectively employed for the prevention and treatment of protein malnutrition in children. Autret and Van Veen (1955) advise that in order to overcome the shortage in supply of milk and other protective foods, attempts should be made to prepare processed protein foods based on vegetable sources such as oilseed meal, and legumes, fortified with vitamins and minerals, which when incorporated at suitable levels in the diets, would supplement to a marked extent the average Indian diets (Doraiswami et al, 1961).

Venkat Rao (1964) puts forth that by suitable blending of two or more protein foods available in the region, it would be possible to obtain a protein blend of high nutritive value. Dunn (1966) conducted a feeding trial with a blend of groundnut protein isolate and Bengalgram flour fortified with vitamins and minerals on preschool children, and found significant increases in the heights and weights. Kurien et al (1969) observed that a protein food based on a blend of peanut and

chickpea flours produced an increase of 4.7 g. in weight per week in rats, when the calorie intake was adequate. Lateef Khan (1969) found that the PER of a number of mixtures of rice, jowar, wheat and bajra with preparations having redgram, Bengalgram and groundnut to be 1.8 and 2.0.

Joseph et al (1960) having determined the amino acid composition of blends of soyabean and sesame in the ratio of 55:45, and of the mixture of groundnut, soya bean plus methionine, found them to be nearly same as that of FAO Reference Protein Pattern. Srinivas et al (1966) found the blend of groundnut flour, soya bean and sesame in the ratio of 40:40:20 to have a PER of 2.3.

Narayanaswamy et al (1971) observed that the incorporation of low cost protein food based on a blend of wheat and soya flour at 10 and 20 per cent so as to provide 1.5 and 3.0g. of extra protein in the diet, brought about a significant increase in growth rate of rats. Pant (1971) indicated that the PER of wheat and pulse diet supplying protein in the ratio of 8:2 was similar to that of lysine supplemented wheat.

Phansalkar (1958 and 1960) indicated that after repletion for 21 days the rates of regeneration of serum proteins with mixtures of wheat, redgram and amaranth were of the same order as that of skim milk, in adult rats. He further indicated that in addition to the above ratio, incorporation

of leaf protein yielded a protein mixture which approached skim milk in regenerating serum proteins.

Supplementation of tapioca with pulses and redgram produced an average weekly increase of 5.4 and 5.6g respectively whereas when tapioca was fed alone the animals died within five weeks (Naryana Rao, 1951). Tasker et al (1962) observed that supplementation of maize - tapioca blend with a high protein food based on a blend of groundnut and bengalgram protein isolate promoted a significant increase in the growth of rats. On the basis of two studies conducted, Tasker (1963) observed that supplementation of a poor tapioca rice diet with a protein food based on 4:3:3 blend of full fat soya flour, groundnut flour and coconut meal resulted in highly significant increase in serum protein and nitrogen storage with PER not significantly different from that of skim milk at 10,15 and 20 per cent level.

C. Biological evaluation of protein quality.

The quality of a protein depends on essential amino acid content and the presence of these amino acids in proper proportion (Howe, 1967). Pomon (1967). states that the quality of a protein depends on its ability to supply essential amino acids in sufficient amounts to fulfil the requirements for maintenance and growth.

Venkat Rao et al (1964) indicate that the quality of protein can be estimated by

1. Methods based on growth and body weight changes
2. Nitrogen balance
3. Carcass nitrogen analysis

and 4. Other methods.

1. Methods based on growth and body weight changes

Protein Efficiency Ratio (PER)

Under carefully controlled conditions Hegsted and Worcester (1947) found that weight gain alone was sufficient to evaluate protein quality without taking into account protein intake. Tasker (1962), Albanese (1963) and Howe (1967) regard that measurement of protein efficiency by gain in body weight is one of the simplest and most popular procedures available. The protein quality therefore is often expressed as Protein Efficiency Ratio which varies directly with protein quality. The National Research Council (1963) states that the rate of growth of weanling rats under standardised conditions provides a reliable measure of the value of dietary protein. Fomon (1967) assumes that PER may provide a generally useful index of the limiting amino acids in the diet and has the major advantages of simplicity, convenience and widespread use of standardised procedures.

The AOAC (1960) has described a standardised technique for determining the PER using a period of 21 to 28 days. The Protein Advisory Group (PAG, 1970) is taking more and more interest in bridging the protein gap of the communities and as one of the measures it studies deeply on different foods, evaluating the protein quality as affected by different factors. The Indian Standards Institution (ISI, 1971) considers PER to be the most feasible parameter for defining protein quality. Since it feels that this method can be utilised for comparison of protein quality, only if the variable factors of the method are the same in the various testing laboratories, it has evolved a standardised procedure for determining PER.

Factors affecting PER:

NRC (1963) and Pomon (1967) indicate that the factors affecting PER assay are (i) age of rat, (ii) length of assay period, (iii) level of protein and (iv) sex of rat. Best conditions include a four week assay period, diets containing 10 per cent protein with sufficient amounts of the other essential nutrients, male rats and *ad libitum* feeding under which conditions, reproducible results can be obtained.

Harte *et al* (1948) put forth that a certain amount of restriction by paired feeding may improve the accuracy of the method.

Bender and Doell (1957) however criticise the PER method on the grounds that

a. No allowance is made for the maintenance requirement of the test animal

b. Result varies with food intake

c. Assumption that gain in body weight is indicative of protein tissue laid down

The NRC (1963) further points out that gain in body weight varies with protein levels and may not be constant in composition for different proteins.

2. Nitrogen balance:

Nitrogen balance is a valuable index for the measurement of nutritive value of protein. It is a very sensitive method for estimating retention of nitrogen in the body (Munro and Allisson, 1964). No substitute can claim to be as satisfactory as nitrogen balance method for evaluating the total nutritive value of proteins. It has long been used to evaluate the quality and quantity of protein in the diet of man (Anderson, 1969). Since differences between biologic value of two proteins is a function of only one variable, namely, protein quality, it follows that biologic value is a suitable index for evaluating differences in protein quality (NRC, 1963). Desikachar et al (1948) state that the biologic assay of protein for the nutritive value is usually done by growth and nitrogen balance method. Protein values have been extensively investigated by growth tests and by nitrogen balance and retention tests (FAO, 1964).

However, the main criticism against the nitrogen

balance method is the short duration of the metabolic period, because it is possible that suboptimal concentrations of a particular amino acid in protein which would ultimately prove to be a handicap to the animal, might pass on without consequence in the nitrogen balance, during the short test period, (Mitchell et al 1945).

3. Carcass analysis

Net Protein Utilisation:

The proportion of the nitrogen retained in the body (NPU) from that consumed through food is known as NPU, which is the most useful indicator of nutritional value of a protein, FAO (1964). NPU in practical evaluation of diets combines quality with quantity, Munro (1964). Measurement of NPU at levels of dietary protein which are adequate for maintenance, provides reasonably constant values for protein quality. NPU operative allows evaluation of dietary protein quality under practical conditions, Platt et al (1964). Allison (1964) states that obviously a more accurate estimation of nitrogen retention in the animal would be obtained by determining carcass nitrogen instead of body weight.

NPU is calculated using the nitrogen intake of each group (I) and their total carcass N (B) including the non protein group (Ik and Bk) using the following formula

$$N P U = \frac{B - Bk + Ik}{I} \times 100$$

The NPU values obtained are designated as NPU (ep) (Miller and Payne, 1959, and NAS - NRC, 1963).

If NPU standardised is required, the protein concentration is substituted for some of the corn starch in the non protein diet formula so as to give between 10 and 15 per cent protein on dry weight basis in the final diet. NPU standardised is calculated from

$$\text{NPU (st)} = \frac{54.\text{NPU}}{54 - P} - 8$$

Where P is the percentage of protein calories (Millen and Payne, 1961 and NAS - NRC, 1963).

Block and Mitchell (1946) observed a good agreement between the results being obtained through NPU method with those reported by other workers using accepted methods, whoing the showing the reliability of the NPU method.

Bender and Miller (1953 and 1955) found that the body constituents including nitrogen to water ratio were constant thus allowing measurement of nitrogen content ofthe body from the water content of the carcass. The relation approximates closely to a straight line given by the equation:

$$Y = 2.92 + 0.02\ X, \text{ where,}$$

$$Y = \frac{N \text{ (in grams)}}{H_2O \text{ (in grams)}} \times 100$$

Dreyer (1957) determined the nitrogen water ratio of 300 albino rats and found the nitrogen values served thereby correlated highly with values obtained by direct kjeldahl determinations. Platt and Miller (1959) noted that NPU determination of various dietary proteins were comparable, when measured at the maintenance levels.

Net Dietary Protein Calories Per cent (NDp Cal%)

FAO (1964) recommends NDp Cal% as a useful system of assessing the protein values of foods and diets. Munro (1964) states that NDP Cal % recognises that the requirement of the consumer for energy will limit the amount of any food stuff that he can eat.

Platt et al (1964) state that NDp cal% takes into account the quantity of protein and its quality in addition to the energy content of the food and thus the relative quantities of carbohydrates, lipids and alcohol. The calorie intake in relation to consumer's needs, the nature and proportion in a diet of many essential accessory food factors and the dietary regimen as the timing of meals and distribution of protein between various meals of the day are all considered in this calculation (index). Thus this method of expressing the nutritive value of a protein in diets can be compared directly with statement of protein requirements in terms of energy.

4. Other methods

Hepatic nitrogen and fat:

Addis et al (1949) confirmed that the liver weight of rats rapidly decreased during short periods of fasting or while consuming diets low in protein. Kesterlits (1944) found that hepatic nitrogen expressed in terms of unit body weight is a good index of the quality and quantity of dietary proteins. Vars and Gurd (1947) developed a method in which the rate of regeneration of heptic proteins in rats was correlated with the nutritive value of dietary proteins.

Campbell and Kosterlitz (1948) evaluated the nutritive value of proteins based on replenishment of labile liver protein in protein fasted adult rats and found that to be a good method. Litwack et al (1954) noted good correlation between protein metabolism and liver fat accumulation. The amount of protein in the diet affected the hepatic fat content.

Harper et al (1955) found that the extent of fat deposition in the liver depended upon the balance as well as absolute quantities of amino acids in the diet. An imbalance between protein and calories in the diet also caused deposition of fat in the livers (Hale and Shaefer, 1952).

III. EXPERIMENTAL PROCEDURE

The experimental procedures pertaining to this study on evaluation of three low cost rice based vegetables food mixtures containing different quantities of protein foods, tubers and green leafy vegetables on albino rats, involved the following steps.

A. Selection of the diets

B. Preparation and analysis of diets

and C. Evaluation of the protein quality of the diets by

1. Protein Efficiency Ratio
2. Hepatic nitrogen and fat contents
3. Nitrogen balance

and 4. Determination of NPU

A. Selection of the diets:

The diets selected for this study were based on the several diet surveys conducted by the Sri Avinasnilingam Home Science College to note the food consumption by rural families in Coimbatore District. From the data gathered through the surveys the composition of the daily dietaries of the rural population was computed, Devadas et al (19[illegible]). The rural diet was found to contain 6.7 per cent protein with a chemical score of 67. This diet

was selected to be the basal diet for this investigation, with a view to evaluate the poor low cost rice based diets.

The composition of the basal diet(diet A) is given in Table I.

TABLE I

PERCENTAGE COMPOSITION OF THE BASAL DIET

Foods	Quantity (g)	Protein (g)
Cereals	69	4.4
Other Ingredients		
Pulses	7	1.7
Green leafy vegetables	3	0.1
Roots and tubers	4	0.1
Other vegetables	5	0.1
Fruits	2	0.0
Milk	8	0.3
Sugar and jaggery	1	-
Oil	1	-
Total	100.0	6.7

Rice, ragi or cholam were consumed as the staple cereals in the different areas. In this study, rice has been considered as the staple. Similarly redgram dhal was included for pulses, amaranthus for leafy vegetables, potato for roots, brinjal for other vegetables and banana for fruits in the formulation of the diet.

Three diet mixtures were formulated to bring up the protein content of the basal diet to 10 per cent utilising the commonly available high protein indigenous food stuffs in different combinations. Their chemical scores were 71,72 and 71. The three experimental diets were designated as B,C, and D. Table II gives the composition of these three experimental diets.

TABLE XI

COMPOSITION OF THE EXPERIMENTAL DIETS

Ingredients	Quantity in g.		
	Diet B	Diet C	Diet D
Rice	35.8	35.3	35.8
Colocasia	6.0	—	—
Horsegram	12.5	13.0	13.5
Sesame	5.0	5.0	5.0
Drumstick leaves	5.0	5.0	—
Groundnut flour	4.5	4.5	4.5
Sweet potato	-	6.0	4.5
Amaranthus	—	—	5.5
Other ingredients as found in basal diet	31.2	31.2	31.2
Total	100.0	100.0	100.0

B. Preparation and analysis of diets:

All the foods needed for the entire study were bought in one lot. All the perishable foods were cleaned and dried to constant weight and powdered. The basal as well as the

three experimental diets needed for the entire study were mixed and preserved in deep freeze.

To ensure an adequate supply of vitamins and minerals to the rats, four per cent mineral mixture and two per cent vitamin mixture were added to the diets (USP XVII). Their composition is given in Appendix I. The diets thus prepared were analysed for their protein content by the macrokjeldahl method (Hawk et al 1965 as modified by NIN) as given in Appendix II. The protein content of the diets as analysed and calculated is given in Table III .

TABLE III

PROTEIN CONTENT OF THE SELECTED DIETS

Diet A		Diet B		Diet C		Diet D	
*	**	*	**	*	**	*	**
6.68	7.00	9.96	11.00	9.93	11.0	9.93	11.2

* Protein content as calculated
** Protein content as analysed

The protein intake of the animals in the study was calculated based on the analysed value.

6. Evaluation of the protein quality of the diets:

1. Protein Efficiency Ratio (PER):

Determination of PER included the following steps.

a. Selection, grouping and care of animals

b. Feeding the animals

and c. Maintaining weight records and calculation of PER.

a. Selection, grouping and care of animals:

Twenty one days old albino rats were separated from their mothers and maintained on stock diets for three days. Forty such weanling male rats were selected for the study and their initial weights recorded. They were divided into four groups of ten each, in such a way that the mean weights of the four groups were similar. The mean weights ranged from 33.96g. to 34.05 g. The groups were designated as Group A,B,C and D corresponding to the diets to be fed. The animals were housed in individual cages which were cleaned daily and each cage was marked with the respective diet number.

b. Feeding the animals:

Group A was fed the basal rice diet while the other three groups were given the test diets B, C and D respectively. Paired feeding technique (Rippon, 1959) was followed in this experiment as recommended by Knox (1956) to eliminate any metabolic adaptation which might be produced by voluntary dietary changes and confirmed by Murray (1948) to be comparable to ad libitum with respect to PER. Water was given ad libitum to all the animals.

To minimise spilling, a weighed quantity of the diet was mixed with water to a thin batter consistency in individual, aluminium cups and steam cooked for 10 minutes. One drop of Cod liver oil was added to each cup after cooking and fed to rats. The left over food was collected on the following day, dried in a hot air oven at 100°C and the dry equivalent obtained. The food consumption was then calculated. Daily food intake records were maintained for a period of 28 days.

6. Maintaining weight records and calculation of PER:

Rats were weighed accurately on alternate days in a rat weighing balance before food was given and the weekly increase in weights were determined for each group. After a period of 28 days the experiment was terminated and PER was determined by the following formula as given by (NAS/NRC, 1963).

$$\text{PER} = \frac{\text{gain in weight of test animal in g.}}{\text{g. of protein consumed}}$$

2. Hepatic nitrogen and fat contents:

The growth and nitrogen balance study was supplemented by a study of the effect of protein quality on the liver, which is considered by Wainio et al (1959) to be the most sensitive tissue to protein depletion.

At the termination of the PER study, the rats were chloroformed and the livers removed. Care was taken to blot out the blood and trim the fatty tissue and stored the livers in the

deep freeze till the day of analysis. The 10 livers in each experimental group were pooled into three groups (3 + 3 +4) and homogenised. The hepatic nitrogen was determined in triplicates by the microkjeldahl method (Hawk et al 1965 as modified by NIN) and fat was estimated by the method of Folch et al (1957).

The initial hepatic nitrogen and fat contents were determined from a group of four weanling male albino rats at the beginning of the study by sacrificing the animals. The mean weight of the animals corresponded to the mean weight of the test groups.

3. Nitrogen balance:

Nitrogen balance index was determined by Mitchell's method (1945). The procedure involved the following steps.

a. Selection of animals.

b. Grouping the animals after feeding the protein free diet for seven days.

c. Feeding the animals for the test.

and d. Analysis of urine and faeces and calculation of the Biological Value.

a. Selection of animals:

Twenty four male albino rats, 100 days old were chosen for the study. Their initial weights were recorded and the animals were housed in individual wire mesh bottomed cages

with funnel attachment, specially devised so as to facilitate collection of faeces and urine.

b) Grouping the animals after feeding the protein free diet for seven days

The selected animals were maintained on protein free diet for a period of seven days, in order to determine the endogenous excretion of nitrogen. The composition of the diet included starch (86%) and fat (8%) with the addition of vitamin and mineral mixtures. About 30 g. of the diet was steam cooked in individual cups and fed to the rats. Intake records were maintained similar to the PER study throughout the experiment. Water was given ad libitum .

After a three day adjustment period with the protein free diet the collection of urine and faeces was done for a period of four days. The urine collected was preserved in the refrigerator in individual bottles with an added layer of toluene. The faeces collected was brushed to remove the hair adhering to it, dried in the oven and preserved for analysis. The volume of urine and the weight of faeces were noted initially. At the end of the depletion period the animals were weighed and the stock diet was fed for a period of four days. The rats were then divided into four groups of six each, ensuring that the mean weight loss of the four groups was similar as recommended by Gangal and Margar (1969) and designated as A, B, C and D corresponding to the diets to be fed.

e. Feeding the animals:

The four groups were fed the different test diets for a period of seven days using the paired feeding technique. Four day collection of urine and faeces were made after an adjustment period of three days with the test diets. The collections were done as described for the protein free diet.

d. Analysis of urine and faeces and calculation of the Biological Value:

The nitrogen content of the individual urine samples as well as the dried and powdered faeces samples collected during the two phases of the study were analysed by microkjeldahl method. Biological Value (BV) was calculated using the formula (NAS/NRC, 1963)

$$BV = \frac{I - (F - FK) - (U - UK)}{I - (F - FK)}$$

Where I = Intake of nitrogen

F = Faecal nitrogen

FK = Endogenous faecal nitrogen

U = Urinary nitrogen

UK = Endogenous urinary nitrogen

Digestibility Coefficient (DC) was calculated using the following formula

$$DC = \frac{\text{Absorbed nitrogen}}{\text{Nitrogen intake}} \times 100$$

4. Determination of NPU:

NPU was determined by the method of Miller (1955), taking body composition into account (Fomon, 1967). The followingwere the steps involved in the determination of NPU.

a. Selection and grouping of animals

b. Feeding the animals and maintaining weight records

and c. Analysis of carcass nitrogen and calculations

a) Selection and grouping of animals:

Forty weanling male albino rats, 25 days old were selected for the study. They were weighed initially and housed in individual cages. The rats were maintained on stock diet for a period of one week after which they were weighed and divided into five groups of eight each according to their weights and designated as A, B, C, D and E.

b) Feeding the animals and maintaining weight records:

The four groups A,B,C and D were fed the basal and test diets and group E was fed the protein free diet, the composition of which is similar to the one given in the nitrogen balance study. Water and food were given ad libitum. The daily food intake as well as the body weights were recorded as done for the previous experiments. The rats were fed for a period of ten days.

e. Analysis of carcass nitrogen and calculations:

At the end of the ten days, the animals were chloroformed, incisions made into the abdominal cavity and the alimentary canal cleared of the contents. The carcasses were laid on a tray, brought to a constant weight. After heating for 48 hours at 105°C in an oven they were weighed and the moisture content of the body calculated.

The moisture free rats were pooled into two groups per diet according to their weights. They were run through a domestic mincer three times and the homogenised carcass was estimated for nitrogen by macrokjeldahl method.

The nitrogen intake (I) of each group, their total carcass N (B), including the non protein group (IK and BK) were calculated and NPU was arrived at by using the formula:

$$NPU\ (op) = \frac{B - Bk + Ik}{I} \times 100$$

NDp Cal% was calculated as follows (NAS/NRC, 1963).

$$NDp\ Cal\ \% = \frac{NPU\ (Op)}{100} \times \frac{25\ N\ \%}{Cals/g}$$

Where N = percentage of nitrogen in the diet, the cals/g represent the metabolizable energy of the diet.

IV. RESULTS AND DISCUSSION

The results of this investigation on the evaluation of three low cost rice based vegetable food mixtures containing different quantities of protein foods, tubers and green leafy vegetables, is discussed under the following headings:

A. Protein Efficiency Ratio

B. Hepatic nitrogen and fat

C. Nitrogen balance

and D. Net Protein Utilisation

A. Protein Efficiency Ratio (PER)

The PER of the three different diet mixtures is discussed under

1. Food and protein intake

2. Body weight gains

and 3. Protein Efficiency Ratio

1. Food and protein intake

The mean total food and protein intake of the four groups of animals fed different diets is given in Table IV with the individual values in Appendix XII.

TABLE IV

THE MEAN TOTAL FOOD AND PROTEIN INTAKE ON DIFFERENT GROUPS OF RATS

Groups	Level of protein in the diet (g/100g diet)	Mean food intake (g)	Groups compared	't' value	Mean protein intake (g)	Groups compared	't' value
A	7.7	287.4± 2.064	A vs B	17.40**	22.1 ± 0.217	A vs B	1.43
			A vs C	18.87**		A vs C	1.95
B	11.1	191.0± 1.881	A vs D	18.33**	21.2 ± 0.204	A vs D	0.13
			B vs C	0.07		B vs C	0.42
C	11.0	190.7± 1.693	B vs D	1.63	20.9 ± 0.176	B vs D	1.97
D	11.2	198.0± 1.539	C vs D	1.95	22.2 ± 0.161	C vs D	2.88**

** Significant at one per cent level

* Significant at five per cent level.

Among the four food mixtures, diet A with its lowest protein content was consumed in higher quantities by the animals in group A and hence the food intake was directly related to the protein content of the diet. Although the food intake of the basal group (A) was much more than the other groups, the mean total protein intake of this diet was more or less similar to that of the other groups, since it contained only 7.7g. per cent. protein. Table IV shows a significantly greater increase in food consumption by group A at one per cent level over the other groups.

Between the three groups, B, C and D there was no significant difference in food consumption, which ranged from 190.7g. for group C to 198.0g. for group D. However when the total protein consumption was taken into account, group C stood last and was significantly lower than group D. The other groups did not show any significant difference amongst themselves with regard to protein consumption.

2. Body weight gains

The mean, initial and final weights, of the different groups of rats are given in Table V with their individual values in Appendix III.

TABLE V

MEAN INCREASE IN WEIGHT OF RATS FED DIFFERENT DIETS

Group	Mean initial weight (g)	Mean final weight (g)	Mean Increase (g)	Groups compared	't' value
A	34.05 ± 0.312	73.90 ± 1.055	39.85 ± 0.020	A vs B	2.32 *
				A vs C	2.69*
B	34.04 ± 0.305	77.46 ± 1.371	43.42 ± 1.225	A vs D	7.16 **
C	33.96 ± 1.029	76.72 ± 1.209	42.76 ± 1.005	B vs C	0.37
				B vs D	1.91
D	34.01 ± 1.015	80.73 ± 0.308	46.72+ ± 0.295	C vs D	2.98*

* Significant at five per cent level

** Significant at one per cent level.

All the groups of rats had increased in their body weight, over a period of 28 days. The mean final weights of the animals varied between 73.90 g. to 80.75 g. among the four groups.

The highest increase in weight was recorded by the group D (46.72 g) and the lowest by the basal diet group A (39.85g), even though the total food consumption was highest in group A. The differences in the weight gain between the groups A and D was significant at one per cent level, and those between A and B, and A and C were significant at five per cent level. Group D was also greater than group C at five per cent level of significance.

When the weight gains were correlated with the food intakes and protein intakes, it is remarkable that although group A had a significantly ($P \leq 0.01$) higher food intake when compared with groups B, C and D, the weight gains of rats fed diet A were significantly lower than that registered by rats fed diets B, C and D. This is a clear indication of the superior quality of the supplemented vegetable protein mixtures. A significantly higher ($P \leq 0.05$) protein intake of group D over that of group C might be the contributory factor for the significantly higher ($P \leq 0.01$) weight gain expressed by group D over that of group C. The better the quality of protein,

and higher its intakes the greater are the weight gains.

3. Protein Efficiency Ratio (PER)

The mean PER values obtained for the different diet groups are presented in Table VI, and the individual values are given in Appendix III.

TABLE VI

PER OF BASAL AND THREE EXPERIMENTAL DIETS

Diets	Mean total protein intake (g)	Mean increase in weight (g)	P E R	Groups Compared	't' value.
A	22.1 ± 0.2168	39.85 ± 0.1974	1.80 ± 0.0200	A vs B	0.40
				A vs C	2.18
B	21.2 ± 0.2025	43.42 ± 1.2250	2.04 ± 0.2450	A vs D	5.96**
C	20.0 ± 0.1761	42.76 ± 1.0050	2.04 ± 0.1000	B vs C	—
				B vs D	0.12
D	22.2 ± 0.1613	46.72 ± 0.2949	2.11 ± 0.0175	C vs D	0.45

** Significant at one per cent level.

Among the four groups of animals, the highest P E R was recorded by the group D (2.11) the protein intake and the weight gain of which ranked first. Groups B and C had similar PER values namely 2.04. The Diet A had the lowest PER value (1.80). The difference between the PER values of groups A and D was significant at one per cent level. With their negligible differences in the chemical scores, the three experimental diets did not show appreciable difference in their PER values.

B. Hepatic nitrogen and fat contents

1. Hepatic nitrogen:

The mean increase in total hepatic nitrogen of the four groups of rats is given in Table VII. The data on individual rats is presented in Appendix IV.

TABLE VII

MEAN INCREASE IN HEPATIC NITROGEN CONTENT OF RATS.

Group	Initial hepatic nitrogen (mg)	Final hepatic nitrogen (mg)	Mean increase in hepatic nitrogen (mg)	Groups compared	't' value	Nitrogen per gram liver weight (mg)	Groups compared	't' value
A	38.34	102.33	63.9 ± 11.910	A vs B	1.06	33.321±2.199	A vs B	2.05
				A vs C	1.05		A vs C	1.96
B	38.34	116.01	77.67 ±13.760	A vs D	0.73	36.681±0.807	A vs D	2.10
				B vs C	9.49		B vs C	0.01
C	38.34	111.22	72.88 ±0.247	B vs D	0.62	36.673±0.998	B vs D	0.22
D	38.34	109.81	71.48 ± 6.916	C vs D	0.38	36.888±0.916	C vs D	0.20

The mean total hepatic nitrogen varied between 102.33 mg. in group A to 116.01 mg. in group B at the end of the growth study. The mean increase in hepatic nitrogen also followed the same pattern. When the four groups were compared for their mean increase in hepatic nitrogen, there was no significant difference between the groups.

When the hepatic nitrogen was expressed as per gram hepatic weight, basal diet A showed the lowest nitrogen content. Among the three experimental groups, although diet D ranked first in PER, it had the least increase in hepatic nitrogen because it had the least hepatic weight gain. But when nitrogen per gram liver weight was taken into account, diet B ranked first among the four groups with 36.88 mg. nitrogen per g. liver.

The statistical analysis revealed that the differences among the groups in these parameters were not significant. As Henry et al (1953 and 1961) had indicated the hepatic nitrogen varied linearly with the quality of protein. In the present study also the higher potentials of diet B may be due to its higher protein quality.

2. Hepatic fat

Table VIII gives the mean total hepatic fat as well as the fat per g. of liver weight for the four groups of rats. The details of the individual values are given in Appendix V.

TABLE VIII

MEAN INCREASE IN HEPATIC FAT CONTENT OF RATS

Group	Initital hepatic fat(mg)	Final hepatic fat(mg)	Mean increase in hepatic fat (mg.)	Groups compared	't' value	Fat per g. liver (m g)	Groups compared	't'value
A	58	396	338	A vs B	2.53	129	A vs B	1.580
				A vs C	2.07		A vs C	1.225
B	58	207	149	A vs D	2.43	65	A vs D	1.383
				B vs C	1.45	80	B vs C	1.420
C	58	241	183	B vs D	0.51		B vs D	0.790
D	58	218	160	C vs D	1.27	73	C vs D	0.630

Table VIII reveals an increase in hepatic fat content in all the groups but the rats fed diet A had double the amount of increase in fat when compared to the groups fed the test diets. Group B which registered the highest increase in hepatic nitrogen had the lowest amount of fat in the livers Neither the difference in the mean increase in hepatic fat, nor the fat per gram liver weight showed any significant difference among the groups.

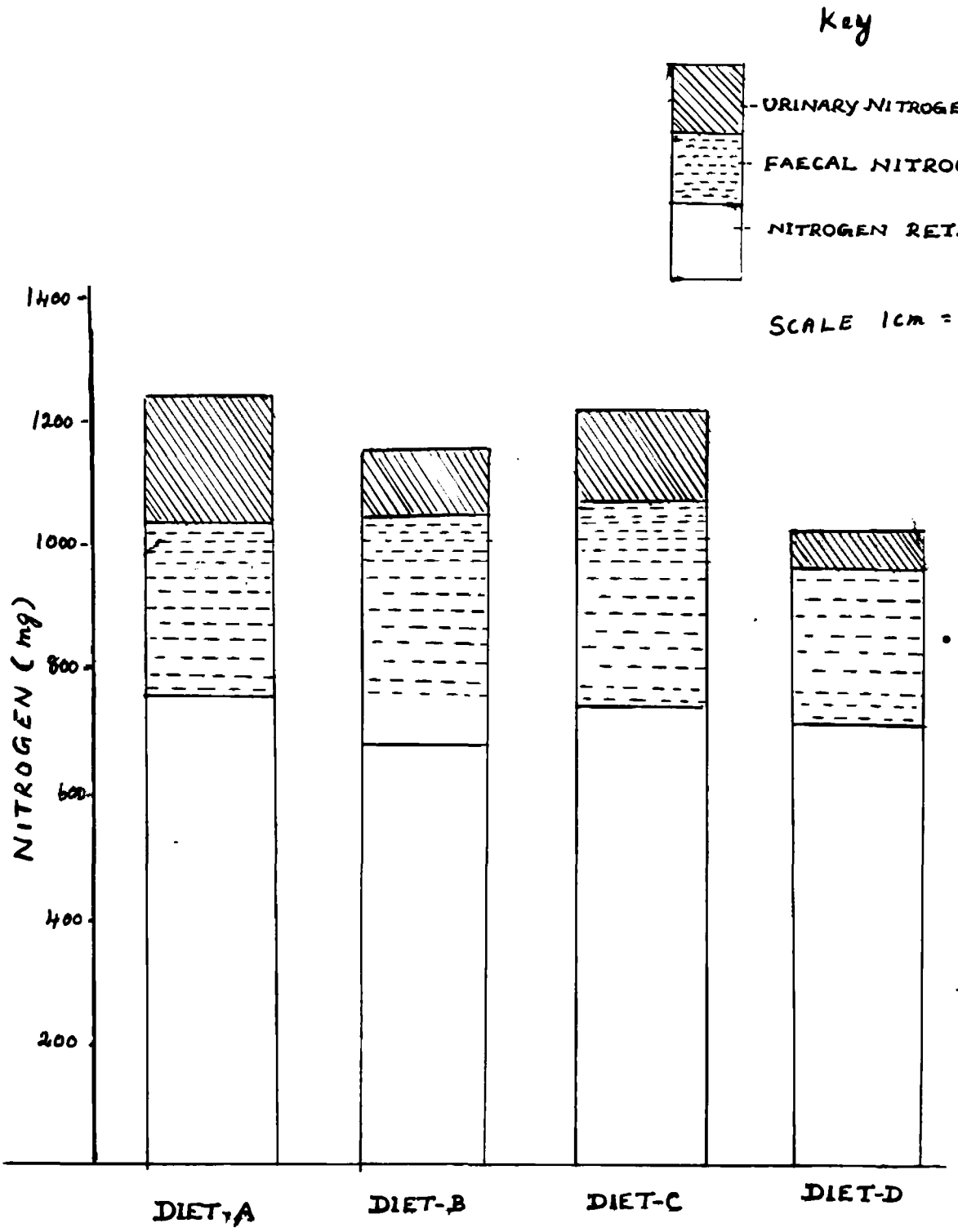
The fact that group A had a higher fat deposition (double that of D) may be due to the fatty infiltration of the liver. Diet A seems to be the least efficient one in this index also.

C Nitrogen balance:

The results of the nitrogen balance study are discussed under: 1) Nitrogen retention, 2) Biological Value (BV) and (3) Digestibility Co-efficient (D.C).

1. Nitrogen retention

The mean daily nitrogen retained by the rats fed different experimental diets is given in Table IX. (Fig 1) The data on individual rats are presented in Appendix VI.



MEAN DAILY NITROGEN RETENTION IN RATS FED DIFFERENT DIETS.

FIG-1

TABLE IX

MEAN DAILY NITROGEN RETENTION IN RATS FED DIFFERENT DIETS

Diet	Total mean nitrogen intake. (mg)	Excretion of N: nitrogen in urine + (mg)	Excretion of N: nitrogen in faeces++ (mg)	Total nitrogen excretion (mg)	Nitrogen retention in the body (mg)	Percentage retention	Groups compared	't' value
A	1227.48	196.08	279.39	475.47	758.21	61.8 ± 2.874	A vs B	0.94
							A vs C	0.22
B	1151.99	109.56	361.10	470.65	681.30	59.3 ± 5.286	A vs D	8.66*
							B vs C	0.86
C	1206.48	135.26	331.92	467.18	739.30	61.5 ± 1.986	B vs D	3.44*
D.	1017.26	68.53	246.17	314.70	702.57	69.0 ± 3.415	C vs D	4.24**

\+ Indicates urinary nitrogen during the experimental period-Endogenous Urinary nitrojen

++ Indicates faecal nitrogen during the experimental period - Endogenous faecal nitrogen

** Significant at one per cent level

* Significant at five per cent level.

Table IX shows that all the animals in the experiment had a positive nitrogen balance. Both the food nitrogen ingested and excreted were highest in group A, and least in group D. However, the retention percentage showed that the animals in group D retained the highest amount of nitrogen ingested. The higher retention recorded by group D may be due to the good quality of the protein ingested. Diet A stood next among the other three groups. This may be attributed to the higher consumption of nitrogen by those animals. However the retention in group D was greater than that in group A at five per cent level. The nitrogen retention by group D was higher than that of group B at five per cent level and group C at one per cent level.

2. Digestibility Coefficient and Biological Value:

Table X shows the BV and DC values of the basal and the test diets and the individual values are given in Appendix VI.

TABLE X

THE BIOLOGICAL VALUE AND DIGESTIBILITY COEFFICIENT OF THE TEST DIETS

Group	B V	Groups compared	't' value	D C	Groups compared	't' value
A	79.33 ± 1.14	A vs B	1.95	77.20 ± 2.09	A vs B	6.98**
		A vs C	1.95		A vs C	4.66**
B	86.27 ± 7.88	A vs D	12.93**	68.55 ± 1.83	A vs D	1.11
C	82.60 ± 3.61	B vs C	0.94	72.45 ± 0.92	B vs C	4.24**
		B vs D	1.31		B vs D	5.97**
D	91.07 ± 2.28	C vs D	4.42**	75.77 ± 1.98	C vs D	3.42 *

** Significant at one per cent level

* Significant at five per cent level.

Among the four food mixtures diet A had the highest D C (77.2) and diet B had the lowest D C (68.6). The increase in DC value of group A over groups B and C were significant at one per cent level. The increases of groups C and D over B were also significant at one per cent level.

The diet D with it's DC of 75.7 stands next to diet A and it was greater than diet C at five per cent level.

As Table X reveals, diet D had the highest Biological Value (91) than the other three groups indicating it's superior quality of protein. Diet B and 86 and C had 86 and 82 as their Biological Value and diet A had the lowest Biological Value (79). The Biological Value of Group D was found to be significantly higher than groups A and C at five per cent level.

The values obtained in this study are in accordance with those reported by Phansalkar (1960) that the Biological Value of cereal proteins varies between 60 to 89 per cent. It is noteworthy that though the Digestibility Coefficient of diet A was the highest, the Biological Value was the lowest. This may be due to the fact that though the digestibility was high the amino acid pattern of this diet may not be as good as the other diets as indicated by its lower chemical score(67). Hence, from the utilization point of view, both amino acid

pattern and digestibility need consideration. Beaton and McHenry (1964) state that variability in digestibility is not a major determinant of the nutritive value of proteins and hence the absence of correlation between the Digestibility Coefficient and the Biological Value obtained for various diets.

D. Net Protein Utilisation (NPU)

Table XI gives the NPU values of the basal as well as the test diets and the ND_p Cal % as derived from the NPU values. The details of the carcass nitrogen and protein intake are given in Appendix VII.

TABLE XI

NPU AND ND_p CALORIES PERCENTAGE OF THE DIETS

Diet	N P U	ND_p Cal %
A	16.13	1.71
B	17.07	2.71
C	21.89	3.44
D	26.53	4.25

The NPU values of four diets, ranged from 16.1 (group A) to 26.5 (group D). Diet D had the highest NPU value following the pattern set by the other indices discussed so far. Diet B had the NPU of 17.07 and C had the NPU of 21.9. The ND_p Cal % of the diets also ranked in the same order. The ND_p Cal % values ranged between 4.2 (group D) to 1.7 (group A) and it clearly

reveals, that among the four food mixtures, diet D had a very good protein quality and the basal diet had the very poor protein quality.

Thus, judged from all the parameters, diet D ranked superior on its protein quality. Diets B and C ranked next in order. Diet A was the least efficient. This higher potency of diet D may be due to better pattern of amino acids and hence the higher Biological Value.

V. SUMMARY AND CONCLUSION

This investigation was undertaken to evaluate a poor rice diet as commonly consumed by the rural people around Coimbatore City containing 6.7 g. protein along with three formulated diet mixtures, supplemented by some commonly avilable high protein indigenous food mixtures, in different combinations at 10 per cent protein level with the rice diet. The diet was evaluated for their Protein Efficiency Ratio, hepatic nitrogen and fat, Biological Value and Net Protein Utilisation on albino rats. The results of the study revealed the following

1. The diets on the basal rice diet (Group A) had a significantly greater food consumption over the other three groups. The differences in food intake between the groups B, C and D were Not appreciable. However when the protein intake was accounted, group D stood first. The difference was significant over group C at one per cent level.

2. A higher increase in weight was recorded by the group D over the groups A and C which were significant at one and five per cent levels respectively. Group A ranked last among the four.

3. Diet A had the lowest PER namely 1.80 and diets B and C had a similar PER value, 2.04. Diet D seemed to be the best in PER with a value of 2.11 which was significant over A at one per cent level.

4. The nitrogen content of the liver as expressed in mg/g. liver weight was more in group D though the differences between the four groups were not statistically significant. The basal diet was the poorest among the four in this parameter.

5. More accumulation of fat was noted in the animals fed diet A. However hepatic fat content did not show appreciable difference among the groups.

6. Diet A was found to have the highest BC(77.2) which was significant over the groups B (68.6) and C (72.3) at one per cent level. The increases of groups C and D over B were also significant at one per cent level.

7. Diet D had the highest BW (91) and diets B,C and A had the BW of 86, 83 and 79 respectively. The BW of the group D was found to be significantly higher than groups A and C at five per cent level.

8. Just like all the other indices NPU was highest for the diet D (26.5)- The groups C, B and A ranked next to the diet D with the NPU values of 21.9, 17.1 and 16.1 respectively. Thus the NDp cal% which were arrived from the NPU also followed the same order of superiority.

It can be concluded that the formulated vegetable protein mixtures were definitely superior to the poor rice diet.

Among the three food mixtures evaluated, diet D stood best in the most of the parameters selected and diets B and C ranked next to diet D. It is remarkable that the main difference in the composition of the diet D from B and C lies in the inclusion of amaranthus in the diet D at 5.5 per cent level. So an additional inclusion of amaranthus to the existing rural diet which has already got green leafy vegetables at three per cent level will improve the quality of the diets to a very great extent, apart from supplementing the diets with pulses and other protein rich sources.

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APPENDICES

APPENDIX I — A

Composition of Mineral Mixture

USP XVII salt Mixture

Grind in a mortar portion of Nacl - 139.3 g with K I - 0.79 g. Similarly grind together remainder of the Nacl with 389.0 g. KH_2PO_4 ; 57.3 g $MgSO_4 7H_2O$; 38.4 g $CaCO_3$ 27.0 g. $FeSO_4 7H_2O$; 4.01g $MnSO_4 H_2O$; 9.548 g $ZnSO_4 7H_2O$; 0.477 g $CuSO_4 5H_2O$ and 0.025 g $CoCl_2 6H_2O$ adding finally the Nacl K I mixture. Reduce the entire mixture to a fine powder.

APPENDIX I-B

COMPOSITION OF VITAMIN MIXTURE

1. Prepare Vitaminised starch 1 g of corn starch to contain.

Vit K (menadione)	--	0.5 mg
Thiamine	--	0.5 mg
Riboflavin	--	1.0 mg
Pyridoxine	--	0.4 mg
Calcium pantothenate	--	4.0 mg
Niacin	--	4.0 mg
Choline	--	200 mg
PABA	--	10 mg
Vit B_{12}	--	2 mg
Folic acid	--	0.2 mg
Biotin	--	0.2 mg
Inositol	--	25 mg

2. Prepare Vitaminised oil - 1 kg of refined groundnut oil to contain the following vitamins.

Vitamin A	--	1000 IU
Vitamin D	--	100 IU
Vitamin E	--	10 IU

Store vitaminised oil in deep freeze.

APPENDIX II

ANALYSIS OF FOODSTUFFS FOR PROTEIN

Principle:

The protein content of a foodstuff is obtained by estimating the nitrogen content of the material and multiplying it by 6.25. This is referred to as crude protein content since the non protein nitrogen present in the material is not taken into consideration. True protein nitrogen can be determined by subtracting NPN (which is estimated by precipitating the protein in the sample with trichloro acetic acid, tungstic acid or copper hydroxide and determining the residual nitrogen in the protein free filtrate) from the total nitrogen.

The estimation of nitrogen is done by kjeldahl method which depends upon the fact that organic nitrogen when digested with sulphuric acid in the presence of a catalyst (selenium oxide, mercury or copper sulphate) is converted into ammonium sulphate. Ammonia liberated by making the solution alkaline is distilled into a known volume of a standard acid which is then back titrated.

Reagents:

1. Concentrated Sulphuric acid - A.R.
2. Digestion mixture - 98 parts $K_2 SO_4$
   2 parts $CuSO_4$
3. 40% NaoH
4. N/10 $H_2 SO_4$ & N/10 NaoH
5. Methyl red indicator: - 0.1. g of the indicator dissolved in 60 cc alcohol and water added to 100 cc

Procedure:

The sample (0.5-2.0g) is weighed into a dry kjeldahl flask. About 5 g of digestion mixture and 20 ml of pure concentrated $H_2 SO_4$ are added to the sample and the mixture digested for 4-5 hours. Glass beads are added to prevent bumping. After the contents of the flask become clear, the digestion is continued for at least one more hour. The contents of the kjeldahl flask are cooled, diluted with distilled water and the mixture made alkaline by adding excess of 40% NaoH. in the presence of litmus paper. A small quantity of pumice powder is added to prevent bumping during distillation. The ammonia liberated is distilled into a receiver containing 25 ml of N/10 $H_2 SO_4$. The excess of acid in the receiver is back titrated against N/10 NaoH using 3 drops of methyl red indicator.

Calculation:

If 'a' g of the sample are taken and if 'b' and 'c' ml of alkali of normality 'd' are required for back titration and to neutralise 25 ml of N/10 H_2SO_4 respectively then the protein content in g per 100 g of sample is equal to

$$\frac{(c - b) \times 14 \cdot 4 \times 6.25 \times 100}{a \times 1000}$$

APPENDIX III

WEIGHT GAINS AND PER OF RATS FED DIFFERENT DIETS

Diet No.	Rat No.	Final weight (g)	Initial weight (g)	Increase (g)	Total food intake (g)	Protein intake (g)	PER
A.	1	78.2	38.2	40.0	292.3	22.5	1.78
	2	77.0	37.0	40.0	277.9	21.4	1.87
	3.	76.5	37.0	39.5	278.5	21.4	1.85
	4	73.9	35.3	38.6	277.8	21.4	1.80
	5	74.7	37.2	70.5	302.2	23.3	1.74
	6	76.0	33.7	39.2	295.0	22.7	1.86
	7	72.7	33.5	39.2	272.0	20.9	1.88
	8	73.5	32.1	40.9	289.7	22.3	1.83
	9	69	31.0	38.0	286.3	22.0	1.73
	10	67.5	28.0	39.5	303.3	23.4	1.69
Mean		73.90	34.05	39.85	287.4	22.1	1.80
B.	1	80.2	38.3	41.9	199.6	22.2	1.89
	2	87.0	37.0	50.0	203.6	22.6	2.26
	3	76.7	36.3	70.4	188.4	20.9	1.93
	4	80.9	35.4	45.5	200.5	22.3	2.04
	5	75.3	34.8	40.5	178.6	19.8	2.05
	6	83.7	34.1	49.6	203.8	22.6	2.19
	7	78.6	33.0	45.6	195.0	21.7	2.10
	8	66.7	32.8	33.9	169.2	18.8	1.80
	9	73.7	30.5	43.2	187.6	20.8	2.08
	10	71.8	28.2	43.6	183.5	20.4	2.14
Mean		77.46	34.04	43.42	192.09	121.21	2.043

Diet No.	Rat No.	Final weight (g)	Inital weight (g)	Increase (g)	Total food intake (g)	Protein Intake (g)	PER
C	1	83.5	38.20	45.3	204.5	22.5	2.01
	2	83.3	37.7	45.6	191.5	21.1	2.16
	3	79.2	36.6	42.6	199.0	121.9	1.95
	4	75.2	35.3	39.9	183.6	20.2	1.98
	5	72.2	34.4	37.8	180.5	19.9	1.90
	6	80.5	33.7	46.8	195.9	21.6	2.17
	7	76.0	33.4	42.6	189.6	20.9	2.04
	8	71.8	32.9	38.9	176.6	19.4	2.05
	9	71.8	30.0	41.8	186.1	20.4	2.05
	10	73.7	27.4	46.3	199.6	22.0	2.10
Mean		76.72	33.96	42.76	1 90.7	20.99	2.04
D	1	83.6	38.9	44.7	198.4	22.2	2.01
	2	85.6	37.8	47.8	200.8	22.5	2.12
	3	84.8	36.3	48.5	206.5	23.1	2.10
	4	78.5	35.0	43.5	201.8	22.6	1.92
	5	79.1	34.7	44.4	201.4	22.6	1.97
	6	78.1	33.7	44.4	192.0	21.5	2.07
	7	80.5	33.5	47.0	202-3	22.7	2.07
	8	76.5	31.5	45.0	182.0	20.4	2.21
	9	80.3	29.6	50.7	203.8	22.8	2.22
	10	80.3	29.1	51.2	190.5	21.3	2.40
Mean		80.73	34.01	46.72	198.8	22.17	2.11

APPENDIX IV

HEPATIC NITROGEN OF RATS FED DIFFERENT DIETS

Diet		Mean Hepatic weight of pooled groups g.	Mean total hepatic nitrogen of pooled groups mg.	N per g. liver mg.
A	a	3.2600	113.6640	36.400
	b	3.1154	97.6990	31.364
	c	2.8148	90.6366	32.198
	Mean	3.0634	102.3332	33.321
B	a	3.1639	117.8236	37.258
	b	3.7083	131.6072	35.862
	c	2.6405	98 .3248	37.244
	Mean	3.1708	116.0052	36.681
C	a	3.1144	111.6200	35.844
	b	2.9094	110.8090	38.078
	c	3.0807	111.2145	36.097
	Mean	3.0350	111.2145	36.673
D	a	3.1940	117.1560	36.680
	b	2.8011	100.3914	35.841
	c	2.9383	111.8904	38.083
	Mean	2.9778	109.8126	36.868

APPENDIX V A

HEPATIC FAT CONTENT OF RATS FED DIFFERENT DIETS

Diet		Mean hepatic weight of pooled groups g.	Fat per 5gm. g.	Mean Total fat of pooled groups g	Fat per gm. liver g.
Initial	a.	1.5045	0.0289	.0870	0.0578
	b.		0.0289	.0870	0.0578
Mean			0.0289	.087 0	0.0579
A	a.	3.2600	0.0521	0.05397	0.1042
			0.0734	0.04786	0.1468
	b.	3.1154	0.0796	0.4960	0.1592
			0.0815	0.5078	0.1630
	c.	2.8148	0.0497	0.2798	0.0994
			0.0491	0.2764	0.0982
Mean		3.0634	0.0642	0.3964	0.1285
B	a.	3.1639	0.0565	0.2310	0.0730
			0.0513	0.1981	0.0626
	b.	3.7083	0.0330	0.2447	0.0660
			0.0305	0.2262	0.0610
	c.	2.6403	0.0502	0.1595	0.0604
			0.0343	0.1811	0.0686
Mean		3.1708	0.0326	0.2068	0.0653
C	a.	3.1144	0.0431	0.2685	0.0882
			0.0425	0.2647	0.0850
	b.	2.9099	0.0406	0.2363	0.0812
			0.0415	0.2415	0.0838
	c.	3.0807	0.0555	0.2187	0.0710
			0.0554	0.2181	0.0708
Mean		3.0350	0.0374	0.2413	0.0795
D	a.	3.1940	0.0590	0.2491	0.0780
			0.0367	0.2344	0.0734
	b.	2.8011	0.0371	0.2078	0.0742
			0.0366	0.2050	0.0732
	c.	2.9383	0.0337	0.1980	0.0674
			0.0366	0.2151	0.0732
Mean		2.9778	0.0366	0.2182	0.0732

APPENDIX VI

DATA ON NITROGEN BALANCE BY RATS FED DIFFERENT DIETS

Diet	No.	N_2 Intake mg.	Urinary N Excretion mg.	Faecal N Excretion mg.	Total mg.	Retention mg.	Percentage
A		1261.56	205.01	229.14	432.15	829.41	66
		1244.32	192.00	298.62	490.62	753,70	61
		1098.94	178.00	266.26	444.26	654.28	60
		1298.52	196.82	301.32	498.14	838.00	65
		1214.76	192.32	290.00	482.32	732.44	60
		1246.78	214.34	291.00	505.34	741.44	59
		1227.48	196.08	279.39	475.47	758.21	61.83
B		1243.20	209.95	367.87	577.82	665.18	54
		1134.86	182.36	362.66	545.02	589.84	52
		1051.39	98.96	357.87	456.83	594.56	57
		1195.00	90.44	352.79	443.23	751.77	63
		1193.47	56.15	359.02	415.17	778.30	65
		1094.03	19.47	366.40	385.87	708.16	65
		1151.99	109.56	361.10	470.66	681.30	59.33

DATA ON NITROGEN BALANCE BY RATS FED DIFFERENT DIETS

Diet No.	N_2 Intake mg.	Urinary Excretion mg.	$\hat{N}_2$ Fecal N_2 Excretion mg.	Total mg.	Retention mg.	Percentage
C	1184.48	162.27	325.60	487.87	696.81	59
	1219.68	133.11	322.82	455.93	763.75	63
	1196.80	146.16	316.83	462.99	733.81	61
	1212.64	148.19	353.24	501.43	711.21	59
	1212.64	111.67	341.99	453.66	758.98	63
	1212.64	110.14	331.04	441.18	771.46	64
	1206.48	135.26	331.92	467.18	739.30	61.50
D	1044.74	89.32	250.30	339.62	705.12	67
	1028.61	55.16	215.19	270.35	758.26	74
	1041.15	51.02	260.43	311.45	729.70	70
	982.02	93.10	273.34	366.44	615.58	63
	967.68	55.69	240.25	295.93	671.75	69
	1039.36	66.86	237.50	304.36	735.00	71
	1017.26	68.53	246.17	314.69	702.57	69.00

APPENDIX VII

BIOLOGICAL VALUE AND DIGESTIBILITY COEFFICIENT OF THE DIETS

Diet		Urinary Nitrogen (mg) I	Endogenous Urinary N (mg) II	I - II (2) mg	Fecal Nitrogen (a) mg	Endogenous fecal (b) mg	a-b (1) mg	Nitrogen intake mg	Nitrogen intake -(1)=X (mg)	Nitrogen intake L-(2) (mg)	BV	DC
A	1	235.62	32.61	203.01	283.53	54.39	229.14	1261.56	1052.42	829.41	80.3	81.8
	2	215.10	23.10	192.00	346.35	47.73	298.62	1284.32	945.70	753.70	79.7	76.0
	3	188.64	10.64	178.00	318.47	52.25	266.26	1098.94	832.68	654.68	78.6	75.8
	4	218.87	22.05	196.82	349.15	47.73	301.32	1298.52	997.20	800.38	80.3	76.8
	5	210.46	18.14	192.32	342.47	52.47	290.00	1214.76	924.76	732.44	79.2	76.1
	6	225.93	11.59	214.34	366.17	75.17	291.00	1246.78	955.78	744.44	77.9	76.7
Mean		215.77	19.69	196.08	334.36	54.96	277.72	1227.48	947.09	752.51	79.3	77.2
B	1	239.350	29.400	209.950	437.320	69.455	367.865	1243.200	875.355	665.385	76.0	70.4
	2.	189.300	6.944	182.360	443.730	111.073	362.657	1134.860	772.203	589.883	76.4	68.0
	3	119.280	20.328	98 .960	432.630	74.760	357.872	1051.390	693.518	594.558	85.7	66.0
	4	126.560	36.120	90.440	463.868	111.075	352.793	1195.200	842.407	751.967	89.3	70.5
	5	67.200	11.046	56.154	441.610	82.590	359.020	1193.470	834.450	778.296	93.2	69.9
	6	41.216	21.756	19.560	435.000	68.530	366.400	1094.020	727.626	708.066	97.0	66.5
Mean		130 48	20·93	109·57	442·36	86·25	361·10	1152·02	790·59	681·35	86.3	68.6

Diet		Urinary Nitrgen (mg) I	Endogenous Urinary N (mg) II	I - II (mg) (2)	Fecal Nitrogen (mg) (a)	Endogenous Fecal(b) (mg)	a - b (1) (mg)	Nitrogen intake (mg)	Nitrogen in take (mg) -(1)=X	Nitrogen intake X- (2) (mg)	BV	DC
C	1	194.880	32.606	162.274	380.000	54.385	325.600	1184.480	858.880	696.606	81.1	72.5
	2	186.210	23.100	133.110	370.550	47.730	322.820	1219.680	896.860	763.750	85.2	73.5
	3	156.800	10.640	146.160	369.050	52.205	316.825	1196.800	879.975	733.815	83.4	73.5
	4.	170.240	22.050	148.190	400.965	47.730	353.235	1212.640	859.410	711.220	82.8	70.0
	5	129.808	18.136	111.672	394.455	52.465	341.990	1212.640	870.650	658.798	75.7	71.8
	6	121.730	11.592	110.138	406.200	75.165	331.035	1212.640	881.605	771.467	87.5	72.5
Mean		154.95	19.687	135.257	386.866	54.946	339.917	1206.480	874.563	722.609	82.6	72.5
D	1	110.880	21.560	89 .320	322.510	72.215	250.295	1044.740	794.445	705.125	88.8	76.0
	2	71.610	16.450	55.160	274.10	58.910	215.190	1028.610	813.400	758.260	93.2	79.1
	3.	79.016	28.000	51.016	312.62	52.205	260.425	1041.150	780.725	729.709	93.5	75.0
	4	122.892	29.790	93.102	327.72	54.385	273.335	982.020	708.685	615.583	86.9	72.2
	5	77.952	22.260	55.692	305.35	65.100	240.250	967.680	727.430	671.738	92.3	75.2
	6	84.000	17.136	66.864	320.10	82.600	257.500	1059.360	861.860	734.996	91.7	77.1
Mean		91.058	22.532	68.525	310.326	64.235	246.165	1017.260	781.090	702.568	91.7	74.3

APPENDIX VIII

NET PROTEIN UTILISATION OF THE DIFFERENT DIETS

Diet	Total carcass nitrogen of test group (mg.) (A)	Total carcass nitrogen of non protein group (mg.) (B)	A - B (mg.)	Food Nitrogen (mg.)	$\frac{A - B}{\text{Food Nitrogen}}$
A	433.55	343.14	90.41	560.40	16.13
B	474.35		131.21	768.40	17.07
C	539.28		196.14	896.82	21.89
D	584.57		241.43	909.98	26.55

APPENDIX X

NET DIETARY PROTEIN CALORIES PER CENT OF THE DIFFERENT DIETS

Diet	N P U	N % of the diet	Calories per gm. of diet	25xN %	$\frac{25 \times N\ \%}{\text{Cal/gm.}}$ (1)	$\frac{N\ P\ U}{100}$ (2)	N D p Cal % (1) x (2)
A	16.13	1.252	2.9	30.8	10.62	.1613	1.71
B	17.07	1.776	2.8	44.4	15.86	.1707	2.71
C	21.89	1.760	2.8	44.0	15.71	.2189	3.44
D	26.53	1.792	2.8	44.8	16.00	.2653	4.25