NUTRITIONAL EVALUATION OF THE SUPPLEMENTARY VALUE OF
LOW COST AND LOCALLY AVAILABLE FOODSNAMELY
HORSE GRAM, FIELD BEANS, SESAME AND
AMARANTHUS TO POOR RICE DIET

P.S. NIRMALA.

A THESIS SUBMITTED TO THE UNIVERSITY OF MADRAS
IN FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY
JANUARY, 1975.

ACKNOWLEDGEMENT

The author acknowledges her deep sense of gratitude to Dr.Rajammal P.Devadas, M.A.,M.Sc.,Ph.D. (Ohio State), Principal, Professor and Head of the Department of Nutrition, Sri Avinashilingam Home Science College, Coimbatore who has stimulated, inspired and encouraged this student of hers towards the culmination of this research. Dr.Devadas's concepts of a long range, concerted and interdisciplinary approach for solving the nutritional problems of India through the use of local foods are reflected in this scientific study. She has been a wonderful mother, guide and mentor throughout, to the author.

Grateful acknowledgements are due to the hundreds of children of varying age groups and their mothers and the women for their full cooperation and participation in the various sections of this study.

The author is immensely thankful to the management of Sri Avinashilingam Education Trust Institutions, for granting permission to undertake the study and for extending generously various facilities such as laboratory, personnel, chemicals and equipment.

LIST OF CONTENTS

Page No.

Page No.

LIST OF TABLES

TABLE NO.		PAGE NO.
XXXXXXVII	INCIDENCE OF SICKNESS IN 400 TARGET CHILDREN	.. 197
XXXXXXVIII	CLINICAL FINDINGS IN TARGET CHILDREN (CLINICAL SYNDROMES)	.. 198
XXXXXXIX	MEAN HAEMOGLOBIN CONTENT IN 100 ml OF BLOOD	.. 199
XXXXXXX	URINARY NITROGEN LEVELS IN TARGET CHILDREN (GRAM/100 ml OF URINE)	.. 200
XXXXXXXI	URINARY CREATININE LEVELS IN TARGET CHILDREN (mg/100 ml OF URINE)	.. 201
XXXXXXXII	RATIONALE FOR SUPPLEMENTAL FOOD QUANTITY	.. 203
XXXXXXXIII	MEAN INITIAL HEIGHT, WEIGHT, AGE, AGE OF MENARCHE AND HAEMOGLOBIN VALUES OF THE SUBJECTS	.. 205
XXXXXXXIV	MEAN NUTRIENT INTAKE OF ADOLESCENT GIRLS WHO PARTICIPATED IN THE STUDY AS REVEALED BY FOOD ANALYSIS (3 DAY FOOD WEIGHMENT SURVEY)	.. 207
XXXXXXXV	MEAN BODY WEIGHT AND HEIGHT CHANGES OF THE TARGET GIRLS (PERIOD OF STUDY 4 MONTHS)	.. 208
XXXXXXXVI	MEAN HAEMOGLOBIN VALUES OF THE GIRLS WHO PARTICIPATED IN THE STUDY	.. 209
XXXXXXXVII	MEAN PCV, MCV, MCH, MCHC AND COLOUR INDEX OF TWO GROUPS OF SUBJECTS SUPPLEMENTED WITH IRON IN THE STUDY	.. 212
XXXXXXXVIII	DAILY TOTAL INTAKE OF VITAMIN A OF THE TARGET CHILDREN	.. 214
XXXXXXXIX	THE EFFECT OF FEEDING 100 g OF CARROTS AND 475 μg OF RETINOL OF SERUM VITAMIN A	.. 215

LIST OF ANNEXURES

LIST OF FIGURES

Figure			Page No.
XX	EFFECT OF A SUPPLEMENTARY NUTRITIONAL SNACK ON BODY HEIGHT GAINS OF SCHOOL CHILDREN (5-8 YEARS OF AGE) OVER A PERIOD OF 10 MONTHS	..	192b
XXI	MEAN FORTNIGHTLY HAEMOGLOBIN CONCENTRATION OF THE ADOLESCENT SCHOOL GIRLS WHO WERE SUPPLEMENTED WITH AMARANTH GREENS OR AN ORAL IRON TONIC	..	211a
XXII	SERUM VITAMIN A VALUES OF POST-ADOLESCENT GIRLS BEFORE AND AFTER AN INTAKE OF 100 GRAMS OF CARROTS (1890 µg OF CAROTENE) AND 475 µg OF RETINOL DAILY FOR A PERIOD OF 20 DAYS	..	216a

I. INTRODUCTION

The last few decades have witnessed tremendous advances in science and technology. Man has split the atom and found his way to the moon. Efforts to harness the scientific knowledge so gained, to augment human welfare, have also increased. India, since her independence, has endeavoured to keep herself in the main stream of technological progress. Giving priority for establishing the requisite facilities for scientific research, she has evolved programmes for application of the results of both generated and adopted research to the welfare of the population. Consequently she has registered massive improvements in health, education, transport, communication and rural development between the year 1947 to 1973 (India 1973). Life expectancy has increased from 32.09 to 57.3 years, the number of primary schools has increased from 2,09,671 to 4,04,418, the route length of the Indian railways has increased from 53,596 Km to 59,790 Km with doubling of the passengers and the net national income calculated at constant prices has doubled. In thousands of villages electric lights, steel plough, water pumps and transistor radios have become part of the land scape ibid. Yet half the population of India suffers from malnutrition and misery.

All these advances have not added to their happiness. The future does not appear bright either (Boerma, 1974). Allevia- tion of hunger and malnutrition is therefore one of the greatest challenges confronting the scientists to day.

The problem of malnutrition in India is largely due to poverty. According to Dandekar (1971), 40 per cent of her population, live below the 'Poverty line'. About a third of the population that is, 189 millions, live in absolute poverty; over 34 per cent eke out a sub human existence in the rural areas with a per capita monthly expenditure of less than 15 rupees, (Fonseca, 1971) (Subramaniam, 1972). On the basis of minimum consumption needs, the per capita annual requirement has been estimated asRs.324/- for the rural and Rs.486/- for the urban areas at 1968-69 prices. Judged by these levels, 55 per cent of the rural and 60 per cent of the urban population would come under the poorer sections of the community living below the Poverty line, Dandekar, (1971). Even if they were to spend 70 to 100 per cent of their income on food, they just cannot afford even the 'lowest' priced balanced diet.

The Indian diet is marked by a preponderance of cereals, which provide 70 to 80 per cent of the dietary protein for the low income groups, (Swaminathan et al. 1972). The surveys carried out by Devadas et al (1971) show that the diets of the rural people are deficient in Calories and in almost all the nutrients. Cantor/AIAO (1973) estimated that one quarter to one third of the Tamil Nadu population consume Calories below the allowances recommended by the Nutrition Expert Group of the Indian Council of Medical Research(ICMR). Portraying the pathetic consequences of malnutrition, Alan Berg (1973) comments, "The light of curiosity absent from children's eyes; twelve-year-olds with the physical stature of eight year olds. Youngsters who lack the energy... . Agonisingly slow reflexes of adults crossing traffic. Thirty year-old mothers who look sixty.... . all reflect inadequate nutrition; all have societal consequences". Kissinger (1975) remarked that no tragedy is more wounding than the look of despair in the eyes of a starving child.

In the midst of the chronically oppressing poverty and its ill-effects, what are the possibilities for combating malnutrition? Improvements in food intake do necessitate economic advancement. However, economic betterment alone cannot guarantee nutritional improvement; it must be accompanied by desirable changes in food combinations, quantities and practices. Increased production and consumption of nutrient-rich animal foods such as egg, milk and meat under the prevailing conditions may not be economically feasible in India. New vistas by are yet to be opened for recommending diets which are adequate and obtainable by the poor man. Improvements in the quality of the

Indian diets can be brought about by increased consumption of the staple foods commonly used along with the addition of legumes and other protein-rich foods, green leafy vegetables and seasonal low-cost fruits such as guava and papaya.

In recent times, a few international organisations have tried to help India to solve her nutritional problems through the use of processed food mixtures such as soy fortified Bulghar Wheat and CSM. However, their efforts have not touched even the fringe of the vast malady. Exotic solutions or ideas may or may not be applicable to our situations. They can never eradicate the entire problem. As the Prime Minister, Indira Gandhi (1974) observed, "There shall have to be an Indian solution with Indian resources for what is essentially an Indian problem". The solution needs to be found through indigenous efforts (Gopalan, 1973). The challenge is therefore to develop the natural resources and evolve low-cost nutritious and acceptable recipes based on local foods to supplement the customary diets. (Devadas et al, 1973). Their nutritional superiority should be first established, in the laboratories and through field trials. The next essential step is educating the masses regarding the use of the proven low-cost foods. While supplying adequate amounts of high quality protein and calories to the increasing population is a tremendous task (Bressani, 1973), some efforts along these lines are being undertaken in some parts of the country. A great deal is yet to be accomplished.

Among the few nutritional formulas developed by different research institutes, Balahar (Kymal, 1973) and Indian MPF, (Doraiswamy et al. 1964, 1965 and Daniel et al. 1967) are some examples. However, these centrally processed foods pose some unsurmountable problems in distribution such as transport, storage and cost (Devadas, 1973). Therefore development and production of nutritional recipes based on inexpensive local foods, involving minimal processing appears to be the solution, as demonstrated by the National Institute of Nutrition, (NIN, 1972), Institute of Rural Health and Family Planning, Gandhigram, (Pisharoti, 1973), CARE, Kerala (Napier, 1973), Sri Avinashilingam Home Science College (Devadas et al. 1974), and others.

A significant increase in cereal grain production from 82.0 million tonnes in 1961 to 107.8 million tons in 1971, was achieved in India during the last decade giving rise to the 'Green Revolution'. (Swaminathan, 1973). The potential of legume grains as sources of greater amounts of protein of improved quality is often discussed, but popularising their role in improving the nutrient intake has been neglected. Research necessary to increase their yield and to correct certain defects in their nutritional and 'food-use' qualities, is yet to be undertaken, (FAO, 1973). There are some encouraging indications that the agronomic and nutrient improvement of legume grains is a possibility (Swaminathan, 1972).

Among the food crops, legumes supply a major part of protein and many other nutrients to a major section of our population. Legume grains contain the highest amount of protein, more than twice the level found in cereal grains. Furthermore, the protein of the legume grains is rich in lysine, but deficient in the sulphur containing amino-acids, in contrast to the cereal grain proteins, which are low in lysine but adequate in the sulphur containing amino acids (Elias *et al.* 1964, Bressani *et al.* 1971). Therefore legume proteins can supplement the rice and other cereal proteins favourably. The role of legumes in crop rotation is also significant. Legumes enrich the soils which are depleted by successive raising of cereal crops. This doubly benificial role of legumes in improving the nutritive value of the diet and maintaining the fertility of the soil warrants more concentrated effort in the cultivation of legumes than taken hither to.

India abounds in varietiesof dark green leafy vegetables, many of which are edible and inexpensive. However, they either remain not utilised or under-utilised. Their easy availability and low cost, have accorded them a low social status. Even nutritionists have not adequately emphasised the importance of edible leaves in human diets, (PAG, 1973).

Among the edible-green leaves, the darker leaves are nutritionally more valuable than the pale green ones. The young tender leaves are more digestible and contain less fibrous material. Nutritionally, many edible leafy vegetables are excellent sources of carotene, folate, niacin, iron, vitamin C and calcium. They are of special importance in the prevention of a avitaminosis-A, which is a major cause of blindness among young children in India. Next to Protein Calorie Malnutrition (PCM) and avitaminoses, iron deficiency anemia is a major health problem among children and mothers in India. Legumes and green leafy vegetables can serve as excellent supplements to the Indian diets in overcoming their deficiencies (Devadas et al. 1973).

The average per capita availability of Calories in India is only 1945 (Gopalan et al. 1971), as against a stated requirement of 2357. In the presence of Calorie deficiency, the protein intake is utilised as a source of energy and thus wasted. The Indian diets are deficient also in several vitamins and minerals. Deficiencies of vitamin A, riboflavin, ascorbic acid, iron and calcium are common. Therefore the diets need to be improved in a number of directions, quantitatively and qualitatively. In this effort, the efficacy of using larger quantities of the many indigenous varieties of cereals and legumes along with green leafy vegetables needs to be explored.

The concept of an indigenous supplementary food is a recent one. A major effort taken in this direction by the Government of India resulted in the large scale production of 'Balahar'. The main problems inherent in such efforts designed for all India consumption are non-confirmity with the local food habits and dietary requirements and problems in transport and distribution. Some commercial organisations have also ventured to produce nutritious food supplements such as 'Balamul'. However, they are expensive and beyond the reach of those segments of the population who need them most. Thus, the efforts of various national agencies in the development of low cost indigenous foods although laudable have not been in conformity with the local food habits, food availability, dietary requirements and purchasing power of the masses. The present study aims at overcoming some of the bottlenecks in the use of indigenous foods, with the following specific objectives:

To develop a series of food formulations utilising several indigenous foods for use in feeding programmes and to promote self reliance

To evaluate the food formulations developed, for their nutritional adequacy and superiority over the basal diets through laboratory trial with albino rats

To assess the acceptability and palatability of the food formulations and their recipes to the target beneficiaries

and To further evaluate the food formulations through a series of human feeding trials.

The studies reported here are some exploratory attempts in utilising selected low-cost local foods, towards the alleviation of malnutrition and under-nutrition, particularly among children. The rice based diets which are commonly consumed in the rural areas around Coimbatore, Tamil Nadu were supplemented with selected low-cost, local vegetable foods in such combinations as to result in higher protein quality. Their growth promoting abilities, iron availability and carotene utilisation have been studied through a series of rat assays and human feeding trials. Each of the series consisted of different combinations of indigenous foods as supplements. The human feeding trialsinvolved different age groups, ranging from the young pre-school child to adult college women. Involving different age groups was necessiated due to the fact that human feeding trialsare laborious, time consuming and requires abundant support and co-operation among the participants in contrast to animal studies.

The first study was on vegetable protein mixtures, formulated with local foods useful for children and their evaluation on albino rats using PER, NPU and NBP cals.% as the criteria. Analysis of tissues and metabolic samples (urine and faecal) were also used as indices for nutritional evaluation. The effect of three selected dietary sources of proteins, namely, a combination of red gram dhal and sesame at a protein ratio of 2:1, defatted fish-flour and skim milk powder, on carotene utilisation in albino rats was also studied.

The next study was with a weaning mixture based on a local cereal, sorghum, and legume, Bengal gram dhal (roasted), evaluated through a feeding trial for six months on young children in the age group 15 to 36 months, in a rural area. In another study, the effect of diets based on a high lysine maize, ordinary maize and skim milk on the nutritional status of children 18-30 months of age, was investigated.

In the next experiment, a nutritious supplement evolved with low-cost indigenous foods, namely, rice (Oryza sativa), cowpea (Vigna catjang), horsegram (Dolichos biflorus), field bean (Dolichos lablab) and sesame seeds was evaluated on school children belonging to a village community. Through another investigation the effect of supplementation of amaranthus, (a green leafy vegetable) carrying 24-30 mg. of iron to adolescent anemic school girls, was compared with the supplementation of the same amount of elemental iron to another identical group.

The last experiment was designed to find out the extent of utilisation of carotene from carrots by a group of college women, through the estimation of serum vitamin A, before and after consuming daily 100 g. of cooked carrots for 20 days.

In all the human feeding experiments, growth rate for the pre school and school children, clinical tests, biochemical analysis of blood and metabolic samples were carried out. The nutritional evaluation of the food supplements, consisted of before and after comparisons of these parameters.

The investigator hopes that the findings of this study on the indigenous food formulations and their evaluation for their nutritive value, palatability, acceptability, growth promoting abilities and cost will stimulate self reliance and reduce the country's dependence on imported foods for the numerous nutritional feeding programmes for pre school and school going children.

II. REVIEW OF LITERATURE

The review of literature relevant to this investigation is presented under the following main headings:

A. The existing diets in Tamil Nadu

B. Use of legumes and green leafy vegetables in supplementing rice and other cereal based diets

C. The use of dark green leafy vegetables in young child feeding

D. Formulation of low-cost indigenous food mixtures for children

E. The role of genetics on the development of highly nutritious products

F. Dietary iron and detection of iron-deficiency anaemia

G. Biological availability of the pro-vitamin-carotenoids.

The Existing Diets in Tamil Nadu

Under the auspices of the ICMR, a number of dietary surveys and studies were conducted between 1960-69. They give an idea of the mean consumption of Calories and nutrients in the different states. Tamil Nadu ranks lowest with regard to the mean consumption of calories and proteins.

Gopalan, et al (1971), and Narasinga Rao, (1971) found that the mean intake of Calories of preschool children was 725, while that of protein was 1.9 g. per kg. of body weight. The protein intake could be considered adequate. The daily calorie intake was very low being the order of 70 to 75 per kg of body weight, as against the 100 K cals/kg which is generally considered adequate for children of this age group. Thus the major bottleneck in the current dietaries of the pre-school children in the poor income groups appear to be Calories and not proteins, (Vinodini Reddy, 1968, Ambalini Bailur, 1970, Swaminathan et al, 1970, and Gopalan, 1970). If the children had been given greater quantity of the same cereal legume based food in amounts that would meet their Calorie requirements, their protein needs would also have been met.

In contrast, the per capita intake of Calories and proteins among adults is highest in Tamil Nadu. The surveys show that adults in Tamil Nadu consume on an average slightly over 2,100 calories and 61.6 g. of proteins per day. The daily Calorie intake of school children (6-15 years of age) is 1560 in Tamil Nadu. Their consumption of protein is 45.7 g. . The average daily Calorie intake of preschoolers (0-5 years of age) is 950 and of proteins 27.8 g. in Tamil Nadu.

The Food Habit survey in South India conducted by the Protein Food Association of India, (1972) also shows that as a result of low income, the greatest sufferers from the nutritional stand point are children aged between 6 and 15 years; while adults are less affected by income levels.

The per capita food consumption in Tamil Nadu was compared by ICMR 1971 based on 115 surveys. Except for cereals, the intake of all other foods was much below the levels recommended by the ICMR. The staple diet consists of rice, and the millets cumbu (Pennisetum typhoideum), Cholam (Sorghum vulgare) or ragi (Eleusine coracana) to which a few grams of Red gram dhal are added in the preparation of sambar. Some amount of Black gram is consumed through iddlies or dosais*. (Protein Associates of India).

Consumption of green leafy vegetables in Tamil Nadu is negligible quantity of 10g. per day. Consumption of other vegetables depends on seasonal availability. The amount is often inadequate, 33 g. on an average. Fats, oils, sugar and jaggery which are sources of calories are consumed in very small quantities, around 8g. Milk and milk products are scarcely taken, even though people are aware of the importance of these foods. The average per capita daily milk consumption, 40 to 60 ml. Meat, fish and eggs are consumed rarely on an average

---

* Idli - Traditional fermented cereal legume break fast preparation
Dosai - Similar to rice pan cake.

11g. per day. Consumption of fish is somewhat better along the coastal areas (PKND, 1974, Devadas et al 1971 and Easwaran, 1971).

A Statewide Food Habit Survey was conducted by the Tamil Nadu Census Department in 1961, based on enquiry, involving recall. The survey recorded a mean daily consumption of 2500 Calories per Consumption Unit. Although this calorie intake is adequate, the mean consumption of foods other than cereals is lower than the recommended levels.

Judged by the monthly consumption expenditure pattern Compiled by the National Sample Survey (Department of Statistics, 1971), nearly 55 per cent of the house-holds spend less than 79 paise which represents the quantified "Poverty line".

When the consumption pattern of the different segments of the population particularly of the vulnerable groups, pre-school children and expectant and nursing mothers is analysed, percentage of Calorie requirements met are far below the recommended allowances among the pregnant and lactating women, (Devadas et al 1971, Easwaran, 1971 (Loc Cit) and children between the ages of 6 months and four years and most particularly children in the 7 to 18 months age group. Protein deficiencies also follow the same pattern.

No special food is provided for weaned infants in Tamil Nadu except perhaps an occasional 'Appam', 'Idly' or Biscuit. Instead of milk, an apology for coffee is given which only depresses the appetite (Raju, 1972).

A fully weaned child in the age range of 7 to 18 months who gets 65 per cent of its Calorie needs fulfilled, fares much better than his counterpart who is still receiving 75 per cent or more of the Calories from breast-milk. This latter category of children is the most deprived of all the sub groups shown. His condition reflects a popular misconception, that the mere existence of breast feeding constitutes adequate nutrition for the child (Devadas, 1974).

Thus an analysis of the earlier surveys indicate the need to increase the intake of Calories and nutrients. If the calorie gap is filled, the protein need is likely to be satisfied also. Of other nutrients, deficiency of vitamin A is crucial.

Use of Legumes and Green Leafy Vegetables in Supplementing Rice and Other Cereal Based Diets

Legumes:

Legumes, also known as pulses, constitute an important article of food, all over the world. Their use is wide-spread in the tropics and the subtropics. They are the main source of protein in some traditional diets. They are consumed in relatively large amounts where foods of animal origin are expensive or in short supply (Roberts, 1973). Although there are over 13,000 species of the family "leguminosae" the following 18 are extensively cultivated for human consumption: Arachis hypogaea (groundnut) Cajanus cajan (pigeon pea), Cicer-arietinum (chick-pea), Dolichos biflorus (horse gram), Glycine max Merr (soya bean), Lablab niger (hyacinth bean), Lathyrus sativus (khesari dhal), Lens esculenta (lentil), Phaseolus mungo (black gram), Phaseolus aureus Roxb (Green gram), Phaseolus vulgaris (kidney bean), Pisum sativum (Pea), Pisumarvense (field pea), and Vicia faba (broad bean), Vigna Catjang (cow-pea). Of these, groundnut and soybean are cultivated primarily for their oil content (FAO, 1973).

There are wide variations in the amounts of legumes eaten, depending upon the agricultural conditions, locations whether rural or urban and income levels (Aykroyd and Doughty, 1964). Certain types of legumes are preferentially cultivated and consumed in certain parts of the world. There appears to be an inverse relationship between the availability of legumes and animal foods. In some Asian countries such as India and Japan, the consumption of legumes tends to increase with increasing income, while, in countries like U.S.A and Italy, the opposite trend is noted (Kurtha and Ellis, 1970).

Chemical Composition and Nutritive Value:

The chemical composition and nutritive value of the more commonly used legumes have been determined by many workers. In general, legumes contain 18 to 25 per cent protein, except groundnut and soyabean, which have a higher content. Legumes are also fair sources of thiamine, riboflavin, niacin and iron, but relatively poor sources of calcium and fat. The dry grain legumes contain little ascorbic acid, while sprouted legumes have an increased content of thiamine, riboflavin and ascorbic acid (Bressani and Elias, 1974).

Nutritionally, legumes are recognised as the highest sources of plant protein, upto 25 per cent in dry grains, with Soy having upto 40 per cent. The limiting essential amino acid in the legume protein, is methionine, while that in cereals is lysine. Legumes are relatively richer sources of lysine, which is the limiting amino acid in cereals and millets, therefore a judicious combination of cereals and legumes, can improve the amino acid balance of the diet as a whole (Aykroyd, 1964). Appropriate cereal - legume mixtures can make the range of essential amino acids more complete. Examples of such mixtures are the Tortillas (corn) and Frijoles (beans) of Mexico; Rice and dhal (legume) of India, and Rice and soy preparation of China (FAO, 1973).

The fact that it is possible to maintain good health on a carefully selected vegetarian diet with dhals, reflexes the nutritional value of legumes. Even severe Protein Calorie malnutrition can be cured by an appropriate mixture of cereal and soy, peanut or other legume protein without adding any animal protein. However, there is a wide variation in the nutritional composition, biological value, flavour and cooking properties of legumes, not only between different legumes, but also between different strains of a single variety (Patwardhan, 1962).

Most legumes, when properly cooked, have a digestibility ranging from 85 to 90 per cent (Srikantia, 1973). Many legumes contain trypsin inhibitors, which are destroyed either by ordinary heating or by more elaborate heat treatment (Borchers and Ackerson, 1950). Germination also improves the biological value of some legumes, by more than 20 per cent.

In addition to trypsin inhibitors, the presence of a variety of toxic substances like haemagglutinins, saponins, alkaloids, cyanogenic glucosides and goitrogenic factors has been reported in a number of legumes. Nevertheless there is little evidence to show that the presence of these factors is associated with frequent or serious untoward developments. Symptoms such as nausea, vomiting and diarrhoea do not occur when these legumes are cooked (Liener, 1962).

Among the toxins present in the legumes, two are of some concern (a) the toxin present in Lathyrus sativus (Khesari dhal), the consumption of which is associated with human neurolathyrism and (b) the toxin present in Vicia faba (broad bean), the consumption of which is associated with favism. The toxic principle in Lathyrus sativus has not been fully understood as yet, but it is believed to be in some way related to the presence in the seed of an unusual amino acid, Oxalyl Amino Alanine (BOAA), (Roy *et al.* 1963). This compound is water soluble and can be removed easily by steeping the seeds in hot water and discarding the steep-water or by parboiling the seeds, a procedure which minimises the loss of water-soluble nutrients, which occurs in the first process.

Haemolytic anaemia, which is a characteristic feature of favism, is sometimes seen in some Mediterranean countries, following the use of uncooked broad beans. Simple heating of the beans renders the seeds safe for human consumption, (Mager *et al.* 1969).

<u>Protein Quality</u>:

The Biological Value of various commonly used legumes has been found to vary between 40 and 78, and their Protein Efficiency Ratio between 0.7 and 2.1, (Patwardhan, 1962).

Not only are these variations noted between legume species, but also between members of the same species. This is as much due to differences in the details of experimental procedure as to the differences in chemical composition of the different varieties of the same legume.

<u>Practical Approaches to Feeding Legumes to Children:</u>

<u>Selection:</u>

The type of legumes to be used for feeding young child will depend initially on those available in the particular area. Selection will then be based on cultural preferences, nutritional composition, culinary attributes, including cooking time and apparent digestibility.

On a world basis, most of the attention has been concluded on the use of three legumes in weaning foods namely, the Chick Pea (Cicer arietinum), ground nut and soy bean. A large number of weaning food mixtures for feeding infants, six to twelve months old or more, have been developed by many workers all over the world including India. Their acceptability tolerance and efficacy in terms of promoting growth have been tested and founds satisfactory. Most of the food mixtures have been developed for commercial distribution containing various types of legumes in different amounts as indicated below:

1. Wheat flour, Bengalgram, lentil, milk and sugar and Superamine (Algeria).

2. Wheat (7), soy or groundnut (1.5), Bengalgram (1.5), Balahar, (India).
3. Groundnut, dried milk, wheat and barley: Lactone (India).
4. Groundnut (3), Bengalgram (1), dry skim milk (1) Multipurpose Food, (India).
5. Soy, sesame, groundnut, fat, sugar SARIMBIH, Indonesia.
6. Wheat (6), Bengalgram (3), milk (1), Laubina (Jordan).
7. Groundnut (3), dry milk (1), Arlac (Nigeria).
8. Millet (4), groundnut (2), dry milk (2), sugar (2), Ladylac, (Senegal).

Figures in Parenthesis indicate the proportions in which the ingredients are used.

The inclusion of milk has always been found to improve the nutritional quality of the mixtures. The amount of legumes in these preparations varies between 20 and 80 per cent, either from a single source or from a combination of two legumes. Only three legumes, soy, Bengalgram and groundnut, repeat themselves in all these preparations.

<u>Problems associated with Inclusion of legumes in the Diets</u>:

Some problems associated with the inclusion of legumes in the diets of infants and children, are endogenous and exogenous toxins, flatulence and cooking time.

Endogenous Toxins:

The presence of various types of toxic substances in legumes has been a vexing problem. Since simple processing procedures like heating, boiling, toasting, puffing and autoclaving which can be employed at the household level, can render the toxins ineffective, it should be possible to eradicate these toxins from legumes before consumption.

Exogenous toxins:

The problem of fungal contamination of groundnut acquires special importance in view of the increasingly greater use of groundnut flour for feeding infants and children. Malnutrition affects adversely the response of children to many toxins. The effects of aflatoxin have been demonstrated to be more severe in malnourished animals. Since most communities which are likely to use groundnut containing feeds are likely to be undernourished also, the question of quality control becomes very important one, (Jaswal, 1961). Strains of groundnut, which though infected with A. Flavus, do not elaborate the toxin, have been identified. The use of such varieties, coupled with improved harvesting and storage practices may control effectively this problem.

Flatulence:

One of the problems associated with the ingestion of legumes is the formation of intestinal gas. The inclusion of beans providing about 25 per cent of Calories in the daily diets has been found to produce 4 to 10 fold increase in gas formation (Steggerda and Dimmick, 1966). There are wide variations in the flatures - producing capacity of legumes, Bengal gram, soy beans, lima beans and navy beans appear to be particularly active. Flatulence arising from the consumption of legumes is an important aspect, from the point of view of acceptability. Many people associate flatus with poor digestibility and hesitate to include legumes in the diets of infants, young children and the sick (Narayana Rao *et al.* 1973).

Studies in man have shown that much of the gas formed is Carbon-di-oxide, (Steggerda, *et al.* 1966). Studies in animals have shown that flatulence is not due to the high fiber content, since indigestion of methylcellulose does not increase flatus. It does not appear to be due to intestinal microbial action either since the use of antibiotics does not always limit gas formation (Kakade and Borchers, 1967). Heated legumes always produce less gas than raw legumes.

Studies on four commonly used legumes in India, namely, Bengal gram (*Cicer arietinum*), red gram (*Cajanus cajan*), black gram (*Phaseolus mungo*) and green gram (*Phaseolus aureus*), have shown that the rate of in vitro amylolysis, which is an index of digestibility of carbohydrates, was lowest in Bengal gram and highest in green gram, with the other two falling in between. The rate of amylolysis is related to the amylose content. Regardless of whether or not the whole legume was used, cooked or raw, or the isolated Carbohydrate was used the trends, were similar. Of these four legumes, Bengal gram has the greatest potential to produce gas, (Ramasastri and Srinivasa Rao, 1969).

<u>Cooking Time</u>:

Many legumes and beans do not cook easily and must be cooked for much longer periods of time than cereals and millets. In these days of fuel shortage it is desirable to develop simple methods to reduce cooking time. Cooking with papain has been reported to cut cooking time by nearly 50 per cent, (Martin *et al.* 1967).

<u>Methods of Preparation</u>:

<u>Boiling and Cooking</u>:

The most common method of preparing legumes for consumption is to dehusk the seeds, soak them in water, cook to a soft them in water, cook to a soft pulp and spice before use. This procedure is simple, and in addition to inactivating the toxic principles in the seed, it enhances also the Biological Value, (Srikantia, 1973).

Roasting and Puffing:

Roasting and puffing of legumes are conventional methods of preparation. These procedures have the advantage that they impart a desirable flavour and taste, in addition to removing the toxic substances. Also, they impart an additional gain in that they do not increase the bulk of the legume, as boiling does. Roasted and puffed legumes may be powdered, stored and used as "Ready-to-Eat" preparations for older infants and young children, (Jelliffe, 1967).

Sprouting:

Many legumes germinate and sprout within 24-48 hours, after the seeds have been soaked thoroughly in water, and spread out. The sprouted seeds can be eaten with or without addition of spices. Sprouting has several advantages. The B-complex vitamins and ascorbic acid increase in contents considerably. The availability of iron is also reported to improve, as a result of a decrease in phytin phosphorus, which is known to inhibit iron absorption. The Carbohydrate moiety also undergoes changes, becoming more digestible.

Germination does not however, remove all the toxic material found in legumes, (Cameron and Hofvander, 1972).

Fermentation:

Fermented preparations from the legumes soy bean, groundnut and black gram, are being extensively used. They are readily accepted and the nutritive value of the seed after fermentation is generally believed to be better than that of the unfermented product (Jelliffee, 1968).

The Use of Dark Green Leafy Vegetables in Young Child Feeding

An immense variety of dark green leafy vegetables exists, many of which are known to be edible. They are inexpensive, high-yielding, already part of the local diet and often easily available, but they frequently have a low status. Among edible green leaves the darker leaves are nutritionally more desirable than the pale green ones; however, the young tender leaves are more digestible and contain less fibrous material (Oomen, 1964, Devadas, 1973).

Nutritional Value:

Nutritionally, many edible leafy vegetables are excellent sources of carotene, folate, niacin, iron, vitamin C and calcium. They are of special importance in the prevention of avitaminosis A, which is a major cause of blindness in young children particularly in some Asian countries. Green leafy vegetables (GLV) are considered as the best supplements for vitamins and minerals and as a minor source of protein when used in home made weaning mixtures. They contain 3 to 5 per cent protein in the fresh state and between 20 to 49 per cent when dried. Moreover, the protein is usually high in lysine and tryptophan. Therefore they are valuable in supplementing the amino acid deficiencies of cereals and in improving the protein quality of cereal-based diets in much the same way as legumes do. They may be eaten as fresh leaves, particularly the young tender leaves, or in a powdered form obtained by sun-drying and/or smoking (Terra, 1964).

<u>Some Common Edible Leaves</u>:

The edible GLV range from leaves of such plants as cassava, sweet potato, colocasia and many others to a variety of different types of amaranthus and wild species (Stanley and Lewis, 1969). Extensive work has been done on cassava leaves, which are stated to contain between 4 per cent protein in fresh young leaves and 30 to 40 per cent protein when dried. (Terra, 1964).

GLV are rich sources of a number of essential nutrients and their inclusion in diets greatly enhances the overall nutritional quality. Although there are wide variations in the chemical composition of the commonly used edible varieties, 100 g of edible portion of GLV generally contain 2.5 g. of protein, 400-1000 mg. of calcium, 20-30 mg. of iron, 3000-10,000 mcg. of carotene, 50-100 mg. ascorbic acid and 150-400 mcg. of riboflavin. The protein concentration is low, and because of the small amounts in which they are consumed, they do not contribute to the total protein intake to any significant extent (Shanley, 1969). Many GLV contain relatively large amounts of oxalic acid which is believed to limit the availability of calcium, (Davidson and Passmore, 1970). Similarly, the availability of iron in GLV is shown to be only between 3 and 5 per cent due to the presence of phytin phosphorus. Not withstanding these factors, GLV provide good amounts of calcium and iron, because of their very high concentrations (Devadas et al. 1971; 1972, 1973). GLV constitute the major, if not the sole, dietary source of vitamin A (β-carotene) for larger segments of preschool children in many developing countries (Pereira and Begum, 1968). There was a significant increase in serum vitamin A after feeding the green leafy vegetables among undernourished pre school children in South India (Lala and Reddy, 1970).

<u>Amounts of GLV that can be fed:</u>

The amount of GLV which must be included in a diet, depends upon whether it is consumed in the raw state or after being cooked. Further more, the nutrient content of GLV is known to be modified by storage and cooking procedures. Cooking does not alter the calcium and iron content of GLV, but lowers significantly the concentrations of most of the vitamins (Kamalanathan et al. 1972). Losses in carotenes are not marked, but ascorbic acid is lost to a great extent, (Kamalanathan et al. 1972). As much as 50-70 per cent of the ascorbic acid originally present may be destroyed by the conventional house-hold cooking methods. After cooking, the riboflavin, folate and ascorbic acid levels may be only one third to one half of the amounts originally present. Cooking under pressure does not seem to modify these losses. Considering all these factors, consumption of 30 g. of raw GLV per day by a preschool child, can provide approximately 150-300 mg., calcium, 6-10 mg. iron, 1000-3000 mcg. carotene; 15-40 mcg. folates, 50-150 mcg. riboflavin and 15-30 mg. ascorbic acid. These amounts to contribute towards meeting a considerable proportion of the daily needs for these nutrients (Oomen, 1967 loc cit).

While consumption of raw GLV has some advantages, one of the important considerations of their use in the raw state, particularly in the rural areas of developing countries, is the risk of introducing bacterial infections and parasitic infestations. Bulk will not pose any problems in the feeding of GLV, since there is considerable shrinkage in volume after cooking (Cameron and Hofvander, 1971, Loc cit).

<u>**Problems Connected with Feeding of Green Leafy Vegetables:**</u>

<u>**Acceptability:**</u>

One of the major problems in including GLV in the diet is the strong food beliefs and prejudices held by many rural communities against GLV. They are believed to be unsuitable foods for infants, children and even expectant and nursing women. GLV are considered to be "cold" foods which predispose the consumer to gastrointestinal upset. In addition, GLV are considered to be 'non prestige' foods. Educating the mother regarding the nutritional qualities of GLV, coupled with demonstration of suitable recipes, is perhaps the most important step to be taken to make her accept GLV (Oomen, 1967, Devadas 1973).

Formulation of lowcost indigenous food mixtures: Possibilities of Improving the Dietary Intake of Children through Indigenous Foods

The immediate solution to the serious and widespread problem of malnutrition among children in India lies in using low cost indigenous nutrient-rich foods. The nutritional status and health of children could be improved considerably if mothers were persuaded to feed their children the required quantities of cereal-legume-mixtures made from the indigenous foods available, supplemented with GLV.

Scarcity and high cost have prohibited the wide use of animal foods by people, in the low economic levels. Successful attempts have been made by several workers to utilise protein rich foods of plant origin such as oil seed meals and legumes as supplement to the poor diets consumed by children (NAS, NRC, 1961, NAS 1966 and Daniel et al. 1967).

Examples of different types of formulation of low cost food mixtures developed in several developing countriesare given by Cameron and Hofvander (1971) as under.

1. Incaparina developed in Guatemala in Central America based on a staple cereal with the addition of vegetable protein concentrate.
2. 'Superamine' produced in Algeria based on wheat and high percentage of precooked peas and lentil which provide most of the protein with a small percentage of skim milk powder.

3. 'Faffa' in Ethiopia which is mixture of cereal (wheat) and a legume (chick pea) with the addition of the vegetable protein concentrate (soy flour) and a small amount (5 per cent) of dried skim milk.

4. Bal-Ahar in India which is a blend of parboiled wheat (Bulgar wheat), edible groundnut flour and and skim milk powder. The protein of the food is about 20-22 per cent its protein Efficiency Ratio is about 1.8 - 2.0 (Kymal, 1973). Due to shortage of milk powder, it has been suggested that Bengal gram flour could be used as a substitute, Balahar as prepared currently has the following composition; 70 per cent parboiled wheat, 15 per cent edible groundnut flour and 15 per cent soy bean flour.

5. CSM supplied by CARE from USA, is a blend of a corn (68 per cent) defatted soya (25 per cent) and skim milk powder (5 per cent).

Apart from scientific considerations, imported feeds have various bottlenecks like cost of transport, storage and distribution. According to Gopalan, (1973) there is no major problem of malnutrition in the country which cannot be solved by locally available feeds.

A suitable cereal-legume-GLV mixture as supplement would help to make up the deficiencies of nutrients in the existing diets of school children (Gopalan, 1973). Some encouraging reports have emerged from these efforts in this direction (Doraiswamy, et al 1965) showed that daily supplement of 53 g. of protein feed based on 4:2:2 blend of groundnut, Bengal gram and sesame flours brought about a highly significant increase in the growth and nutritional status of children. The basal diet consumed by the children was deficient in protein, calcium, vitamin A and riboflavin when compared with the allowances recommended by the ICMR.

Bhagavan et al (1962) conducted a feeding trial with a feeding trial with a blend of cereal grains, legume seeds, oil seed meals, leaf protein and isolated plant proteins. The results of this vegetable protein mixture compared well with that of milk protein.

Realising the importance of self reliance, the Government have stressed the need to use local foods with simple processing, capable of being prepared by the village communities.

Hence, utilising indigenous protein-rich sources is a right step in fulfilling the protein gap. The main sources of protein for such combinations are the cereals, legumes, oilseed meals, leaf proteins, yeast and isolated plant proteins (Scrimshaw and Bressani, 1960; Anfour et al, 1965; Register et al 1967 and Yadav and Bharadwaj, 1971 Autret et al. 1955).

Various blends of protein mixtures have been formulated and evaluated in several laboratories in India. Most of these low-cost vegetable protein mixtures have been prepared by using flours and concentrates maintaining the cost of the final product somewhat similar to that of the staple foods (Srikantia and Gopalan, 1960, Gupta, 1972). Studies carried out during the last few years at the Central Food Technological Research Institute (CFTRI), National Institute of Nutrition (NIN) and Sri Avinashilingam Home Science College have shown that low cost vegetable food mixtures containing adequate amounts of protein of fairly high Biological Value are quite effective in overcoming the problem of malnutrition (Phansalkar et al. 1958, Venkata Rao et al. 1964, Nirmala et al. 1966, Register et al. 1967, Prasanna et al. 1969, Kamalanathan et al 1970, Devadas and Jamala, 1971 and Gopalan et al. 1973).

Srikantia and Gopalan (1960) formulated protein mixtures with Bengalgram flour, groundnut flour and sesame flour in various proportions as a supplement for children suffering from Kwashiorkor. Tasker et al (1960) noted that the vegetable protein mixtures were good supplements to poor diets. Protein foods based on blends of groundnut, soyabean and sesame flour with a protein content between 45 to 48 per cent when fed at 16 per cent level were as effective as skim milk powder in promoting growth in rats (Krishnamurthy et al. 1960). Daniel et al (1964) reported the effectiveness of low cost balanced foodsbased on blends of ragi or maize, groundnut, Bengal gram, soyabean and sesame flours fortified with limiting amino acids on weaned infants. Kamalanathan et al (1970) tested a protein food mixture based on groundnut meal, sesame meal and horsegram on preschool children. The increases in height, weight and haemoglobin level of the children receiving the blend were significantly higher than those of the control group. Prasanna et al (1965) proved that the blends of vegetable protein mixture were as effective as skim milk in treating Kwashiorkor. When pre-school children, suffering from Kwashiorker were treated with a blend of soya flour, pea nut flour and sesame flour, they showed higher digestibility (78.3 per cent) and nitrogen retention (58.8%) than normal children. A seven month feeding trial with groundnut protein isolate and bengal gram flour supplemented with vitamins and minerals havebeen conducted by Dumm et al (1966) using children 1-5 years of age. Eighteen children received the supplement and eighteen others served as 'controls'. A significant increase was seen in the height of the children on the protein food supplement compared to an isocaloric control group. Dumm et al (1966) reported a feeding trial conducted on the preschool children with a blend of groundnut protein isolate and bengal gram flour known as K.P.F. Statistically significant deferences in heights and weights were seen in the experimental children over the control

Deshmukh and Sohonie (1965) found green gram to be a good supplement to cereals and reported that the essential amino acid composition of greengram was comparable with those reported for other members of the dicotyledenous family. A protein rich mixture of vegetable foods consisted of 47 per cent autoclaved chick peas, 35 per cent defatted sesame flour, 18 per cent heat processed low fat soya bean flour and 30 per cent protein was developed by Guggenheim and Szmelcman (1966). The vegetable protein mixture was well accepted and tolerated by infants and feeding trials resulted in weight increase and retention of nitrogen.

Prasanna et al (1969) studied the net protein utilisation of an infant food containing 70% of plant protein and 30% of millet protein and found that the Net Protein Utilisation (N.P.U.) was comparable to that of the formula based on buffalo milk. Feeding trials with weaned infants have shown that incorporation of vegetable protein mixture in their diets brought about a marked improvement in their growth and general health, (Swaminathan 1968). Devadas and Jamala (1971) demonstrated the efficacy of a weaning mixture based on maize-greengram-groundnut flour to preschool children. Heredia et al (1971) reported an infant feeding trial with a weaning food supplement. A weaning food supplement and the supplement fortified with methionine were fed to two groups of children aged six months to provide one third to one quarter of daily calories, for a period of one year. A control group of children were given an equal quantity of a milk food. The differences in heights and weights of the children on the weaning food and those given the milk food were not of statistical significance. Subba Rao and Ramamurthi Rao (1972) found peanut flour to be a good supplement to raise the protein level of various special foods for feeding infants. Peanut protein isolate contains about 90 per cent protein and is free from fibre, Madhavi et al (1973) have evaluated the feasibility of supplementing the home diets of preschool children with a vegetable protein mixture based on wheat, groundnut, Bengal gram, sugar and dry skim milk.

Devadas (1972) conducted a feeding trial for a period of one year with the locally available low cost protein rich food on the pre-school children of 1-3 years. A significant increase was seen in the heights and weights of the children fed with the infant food over the control children. Gopalan (1972) had developed, 'Hyderabad mixture' based on wheat, groundnut and green gram.

Thus the use of vegetable protein mixtures had been extensively studied from different analysis in terms of their efficacy in promoting growth of children.

The Role of Genetics on the Development of Highly Nutritious Products

During this decade research has achieved a major break through in plant genetics and a formidable approach to the development of highly nutritious food products, particularly among cereal grains and legumes which are more commonly consumed by the great majority of the world population. This approach could be considered as genetic fortification. Mertz, Bates and Nelson (1964) reported that a strain of maize homozygous of the recessive mutant gene, Opaque-2, had a significantly higher lysine and tryptophan content than the normal maize.

In the case of Opaque-2 maize gene an increase in the essential amino acids, lysine and tryptophan, has been achieved by reducing the concentration of alcohol soluble proteins and increasing the concentration of sodium hydroxide protein fraction which is richer in the two amino acids specified above. Opaque-2 gene caused a reduction in the amino acid leucine which, being in high concentration in normal maize protein, is associated with the low protein quality of normal maize and the development of Pellagra due to niacin deficiency, (Kies and Fox, 1972, Bressani _et al._ 1969). In terms of nutritional value, Opaque-2 maize has a Biological Value of 92 and 72 per cent in human adults and children respectively, as compared to egg protein with a value of 96 per cent for adults, and milk with 80 per cent for children, (Bressani et al. 1969 and Young et al. 1971).

In India, attention has been paid to the improvement of protein quality through the use of Opaque-2 and floury-2 genes, both of which have been incorporated into the elite composites and the parental inbred lines of promising hybrids, (Swaminathan et al. 1969). On chemical analysis the opaque-2 composites were found to contain 100 per cent more lysine and 60 per cent more tryptophan than their normal versions. Three opaque-2 composites namely Shakti, Rattan and Protina were released in 1971. The nutritional superiority of the Opaque-2 maize composite has been established from the comparative feeding trials carried on rats, chickens and young children (National Commission on Agriculture, 1973). The PER of Opaque-2 maize and normal maize as determined from rat feeding trials were found to be 3.30 and 1.20 respectively, (Mertz et al. 1965). Supplementary child feeding studies have shown that Opaque-2 maize protein is comparable with milk, in terms of weight gains (National Commission on Agriculture, 1973, Swaminathan et al. 1972).

Feeding Trials with Opaque-2 Maize in Overcoming Malnutrition:

De Souza (1972) showed that the protein quality of Opaque-2 maize was superior to that of ordinary maize through rat assays. The protein value of Opaque-2 maize was reported to be 90 per cent of that of milk by Clark (1968). The efficiency of Opaque-2 maize for rehabilitating undernourished children was proved by Abraham (1971). Harpstead et al (1968) have reported the recovery of children from advanced stages of malnutrition, when Opaque-2 maize was fed in traditional dishes. Clark et al (1968) conducted a nitrogen balance study with six young adults which showed that a total intake of 300 to 350 grams of Opaque-2 maize was adequate for nitrogen equilibrium. Only two studies have been reported in India on the use of Opaque-2 maize in human feeding trials. Swaminathan (1971) has described a trial in young children near Delhi. Children who were fed with Opaque-2 maize, had gained more weight than children who got their protein through skim milk. At NIN, (1974) a clinical trial wasundertaken in which Opaque-2 maize was fed to children suffering from Kwashiorkor. The results indicated that Opaque-2 maize can successfully replace other vegetable foods which have so far been used in the treatment of Protein Calorie Malnutrition.

Dietary Iron and Detection of Iron-Deficiency Anemia

There may be 200 million people with iron deficiency in the world today. If this deficiency is of dietary origin, there must be evidence that an unusual dietary situation exists. Adult men and women in India have a dietary iron intake of about 18 mg/day. In infancy, a more circumscribed problem exists where the child outstrips his iron reserves after 3-6 months; his dietary iron is usually very limited due to the low iron content of milk and the restricted amounts of other foods ingested (Sood, 1967; Apte 1971).

Iron deficiency may be defined in a manner applicable to the individual subject and/or to the population at large. Anemia in the individual is defined as a decrease in hemoglobin concentration below the physiologic norm, i.e., a depression below the level, found when there is no limitation on hemoglobin synthesis, (FAO/WHO 1972). In the past, difficulties have arisen by attempting first to identify anemic subjects, and then to determine the cause of their anemia. The problem is that it is not possible to determine anemia in a given individual by determination of hemoglobin concentration. The use of an arbitrary cut-off point for the hemoglobin value ignores the large overlap between normal and abnormal values, (WHO/FAO, 1972). The only direct way to identify anemia withcertainity in the individual subject is to administer iron and determine whether the hemoglobin level is increased, (Garby et al, 1969).

The detection of true iron deficiency can be approached only through an examination of aspirated bone marrow or by the evaluation of iron absorption, since the absorption bears a reciprocal relationship to iron stores, Kuhn et al (1968). It has recently been suggested that Cobalt absorption may be useful for the detection of iron depletion since since it parallels iron absorption, (Sorbie et al 1971).

Prevalence of Iron Deficiency:

Iron deficiency can be identified at three levels (WHO/FAO; 1972). In the first category are individuals who have depleted iron stores but no anemia and no abnormality in other laboratory parameters or iron deficiency. In the second category are individuals with iron-deficient erythropoiesis whose deficiency is identifiable by laboratory measurements of transferrin saturation, red-cell protoporphyrin and red cell indices. These may be normal or only slightly depressed. In the third category are the individuals who have iron deficiency and a detectable anemia. Using these three categories one finds from the literature that about 30% of menstruating women have iron depletion, 15 per cent iron-deficient erythropoiesis and about 7 per cent iron-deficiency anemia. A prevalence of about 35 per cent iron deficient erythropoiesis in pregnant women bears this out, since normal iron stores are required in pregnancy if iron deficiency is to be avoided, (WHO/FAO 1968, Cook et al 1971). The majority of infants have no iron stores, about 50 per cent have iron-deficient erythropoiesis, and 25 per cent have iron deficiency anemia if no supplements are provided. Normal men, by contrast, rarely have iron deficiency, consistent with their favourable iron balance.

Most nutritional deficiency anaemia is mild and bears no identifiable symptoms, Elwood et al, (1969). However, when mild iron-deficiency anaemia is common, severe and potentially hazardous anemia will occur more frequently.

Amaranth as a Source of Iron:

Amaranth, one of the green leafy vegetables is an excellent source of iron as evidenced by its high iron content (25.5 mg/100g.) (I.C.M.R. 1966). Mccarrison (1956) emphasized that green leafy vegetables form one of the three classes of protective foods. They are rich in mineral elements and vitamins which are lacking in cereal grains.

As sources of iron the green leafy vegetables are extremely important since they are less expensive and available throughout the year, (James and Baden, 1967). The highest values of phytic acid are found in cereal and cereal products, nuts and legumes which in turn, interfere with iron absorption, whereas green leafy vegetables are low in the phytic acid content. But they have a large amount of oxa-lates (Hussain and Patwardhan, 1959).

Anaemias are common in areas where starchy roots are the staple foods and green leafy vegetables are eaten only in small amounts (F.A.O. 1954, Saroja, 1966). Hence their consumption of leafy vegetables are promoted in such areas through every possible nutrition education programmes. Although green leafy vegetables contain a low percentage of available iron, yet bulk for bulk, they furnish much more available iron than other foods (Saroja, 1966, Anandhan et al. 1967).

Devadas et al. 1973 has reported that supplementation of amaranthus to school children had significantly increased their haemoglobin content. Apart from their iron content amaranth greens are excellent sources of carotenes which are the precursors of vitamin A, and folic acid, calcium and ascorbic acid, and folic acid contents. The folic and content might be mostly lost during the cooking process.

Biological availability of Provitamins mainly Carotenoids

Vitamin A - Nutritional Status:

Vitamin A deficiency is one of the most devastating public health problems in India. The general effect of vitamin A deficiency in childhood is impairment of growth. The more specific effects are seen in the eye in the form of xerosis of the conjunctiva and Bitot's spots Bitots spots have been recorded that in five to 11 per cent of children in Southern and Eastern India (Gopalan, 1957 and Pereira *et al.* 1967).

Foods containing preformed vitamin A are expensive and beyond the reach of the poor. Hence Carotene derived from plant foods seeds to be the chief source of vitamin A in the diet of many population groups in India, (Rao and Rao, 1970). Therefore, the efficiency of absorption and utilisation of carotene from the vegetable sources becomes important. The most rational method for the control and prevention of vitamin A deficiency is the improvement of diets through inclusion of carotene rich foods such as carrots, green leafy vegetables and yellow fruits.

The true vitamin A value of the vegetables depend on their alpha and beta carotene contents and their availability to the body. It is essential, therefore to know the true vitamin A activity of the carotinoids present in the foods, in order to assess their value as a source of vitamin A. Carrots, amaranthus, papaya, cabbage, yellow pumpkin, apricots and spinach are especially rich in carotenes.

In most of the balance studies in man only the total carotenes present in the vegetable food sources have been measured. A few investigators have substracted basal carotenoid excretion which, according to Kawaguchi and Fujita (1956), is a common source of error. Furthermore, in some cases carotenoid extraction from the faeces may have been incomplete (Kawaguchi and Fujita, 1956). It is also possible that the entire amount of the biologically active carotenoids were also initially extracted from the food by the procedures used, (Ahrendinger and Hinds, 1969). Another problem is the detailed carotenoid composition of many common foodstuffs is still unknown (Mc Laren, 1966).

No information has been reported on the microbiological distruction of the Carotenes in the gastro-intestinal tract. In 1970, Rao and Rao separated the faecal pigments on a chromatographic column and found bands that were not in the carotene sources fed. They suggested that these compounds may have been formed by the intestinal bacteria.

Rao and Rao (1970) have reported two balance studies in which basal carotenoid excretion had been determined initially. In their first study four young adult males eating diets low in fat absorbed 33, 36, 46 and 98 per cent of the total carotenes from the mixed vegetables, carrot, papaya and amaranth respectively. The differences appeared to be related to the amount of β-carotene and possibly the fiber content of the foodstuffs. They calculated that 20% of the total carotenes in carrots were β-carotene. In the second study, four under-nourished pre-school children absorbed 75% of the total carotenes and 70% of the β-carotene from 50g. of amaranth (Lala and Reddy, 1970).

A number of investigators have reported that provitamin A absorption especially from carrots, is directly proportional to particle size Kreula and Virtanen (1939), Van Zeben (1946), Kreula (1947), Van Zeben and Hendricks, (1948), Hume and Krebs, (1949), and Leonardi (1947) suggested that absorption of the carotene from vegetables is possible only after the destruction of the vegetable cell membranes. Wilson et al (1937), concluded that Carotene from raw carrots or cooked spinach was absorbed equally well. Erikson and Haygaard (1941) reported that cooking increased carotene absorption from both carrots and spinach. Callison and

Greath-Hoiles (1947) found that there was no difference in the biological value of frozen carrots, cooked or raw. Kreula, (1947) and Wilbrand (1950) reported that the percentage of carotene absorbed was inversely proportional to the amount ingested. The Joint FAO/WHO group (1967) reviewed the results of ten studies on the "apparent absorption" of carotene in oil (Heymann, 1936, Van Eekelen and Pannevis, 1938, Wilson, 1940, Wagner, 1940, Wald et al. 1941, Van Zeben, 1946, Kreula, 1949, Hume and Krebs, 1949, Kaguchi and Fujita, 1956). The 77 subjects absorbed 25 to 98 per cent of the trial doses. Regardless of the age of the subjects, Carotene in oil appeared to be more readily absorbed than Carotene from vegetable sources. Rao and Rao (1970) reported that crystalline β-carotene was completely absorbed by four adult subjects.

In 1966, Goodman and his associates compared the absorption of labeled β-carotene and retinol by measuring the amount of radio-activity recovered in the thoracic duct lymph. Vitamin A precursors may be converted to retinol in the intestinal wall or absorbed "in tact". The subjects absorbed 8.74 and 16.7 per cent of the β-carotene within 22 hours after it was administered, and 22 to 30 per cent of the radio activity was in the form of β-carotene. After feeding retinol at 14°C, a total of 21.5 per cent of the radio activity was recovered in the lymph during the next 20 hours. Blomstrand and Werner (1967) administered labled Carotene to four subjects and recovered 8.7 to 52.3 per cent of the radio-activity. Unchanged labeled carotene comprised 1.7 to 46.9 per cent of the radio activity absorbed. When pre-formed Vitamin A was administered, 6.7 to 66.9 per cent of the radio activity was recovered.

__Presence of Other Dietary Components:__

__Fat:__

Dietary lipids do not appear to be as important for utilising pre-formed vitamin A as they are for the provitamins (Wilson _et al_ (1957). Recently, Lima _et al_ (1967) and Figueira _et al_ (1969) concluded that dietary fat is not essential for vitamin A absorption since young children on low fat diets, supplemented with vitamin A and dried skim milk, had normal plasma values. On the other hand, in pre-mature infants, the amount of vitamin A absorbed appeared to parallel the amount of fat absorbed (Healey _et al_ 1947).

The type of dietary fat may also influence vitamin A absorption. Animal experiments suggest that unsaturated fatty acids affect vitamin A utilisation (Rubin and deRitter, 1954).

Several investigators have reported that dietary fat enhanced Carotene absorption in humans (Wilson *et al.* 1937, Lombardi, 1947 and Kawaguchi and Fujita, 1956). Roels, et al (1958) found that African boys receiving 200g. of carrots along with their normal diet absorbed only 5 per cent of the carotene, but a daily supplement of 18g olive oil raised the absorption to 25 per cent and serum levels of both carotene and vitamin A increased rapidly.

Animal experiments indicate that both the amount (Bonger *et al* 1969) and type of fat have a deciding effect on carotene utilization (Thompson, 1964). Carotenes are poorly utilised when indigestible fats are included in the diet, (Moore, 1957, Daniel, 1955 and Thompson, 1964).

Protein:

Experimental evidence on the close relationship between vitamin A utilization (absorption, transport and storage) and the quantity and quality of dietary protein has been recently reviewed by Moore (1960), Gershoff (1965), Mahadevan *et al* (1965) and Arroyave (1969). Moore (1960) suggested that a very low intake of protein can result in poor utilization of vitamin A because of fatty infiltration of the liver and the consequent deterioration of digestion and absorption processes. In Arroyave's view (1969) the metabolic processes that are affected probably depend on the dietary protein level and the duration of the diet.

In 1959, Arroyave *et al* reported that the oral administration of 75,000 μg vitamin A palmitate to children with Kwashiorkor had effect on their plasma vitamin A values. However, when the test load was administered, after supplementing their diets with skim milk for 3 to 5 days, the serum vitamin A values had increased. The investigators were not sure if the initial response was due to mal-absorption, abnormal transport or both. Later (1961) they observed that even without vitamin A in the diet, protein supplements caused an increase in the plasma values with vitamin A reserves in the liver of those children and that the plasma values of those children with scant, vitamin A stores did not change. Gopalan *et al* (1960) and Kamo *et al* (1968) have also reported similar experiences from India and Africa, respectively.

Reports on the effects of protein intake on the utilization of the provitamins A are conflicting. Gronowska-Senger and Wolf (1970) found that feeding young men a protein-free diet for 8 or 9 days had no effect on the specific activity of intestinal carotene dioxygenase. Murich *et al* (1964) fed 37 healthy girls, 7 to 10 years of age, varying levels of protein from plant and animal sources and a constant amount of vitamin A activity but the proportion of "plant vitamin A" varied. In two of the three experiments, the plasma Carotene values were higher when the girls were given the high protein diet. Multiple regression analysis of the data showed that the serum carotene levels tended to be influenced by protein from animal sources and serum vitamin A, by protein from plant sources.

Some other investigators have not observed any effect of dietary protein on the apparent absorption and utilization of pro vitamins by humans. Roels *et al* (1963) reported that administering Carotene-rich palm oil (13,500 IU daily) cured night blindness in 3 to 13 years old, Indonesian boys. After treatment, their serum vitamin A levels were the same as those of boys receiving 2,000 I.U. vitamin A Acetate (600 µg retinol) and did not increase when 2 g. skim milk/kilogram body weight were added to the experimental diets. From faecal carotene excretion and serum Carotene and vitamin A values, Mosebette (1955) found that, adding protein in the form of fat-free milk to the diets of six normal pre-adolescent girls, 8 to 11 years old, had no effect on their utilization of 3,6000 µg. carotene from sweet potatoes. Recent data of Lala and Reddy (1970) also suggest that a mild degree of Protein-Calorie-Malnutrition does not impair carotene absorption. Protein intake has a direct effect on intestinal protein biosynthesis and the intestinal enzyme, carotene dioxygenase (Gronowska Senger and Wolf, 1970).

III. EXPERIMENTAL

This investigation, "Nutritional Evaluation of the Supplementary Value of Selected Low Cost Local Foods to Poor Rice Diets" involved the following series of experiments:

A_1. Formulation of a series of Cereal-legume mixtures, useful for school children based on local foods and their evaluation on albino rats;

A_2. Finding out the optimum ratio of Protein in a sorghum-green gram mixture, and

A_3. Testing the suitability of a bioassay technique for the evaluation of the nutritive quality of Protein, a cereal-legume-oil seed mixture also formed part of this series.

B. The effect of three dietary proteins on carotene utilization by albino rats.

C. Evaluation of a weaning mixture based on a local foods through a feeding trial on Young Children for six months.

D. Investigation of the effects of diets based on high lysine maize, on the nutritional status of children 18-30 months of age.

E. Evaluation of a nutritious supplement evolved with indigenous foods on 5 to 7 years old school children

F. Supplementation of amaranthus, (a green leafy vegetable) to anemic adolescent school girls.

G. Determination of the availability of B-carotene from carrots to women college students.

All the other experiments were conducted on human beings - pre school children, adolescent girls and college women.

The procedures used for the different experiments are discussed in detail in the following pages.

A. A series of cereal legume mixtures useful for school children based on local foods and their evaluation on albino rats:

Materials and Methods:

Protein rich food mixtures were formulated based on locally available vegetable foods for feeding school children. The food mixtures were evaluated through animal assays using PER, NPU, NBP and BV as the criteria. The following steps comprised the experiment.

1. Formulation of the Protein rich food mixtures.
2. Selection of six protein rich mixtures for the evaluation of protein quality
3. Conducting animal assays.

1. Formulation of the Protein-rich food mixtures:

Formulation of the protein rich food mixtures involved the following steps:

a. Basis for the selection of the Ingredients for the formulations.

b. Formulation of the protein rich food mixtures.

c. Chemical analysis of the nutrients in the formulations.

d. Calculation of the amino acid pattern of the protein of the formulations.

a. Basis for the selection of the ingredients of the formulations:

The major cereals grown in Tamil Nadu are rice (Oryza sativa), bajra (Pennisetum typhoideum), jowar (Sorghum vulgare) and maize (Zeamays). Rice is the most commonly desired and popular cereal. But rice is always in short supply and costs more. Maize is one of the crops which utilise solar energy most efficiently. Unlike rice, maize can be grown with considerably less water. In availability, protein quality and cost, maize offers many advantages. It is a crop which can be grown successfully in areas like Coimbatore District, where the rainfall is insufficient for rice, but too much for jowar, ragi and bajra. Hence maize was selected as the main cereal for the food formulations in this study.

In Coimbatore district, hybrid maize cultivation is currently in progress. The production figures of maize for Coimbatore were 6040 tons in 71-72. For the entire state of Tamil Nadu, the total production of maize was 15,300 tons for the same year (National Commission of Agriculture, 1973).

The need for increasing the production of legumes wherever they are grown in India is crucial. Among the legumes, horsegram (Dolichos biflorus) green gram (Phaseolus aureus roxb) red gram (Cajanus cajan) and cowpea (Vigna catjang) are being cultivated in Coimbatore district. The proportion of areas cultivated during 1973 are given below (Tamil Nadu Nutrition Project, CARTCR/AIAC Report, 1973).

Legumes		Proportion of area%
Cowpea	..	3.0
Green gram	..	5.9
Horse gram	..	80.0
Red gram	..	9.0

Interms of cost, Red gram is the most expensive and horsegram the least. Hence cow pea (Co variety), Horsegram and green-gram (Anjagam variety) were selected for use in this study because of their high protein content and low cost.

b. Formulation of the mixtures:

Twenty nine combinations (Annexure I) were achieved with maize as the main cereal in all the formulations and any two of the following legumes with or without one of the oil seed group.

Legumes

Bengal Gram (Cicer arietinum)

Cow pea (Vigna Cajang)

Green gram (Phaseolus aureus Roxb)

Horse gram (Dolichos biflorus)

Redgram (Cajanus cajan)

Oil seed

Ground nut roasted/groundnut cake (Arachis hypogaea)

Sunflower seed/sunflower seed cake (Helianthus annus)

c. Chemical analysis of the nutrients with the formulations.

d. <u>Amino acid profile of the mixtures:</u>

The amino acid profile of the 29 mixtures, thus formulated were calculated using the Food Composition Tables of the ICMR, 1971.

2. <u>Selection of the mixtures for the evaluation of protein quality:</u>

Out of the 29 mixtures, the following combinations were selected for the evaluation of protein quality. The figures in the parenthesis give the proportions in which they were combined.

1. Maize, cowpea, greengram, sunflower seed (6:1:1:2)
2. Maize, horse gram, green gram (6:2:2)
3. Maize, cowpea, green gram (6:2:2)
4. Maize, horse gram, cowpea, sunflower seed (6:1:1:2)
5. Maize, green gram, sunflower seed (6:2:2)
6. Maize, cowpea, horsegram, groundnut (6:1:1:2)

The calorie and protein content and cost of the six cereal -legume, and oilseed mixtures selected are presented in Table I.

TABLE I

CALORIES, PROTEIN AND COST OF THE SIX CEREAL-LEGUME OIL SEED MIXTURES

Formulations (1)	Ingredients (2)	Amount g. (3)	Calories (4)	Protein g. (5)	Cost** Rs.Ps. (6)
Maize-Cowpea Greengram, Sun-flower seed	Maize	60	205	6.7	0.06
	Cowpea	10	32	2.4	0.03
	Greengram	10	33	2.4	0.03
	Sunflower seed	20	124	4.0	0.07
	Total	100	394	15.5	0.19
Maize-Horsegram Greengram	Maize	60	205	6.7	0.06
	Horsegram	20	64	4.4	0.03
	Greengram	20	67	4.8	0.06
	Total	100	336	15.9	0.15
Maize-Cowpea-Greengram	Maize	60	205	6.7	0.06
	Cow pea	20	65	4.8	0.06
	Greengram	20	67	4.8	0.06
	Total	100	339	15.3	0.18
Maize-Horsegram Cowpea-Sunflower Seed	Maize	60	205	6.7	0.06
	Horsegram	10	32	2.2	0.02
	Cowpea	10	32	2.4	0.03
	Sunflower seed	20	124	4.0	0.07
	Total	100	393	15.3	0.18
Maize, Greengram Sunflower seed	Maize	60	205	6.7	0.06
	Greengram	20	67	4.8	0.06
	Sunflower seed	20	124	4.0	0.07
	Total	100	396	15.5	0.19
Maize, Cowpea Horsegram Groundnut	Maize	60	205	6.7	0.06
	Cowpea	10	32	2.4	0.03
	Horse gram	10	32	2.2	0.02
	Groundnut (Roasted)	20	114	5.2	0.08
	Total	100	383	16.5	0.19

** At prices prevailing in Coimbatore in April, 1974.

The amino acid content of the selected formulations are given in Annexure II. A comparison of the amino acid pattern of the formulations with the requirements specified by FAO/WHO (1972) for school going children is given in Table II.

TABLE II

AMINO ACID PATTERN OF THE SIX FORMULATIONS IN COMPARISON WITH THE REQUIREMENTS SPECIFIED BY FAO/WHO, (1972) FOR SCHOOL GOING CHILDREN

Mg. of Amino Acid/g. of Protein

Amino Acid	Requirements recommended by FAO/WHO 1972	Formulations					
		A	B	C	D	E	F
Isoleucine	25	43	49	45	43	45	42
Leucine	44	91	97	94	91	91	85
Lysine	53	45	59	55	53	46	45
Methionine Cystine	25	17 14 (31)	15 15 (30)	16 13 (29)	17 16 (33)	17 14 (31)	14 15 (29)
Phenylalanine Tyrosine	25	48 30 (78)	53 21 (74)	52 31 (83)	49 28 (77)	49 26 (75)	59 33 (92)
Threonine	31	39	39	39	40	39	37
Tryptophan	6	10	9	9	10	9	9
Valine	24	49	53	49	51	59	49

It is noteworthy that all the six cereal-legume-oilseed formulations met the requirements suggested by FAO/WHO, 1972 for school going children, except that the formulations A, E and F were slightly lower only in their lysine content.

3. Conducting the animal assays:

The procedures adopted for conducting the experiments included:

a. Preparation of the diets
b. Selection, allotment and care of rats
c. Recording feed intake
d. Weighing the rats
e. Autopsy
f. Estimation of carcass nitrogen
g. Analysis of the liver
h. Conducting the nitrogen balance study and estimation of urine, faeces and food for nitrogen content
i. Nutritional evaluation of the cereal-legume and oil seed food mixtures
j. Bioassay evaluation of an mixture of horsegram and sesame seeds.

4. Preparation of the diets:

As suggested by the National Research Council, National Academy of Sciences (NRC) (1963), 10 per cent protein and 15 per cent fat levels were used for studying the protein quality of the six formulations evolved out of indigenous foods. The composition of the six diets used in the animal experiments are given in Table III.

TABLE III

COMPOSITION OF THE VARIOUS DIETS

Constituents	Diets-grams						
	SM	A	B	C	D	E	F
Cereal-pulse-Oil seed Mixture	0.0	64.5	62.8	61.3	65.4	64.5	60.6
Skim Milk	26.3	--	--	--	--	--	--
Groundnut oil	15.0	2.2	12.3	12.3	12.2	2.2	14.6
Salt mixture	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mixture	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Starch (Corn)	53.7	28.3	19.9	21.4	17.4	28.3	19.8
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

SM .. Skim milk
A .. Maize-cowpea-greengram-sunflower seed
B .. Maize-horsegram-greengram
C .. Maize-cowpea-greengram
D .. Maize-horse gram cowpea-sunflower seed
E .. Maize-greengram-sunflower seed
F .. Maize-cowpea-horsegram-groundnut

As indicated earlier all these formulas had 10 per cent protein and 15 per cent fat.

All the raw food ingredients were procured in one lot to last for all the experiments. The dry ingredients were roasted for 10 minutes and ground to a fine flour in a mill. All the powdered foods were analysed individually for protein content.

The constituents of the diets were then mixed uniformly without lumps sieved, placed in air tight bottles and stored in the refrigerator. Groundnut oil was used as the source of fat and amalar starch for making up the diets. Salt mix-ture and vitamin mixture of the composition given in Annexure III were added at five per cent and one per cent levels respectively.

b. Selection, allotment and care of rats:

Rats of Norwegian strain, 21 days old, bred in the college laboratories were used to determine the PER, and thirty days old rats were used for the determination of NPU and NDP cals%. Littermate allotments allotment was made and the rats were housed in individual cages with wire screen bottoms. Weighed quantity of each diet was made into a thick paste with hot water to minimise spilling and given in aluminium cups. Drinking water was supplied in glass bottles adlibitum.

c. Recording food intake:

Uneaten food from the individual cages was collected every morning, placed in a watch glass a nd dried in a hot air oven at 80°C, until completely dry and stored in polythene bags. At the end of the feeding period the quantity of uneaten food was substracted from the quantity of food supplied, to calculate the food intake and recorded.

d. <u>Weighing the rats:</u>

The rats were weighed daily early in the mornings before supplying food and water, in an 'Ohaus triple Beam Animal Balance' to the nearest 0.1g.

e. <u>Autopsy:</u>

After an experimental period of 21 days for PER, and of 10 days for NPU and NDp Cal%, the rats were sacrificed by chloroforming. The livers were removed immediately frozen and stored at - 4°C for estimating the nitrogen content.

f. <u>Estimation of carcass nitrogen:</u>

For the carcass nitrogen analysis, the body cavities were opened and the contents of the stomach and caecum were removed. The carcass was then dried at 105°C for 48 hours and at 80°C for 30 minutes and cooled in a desiccator. Heating at 80°C and cooling were repeated until constant weights were obtained. The dried carcass was powdered and aliquots were analysed for nitrogen content, using the micro-digestion and micro-distillation procedures (NIN, 1971).

g. Analysis of the liver:

The whole liver was homogenised with five times its weight of distilled water and aliquots were taken for the determination of nitrogen.

h. Conducting the nitrogen balance study and analysis of urine and faeces:

The procedures for conducting the nitrogen balance study involved the following steps:

i. Selection of animals

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

iii. Duration of the feeding

iv. Analysis of urine and faeces

v. Statistical analysis.

i. Selection of animals:

Male albino rats, 100 days old were chosen for the study. Their initial weights were recorded and they were housed in individual wire mesh bottomed cages with specially devised funnel attachment, to facilitate collection of urine and faeces.

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

The selected animals were maintained on a protein free diet for a period of seven days, in order to determine the endogenous excretion of nitrogen. The composition of the diet including starch and fat with the addition of mineral and vitamin mixtures. Intake records were maintained similar to the PER study throughout the experiments. Water was given adlibitum.

After a three day adjustment period with the protein free diet urine and faeces were collected for a period of four days. The urine collected waspreserved 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 animalswere weighed and the stock diet was fed for a period of four days. The rats were then divided into groups ensuring that the mean weight loss of the groups was similar and designed as A, B, C, D, E and F corresponding to the six diets to be fed.

iii. Duration of the feeding:

The six groups were fed the different test diets for a period of seven days for NPU, NDP cal % determinations. Four day collection of urine and faeces were made after an adjustment period of three days with the test diets. The collections were done using procedures described for the protein free diet. For PER, the feeding was continued till 28 days.

iv. Analysis of urine and faeces:

The nitrogen content of the individual urine samples as well as the dried and powdered faeces sample collected during the two phases of the study were analysed by the microkjeldahl method.

v. Statistical Analysis:

Analysis of variance was used for the statistical appraisal of results. Comparisons, each with one degree of freedom were used to test the differences in the means of the treatments. Only ratios which had a probability of 0.05 to 0.01 or less of being as large as the calculated value by chance alone were considered to be significant. For the bioassay series and the nitrogen balance study the regression lines were calculated using regression equations (Snedecor, 1959).

A.2. Procedure for the Nutritional evaluation of cereal legume mixtures:

This study was framed to find out how much of green gram supplemented to a sorghum diet would result in a maximum PER value.

Eleven diets for growing rats were prepared. They contained equal amounts of protein, derived in different proportions from sorghum and greengram. The composition of the diets used in the study is shown in table IV.

TABLE IV

COMPOSITION OF THE DIETS CONTAINING SORGHUM AND GREEN GRAM IN DIFFERENT PROPORTION

Constituents	Diet-grams						
Source of Protein fraction Sorghum Green gram	A 100% / 0%	B 80% / 20	C 60% / 40	D 50% / 50	E 40% / 60	F 20% / 80	G 0% / 100
Sorghum	85.0	80.0	60.0	50.0	40.0	20.0	0
Greengram	---	8.0	16.0	21.0	25.0	33.0	42.0
Groundnut oil	5.4	5.4	5.7	5.7	6.1	6.3	6.5
Salt mixture	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mixture	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Starch corn	4.6	1.6	13.3	18.3	23.9	35.7	46.5
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Protein %	8.5	10	10	10	10	10	10
Fat %	7	7	7	7	7	7	7

A.3. Procedure of bioassay evaluation of the proteins from a mixture of horse gram and sesame seeds:

An experiment was designed to determine the nutritive value of a combination of horse gram and sesame at a ratio of @ 4:1 by bioassay technique comparing it with a known standard protein, skim milk powder.

Weanling albino rats of the Norwegian strain, 23 days old, weighing 31 grams, were selected for the growth study. They were divided into eight groups each containing six littermates. Four groups were fed the horsegram-sesame mixture at 9, 11, 13 and 15 per cent concentrations and the other four skim milk proteins at 7, 9, 11 and 13 per cent levels. These levels were chosen to get equal response of body weight gains in both the series of diets.

Horse gram and sesame proteins were taken in the proportion of 4:1 to have a balanced amino acid pattern. Sesame was added to makeup the methionine deficit in horsegram. The horsegram was cleaned and powdered with husk and the sesame was dehusked by soaking overnight in water and drying. The protein content of both horsegram and sesame was analysed. Sago powder served as a source of carbo hydrate and groundnut oil as the source of the 10 per cent fat in the diets. The individual components were mixed by hand, free of lumps. Table V gives the composition of the experimental and skim milk diets.

TABLE V

COMPOSITION OF THE EXPERIMENTAL AND SKIM MILK DIETS

Constituents	grams in one kilogram							
	Experimental				Skim milk			
	A	B	C	D	E	F	G	H
Horsegram Powder	327.2	400.0	472.7	545.4	—	—	—	—
Sesame	98.3	120.2	142.0	164.0	—	—	—	—
Skim milk powder	—	—	—	—	184.2	236.8	289.4	342.0
Groundnut oil	54.7	48.1	38.6	29.0	100.0	100.0	100.0	100.0
Salt mixture	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Sago powder	179.8	391.7	306.7	221.6	675.8	623.2	570.6	518.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0

Key to codes:
A .. Horse gram + Sesame
B .. Horse gram + Sesame
C .. Horse gram + Sesame
D .. Horse gram + Sesame
E .. Skim Milk
F .. Skim Milk
G .. Skim Milk
H .. Skim Milk

	A	B	C	D	E	F	G	H
Protein percent (by calculation)	9.0	11.0	13.0	15.0	7.0	9.0	11.0	13.0
by analysis	9.4	11.0	13.6	15.8	7.9	9.3	11.1	13.6

All these diets had 10 per cent fat

Weighed quantity of each diet was made into a paste and cooked by steaming method for 15 minutes to minimise spilling. The cooked diet was fed adlibitum with vitamin mixture, vitamin B complex and cod liver oil daily. The method of recording the food intake and autopsy are described in the earlier procedure.

The whole liver was homogenised with five times its volume of 0.1N Phosphate buffer (pH 7.2) and triplicate samples were taken for the various determinations. Hepatic succinic dehydrogenase activity was measured by using the method of Srinivasamurthy and Swaminathan (1955). Hepatic nitrogen determinations were made with homogenised liver samples by the microkjeldahl method (AOAC, 1960).

The regression of a gain in body weight in grams to total nitrogen intake or protein intake were calculated for each dietary protein.

The residual sum of a squares was computed by fitting separately to each protein, an equation of the form $y=a+b\,x$ where 'y', is the response (body weight) and 'x' is the amount of protein or nitrogen actually consumed by each animal.

Analysis of variances with non orthogonal comparisons was carried out in the case of body weight, PER, hepatic weight, hepatic nitrogen, protein intake and hepatic XDH activity.

B. <u>Procedure for the evaluation of dietary proteins on carotene utilization by Albino Rats:</u>

This study was designed to investigate the carotene protein interrelationship using three dietary proteins of varying qualities namely skim milk, fish flour and a mixture of red gram and sesame as supplements to a basal rice diet, fed at 15 per cent level.

The dietary proteins tested were a combination of red-gram and sesame proteins in the ratio of 2:1 fish flour and skim milk powder. All these protein sources were supplemented to a poor rice diet so that the total protein content was 15 per cent. Table VI gives the composition of the experimental diets.

TABLE VI

COMPOSITION OF THE EXPERIMENTAL DIETS

Constituents	g. in one kilogram of diet			
	Rice	Rice + Redgram dhal + Sesame	Rice + Fish flour	Rice + Skim Milk powder
Rice	800	390	500	500
Red gram dhal	—	360	—	—
Sesame	—	210	—	—
Fish flour	—	—	150	—
Skim milk powder	—	—	—	300
Groundnut oil	100	—	100	100
Salt mixture	40	40	40	40
Starch	60	—	210	40
Total	1000	1000	1000	1000
Protein (per cent by calculation)	5.20	14.96	16.60	15.30

__Selection and grouping of experimental animals:__

Twenty two days old weanling littermate rats of Norwegian strain weighing on an average 37 to 40g., were selected for this experiment. The diet of their mothers during lactation was partially restricted in vitamin A, in order to minimise the hepatic storage of this vitamin in the weanlings. Each littermate group contained five rats. They were intially fed the vitamin A deficient diet. After 30 days of depletion one rat was selected randomly for analysis of the hepatic stores in a depleted condition and the remaining four were assigned to the different protein diets.

The care of animals and feeding are described in the earlier section of this report.

Alphatocopherol in groundnut oil and water soluble vitamin supplements were added to the cooked food. Feed intake was recorded daily. Vitamin A was provided during the repletion period in the form of carotene, which was supplied by dry carrot powder in groundnut oil. The weights of the animals were recorded once in two days.

AUTOPSY:

After the initial vitamin A depletion period of 30 days, one out of the five depleted litter mates was sacrificed in order to estimate the hepatic vitamin A at the end of the depletion period. The remaining four rats were assigned to the experimental repletion diets for a period of 28 days and sacrificed on the 29th days 24+1 hours after the last carotene dose. Blood sample of about four ml. was collected by the heart puncture, and livers were removed and weighed immediately.

The individual livers were saponified separately and stored in frozen condition (-20°C) for a period of less than seven days until the time of vitamin A extraction and determination. The blood was centrifuged and the serum separated, frozen and stored till it was used for the analysis. The method is given in Annexure IV.

Feeding:

The food cooked on a waterbath was cooled and 500mg. of water soluble vitamin mixture in sucrose and two drops of alpha-tocopherol supplement were added. In another dish 1 ml. of the carotene supplement was poured. The rats were given the carotene supplement first and then the respective diets. The animals invariably consumed the carotene supplement within five or seven minutes after serving.

Analysis:

Vitamin A determinations were done by the method of Jacobs (1956) using the Carr Price colour reaction. The total protein and the protein fractions were analysed by the method of Jhonson and Gibson, quoted by Kleiner and Dotti (1962) Analysis of Variance was used for the statistical analysis (Snedecor 1956). The method is given in Annexure V.

e. **Procedure for the evaluation of a weaning mixture based on local foods:**

The present study was undertaken to evaluate a weaning mixture based on locally available (in Perur Block, Coimbatore district) cereals and legumes namely, sorghum, roasted Bengal gramdhal, roasted groundnuts and jaggery through a feeding trial on 25 preschool children. Acceptability of the weaning mixture, its shelf life and the knowledge of mothers about the components of the weaning mixtures were also studied.

Materials and Methods

Developing the weaning food mixtures:

The information gathered on locally available foods in the experimental village showed that sorghum was the largest grain crop next to the cultivation of maize and rice. Distribution of area under cerealsin the experimental village were given in Table VII.

TABLE VII

DISTRIBUTION OF AREA UNDER CEREALS IN THE EXPERIMENTAL VILLAGE

Name of the cereal	Percentage of area in the village
Paddy	0.5
Sorghum	74.0
Ragi	2.3
Maize	8.2
Varagu / Thinai / Samai	15.0
Total	100.0

Ragi, Thinai, Varagu were also the other millets. Six ready to consume weaning food mixtures were formulated utilising either one of the cereals, namely, sorghum, ragi or maize, one legume, either green gram dhal or Bengal gram dhal, roasted groundnuts and jaggery. The factors which were considered in the selection of the food ingredients were availability of local food ingredients and low cost.

Shelf life and acceptability trials were carried out with the food mixtures developed.

Table VIII gives the composition and nutritive value of the different RTC cereal - legume weaning mixtures developed.

TABLE VIII

COMPOSITION AND NUTRITIVE VALUE OF THE CEREAL (PULSE) LEGUME WEANING MIXTURES

S. No.	Weaning Mixtures	Amount g.	Calories	Protein g.	Cost** Serving Rs. Ps.
1.	Sorghum + Bengalgram + groundnut	100	390	12.9	0.18
2.	Sorghum + Greengram + Groundnut	100	384	14.1	0.19
3.	Ragi + Bengalgram + groundnut	100	380	12.4	0.18
4.	Ragi + Greengram + groundnut	100	351	12.9	0.19
5.	Maize + Bengalgram + groundnut	100	386	13.8	0.18
6.	Maize + greengram + groundnut	100	381	14.3	0.19

** Prevailing prices in Coimbatore, 1973.

2. <u>Conducting quality control Tests:</u>

Table IX represents the quality control tests for Sorghum based weaning mixture Compared with the ISI specification.

TABLE IX

RESULTS OF THE QUALITY CONTROL TESTS FOR SORGHUM BASED WEANING MIXTURE COMPARED WITH I.S.I. SPECIFICATION (quantity/100g.)

ITEM	Moisture	Total Protein	Fat g	Total Carbohydrate g	Total ash g	Calcium g.	Iron mg	Crude fibre content on dry basis g	Vitamin A I.U.	Thiamine mg	Riboflavin mg	Nicotinic acid mg
Sorghum bengal gram mixture	8.4	13.0	7.0	68.7	1.7	153.5	7.9	1.3	165.2	0.543	0.475	9.373
Requirement ISI specifications	6.0	14.0	7.5	45.0	5.0	1000	10.0	1.0	1500	0.500	0.600	5.000

Negligible differences were noted in the protein, ash, calcium and iron content of the weaning mixture and ISI specifications only in the case of calcium the difference is high.

Determination of NPU and NDP calories per cent:

A series of animal assays were conducted to determine the Biological value of the protein in the sorghum based weaning mixtures, Devadas and Jamala (1970). The NPU and NDP cal per cent of the mixtures were 61.0 ± 3.36 and 6.1 ± 0.34 respectively.

All these mixtures were tested on selected pre-school children for their acceptability and digestibility and found acceptable. Among the six infant weaning food mixtures thus evaluated, the most acceptable one was the sorghum, roasted Bengalgram dhal mixture. It was selected for the final feeding trial from which 390 calories and 12.9 g. of protein per serving of 100 gram.

Preparation of the sorghum-bengalgram mixture

Roasted sorghum flour	37.5 g.
Roasted bengalgram dhal	25.0 g.
Roasted groundnut	12.5 g.
Jaggery	25.0 g.

1. The above cereal, legume and oilseed were powdered together
2. Mix the powder with powdered jaggery.

4. Conducting the feeding trial:

A mixture of Corn Soya milk (CSM) and jaggery was selected for comparison in the feeding trial because of its nutritive value, (Graham et al. 1971), acceptability and free supply from CARE.(one hundred grams of CSM mixture provided 373 calories and 20 g. protein.)

Acceptability trials with the developed weaning mixtures:

The objectives of the study was to determine preferences of a rural community for different cereal, legume and oilseed combinations using various indigenous sources.

The products were tested among children in two villges namely Jothipuram in Periananaickenpalayam Block and Vedapatti in Perur block.

In one series all the six formulations were tested on 20 preschool children. Their responses were analysed.

In another series, with all the six formulations three preparations namely laddu, payasam and uppuma were prepared and tested, 20 pre-school children formed the testing panel.

5. Standardising food preparations:

With the developed weaning food mixture laddu, payasam and uppuma or pongal were prepared.

a. **Laddu:**

Add 2 tea-spoons of boiled water to 20 g. of the prepared weaning food mixture and made into balls.

Serving 1.

b. **Payasam:**

Boiled the water, sprinkled the flour, mixed with a spoon. A proportion of 25 grams dry weaning mixture can take 75 ml. water to obtain a thick porridge like consistancy.

c. **Uppuma or pongal:**

1. Boiled the water
2. Sprinkled 100 g. prepared dry weaning mixture.

Sorghum	37.5 g.
Roasted bengal gram dhal	25.0 g.
Roasted groundnut	12.5 g.
Jaggery	25.0 g.

3. Added a table spoon quantity of oil
4. Cooked till soft

Serving : 4

6. **Testing the acceptability**

1. The first series had a randomised complete block design when all the subjects tasted all the six formulated weaning mixtures.
2. The second series consisted of three products.

Most of the children tasted only one product the older children were able to test 2 or 3 products at a time.

One standardised teaspoonful was placed on the (parents) mothers palm. This was fed to the child. The investigator judged whether the child liked or disliked the product from 2 behavioural responses:

a. Facial expression of the child

b. Whether he voluntarily opened the mouth for the second mouthful (liked) or not (disliked)

From these observations the number of children who liked and disliked different weaning mixtures, was analysed. similarly the number of ch ildren who 'liked' and 'disliked' each products namely laddu, uppama or pongal and payasam was observed.

7. Shelf life studies:

The sorghum based weaning food mixture was packed in polythene bags and were stored in plastic cans and left in wall shelves (at 37°C) at room temperature, for a period of nine months. The control samples were kept at 0°C in a refrigerator. Samples stored at room temperature (at 37°C) were analysed once in three months for free fatty acids, peroxide value, vitamin A and thiamine Free Fatty Acid (FFA) and peroxide value were determined according to Krishnamurthy et al (1960), Vitamin A and thiamine contents according to A.O.A.C. methods. The organoleptic quality of the weaning food mixture was tested by a panel of judges. The score system adopted was similar to that of Chandrasekhar *et al* (1960). A product having a score of 2 or less was considered acceptable.

1. **Selection of Children:**

A socio economic survey (Annexure VI) was done to ascertain the number of children of the age group 18 months to 36 months in the village. Their family size, caste, religion, occupation of the father, educational level of the parents and income of the family was also noted.

Seventy five children were selected for the experiment from the village Vedapatti. One group of 25 children were given the sorghum based weaning food mixture, to the other group of 25 children, 100 grams of the CSM supplement were given. A control group of 25 children who did not receive any food ration.

2. **Distribution of food:**

For the feeding, a "Take home dry food distribution system was followed. The prepared mixture was distributed to the mothers and they were instructed to feed the days' ration to the child in any form. The food was distributed at the rate of 50 g. per child per day for the target children weighing below 7 kg and 100 g per day for children weighing above 7 kg. to be fed in four servings. The dry food in one kg packets was then distributed everymonth for the target children. Frequent home visits and repeated cooking demonstrations facilitated the take home dry food available to the target child. The feeding trial was carried out for a period of six months. The amount of the dry food, prepared by the mother as per the directions was recorded daily to quantify the food intake of the child.

3. Diet survey:

The distributed dry food was intended to increase the total nutrient intake of the target children. A food habits survey and dietary intake survey was carried out in all the target children and their households. This data wasused to evaluate whether or not the test children were experiencing significant net gains in food intake. The questionnaire and schedule used for this purpose are given in Annexure VII.

Food weighment survey:

Since food weighment surveys are the most accurate for finding out the food consumed, a three day food weighments survey was conducted to assess the home food intakes of the children and to assess their nutritional status. Ten children in the three experimental groups were selected randomly.

The investigator stayed in the village for three days for carrying out the weighment from the early morning to night. All the raw foods used in the family were weighed and their cooked weights were also noted a with a lever balance up to the accuracy of 1 gm and entered in the food weighment sheet (Annexure VIII).

The individual consumption of food by the children was weighed and corrections were made for the plate waste of individual subjects. The nutrient intake of the children for the three days was calculated separately from the collected data, in order to see the day today variations. This survey was done before and after the feeding trial.

5. Anthropometric measurements:

The growth rate was assessed in terms of body weights and heights which were recorded at monthly intervals. Head and chest circumference were recorded both at the initial and final stages of the study. Records of illness were also maintained.

a. Body weights:

Weights were recorded every month with an Avery platform scale (lever type) which was corrected for zero errors and standardised after every six weighings with 10 kg. or 5 kg.

standard weights. Each child was weighed without clothes accurately to the nearest 50g. The same balance was used throughout the study.

b. Body heights:

For children of the age group 6 to 30 months measurements of standing heights is impossible, hence crown to heel measurement was taken using a specially constructed 'Infantometer'. This is a wooden board about 5' long with a fixed head board and a sliding foot piece, a steel tape measure is fitted along each side. The infant is laid on the board with his or her head positioned firmly against the fixed board. The sliding foot piece is then moved to obtain firm contact with the child's heel. Readings were taken at the nearest 0.1 C.M.

c. Head circumference:

Head circumference was measured with a fibreglass tape measure passing it over the supra orbital ridge and occipital prominence. Readings were taken to the nearest 0.1 c.m.

e. Chest circumference:

The average of the inspired and expired chest measurements to the nearest 0.1 c.m. was taken with a fibre glass tape with the child sitting on his mother's lap.

e. **Clinical assessment:**

An experienced pediatrician assessed the clinical status of all the target children before and after the study. The proforma used for the clinical assessment is given in Annexure IX.

Haemoglobin estimation on the finger prick blood samples was carried out using cyanmethaemoglobin method.

The other criteria used in the evaluation of the supplement were biochemical analysis of the blood and urine samples, acceptability of the weaning mixture, records on house visits and cost analysis of the supplement.

f. **Investigation of the Effects of Diets Based on (Opaque-2 maize) High lysine Maize on the nutritional status of children 18-30 months of age:**

This study was designed to ascertain the effect of feeding children 18-30 months of age, diets with a high lysine maize, ordinary maize and skim milk on the nutritional status of the target children. The home diets of the children were based mainly on rice.

1. Selection of the villages
2. Socio economic and dietary survey
3. Number and grouping of children.

1. **Selection of the villages:**

Four villages (Velandipalayam, Jothipuram, Koundampalayam and Periananaickenpalayam) in Perinaickenpalayam block, Coimbatore district were selected for the study because of good rapport and easy accessibility. No other research projects or feeding programmes were in operation in these villages.

2. **Socio economic survey:**

All the families in the four villages were enumerated and a socio economic survey was done using a specially designed three questionnaires (Annexure VI).

3. **Number and grouping of children:**

Two hundred children between 18-30 months belonging to poor socio-economic status were selected in the four villages for the study. They were divided into four groups of 50 children each. Each village with its centre for feeding was used as a cohort.

Evaluation:

The composition of the four experimental diets per day per child were given in Table X.

TABLE X

COMPOSITION OF THE FOUR EXPERIMENTAL DIETS G/DAY/CHILD

Group I (Opaque-2 Maize) (g)		Group II (Ordinary Maize) (g)		Group III (Skim Milk) (g)		Group IV control
Maize	92	Maize	100	Skim Milk	22	
Corn starch	8	Amaranth		Tapioca	80	
Amaranth/ Carrot/ Tomato	25	Amaranth	25	Amaranth	25	Nil
Oil	5	Oil	5	Oil	15	
Jaggery	20	Jaggery	20	Jaggery	20	

Calculating the supplementation to meet one third of the daily allowances recommended. The experimental dietswere planned to provide 400 calories and 10g. protein. In order to increase the vitamins and mineral intake, 25 g. of carrots/ papaya/tomato/green leafy vegetables are included in the preparations daily.

Composition of the experimental diets:

The composition and the nutritive value of the experimental diets are given in Table XI.

TABLE A1

COMPOSITION, NUTRIENT CONTENT OF DIETS g/CHILD/DAY
Opaque - 2 Maize Diet

Foodstuff	Amount g	Calories	Protein g	Calcium mg	Iron mg	Carotene µg	Thiamine mg	Riboflavin mg	Vitamin C mg
Opaque-2 Maize	92	315	8.8	9.2	1.8	82.8	0.386	0.092	—
Corn starch	8	28	0.1	—	—	—	—	—	—
Amaranth/ carrot/tomato	25	11	1.0	99.2	6.4	1380.0	0.008	0.075	24.8
Jaggery	20	77	0.1	16.0	2.3	34.0	0.004	0.008	—
Oil	5	—	—	—	—	—	—	—	—
		476	10.0	124.4	10.5	1496.8	0.398	0.175	24.8
RDA ICMR 1978		1200	18.3	400-500	15-20	1000	0.600	0.700	30.50

ORDINARY MAIZE DIET

Foodstuff	Amount g	Calories	Protein g	Calcium mg	Iron mg	Carotene µg	Thiamine mg	Riboflavin mg	Vitamin C mg
Ordinary maize	100	342	8.7	10.0	2.0	90.0	0.420	0.100	—
Amaranth/carrot tomato	25	11	1.0	99.2	6.4	1380.0	0.008	0.075	24.8
Oil	5	45	—	—	—	—	—	—	—
Jaggery	20	77	0.1	16.0	2.3	34.0	0.004	0.008	—
		475	9.8	125.2	10.7	1504.0	0.432	0.183	24.8

SKIM MILK DIET

Skim Milk	22	78	8.5	301.4	0.31	—	0.099	0.361	1.1
Tapioca/ Potato	90	126	0.6	40.0	0.72	—	0.040	0.080	20.0
Amaranth/carrot tomato	25	11	1.0	99.2	6.4	1380	0.008	0.075	24.8
Oil	15	135	—	—	—	—	—	—	—
Jaggery	20	77	0.1	16.0	2.3	34	0.004	0.008	—
		427	10.0	456.6	9.73	1414	0.151	0.524	45.9

Amaranthus - Monday, Thursday;
Tomato - Tuesday, Wednesday;
Carrot - Saturday
Papaya - Friday

It was observed that children could consume at one time only 25 g. of food on an average, since 25 g. raw food become more than 75g. as diets based on cereals become bulky on cooking.

6. **Standardisation of the receipes based on maize:**

a. **Maize diets**

The following receipes incorporating the maize and other ingredients were standardized and prepared:

I	Laddu
II	Payasam
III	Uppuma
IV	Uppuma with greens
V	Tomato-bath
VI	Lime bath
VII	Vegetable bath preparation (carrot/beans/greens)
VIII	Tamarind bath

7. **Acceptability test:**

Acceptability tests were carried out on the recipes standardized on selected (25) children, who were not included in the subsequent studies.

Taste attributes are important in determining the food commodity to be programmed. Therefore in order to give different tastes, instead of one monotonous preparation, varieties of preparations were tried. Children were found to relish the differences in taste. The sweet preparations laddu and payasam sweet were universally liked by all the children.

8. <u>Frequency of serving and record and food intake</u>:

The following table XII gives the type and amount of food given per child per day.

TABLE XII

FREQUENCY AND AMOUNTS OF FOOD GIVEN PER CHILD PER DAY

Morning snack	Lunch	Evening snack
Maize - 20 g	Maize - 50-60 g	Maize - 20 g
Laddu	Uppuma with greens or uppuma with carrot or tomato bath/lime bath/ tamarind bath	Laddu Carretstick-15 g

The quantity of 90-100 g. maize was split up into three servings as morning snack, lunch and evening snack.

By using a serving spoon, the cooked food was served on small plates and given to the child. The amount consumed by each child was noted. Care was taken to note that the children consumed the supplements served. Wastage was recorded and found to be minimum, a record of the daily attendance of children participating in the feeding trial was maintained.

b. <u>Skim milk diet:</u>

The skim milk diet was divided into three servings: (The proposed quantity of 22 g skim milk was split up into 2 servings).

Morning snack: Milk/payasam

Lunch : Potato with greens kootu/Tapioca with greens kootu

Mid afternoon snack: Milk/payasam.

8. <u>Evaluation of the nutritional status of children:</u>

a. <u>Weights and heights:</u>

The nutritional status of the target children was evaluated through anthrepometric measurements, biochemical investigations, haemoglobin estimation clinical assessment and metabolic balance studies.

The growth rate was assessed in terms of body weight and heights which were recorded at monthly injervals using procedures described earlier.

b. **Clinical examination:**

Clinical examinations were carried out in the beginning and at the end of the study by a trained paediatrician. The paediatrician examined the hair, face, eyes, lips, tongue, teeth, gum, skin, nail, subcutaneous tissue and muscle skeletal system of the children. She recorded also symptoms of nutritional deficiency, using the schedule given in Annexure (VII).

c. **Estimation of Haemoglobin and faecal examination:**

In each group, 20 children were randomly selected for Haemoglobin estimation and faecal analysis for possible worm infestations at the beginning and the end of the study.

d. **Urinary nitrogen and creatinine ratio:**

Analysis of creatinine and nitrogen content of the early morning samples of urine was carried out at the beginning and at the end of the experiment for the children in the three experimental groups. For the creatinine estimation Folin's method was used Annexure (X) and for nitrogen estimation micro-kjeldahl method was used (Annexure XI).

e. **Incidence of sickness:**

The incidence of sickness in children was recorded all through the study to see whether or not supplement had any ill effects on the children. A record was kept on the reason of sickness and its duration.

f. **Nitrogen balance studies:**

Three day nitrogen balance studies were carried out on six randomly selected children on each diet. The method adopted is given in Annexure XII. Samples of the cooked foods in proportion eaten by each child, were weighed out daily and homogenised. Aliquots were taken for the estimation of dietary protein, by the micro Kjeldahl method.

Urine and faeces were collected for 3 days. The 24 hour composites of stool were homogenised and analysed for nitrogen. The nitrogen content of 24 hours urine collection was also analysed. Each day's collection of urine and faeces was analysed separately.

2. **Evaluation of a Nutritious Supplement Evolved with Indigenous Foods on 5 to 7 years Old School Children:**

This study was carried out in rural community at Vedapatti village. Two hundred children were fed a nutritious supplement for a period of ten months. A similar group of 200 children belonging to another village nearby namely Sandapalayam served as the control. The criteria used in the evaluation of the supplement were, anthropometric measurements, clinical and biochemical examinations. School attendance, performance, curricular and co-curricular activities and incidence of sickness of children were also noted. The experimental steps comprised

1. Selection of the village
2. Socio economic survey
3. Selection of the children
4. Conducting the Bench mark survey
5. Selection of the supplement
6. Evaluation of the supplement.

1. <u>Selection of the village</u>:

Six villages namely Vedapatti, Vedapatti Cherri, Vanniampalayam, Kurumbapalayam, Jambialagam palayam and Andipalayam in Perur Block and Samiapalayam in Periannaicken palayam block of Coimbatore district, were selected for the study because of the good rapport already established in these villages. These villges were difficult to reach, since very few buses were running in this route.

2. <u>Socio economic survey</u>:

All the families in the villages were enumerated and a socio-economic survey was done using the questionnaire given in Annexure VI. The date on the socio economic survey of the individual villages are given in Annexure XIII.

3. <u>Selection of the children</u>:

Since it was considered desirable to have as large a sample aspossible for the human feeding trial, all the children studied in the Ist to IVth Classes in the experimental village namely Vedapatti, were enrolled as the participants in the feeding trial. Out of children in the school participated only 200 children of the age 5-8 years were selected for the collection of data. Similarly, another set of 200 children who were identical in the age and sex with that of the children in the experimental group were selected to serve as control from a nearby village namely Sandampalayam.

2. Conducting the benchmark survey:

A baseline information on the socio economic status, dietary intakes and food practices of the children in both the groups was collected.

3. Selection of the supplement:

The following were the criteria applied to the selection of the supplement.

Surplus and availability of the raw food commodities at the village level:

The prime objective of this study was to utilise the indigenous foods available at the village level in developing a nutritious snack in order to convince the rural mother that it is entirely within her economic and ecological capability to prepare nutritious snacks herself on a regular and continuing basis for her child. The vegetable protein mixture was formulated out of the locally available indigenous foods - rice, horsegram, cowpea, fieldbean dry and sesame. Since rice is the staple food commodity of this region and was considered very prestigeous food, and "culturally super" it was included in the supplement. Horsegram, cowpea, and field beans were selected for their high protein content and low cost. Sesame was selected for its calorie and protein contributions.

Although these foods were available in the village level in abundance, the rural homemakers were not using them. Therefore this cereal-legume-oilseed-vegetable combination, was developed to provide a good amount of protein and calories.

Cost:

Animal foods are very expensive and beyond the reach of the villager to meet his protein requirement. As to be within the economic means and at the same time furnish adequate calorie and protein contents these low cost indigenous foods were selected. The following Table XIII gives the cost of the supplement per child per day.

TABLE XIII

COST OF THE SUPPLEMENT PER CHILD PER DAY

Ingredients		Amount (g)	Cost* Rs. Ps.
Rice		20	0.05
Cowpea		20	0.05
Field Beans dry		15	0.05
Horsegram		10	0.03
Gingelly seeds		5	0.03
Groundnut oil		15	0.10
Greens Amaranthus		30	0.01
	Total	115	0.32

* Prevailing prices in Coimbatore in June 1973

Note: on the days when payasam was prepared 20g. of tomato (0.05ps) and 25g of jaggery (0.06) to be included instead of oils and greens.

The cost of the CARE Food commodities per child/day are listed for comparison.

TABLE XIII(a)

A COMPARISON OF THE COST OF THE CARE FOOD COMMODITIES AND THE SUPPLEMENT

Ingredients	Amount (g)	Cost Rs.Ps.
CSM	42	7.9
Bulgur wheat	56	8.0
Salad oil	7	2.6
Rice and other ingredients (through local contribution)	60	15.0
Total	165	33.5

Nutritive Value:

In the selection of the indigenous foods their nutritive value was kept in mind. Table XIV and XV give the nutritive value of the supplement prepared in the form of uppuma or sundal and payasam.

Standardisation of the supplement:

The ingredients used in the supplement were dry roasted and stored in plastic tins. The supplement observing was formulated all the safety measures. The ingredients were made into various forms, coarse grain and flour that could be helpful in making various preparations.

Formulation of recipes out of the formulated mixtures:

Taste attributes should probably be an important consideration in determining the food commodity to be programmed. So to give different taste instead of the monotonous preparation varieties were tried.

Out of the formulated mixtures 11 receipes were developed. In the following ways the recipes were tried.

Out of the formulated mixtures 11 receipes were developed. In the following ways the recipes were tried.

1. Uppuma with greens
2. Pongal
3. Puttu
4. Adai
5. Dosai
6. Iddly
7. Tomato bath preparation
8. Vegetable bath preparation
9. Lime bath preparation
10. Tamarind bath preparation
11. Sambar bath preparation

All the 11 recipes were found to be acceptable. Out of the 11 recipes, six most acceptable and commonly used ones namely uppuma with greens, tomato bath, vegetable bath, lime bath, tamarind bath and Pongal were selected and standardised.

<u>Acceptability trials</u>:

There is considerable literature to support the conclusion that unless a product is acceptable, it is not likely to be fed to the target child. The taste and flavour of a product will also contribute to the acceptability of it.

It was also necessary to ensure to the extent possible that the ingredients used were not identified with negative characteristics, eg. too flatulent, causes indigestion or of low status. These factors were also considered in the selection of the ingredients for the formulations, that their taste and flavour are acceptable and are not associated with any negative characteristics.

With the standardised recipes acceptability and digestibility trials were carried out on selected 400 children of the age group 5-10 years.

Quantity of other food stuffs used per child per meal apart from the cereal pulse mixtures are listed below.

Food item	Amount (g)
Vegetable protein mix	100
Greens	50
Jaggery	10
Papaya/Tomato/Carrot	20
Oil	8

Source: (Devadas, 1971)

Three recipes namely: Uppuma, Sundal and Payasam were prepared. These recipes were standardised and the method of preparation are given in Annexure XIV. With the standardised recipes of uppuma, sundal and payasam, acceptability tests it were carried out on selected school children. Through the acceptability tests it was revealed that the supplement was relished by the children. There was no incidence of digestive disturbances in children. Children had no vomitting and relished the food very much.

TABLE XIV

COMPOSITION AND NUTRITIVE VALUE PER SERVING OF SUNDAL OR UPPUMA

Ingredients	Amount g	Calories	Protein g	Calcium mg	Iron mg	Retinol μg	Thiamine mg	Riboflavin mg	Vitamin C mg
Rice	20	69	1.3	1.8	0.8	—	0.040	0.010	—
Cowpea	20	65	4.8	15.4	1.2	0.50	0.102	0.040	—
Field bean dry	15	52	3.7	9.0	0.4	—	0.078	0.024	—
Horse gram	10	32	2.2	28.7	0.8	1.75	0.042	0.020	0.1
Gingelly seeds	5	28	0.9	72.5	0.5	0.75	0.050	0.017	—
Oil-groundnut	15	135	—	—	—	—	—	—	—
Greens Amaranthus	30	14	1.2	119.1	7.7	414.0	0.009	0.090	29.7
Total	115	395	14.1	246.5	11.4	417.0	0.321	0.201	29.8

TABLE XV

COMPOSITION AND NUTRITIVE VALUE PER SERVING OF PAYASAM

Ingredients	Amount g	Calories	Protein g	Calcium mg	Iron mg	Retinol µg	Thiamine mg	Riboflavin mg	Vitamin C mg
Rice	20	69	1.3	1.8	0.8	—	0.040	0.010	—
Cowpea	20	65	4.8	15.4	1.2	0.50	0.102	0.040	—
Field Beans dry	15	52	3.7	9.0	0.4	—	0.078	0.024	—
Horse gram	10	32	2.2	28.7	0.8	1.75	0.042	0.020	0.1
Sesame	5	28	0.9	72.5	0.5	0.75	0.050	0.017	—
Jaggery	25	96	0.1	20.0	2.9	10.50	0.005	0.010	—
Tomato	20	4	0.2	5.6	0.1	17.50	0.024	0.012	5.4
Total	115	346	13.2	157.0	6.7	31.0	0.0341	0.133	5.5

Amaranthus/tomato were added to uppuma or sundal and payasam respectively to meet the retinol and vitamin C requirements of the children.

Various studies have demonstrated that the critical bottleneck in school childrens diet are Calories and Vitamin A. Therefore 346-395 calories were to be delivered to the school children through the nutritious supplement evolved.

Amino acid composition:

The amino acid profile of the supplement in comparison with those of FAOs provisional pattern, cow's milk and CSM are presented in Table XVI.

TABLE XVI

SELECTED ESSENTIAL AMINO ACID PROFILE OF THE SUPPLEMENT

Amino Acid	Grams of Amino Acid per 100g of Protein			
	1973 FAO reference pattern	Cow's Milk	C.S.M.	Supplement
Lysine	7.5	7.8	5.0	5.7
Threonine	4.4	4.4	3.7	3.2
Methionine	1.7	1.6	2.0	1.2
Tryptophan	0.4	1.4	1.1	0.8

The amino acid content of the supplement (vegetable protein mixture) did not compare favourably with the FAO reference pattern except in Tryptophan. In spite of the fact that several ingredients of the supplements such as horse-gram, cowpea, field beans and sesame which are rich in either lysine, threonine, methionine or tryptophan. The home diets consumed by the target child were derived from cereals, legumes and small quantities of milk which would have significantly improved the total amino acid intake of the children.

Acceptability:

There is considerable literature to support the conclusion that unless a product is acceptable to the mother, it is not likely to be fed to the target child. The taste and flavour of a product will also contribute to the acceptability of it.

It was also necessary to ensure to the extent possible that the ingredients used were not identified with negative characteristics, such as too flatulent, causes indigestion or of low status. These factors were also considered in the selection of the ingredients for the supplement. Their taste and flavour were acceptable and are not associated with any negative characteristics. Thus, rice was selected because of its prestige value, and the legumes that they will not cause any digestive troubles.

Feeding the children with the supplement:

The feeding trial was started with the 200 children (Experimental) irrespective of whether they participated in the school lunch programme or not. The supplement was served daily at 3.30 p.m. as uppuma or sundal or payasam for six days per week.

Care was taken to see that the children consumed the supplement served. The record of the daily attendance of children participating in the feeding trial was also maintained along with the exact quantities of the supplement consumed and the plate wastages if any.

The feeding trial was carried out for a period of 10 months.

Evaluation of the Supplement

Nutritional assessment of school children:

In the process of evaluation of the nutritious supplement to school children, various parameters were used to assess the nutritional status of the school children.

A. **Socio Economic and Food habit survey:**

A socio economic and food habit survey was conducted for all the 400 children (200 experimental and 200 control) to study their socio-economic study and living conditions and to study their food habits. The food habits and dietary practices at various income levels were studied. The pro-forma used is given in Annexure VI.

b. **Food weighment survey:**

A three day food weighment survey was conducted to assess the home food intakes of 10 randomly selected children in the experimental group and ten children in the control group respectively. The proforma used is given in Annexure VIII.

c. **Anthropometric measurements:**

A longitudinal and cross sectional study of the heights and weights taken of the children in both the groups were carried out.

The heights were taken in cms with the help of the stadiometer and weights taken in kilograms with help of the Avery beam balance.

Standardising Heights:

For taking heights the subject was asked to stand barefoot on the centre of the platform fully erect with heels together, legs, back and neck straight and eyes horizontal.

The horizontal level was lowered so as to rest firmly on the crown of the head. The reading was measured to the nearest 0.5 cm.

Weight:

For recording the weights the scales should rest firmly on a horizontal base. Before each period of examination the balance arm was checked with no load on the scale to verify whether the arm was horizontal when the scale reading was zero. If it was not zero the screw head was adjusted at the left hand side so that the arm was horizontal with zero load.

Once in three weighings the scale was checked with standard weights of 20 kg and 40 kg. and in order to have no error.

When reading the scale, care was taken to see that the movable weight resting in the slots at 20 kg. was put accurately in the slot.

The subject was asked to stand steadily in the centre of the platform while being weighed.

4. **Attendance in school:**

A daily record of the attendance in school was maintained through out the study to see the regularity of the children in attending the school and their interest in studies.

e. <u>Participation of the children in the curricular and extra curricular activities</u>:

A record of the participation of the children in the curricular and extra curricular activities was kept throughout the study. These records were kept to see how far the children are improving in their academic abilities and physical activities. Their enthusiastic participation in games was also noted.

f. <u>Incidence of sickness</u>:

The incidence rate of sickness among the target children were recorded everymonth.

g. <u>Clinical assessment</u>:

Clinical examinations were carried out on all the 400 children in both the groups using the modified ICMR schedule given in Annexure IX.

h. <u>Biochemical tests</u>:

1. <u>Haemoglobin estimation</u>:

Haemoglobin estimation on all the 400 children of both the groups was carried out from finger prick sample. The initial and final levels of haemoglobin were estimated using cyanmethaemoglobin method.

2. **Urinary creatinine and Nitrogen ratio:**

The early morning urine samples of 50 randomly selected children (25 in experimental and 25 in control) were analysed for the creatinine and nitrogen content using Folin's method and microkjeldahl method respectively.

F. **Supplementation of Amaranthus to Anaemic Adolescent School girls:**

The study was designed to find the effect of supplementation of 100 g of Amaranth containing of 24.3 Mog. of iron compared with the effect of an oral iron tonic containing equal quantity of the mineral given to another group of similar girls.

1. **Selection and grouping of subjects:**

Adolescent girls between 13 and 16 years of age studying in Sri Avinashilingam Home Science Girls School and residing in the school hostel were selected for the study. For 84 adolescent girls, heights, weights and estimation of haemoglobin using the finger tip blood using the Oxy haemoglobin method, were determined. Those with haemoglobin values below 11.5 per 100 ml were considered anaemic, (WHO 1959). They were 24 in number. Faecal specimens of all these girls were examined by the MIF staining method (ICMR) to detect out hook worm infestation if any. All the girls had attained puberty since two years or earlier. A physician evaluated the subjects as healthy otherwise. The 24 anaemic girls were randomly assigned to two experimental groups A and B, of 12 each. Another group of 12 non anaemic girls were selected as a control group.

Dietary treatments:

No attempt was made to restrict or change the hostel menu throughout the experimental period. The girls in group A were fed cooked amaranth 100g (fresh weight) mashed with added salt and chillies and served along with lunch. This was the maximum amount of amaranth the girls could consume when cooked and served. The girls in group B were supplemented with an iron tonic (colloidal iron hydroxide) to supply 25 mg of iron daily. The control group C was given none of the above supplements. To the knowledge of the investigator none of the subjects consumed any iron tonics during the course of the experimental elsewhere.

The amaranth used for supplementation waspurchased from the same garden and analysed from time to time for iron content. No appreciable variations were observed. The mean iron content of this supplement was 24.3 mg/100 g. The mean iron content of the tonic was 22.3 mg/100 gm. No palatability difficulties were encountered during the experiment. Furthermore, no intestinal troubles such as diarrhoea and vomiting occurred.

The hostel diet was analysed for its protein, iron and calcium contents. A three day weighment survey was carried out for this purpose.

Indices of evaluation:

The heights, and weights and haemoglobin content of the girls were measured every fortnight. For the haemoglobin determination, each time a standard every fortnight. For the haemoglobin determination each time a standard curve was drawn with fresh blood samples of the rat whose heme iron content was estimated at the time by Wong's method (Hawk *et al.* 1954). At the end of the experiment 2 ml. of venous blood was drawn from subjects and Packed Cell volume and the RBC count were obtained using micro haematocrit tubes and the neubar ruled counting chambers (ICMR, 1963). MCH, MCV and MCHC, and colour index were calculated using the above values.

G. Determination on the availability of β-Carotene in a human feeding trial:

The present investigation was undertaken to determine the availability of β-carotene from carrots to women college students.

Twenty one college students of the age group 20 to 24 years, of Sri Avinashilingam Home Science College, Coimbatore, were selected for the study. They were divided into three groups. Ten of them formed the experimental group who were fed carrot supplementation and five of them formed the control group, with no supplementation for the study. The remaining six members formed the retinol supplementation group.

Diet Consumed by the Subjects:

Calculations of the daily vitamin A intake of both animal and vegetable sources from a dietary record sheet kept for the entire 20 days by each subject who were residing in the hostel were done. From the record of food intake, the average vitamin A (microgram of retinol) intake per head per day was calculated using the tables of food composition table, (ICMR, 1971). The mean daily food intake and vitamin A (retinol) content of the diet of the 20 subjects are given in table XVII. The average vitamin A, Calorie, protein and fat intake per head per day was calculated using the ICMR Food Composition Tables. The cooked food samples were also analysed for its vitamin A, and carotene contents.

Before the beginning of the feeding schedule, five ml. of venous blood sample was drawn from the forearm of each subject, for vitamin A determination in serum.

TABLE XVII

MEAN DAILY FOOD INTAKE AND VITAMIN A (RETINOL) CONTENT OF THE DIET OF THE 21 SUBJECTS

Foodstuffs	Mean daily intake g.	Retinol Content µg
Cereals	315	4
Pulses	45	14
Roots and tubers	30	2
Leafy vegetables	10	138
Other vegetables	30	15
Fruits	40	5
Milk and milk products	250	120
Sugar	25	—
Oil	20	—
Total		298

Carrots which are a source of biologically utilisable carotene were fed to ten subjects as a supplement to their regular hostel diet. Hundred grams of carrots were cooked in a pressure cooker for five minutes and fed to each subject at lunch time. The cooked carrots mixed either with a little sugar or with salt & pepper. The cooked carrots provided about 1890 μg of carotene (475 μg of retinol equivalent). This level was chosen to represent along with the basal diet (298 μg of retinol equivalent) the ICMR recommended allowance for vitamin A for adult women, namely 750 μg. Five subjects who did not receive the carrot supplement served as controls. Another group of six subjects were fed daily 475 μg of retinol "Propalin" a commercial vitamin A preparation containing 24,000 μg of retinol per gram carefully diluted in coconut oil to obtain the required dilution. The amount of retinol was equivalent to 1890 μg of carotene which was present in 100 g of carrots. All the supplementation lasted for 20 days. Same controls were used for both the supplementary studies. No other sources of vitamin A in the form of tonics or pills were consumed by the subjects during the course of the experimental period.

Serum vitamin A levels before and after supplementation was taken as an index of the utilization of carotene and vitamin A, consumed by the subjects. The method is given in Annexure XV.

Same controls were used for both the supplement study. Among the ten students who formed the experimental group for carrot supplementation, vitamin A activity could be measured only for six students during final analysis.

IV. RESULTS AND DISCUSSION

A. Formulation of a series of cereal - legume mixtures useful for children based on local foods and their evaluation on albino rats:

The protein quality of maize diets with selected indigenous foods namely cowgram, greengram, horsegram, sunflower seeds, and groundnuts in different combinations was studied on albino rats using body weight changes, PER, NPU and NDP calories per cent as criteria.

Growth and PER

Table XVIII gives the body weight gains of rats on the six food formulations (mixtures) and the PER of the diets. The analysis of variance is given in Annexure XVII.

TABLE XVIII

MEAN BODY WEIGHT GAINS OF RATS ON THE SIX FORMULATIONS, AND THE PER OF THE DIETS

(No. of rats in each group: 8)

Code Number	Source of Protein	Mean Increase in body weight for 21 days	Mean Protein Intake	PER
S	Skim Milk	85.60 ±[a] 4.10	26.6 ± 0.50	5.01 ± 0.07
M	Maize	13.76 ± 6.52	15.66 ± 0.36	0.87 ± 0.06
A	Maize - Cowgram - Green gram - Sunflower seed	49.25 ± 2.45	21.05 ± 0.82	2.34 ± 0.60
B	Maize - horse gram - green gram	54.01 ± 2.48	25.30 ± 0.69	2.24 ± 0.02
C	Maize - cowgram - green gram	60.94 ± 1.77	26.70 ± 0.24	2.59 ± 0.04
D	Maize - horse gram - cowgram - sunflower seeds	70.41 ± 1.66	26.22 ± 0.36	2.72 ± 0.07
E	Maize - green gram - Sunflower seed	53.38 ± 1.54	21.84 ± 0.94	2.44 ± 0.72
F	Maize - cowgram - horse gram - groundnut	60.44 ± 2.34	23.05 ± 0.82	2.64 ± 0.05

a ± Standard error
S = Skim Milk
M - Maize

The body weight gains and the PER values were highest for the rats on the skim milk diets namely 85.60 g. and 3.01 and lowest for the basal maize diet, 13.76 g. and 0.87. The body weight gains and the PER values for all the six food formulations were significantly higher than the basal maize diets. That the PER values were greater than 2.2 which is an indication that the protein quality of all the six food mixtures was satisfactory as per FAO requirements, (1971). Girija Bai and Devadas (1972) and Devadas (1973) reported similar PER values for vegetable protein mixtures.

The NPU and NDP calories % of the six food formulations are given in Table XIX.

TABLE XIX

NPU AND NDP CALORIES % OF THE SIX FORMULATIONS

Code	No.	Formulations	NPU	NDP Cals %
		Skim milk	70	11.41
I	A	Maize cowpea-greengram, sunflower seed	66	6.95
II	B	Maize - horsegram - greengram	65	9.23
III	C	Maize - cowpea - greengram	65	5.06
IV	D	Maize - horsegram - cowpea - Sunflower seed	59	5.74
V	E	Maize - greengram - sunflower seed	62	6.27
VI	F	Maize - cowpea - horsegram, groundnut	61	7.15

The NPU values of the six formulations ranged from 59 to 66 and that of the NDP calories per cent from 5.06 to 9.23. Skim milk which was the reference standard protein had the highest NPU value namely 70 and NDP Cal per cent 11.4 also ranked in a similar order. The NPU and NDP cal% values for the various food formulation were satisfactory when compared with the values reported for vegetarian diets (FAO, 1965) have been reported by Guttikar et al (1965) also reported Girijabai and Devadas (1972) and Devadas et al (1973) similar NPU values and NDP cal% for vegetable protein foods.

Cost analysis of the six formulations in comparison with C.S.M. are represented in Table XX. The cost of the 100 grams of the food formulations asper the prevailing prices during October 1974, were comparable to the cost of the imported food items such as C.S.M., bulgar wheat etc. At present the storage and transport cost of these foreign foods are very high, (Pingale; 1973).

TABLE XX

COST ANALYSIS OF THE SIX FORMULATIONS AS PER THE PREVAILING PRICES ON OCTOBER '74, AT COIMBATORE, TAMIL NADU

	Formulations	Amount (g)	Cost per child Paise a
* CSM			
	Bulgar wheat	56	7.9
	CSM	42	8.0
	Salad oil	7	2.6
	Total		18.5
A	Maize, cowpea, greengram, sunflower seed	100 g.	0.19
B	Maize, Horsegram and greengram	100 g.	0.15
C	Maize, Cowpea, greengram	100 g.	0.18
D	Maize, Horsegram, cowpea, sunflower seed	100 g.	0.18
E	Maize, greengram sunflower seed	100 g.	0.19
F	Maize, Horsegram, Cowpea, Groundnut	100 g.	0.19

* Source: Pingale, S.U. 1973 — Cost availability of raw materials for feeding programme - a forecast in Report of the Hyderabad Workshop, Protein Foods and Nutrition Development Association, p.81.

a 100 paise make one rupee which is equivalent to 0.131$ in U.S.A.

Cost analysis of the indigenous food formulations show that the indigenous food mixtures are comparable with the cost of CSM, Bulgar wheat and salad oil.

Organoleptic Acceptability:

After testing the various formulations prepared in the form of various recipes for appearance, texture on adults and children, it was found that all the recipes were very acceptable to the target children. Children relished the preparation and did not have any digestive problems or vomitting after the consumption of the supplement. The addition of sweetening agents such as Jaggery in the range of 15 to 20 per cent were acceptable to pre school and school children in dry food products. However in semi-solid cooked foods (which increase in volume by three or four times after cooking the level of sweetening agents had to be increased to 40 to 50 per cent. It was found that higher levels of fat content increased acceptability. In these trials it was also established that it was virtually impossible to produce an acceptable recipe without including some oil.

Bulk consumption:

Once we know how much food a child requires how do we know whether a child can consume that amount at one time?

In order to gain an insight in to the consumption capacity of the pre-school and school child, experiments were conducted on a group of 30 children ranging in age from two to five years, and six to nine years. These children were fed the various food preparations. Everyday the amount of food each child was able to consume was noted. These observations are presented, in Table XXI A. The 'payasam' and 'uppuma' preparations became very bulky as compared to the laddu preparation.

TABLE XXI A

THE QUANTITIES OF THE VARIOUS COOKED AND RAW FOODS THAT COULD BE CONSUMED BY THE TARGET CHILDREN AT ONE SITTING

Target Children	Recipe	Raw Wt. g.	Cooked Wt. g.
Preschool (3 to 4 years)	Uppuma	75	225
	Payasam	50	250
	Laddu	75	187
School (6 to 9 years)	Uppuma	100	350
	Payasam	80	400
	Laddu	100	250

The formulations for the feeding programmes could be prepared in the form of vegetable uppuma/payasam/laddu. The important processing stages are:

1. Dehusking of the cereals
2. Pre-roasting of cereals and pulses

and 3. Fortification with vitamins and minerals.

The processed dry (raw) formulations should be in the form of small granules like wheat rava, (corngrits) so that they can be used for preparations like uppuma, and the final recipe will not become lumpy.

Thus the organoleptic evaluation was also done and the various food preparation (dishes) made were found to be palatable and whole some. All the cereal legume oil seed ingredients were roasted to increase their cooking qualities, acceptability and to overcome trypsin or other possible inhibitors. As a result of all these varied tests formula II, and III. Consisting of Maize horsegram and cow gram or greengram with the addition of suitable vitamins and minerals to fill the existing nutritional gaps in the diets of pre-school and school age children, have been selected.

The reasons for the selection of these two formulae are:

a. They are the simplest as it has only 3 ingredients as compared to other formulae which have 5 or more.

b. They have a high percentage of maize which makes it truly indigenous to Tamil Nadu.

c. Their other ingredients green gram and horsegram or cow gram is also grown in Tamil Nadu; although these legumes are not grown in sufficient quantities to be useful immediately, but the potential is there and can be developed if the need arises.

d. They are the cheapest among the five formulae.

e. The caloric fic value of 365 for a ration of 100 grams is able to fill the existing caloric and protein gaps in the dieptans of the preschool diets.

Table XXI B gives the recommended rations for the various target groups of the population, based on the deficiencies of these groups.

TABLE XXI B

RATIONALE FOR SUPPLEMENTAL FOOD QUANTITY

Age Group	Calorie Deficit	Protein Deficit	Basis of Information	Food supplement	Calories	Protein	Cost per ration (paise)
Very Young Preschool children	300	Nil	Devadas, 1973	50	197	7.6	0.09
Preschool children	352	Nil	Devadas, 1973	75	295	11.5	0.14
School children	387	Nil	Devadas, 1973	100	393	15.3	0.18
Pregnancy	1521	18	Devadas and Easwaran, 1971	350	1376	53.5	0.63
Lactation	1164	17	..	300	1179	45.9	0.54

The formulation made out of Maize, cow gram, green gram mix can be used in appropriate quantities for feeding of mothers, pre school children and school children. It might be neither necessary from the nutritional considerations, nor desirable from practical point of view, to device separate formulations for mothers and preschool children. Thus this experiment was designed with a view to reduce Tamil Nadu's dependence on imported foods for the school and preschool Nutrition Programmes. The aim of the project was to develop food formulations that are indigenous to Tamil Nadu, that could replace the 'care' foods in part or in whole to give each beneficiary child 394, Calories of energy and 15.5 g of protein. This target was reached, since the nutritive value, palatability and acceptability along with the costs of the ingredients and processes have been established through this study.

A_2. Nutritional Evaluation of Sorghum-Greengram Diets:

This experiment was designed to find out how much of green gram supplemented to a sorghum diet would result in maximum PER value.

The PER value and lysine and methionine content of the various formulations are given in Table XXII.

TABLE XXII

PROTEIN INTAKE, BODY WEIGHT GAIN AND PER VALUE LYSINE AND METHIONINE CONTENT OF THE VARIOUS FORMULATIONS WITH DIFFERENT PROPORTIONS OF SORGHUM AND GREENGRAM

Protein ratio of the formulations Sorghum: GG	Greengram gm.	Protein intake in foods	Average weight gain	PER	Amino Acid Contents(g) Lysine	Methionine
100	0	26.5	23	0.87	0.2117	0.1411
80	20	27.5	38	1.38	0.3405	0.1574
60	40	30.5	63	2.10	0.4320	0.1488
50	50	25.5	52	2.04	0.4954	0.1475
40	60	26.30	40	1.52	0.5412	0.1432
20	80	28.91	22	0.76	0.6327	0.1346
0	100	9.50	15	0.77	0.7414	0.1291

The diets in Table XXII contained equal amounts of protein, but derived in different proportions from sorghum and green gram.

Maximum protein value was obtained when 60 per cent of the protein in the diet were derived from sorghum and 40 per cent from greengram (Fig.I). These figures corresponded to 75 g. of sorghum and 25g. of greengram in a mixture of 100 gram of the feed.

In amino acid contents lysine appears to be the main limiting amino acid when sorghum provides from 50 per cent to 100 per cent of protein of the diet. On the other hand, methionine becomes the defficient amino acid when greater proportion of protein isprovided by green gram.

In this experiment the optimum proportion of sorghum to green gram was of 3:1 ratio. The results of various nutritional surveys carried out in Coimbatore, Tamil Nadu State and the calculation of ratio of cereal to pulse as consumed by the population is given in Table XXIII.

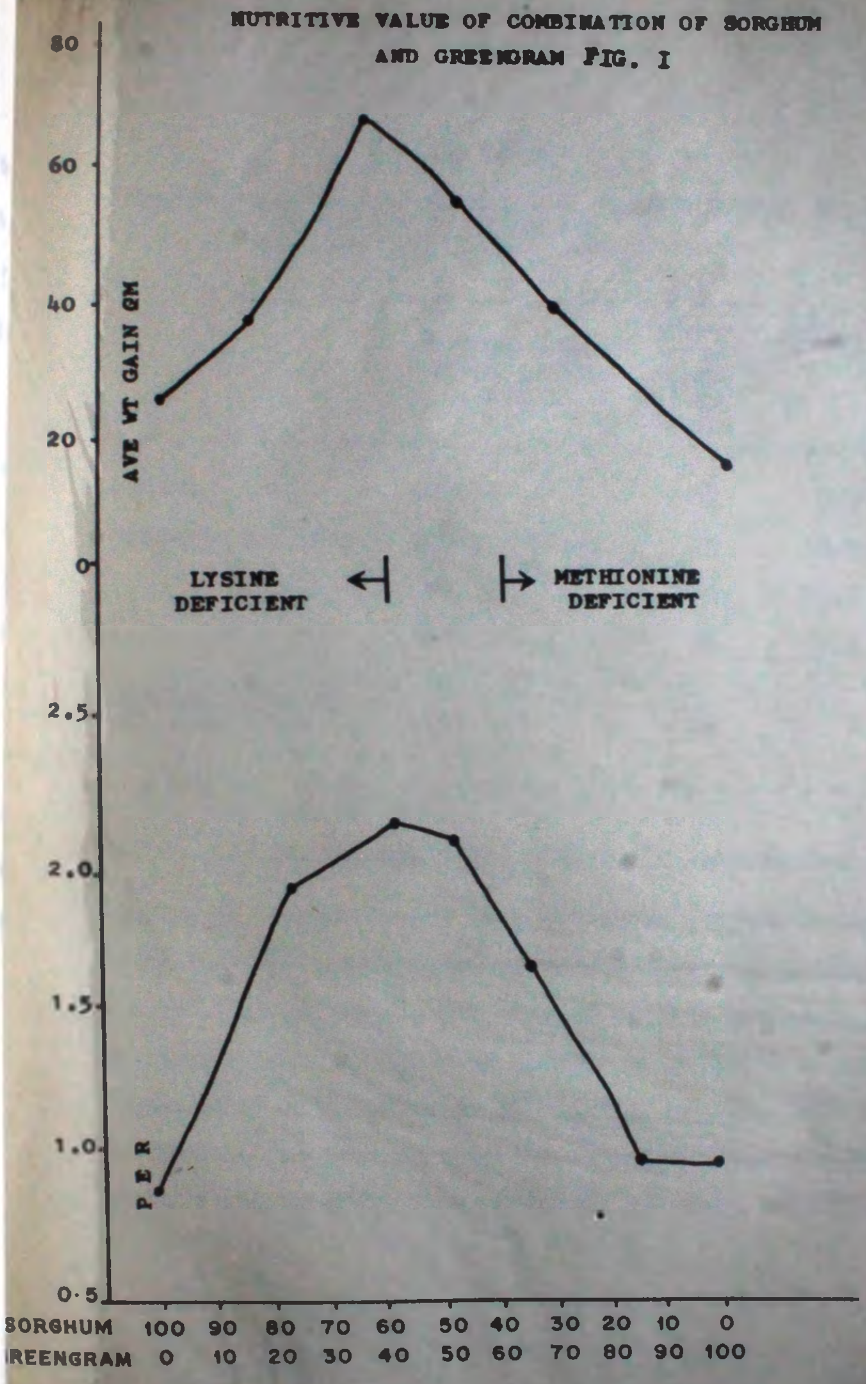

NUTRITIVE VALUE OF COMBINATION OF SORGHUM AND GREENGRAM FIG. I
80
60
40
20
0
AVE WT GAIN GM
LYSINE DEFICIENT
METHIONINE DEFICIENT
2.5
2.0
1.5
1.0
0.5
PER
SORGHUM 100 90 80 70 60 50 40 30 20 10 0
GREENGRAM 0 10 20 30 40 50 60 70 80 90 100

TABLE XXIII

AMOUNT AND RATIO OF CEREALS AND LEGUME CONSUMED PER DAY IN COIMBATORE DISTRICT
(Devadas 1974 - CR-O, 1973)

	Sorghum g/day or any other cereal		Green gram or any other legume	Cereal/legume ratio
Adults	423	Devadas et al (1972)	50	8.5 : 1
Children 3-4 years	281		24	11.7 : 1
	295		26	11.3 : 1
	277		15	18.5 : 1
Optimum ratio as per this study	75		25	3 : 1

The present dietaries this ratio ranging from 8 to 18.5. In all the cases, consumption of sorghum or cereals predominate. Adults consumed more sorghum and greengram compared to children. The protein quality increases to a maximum when the ratio of sorghum or (cereal) to pulse (greengram) consumption is of 75/25 g. or 3:1. This is of particular interest in evolving simple indigenous foods, to replace the imported food mixtures, having a single locally grown cereal like sorghum and a legume green gram has the following two advantages.

1. Ready availability of raw materials in Coimbatore district, of Tamil Nadu State.
2. 100 grams of the mixture would supply adequate proteins and calories to the target school child, so that present dietary deficiencies could be made up.
3. The PER value is above 2.0.
4. The mixture is least expensive and simple in having only two ingredients namely a cereal and legume so it is adapatable for teaching and demonstration purposes among the rural communities.

4.3. <u>Bioassay Evaluation of the Proteins from a mixture of Horse-gram and Sesame seeds:</u>

A vegetable protein mixture of horsegram and sesame in the protein ratio of 4:1 was evaluated using the bioassay technique, the results of which are presented below. The mean body weight of rats fed diets of varying sources and concentrations of proteins are presented in Table XXIV.

TABLE XXIV

MEAN BODY WEIGHTS OF RATS FED DIETS OF VARYING SOURCES AND CONCENTRATIONS OF PROTEINS

Group	Source of protein in the diet	Percentage of protein in the diet	Body Weight		
			Initial (g)	Final (g)	Increase (g)
A	Horse gram + sesame	9	28.7±0.83	42.4±1.41	13.7±0.80
B	-do-	11	27.8±2.01	49.9±0.51	22.1±1.38
C	-do-	13	27.2±0.73	49.7±2.84	22.5±1.87
D	-do-	15	27.2±1.73	64.5±2.88	37.3±2.25
E	Skim Milk	7	27.1±1.25	55.1±2.61	28.0±0.78
F	-do-	9	28.1±1.36	68.4±3.94	40.3±0.54
G	-do-	11	28.5±2.30	83.0±4.28	54.6±0.54
H	-do-	13	29.0±1.70	91.4±2.13	62.4±2.25

a Mean : ± Standard error.

The growth rate responses for the skim milk protein diets were greater than those for horsegram - sesame mixtures for all the concentrations. Analysis of variance for weight gain were given in Annexure XVIII.

Table XXV . gives the mean food and protein intake of rats fed diets of varying protein contents for 20 days and the PER of the different food mixtures.

TABLE XXV

MEAN FOOD AND PROTEIN INTAKE OF RATS FED DIETS OF VARYING PROTEIN CONCENTRATES FOR 28 DAYS AND THE PER OF THE DIETS

Group	Food Intake	Protein Intake	PER
A	133.0±13.21[a]	11.97±0.76	1.05±0.091
B	164.5±8.25	18.09±1.24	1.10±0.095
C	148.0±11.29	19.24±0.51	1.16±0.152
D	160.5±14.39	24.07±1.11	1.38±0.101
E	138.4±9.07	9.69±1.77	2.62±0.081
F	161.3±6.59	14.52±1.98	2.84±0.118
G	169.3±15.37	18.59±1.30	2.94±0.107
H	165.2±6.37	21.63±1.09	2.92±0.097

a Mean: ± Standard error.

The food intake of rats did not vary much except in rats fed 9 per cent horse gram - sesame mixture diet and 7 per cent skim milk diets, in which the consumption was less than in others. Therefore the protein intake of the different dietary groups varied only due to the difference in the concentration of protein of the diet. The analysis of variance for protein intake is given in Annexure XIX.

The mean PER values (Table XXV) increased from 1.05±0.094 to 1.38±0.101 for horsegram sesame mixtures, when protein content of the diets was increased from 9 to 15 per cent. For skim milk, the corresponding PER values increased from 2.62±0.081 to 2.94±0.10, when protein content was raised from 7 to 11 per cent. There after the PER values remained constant even when the protein content was further increased to 13 per cent. Analysis of variance for PER is given in Annexure XX.

TABLE XXVI

MEAN LIVER WEIGHTS TOTAL HEPATIC NITROGEN AND HEPATIC SDH ACTIVITY OF RATS FED DIETS OF VARYING PROTEIN QUANTITY

Group	Fresh liver weight (mg)	Total hepatic nitrogen (mg)	Hepatic SDH Activity Mmg. of triphenyl tetrazolium chloride reduced per g./30 minutes
A	2.69±0.254[a]	64.3±7.97	835[a]±41.15
B	3.31±0.291	95.3±8.96	1064±38.78
C	3.83±0.284	137.8±10.56	1354±85.60
D	4.09±0.178	154.5±11.21	1683±89.08
E	3.00±0.308	60.2± 5.25	954±36.07
F	3.75±0.357	113.6±11.90	1182±46.66
G	4.82±0.379	175.1±13.50	1564±65.50
H	4.81±0.221	218.8±16.21	18.62±71.68

a Mean : ± Standard error

There were no significant differences in the hepatic weights of the eight groups of the experimental animals. But significant differences were found in the total hepatic nitrogen content and hepatic nitrogen per g. of fresh tissue among the groups fed different levels of protein. The lowest value of 60.2±5.25 mg. of hepatic nitrogen was found in rats fed 7 per cent skim milk and highest value 218.8±16.2 mg. in rats fed 13 per cent skim milk diet. As the protein concentration was increased from 9 to 15 per cent in heterogeneous diets and from 7 to 13 per cent in skim milk diets, the total hepatic nitrogen values also increased from

64.3±7.97 to 154.5±11.2 mg. of nitrogen and from 60.2±5.25 to 218.8 ±16.2 mg. of nitrogen respectively. Thus the picture of hepatic nitrogen concentration was different from that of growth and PER indices. In the case of PER even the lowest level of 7 per cent skim milk gave a better response than the vegetable protein mixture.

The Hepatic Succinic Dehydrogenase (SDH) activity was increased with the increase in the level of protein and quality of protein. The analysis of variance for SDH activity is given in Annexure XXI. The rats on the skim milk diet had higher hepatic SDH activity at all levels than those on the horsegram and sesame mixture. Thus higher hepatic nitrogen contents correlated with higher hepatic SDH enzyme activities.

<u>Log dose response</u>:

Analysis of the data and the log dose response curves using body weight gains as the response, and log nitrogen intake or percentage of dietary protein as a measure of dosage failed to satify the requirements of a satisffactory assay. (Fig.II and III). However, calculation of slopes ratio using slopes of the regression lines relating actual protein or nitrogen intakes as dose and body weight gain as response provided useful data. The best fit of the data was obtained by plotting gain in body weight against nitrogen intake.

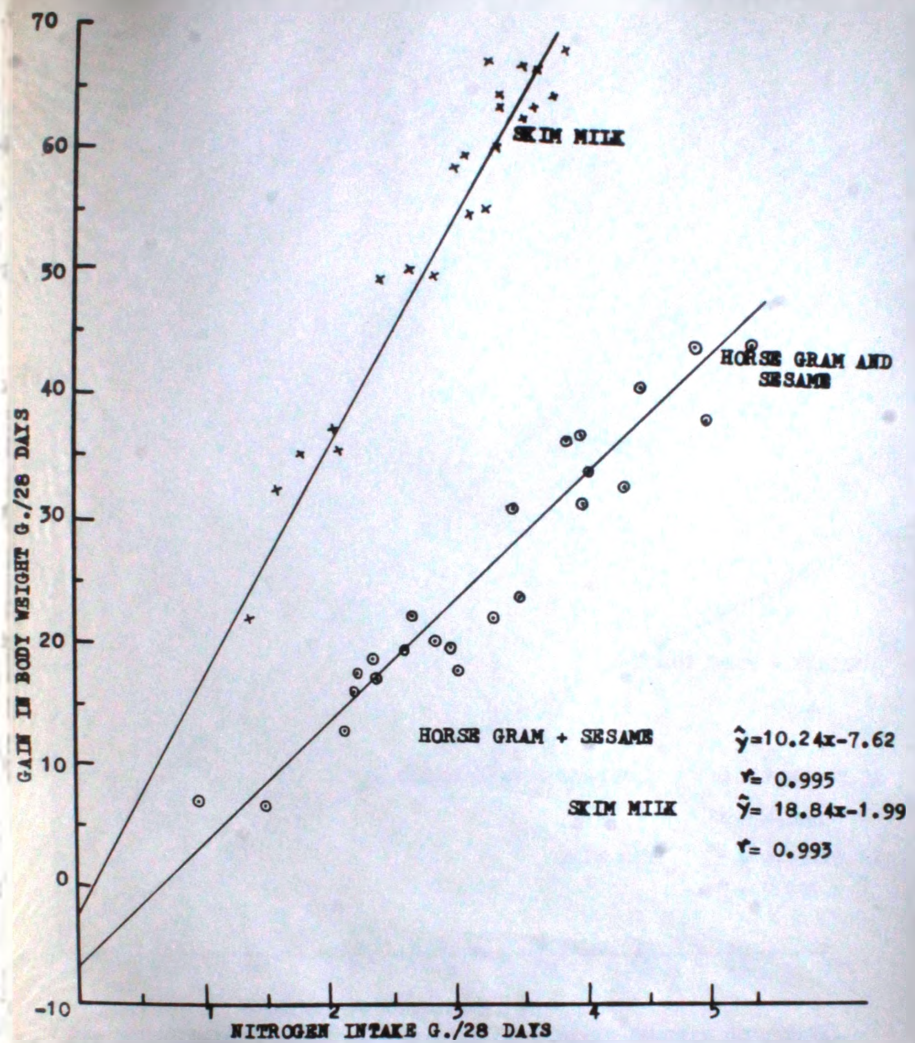


FIG. [illegible] REGRESSION OF WEIGHT GAIN ON NITROGEN INTAKE OF RATS ON DIETS DIFFERING IN SOURCES AND AMOUNT OF PROTEINS

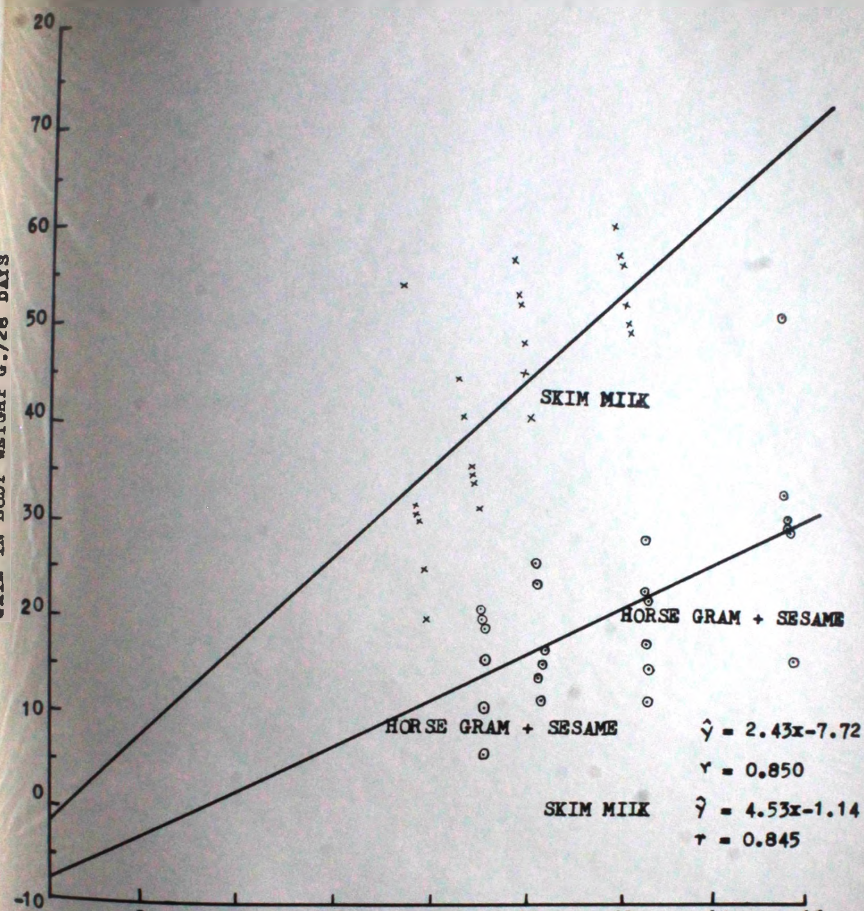


FIG.III. REGRESSION OF WEIGHT GAIN ON DIETARY PROTEIN CONCENTRATIONS OF RATS ON DIETS WITH DIFFERENT SOURCES OF DIETARY PROTEINS

This gave higher correlation coefficients, small errors on the slopes of the regression lines and intercepts which approached those found experimentally.

The Relative growth index which is an expression for the relative nutritive value of dietary proteins, is the slope of the regression between dose and response expressed as the percentage of the slope obtained with the protein of reference quality of maximum nutritive value. Using this index and skim milk as a reference protein of 100, the relative nutritive value based on body weight gain of horse gram, sesame mixture was 53.6.

TABLE XXVII

REGRESSION OF GAIN IN BODY WEIGHT ON TOTAL NITROGEN AND PROTEIN INTAKE AND DIETARY PROTEIN LEVEL

Protein	Regression equation	Correlation Co-efficient xy
	Regression equations	
	y = gain in body weight in g/28 day; x = total dietary protein or nitrogen intake in g/28 day or dietary protein concentration.	
Protein		
Horsegram - sesame mixture	1.567 x - 6.31 = y	r = 0.945
Skim milk powder	2.923 x - 0.66 = y	r = 0.999
Nitrogen		
Horsegram - sesame mixture	10.240 x - 7.62 = y	r = 0.995
Skim milk powder	18.840 x - 1.99 = y	r = 0.993
Dietary Protein Concentration		
Horse gram - sesame mixture	2.412 x - 7.72 = y	r = 0.850
Skim milk powder	4.530 x - 1.14 = y	r = 0.845

B. **The Effects of Three Dietary Protein on Carotene Utilization by Albino rats:**

This study was designed to investigate the carotene protein interrelationship using three dietary proteins of varying qualities namely skim milk, fish flour and a mixture of red gram and sesame as supplements to a basal rice diet fed at 15 per cent level. This level was chosen since vegetable protein mixtures are inadequate for growth at 10 per cent or lower level.

Four groups of weaning male rats, depleted of vitamin A for 30 days were fed ad libitum four experimental diets, supplemented with 90 mcg of carotene for 28 days. The carotene source was dry carrot powder suspended in groundnut oil.

The criteria for evaluating the carotene utilization on different dietary proteins, were growth rate, hepatic storage of vitamin A and total serum proteins and protein fractions.

Body weight:

The growth performance of the experimental animals on to the four diets is given in Table and Figure IV. As far as body weight changes are concerned growth performance was best

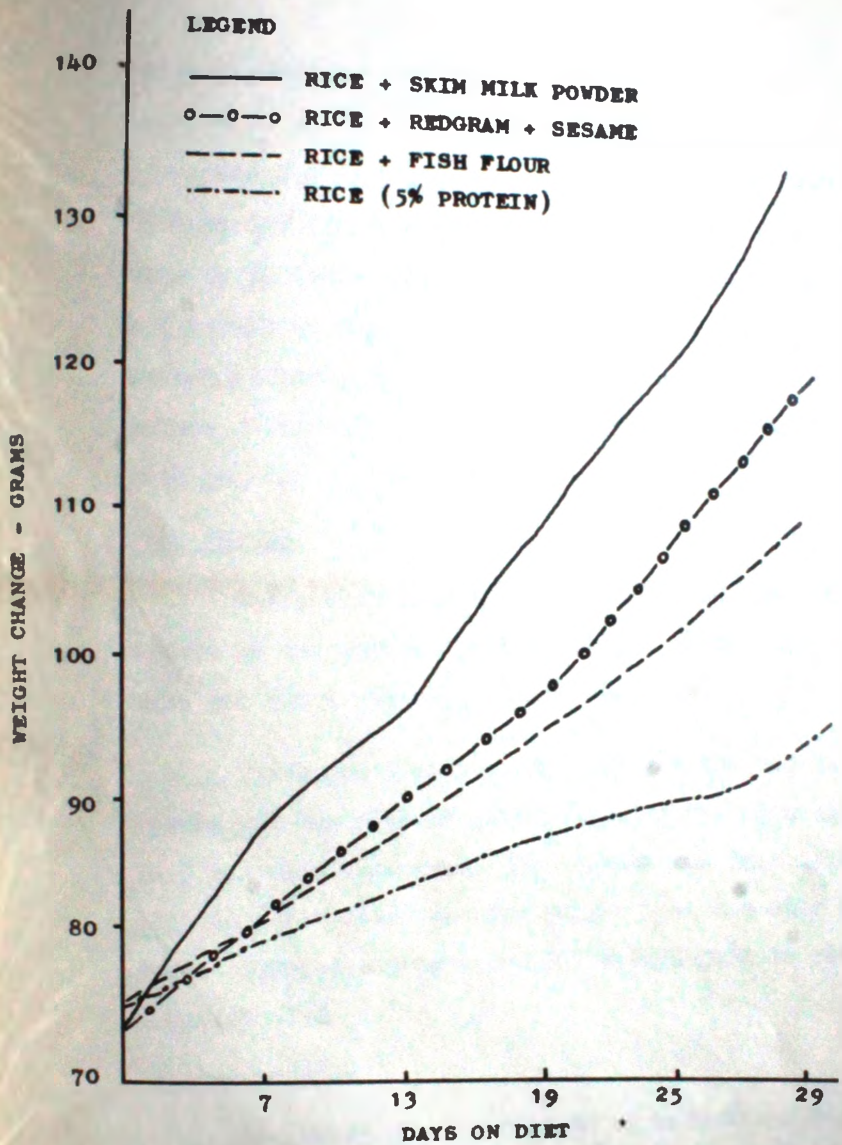


FIG. IV: AVERAGE WEIGHT GAIN DURING A 28 DAY PERIOD OF RATS ON DIETS DIFFERING IN SOURCE OR AMOUNT OF NITROGEN

(61±5.1 g/28 days) in the group fed the skim milk protein and lowest in the rice diet (18±1.09/28 days) Combination of sesame and dhal produced a growth rate of 44±3.5 g. in 28 days and fish meal 31±2.4 g. during the same period. Thus is in terms of growth promoting ability, the proteins are ranked in the following order, skim milk, red gram dhal sesame mixture, fish meal and 5 per cent rice protein. The growth pattern did not correlate with the calculated amino acid pattern of the diets.

Food intake:

There was no marked differences in the total food intake of the various dietary groups except for animals which were fed the 5 per cent rice diet.

There was a correlation between the higher food intake and the rate of growth of rats fed skim milk protein. In 5 per cent rice diet, food intake was less than that on all the other diets and the weight gain was also significantly less. Analysis of variance for weight gain is given in Annexure XXII.

Hepatic weight:

The fresh hepatic weights of animals on the different diets did not vary significantly. In general, the liver weights were proportional to the body weights. The hepatic weight of the rats fed the 5 per cent rice diet was the lowest (Table XXVIII) (4.08 ± 0.123 g) while that of the skim milk diet was the highest (4.57 ± 0.381) while those of the other groups of animals, were in between these two limits. Analysis of variance for hepatic vitamin A per g. of liver is given in Annexure XXIII.

Hepatic storage of vitamin A:

Measurement of the hepatic vitamin A is one of the most widely used criteria for evaluating vitamin A utilization as affected by dietary protein quality. Mean hepatic vitamin A both total and concentration of groups of rats on a fixed dose of carotene (90 mcg/day) and diets differing in source and amount of nitrogen Fig. V.

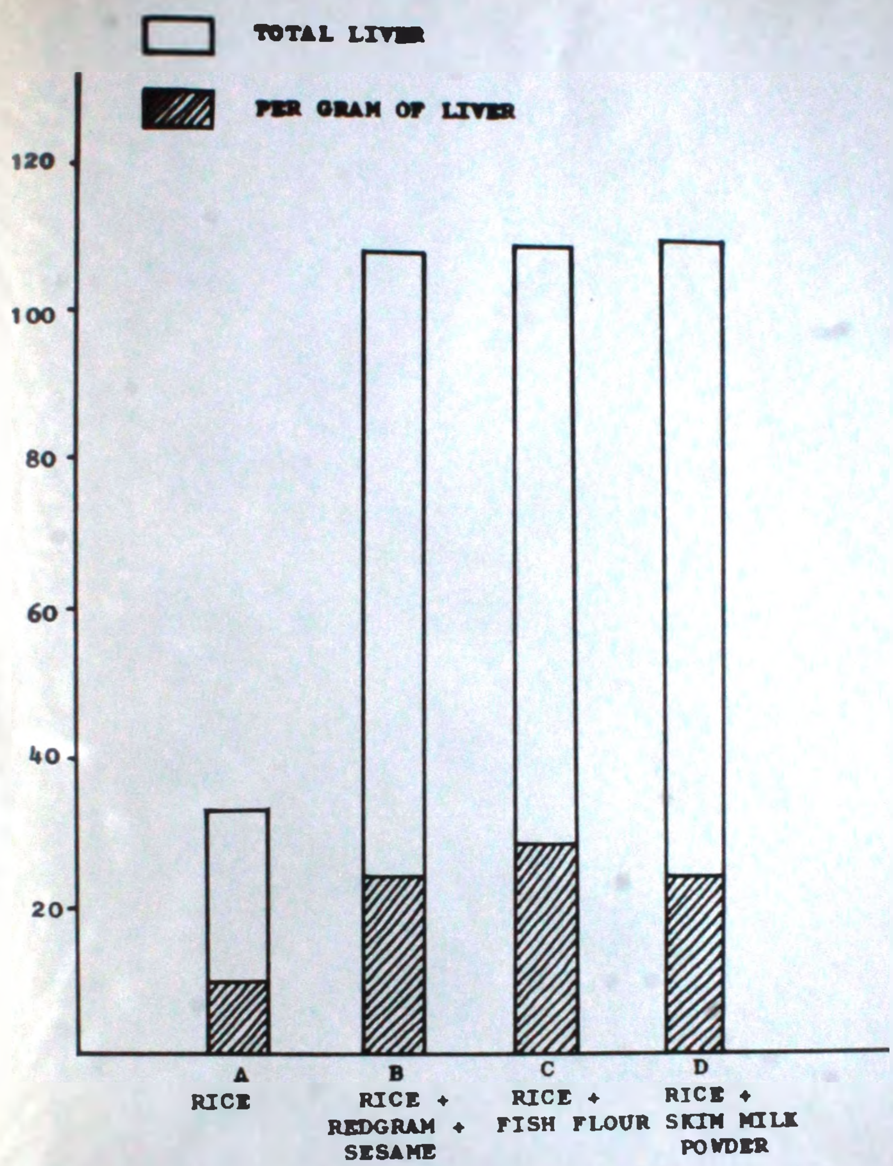


FIG. V : MEAN HEPATIC VITAMIN A BOTH TOTAL AND CONCENTRATION OF GROUPS OF RATS ON A FIXED DOSE OF CAROTENE (90 mcg/DAY) AND DIETS DIFFERING IN SOURCE AND AMOUNT OF NITROGEN

TABLE XXVIII

Mean values for body weight, food intake, liver weight and hepatic vitamin A of young Vitamin A depleted Rats fed daily 90 mcg Carotene for 28 days.

Diet	No. of Rats	Body weight Initial (g)	Body weight Final (g)	Weight Gain (g)	Total food intake (g)	Total protein intake (g)	Liver weight (g)	Hepatic vitamin A (mcg of retinol) Total	Hepatic vitamin A (mcg of retinol) Per gram liver
A	6	75 ± 5.2[a]	93 ± 4.1	18 ± 1.0	262 ± 5.3	13	4.08 ± 0.123	32 ± 5.7	8 ± 1.2
B	6	74 ± 6.3	117 ± 8.0	43 ± 3.5	274 ± 8.6	41	4.49 ± 0.274	106 ± 5.7	23 ± 4.8
C	6	76 ± 6.7	108 ± 6.5	32 ± 2.4	278 ± 12.6	42	4.16 ± 0.007	107 ± 6.0	26 ± 5.3
D	6	74 ± 5.9	134 ± 7.6	60 ± 5.1	286 ± 5.0	43	4.57 ± 0.381	108 ± 12.0	24 ± 2.0

a = Standard error of the mean.

The animals fed a 5 per cent rice diet had significantly lower amount of vitamin A stores per g. of liver than all the other groups .8±1.2 mcg. There were no significant differences in the hepatic vitamin A concentration among the animals fed the other diets.

Serum proteins:

The total serum protein and fractions of the animals fed a vitamin A deficient diet for 30 days and the experimental diets which were supplemented with 90 mcg. carotene for 28 days is given in Table XXVIII *a*.

The total serum protein and fractions of the animals fed a vitamin A deficient diet for 30 days and the experimental diets A, B, C and D which were supplemented with 90 mcg. carotene for 28 days is given in Fig. VI.

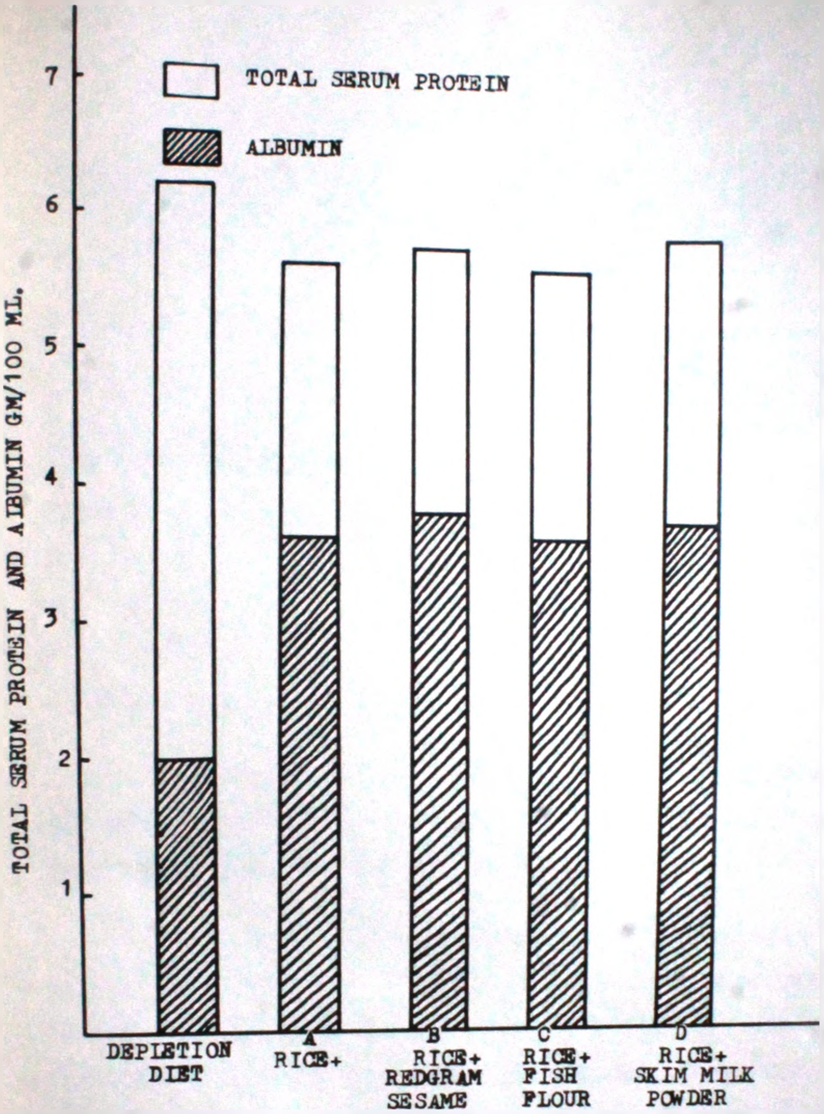


FIG.VI TOTAL SERUM PROTEIN AND SERUM ALBUMIN AND SERUM GLOBULIN OF RATS ON THE DIFFERENT DIETS

TABLE XXVIII

TOTAL SERUM PROTEINS, ALBUMIN AND GLOBULIN VALUES, EXPRESSED AS g/100 ml. FOR ANIMAL DEPLETED OF VITAMIN A FOR 30 DAYS AND OF ANIMALS REPLETED BY A DAILY (90 mcg) CAROTENE SUPPLEMENTATION FOR 28 DAYS ON THE VARIOUS EXPERIMENTAL DIETS

Diet	Total Serum protein	Serum albumin	Serum globulin
Depleted animal (on 30th day of depletion)	6.16	2.09	3.47
A (on 28th day of) repletion	5.61	3.52	2.09
B "	5.72	3.61	2.11
C "	5.62	3.54	2.08
D "	5.81	3.52	2.29

Standard error calculations have not been given since the mean value were for a triplicate pooled samples of blood from the various experimental groups.

There was a slight increase in the total protein (6.16g/100 ml.) in the vitamin A depleted animals as measured on the 30th day of depletion. The total serum proteins on the repleted animals by carotene supplements as measured on the 28th day of repletion was comparable to that of animals on stock diets (5.7g/100ml.) but lower than that of vitamin A depleted animals. The total serum proteins of the rats fed the red gram, sesame combination, fish meal or skim milk did not differ from each other and the rats depleted with a 5 per cent rice diet also had similarserum protein values.

The serum albumin values was slightly lower (2.69/100ml.) in the depleted animals as measured on the 30th day of depletion. Serum albumin of the repleted animals with carotene supplementation on the different dietary proteins did not differ among themselves but were higher than that of the depleted animals.

Serum globulin value of the vitamin A depleted rats were higher than that of the repleted, ones, where as there was no difference in the repleted groups which were fed the various experimental diets.

M McLaren (1965) found a rise in the serum globulin level and a decrease in the serum albumin levels in the vitamin A deficiency rats.

G. <u>The Evaluation of a Weaning Mixture Based on Local Foods:</u>

This study aimed at evaluating a weaning mixture based on local cereals, legumes and an oil seed through a human feeding trial. Acceptability of the weaning food mixture, its shelf life and the knowledge of mothers about the components of the weaning mixture were also studied. The findings are presented in the following order:

1. Demographic, social and economic characteristic of children
2. Food and nutrient intake of the selected children
3. Calorie and protein intake of the target children including the supplement
4. Acceptability of the weaning mixture
5. Growth rate of the target children
6. Episodes of illness
7. Biochemical estimation in blood and urine samples of the target children
8. The Clinical syndromes of malnutrition
9. Mothers attitudes and views regarding the weaning mixture.

The monthly income of the families of the target children isin Table XXIX.

TABLE XXIX

INCOME OF THE FAMILIES OF THE TARGET CHILDREN

Range in Rupees	Number of Families			Total	Percentage
	A	B	C		
0 - 100	16	18	16	50	66.6
101 - 200	8	8	7	23	30.7
201 - 300	-	-	2	2	2.7
Total	24	26	25	75	100.0

The monthly income of 50 families out of 75 was below Rs.100 per month, showing that the majority of the children were from low income families. Cooli and agricultural day labour was the main occupation for 47 families, (62.6 per cent).

The following table XXX represents the distribution of household.

TABLE XXX

DISTRIBUTION OF HOUSEHOLDSBY HOUSEHOLD SIZE

Number in Family	Per cent of Households	
	Experimental households	Control households
3	33.14	31.85
4	19.26	19.54
5	17.18	17.65
6	12.94	14.66
7	7.75	7.23
8 and above	9.73	9.07

The smaller the family, the better is their nutritional status. Limitation offamily size to a smaller number of children isbound to increase the quantum of food available to each member of the family. Limited income is better utilized when the family size is small.

It is revealed from the Table XXX that 33 per cent of the household have a family size of 3 and 19 per cent have family size of four.

Table XXXI presents the distribution of the households of the target children and those in the control groups by the occupation of the heads.

TABLE XXXI

DISTRIBUTION OF HOUSEHOLDS BY OCCUPATION

Occupation	Percent of households	
	Experimental total	Control Total
Land owner - cultivator	3.4	3.2
Money lender	13.3	14.4
Agricultural labourer, cooli, brick layer	73.4	73.8
Village artisans, carpenter, blacksmith, painter mason, goldsmith	2.4	3.9
Industrial worker	3.5	2.1
Others	4.0	2.6

The majority of the heads of families in both groups were agricultural labourers, coolies and brick layers.

Food Intake of the Target Children:

Breast feeding and weaning practices showed that supplementary liquid and solid foods were usually not introduced during this period. At the age of 12 months, 59 per cent of the infants had not yet been given any solid foods and of these 26 per cent had not even been introduced to liquid supplements. At this age 18 per cent of the children had not received any other food than breast milk. It is recognised that breast milk alone cannot fulfil the nutrient requirements of a growing child beyond six months of age. Yet one fourth of the infant population at the age of one year had not received any additional food and another one fourth had received an inadequate supplementary diet. Fig. VII represents the percentage of the target children introduced liquid supplements, solid supplements, at varying months of age.

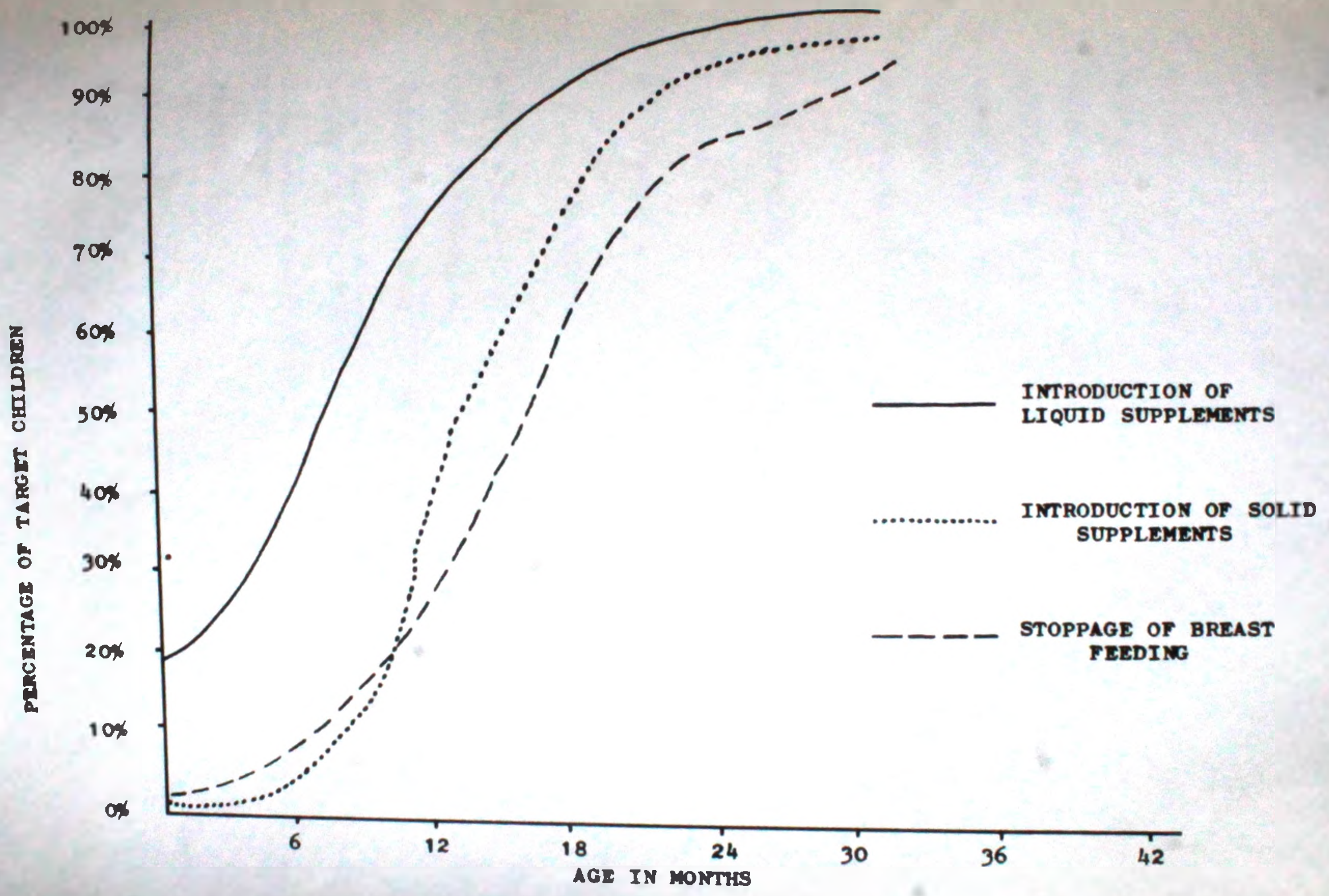
<u>Calorie and protein intake of the children:</u>

The mean calorie and protein intake of the target children are presented in Table XXXII.

The protein intake of the target children was slightly higher than the allowances recommended by the ICMR (1968). On the other hand the intake of calories fall short by 200 to 300 when compared to the allowances recommended even on the basis of the existing low body weights; If calculated on ideal body weight basis the short fall would be even greater.

In the current dietaries of pre-school children belonging to the poor income groups, the primary bottleneck. This appears to be calories and not protein. The PCM observed

TRENDS IN BREAST FEEDING AND SUPPLEMENTATION FIG. VII



in the target children must have been conditioned to a large extent by the poor calorie intake. This factor was taken into consideration in the formulation and fixing of the quantities of the supplements for the target children. Accordingly the amount of weaning mixture for the target children, was planned to supply 390 calories and 12.9 gram of protein per child per day.

TABLE XXXII

MEAN CALORIE AND PROTEIN INTAKE OF THE TARGET CHILDREN (THREE DAY FOOD WEIGHMENT)

Sample size: 10 children in each group

Nutrients	Home Diets		
	Groups		
	A	B	C
Total Calories/day	773±150	740±143	798±107
Calories/Kg. body weight	77±14	78±16	77±18
R.A.* Based on actual body weights (ICMR 1971)	900	900	900
R.A. Based on ideal body weights	1200	1200	1200
Protein (g)	17.1± 3.8	19.3± 3.0	22.3± 8.0
RA, ICMR, 1971		17-20	

* R.A. Recommended Allowances for Indians.

Mineral and vitamin intake of the target children:

The mean intake of minerals and vitamins is presented in Table XXXIII.

TABLE XXXIII

MEAN INTAKE OF MINERALS AND VITAMINS BY THE TARGET CHILDREN
(THREE DAY WEIGHMENT)

Sample size: 10 children each group

Nutrients	ICMR R.A. 1971	Home Diets		
		Groups		
		A	B	C
Calcium (mg)	400-500	159	191	179
Iron (mg)	15-20	8.4	9.3	8.6
B Carotene (µg)	1000	367	302	415
Thiamine (mg)	0.600	0.464	0.427	0.539
Riboflavin (mg)	0.700	0.303	0.176	0.266
Vitamin C (mg)	30-50	31.9	31.0	23.4

* Recommended allowances for Indians.

The intake of minerals and vitamin, B Carotene, thiamine and riboflavin by the selected children in the three groups were far below the allowances recommended by the ICMR, 1971.

Calories and protein intake of children as improved by the supplement:

The mean calories and protein intake of children per kilogram of body weight after supplementation is given in Table XXXIV.

TABLE XXXIV

MEAN CALORIES AND PROTEIN INTAKE OF THE TARGET CHILDREN PER KG. OF BODY WEIGHT

Nutrients	Group A		Group B		Group C
	Home diet	Home diet + supplement	Home diet	Home diet + supplement	Home diet
Calorie/kg. body weight	77± 14	106±22	78± 16	93±24	77±18
Protein g/kg body weight	1.7± 0.5	2.5±0.4	1.6±0.6	2.3±0.7	2.1±0.4

The total dietary intakes of the supplemented children, indicated that the 'take home dry food' was a real supplement and not a substitute. The mean Calorie and protein intake of the children per kilogram body weight receiving the weaning mixture was higher than that of the children receiving CSM, and of the children in the control group. This is due to the fact that the weaning mixture was more acceptable and palatable as revealed by the acceptability tests discussed elsewhere in this report.

Acceptability of the six weaning mixtures:

Acceptability and digestibility trials were carried out with six formulations on children 15-36 months old. The percentage scores of likes and dislikes of the target children with different formulations are represented in Table XXXV.

TABLE XXXV

PERCENTAGE SCORES OF LIKES AND DISLIKES OF THE TARGET CHILDREN WITH REGARD ACCEPTABILITY OF THE SIX WEANING MIXTURES

No. of children: 10

S.No.	Mixtures	Liked	Disliked	Uncertain
1.	Sorghum Bengalgram Groundnut Mixture	90	7	3
2.	Sorghum-greengram-groundnut Mixture	85	10	5
3.	Ragi-Bengal gram - groundnut mixture	70	20	10
4.	Ragi-greengram - groundnut mixture	65	30	5
5.	Maize-Bengalgram, - groundnut mixture	70	24	6
6.	Maize greengram - groundnut mixture	65	25	10

Thus the weaning food formulations made out of sorghum, bengalgram dhal and groundnut mixture were found to be highly acceptable.

<u>Cost Analysis</u>

The following table XXXVI illustrate the cost analysis of the weaning mixture and CSM.

TABLE XXXVI

COMPARATIVE COSTS OF SUPPLEMENTARY WEANING FOODS

Name of Food	Each Ration*		Calories			Protein
	Weight in g.	Cost ps.	Number	Cost per calories	Number(g)	Cost per (g)
Sorghum-Bengal gram mix	100	18	390	0.046	12.9	1.4
CARE BW 56g CSM 42	98	16.3	344	0.047	14.67	1.3

* as per prevailing prices at Coimbatore in 1972.

The cost analysis showed that the indigenous weaning food mixture and the Foreign food commodities were similar in its price range. The indigenous weaning mixture had the added advantage that there is not much cost involved with transport and storage unlike the imported foods.

Nutritional value of Sorghum based weaning mixture in comparison with other Commercial weaning foods (Baby foods) were presented in Table XXXVII.

TABLE XXXVII

NUTRITIONAL VALUE OF SORGHUM BASED WEANING MIXTURE IN COMPARISON WITH OTHER COMMERCIAL WEANING FOODS (baby foods)

Contents	Units	Sorghum based weaning mixture	Farex	Pree-tinex	Proti-nules
Calories	-	390	375	400	335
Protein	g	12.0	13	16.8	2.4
Fat	g	7	3	-	-
Calcium	mg	153.5	750	400	-
Iron	mg	7.9	20	-	25
Carotene	mg	88	-	-	-
Thiamine	mg	0.543	-	2.0	-
Riboflavin	mg	0.475	-	3.28	2.0
Vitamin A	Iu	165.2	-	4000	5000
Nicotinic acid	mg	9.373	-	20	20
Price structure for 100 grams (value in Rs.)	Rs.	0.30	2.09	5.00	4.90

In comparison with cost the Indigenous mixture was very much low and also within the reach of the common man. The commercial foods are too expensive and beyond the reach of the common man. From the nutritional stand point this mixture might be worthwhile for the vulnerable sections of the populations who belongs to the poor socio-economic status.

TABLE XXXVIII

RATIONALE FOR SUPPLEMENTAL FOOD QUANTITY RECOMMENDATIONS

Age Group	Calorie Deficit	Protein Deficit (g)	Basis of Information	Recommended Food supplement	Calories provided	Proteins provided (g)
Very young children (Below 3 years)	300	Nil	Present study	50 g	195	6.4
Preschool child (3-5 yrs)	352	Nil	Devadas (1973) *et al*, Survey	100 g	390	12.9
School children (6-10 years)	387	Nil	Devadas (1973) Survey	120 g	378	15.5
Pregnant women	1521	18	Devadas and Easwaran 1971	400 g	1560	51.6
Lactating women	1164	17		300 g	1170	38.7

Source: ORG Survey P.46 (1972)

Devadas R.P. and Easwaran P. 1971

Nutrient intake of selected vulnerable groups in Coimbatore District. A paper presented at the IBP/H.A. Meeting in Blantyre Malawi, 5th - 12th April, 1971, unpublished.

The weaning food formulations can be of use for bridging the calorie gap of the vulnerable sections of the Indian population namely preschool, pregnant and lactating women which are of low cost and indigenous food mixtures, one more advantage is that they would be able to prepare the same in their homes even after the feeding programme is with drawn. The mothers can be convinced about the nutritional values of the local foods and they may be taught how to prepare the weaning foods through simple cooking procedures.

Acceptability of the weaning food as compared to C.S.M.:

Results of the acceptability tests with sorghum - based weaning mixture and C.S.M. are presented in Table XXXIX.

TABLE XXXIX

RESULTS OF THE ACCEPTABILITY TESTS
(Sample size: 25 in each group)

Group	Number of children							
	Liked very much		Liked		Fairly liked		Not liked	
	Number	%	Number	%	Number	%	Number	%
A (weaning mixture	10	40	6	24	9	36	-	-
B (CSM)	-	-	6	24	12	48	[illegible]	28

There was no 'not liked' score in group A where as in group 'B' 28 per cent of the children had a dislike to the C.S.M. supplement. The C.S.M. jaggery mixture food packets were received for distribution every month from CARE, Tamil Nadu and they were at times heavily infested with weavils. Therefore children did not like it generally. The imported foods like CSM have to be stored for six months or more during shipping and weavil infestation is a big problem in their storage.

On the other hand children relished the sorghum based weaning mixture, which was made from fresh ingredients and did not have any digestive problem as indicated by the absence of signs such as nausea, vomiting, diarrhoea or flatulence for a period of 15 days. The quantity of the weaning mixture that could be consumed by the target children of age 1½ to 2½ years,was found to be around 25g. (raw weight) in one serving. This is because sorghum-legume based mixture increased in its bulk on cooking to nearly three times.

Out of the several recipes tested children liked the laddu and uppuma to the maximum. These were also easy for the mothers to prepare at home.

Shelf life studies:

The weaning food was prepared and packed in polythene bags which were stored in plastic cans with screw lids and at (37° C) room temperature in a wall shelf for a period of 6 months. Control samples were kept at 0° C in a refrigerator. Samples stored at (37°C) room temperature were analysed once in three months for free fatty acids, and peroxide value. (Krishnamurthy *et al*, 1968). The organoleptic quality of the weaning food was tested by a panel of 6 judges. The score system adopted was similar to that of Chandrasekhara *et al* (1968). A product having a score of 2 or less was considered acceptable. The results are given in Table XXXIX a.

TABLE XXXIX a

SHELF LIFE OF THE WEANING FOOD MIXTURE (Sorghum based)

Constituents	Initial	3 months	6 months
FFA (as % of oleic acid)	1.6	3.8	6.3
Peroxide value*	3.4	5.6	8.4

* (Millimole of Thio per g. of fat)

The total plate count of the product was 27,000 per gram which is less than the limits prescribed in the Indian Standards. The weaning food (product) was free from E.coli, salmonella and anaerobic pathogens.

The acceptability and the organoleptic qualities of the weaning food was good at 3 and 6 months of shelf life.

Growth rate:

The mean height and weight of children fed sorghum based weaning mixture and C.S.M. in comparison with the control group are presented in Table XXXX.

TABLE XXXX

MEAN HEIGHTS AND WEIGHTS OF CHILDREN ON SORGHUM BASED WEANING FORMULATIONS AND C.S.M.

Number of children: 25 in each group (Age: 24 to 36 months)
Period : 6 months

Group	Initial	Final	Difference
	Mean heights - cm.		
A (weaning mixture)	80.7±3.05	84.3±3.20	3.6±0.67**
B (CSM)	77.8±4.16	82.1±3.57	4.3±0.95**
C (Control)	81.1±4.38	83.4±3.62	2.3±0.55
	Mean weights - kg.		
A (weaning mixture)	9.49±1.48	11.15±0.92	1.66±0.59*
B (CSM)	8.86±1.17	10.44±1.21	1.58±0.28*
C (Control)	9.61±0.57	10.28±0.88	0.67±0.45

** Significant at one per cent level.

* Significant at 5 per cent level.

There was a significant increase in body heights and body weights of the children given the sorghum based weaning mixture and CSM when compared to the control group. The differences in increases in body weight gains and heights, between the groups on the weaning mixture and C.S.M. were not significant. There were no differences between in the growth rates of boys and girls in all the groups.

Both the experimental groups A and B had registered greater increases in heights and weights when compared to the control group of children. A scatter diagram Fig VIII & IX represents the individual heights and weights of the target children of the three groups.

Incidence of malnutrition as indicated by the body weights of the target children:

The body weight of the target children as per cent of the ICMR standard are represented in Table XXXXII.

TABLE XXXXII

BODY WEIGHTS OF THE TARGET CHILDREN AS PER CENT OF THE ICMR STANDARD

Percentage of the children

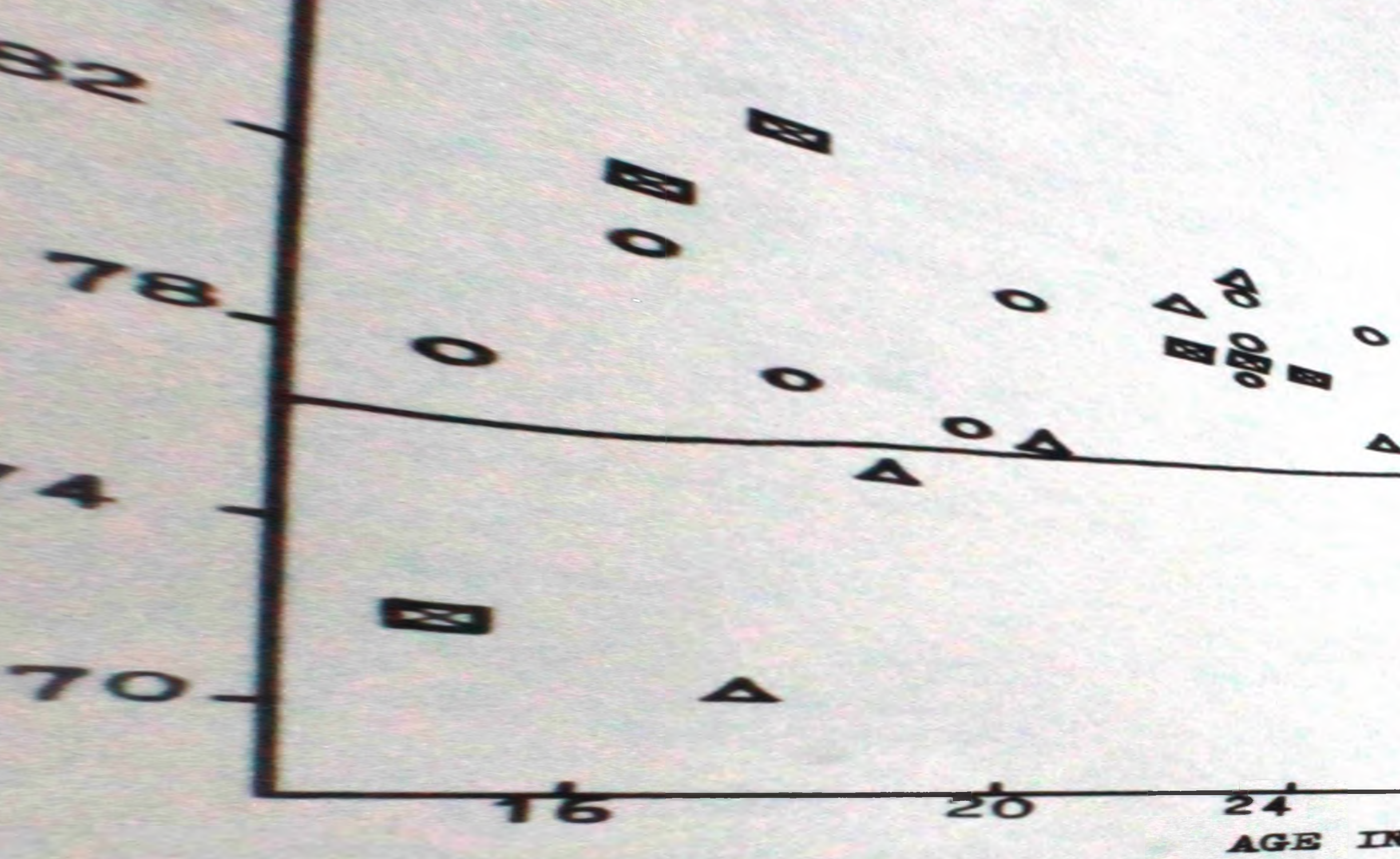
Weight as a percentage of the ICMR standard		Group A		Group B		Group C	
		Initial	Final	Initial	Final	Initial	Final
Normal	90 & above	53.8	74.8	65.3	79.7	71.5	66.2
First degree Malnutrition	80-90	40.0	23.0	29.6	18.9	26.5	23.4
Second degree Malnutrition	70-80	7.2	2.2	5.1	1.4	2.0	9.6
Third degree Malnutrition	Below 70	Nil	Nil	Nil	Nil	Nil	0.8

The body weights of the supplemented and unsupplemented children when expressed as a percentage of ICMR standard showed an upward shift at the end of the feeding trial in the groups

9.

9.

9.



SCATTER D
GROUPS O

A and B, when compared with group C who received no supplementation. The incidence of third degree malnutrition was almost absent in all the target children. More children in group A and B had shifted to the normal body weight category at the end of the study whereas in the control group children shifted back from normal category to first and second degree malnutrition.

Head and Chest Circumference:

The mean head and chest circumference of the children are presented in Table XXXXIII.

TABLE XXXXIII

MEAN HEAD AND CHEST CIRCUMFERENCES; NUMBER OF CHILDREN: 25 in each group
Period: 6 months

Groups	Mean head circumference (cm)		Mean chest curcumferences (cm)	
	Initial	Final	Initial	Final
A (weaning mixture)	43.1±2.308	44.1±2.605	43.9±2.883	44.9±2.070
B (CSM)	42.3±3.204	43.2±3.116	43.0±1.820	43.8±2.435
C (Control)	43.9±3.67	44.5±1.980	44.7±2.960	45.3±2.580

There was no significant differences in the measurements of the head and chest circumference of the children between the three groups. This may be due to the fact that the supplementation lasted only for a period of 6 months which may be too short a period for this anthropometric index to show differences between the supplemented and unsupplemented groups.

<u>The episodes of illness</u>:

The monthly incidence of major episodes of illness among the target children in the three groups are presented in Table XXXXIV. The duration of individual episodes of illness were from 4 to 5 days, on an average.

TABLE XXXXIV
EPISODES OF ILLNESS AMONG THE TARGET CHILDREN
25 children in each group

Groups	Types of illness (average incidence/month)		
	Cold, fever and cough	Diarrhoea	Small pox, measles and whooping cough
A	18	10	2
B	20	13	1
C	23	17	3

There were no differences seen in the incidence of sickness among the three groups of children except that the incidence was high in the control group. The monthly episodes of illness were of the order of 90 to 95 per cent in all the three groups of children. Absence of protected water supply, poor sanitation and hygiene in and around the homes of the target children, might have contributed to the illness. Lack of health care was a big problem in the feeding programme. The mothers were anxious to have facilities for health care of their children. They requested often for medical help, when the investigator did house visits to record food intake and other details. If the mass feeding programmes are to fulfil their objectives it is imperative that the necessary facilities of health care and immunization are ensured.

Biochemical estimation of urinary nitrogen, creatinine and their ratio:

The early morning samples of urine were collected and analysed for their nitrogen and creatinine content. The nitrogen: creatinine ratios were calculated. These data are presented in Table XXXXV.

TABLE XXXXV

MEAN NITROGEN CREATININE/RATIO

Number of children: 25 in each group

Period of supplementation : 6 months

Group	Period	Creatinine mg. per 100 ml.	mg.nitrogen/mg. creatinine ratio
A	Initial	4.16	13.3
	Final	63.0*	16.2*
B	Initial	42.9	14.0
	Final	66.5*	16.6*
C	Initial	44.5	14.0
	Final	42.8	15.6

* Significantly greater than control value (P.0.05)

There was an increase in the creatinine content of urine, and the ratio of mg. of urinary nitrogen/mg. creatinine in Group A, and B was also increased. The controls showed no change.

Haemoglobin estimation:

The haemoglobin content of the finger prick samples of blood of the children estimated at the beginning and at the end of the study are presented in Table XXXXVI.

TABLE XXXXVI

MEAN HAEMOGLOBIN CONTENT OF THE TARGET CHILDREN

Number of children: 75 in each group
Period of supplementation : 6 months

Group	Mean Value (grams per 100 ml)		
	Initial	Final	Difference
A	7.8±1.2	9.7±0.9	1.9±1.04
B	8.1±1.4	9.4±1.0	1.3±0.45
C	8.8±1.2	9.9±1.4	1.1±0.32

WHO (Average) 10.89 g/100 ml.

Judged by the WHO standards all the 75 target children in the three groups were anaemic to start with. At the end of the study, there was slight improvement in the haemoglobin level in all the three groups. But there were no significant differences among the various groups for this index.

Table XXXXVII gives the percentage classification of target children according to the severity of anemia.

TABLE XXXXVII

PERCENTAGE CLASSIFICATION OF TARGET CHILDREN ACCORDING TO SEVERITY OF ANEMIA

Group	Normal		Mild*		Moderate*		Severe*	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
A	10.6	17.2	55.1	63.4	21.4	14.5	11.9	5.1
B	15.7	24.1	52.4	52.1	21.6	13.4	10.6	8.3
C	12.4	15.6	55.6	56.1	22.1	21.2	10.3	7.3

* A deficit of up to 2g/100 ml. of haemoglobin from the normal mean (WHO standards) is considered as mild, 2 to 4g/100 ml as moderate, 4g or more/100 ml as severe anemia.

Clinical examination:

The results of the clinical assessment are given in Table XXXXVIII.

TABLE XXXXVIII

Clinical Examination of Children:
No.of children 25 in each group.
Period: 6 months

Symptoms	Number of children manifesting the symptoms					
	Group A		Group B		Group C	
	Ini-tial	Fin-al	Ini-tial	Fin-al	Ini-tial	Fin-al
Protein Caloric						
Mild	10	3	12	10	15	10
Moderate	4	-	4	1	6	4
Severe	-	-	-	-	-	-
Vitamin A deficiency prevalence	4	2	6	3	3	3
Vitamin B complex deficiency prevalence	5	2	4	2	3	4
Anemia (mild form) as judged by palar of the skin and eyelids	12	10	14	11	13	8

The number of children who manifested the various clinical symptoms was found to decrease in the two experimental groups at the end of the study.

The supplementation of the weaning mixture was effective in bringing down the number of mild protein Caloric Malnutrition syndromes, However Anemia continued to be present even after the supplemental feeding.

Home visits:

The mothers of the target children were interviewed to know whether or not they would be able to prepare the weaning formula in their homes and feed to their children after the feeding trial was over. Thirty four mothers whose monthly income fell below Rs.100 expressed their willingness but that it was beyond their economic capacity to buy the raw ingredients needed for the supplement and to prepare the feed. Out of the 16 mothers whose monthly income was Rs.101-200, 13 mothers were willing to prepare the supplement at their own cost and feed the children. The cost of the weaning mixture was an important bottleneck in the follow up of the feeding programmes.

B. Effects of Supplements Based on Opaque - 2 Maize, Ordinary Maize and Skim milk on the Nutritional Status of Preschool children 18-30 months of age

This study was designed to ascertain the effect of feeding children 18-30 months old, diets based on high lysine maize, ordinary maize and skim milk, on their nutritional status. The home diets of the children were based on rice. Opaque-2 maize has a higher lysine content than ordinary maize and may play an important role as a rich and low-cost source of good quality protein and Calories which are limiting in the diets of preschool children at present. The results of this study are presented in the following order and discussed.

General background of the families:

There was 1522 families, in the selected villages, of these 863 were nuclear and 659 joint families. The total number of members in these families was 6866, out of whom 3562 were males and 3304 females.

Table XXXXIX gives the caste wise distribution of the families of the four villages.

TABLE XXXXIX

CASTE OF FAMILIES OF THE FOUR VILLAGES

Caste	Experimental Groups			Control Group
	A	B	C	
[illegible]				
Gounder	44	27	34	29
Naidu	222	59	76	42
Devar	13	13	18	27
Vellalar, Pillai, Mudaliar	13	25	14	36
Brahmins	6	35	15	22
Chettiar	23	31	64	19
Nadar	13	6	21	9
Muslims	3	3	9	13
Malayali	10	19	21	10
Christian	11	11	13	5
Naicker	-	5	13	12
Dhobi, Barber, Asari	38	28	30	37
Harijan	1	4	3	8

The monthly income ranges of the families is given in Table XXXXX

TABLE XXXXX

INCOME RANGES OF THE FAMILIES

Income ranges Rupees	Experimental Villages			Control
	A	B	C	
Upto 100	78	22	41	98
101 to 200	258	78	94	231
201 to 300	427	135	124	49
301 to 400	75	21	45	51
401 to 500	77	17	21	9
501 to 600	53	24	14	9
601 to 700	16	5	5	8
701 to 800	4	2	3	5
801 to 900	2	-	3	1

The monthly income of the majority of the families in the experimental villages was upto Rs.300. For most of the families in the control group, the income was upto Rs.200.

The following Table XXXXXI shows the households classified by the occupation of the head of the families.

TABLE XXXXXI

DISTRIBUTION OF THE HOUSEHOLDS BY OCCUPATION

Occupation	Experimental Groups A	B	C	Control Group
Cooli-agricultural labourers	54	28	104	69
Industrial labourers	198	159	114	57
Salaried teacher	47	58	9	11
Business	26	31	13	35
Land Owners	17	12	24	9
Clerk, Electrician, Driver, Tailor, Nurse	13	18	21	18
Dhobi	8	3	5	8
Goldsmith	3	1	-	-
Gramsevak	-	1	-	-
Weavers	-	-	23	129

The majority of the families in the experimental villages were Industrial labourers. Most of the families in the control village was weavers.

Table XXXXXII gives the educational background of the family members.

TABLE XXXXXII

EDUCATIONAL BACKGROUND OF THE FAMILY MEMBERS OF THE FOUR VILLAGES

Educational Level	Experimental group A	Experimental group B	Experimental group C	Control group
Primary school	418	235	343	686
Middle school	309	179	261	434
High school	224	293	219	301
College	35	123	108	83
Illiterate	848	90	496	387

The illiteracy in all the villages were considerable. Majority of the fathers had low educational attainments (Primary School).

<u>Mean nutrient intake of the target children:</u>

Mean nutrient intake of the target children along with the supplement are presented in Table XXXXXIII. The composition and amounts of the supplements as presented in Table 41 experimental section.

The Calorie content of home diet of children was deficient. It was made up 30 per cent by the supplement. The mean intake of Calcium, retinol, riboflavin and vitamin C through the home diet was below the allowances recommended by the ICMR, 1971. Supplementation of the diets had helped in narrowing the gaps.

Incidence of sickness:

The monthly incidence of sickness was found to be 60 per cent. The main ailments were fever, cold, cough, diarrhoea and dysentry, chicken pox and measles. The main duration of acute episodes of sickness was between five to seven days in a month.

TABLE XXXXXIII

MEAN DAILY NUTRIENT INTAKES OF THE TARGET CHILDREN (3 DAY FOOD WEIGHMENT SURVEY) (No.of Children Surveyed) 15 IN EACH GROUP

Nutrients	R.A., I.C.M.R. 1971	Opaque-2 Maize Diet: Home Diet	Opaque-2 Maize Diet: Supple-ment	Opaque-2 Maize Diet: Total	Ordinary Maize Diet: Home Diet	Ordinary Maize Diet: Supple-ment	Ordinary Maize Diet: Total	Skim Milk Group: Home Diet	Skim Milk Group: Supple-ment	Skim Milk Group: Total	Control
Calories	1200	809	476	1285	775	475	1240	832	427	1259	805
Protein (g)	18.3	18.8	10.0	28.8	16.1	9.8	25.9	20.3	10.0	30.1	21.2
Calcium (mg)	400-500	163.4	124.4	287.8	130.2	125.2	255.4	453.1	456.6	909.7	185.7
Iron (mg)	15.20	10.0	10.5	20.5	11.6	10.7	22.3	20.3	9.7	30.0	7.2
β Carotene (μg)	1000	254.0	1496.8	1750.8	77	1504.0	1581	20.2	1414	1616	12.7
Thiamine (mg)	0.6	0.46	0.398	0.853	0.479	0.432	0.911	0.396	0.151	0.547	0.444
Riboflavin (mg)	0.7	0.239	0.175	0.414	0.297	0.183	0.480	0.333	0.524	0.847	0.316
Vitamin C (mg)	18.5	24.8	43.3	10.0	24.8	34.8	5.9	45.9	45.9	51.8	73.1

Anthropometric measurements:

The growth of children in the three experimental groups was assessed in terms of their body heights and weights at regular intervals. Table XXXXXIV gives these data.

TABLE XXXXXIV

MEAN INITIAL AND FINAL BODY WEIGHTS AND HEIGHTS OF THE TARGET CHILDREN

No. OF CHILDREN IN EACH GROUP : 50
DURATION - 6 MONTHS

Diet Group	Age in months	Weight in kg. Ini-tial	Final	Diff-erence	Height in cm. Ini-tial	Final	Diffe-rence
Opaque-2 Maize	12-17	7.84	9.40	1.56**	72.5	76.2	3.7**
Ordinary Maize	-do-	8.15	9.45	1.30**	73.1	76.3	3.2**
Skim Milk	-do-	8.16	10.15	1.99**	73.3	78.2	4.9**
Control	-do-	8.00	8.67	0.67	72.8	75.3	2.5
Opaque - 2 Maize	18-24	9.59	10.90	1.31**	80.8	85.1	4.3**
Ordinary Maize	-do-	9.48	10.42	0.94**	80.6	84.5	3.9**
Skim Milk	-do-	8.80	10.50	1.70**	80.6	85.6	5.0**
Control	-do-	9.20	9.81	0.61	80.7	83.7	3.0
Opaque - 2 Maize	25-30	11.42	12.30	0.88*	86.8	90.2	3.4*
Ordinary Maize	-do-	10.86	11.69	0.83*	86.2	89.8	3.6*
Skim Milk	-do-	11.10	12.02	0.92*	87.4	91.5	4.1**
Control	-do-	10.94	11.45	0.51	87.0	89.1	2.1

* Significant at (P 0.05)
** Significant at (P 0.01)

There was an increase in the mean heights and weights of the target children who participated in the feeding trial. All the groups who received the different supplements and registered significant increments in heights and weights when compared with the control group.

The heights and weights of the children in the high lysine Maize (Opaque - 2 Maize) group ranked next to the skim milk group. But the differences between these two groups in this was not significant.

Biochemical estimation of haemoglobin:

The haemoglobin content of the finger prick samples of blood of the children were estimated at the beginning and at the end of the study is presented in Table XXXXV.

TABLE XXXXV

MEAN HAEMOGLOBIN CONTENT IN 100 ml. BLOOD; (DURATION 6 MONTHS)
No. OF CHILDREN IN EACH GROUP 50

Group	Initial	Final	Difference
Opaque - 2 Maize	5.8	7.7	1.9
Ordinary maize	6.1	7.7	1.6
Skim milk	6.0	8.1	2.1
Control	6.0	7.3	1.3

WHO (Average) 10.8 g/100 ml.

All the 200 children were anaemic to start with. At the end of the study, there was only slight improvement in the haemoglobin levels in all the groups. There was no significant differents in the haemoglobin increment among the various groups of children. The iron intake (20 mg./day) of the target children was more than the requirements, specified by the ICMR (1968). Perhaps the dietary iron was poorly available. It is pertinent to recall that none of the children had hook worm infestations as evidenced through afecal analysis. The slow responses of blood Haemoglobin concentrations to nutritional improvement has been noticed in this laboratory (Devadas, *et al.* 1972).

Nitrogen balance study:

The Nitrogen balance data obtained on the target children fed various diets are reported in Table XXXXVI.

TABLE XXXXVI

NITROGEN BALANCE DATA ON THE TARGET CHILDREN (3 DAY PERIOD; 6 CHILDREN IN EACH GROUP)

	N Intake g/day	Nitrogen excretion g/day		Average daily balance g/day
		Urinary	Faecal	
High lysine maize	4.52±0.54	1.54±0.21	1.37±0.31	1.61±0.51
Ordinary maize	4.08±0.53	1.71±0.25	1.79±0.33	0.81±0.34
Skim milk	4.55±0.46	1.30±0.31	1.37±0.34	1.88±0.24

Holt and his colleagues, (1965) observed that in a growing child nitrogen retention continued although at a lesser rate, even on deficient diets. According all the three groups of children who took part in this study were in positive nitrogen balance, though their rates of growth were significantly different. The differences in nitrogen retention between the three groups of children were not of statistical significance. Knapp et al. 1973 also found that Nitrogen balance data on various dietary proteins and protein combination fed to young children did not show any significant differences. The results of this study is thus similar to the previous reports.

Table XXXXXVII gives the nitrogen balance data per kg. of body weights.

TABLE XXXXXVII

NITROGEN RETENTION IN CHILDREN FED THREE DIFFERENT SUPPLEMENTS

Diet	Dietary Calories K cal./kg	Dietary proteins g/kg.	Nitrogen retention mg/N/kg. body weight
High lysine maize	110 (100-124)	2.37 (2.24-2.60)	166 ([illegible]-210)
Ordinary maize	115 (93-128)	2.84 (2.16-3.02)	160 (102-[illegible]4)
Skim Milk	105 (98-129)	2.52 (2.21-2.89)	169 (105-214)

At a level of 2.5g/kg. body weight of protein, in the three groups, nitrogen was retained upto a level of 165 mg/kg. of body weight. The NPU of these diets was approximately 55. The above levels in terms of the reference protein, would be between 1.1 and 1.3g/kg.

A positive nitorgen balance of 30 mg/kg. of body weight has been shown to be sufficient to promote satisfactory growth (NIN, 1973). All the nine children studied retained much more than 30 mg/kg. of body weight at an intake of 2.5g/kg. (1g. reference protein) of body weights, which is very close to the protein requirements computed by FAO/WHO Expert group (1973) employing the factorial approach.

Clinical examination:

The clinical picture of the target children at the beginning and at the end of the study is presented in Table XXXXVIII.

TABLE XXXXVIII

CLINICAL PICTURE OF THE TARGET CHILDREN

Symptoms	Opaque-2 maize		Ordinary		Skim milk		Control	
	Ini-tial	Fin-al	Ini-tial	Fin-al	Ini-tial	Fin-al	Ini-tial	Fi-nal
Protein Calorie Malnutrition Mild	10	8	12	9	11	7	12	10
Moderate	3	-	3	1	2	-	3	1
Severe	nil	nil	nil	nil	nil	nil	nil	nil
Vitamin A deficiency	4	2	5	3	3	1	4	4
Deficiencies of Vitamin B Complex	7	4	8	5	7	2	8	7
Anemia	21	15	19	16	20	12	20	18

The most predominant clinical syndromes were mild forms of Protein Calorie Malnutrition and anaemia, Vitamin A deficiency and B Complex deficiency symptoms were also present to a considerable extent. The number of children manifesting the various symptoms of deficiency diseases had decreased in the three experimental groups at the end of the study in comparison with the control group.

3. The Results of the Experiment on the Evaluation of an Indigenous nutritious supplement to School Children

A study was designed to evaluate a nutritious supplement to school children belonging to a village community.

Calorie and protein intake of the target school children:

The mean calorie and protein intake of the target children is presented in Table XXXXXIX

TABLE XXXXXIX

MEAN CALORIE AND PROTEIN INTAKE PER DAY PER CHILD

Nutrients	R.A. ICMR 1971	Supplemented			Unsupplemented group
		Home Diet	Supplement	Total Daily intake	Home Diet
Calories	1500-1800	1263	395	1658	1259
Protein(g)	22-33	27	14	41	28

The 20 per cent deficit in the calorie content of home diets of children was made up by the supplement of 150 grams which provided 395 calories and 14 grams of protein.

Mineral and vitamin intake of the children in the experimental group:

The mean consumption of mineral and vitamins by the target children studied is given in Table XXXXXX.

TABLE XXXXIX

MEAN CONSUMPTION OF MINERALS AND VITAMINS BY THE TARGET SCHOOL CHILDREN

Nutrients	R.A. ICMR 1971	Supplemented Group			Unsupplemented Group
		Home Diet	Supple-ment	Total	Home Diet
Calcium (mg)	400-500	212-	247	459	242
Iron	15-20	18	12	30	19
Retinol equivalent(µg)	300-400	63.5	417.1	480.6	89.5
Thiamine (mg)	0.8-1.0	0.816	0.321	1.137	0.854
Riboflavin(mg)	0.8-1.0	0.350	0.201	0.551	0.382
Vitamin C(mg)	30-50	21	30	51	20

The mean intake of calcium, retinol, riboflavin and vitamin C in the home diets were found to be below the recommended allowances of the ICMR. Supplementation of the diets of the children had helped in bridging the gap existing in the intake of all the nutrients except riboflavin.

The inclusion of green leafy vegetable (Amaranth tender) in the supplement elevated the iron intake, but how much of the iron was available to the body is to be investigated.

Anthropometric measurements:

The growth of the children of both the supplemented and unsupplemented group was assessed in terms of their body weights and body heights taken at intervals.

Heights and Weights Analysed According to sex of Children:

Table XXXXXII shows the mean increments in the heights of the children when viewed sexwise.

The difference between the increments of supplemented and unsupplemented groups were statistically significant for both the parameters.

TABLE XXXXXXI

THE MEAN INCREMENTS IN HEIGHTS AND WEIGHTS OF CHILDREN ACCORDING TO SEX

Group	No.of children	Initial	Final	Difference	't' value	No.of children	Initial	Final	Difference	't' value
		Mean Height (cm)								
Supplemented	107	111.15	117.33	6.18±0.1303	15.14**	93	109.04	115.39	6.35±0.1410	15.54**
Unsupplemented	107	112.24	115.80	3.56±0.1145		93	109.19	113.42	3.66±0.1212	
		Mean Weight (kg)								
Supplemented	107	17.00	18.95	1.95±0.7044	6.26**	93	16.25	18.30	2.05±0.7511	17.40**
Unsupplemented	107	17.05	18.39	1.344± 0.7142		93	16.27	17.47	1.20± 0.4460	

** Significant at 1% level.

Heights and Weights of Children Analysed According to Age:

A comparison of the mean increases in heights and weights of children according to their age range is presented in Table XXXXXXII.

In each age group, the increases in heights and weights of the supplemented group were compared with the corresponding increases in the unsupplemented group. In all the age groups the differences in increases in heights and weights between the two groups (supplemented and unsupplemented) were statistically significant. Fig X and XI represents body weight and height gains of school children in comparison with the control group.

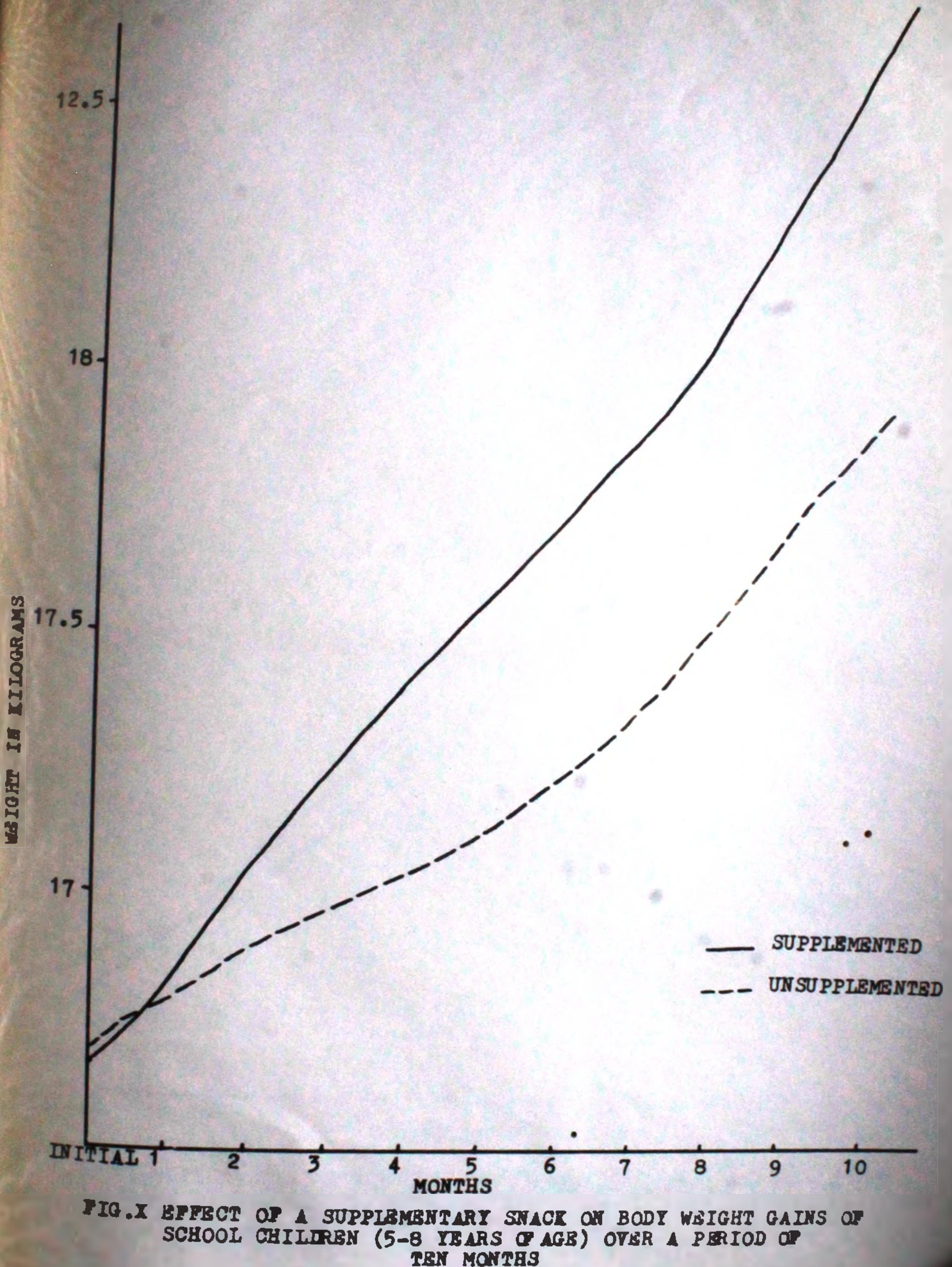


FIG.X EFFECT OF A SUPPLEMENTARY SNACK ON BODY WEIGHT GAINS OF SCHOOL CHILDREN (5-8 YEARS OF AGE) OVER A PERIOD OF TEN MONTHS

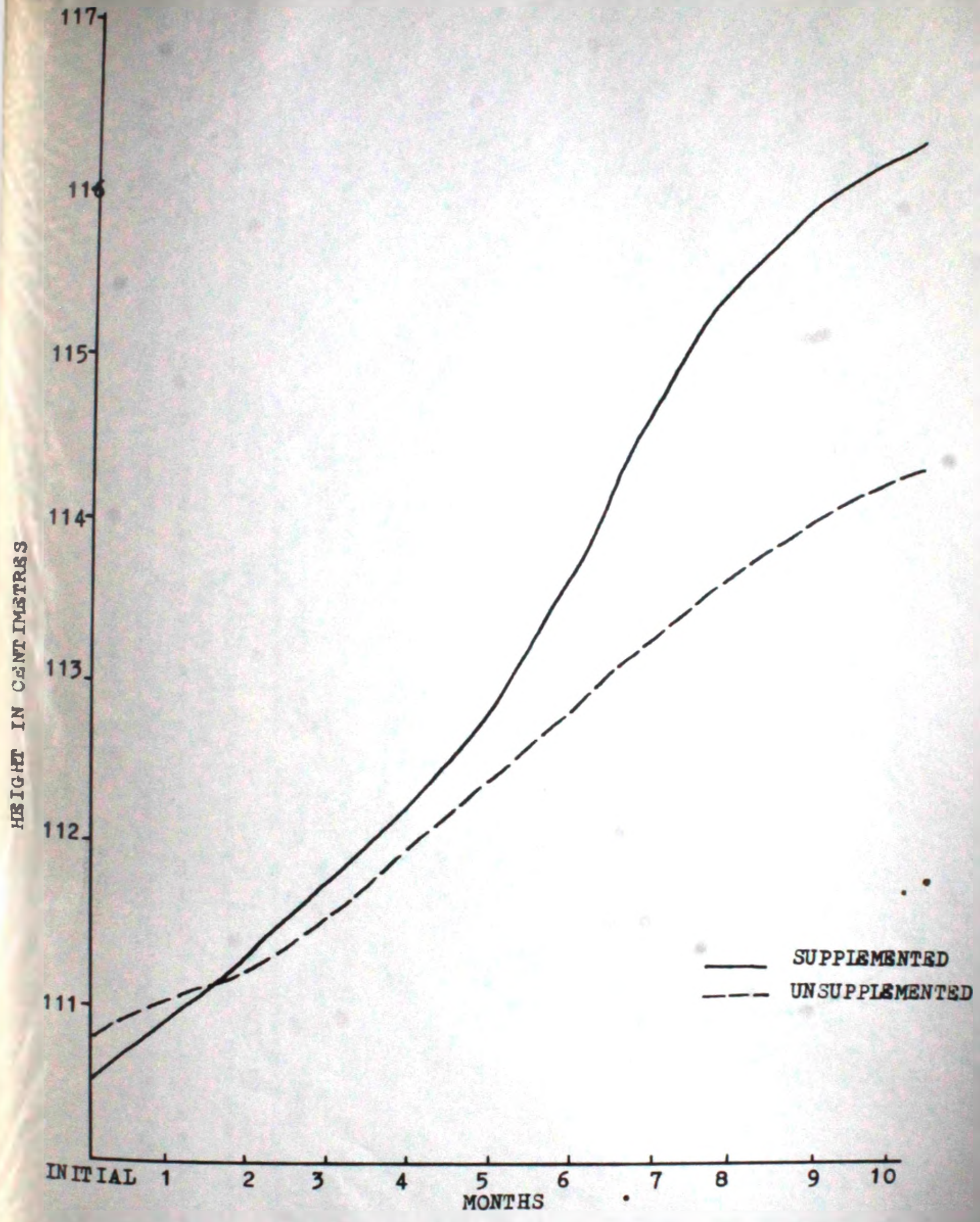


FIG. XI EFFECT OF A NUTRITIONAL SUPPLEMENTARY SNACK ON BODY HEIGHT GAINS OF SCHOOL CHILDREN (5-8 YEARS OF AGE) OVER A PEIOD OF TEN MONTHS

TABLE [illegible]

THE MEAN INCREASES IN HEIGHTS AND WEIGHTS OF CHILDREN ACCORDING TO THEIR AGE

Group	No. of children	Age (566 yrs.) Initial	Final	Difference	't' value	No. of children	Age (6-7 yrs.) Initial	Final	Differen-ce	't' value	No. of children	Age (7-8 yrs) Initial	Final	Differen-ce	't' value
							Weight in kg.								
Supplemented	53	14.89	16.81	1.92± 0.10	6.965**	68	16.23	18.05	1.82± 0.95	2.48*	79	18.21	20.39	2.18± 0-08	11.02**
Unsupplemented	53	16.08	15.11	0.97± 0.08		68	16.13	17.62	1.49± 0.89		79	19.50	18.22	1.28± 0.08	
Supplemented	53	104.48	111.16	6.68± 0.16	10.52**	68	108.52	115.05	6.53± 0.17	10.75**	79	115.41	121.16	5.75± 0.13	5.36**
Unsupplemented	53	104.69	108.75	4.06± 0.13		68	108.81	112.59	3.78± 0.20		79	116.60	119.80	3.20± 0.09	

** Significant at 1% level.
* Significant at 5% level.

Attendance in School:

Table XXXXXXIII gives the monthly attendance of children in the school.

TABLE XXXXXXIII

CONSOLIDATED SCHOOL ATTENDANCE FOR 10 MONTHS

Group	Number of children Percentage of Attendance			
	80-85	85-90	90-95	95-100
Supplemented	4	3	24	169
Unsupplemented	2	8	25	165

The school attendance of children in both the groups was good. Even during the busy harvest seasons there were no serious fall in attendance in the school. The similarity in school attendance for both the supplemented and unsupplemented groups of children makes valid further comparisons of school academic performance and their participation in co-curricular groups activities.

The attendance of the children taking the supplement was also noted. The supplement was fed on six days of the week, except on sundays.

TABLE XXXXXXIV

PERCENTAGE OF ATTENDANCE FOR THE SUPPLEMENTARY FEEDING

75 - 80	85 - 90	90 - 95	95 - 100
2	8	48	142

The average attendance of children was 80 to 90%. This revealed the enthusiastic and interested participation of the children in the feeding trial. Further, there were very little plate wastage and the supplement was greatly relished by the target children.

The academic performance of the children in both the groups were studied every month as part of the evaluation. The marks obtained every month in Tamil, Mathematics, General knowledge, History and Geography tests by each child were recorded. From the total marks obtained the percentage of marks obtained was calculated as given in the Table XXXXXXV.

TABLE XXXXXXV

ACADEMIC PERFORMANCE OF TARGET CHILDREN

Group	Number of School Children — Percentage of consolidated marks obtained in 5 subjects			
	Below 35%	35-50%	50-60%	65-80 and above
Supplemented (200 children)	11	73	81	35
Unsupplemented (200 children)	14	86	78	22

In general, the academic performance of the children in the supplemented group was slightly better. However, the difference was not statistically significant, except in the range of 65 to 80% marks. In this range, the supplemented groups of children fared better.

Participation of the children in the co-curricular activities:

The co-curricular activities of the children noted in both the groups are presented in Table XXXXXXVI.

TABLE XXXXXXVI

PARTICIPATION OF CHILDREN IN CO-CURRICULAR ACTIVITIES

Groups	Number of children - 20					
	Singing	Dancing and Dramatics	Gardening	Picture collecting	Sports running	Total
Supplemented	20	22	50	15	93	200
Unsupplemented	17	18	54	13	98	200

There was no significant difference between the two groups in regard to participation in co-curricular activities.

Incidence of sickness in children:

The incidence of sickness among children in both the groups was studied throughout the experimental period and the results are given in Table XXXXXXVII

TABLE XXXXXXVII

INCIDENCE OF SICKNESS IN 400 TARGET CHILDREN

Months	Number of school children – Incidence of Sickness											
	Fever		Cold & Cough		Diarrhoea		Small pox		Chicken pox		Total	
	S*	US**	S	US	S	US	S	US	S	US	S	US
1	12	17	14	8	2	7	1	2	-	1	29	35
2	23	18	7	4	5	6	1	1	-	0	36	29
3	30	29	8	13	6	8	2	-	-	-	46	50
4	31	43	2	2	2	3	-	3	-	-	35	51
5	17	14	9	11	1	3	1	-	-	-	28	28
6	9	9	5	4	1	-	-	-	-	-	16	13
7	6	9	4	7	2	3	-	-	-	-	12	19
8	13	16	7	6	-	-	-	-	-	-	20	23
9	35	30	19	21	4	2	2	2	1	-	61	55
10	12	14	1	11	2	-	-	-	-	-	15	25
								Total			298	328

* Supplemented
** Unsupplemented

The incidence of sickness was high in both the groups of children. The differences in the incidence of sickness between the groups was not significant. This data shows the need for health care in the elementary schools in our state.

Clinical assessment:

The results of the clinical assessment carried out at the beginning and at the end of the study period in both the groups are given in Table XXXXXXVIII.

TABLE XXXXXXVIII

CLINICAL FINDINGS IN TARGET CHILDREN (CLINICAL SYNDROMES)

Groups	Vitamin A deficiency		Angular stomatitis		Anaemia (Pallor)		Spongy bleeding gums	
	I*	F*	I	F	I	F	I	F
Supplemented	12	4	26	8	14	14	12	5
Unsupplemented	16	16	14	9	17	17	18	18

*I - Initial
*F - Final

To start with, children in both the supplemented and unsupplemented groups had the following clinical syndromes: vitamin A deficiency, angular stomatitis, anaemia, caries, mottled enamel and spongy bleedings gums. But at the end of the study period the incidence of vitamin A deficiency, angular stomatitis and spongy bleeding gums in the supplemented group had been reduced considerably. However no such improvement was evident among the unsupplemented group.

Children in both the groups were anaemic before and after the study period. Although the iron requirement of both the groups of children was met, the children were still anaemic posing the question how much of the iron was available to the body.

Biochemical analysis:

Haemoglobin:

The results of the haemoglobin content of the finger prick samples of the children in both the groups ~~as~~ estimated at the beginning and the end of the study are given in Table XXXXXXIX.

TABLE XXXXXXIX

MEAN HAEMOGLOBIN CONTENT IN 100 ml OF BLOOD

Groups	Mean Value (grams/100 ml) Initial	Final	Difference	't' value
Supplemented	9.90 ± 1.78	10.74 ± 1.24	0.84	3.865**
Unsupplemented	9.85 ± 1.00	10.31 ± 1.14	0.46	

** Significant at 1 per cent level.

The findings reveal that the children in both the groups were anaemic to start with. At the end of the study there was a slight improvement in both the groups. Nevertheless haemoglobin levels of both the groups was below the WHO standards of 11.5 g/100 ml. for the age group 5-8 years (Jelliffee,1966). But the differences between the groups was not statistically significant. The inclusion of 30 grams of tender amaranth in the supplement per child per day, had contributed 8 mgs. of iron, but how much, of it was available to the body to be studied.

Urinary nitrogen and creatinine:

Early morning urine samples of 25 randomly selected children for both the groups were collected and analysed for their nitrogen and creatinine content. The data are presented in Table XXXXXXI.

TABLE XXXXXXI

URINARY NITROGEN LEVELS IN TARGET CHILDREN (g/100 ml of Urine)

Group	No.of children	Initial (g)	Final (g)	Difference (g)	't' value
Supplemented	25	1.728 ± 0.100	2.453 ± 0.107	0.725 ± 0.089	5.613**
Unsupplemented	25	1.680 ± 0.080	1.890 ± 0.085	0.210 ± 0.020	

At the end of the study, the supplemented group had higher total urinary nitrogen content. This shows that the protein intake and lean body mass were higher in the supplemented group children.

The urinary creatinine content of the children in both the groups are given below in Table XXXXXXXI

TABLE XXXXXXXI

URINARY CREATININE LEVELS IN TARGET CHILDREN (mg/100ml.of Urine)

Group	No.of children	Initial (mg)	Final (mg)	Difference (mg)	't' value
Supple-mented	25	48.84 ± 3.487	68.36 ± 4.744	19.52 ± 0.989	5.223**
Unsupple-mented	25	40.04 ± 2.065	51.52 ± 2.333	11.148 ± 1.158	

** Significant at 1% level.

The difference between the groups was statistically significant at 1% level. The supplemented group had higher creatinine content again shows that the lean body mass of the supplemented groups of children was higher. This correlates well with the higher body weights and heights of the children of the supplemented groups.

From the findings of this study it can be concluded that it is possible to overcome the present 20 per cent deficit in the calorie content of the diets of the school children by supplementation of this nutritious snack made of low cost indigenous foods. The inclusion of green leafy vegetables - Amaranth tender in the daily diet of the school children could overcome the vitamin A deficiency prevalent among the school children. The results of this study showed that the nutritional status of the school children could be improved with the supplementation of their diets with this nutritious snacks made of local foods.

The following table XXXXXXXII illustrates the rationale for supplemental indigenous food quantity for various age groups.

TABLE XXXXXXXII

RATIONALE FOR SUPPLEMENTAL FOOD QUANTITY

Age Group	Calorie Deficit	Protein Deficit	Basis for Information	Food supple-ment	Calories	Protein	Cost analysis Rs. p.
Pre-School children	352	Nil	Devadas,1973	75	257	9.2	0.21
School children	387	Nil	Devadas,1973	115	395	14.1	0.32
Pregnancy	1521	18	Devadas and Easwaran,1971	420	1442	51.3	1.10
Lactation	1164	17	Devadas and Easwaran,1971	345 g	1185	42.1	0.96

Calorie and Protein deficit can be make up by the addition of the indigenous supplements in appropriate quantities which are of low-cost.

F. **Nutritional Evaluation of Supplementation of Iron From Amaranthus to Anaemic Adolescent School Girls**

The study was designed to find the effect of supplementation of 100 g. of Amaranth containing of iron 24.3 mg. compared with another group of similar girls who were given an oral iron tonic containing equal quantity of the mineral.

Thirty six anaemic girls with haemoglobin values less than 11.5g. per cent were chosen from a group of 81 adolescent school girls of 13 to 15 years of age who were residing in a hostel.

They were randomly assigned to the experimental groups who were given the Amaranth (Group A) or iron tonic (Group B) supplements, and one control group (Group C) who received no iron supplement in addition to the hostel diet which contained 15.37 mg. of iron.

Measurements of Heights, weights and haemoglobin estimation for all the subjects were done every fortnight. PcV, McV, McH, cHc and colour index values were determined for the blood samples and used as the indices of final evaluation.

Height, Weight and Haemoglobin Values:

Table XXXXXXXXIII gives the mean initial height, weight, age, age of menarche and haemoglobin values of the subjects.

The adolescent girls of the present study were comparable with similar adolescent girls in various parts of India but were below the Western standards in their heights and weights. The mean age of monarche (13.8 ± 0.50) of the adolescent girls of the present study was similar to the age of monarche reported by ICMR (1966) for rural girls of Madras and Kerala States, namely, 14.16 and 14.42 respectivily. The girls of the present study were also from rural homes.

TABLE XXXXXXXIII

MEAN INITIAL HEIGHT, WEIGHT, AGE, AGE OF MONARCHE AND HAEMOGLOBIN VALUES OF THE SUBJECTS

No. of subjects	Code	Source of supplementation	Height cm.	Weight cm.	Age yrs.	Age of monarche yrs.	Hb g/100 ml blood
12	A	Amaranth	155± 1.8 a	42.1± 1.47	15.6± 0.14	14.84± 0.70	9.76± 0.132
12	T	Tonic	140± 2.7	38.4± 1.84	15.9± 0.77	14.9± 0.34	9.91± 0.426
12	C	Nil	155± 2.1	46.3± 1.35	15.2± 0.20	13.8± 0.50	11.19± 0.044

a Mean ± Standard error

So menstruation might not be a contributory factor in iron deficiency anaemia for the subjects of the present study.

All the faecal samples of the adolescent girls examined for ova revealed absence of hook worm infestation. So anaemia due to parasites gets ruled out for the subjects of the present study.

Dietary Intake

Table XXXXXXXIV shows the nutrient intake of the school girls who participated in the study.

TABLE XXXXXXXIV

MEAN NUTRIENT INTAKE OF ADOLESCENT GIRLS WHO PARTICIPATED IN THE STUDY AS REVEALED BY FOOD ANALYSIS

(3 day weighment survey)

Groups	Source of iron supplement	Protein (g)			Iron (mg)			Calcium (mg)			Ascorbic acid (mg)		
		Hostel diet	Supplement	Total	Hostel diet	Supplement	Total	Hostel diet	Supplement	Total	Hostel diet	Supplement	Total
A	Amaranth	48.5	3.02	51.52	15.4	23.4	38.8	472	360	832	14.8	16.5	31.3
B	Tonic	49.1	Nil	49.1	14.8	24.3	39.1	482	Nil	482	15.4	Nil	15.4
C	No supplements (control)	49.4	Nil	49.4	16.1	Nil	16.1	[illegible]	Nil	475	16.5	Nil	16.5

All the children had received adequate amount of protein. The group of girls whose diets were supplemented with Amaranthus had a higher intake of calcium, and Ascorbic acid compared to the other two groups of girls namely, C and T. The Ascorbic acid intake of all the three groups of girls were below the requirements.

Height, Weight Changes:

All the adolescent girls in the three groups exhibited no significant changes in heights or weights during the study which lasted for four months indicated that they had stopped growing rapidly (Table XXXXXXXV). The experimental period of four months might be very short to assess growth if any had occurred. The adolescent girls who participated in the study had reached menarche one year earlier and perhaps they might have had their growth spurt then.

TABLE XXXXXXXV

MEAN BODY WEIGHT AND HEIGHT CHANGES OF THE TARGET GIRLS
(Period of study 4 months)

Code	Source of iron supplement	Height cm. Initial	Height cm. Final	Weight kg. Initial	Weight kg. Final	Height changes cm.	Weight changes kg.
A	Amaranth	148.0± 2.72[a]	148.4± 2.45	42.4± 1.47	44.2± 1.00	0.142	1.001
T	Tonic	154.8± 1.78	155.2± 1.12	38.4± 1.84	41.6± 2.16	0.109	1.240
C	No supplement (control)	154.6± 2.09	155.1± 1.38	46.3± 1.35	46.8± 1.41	0.174	0.207

a Mean ± Standard error

TABLE XXXXXXXVI

MEAN HAEMOGLOBIN VALUES OF GIRLS WHO PARTICIPATED IN THE STUDY

Code	Source of supplementation	Total iron intake mg/day	Hb.values g per 100 ml. blood Initial	Final	Increase
A	Amaranth	38.8	9.76±0.132a	11.36± 0.275	1.60±0.056
T	Tonic	38.7	9.91±0.426	10.80± 0.137	0.89±0.115
C	No supplementation	15.4	11.19±0.044	11.05± 0.007	Nil

a Mean ± Standard error.

Haemoglobin Values:

Table XXXXXXXVI gives the mean initial and final haemoglobin values of the subjects in the three groups. It may be remembered that the target girls were selected on the basis of their low haemoglobin values at the beginning of the study.

As could be seen from Table XXXXXXXVI except in the control group, both the group of girls given either amaranth greens or iron tonic supplementation showed increases in haemoglobin concentrations, 1.60±0.056 and 0.89±0.115 grams per cent respectively.

Adolescent girls who consumed amaranth as a source of iron (Group A) showed significantly higher haemoglobin values over the group given oral iron tonic. The details of the analysis of variance is given in Annexure XXIV. The girls fed tonic showed increases in haemoglobin values over the unsupplemented group, but the increase was lower than that of the subjects who were given amaranth. This might be due to the higher availability of iron from amaranth because of its calcium content, or due to other haemopoietic factors like folic acid and vitamin C, present in the greens. It may be pertinent to recall that iron supplementation raised the haemoglobin levels in infants with a birth-weight between 2.5 kg. and 3.18 kg. Iron supplementation also raised the haemoglobin levels in males from a good social background and of those who gained more weight. (Burman,1972).

The group fed the amaranth supplement showed during the course of the experimental period higher haemoglobin values as could be seen in Figure XII.

Though the haemoglobin values increased in both the supplemented groups within a four month experimental period none of the values reached the accepted WHO standards of 14g. per 100 ml. The girls who received amaranth attained a haemoglobin value of 11.36±0.275 which approached the WHO standard for absence of anaemia.

Red Cell Indices:

Table XXXXXXXVII gives the PCV, MCV, MCH, MCHC and colour index of the two groups supplemented with iron at the end of the study.

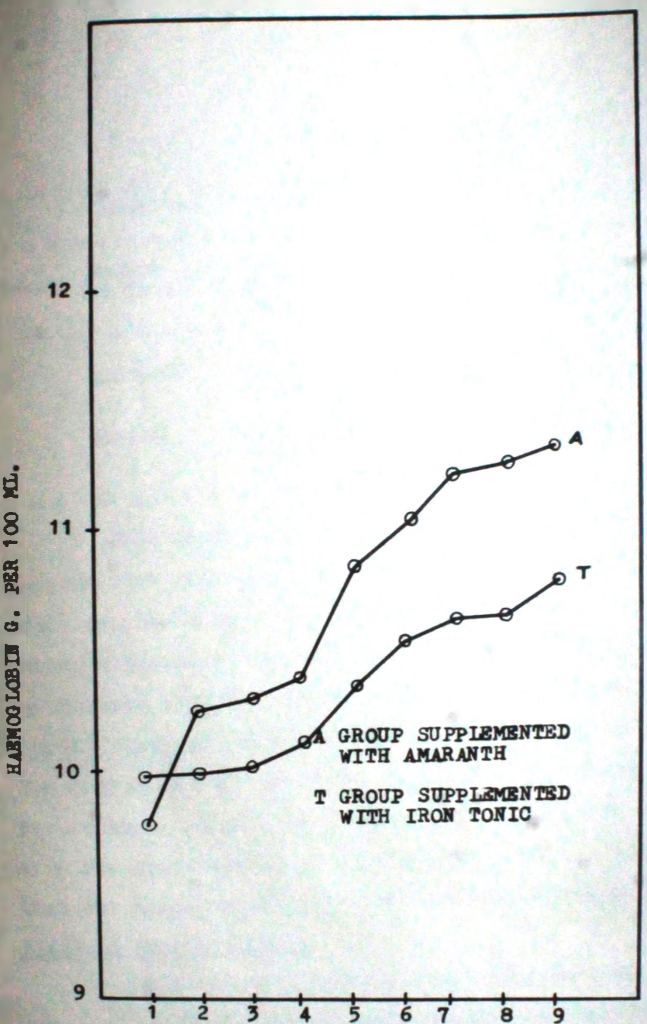


FIG. XII MEAN FORTNIGHTLY HAEMOGLOBIN CONCENTRATION OF THE ADOLESCENT SCHOOL GIRLS WHO RECEIVED SUPPLEMENTS OF AMARANTH GREENS OR AN ORAL IRON TONIC

TABLE XXXXXXXVII

MEAN PCV, MCV, MCH, MCHC AND COLOUR INDEX OF TWO GROUPS OF SUBJECTS SUPPLEMENTED WITH IRON AT THE STUDY

Group	Source of iron	PVC per-cent	MCV cum	MCH γγ	MCHC Per-cent	Colour index
A	Amaranth	43.46± 1.447	75.2± 9.90	18.16± 2.980	24.30± 0.707	0.63± 0.004
T	Tonic	30.73± 1.956	67.3± 7.20	22.002± 1.580	33.66± 2.000	0.77± 0.055

From Table XXXXXXXVII, it is observed that the subjects supplemented with amaranth (Group A) had higher PCV than the group supplemented with oral iron tonic. Subjects fed amaranth greens attained PCV values equal to that of the values reported by Wintrobe (1956) and WHO (1959) (37.42 per cent). The values for MCV 75.2±9.90 cum were also comparable to the reported values. The MCHC values of the group of girls fed amaranth were below the standard values. The colour index values of both the group of girls supplemented with iron were below 1. This indicated that the subjects were not suffering from megaloblastic anaemia.

<u>Clinical Manifestations:</u>

There was no significant correlation between clinical symptoms and the low haemoglobin values in this study. The girls with low haemoglobin values looked healthy and carried on their normal activities.

G. **The Results of the Investigation Undertaken to Determine the Availability of β Carotene from Carrots to Women College Students are Presented Here:**

The objective of the study was to find out the utilisation of β carotene from carrots by the post adolescent women college students. Serum vitamin A levels before and after carrot supplementation were considered as the criteria for this utilisation. One 100 grams of cooked carrots which contained 1890 micrograms of retinol were fed to ten subjects daily. Five of them who did not receive the carrot supplement formed the controls. This amount of carotene along with basal diet was found to fulfil the ICMR recommended allowances for the subjects of the study. Another group of similar women were supplemented with 475 micrograms of retinol daily for twenty days. The daily total intake of vitamin A of the target women is presented in Table XXXXXXXVIII.

TABLE XXXXXXXVIII

DAILY TOTAL INTAKE OF VITAMIN A OF THE TARGET CHILDREN

Group	Daily intake of Vitamin A: Basal Diet	Supplement	Total μg of retinol equivations
Control	298 μg of retinol (equivals)	Nil	298 μg of retinol
Carrot supplementation (1890 μg of carotene)	298 μg of retinol equivals	* 475 μg retinol equivals	773 μg of retinol
Retinol supplementation propalin**	298 μg of retinol equivals	475 μg of retinol	773 μg of retinol

(* 100 gram of cooked carrots provided 1890 μg of carotein which is considered equivalent to 475 μg of retinol)

** Propalin From - Glaxo Laboratories - A commercial Vitamin A preparation containing 24,000 μg of retinol per gram carefully diluted in coconut oil to obtain the required dilution.

Levels of vitamin A in the serum of the young women before and after supplementation of 100 grams of cooked carrots which supplied 6890 μg of β carotene for a period of 20 days are presented in Table XXXXXXXIX.

TABLE XXXXXXXXX

THE EFFECT OF FEEDING 100 g. OF CARROTS AND 475 µg OF RETINOL ON SERUM VITAMIN A

Period of study - 20 days

Groups	Supplement	No. of subjects	Serum vitamin A µg /ml. Initial	Final	Difference
I	(Cooked Carrots 100 gram)	10	31.90± 6.4	34.90± 4.3	3.00± 2.4
II	(Retinol 475 µg)	6	37.10± 2.5	45.28± 1.6	8.18±1.8
III	(Control)	5	30.96± 4.1	31.80± 3.8	0.84

* ± Standard Deviation

Levels of vitamin A in the serum of the control group of women students who did not receive any supplement, showed no significant change during the 20 days period of study, whereas the All 10 women who were fed 100 gram of cooked carrots in the experimental group for 20 days shown a significant change. Further more the group of six women who were fed 475 µg of (preformed vitamin A) retinol, showed a more significant increase in serum vitamin A levels at the end of the 20 days of study. This mean increase of 8.1 µg/100 ml. was larger than the increase 3.00 µg /100 ml. for feeding carrots.

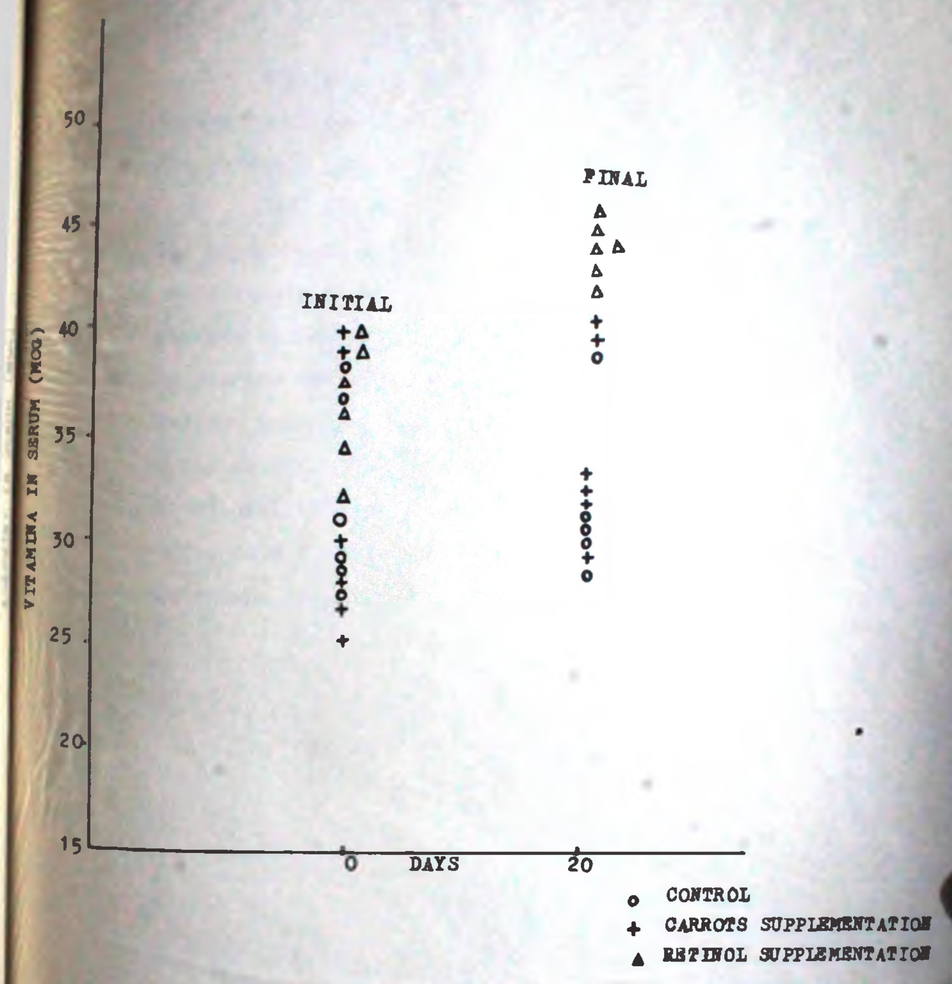


FIG. XIII SERUM VITAMIN A VALUES OF POST ADOLESCENT GIRLS BEFORE AND AFTER AN INTAKE OF 100 GRAMS OF CARROTS (1890 /UG OF CAROTENE) AND 475 /UG OF RETINOL DAILY FOR A PERIOD OF 20 DAYS

The mean increase in serum vitamin A level for the feeding of preformed vitamin A was significantly higher than the increase for carrot supplement Fig XIII.

The results of this supplementation study showed that the adult college women registered a significant increase in serum vitamin A levels after consuming 100 grams of cooked carrots for 20 days. Similar observations had been reported by Pereira and Begum (1968) and Lala and Reddy (1970). While Pereira and Begum found the increase in serum levels of children after feeding for three months, the present study proved the beneficial effects in as short a period as 20 days. This indicates that β carotene from carrots was not only absorbed but also converted to vitamin A.

V SUMMARY AND CONCLUSION

The studies reported here are some exploratory attempts in utilising selected low cost foods such as Maize, Sorghum, (cereals), cow pea, Horse gram, Field beans (legumes), sesame, groundnuts and Sunflower seeds (oil seeds) and amaranthus and carrots, (vegetables) grown in Coimbatore district, Tamil Nadu State towards the alleviation of malnutrition and under nutrition, particularly among children. While rice rice is the predominant and prestigious cereal in the Tamil Nadu state, other millets such as Maize and Sorghum are locally grown, and of low cost (1/3 the cost of rice) which would suit the purse of the common man. Further these millets also contribute more quantity of protein, minerals and vitamins when compared to rice. Hence these indigenous foods at low cost are evaluated for their nutritional value.

A series of food formulations incorporating several low cost indigenous foods in different combinations were developed and evaluated for use in community feeding programmes, with the specific objective of promoting self reliance. Each of the series of food mixtures evaluated in this study consisted of different combinations of indigenous foods as supplements. The human feeding trials involved different age groups, ranging from the young preschool child to adult college women. Involving different age groups was necessitated due to the fact that human experiments are laborious, time consuming and requires abundant support and cooperation among the participants in contrast to animal studies. While growth promoting abilities were evaluated through a feeding trial among children, evaluation of iron and vitamin A availabilities from the selected foods required drawing out frequent and considerable blood samples and this was difficult to get from the young children. Hence these studies were carried out on older age groups. The nutritive efficacy of the recipes of the food formulations which were found acceptable were further evaluated through a series of human feeding trials.

The first study was on vegetable protein mixtures formulated with the local foods namely, maize, cow gram, horse gram, groundnut and sunflower seeds, which will be useful for preschool and school going children.

The formulations were first evaluated on albino rats using PER, NPU and NDP Cals per cent as criteria. The PER of the formulate ranged from 2.306 $\pm$ .01216 to 2.575 $\pm$ 0.0926 and NPU values ranged from 69 to 76. These values are reasonable according to the standards specified by the PAG and ISI for weaning foods. Furthermore the NDP Cals per cent value for the various food formulations were satisfactory, when compared with the values reported for vegetarian diets by FAO/WHO (1974). The cost analysis of the formulation showed that for a ration of 100 grams of the food mixture, the costs ranged between 15 to 19 paise per child per day, which was lower than the cost of any processed or imported foods commonly used in feeding programmes, and the prices of which are exorbitant due to transport and storage charges. Organoleptic evaluation was carried out on selected food preparations (recipes) in which the formulated food mixtures had been incorporated. All the recipes tested were found to be palatable and wholesome. The series of maize based food formulae thus evolved along with vitamin and mineral fortification is recommended for use in feeding programmes and other nutrition intervention projects to fill the existing nutritional gaps in the diets of the pre-school and school children.

In the next experiment, nutritional evaluation of a sorghum - green gram mixture was carried out. The experiment was designed to find out how much of green gram supplemented to a sorghum diet would result in maximum PER value. Such an approach would help to evolve simple food formulations involving only two ingredients, namely, a local cereal sorghum and a legume, green-gram. Maximum protein value was obtained when 60 per cent of the protein in a diet were derived from sorghum and 40 per cent from green gram. The protein quality increased to the maximum when the proportion of sorghum or cereal component to the legume (green gram) component was in the ratio of 3:1. This findings is of particular interest in evolving simple low cost indigenous food mixtures, to replace the more expensive food supplements which are used at present in the on going nutrition programmes.

A mixture of horse gram and sesame in the protein ratio of 4:1 was next evaluated using a bioassay technique. The mean PER values increased from 1.05±0.091 to 1.38±0.101 for horse gram - sesame mixture when the protein content of the diet was increased from 9 to 15 per cent. Significant differences were noted in the total hepatic nitrogen content and hepatic nitrogen per g. of fresh tissue among the groups fed different levels of protein. The hepatic Succinic Dehydrogenase (SDH) activity also increased with the increase in the level and quality of protein. The Relative Growth Index (RGI) which is an expression used for the relative nutritive value of dietary proteins, is the slope of the regression between dose and response expressed as a percentage of the slope obtained with the protein of reference quality or maximum nutritive value. Using this index and skim milk as a reference protein with 100 as value, the relative nutritive value based on body weight gain of horse gram - sesame mixture was found to be 53.6. Such protein quality evaluations are helpful and necessary to evolve a series of vegetable protein mixtures, using indigenous food sources such as oil seeds, legumes and cereals for use in supplementing feeding programmes.

The effect of three selected dietary sources of proteins (1) a combination of red gram and sesame at a protein ratio of 2:1, (2) defatted fish flour and (3) skim milk powder, on carotene utilisation in albino rats was next studied in comparison against a basal rice diet. The criteria for evaluating carotene utilisation with the different dietary proteins, were growth rate, hepatic storage of Vitamin A, total serum proteins and protein fractions. Combination of red gram and sesame produced a growth rate of 44 ± 3.5 g in 28 days, while fish meal stimulated

31 ± 2.4 g during the same period. The growth promoting utility was best (60 ± 5.1 g/28 days) in the groups fed the skim milk protein and lowest in the rice diet (18 ± 1.09/28 days). The animals on a 5 per cent rice diet had the lowest amount of vitamin A per gram of liver (8 ± 1.2 mcg/g of liver). The hepatic vitamin A per g of liver and the total hepatic vitamin were similar in all the three experimental groups and were significantly higher than those of the rats on poor rice diets. Thus improvement of dietary protein quality, improved not only growth rates, but also helped in better dietary carotene utilisation. This finding is significant since along with Protein Calorie Malnutrition, hypovitaminosis A is a public health problem among Indian children.

Another study was aimed at evaluating a weaning mixture based on a local cereal (Sorghum) a legume (Bengal gram) and oil seed (groundnut) through a human feeding trial. The growth rates of the preschool children who were taking part in the feeding trial were assessed through their body weights and body heights. Children receiving the supplement had registered significantly higher increases in body weights and body heights within a period of six months when compared to control group of children. The weaning mixture as a supplement was effective in bringing down the number of children with mild protein calorie malnutrition syndrome, and also in shifting severe anaemia to mild and moderate degrees of anaemia. The shelf life studies showed that the above weaning mixture had a satisfactory shelf life for a period of 4 to 6 months and the cost of the 80 grams of the above mixture was 15 paise per child per day as per the prevailing prices on October 1973.

The effects of food supplements based on opaque-2 maize, ordinary maize and skim milk on the nutritional status of pre-school children 18-30 months of age was studied in the next experiment. All the groups who received the different supplements had registered significant increments in heights and weights, when compared with the control group who did not receive any supplement. The heights and weights of the children on the high lysine maize Opaque-2 maize) diet ranked next to the skim milk group. Nitrogen balance data on these diets did not show any significant differences among opaque-2 Maize, ordinary maize and skim milk. The clinical picture of the target children revealed that the most predominant clinical syndromes were mild forms of protein calorie malnutrition and anaemia. The number of children manifesting the various symptoms of deficiency diseases decreased in the three experimental groups at the end of the study in comparison with the control group. In the age group of 12-17 months the increments in heights and weights for opaque-2 maize was greater (3.7 cm and 1.56 kilogram) than ordinary maize (3.2 cm and 1.30 kilogram) which were significant at p 0.05 levels. Similar trends were found in the other two age groups. Thus opaque - 2 maize was found to be better than ordinary maize in promoting the growth of pre-school children. This finding is of great significance in the Indian context, since opaque-2 maize can play an important role as a rich and low-cost source of good quality protein and calories. Both these are lacking predominantly in the present dietaries of Indian children.

Another study was designed to evaluate a nutritional supplement made of low cost indigenous foods such as cow-pea, horse gram, field beans, sesame and rice. School going children in the age range 6-9 were selected as the subjects for study. The findings revealed the following: The mean Calorie and protein values of home diets were 1263 and 27.1 g for the target children. The home diets were deficient in Calcium and Vitamin A. The consumption of the nutritional supplement made up the 20 per cent of the deficit in Calories. The inclusion of tender amaranthus in the formulation, provided a good amount of the micronutrients calcium and Vitamin A. The mean increase in the body weights of the children in the supplemented group was 1.99±0.529 kg over a period of 10 months. This increase was greater than the 1.27±0.050 kg. registered by the unsupplemented group for the corresponding period. The mean increase in the heights of the children in the supplemented group was 6.2±0.1132 cm. which was greater than 3.6±0.0844 cm registered by the unsupplemented group. At the end of the study, the incidence of vitamin A deficiency, angular stomatitis and spongy bleeding gums in the supplemented group had been reduced. The mean increase in the haemoglobin content of the capillary blood of the children of the supplemented group was 0.84g/100 ml-while the corresponding increase in the unsupplemented group was 0.46g/100 ml. The mean increases in the urinary nitrogen and creatinine levels were higher in the supplemented groups being is 0.72g/100 ml and 19.52mg/100 ml respectively, 0.21 g per 100 ml and 11.48 mg/100 ml respectively in the unsupplemented group indicating that the protein intake and lean body mass were higher in the supplemented group as against the unsupplemented group. Thus it is possible to overcome the present 20 per cent deficit in the Calorie content of the diets of school children by supplementation with a nutritious snack made of low cost indigenous foods. The inclusion of green leafy vegetable-amaranthus in the daily diet of the school children helps to overcome the deficiencies of vitamin A and iron. The cost analysis of the supplements showed that the total cost per school child per day was 20 paise in October 1974, which was very economical when compared to the huge costs involved in the transport and storage of the imported foods like CSM.

In the next study the supplementation of iron from amaranthus to anaemic adolescent school girls whose growth spurt (menarche) had occurred two years previous to this study was evaluated. Anaemic adolescent girls were selected for the supplementation, since iron deficiency is predominant in this age group in South India and anaemia could be reverted within a period of four months through dietary supplementation. The basal diet contained 15.4 mg of iron per day; the amaranth or iron tonic supplement contributed 24.3 mg of iron per head per day. The girls in the control group who did not receive any iron supplementation showed no increase in haemoglobin values at the end of four months. Increase in Haemoglobin values of the group fed amaranth were higher than those fed the iron tonic (0.89 g/100 ml and 1.60 g/100 ml). The green leafy vegetables were effective in increasing the Haemoglobin values of adolescent girls who were anaemic in the beginning.

In the last study, the availability of β-carotene from carrots to women college students was investigated. College students were selected for this study since it involved drawing out frequent and considerable quantities of blood samples from the subjects. Levels of vitamin A in the serum of young college women before and after supplementation of 100 grams of cooked carrots, which supplied 6890 μg of β carotene per day for a period of 20, days were compared with a similar group of women who were fed 475 μg of retinol during the same period. The results of this study showed that the levels of vitamin A in the serum of the control group of women, who did not receive any supplement manifested no significant change after the 20 days study period, whereas all the 10 women who were fed carrots had shown a significant rise (3.00 μg/ml) in serum vitamin A levels. Furthermore the group of six women who were fed 475 μg of preformed retinol showed a more significant increase of 8.1 μg/100 ml in serum vitamin A level. The total amount of vitamin A contained in the basal diet and the supplement met the requirement of this nutrient for this age group as specified by ICMR. Thus the existing deficiency of vitamin A in the Indian diets could be effectively made up though the use of local vegetable sources such as carrots and papaya if given in adequate quantities.

This series of experiments hasthus brought out valuable information on the possibility for evolving low cost indigenous food formulations for nutritional improvement. Effort is now needed to produce these formulations on large scale for the nutritional feeding programmes and for popular consumption in our country.

B I B L I O G R A P H Y

1. Abraham, J and Prusso, P. 1971.	Opaque Maize-2 in Columbia Nutrition News letter, 9, pp 1-14.
2. Alan Berg, 1973	The Nutrition Factor. The Brookings Institution Washington, p.9.
3. Almendinger, R. and Hinds, F.C. 1969	Apparent Carotenoid Increases in the Digestive Tract of Beef Cattle, J.Nutr., 97, p.13.
4. Ambalini Bailur, 1970	Studies on nutrition of Pre-school children, A Decade of Progress 1961-1970, National Institute of Nutrition, Hyderabad, India.
5. Anandam, K., Susheela, A. and Devadas, R.P. 1965	Effect of incorporation of leafy and non-leafy vegetables in the school lunch on the growth and nutritional status of children. J.Nutr.Dietet. 2, 202-205.
6. Apte S.V., 1971	Observations on iron requirements Proceedings of the Nutrition Society of India 10: 49-53.
7. Arroyave, G., Viteri, F., Behar, M. and Scrimshaw, N.S. 1959	Importance of Intestinal Absorption of Vitamin A palmitate in Severe Protein Malnutrition (Kwashiorkor) Amer. J. Clin. Nutr., 7, p.105.
8. Arroyave, G. 1969	Interrelations Between Protein and Vitamin A and Metabolism. Amer.J. Clin.Nutr., 22, p.1119.
9. Asfour, R.Y., Tannous, R.I.Y., Sabry, Z.I. and Cowan, J.W. 1965	Protein rich Food Mixtures for Feeding Infants and Young Children - II. Preliminary Clinical Evaluation with Laubina Mixture, Amer.J.Clin.Nutr. 17, p.148.

10. Autret, M., Pharon, D. and Van A.O.V. 1955 — Possible sources of proteins for Child Feeding in Under-developed countries. The American Journal of Clinical Nutrition, 3, 234.

11. Aykroyd, W.R. and Doughty, J. 1964 — Legumes in human Nutrition FAO Nutritional Studies, No.19, Rome, Italy.

12. Berger, S., Kerycka, M., Milner, M., Bialek, T., and Chabrowski, K. 1969 — Studies on Availability and Mechanism of Carotene and Vitamin A Utilisation from Different Dietary Sources and Under Different Experimental Conditions, Final Report from Warsaw Agricultural University (June 1964 - June 1969), Warsaw, Poland.

13. Bhagavan, R.K., Doraiswamy, A.R., Subramanian, N., Narayana, M., Swaminathan, M., Bhatia, D.S., Sreenivasan, A. and Subrahmanyan, V. 1962 — Use of Vegetable Protein in the Treatment of Protein Malnutrition - Kwashiorkor. American Journal of Clinical Nutrition, 11, p.127.

14. Bhatia, D.S., Ramanathan, L.A., Prasad, M.S. and Vijayaraghavan, P.K. 1967 — Use of Papain in the Preparation of Quick Cooking Dehydrated Pulses and Beans Food Tech., 21, 105.

15. Blomstrand, R. and Werner, B. 1967 — Studies on the Intestinal Absorption of Radio Active B-Carotene and Vitamin A in Man. Conversion of B-Carotene into Vitamin A. Scand.J.Clin.Lab. Invest., 19, p.339.

16. Borchers, R. and Ackerson, C.W. 1950 — The Nutritive Value of Legume seeds X. Effect of Autoclaving and Trypsin Inhibitor Test for 17 Species. Journal of Nutrition, 41, 339.

17. Bressani, R., Elias, L.G. and Gomez Brenes, R.A. 1969. — Protein quality of opaque-2 Corn evaluation in rats. J.Nutr., 97, 173

18.	Bressani, R. and Elias, L.G. 1974	In Legume Foods (A.A. Altschul, Editor) Academic Press, Chapter 7.
19.	Bressani, R., Elias, L.G. and Navarrete, D.A. 1961	Nutritive Value of Central American Beans, IV. The essential amino acid content of samples of black beans, red beans and cow peas of Guatemala, J. Food. Sci., 26, p.525
20.	Bressani, R. 1973	Legumes in Human Diets and How they might be improved. Nutritional Improvement of food legumes by Breeding. Proceeding of a symposium sponsored by PAG, held at the Food and Agriculture Organization, Rome, Italy, 3-5 July 1972 and PAG statement 22. Upgrading Human Nutrition through the improvement of Food legumes. Protein Advisory Group of the United Nations System, United Nations, New York 10017, p.15
21.	CANTOR/ATAC, 1973	The Tamil Nadu Nutrition Study. An Operations Oriented Study of Nutrition as an Integrated System in the State of Tamil Nadu, Vol.1., pp.81, 82, 144, 178, 192, 193.
22.	Clark, H.E. 1968	Meeting Protein Requirements of man. Journal of American Dietetic Association, 52, pp.475-479.
23.	Cook, J.D., Alvarado, J., Gutnisky, A., Jamra, M., Labardini, J., Layrisse, M., Linares, J., Loria, A., Maspes, V., Restrepo, A., Reynafarje, C., Sanchez Medal, L., Velez, H. and Viteri, F.	Blood 38: p. 591-603.

24. Dandekar, V.M. 1971 — Poverty in India, Economic and Political Weekly, Bombay.

25. Deuel, H.J. 1955 — The Lipids, Biochemistry, Interscience Publishers, New York and London, Vol.2, p.919.

26. Daniel, V.A., Leela, R., Venkat Rao, S., Narinarasa, K., Indiramma, K., Swaminathan, M., and Parpia, H.A.B. 1964 — Mutual Amino-acid Supplementation of Proteins. II. Nutritive Value of Proteins of Blends of Wheat, Groundnut, Soybean, Bengal gram, Sesame and Skim-milk Powder Fortified with Limiting Amino-acids, J.Nutr. Dietet., 1, p.293.

27. Daniel, V.A., Subramanya, Urs, M.K., Desai, B.L., Venkatarao, S., Rajalakshmi, D., Swaminathan, M., and Parpia, H.A.B. 1967 — Studies on Balanced Foods for Weaned Infants. The Journal of Nutrition and Dietetics, 4, p.183.

28. Davidson, L.S.P., and Passmore, R. 1970 — Human Nutrition and Dietetics, Williams and Wilkens and Co., Baltimore, p.899.

29. Deshmukh, A.D. and Sohonie, K. 1965 — Proteins of Green-gram-Amino Acid Composition J.Nutr. Dietet., 2, p.183.

30. Devadas Rajammal P., and Jamala, S. 1971 — Studies on Infant Weaning Foods - Final ICMR Report of the Studies on Infant Weaning Foods.

31. Devadas Rajammal P., and Easwaran P. 1971 — Nutrient intake of selected Vulnerable groups in Coimbatore District. A paper presented at the IBP/H.A. Meeting in Blantyre Malawi, 5th - 12th April.

32. Devadas, R.P., Baby Anuradha and Sharadambal, S. 1972 — Evaluation of an applied nutrition feeding programme on the nutritional status of women. Ind.J.Nutr. Dietet., 9, pp.143-148.

33. Devadas Rajammal P., Vasanthi, M.N.M., Sharadambal, B. 1972	The Nutritional status of selected pre-school children in an Applied Nutrition Programme. Ind.J.Nutr.Dietet.9, pp.1-4.
34. Devadas Rajammal P. 1973	An infant Food Developed by Sri Avinashilingam Home Science College for Women, Coimbatore, Kuzhandai Amudhu, Social Welfare, XXI,6, 29.
35. Devadas Rajammal, P 1973	Use of local foods for formulating low cost Nutritious Food mixtures, Home made weaning foods and Multimixes. A paper presented during the Nutrition Training for Field Officers of CARE from Kerala, Tamil Nadu, Mysore and Andhra Pradesh from July 16 - Aug. 7, 1973 at Sri Avinashilingam Home Science College, Coimbatore 641011 (unpublished)
36. Devadas Rajammal P., Murthy, N.K., Elizabeth N.J., and Vasantha P.S. 1973	Nutritional status and nutrient intake of children in the group of 0-30 months in a rural area. Indian J.Nutr.Diet., 10, p.173,177.
37. Devadas Rajammal P., Usha Chandrasekar and Kumari, K.S. 1973	Availability to school children of iron from amaranthus cooked in two different utensils Ind. J.Nutr.Dietetics 101, pp.223-229.
38. Devadas Rajammal P., Usha Chandrasekhar, and Prema Kumari,S. 1973	Availability of iron from green-leafy Vegetables as compared to iron salts for school children, IndianJournal of Medical Research, 61, 270-277.
39. Devadas, R.P., Usha Chandrasekar, Jamala, S., and Murthy, N.K. 1974.	Nutritional evaluation of Maize based indigenous infant food, "Kuzhandai Amudhu" Ind. J.Nutr. Diet. 11, 257-293.

40. Doraiswamy, T.R., Chandrasekara, M.R., Subbarao, B.H., Sankaran, A.N. and Swaminathan, M. 1964. — The Effects of Supplementing the Diet of Undernourished Weaned infants and Preschool children with a Spray Dried Protein Food Based on Groundnut Protein Isolate and Skim-milk Powder on the Growth and Nutritional Status, Central Food Technological Research Institute, Mysore.

41. Doraiswamy, T.R., Parthasarathy, H.N., Tasker, P.K., Sankaran, A.N., Rajagopalan, R., Swaminathan, Sreenivasan, A. and Subramanyam, V. 1965 — Effect of supplementing protein food on the growth of children. The Indian Journal of Nutrition and Dietetics, Vol.2, No.2, p.71.

42. Daum, M.R., Rao, B.R.H., Jesudian, G., and Benjamin, V. 1966 — Supplemented groundnut protein isolate in pre-school children II. Journal of Nutrition and Dietetics, 3.

43. Ehrlich, S.T., Farthing, B.R. and Moschette, D.S. 1964 — Metabolic Patterns in Pre-adolescent Children II. Response of Vitamin A and Carotene Serum levels to Dietary protein and Vitamins J.Nutrition, 84, p.389.

44. Elias, L.G., Colindres, R. and Bressani, R. 1964 — The Nutritive Value of Eight Varities of Cow Peas (Vigna Sinesis) Journal of Food Science, 29, p.118.

45. Elwood, P.C., Waters, W.E., Green, W.J.W. and Sweetnam, P. 1969 — J.Chronic Dis.21: 615 - 620.

46. Eriksen, B. and Haygaard, A. 1941 — The Absorption of Carotene in Man. Klin. Wochenschr. 20, p.200 - Cited in: Chem. Abstr. 36. (Abstr.No.7075) 1942.

47. FAO
1954
Minerals and Vitamins for International use. F.A.O. Rome, Italy.

48. FAO/WHO
1968
FAO/WHO Scientific group, WHO Technical report series Number:405

49 FAO/WHO,
1971
Specification for the Identity and Purity of Some Extraction Solvents and Certain Other Substances. FAO Nutrition, Series No.4813, WHO/Food Add/70, p.40

50. FAO/WHO,
1967
Requirements of Vitamin A, Thiamine, Riboflavin and Niacin Tech. Report series No. 362, Rome.

51. Figueira, F., Mendonca, S., Rocha, J., Azavedo, M., Bunce, G.E. and Reynolds, J.W.
1969
Absorption of Vitamin A by Infants Receiving Fat-free or Fat-containing Dried Skim milk For Formulas. Amer.J.Clin. Nutr., 22, p.588.

52. Fronseca, A.J.(Editor),
1971
Challenge of Poverty in India Vickers Publications, Bombay, London, p.12.

53. FAO/WHO
1972
Nutritional Anaemias. Technical Report series No.503

54. Garby, L., Irnell, L., and Warner, I.
1969
Acta, med. Scand, 185; 113-117.
Iron Deficiency in Women of Fertile Age in a Swedish Community.

55. Gershoff, S.N.
1964
Effects of Dietary Levels of Macronutrients on Vitamin Requirements, Federation Proc. 23, p.1077.

56. Gopalan, C., Venkatachalam, P.S. and Belavady, B.
1960
Studies of Vitamin A Deficiency in Children, Amer.J.Clin. Nutr. 8, p.833.

57. Gopalan, C.
1970
Some Recent Studies in the Nutrition Research Laboratories, Hyderabad. The American Journal of Clinical Nutrition, 23, 1, 35-51.

58. Gopalan, C., Balasubramanian, S.C., Ramasastri, B.V., and Visweswara Rao, K.
1971
Diet Atlas of India. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India.

59. Gopalan, C.
1972
Report for the Period 1st October, 1970 to 31st December, 1971. Indian Council of Medical Research, National Institute of Nutrition, Hyderabad-7

60. Gopalan
1973
World Review of nutrition and Dietetics Vol.16, S.Karger, Basel, Munchen-Paris-London New York. p.104.

61. Gopalan, C.
1973
Report of the Task Force on Nutrition (National Committee on Science and Technology) Proceedings of the Nutrition Society of India, NIN,ICMR, No.13, pp.3-24.

62. Gopalan, C.
1973
Report of the Task Force on Nutrition (National Committee on Science and Technology) Proceedings of the Nutrition Society of India, No.13, pp.3-24.

63. Gronowska-Senger, A., and Wolf, G.
1970
Effect of Dietary Protein on the Enzyme from Rat and Human Intestine Which Converts β-Carotene to Retinol, J. Nutrition, 100, p.300.

64. Guggenheim, K., and Szmelcman, S. 1966 — A protein rich mixture from Vegetable sources; Assessment of nutritive Value on Animals, infants and invitro. Paper presented at the VIIth International Congress of Nutrition, Hamburg, 3-10, VIII., 78

65. Gupta, S.M. 1972 — The Low Cost High Protein Mixtures - Prospects and Problems. J. Food Sci.Technol., 9, pp.3-8.

66. Gattiker, M.N., Parameswaran, M., Doraiswamy, T.R., Narayana Rao, M., and Swaminathan, M. 1965 — Effect of supplementary protein food based on a blend of groundnut, bengal gram and sesame on the retension of nitrogen, calcium and phosphorus in undernourished children subsisting on an inadequate diet. Journal of Nutrition and Dietetics 2, 73-77.

67. Girija Bai R. and Rajammal P.Devadas 1972 — Nitrogen Balance in College Women in indigenous Diets. Ind.J.Nutr. Dietet. 11, pp.1-9.

68. Harpstead, D.D., Pradilla, A., and Sarria, D. 1968 — Agronomy Abstract p.66

69. Heymann, W. 1936 — Absorption of Carotene Amer.J.Dis.Child 51, p.273.

70. Holt, L.E.(Jr.) and Snyderman, S.E. 1965 — Protein and amino acid requirements of infants and children. All India Part I-A.

71. Hume, E.M. and Krebs, H.A. 1949 — Vitamin A Requirement of Human Adults. An Experimental Study of Vitamin A Deprivation in Man, Medical Research Council, Special Report Series No.264, H.M. Stationery Office, London, pp.145.

72. Henley, T.H.M., Dann and Golden, W.R.C. 1944 — Reserves, Absorption and plasma levels of Vitamin A in premature infants. Amer.J. Dis. Child-68: 257.

73. Hussain, R., and Patwardhan, V.N. 1959 — The influence of phytate on the absorption of Iron. Indian Journal of Medical Research 47., 876.

74. India 1973 — Measures of Fertility and Mortality in India. Sample Registration Bulletin Vol.VI No.3 and 4. Vital statistics Divisions, office of the registrar general, Government of India.

75. Indira Gandhi 1974 — Editorial, Home Conference YOJANA, Vol.XVIII No.20, p.3

76. James, J., and Reedon, J.R. 1967 — Present knowledge of iron and copper. Nutrition Reviews 25., 321.

77. Jelliffee, D.B. 1967 — Approachesto Village Level Infant Feedings I. Multi-mixes as Weaning Foods. J. Trop. Pediatr. 13, p.46.

78. Jelliffe, D.B. 1968 — Infant Nutrition in the Subtropics and Tropics WHO-Monograph Series No.29, World Health Organisation, Geneva, Switzerland, p.56.

79. Kakade, M.L. and Borchers, R. 1967 — Gastrointestinal Gas Production in Rats Fed Raw and Heated Navy Beans with and without Added Antibiotics, Proc.Soc. Exp. Biol.Med. 124, p.1272.

80. Kamalanathan, G., Gita Balinakshi and Devadas, R.P. 1970 — Effect of a Blend of Protein Foods on the Nutritional Status of Pre-school Children in a Rural Balwadi, Ind.J.Nutr. Dietet., 7, p.288

81. Kamalanathan, G., Saraswathi, G., Devadas Rajammal P. 1972 — The Indian Journal of Nutrition and Dietetics, Vol.9, No.4 p.202-205.

82. Kawaguchi, T. and Fujita, A 1956 — Studies on the Absorption of Carotene in Man. J.Vitaminol., 2, p.115.

83. Kies, C. and Fox, H.M. 1972 — Interrelationships of leucine with lysine, Tryptophan, and niacin as they influence protein value of Cereal grains for humans. Cereal Chem. 49, 223.

84. Kissinger, H.A. 1975 — To Free Mankind from the Scourage of Hunger' Span, 16, 2, p.5

85. Konno, T., Hansen, J.D.L., Truswell, A.S., Wood-Walker, R. and Becker, D. 1968 — Vitamin-A Deficiency and Protein-Calorie Malnutrition in Cape Town, S.Afr.Med.J.42, p.950.

86. Kreula, M., and Virtanen, A.I. 1939 — Absorption of Carotene From Carrots in Humans, Upsala Lakareforen. Forh., 45, p.357.

87. Kreula, M.S. 1947 — Absorption of Carotene from carrots in man and the Use of the Quantitative Chromic oxide Indicator Method in the Absorption Experiments, Bio.Chem. J.41, p.269.

88. Krishnamurthy, K., Ramakrishnan, T.N. Rajagopalan, R., Swaminathan, M. and Subramanian, V. 1960 — Studies on the Nutritive Value of Composite Protein Foods Based on Blends of Groundnut, Soybean and sesame flour, Food Sci.9, p.87.

89. Kuhn, I.N., Monsen, E.R., Cook, J.D., and Finch, C.A. 1968 — Iron Absorption in Man, Am. J. Lab. Clin. Med. 71, p.715-721.

90. Kurtha, A.N. and Ellis, F.R., 1970 — The Nutritional, Clinical and Economic Aspects of Vegan Diets. Pl.Fds. Human Nutrition, 2, p.13.

91. Kymal, P.K. 1973 — Balahar, Present Experience and Future Projection, Proceedings of the Nutrition Society of India, No.15, pp.32-35.

92. Lala, V.R. and Reddy, V. 1970 — Absorption of β Carotene from Green Leafy Vegetables in Undernourished Children, Amer. J.Clin. Nutr., 23, p.110.

93. Layrisse, M. and Martinez-Torres, C. 1972 — Am.J.Clin.Nutr.25: p.401-411. Model for Measuring the Dietary Absorption from Heme Iron Test with a Complete Meal.

94. Leiner, I.E. 1962 — Toxic Factors in Edible Legumes and their Elimination. American Journal of Clinical Nutrition, 11, 281.

95. Leonhardi, G. 1947 — Uber die Resorption von Karotin aus Gemüsen beim Menschen. Z. Mitteilung d. Gesamte Inn. Med., 2, 376, Cited in Chem. Abstr. 42: (Abstr.No.3473),1948.

96. Lian, O.K., Lian Tjay Tie, Rose, C.S., Prawiranegara, D.D. and Gyorgy, P. 1967 — Red Palm Oil in the Prevention of Vitamin A Deficiency. A Trial on Pre-school Children in Indonesia, Amer.J.Clin. Nutr., 20, p.1267.

97. Madhavi, V., Prahalad Rao., N Mathur, Y.C., and Reddy, Y.R. 1973 — Effect of Substituting Processed Soya Flour for Dry Skim milk in the Protein Packet in the Treatment of Protein-Calorie Malnutrition in Pre-school Children - A Preliminary Observation. Nutrition Society of India, Sixth Annual Meetings, Scientific Programme and Abstract of Papers. National Institute of Nutrition.

98. Mager, J., Razin, A. and Hershko, A 1969 — Toxic Constituents of Plant Food Stuffs (I.E. Liener, Editor). Academic Press, New York, p.294.

99. Mahadevan, S., Malathi, P. and Ganguly, J. 1965. — Influence of Protein on Absorption and Metabolism of Vitamin A, World Rev. Nutr. Dietet., 5, p.209.

100. Matoth, Y., Elian, M. and Gruenberg, A 1968 — Evaluation of a protein rich mixture based on Vegetable foods of middle eastern countries. Journal of Clinical Nutrition, 21, 3, 226, 229.

101. Max Milner 1974 — 'Need for improved Plant Proteins in World Nutrition'. Journal of Agricultural and Food Chemistry. An American Chemical Society Publication Vol.22, No.4, p.548-549.

102. Mc Carrison, R. 1956 — Food. Macmillan and Co., Limited, London.

103. Mclaren, D.S. 1966 — Present Knowledge of the Role of Vitamin A in Health and Disease. Trans. Roy.Soci.Trop. Med. Hyg. 60, p.436.

104. Mertz, E.T., Bates, L.S. and Nelson, A.E. 1964 — Mutant gene that changes protein Composition and increases lysine content of maize endosperm. Science, 145, 279.

105. Moore, T. 1960 — Vitamin A and Proteins. Vitamins and Hormones, 18, p.431.

106. Moore, T. 1957 — Vitamin A, Elsevier Publishing Co., Amsterdam, p.645.

107. Moscanatte, D.S. 1955 — Metabolic Studies with Pre-adolescent Girls. I.Utilization of Carotene, J.Amer. Diet. Ass. 31, p.37.

108. Napier,G.W. 1975 — The scope and possibilities for the use of the Kerala Indigenous food (KIF) Proceedings of the Nutrition Society of India, 15,p.36-39.

109. Narayana Rao, M., Swarpalakas, K.S., Sundaravalli,A.S. and Doraiswamy, T.R. 1973. — Flatus Production in Children Fed legumes Diets, PAG Bulletin 3, 53.

110. NAS, NRC, 1961 — Meeting Protein Needs of Infants and Children, Publ.843, National Academy of Science, National Research Council, Washington, D.C. p.45-55.

111. NAS, NRC, 1966 — Preschool Children - Malnutrition Primary Deterrent to Human Progress, Publ. 1282, National Academy of Sciences, National Research Council, Washington, D.C. p.34, 59.

112. National Commission on Agriculture 1973 — Maize. Report of the study team of National Commission on Agriculture, New Delhi, p.66-72.

113. Nelson de Souza and Batra de Oliveriro J.E. 1972 — Nitrogen Balance Evaluation of Hybrid and opaque-2 Corn Varieties on rats Nutrition Reports international 6, pp.69-74.

114.	NIN 1972	Annual Report of the National Institute of Nutrition, Hyderabad, India.
115.	NIN 1974	Annual Report, National Institute of Nutrition Hyderabad pp.79-81.
116.	Nirmala, P.S., Swarnam, S. and Devadas Rajammal, P. 1966	Diet and Nutritional Status of Expectant Mothers from Low Income Families, J. Nutr. Dietet., 3, 129.
117.	Nutrition Reviews, 1965	Evaluation of Peanut Mixture, 23, p.75-78.
118.	Oomen, H.A.P.C., 1964	Vegetable Greens a Tropical Underdevelopment Chronica Horticultural, 4, 3.
119.	Oomen, H.A.P.C., 1967	Inadequate Consumption of Leafy Vegetables. Proceedings of the 7th International Congress of Nutrition, Hamburg, 1966.
120.	PAG, 1973 PAG, 1973	PAG Bulletin, 3, 14 PAG Bulletin, 3, 25.
121.	PAG, 1973	PAG Statement (No.22) on upgrading human nutrition through the improvement of Food legumes, PAG Bulletin, Vol.IIII, No.2, p.1
122.	Panemangalore, M., Balaji, A.M., Narayana Rao, M., Rajagopalan, R., Chandrasekhar, B., Swaminathan, M., Sreenivasan, A. and Subramanyan, V. 1964.	Studies Based on a Processed Food Based on a Blend of Groundnut Flour and Full Fat Soy Flour Fortified with Essential Amino-acids, Vitamins and Minerals-II, Amino - acid Composition of Nutritive Value of Proteins J. Nutr.Dietet. 1, p.4.

123. Patel, B.P. 1970 — The Genesis and Problems of our Population Problems, Government of India, p.5.

124. Patwardhan, V.N. 1962 — Pulses and Beans in Human Nutrition, American Journal of Clinical Nutrition, 11, 12.

125. Pereira, S.M. and Begum, A. 1968 — Studies in the Prevention of Vitamin A Deficiency, Indian Journal of Medical Research, 56, 563.

126. Pereira, S.M., Jesudian, G., Shanta Murthy, R. and Benjamin, V. 1968 — Trials with an infant food supplement based on groundnut flour in the diets of young children. Journal of food Science and Technology, 5, 133.

127. Pereira, S.M., Sheila Jones, Mathews and Jesudian, G. 1971 — Infant feeding trials with a weaning supplement. Journal of food science and Technology, 8, 113-116.

128. Protein Foods and Nutrition Development Association of India, (PFND) 1973 — 'Better Foods for Better Nutrition' Report of the Workshop held in at Hydrabad. pp.7-28.

129. Phansalkar, S.V., Ramachandran, M. and Patwardhan, V.N. 1958 — Nutritive Value of Vegetable Proteins II. The Effect of Vegetable Protein Diets on the Regeneration of Haemoglobin and Plasma Proteins in Protein Depleted Rats. Ind.J. Med. Res., 46, p.333.

130. Pickaroti 1973 — Experiences with local marketing systems for Promoting Low Cost Weaning foods. Proceedings of the Nutrition Society of India. 15, p.106-110.

131.	Pingale, S.K. 1973	Cost availability of raw materials for feeding programme- a forecast in report of the Hyderabad Workshop, N.I.N. p.81.
132.	Prasanna, H.A., Rama Rao, G. and Chandrasekharan, N.R. 1965	Annual Report, Abstract, 13, CFTRI, Mysore.
133.	Prasanna, H.A., Rama Rao. G., Desai,B.L.M. and Chandrasekar,N.R. 1969	Use of a Spray Dried Food Based on Coconut in the Treatment of Protein Malnutrition, J. Food Sci.Technol., 6, p.187-188.
134.	Ramasastri, B.V. and Srinivasa Rao, P. 1969	Some Studies on the Nutritive Value of Rice Varieties and Pulses, Proc. Nutr. Soc., India, 7, 13.
135.	Rao, N.G. and Rao, B.S.N. 1970	Absorption of Dietary Carotenes in Human Subjects, Amer.J. Clin.Nutr., 23, p.105.
136.	Raju, B.V. 1972	Unpublished communications.
137.	Register, U.D., Mitsuko, I., Thurston, C.E., Vyhmeister,I.B. Dysinger,P. Blankenship, J.W. and Horning, M.C. 1967	Nitrogen Balance Studies in Human Subjects on Various Diets, Amer.J.Clin.Nutr. 20, pp.753-755.
138.	Registrar General, Government of India. 1973	'Measures of Fertility and mortality in India' Registration Bulletin Vol.XI.No.3,and 4 Vital statistics division, Office of the Registrar General, Government of India.

139. Ritchie -Calder, 1974
'Misery go-round', World Health, World Health Organization, Sep., p.3.
Avenue Appia. 1211, Geneva 27, Switzerland.

140. Roels, O.A., Trout, M. and Dujacquier, R. 1958
Carotene Balance on boys in Ruanda where Vitamin A Deficiency is Prevalent. J. Nut. 65, p.115.

141. Roels, O.A., Djaeni, S., Trout, M.E., Lauw, T.G., Health, A., Poey, S.H., Tarwotjo, M.S. and Suhadi, B. 1963
The Effect of Protein and Fat Supplements on Vitamin A - Deficient Indonesian Children, Amer.J.Clin.Nutr.12, p.380.

142. Roy, D.N., Nagarajan, V. and Gopalan, C. 1963
Production of Neurolathyrism in Chicks by the Injection of Lathyrus Sativus Concentrate Current Science, 32, 116.

143. Rubin, S.H. and De Ritter, E. 1954
Vitamin A Requirements of Animal Species, Vitamins Hormones, 12, p.101.

144. Saroja, S. 1966
Availability of iron in some green leafy vegetables. Indian Journal of Home Science 1. p.8-15.

145. Senecal, J. 1961
Studies on the Use of Peanut Flour in Infant Feeding, In progress in Meeting Protein Needs of Infants and Preschool Children, National Academy of Science/National Research Council Publication 843, Washington, D.C., U.S.A. p.119.

146. Roberts, L.P. 1973
Nutrition Realities in the lower Income Countries. Nutrition Reviews 31, 201, 206.

147. Scrimshaw, N.S. and Bressani, R. 1960 — Vegetable Protein Mixtures for Human Consumption, Fifth International Congress of Nutrition, Sept., 1-7, 1960, Washington, D.C. Panel III, Proteins and Amino Acids in Nutrition, p.20.

148. Shanley, B.M.G. and Lewis, O.A.M. 1969 — The Protein Nutritional Value of Wild Plants Used as Dietary Supplement in Natal South Africa, Pl. Fds. Hum. Nutrition, 1, 253.

149. Simlos, A. 1949 — Vitamin A and B Carotene Beim Gesunden Saugling. Ein Beitrag Zur Frage Über Bilanz. Int. Z. Vitamin Forch, 21, p.151.

150. Sood, S.K. 1967 — Iron deficiency anaemia. Proc. Nutr. Soc. 2, 34.

151. Sorbie, J., Olatunbosun, D., Corbelt, W.E.W., and Valberg, L.S. 1971 — Can. Med. Associ. J. 104:777-782.

152. Srikantia, S.G. 1973 — Use of Legumes and Green Leafy Vegetables in the Feeding of Children, A Review of Experience in PAG Bulletin 3, 31.

153. Srikantia, S.G. and Gopalan, C. 1960 — Clinical Trial with Vegetable Protein Food in Kwashiorkor, Indian Journal of Medical Research, 58, 5, pp.637-643.

154. Steggerda, F.R. and Dimmick, J.F. 1966 — Effect of Bean Diets on Concentration of Carbondioxide in Flatus, American Journal of Clinical Nutrition, 19, 20.

155. Subba Rao, B. and Hanumantha Rao, R. 1972 — Effect of Lipid Protein Interaction on Nutritive Value of Groundnut Protein, Ind. J. Nutr. Dietet., 9, p.33.

156. Swaminathan
1968

Production of Nutritious Food supplement for feeding Infant and Pre-school Children Indian Council of Child Welfare - Children's Annual, 82-85.

157. Swaminathan, M.C., Hanumantha Rao, D. Visweswara Rao, K., Narasimhan, M.V.V.L. and Naranasimmi Damo.
1970

An Evaluation of the supplementary feeding programme for Pre-school Children in the Rural areas around Hyderabad city. Indian Journal of Nutrition and Dietitics, 7, 342-349.

158. Swaminathan, M.S.
1971

Research trends in crop Improvements World Science News, 9, p.27.

159. Swaminathan, M.S.
1973

Our Agricultural Future. Sardar Patel Memorial Lectures. All India Radio.

160. Tasker, P.K. Krishnamurthy, K., Rajagopalan, R., Swaminathan, M. and Subramaniam, V.
1960

Supplementary Value of Composite Protein Foods Containing a Blend of Coconut Meal, Groundnut Flour and Bengal gram to a Poor Rice Diet. Food Sci. 9, p.85.

161. Terra, G.J.A.
1964

The Significance of Leafy Vegetables, Especially of Cassar, in Tropical Nutrition, Trop. Geog. Med.2, 97.

162. Thompson, S.Y.
1964

Factors Affecting the Absorption of Carotene and its Conversion into Vitamin A. Exp. Eye Res.3, p.392.

163. UNICEF
1973

Statistical profile of children and youth in India, United Nations Children's Fund(UNICEF) New Delhi, p.40

164.	Van Eekelen, M. and Pannevis, W. 1938	Absorption of Carotinoids from the Human Intestine. Nature, 141, p.203.
165.	Van Zeben, W. 1946	The Absorption of Carotene by Man. Vitamin forsch. 17, p.74.
166.	Van Zeben, W. and Hendriks, T.F. 1948	The Absorption of Carotene from Cooked Carrots Int. Z. Vitamin Forsch. 19, p.265.
167.	Venkat Rao, S., Daniel, V.A., Leela, R., Hariharan, K., Swaminathan, M. and Parpia, H.A.B. 1964	Effect of supplementation of blends of groundnut, soy bean, sesame and coconut with the limitary amino acids in the nutritive value of the proteins. Journal of Nutrition and Dietitics, 1,3, 184-187.
168.	Vinodine Reddy 1968	Nutritional status of Indian Children, Swasth Hind. XII 335-343.
169.	Wagner, K.H. 1940	Die Experimentalle Avitaminose A beim Menschen. Hoppe Seyler's Z. Physiol. Chem. 264. p.153.
170.	Wald, G., Carroll, W.M. and Sciarra, D. 1941.	The Human Excretion of Carotenoids and Vitamin A, Science, 94. p.95.
171.	Wildbrand, U. 1950	Untersuchungen über die Karotin - resorption under besonderer, Berücksichtigung der Resorption be Unterernahrten, Med. Monatsschr. 4, p.924.
172.	Wilson, H.E.C., Gupta, M.D. and Ahmad, B. 1937	Studies on the Absorption of Carotene and Vitamin A in the Human Subject. Indian. J. Md. Res. 24, p.807.

173. With, Z.K. 1940 — Absorption, Metabolism and Storage of Vitamin A and Carotene, Oxford University Press, London, p.263.

174. Yadav, N.R. and Bharadwaj, U.R. 1971 — Biochemical Studies on Indian Wild Legumes, I. Chemical and Nutritional Constituents of Some Indian Wild Legumes, Ind.J.Nutr.Dietet. 8, p.1

175. Young, V.R., Ozalp, I., Cholakos, B.V. and Scrimshaw, N.W. 1971 — 'Feeding Trials with opaque - 2 maize' Journal of Nutrition, 101, p.1475.

[illegible]

ANNEXURE I

TWENTY NINE FORMULATED FOOD MIXTURES

S.No.	Mixtures	Proportion
1.	Maize Bengalgram groundnut Mix	6:2:2
2.	Maize Bengalgram groundnut Mix	7:2:1
3.	Maize Redgram groundnut Mix	6:2:2
4.	Maize Redgram groundnut Mix	7:2:1
5.	Maize Greengram groundnut Mix	6:2:2
6.	Maize Greengram groundnut Mix	7:2:1
7.	Maize Horsegram groundnut Mix	6:2:2
8.	Maize Horsegram groundnut Mix	7:2:1
9.	Maize Cow pea groundnut Mix	6:2:2
10.	Maize Cow pea groundnut Mix	7:2:1
11.	Maize Field bean groundnut Mix	6:2:2
12.	Maize Field bean groundnut Mix	7:2:1
13.	Maize Cow pea Horsegram groundnut Mix	6:1:1:2
14.	Maize Horsegram greengram Mix	6:2:2
15.	Maize Cow pea greengram Mix	6:2:2
16.	Maize Bengalgram Sunflower seed Mix	6:2:2
17.	Maize Bengalgram Sunflower seed Mix	7:2:1
18.	Maize Redgram Sunflower seed Mix	6:2:2
19.	Maize Redgram Sunflower seed Mix	7:2:1

S.No.	Mixtures	Proportion
20.	Maize Greengram Sunflower seed Mix	6:2:2
21.	Maize Greengram Sunflower seed Mix	7:2:1
22.	Maize Horsegram Sunflower seed Mix	6:2:2
23.	Maize Horsegram Sunflower seed Mix	7:2:1
24.	Maize, Cowpea Sunflower seed Mix	6:2:2
25.	Maize, Cowpea Sunflower seed Mix	7:2:1
26.	Maize, Fieldbean Sunflower seed Mix	6:2:2
27.	Maize, Fieldbean Sunflower seed Mix	7:2:1
28.	Maize, Cowpea Greengram Sunflower seed Mix	6:1:1:2
29.	Maize, Horsegram, Cowpea Sunflower seed Mix	6:1:1:2

ANNEXURE II

AMINO ACID PROFILE OF THE SELECTED FORMULATIONS (g%)

Formulations	Amino acids (g)											
	Arg	His	Lys	Try	Phe	Tyr	Met	Cys	Thr	Leu	Ileu	Val
Maize, Cowpea, Greengram Sunflower seed	0.9808	0.4085	0.7020	0.1498	0.7452	0.4596	0.2697	0.2178	0.6104	1.4057	0.6661	0.7659
Maize, Horsegram Greengram	1.0668	0.4353	0.9330	0.1381	0.8460	0.3331	0.2389	0.2444	0.6145	1.5409	0.7856	0.8408
Maize, Cowpea Greengram	1.0179	0.4559	0.8989	0.1428	0.8255	0.5107	0.2591	0.2147	0.6302	1.5313	0.7335	0.8055
Maize, Horsegram Cowpea, Sunflower seed	0.9754	0.4101	0.7084	0.1514	0.7445	0.4212	0.2636	0.2406	0.6146	1.3980	0.6619	0.7803
Maize, Greengram sunflower seed	1.0107	0.3966	0.7127	0.1459	0.7560	0.4092	0.2657	0.2110	0.5984	1.4143	0.6963	0.7691
Maize, Cowpea, Horsegram, Groundnut (roasted)	1.2173	0.4284	0.7489	0.1429	0.8181	0.5395	0.2361	0.2483	0.6065	1.4884	0.6851	0.8042

Arg = Arginine
His = Histidine
Lys = Lysine
Try = Tryptophan

Phe = Phenylalanine
Tyr = Tyrosine
Met = Methionine
Cys = Cystine

Leu = Leucine
Thr = Threonine
Ileu = Isoleucine
Val = Valine

ANNEXURE III

COMPOSITION OF MINERAL AND VITAMIN MIXTURE USED FOR RAT ASSAYS

Composition of Mineral Mixture

Calcium Carbonate	..	38.1400
Cobalt Chloride	..	00.0023
Cupric Sulphate	..	00.0477
Ferrous Sulphate	..	2.7000
Magnesium Sulphate	..	5.7300
Manganese Sulphate	..	0.4010
Potassium Iodide	..	0.0790
Potassium Phosphate	..	38.9000
Sodium Chloride	..	13.9300
Zinc Sulphate	..	0.0548
		99.9848

Composition of Vitamin Mixture

Vitamin A	..	1000.0 I.U
Vitamin D	..	100.0 I.U.
Vitamin E	..	10.0 I.U.
Vitamin K	..	0.5 mg
Thiamine	..	0.5 mg
Riboflavin	..	1.0 mg
Pyridoxine	..	0.4 mg

Niacin	..	4.0 mg
Pantothenic acid	..	4.0 mg
Choline (50% solution in oil)	..	200.0 mg
Inositol	..	25.0 mg
PABA (Para Amino Benzoic Acid)	..	10.00 mg
Vitamin B_{12}	..	2.0 mg
Biotin	..	0.02 mg
Folic acid	..	0.20 mg
Corn Starch	..	734.38 G

ANNEXURE IV

DETERMINATION OF HEPATIC VITAMIN A

Principle:

Antimony trichloride in chloroform gives a blue colour with the Vitamin A.

Reagents needed:

1. 60% Potassium hydroxide
2. Antimony trichloride solution. 25g of antimony trichloride was dissolved in 100 ml. of chloroform.
3. Vitamin A standard

   Vitamin A standard solution contained 25 I.U. per ml.
4. Petroleum ether (60-80°)
5. Acetic anhydride
6. 95% alcohol
7. Chloroform (moisture free)

Procedure:

The whole liver was saponified in alcoholic alkali (5ml of alcohol/g. liver and 0.5 ml. of 95% alcohol) for 20 minutes or until the tissues are completely degenerated. The solution was extracted thrice with petroleum ether (60-80°C) and dried through anhydrous Sodium sulphate and evaporated over a water bath until dry. This was dissolved in 15ml of chloroform.

<u>Calculation</u>:

Blank- 1ml of chloroform plus 5 ml of antimony trichloride solution plus 2 drops of acetic anhydride.

Unknown - 1ml of chloroform extract in chloroform plus 5 ml of antimony trichloride solution plus 2 drops of acetic anhydride.

The optical density readings were converted to mcg. of Vitamin A acetate from a standard curve prepared by making quantitative dilutions of crystalline Vitamin A acetate. These readings were further converted to equivalent weight of retinol Vitamin A alcohol.

ANNEXURE V

DETERMINATION OF PLASMA AND SERUM PROTEIN (KLEINER AND DOTTI 1962)

Reagents:

1. Sodium Sulfate 30%
2. Trichloro acetic acid 20%
3. Sodium hydroxide 10%
4. Folin-ciocalteu phenol reagent.
5. Tyrosine standard solution 0.2 mg/ml (20mg/100 ml in N/10 HCl)
6. Sodium carbonate saturated solution.

When serum is used, the fibrinogen determination is omitted. In this case one ml. of serum is diluted with 1.5 ml distilled water and 7.5 ml of 30% sodium sulfate solution to precipitate globulin.

Globulins:

The globulins are removed from the supernatant fluid. Transfer 2 ml of this supernatant liquid to a clean dry test tube. Add 3 ml. of 30% Sodium sulfate solution, then mix by inversion, and place in the incubator at 37°C for at least 10 minutes. Filter through pleated filter paper into a clean dry test tube, returning the filtrate to the paper for re-filtration until it becomes clear. Reserve the filtrate which contains the albumin. Discard the precipitate (globulin)

Albumins:

The albumins are precipitated from the globulin free filtrate with trichloro acetic acid.

Transfer 2 ml of globulin free filtrate to a clean dry 15 ml centrifuge tube. Add 3 ml of water and one ml of 20% trichloroacetic acid. Mix by inversion. Allow a few minutes for the albumin precipitate to floculate and centrifuge. Pour off and discard the supernatant liquid and wipe the tip of the tube with filter paper. Retain the albumin precipitate. Set tube containing albumin aside until later.

Total Protein:

Total protein is determined on untreated plasma. Into a clean, dry test tube introduce 0.5 ml of 10% sodium hydroxide solution. From a pipette graduated to contain 0.1 ml discharge into the tube exactly 0.1 ml of plasma. Rinse the pipette by drawing up and draining out the alkaline solution 2 or 3 times after adding the plasma. Finally blow out any liquid remaining in the pipette.

To the tubes containing the albumins precipitate add 0.5 ml of 10% sodium hydroxide. Heat them and the total protein tube in a boiling water bath for 10 minutes. Finally remove the tubes from the water bath and add 7.5 ml of distilled water to each.

Standard:

Introduce into another tube 2 ml of the standard tyrosine solution (0.2 mg/ml) and add 6 ml of distilled water.

To each of the tubes add 1 ml of folin-ciocalteu phenol reagent and 3 ml of a saturated solution of sodium carbonate. Mix by inversion. Allow 10 minutes for colour to develop; then compare in a colorimeter with the standard set on 10 mk.

ESTIMATION OF PROTEIN BY KJELDAHL'S METHOD

Reagents used:

1. Concentrated sulphuric acid
2. Concentrated sodium hydroxide solution
3. 0.1N Hydrochloric acid
4. 2% boric acid
5. Mixed Indicator
6. Zinc dust
7. Potassium sulphate
8. Copper sulphate
9. Porcelain bits

Procedure

Weighed 3 g of the sample and transferred to a kjeldahl flask. Added 2 g of copper sulphate crystals and 10g of potassium sulphate. Added slowly 30 ml of concentrated H_2SO_4.

Inserted a glass funnel with a small watch glass into the mouth of the flask to act as a condenser. Digested the contents of the flask by boiling on the electric furnace.

Continued boiling with low heat until the mixture was clear. Cooled the flask and diluted the mixture with 200 ml of cold water and shook thoroughly.

Pipetted out 20 ml of 2% boric acid solution into a beaker. Added 2 to 3 drops of mixed indicator. Placed the flask under the condenser.

Added 70 to 100 ml of concentrated sodium hydroxide to the digestion flask slowly down the sides of the flask. Added 1/4 teaspoon of zinc powder and connected the flask to the condenser. Distilled for about 30 minutes and checked the completion of distillation by testing a drop of the distillate with litmus paper. The beaker was removed from the condenser and the basic solution was titrated against 0.1 N HCl.

Calculation:

a) Amount of HCl used for tiltration = X ml.

b) X x Normality of HCl = N. equivalent of NH_3 = mg of N_2.

Mg of N x 6.25 = mg of protein in the sample. X × 14 = mg of N_2

ANNEXURE VI

QUESTIONNAIRE TO ELICIT INFORMATION ON SOCIO ECONOMIC BACKGROUND OF THE FAMILIES

I 1. Serial Number:

2. Sample Village:

II House Hold Particulars:

a. Name of the head of the family interviewed:

b. Person interviewed
(relation to the head of the family)

c. Religion: 1. Hindu
2. Christian
3. Muslim
4. Others - Specify

d. Caste

e. Type of the family 1. Nuclear
2. Joint

III. Please give the details of your household members and their demographic particulars:

S. No.	Name	Relation to the head of the family	Age in complete years	Marital Status	Age at first child birth	Age at marriage	Education	Occupation	Income	Number of children alive	Number of children dead	

ANNEXURE VII

SRI AVINASHILINGAM HOME SCIENCE COLLEGE FOR WOMEN
COIMBATORE 641 011

FOOD HABITS AND DIETARY SURVEY SCHEDULE

Part: I Household Particulars

Quarter			
1	2	3	4

Pr	In./Inf.	0.6	6-15	N

Rural/Urban

I. Family Characteristics and Consumption:

1. Identification of sample household:

   1. Serial Number ....
   2. Sample Village/Block ....
   3. Village Category/Stratum ......
   4. Sub sample ....
   5. Taluk/Town ....
   6. District ....
   7. Street ....
   8. House Number ....

2. Household Particulars:

   a) Name of the head of the family interviewed . . . .

   b) Person interviewed (relation to the head of the family) . . . .

   c) Household religion: 1 ▱ Hindu 2 ▱ Christian 3 ▱ Muslim 4 ▱ Others (Specify)

   d) Occupation of the head of the family . . . . . .(f) Type of family: 1. ▱ Nuclear 2. ▱ Joint

   e) Caste and subcaste of the household: Caste .... Subcaste ....

   g) Is the family living in rented or owned house? 1. ▱ Rented; 2 ▱ Owned; if owned, value of house ....

   h) Land owned (inacres) ....; Value in Rs...........

3. (a) Please give the details of your household members and their demographic particulars;

Sl. No.	Relation to head of family	Sex Male - 1 Female - 2	Age in completed years	Marital status	Age at 1st child birth	General Education (Standard completed)	Occupation (last month)	No. of children alive	No. of children dead	Age at marriage	P/L

3. (b) Has any member of the family left or arrived last month?
1 [ ] Yes; 2 [ ] No.
If yes, please indicate the sl. No. of the family member and period of stay in each case.

Arrived		Departed	
Sl. No.	No. of days stayed	Sl. No.	No. of days stayed away

4. Please give details of purchases/reciepts of food and non-food articles in your household.

Description of Item	Local Unit	Standard Unit	Conversion factor Local/Std. Units	Time when last purchased/obtained	Quantity purchased/obtained	Where and how purchased/obtained	Price per local unit in retail market	Total Cost	How long the quantity purchased/obtained will last lasted	Normal frequency of purchases/receipts, etc.	Estimated consumption for 30 days
1	2	3	4	5	6	7	8	9	10	11	12
CEREALS	x	x	x	x	x	x	x	x	x	x	x
Raw Rice		kg									
Boiled Rice		kg									
Wheat		kg									
Rava		kg									
Maida		kg									
Jowar		kg									
Bajra		kg									
Maize		kg									
Ragi		kg									
Tapioca		kg									
Sago		kg									
Others (specify)		kg									
PULSES	x	x	x	x	x	x	x	x	x	x	x
Red gram		kg									
Black gram		kg									
Bengal gram		kg									
Green gram		kg									
Horse gram		kg									
Others (specify)		kg									

1	2	3	4	5	6	7	8	9	10	11	12
MILK & MILK PRODUCTS	x	x	x	x	x	x	x	x	x	x	x
Milk		lit									
Ghee		kg									
Curd		lit									
Butter		kg									
Buttermilk		lit									
Baby food		kg									
EDIBLE OIL	X	x	x	x	x	x	x	x	x	x	x
Gingelly oil		lit									
Groundnut oil		lit									
Vanaspathy		kg									
Coconut oil		lit									
Others (specify)											
MEAT & MEAT PRODUCTS											
Mutton		kg									
Chicken		kg									
Pork		kg									
Beef		kg									
Egg		No									
Fish (fresh)		kg									
Fish (fried)		kg									
VEGETABLE	x	x	x	x	x	x	x	x	x	x	x

1	2	3	4	5	6	7	8	9	10	11	12
FRESH FRUITS	x	x	x	x	x	x	x	x	x	x	x
DRY FRUITS	x	x	x	x	x	x	x	x	x	x	x
SUGAR	x	x	x	x	x	x	x	x	x	x	x
Sugar (crystal)		kg									
Jaggery		kg									
Khandasari		kg									
Others (specify)		kg									
<u>Salt & Spices</u>		x	x	x	x	x	x	x	x	x	x
Salt											
Turmeric											
Black pepper											
Dry chillies											
Green chillies											
Garlic											
Tamarind											
Ginger											
Coris											
Mustard											
Fenugreek											
Cuminseed											
Cloves											
Cardamom											
BEVERAGE & REFRESHMENTS	x	x	x	x	x	x	x	x	x	x	x

4.(a) Also please tell us your expenditure on non-food items during the last month

Non-food items	Expenditure last month (in Rs.) Cash	Credit	Non-food items	Expenditure last month (in Rs.) Cash	Credit
Pan Supari (133-135)			Durable goods (190-240)		
Tobacco (137-144)			House Rent		
Intoxicants(146-151)			Education (247-251)		
Fuel & light (153-164)			Medicine (252-257)		
Clothing (166-182)			Other Servi-ces(259-312)		
Foot wear (185-188)			Non-food (sub-total)		

5.(a) Please tell us between which figures your household monthly Income falls:

0-200 /__7 % in kind 201-400 /__7 % in kind 401-800 /__7 % in kind

0-50 /__7 % 201-300 /__7 401-600 /__7

51-100 /__7 301-400 /__7 601-800 /__7

801 and above /__7 % in kind

801 and above /__7

Unwilling to disclose /__7

Unable to specify /__7

(b) Interviewer'sopinion of household income given above.

Gross underestimate /__7 apparently accurate /__7

Gross overestimate /__7

(e) If any member of the household borrowed money last month, please indicate the amount and purpose.

Amount . . . . Purpose . . . .

6.(a) Please indicate the source of water supply for the village and the house.

	In the village	In the house
No supply	☐	☐
Well water	☐	☐
Tap water	☐	☐
Tank or river water	☐	☐

(b) i) Is the village electrified? ☐ Yes; ☐ No

ii) Is your house electrified? ☐ Yes; ☐ No

7. Has some one been ill in your house during the previous 30 days?
☐ Yes; ☐ No

If yes, please give the following particulars?

Serial no of the household member	Nature of illness	Duration of illness	Attendance Type	Attendance Frequency	Cost (in Rs.

8. Has some death occurred in your house during the one year upto the date of interview?

▱ Yes ▱ No

If Yes, please give the following particulars:

S.No.	Age	Sex	Cause of death

9. (a) Institution/Type/Qualification of medical help:

Institution	Type Allo-1, Ayur-2, Homeo-3	Qualification
Hospital		
Dispensary		
Private Doctor		
Midwife		

9. (b) Please indicate the distance of the nearest medical aid from your house (in kms) .....

II. DIETARY HABITS: 1. Please tell us if the family is:

1. ▱ Vegetarian 2. ▱ Vegetarian but takes egg.

3. ▱ Non-vegetarian.

If answer is (2) or (3) ask the following details for each member of the family:

Source	Food Item	Serial No. of the family members taking different food items									
		1	2	3	4	5	6	7	8	9	10
Prepared at home	Egg										
	Fish										
	Meat										
Purchased from outside	Egg										
	Fish										
	Meat										

2.(a) If some members of the family are eating outside, please give the following details:

Serial number of the household member	No. of times food taken outside per week	Place of eating	Reason	Foods consumed	Average purchased price per week (in Rs.)

2.(b) Do the women and/or children of the household receive any food at Anganwadi, School, Maternal and childcare centres, Urban bread distribution centres or at any other place. Please give details:

Sl.No.of household member	No.of time food taken received per week	Distribution-centre	Reason	Foods Recei-ved	Cost per week (if any) in Rs.

3. Please tell us the food prepared and consumed in your house the previous day and by whom in the family were these consumed. If not consumed please say if saved for the next meal or destroyed.
Time Codes. 0 ▱ Pre-prepared 1 ▱ Morning 2 ▱ Noon 3 ▱ Evening 4 ▱ Nights

Time of preparation (code)	Food Item Prepared/ Purchased	Quantity Prepared/ Purchased		Principal Ingredients used						Time of consumption (code)	Quantity consumed by each member of the family (in units as in col.3)									Balance Unit (4-20)	
				Ingredient			Ingredient				Serial No. of the household member (as in Block 3)										
		Unit	Qty. in units as in (3)	Ingredient	Unit	Quantity	Ingredient	Unit	Quantity		1	2	3	4	5	6	7	8	Total	Saved for the next meal	Destroyed
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
		9																			

Purchased from outside

271.

4. Was the previous day? 1 /__/ Festival; 2 /__/ Celebrations;
3 /__/ Fast; 4 /__/ Morning;
5 /__/ Any other event; 5 /__/ Normal

5. If you are avoiding any foods in the household, please tell us the reasons for avoiding them?

Food Item	Reasons								
	Heavy	Cold	Gas producing	Religious	Traditional	No liking	Not good for health	Not economical	Others (specify)

6. i) Do you preserve fruits or vegetables at home or buy from outside
0 /__/ Do not preserve at home or buy from outside
1 /__/ Preserve at home
2 /__/ buy from outside
3 /__/ Both

ii) If preserved at home please state the form in which preserved.
1 /__/ Jams 2 /__/ Pickles
3 /__/ Dried forms
4 /__/ Others (specify)

iii) What are the foods you commonly preserve?

------- ------- -------
------- ------- -------

iv) Also name the preserved foods purchased from outside?

1 [ ] Jams & Jellies
2 [ ] Pickles
3 [ ] Dehydrated or dried vegetables
4 [ ] Tinned vegetarian;
5 [ ] Tinned non-vegetarian.
6 [ ] Tinned Milk
7 [ ] Others (specify)

7. Have you heard the words 'Vitamin', 'Minerals', 'Calorie', 'Proteins', 'Calcium', and 'Iron'? If so Please name the source of information and the foods in which they are present.

Nutrient	Source of Information	Foods in which present
Vitamins		
Minerals		
Calories		
Protein		
Calcium		
Iron		

ANNEXURE VIII

SRI AVINASHILINGAM HOME SCIENCE COLLEGE, COIMBATORE 11

FOOD AND NUTRIENT INTAKE 3 - DAY WEIGHMENT ON SELECTED TARGET CHILDREN

Name of the Investigator . . . .
Dates of Survey: . . . .
Name of the Head of the Family . . . .
Name of the Subject . . . .
Age: . . . .

Door No. . . . .
House No. . . . .
Name of the Village . . . . .
Name of the Block . . . . . .

Name of the Meal	Menu	Weight of raw ingredients (g)	Weight of Cooked food (g) (in terms of Vessels)	Wt. of Cooked food (g)	Amount consumed by the individual (in terms of Vessels)	Amount consumed by the individual (g)	Whether the food is purchased or cooked at home	Raw equivalents consumed by the Individual (g): (Wt. of raw ingredients g x Individual consumption g) / Total Cooked food g

274

ANNEXURE IX

SRI AVINASHILINGAM HOME SCIENCE COLLEGE FOR WOMEN
COIMBATORE: 641011

NUTRITION ASSESSMENT SCHEDULE

S.No. — **Date of Assessment**: I Initial, II Final

Name of the village/Block

Name of the child:

Name of the head of Family:

Boy/ Girl ———————— **Age:** ———

Clinical Examination	I	II

ANTHROPOMETRY

1.1: Length (cm) : ——————————
1.2: Weight (kg) : ——————————
1.3: Head circumference (cm) : ——————————
1.4: Chest circumference (cm) : ——————————

CLINICAL EXAMINATION

Hair

2.1: Loss of lustre : ——————————
2.2: Thinning : ——————————
2.3: Sparse : ——————————
2.4: Discoloured : ——————————
2.5: Easily plucked : ——————————

Face

3.1: Moonface (Puffy cheeks) (Baggy cheeks) : ——————————
3.2: Nasolabial dyssebacea : ——————————

Clinical Examination		I	II
Eye			
4.1: Pigmentation of the conjunctival	:		
4.2: Conjuctival xerosis	:		
4.3: Bitot's spots	:		
4.4: Corneal xerosis and keratomalacia	:		
4.5: Corneal opacity	:		
4.6: Night blindness	:		
4.7: Photophobia	:		
Lips			
5.1: Angular stomatitis	:		
5.2: Cheilosis	:		
Tongue			
6.1: Red and raw	:		
6.2: Papillae - atrophic	:		
6.3: Papillae - Hypertrophic	:		
Teeth			
7.1: Caries	:		
7.2: Mottled enamlel	:		
Gums			
8.1: Spongy - bleeding	:		
Skin			
9.1: Follicular Hyperkeratosis (Phrynoderma)	:		
9.2: Mosaic dermatosis	:		
9.3: Pellagrous dermatosis	:		

Clinical Examination		I	II
9.4: Crazy-Pavement dermatosis	:		
9.5: Petechiae and ecchymoses	:		
9.6: Pigmentation at knuckles, fingers, toes	:		
9.7: Flexural lesions	:		
Nail			
10.1: Koilonychia finger nails	:		
Subcutaneous Tissue			
12.1: Craniotabes	:		
12.2: Beading of ribs	:		
12.3: Knock-knees Bow-legs	:		
12.4: Epiphyseal enlargement	:		
12.5: Frontal & Parietal bossing	:		
Gastro-Intestinal System			
13.1: Enlargement of spleen	:		
13.2: Enlargement of Liver	:		
Nervous System			
14.1: Numbness & tingling of extremeties	:		
14.2: Burning feet	:		
14.3: Tenderness of calf muscles	:		
14.4: Loss of knee/ankle/jerks	:		
Laboratory investigations	:		
Motion examination	:		
Haemoglobin by Cyanmeth-haemoglobin	:		
Recent Episode of (1) Respiratory illness	:		
(2) Gastro intestinal infections Pallor	:		
GENERAL REMARKS:			
Final assessment of general nutritional status	:		

ANNEXURE X

ESTIMATION OF URINARY CREATININE

Principle:-

Creatinine forms a coloured complex with picric acid in the presence of strong alkali. This picrate of creatinine has the absorption maxima at 540 mm in the alkaline medium.

Procedure:

One ml of urine is diluted with distilled water to 100 ml, mixed well and a 5 ml. aliquot is pipetted into a test-tube. To this is added 2.5 ml of 1% picric acid followed by 0.5 ml of 10% NaOH. The contents of the tube are then mixed well. Colour is allowed to develop for 20 minutes. The volume is then made up to 10 ml with water and the intensity of the yellow colour developed is read at 540 mm in klett Summerson Colorimeter. A blank is prepared using 5 ml of water instead of urine.

A standard curve is prepared by using dilute creatinine standard solution (range 0 to 50 mg). The amount of creatinine in the sample is calculated from this standard graph.

Reference:-

ICMR 1971 — A manual of laboratory techniques, National Institute of Nutrition, Indian Council of Medical Research, Tarnaka, Hyderabad-7

ANNEXURE XI

ESTIMATION OF URINARY NITROGEN

Microkjeldhal method:

Principle

The nitrogenous material is digested with concentrated sulphuric acid and the ammonium sulphate formed is converted to ammonia which is estimated by the usual acid base titration.

Procedure:

Into a microkjeldhal flask is pipetted a known aliquot of the sample containing not more than 1mg of nitrogen. To this is added 1 ml of concentrated sulphuric acid and a glass bead. The mixture is digested by heating till the brown colour almost disappears. The kjeldhal flask is removed, then slightly cooled and a few drops of hydrogen peroxide are added and heated until the contents of the flask become crystal clear. The flask and the contents are then allowed to cool.

The contents of the flask are transferred quantitatively to the distillation set with washings of about 3-4 ml water. About 10 ml of concentrated solution of sodium hydroxide is allowed in and the steam distillation started. The ammonia liberated is collected into 5 ml of the absorption mixture.

The distillation is continued till the volume of the distillate is about 15 ml. After complete distillation the absorption mixture is titrated with N/100 H_2SO_4 the end point of the reaction being faint green colour. The volume of N/100 H_2SO_4 needed for titration is noted down.

A blank is also run simultaneously with the sample with the same volume of concentrated H_2SO_4 added and digested similarly.

Calculation:

The nitrogen content of the original sample is calculated from the equation 1 ml of N/100 H_2SO_4 = 1.14 mg of nitrogen.

Reference:

ICMR 1971 A manual of laboratory techniques, National Institute of Nutrition, Indian Council of Medical Research, Taranaka, Hyderabad-7.

ANNEXURE XII

PROCEDURE FOR NITROGEN BALANCE STUDY:

Analysis of food, urine and faeces:

One tenth of all the foods consumed by the selected subjects during the eight experimental periods was weighed, homogenised in waring blender, made up to volume, and one tenth was taken for analysis of protein, calcium or iron estimation.

Urinary creatinine was determined within 6 hours of collection, following the method of Jaffe (Klenier and Botti, 1962) Urinary Nitrogen and Calcium was estimated in diluted samples using the micro Kjeldahl method, (Hawk et al 1954)

The 24 hours of urine and faeces were collected (6 A.M. to 6 A.M.) for three consecutive days per subject. Nitrogen estimations were done either on the same evening or the next day morning within 10 hours after the collection time; Triplicates of 5 ml of the urine samples and two grams of the faecal samples were used for nitrogen estimation.

ANNEXURE XIII

CONSOLIDATED DATA ON THE SOCIO ECONOMIC SURVEY OF THE INDIVIDUAL VILLAGES

Village	:	Vedapatti
Total families	:	384
Total members in the family	:	1916
Male	:	1018
Female	:	898
Type of family		
Nuclear	:	246
Joint	:	138
Education		
Balwadi	:	Nil
I class	:	161
II class	:	47
III class	:	165
IV class	:	174
V class	:	44
VI class	:	21
VII class	:	49
VIII class	:	25
IX class	:	53
X class	:	14
XI class	:	43

Occupation

Type of work	Number of families
Cooli	226
Farm labourer	29
Farmer	18
Thandal	1
Farm owner	16
Agriculturist	4
Mill worker	16
Clerk	8
Shop keeper	7
Mill labourer	11
Teacher	5
Electrician	3
Office worker	3
Dhobi	4
Peon	2
Milk supplier	2
Factory worker	2
Flower seller	2
Supervisor	2
Work shop worker	2
Cycle shop worker	2
Tree climber	2
Merchant	2
Watchman	2
Poojari	2
Headmaster, Superintendent, Masthiri, Bill Collector, Black-smith, Goldsmith, Barber, Driver Line man, Postman, Line inspector	11
Total:	384

Income

Income range in Rs.	No.of families
Below 50	30
51 - 100	246
101 - 150	51
151 - 200	44
201 - 250	8
251 - 300	1
301 - 350	1
351 - 400	1
401 - 450	Nil
451 - 500	Nil
500 and above	2
Total	384

Caste

Caste	Number of families
Adhi Dravidar	148
Gounder	60
Naidu	46
Pillai	26
Pandaram	38
Harijan	11
Asari	10
Parayar	4
Mudaliar	4
Vannan, Nadar, Kooper, Chettiar	16
Kuravar	3
Navidar, Muslim, Konar, Gowder, Valluvar, Devar, Palavar, Nayakkar, Devandhirar	18
Total	384

Mother tongue

	No.of families
Tamil	318
Kannadam	46
Telugu	16
Malayalam	4
Total	384

Age

Age Range years	Sex	Number
Below 1 year	M	16
	F	18
1 - 3 years	M	84
	F	38
4 ,,	M	36
	F	43
5 ,,	M	56
	F	37
6 ,,	M	84
	F	56
7 ,,	M	46
	F	49
8 ,,	M	64
	F	70
9 ,,	M	23
	F	26
10-12 years	M	46
	F	39
13-15 ,,	M	101
	F	78
16-18 ,,	M	90
	F	76
19 and above	M	364
	F	362
Presence of Father and Mother:		
Father	-	361
Mother	-	356

Village – Andipalayam

		Number
Total families	:	53
Total members in the family	:	263
Male	:	140
Female	:	123
Type of family		
Nuclear	:	37
Joint	:	16
Education:		
I Class	:	12
II Class	:	6
III Class	:	12
IV Class	:	8
V Class	:	5
VI Class	:	6
VII Class	:	3
VIII Class	:	–
IX Class	:	4
X Class	:	3
XI Class	:	–
P.U.C.	:	–

Occupation:

Type of work		No.of families
Cooli	:	37
Mill worker	:	6
Lorry Driver	:	2
Shop keeper	:	2
Milk seller	:	2
Manual work	:	1
Tailor	:	1
Manager	:	1
Betel leaves seller	:	1
	Total	53

Income

Income range (in Rs.)		No.of families
Below 50	:	6
51 - 100	:	14
101 - 150	:	18
151 - 200	:	10
201 - 250	:	4
251 - 300	:	-
301 - 350	:	-
351 - 400	:	1
401 - 450	:	-
451 - 500	:	-
500 and above	:	-
	Total	53

Caste:

Caste		No.of families
Devar	:	38
Gounder	:	9
Karavar	:	2
Nadar	:	2
Udayar	:	1
Nair	:	1
	Total	53

Mother tongue

		No.of families
Tamil	:	47
Kannadam	:	6
	Total	53

Age:

Age range	Sex	Number
Below 1 year	M	5
	F	6
1 - 3 yrs.	M	13
	F	6
4 years	M	2
	F	2
5 years	M	6
	F	5
6 years	M	5
	F	6
7 years	M	1
	F	5
8 years	M	-
	F	-
9 years	M	14
	F	9
10 years	M	12
	F	X11
12 years	M	X11
	F	11
13 - 15 years	M	5
	F	8
16 - 18 years	M	6
	F	5
19 years and above	M	68
	F	60

Presence of father and mother

Father	..	49
Mother	..	52

Village - Kurumampalayam

Total families	..	183
Total members in the family	..	818
Male	..	404
Female	..	414
Type of family:		
Nuclear	..	135
Joint	..	48

Education

Balwadi	..	7
I Class	..	15
II Class	..	12
III Class	..	23
IV Class	..	28
V Class	..	48
VI Class	..	22
VII Class	..	17
VIII Class	..	18
IX Class	..	10
X Class	..	10
XI Class	..	23
P.U.C.	..	5
III B.A.	..	1

Occupation

Type of work		Number of families
Cooli	..	79
Mill worker	..	37
Farm labourer	..	9
Workshop worker	..	5
Wireman	..	5
Milk seller	..	5
Watchman	..	4

contd..

Type of work		Number of families
Shop keeper	..	3
Tea Shop worker	..	3
Agriculturist	..	3
Workshop keeper	..	3
Asari	..	2
Teacher	..	2
Driver	..	2
Business	..	2
Clerk	..	2
Farm labourer	..	2
Attender	..	1
Tailor	..	2
Printer	..	1
Electrician	..	1
Woodseller	..	1
Officer	..	1
Carpenter	..	1
Bill Collector	..	1
Puffed rice seller	..	1
Village munsif	..	1
Peon	..	1
Oil seller	..	1
Barber	..	1
Hotel owner	..	1
	Total	183

Income

Income range in Rs.		Number of families
Below 50	..	21
51-100	..	46
101-150	..	48
151-200	..	40
201-250	..	13
251-300	..	6
301-350	..	2
351-400	..	2
401-450	..	2
451-500	..	1
Above 501	..	2
		183

Caste		Number of families
Gounder	..	83
Gowder	..	38
Harijan	..	22
Naidu	..	10
Asari	..	7
Nadar	..	3
Chettiar	..	3
Nair	..	2
Pillai	..	3
Mudaliar	..	2
Vannar	..	2
Barber	..	2
Brahmin	..	1
Dhevar	..	1
Anuppar	..	1
Muslim	..	1
Naicker	..	1
Pandaram	..	1
	Total	183

Mother Tongue		Number of families
Tamil	..	56
Malayalam	..	2
Kannadam	..	112
Telugu	..	13
	Total	183

Age range:

Age range	Sex	Number
Below 1 yr.	M	13
	F	21
1 - 3 yr	M	28
	F	33
4 yrs.	M	4
	F	5
5 yrs.	M	11
	F	11
6 yrs.	M	10
	F	13
7 yrs.	M	9
	F	12
8 yrs.	M	13
	F	4
9 yrs.	M	8
	F	5
10 - 12 yrs.	M	29
	F	41
13 - 15 yrs.	M	33
	F	28
16 - 18 yrs.	M	25
	F	19
19yrs. and above	M	221
	F	221

Presence of father and mother

Father	..	179
Mother	..	168

Village Nambiyalagan Palayam

		Number
Total families	..	62
Total members in the family	..	274
Male	..	139
Female	..	135

Type of family:

Nuclear	..	40
Joint	..	22

Education

I Class	..	—
II Class	..	1
III Class	..	8
IV Class	..	—
V Class	..	8
VI Class	..	14
VII Class	..	4
VIII Class	..	4
IX Class	..	4
X Class	..	9
XI Class	..	9
P.U.C.	..	1

Occupation

Type of work		Number of families
Cooli	..	48
Mill worker	..	9
Shop keeper	..	2
Puffed rice seller	..	1
Tailor	..	-
Watchman	..	1
Clerk	..	1
	Total	62

Income

Income Range in Rs.		Number of families
Below 50	..	5
51 – 100	..	17
101 – 150	..	18
151 – 200	..	12
201 – 250	..	5
251 – 300	..	3
301 – 350	..	2
351 – 400	..	–
401 – 450	..	–
	Total	62

Caste

Caste		Number of families
Bannadi	..	30
Harijan	..	15
Devanthirar	..	13
Pallar	..	4
	Total	62

Mother tongue		Number of families
Tamil	..	62

Age

Age range	Sex	Number
Below 1 year	M	2
	F	4
1 – 3 yrs.	M	8
	F	7
4 yrs.	M	3
	F	4

Age range	Sex	Number
5 yrs.	M	4
	F	3
6 yrs.	M	4
	F	4
7 yrs.	M	3
	F	3
8 yrs.	M	1
	F	3
9 yrs.	M	2
	F	3
10 - 12 yrs.	M	9
	F	14
13 - 15 yrs.	M	15
	F	9
16 - 18 yrs.	M	12
	F	13
19 and above	M	76
	F	68

Presence of father and mother:

Father	60
Mother	57

Village: Vanniampalayam

		Number
Total families	..	196
Total members in the family	..	1071
Male	..	555
Female	..	516
Type of family		
Nuclear	..	113
Joint		83

Education

Balwadi	2
I Class	18
II Class	33
III Class	43
IV Class	26
V Class	43
VI Class	39
VII Class	14
VIII Class	34
IX Class	20
X Class	15
XI Class	20
PUC	13
B.Com	1
B.A.	2

Occupation

Type of work	Number of families
Cooli	121
Mill worker	58
Workshop worker	7
Carpenter	4
Farm Labourer	2
Teacher	1
Shop owner	1
Driver	1
Total	196

Income

Income range in Rs.	Number of families
Below 50 -	12
51 - 100	28
101 - 150	67
151 - 200	28
201 - 250	20
251 - 300	11
301 - 350	8
351 - 400	11
401 - 450	8
451 - 500	Nil
500 and above	3
Total	196

Caste

Caste		Number of families
Harijan madheri	..	4
Harijan Panadi	..	113
Devar	..	34
Deventhirar	..	11
Gowder	..	11
Kavaranaidu	..	11
Asari	..	5
Vellala Gounder	..	3
Pandaram	..	2
Pillai	..	1
Asari	..	1
	Total	196

Mother tongue		Number of families
Tamil	..	143
Telugu	..	50
Malyalam	..	2
Kannadam	..	1
	Total	195

Age

Age range	Sex	Number
Below 1 yr.	M	9
	F	10
1 - 3 yrs.	M	43
	F	41
4 yrs.	M	3
	F	16
5 yrs.	M	47
	F	38
6 yrs.	M	40
	F	31

Age range	Sex	Number
7 yrs.	M	51
	F	35
8 yrs.	M	11
	F	23
9 yrs.	M	15
	F	12
10 - 12 yrs.	M	37
	F	35
13 - 15 yrs.	M	36
	F	30
16 - 18 yrs.	M	29
	F	14
19 and above	M	234
	F	231

Presence of father and mother

Father	..	188
Mother	..	185

Village: Vedapatti Cherri

		Number
Total families	..	117
Total member in the family	..	534
Male	..	273
Female	..	261

Type of family:

Nuclear	..	77
Joint	..	40

Education | **Number**

Education		Number
Balwadi	..	2
I Class	..	3
II Class	..	4
III Class	..	12
IV Class	..	6
V Class	..	9
VI Class	..	3
VII Class	..	7
VIII Class	..	6
IX Class	..	4
X Class	..	3
XI Class	..	3
P.U.C	..	3

Occupation

Type of work		Number of families
Cooli	..	94
Farm labourer	..	1
Mill worker	..	15
Shop keeper	..	1
Dhoby	..	2
Headmistress	..	1
Peon	..	3
	Total	117

Income

Income range in Rs.		Number of families
Below 50	..	16
51 - 100	..	61
101 - 150	..	29
151 - 200	..	8
201 - 250	..	1
251 - 300	..	1
301 - 350	..	Nil
351 - 400	..	Nil
401 - 450	..	Nil
451 - 500	..	Nil
501 and above	..	1
	Total	117

Caste

Caste		Number of families
Adhi Thiravidar	..	51
Gounder	..	2
Naidu	..	1
Harijan	..	61
Christian	..	2
	Total	117

Mother tongue		Number of families
Tamil	..	112
Telugu	..	1
Kannadam	..	4
	Total	117

Age

Age range	Sex	Number
Below 1 year	M	7
	F	5
1 - 3	M	17
	F	15
4 yrs.	M	2
	F	4
5 yrs.	M	8
	F	9
6 yrs.	M	4
	F	9
7 yrs.	M	10
	F	8
8 yrs.	M	7
	F	4

Age Range

Age Range	Sex	Number
9 yrs.	M	6
	F	1
10 - 12 yrs.	M	22
	F	23
13 - 15 yrs.	M	16
	F	20
16 - 18 yrs.	M	13
	F	11
19 and above	M	161
	F	152

Presence of father and mother

Father	..	100
Mother	..	114

Village: Sundapalayam

		Number
Total families	..	198
Total members in the family	..	956
Male	..	468
Female	..	488

Type of family:

Nuclear	..	172
Joint	..	26

Education

Balwadi	..	18
I Class	..	34
II Class	..	45
III Class	..	32
IV Class	..	22
V Class	..	27
VI Class	..	26
VII Class	..	17
VIII Class	..	14
IX Class	..	10
X Class	..	17
XI Class	..	31
P.U.C.	..	5
B.A.	..	6
M.A.	..	3
B.Sc. B.L.	..	1
L.M.S.	..	1

		Number
B.Sc.	..	3
Polytechnique	..	1

Occupation

Type of work		Number of families
Mill worker	..	31
Coole	..	99
Tailor	..	7
Driver	..	4
Teacher	..	13
Shop keeper	..	13
Food carrier	..	1
Carpenter	..	1
Farm labourer	..	14
Agriculturist	..	2
Revenue Inspecter	..	2
Weaver	..	1
Climber	..	3
Postman	..	1
Painter	..	1
Cinema operater	..	1
Compounder	..	1
Watchman	..	2
Conductor	..	1
	Total	198

Income

Income range in Rs.		Number of families
Below 50	..	67
51 - 100	..	91
101 - 150	..	32
151 - 200	..	2
201 - 250	..	1
251 - 300	..	1
301 - 350	..	1
350 - 400	..	Nil
401 - 450	..	Nil
451 - 500	..	2
500 and above	..	1
	Total	198

Caste

Caste		Number of families
Naidu	..	29
Gounder	..	55
Nadar	..	35
Pillai	..	4
Maharashtra	..	1
Malayali	..	8
Harijan	..	15
Bhramin	..	4
Chettiar	..	4
Marathi	..	1
Dever	..	8
Asari	..	10
Adhi Thiravider	..	8
Konar	..	4
	Total	198

Mother tongue

Tamil	..	141
Telugu	..	28
Malayalam	..	8
Kannadam	..	18
Hindi	..	3
	Total	198

Age

Age range	Sex	Number
Below 1 year	M.	24
	F	35
1- 3 years	M	19
	F	22
4 yrs.	M	12
	F	27
5 yrs.	M	18
	F	15

Age range	Sex	Number
6 yrs.	M.	24
	F	23
7 yrs.	M	24
	F	22
8 yrs.	M	19
	F	23
9 yrs.	M	26
	F	22
10 - 12 yrs.	M	30
	F	23
13 - 15 yrs.	M	24
	F	24
16 - 18 yrs.	M	24
	F	16
19 and above	M	224
	F	236

Presence of father and mother:

Father	..	187
Mother	..	190

ANNEXURE XIV

THE METHOD OF PREPARATION OF THE THREE RECIPES (SUNDAL, UPPUMA AND PAYASAM)

I **Sundal:**

1. Clean the ingredients.
2. Soak the legumes and rice in water for a period of four hours.
3. Boil the water.
4. Add the soaked ingredients to the boiling water.
5. Cook till soft.
6. Remove from fire.
7. Heat the oil in the pan.
8. Season with mustard, onion and chillies.
9. Add the cooked ingredients, stir.
10. Add the washed greens, mix.
11. Cook for five minutes.
12. Remove from fire.

II **Uppuma:**

1. Clean the ingredients.
2. Powder them into granules.

3. Roast the powder on a slow fire.
4. Heat the oil.
5. Season with mustrad, chopped chillies and onion for 5 minutes.
6. a. Add the boiled water, stir.
   b. Add salt mix.
7. Sprinkle the powder granules little at a time.
8. a. Stir well to avoid lumping
   b. Add the washed greens, stir.
9. Cook for 20 minutes till soft.
10. Remove from fire.

III <u>Payasam</u>:

1. Clean the ingredients.
2. Powder them.
3. Roast on a slow fire.
4. Boil the water
5. Sprinkle the roasted powder, little at a time, stir.
6. Add the jaggery powder, stir well.
7. Cook till soft.
8. Remove from fire.

ANNEXURE XV

ESTIMATION OF HAEMOGLOBIN CONTENT CYANMETHEMOGLOBIN METHOD

Cyanmethemoglobin method measures not only oxy-haemoglobin, but also carbonmonoxide haemoglobin and methemoglobin except sulph haemoglobin. With filter type photoelectic colorimeters the single relatively broad band of cyanmethemoglobin in the green spectral region has a distinct advantage.

Procedure

Exactly 5 ml of Drapkin's diluent solution is measured into a dry test tube from a burette or a pipette with suction bulb.

Exactly 0.02 ml of blood is tranferred from a standardised Haemoglobin pipette into the diluent solution. Usual care in filling and cleaning of loaded Haemoglobin pipette must be observed.

The pipette is rinsed three times with the diluent solution without allowing the formation of air bubbles in the solution.

The blood and the diluent are thoroughly mixed by rotating the tube.

10 minutes time is allowed for the formation of the cyanmethemoglobin.

5 ml of diluent solution is used as blank.

With green filter No 540 the readings are taken in a photoelectoric colorimeter.

Calibration procedure

Total blood iron is determined by Wongs method. This determination would give absolute amount of Haemoglobin.

Exactly 0.02 ml of this known blood sample is measured as above into 5.0,7.5,10.0, 12.5 and 15.0 ml, respectively of diluent solution and mixed by rotating the tubes. These solutions are now equivalent to blood samples containing respectively 100, 67, 50, 40 and 30 percent of that of the original solution.

The intensity of the colour is read using green filter No 540 against diluent as blank set at zero O.D.

On a graph paper a standard graph is drawn using these haemoglobin concentration and corresponding density values.

Reference

ICMR 1971 — A manual of laboratory techniques, National Institute of Nutrition, Indian Counfil of Medical Research, Taranaka, Hyderabad - 7.

ANNEXURE XVI

Analysis of the serum Vitamin A level:

5 ml of venous blood samples was drawn from the forearm of each subject for Vitamin A determination in serum. The Vitamin A was extracted with pure xylene and readings were taken on a fluorometer at 350 mμ excitation and 490 mμ emission before and after irradiation with ultraviolet light.

Estimation of serum Vitamin A by Microfluorometric Procedure:

Principle:

Protein bound vitamin A is released and vitamin A esters hydrolysed from serum by hot alcoholic potassium hydroxide. The vitamin A alcohol formed is extracted with xylene. The xylene layer is separated and excited at 350 mμ and the flourescence measured at 490 mμ. Nonspecific flourescence if any is measured at the same wavelenghts following ultraviolet destruction of vitamin A in the xylene extract and substracted from the total flourescence to yield the net fluorescence due to vitamin A. The vitamin A content of the sample is obtained by comparing the net fluorescence with that produced by standard vitamin A.

Materials and Methods:

Water, double distilled from all glass apparatus was used to prepare all solutions. All chemicals used were of analytical quality and were commercially obtained. All solvents were of reagent grade. Alcohol was further purified by fractional distillation. Vitamin A was obtained from Glaxo Laboratories Ltd., Bombay.

Flourescence measurements were carried out in a coleman Fluorometer.

Procedure:

5 ml of venous blood was collected from the forearm in 10 ml test tubes and allowed to clot. It was then centrifuged and 0.5ml serum was transferred into 10 ml centrifuge tubes. 0.5 ml of 1N alcoholic potassium hydroxide prepared the same day by dilution of 19N potassium hydroxide in water with 95 per cent ethanol, was added to the serum aliquot, and mixed well. The tubes are then kept in a hot water bath, maintained at 60° C for 20 minutes to extract and hydrolyse vitamin A esters to alcohol. The tubes were cooled and the Vitamin A alcohol was extracted with 6 ml of xylene, by vigorously mixing the two phases for 20 seconds. The upper xylene layer was separated by centrifugation and transferred to the cuvettes. Flourescence was measured in the fluorometer at 490 mμ with excitation at 350 mμ (uncorrected) The cuvettes were then covered with glass beads (5 mm diameter) to avoid evaporation, and

irradiated for 10 minutes in an irradiation chamber, Fleurescence readings were again taken following irradiation and the net fleurescence destroyed by ultraviolet irradiation was calculated.

A series of standards of vitamin A containing 0.12–0.43µg in 0.5 ml, was treated in similar manner to the serum sample. A standard curve was constructed relating the fluorescence of these standards with the concentration and was used for estimating the vitamin A content of the unknown samples

ANNEXURE XVII

ANALYSIS FOR VARIANCE FOR BODY WEIGHT INCREASES OF RATS FED THE FOOD FORMULATIONS

Source of variation	Degrees of freedom	Sum of squares	F ratio
Total	74	14630	76.72**
Treatment	7	13007	
Error	67	1623	

Group code	$\bar{X}$	$\bar{X}$ - 13.8
S		
I	85.6	71.8
II	40.3	26.5*
III	54.0	47.1*
IV	60.9	39.6*
V	70.4	56.6*
VI	53.4	39.6*
M	60.4 13.8	46.6*

* Significant at 5% level

** Significant at 1% level

S - Skim milk group

M - Maize

ANNEXURE XVIII

ANALYSIS OF VARIANCE FOR WEIGHT GAIN OF RATS FED DIETS OF VARYING SOURCES AND CONCENTRATIONS OF PROTEINS
(NON ORTHOGONAL COMPARISON)

Source of variation	Degrees of freedom	Sum of squares
Total	47	12655.31
Treatment	7	12057.00**
Error	40	598.31
A Vs E	1	615.37**
B Vs F	1	1293.80**
C Vs G	1	3088.02**
D Vs H	1	1885.01**
A Vs F	1	2205.23**
B Vs G	1	5612.41**
C Vs H	1	4800.00**
C Vs E	1	86.4*
B. Vs E	1	269.8**
A+B+C+D Vs E+F+G+H	2	6931.21**

* Significant at five per cent level

** Significant at one per cent level

ANNEXURE XIX

ANALYSIS OF VARIANCE FOR PROTEIN INTAKE OF RATS FED DIETS OF VARYING PROTEIN CONTENTS FOR 28 DAYS

(NON ORTHOGONAL COMPARISON)

Source of variation	Degrees of freedom	Sum of squares
Total	47	856.09
Treatment	7	848.48**
Error	40	7.61
A Vs E	1	15.61
B Vs F	1	38.35**
C Vs G	1	1.26
D Vs H	1	17.86
A Vs F	1	19.51
B Vs G	1	0.75
C Vs H	1	17.86
A+B+C+D Vs E+F+G+H	2	30.04*

* Significant at five per cent level

** Significant at one per cent level

ANNEXURE XX

ANALYSIS OF VARIANCE FOR PER OF RATS FED DIETS OF VARYING SOURCES AND CONCENTRATIONS OF PROTEINS AT DIFFERENT LEVELS FOR 28 DAYS

Source of variation	Degrees of freedom	Sum of squares
Total	47	35.90
Treatment	7	29.29
Error	40	6.61
A Vs E	1	10.50
B Vs F	1	8.80
C Vs G	1	9.51
D Vs H	1	7.08
A Vs F	1	9.39
B Vs G	1	10.15
C Vs H	1	9.29
A+B+C+D Vs E+F+G+H	2	17.85

* Significant at five per cent level

** Significant at one per cent level

ANNEXURE XXI

ANALYSIS OF VARIANCE FOR HEPATIC SDH ACTIVITIES OF RATS FED DIETS OF VARYING CONTENTS AND SOURCES OF PROTEINS

(Non Orthogonal comparison)

Source of variation	Degrees of freedom	Sum of squares
Total	47	2852949.3
Treatment	7	2784387.3**
Error	40	68563.0
A Vs E	1	22082.6**
A Vs F	1	20650.6**
C Vs G	1	66360.1**
D Vs H	1	47082.6**
A Vs F	1	182701.5**
A Vs C	1	375000.1**
C Vs E	1	306588.1**
A+B+C+D Vs E+F+G+H	2	147737.0**

** Significant at one per cent level

ANNEXURE XXII

ANALYSIS OF VARIANCE FOR WEIGHTS GAIN OF RATS FED DAILY 90 mcg. CAROTENE FOR 28 DAYS

Source of variation	Degrees of freedom	Sum of squares
Total	23	7316
Treatment	3	6173**
Error	20	1143
A Vs B	1	252*
A Vs C	1	533**
A Vs D	1	2160**
B Vs C	1	469**
B Vs D	1	936**
C Vs D	1	2730**

* Significant at five per cent level

** Significant at one per cent level

ANNEXURE XXIII

ANALYSIS OF VARIANCE FOR HEPATIC VITAMIN A PER g. OF LIVER OF RATS FED DAILY 90 mcg. CAROTENE FOR 28 DAYS

Source of variance	Degrees of freedom	Sum of squares
Total	23	6172
Treatment	3	5425**
Error	20	747
A Vs B	1	850**
A Vs C	1	901**
A Vs D	1	900**
B Vs C	1	0.8
B Vs D	1	0.6
C Vs D	1	3

* Significant at five per cent level

** Significant at one per cent level

ANNEXURE XXIV

ANALYSIS OF VARIANCE FOR INCREASE IN HAEMOGLOBIN CONCENTRATION OF THE SUBJECTS OF DIFFERENT GROUPS

Source of variation	Degrees of freedom	Sum of squares
Total	35	22.64
Treatment	2	8.39**
Error	33	14.25
A Vs T	1	2.35*
A Vs C	1	3.55**
T Vs C	1	11.60**

* Significant at 5 per cent level

** Significant at 1 per cent level