BIOLOGICAL VALUE OF VEGETABLE PROTEINS AVAILABLE IN PAKISTAN AS DETERMINED BY RAT FEEDING EXPERIMENT

By

JAMEELA JAN

Bachelor of Science
University of Peshawar
Peshawar, West Pakistan
1962

Master of Science
University of Punjab
Lahore, West Pakistan
1964

Submitted to the faculty of the Graduate College
of the Oklahoma State University
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Thesis Approved:

Helen F. Barbour
Thesis Adviser

June Cozine

N N Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

The main food problem in Pakistan is that of protein malnutrition, especially in children, expectant and nursing mothers.

Pakistan is a developing country with many economic problems and with a large population to feed. The food situation is very poor and consequently national health is affected. Since malnutrition is wide spread in Pakistan, some of the reasons for this are thought to be as follows:

1. low income per capita,
2. high birth rate,
3. low food production due to outdated methods of agriculture, water logging and salinity of soil,
4. uncertain water situation,
5. availability of animal protein which is not sufficient to meet the needs of the people.

Protein malnutrition is wide spread among young children, especially two to four years of age, in most technically developing countries of the world. The prevention depends on the availability of sources of good quality protein, but protein containing foods of animal origin such as milk, meat, cheese and eggs are often beyond the economic reach of the segment of the population needing them most.

Therefore better use of vegetable protein appears to be the practical solution to the problems of protein malnutrition for many countries (36).

The estimation of the Food and Agriculture Organization regarding the intake of protein and calories in the developing countries, including Pakistan, by food produce sheet method indicates that average diets are insufficient in quantity and defective in quality. Total per capita protein supplies may be below 60 grams daily and those of animal protein less than ten grams. This reflects the low productivity of such foods as milk, meat, eggs and fish (26).

There are other reasons for protein malnutrition such as lack of knowledge of nutrition, resulting in incomplete utilization of locally available foods and detrimental cooking practices.

Pakistan has a good production of legumes, cereals and seeds which are cheap sources of protein with a wide variety of essential amino acids. Therefore greater stress should be placed on the use and consumption of plant proteins in such countries that their amino acids supplement each other.

Protein malnutrition can be prevented to a considerable extent by using combinations of plant proteins in the diet. Plant protein mixtures should be incorporated in the diet in such a way that they are palatable to the masses of people. For this reason more ways of using plant protein mixtures in regular diets should be investigated.

Assumptions on which this research is based include:

1. Mixtures of vegetable protein can be formulated which contain adequate amounts of the essential amino acids.

2. White rats fed mixtures of animal or vegetable proteins will exhibit sufficient growth in four to five weeks to demonstrate the efficiency of the protein.

3. Four rats per diet is a sufficient number to permit the investigator to draw conclusions which establish the value of the diets in relation to their protein content.

4. The amount of essential amino acids needed daily by the rat are known.

5. The amount and kind of nutrients required daily for growth and health of white rats are known.

In this study the author plans to test the following hypothesis:

1. Vegetable protein mixtures will support growth and maintain health in growing rats.

2. A vegetable mixture of proteins that supplement each other in relation to essential amino acids sufficiently well to support life and growth does not contain the protein efficiency ratio of the control diet.

3. The addition of 20 percent of whole fish flour to a vegetable protein mixture will produce a protein efficiency ratio essentially equivalent to that in the control diet.

This study has been undertaken to formulate plant protein mixtures using some of the typical cereals and legumes commonly eaten in Pakistan in such a way that they provide all the essential amino acids in sufficient amounts to maintain health and growth.

It is planned to use four diets, two of which contain animal protein. Of these two diets one is to serve as a control diet while the other will serve as an experimental one containing a small amount of fish flour in addition to vegetable proteins. The other two diets used in this experiment contain only vegetable protein mixtures.

Sixteen albino male rats of weanling age and within 5 grams of the same weight were placed in groups of four per diet.

It is anticipated that the study will require four to five weeks to demonstrate the results of the variation of protein sources.

CHAPTER II

REVIEW OF LITERATURE

Importance of Amino Acids and Proteins

Every living organism must have an adequate source of protein in order to grow and maintain itself. In 1928 the word protein was coined from a Greek word meaning "to take first place." This word describes the nitrogenous substances which are the basis for the different proteins. Protein is considered first since, after water, it composed the greatest proportion of the body tissue and is an indispensable constituent of every living cell (8).

Protein may be used as a source of energy. This has been shown experimentally during fasting and when the diet contains insufficient carbohydrate and fat for fuel; at such times the quantity of protein available for tissue growth or repair is reduced. When either carbohydrate or fat, or both, are increased under the circumstances, the use of protein as a fuel is lessened or ended and nitrogen excretion is diminished. As energy for the body is more economically supplied by the other food stuffs, so the use of protein as fuel food has been likened "to burning rose wood rather than pine for warmth." (8, 103).

Protein has regulatory functions such as regulation of osmotic pressure and of water balance within the body and also in maintaining hydrogen ion concentration. The proteins are important in this way because they are amphoteric substances which can combine with acid or base and act as buffers. In many instances protein acts as a precursor of body hormones such as tropic hormones of the pituitary gland, thyroxin, adrenalin and insulin. In the same way digestive enzymes and those concerned in oxidation-reduction activity are each composed of proteins.

Some twenty two or more different amino acids are needed for the growth of new tissues, for the maintenance of established cellular constituents and for other metabolic requirements. The function of a dietary protein is to supply the nitrogen for the symthesis of a variety of nitrogen-containing compounds (heme, creatin, hexoseamine) and to provide those amino acids that cannot be synthesized in adequate amounts by the body. These are called essential or indispensable. Rose defines (29, 109) an essential amino acid as "one which cannot be synthesized by the animal organism out of materials ordinarily available at a speed commensurate with the demands for normal growth."

The amino acids required for growth are needed mainly for the synthesis of the protein molecules entering into the structure of protoplasm. For this function the simultaneous presence in the tissues is required of all of the amino acids that the body cannot manufacture itself from dietary constituents. The absence of any one essential amino acid will block the synthetic process (22).

The kind and amount of amino acids in a protein determine its nutritive or biological value. The amino acid composition of animal muscle, milk and eggs are similar though not identical, to the amino acid composition of human tissues (22).

Amino acids are distributed through out the body and serve as the substance for the formation of the many characteristic tissue proteins and of the multitude of non-protein nitrogenous components of the cell. Thus amino acids occupy a unique position in nutrition.

The first one of the amino acids was discussed over 125 years ago and the last of the twenty two was isolated and identified in 1935 by W. C. Rose (30, 631). According to him there are eight amino acids which are essential for maintenance of nitrogen equilibrium in man. The food eaten must contain these amino acids because they can not be manufactured in the body rapidly enough to supply the need for them.

Classification of Amino Acids with Respect to Their Growth Effect

The various amino acids which are considered as essential and non-essential for human beings are as follow: (8, 102).

Essential	Non-Essential
Lysine	Glycine
Tryptophan	Alanine
Phenylalanine	Serine
Leucine	Norleucine
Threonine	Aspartic Acid
Methionine	Glutamic Acid
Isoleucine	Hydroxy Glutamic Acid

Essential	Non-Essential
Valine	Proline
	Hydroxy Proline
	Citruline
	Tyrosine
	Cystine
	Arginine
	Histidine

A good quality protein, or complete protein, is one that supplies all of the essential amino acids in sufficient quantities and in proper ratio for growth and maintenance.

Tryptophan and lysine. - Osborn and Mendel (25) experimented with animals and found that lysine is indispenasble for the function of growth. In the same way they demonstrated the essential nature of tryptophan. Willcock and Hopkins (40) have shown in 1906 that the protein zein has no power whatsoever of maintaining growth in the young animal. Further, they reported that the addition of tryptophan to a zein ration is insufficient to convert such loss (in weight) into equilibrium or gain although the duration of life is thereby prolonged.

The supplementation of a zein diet with another protein containing adequate quantities of lysine and tryptophan resulted immediately in growth. Undoubtedly, both lysine and tryptophan are indispensable dietary components. In the absence of either, nutrition fails and eventually death results.

Cystine and Methionine. - These are the only known protein components which contain sulfur. Cystine as demonstrated by Osborn and

Mendel, (25) caused increases in body weight of animals. The proteins of the cowpea and of lentils are similarly enhanced in nutritive value by the addition of cystine.

Histidine and Arginine. - It has been observed (29) that when arginine and histidine are removed from acid hydrolyzed casein the resulting material, though supplemented with tryptophan, is inadequate for maintenance or growth. But if either arginine or histidine is added to the ration no loss in weight occurs and growth may be renewed.

From these studies it is concluded that either of the two amino acids are interchangeable with tryptophan in metabolism, but that at least one must be present in the food for man.

Tyrosine and Phenylalanine. - It is believed that these two amino acids are interchangeable, at least in part, but that one must be included in the food. Hopkins (17) found that the rat which received cystine, tyrosine, lysine, tryptophan and histidine as the only sources of nitrogen manifested a remarkably slow loss of weight and long maintenance of apparent health. When, however, leucine, alanine, glycine and glutamic acid were fed in place of the previous mixture, the losses in weight were rapid and the animal soon succumbed.

It was proved that in the absence of leucine, isoleucine and norleucine the diet was quite incapable of meeting the needs of the organism. The animals declined in weight very rapidly. When both leucine and isoleucine were present, normal growth occurred. It has been observed that if one administers methionine at a level which permits maintenance or only slow growth, the addition of cystine markedly improves the quality of the food. Thus cystine stimulates growth when methionine is furnished in inadequate quantities but not when it is entirely excluded from the diet. Animals deprived of valine experience a profound nutritive failure with rapid decline in weight, loss of appetite and death (31).

Composition of Protein

Protein has been defined as an extremely complex nitrogenous, organic compound which is found in all animal and vegetable cells. It constitutes a major part of the living protoplasm of these cells.

Most plants make their own protein by combining the nitrogen from nitrogen-containing materials in the soil with carbon dioxide from the air and with water. Legumes which include beans, peas, and peanuts, can use the nitrogen directly from the air for combining with the other substances to make protein (22).

Animals and human beings have to get proteins from plants and other animals since they cannot use simple raw materials for building their proteins. The proteins once eaten, are digested into smaller units and rearranged to form the many special and distinct proteins needed by man. Plants are the basic factory of protein. All proteins come directly or indirectly from plants. The farm animals convert plant proteins into animal proteins for human consumption.

Proteins are made up of chemical units called amino acids. The kinds and amounts of amino acids in a protein determine its nutritive value. Animal proteins can supply all of the amino acids in about the same proportion in which they are needed by the human body.

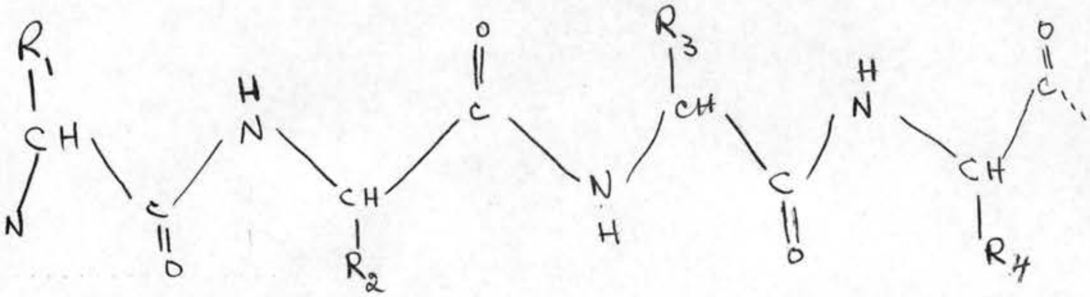
They are considered as proteins of high nutritive value. Proteins from fruit, vegetables, grains and nuts supply important amounts of many amino acids but they do not supply as good a combination as do animal proteins. Therefore their biological value is lower (21).

The Structure of Proteins

The name protein (Greek: Proteus to occupy first place) is well chosen; all the basic functions of life depend on specific proteins. Indeed there is no form of life without protein; they are present in all cells; they make up the contractile elements; the enzymes that catalyze the release of energy for maintenance of life; and they are present in blood where they have a transport function (19).

The chemical structure of proteins is quite simple as numerous amino acids are joined to form peptide linkages.[1] When a peptide chain is extended by more and more amino acids until a chain length of from one hundred to several thousands amino acid residues is reached it is classified as a protein.

A part of a peptide chain may be represented by the following structural formula (19, 43):



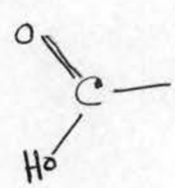
[1]A peptide linkage is a combination of two or more amino acids.

The sequence of amino acids determine the primary structure; when several hundred amino acids join to form an extended chain molecule, it is easy to imagine that such a molecule may be arranged in space in various ways. For example it may retain a straight linear form or bunch up randomly in a ball, or arrange itself in a highly ordered helix (screw shape). These arrangements are called the secondary structure. When several chains line up and form agregates as globulin, a soluble protein does, chains with their own secondary structure orient themselves into larger molecules of definite shape to form tertiary structures of proteins. (19).

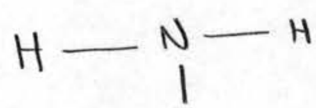
The Structure of Amino Acids

During hydrolysis proteins break down into amino acids while in the organism they are built up from amino acids.

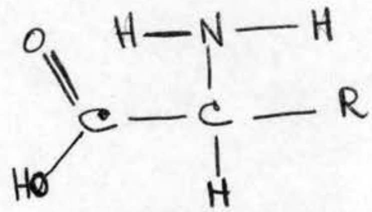
The name indicates that amino acids possess two characteristic functional groups, the amino group and carboxylic group. The carboxylic group has the following structure (21, 64):



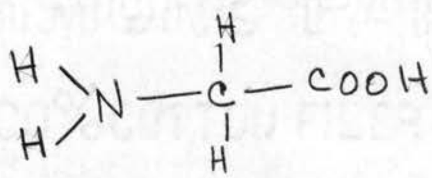
The amino group has the structure presented below:



This amino group is attached to a carbon which, in turn is attached to the acid group. So the chemical structure common to all amino acids is the following arrangement (21, 64):



One amino acid differs from another in the chemical group that is attached to the same carbon atom as the amino acid where the R appears above. The attachment may be as simple as a lone hydrogen atom to make the amino acid glycine which is the only amino acid with out a carbon containing substituent in the R position. For example (19, 43):



It may be a certain chain of carbon and hydrogen atoms to make leucine, a chain plus another amino group to make lysine, or a chain that includes an atom of sulfur to make methionine. Adding a more complicated arrangement such as a ring of carbon and hydrogen atoms, would make phenylalanine; two rings with some iodine attached would make thyroxine.

Amino Acids and Protein Requirements

In assessing the protein requirement of man or of any animal, the quantitative demands as well as the qualitative needs must be considered (15).

Quantitative Requirements. - Protein malnutrition is mostly prevalent in countries with a short supply of animal protein. The protein requirement in these countries has to be met largely from vegetable sources such as legumes, wheat, rice and maize. In these foods the protein content is low and incomplete in relation to amino acids so that large amounts have to be eaten to meet the requirements. Also care must be taken to include those foods in which the amino acid content of one supplements another. The greatest need for protein is during rapid growth periods. During pregnancy and lactation an increased amount of approximately 20 grams and 40 grams respectively should be added to the usual requirement (15).

The protein requirement during the whole life span is not the same since it changes with age, sex and state of health. Given below are the Recommended Daily Dietary Allowances for protein for a standard man and woman in the United States of America (12, vii):

Age & Sex	Weight (Kg)	Height (cm)	Protein (Grams)
Man			
18-35 years	70	175	70
35-55 years	70	175	70
55-75 years	70	175	70
Woman			
18-35 years	58	163	58
35-55 years	58	163	58
55-75 years	58	163	58

The Recommended Dietary Allowance of protein for adults in the

United States of America has been one gram of protein per kg. per day since the original allowances were established in 1943.

The Committee on Amino Acids (10) of the Food and Nutrition Board and the FAO Committee on Protein Requirements (26) established the minimal need for adults to be between 0.3 and 0.35 grams per kg. per day when the diet contained protein of maximal nutritive value.

During pregnancy and lactation the growth of the fetus and other tissue clearly represent an additional protein requirement of the mother. Given below are the amounts of protein recommended during pregnancy and lactation (12, p. vii).

Age	Weight	Height	Protein
Woman	kg	cm	Gms
18-35 years	58	163	58
Pregnancy (2nd and Timester)			+20
Lactation			+40

An additional protein allowance of 20 grams per day does not appear unreasonable because of the growth of the child. The body of the new born infant contains about 2 percent nitrogen. Therefore approximately 450 grams of protein must be accumulated largely during the second and third trimester of pregnancy. During lactation the amount of protein is increased by 40 grams because a liter of breast milk contains approximately 12 grams of protein and the milk production may exceed a liter per day.

During the first few years of life growth is quite rapid which requires nitrogen for the formation of new tissues. Therefore the protein intake should be liberal. The Recommended Daily Dietary Allowance for protein set by the Food and Nutrition Board (12, vii)for infants and children of the United States is as follows:

Age & Sex	Weight Kg	Height cm	Protein Gms
Infants (up to 1 year)	8		x 2.5 Kg ∓ .5
Children			
1-3 years	13	87	32
3-6 years	18	107	40
6-9 years	24	124	52
Boys			
9-12 years	33	140	60
12-15 years	45	156	75
15-18 years	61	172	85
Girls			
9-12 years	33	140	55
12-15 years	47	158	62
15-18 years	53	163	58

It has been demonstrated by experiments that the protein metabolism of young children is the same as of an adult except for the larger amount required for growth.

Qualitative Requirement: - According to Rose (30) there are eight so called essential amino acids which can not be manufactured by the human body and must be supplied by protein food to enable tissue synthesis to take place. The requirement for each one of these essential amino acids for infants, females and adult males are given below (24):

Amino Acids	Infants MgKg	Female MgKg	Adult Male MgKg
Histidine[1]	3.2	-	-
Isoleucine	90	450	700
Leucine	150	620	700
Lysine	90	500	800
Phenylalanine	90	220	1110 (300 if tyrosine present)
Methionine	65	305	200 (if cystine present)
Threonine	60	205	500
Tryptophan	22	157	250
Valine	93	650	800

Animal proteins, rich in essential amino acids, are usually high in biological value while proteins of vegetable origin are known to be relatively lacking in one or more essential amino acids. Therefore the fulfillment of the protein needs is relatively dependent upon a combination of foods which supply the essential amino acids in adequate amounts.

When a protein of high biological value such as is present in egg, milk, and meat is the source of the essential amino acids, a daily intake of 0.35 grams per kg/day is adequate.

Factors affecting the protein requirements of an individual in-

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[1]Histidine is required by the human body during growth only.

clude the diet, time factor and physical health of the individual.

Nitrogen balance is a tool that has been used by many workers in estimating the part played by non-protein calories in the establishment of nutritional status. Swanson (38) found that the total energy balance and the nitrogen balance are interwoven. When the diet supplies more than 50 percent of the needed calories, the internal reserve that an animal in a reasonably good state of nutrition possesses in its hepatic and plasma protein acts like a safety valve. These proteins, when drawn upon in times of emergency, may provide sufficient energy to satisfy caloric needs and thereby permit utilization of food nitrogen. With the first restriction of calories an animal whose internal reserves are low in protein slips immediately into positive balance but then suddenly and rather dramatically drops into negative balance. Swanson (38) found with humans that while 1500 calories will support retention on a low protein intake approximately the critical level of 900 calories is ample when the protein intake is doubled. In rehabilitation addition of protein to rations ranging in energy value from 2000 to 3000 calories per day exert no favorable influence on the retention of nitrogen.

The time factor influences nitrogen utilization. The distribution of the amino acids among the daily meals is a factor to consider in meeting the protein needs. In building body proteins whether for growth or replacement, the body uses all of the essential amino acids plus the non-essential ones. All the essential amino acids should be available simultaneously for the body to utilize them fully (37).

Geiger (13) has found that an animal may not grow well even when he receives adequate amounts of all of the amino acids. Unless the amino acids or supplementary proteins are fed simultaneously, retardation of growth occurs.

Leverton and Gram (23) advise consideration of distribution of quality and kind of protein among the three meals as a means of securing the best retention of nitrogen. With fourteen college women on a controlled diet better retention of nitrogen was secured when eight ounces of milk were given at breakfast than when no animal protein was included at this meal.

The vitamins and minerals also are important in protein synthesis. It is thought that potassium is necessary for the effective utilization of amino acids. In certain pathological conditions such as burns (skin) and disease of the kidneys there may be an exceptionally liberal amount of protein required because there is more tissue destruction and loss of protein (7).

Protein Deficiencies in Developing Countries

Multimillions of people throughout the world suffer from chronic malnutrition and countless infants die every day due to lack of proper diet (35).

A statistical calculation of net food supplies per capita available at the retail level provides some indication of quantitative and qualitative adequacy of diets, even though they are subject to considerable errors of estimation and moreover hide the differences in food available in various groups in the world because of such factors as location, economic status, age and sex. The following table shows the amount of calories and protein available per capita in some economically developed and underdeveloped countries (14, 17):

CALORIES AND PROTEIN AVAILABLE PER DAY

Countries	Calories	Total Protein (Gms)	Protein (Animal) (Grams)
New Zealand	3380	150	71
United States	3100	94	66
Sweden	2950	85	57
United Arab Republic	2590	77	15
Mexico	2250	58	13
Pakistan	2000	47	8
South India	1840	45	5

Countries such as Bolivia, Chile, Columbia, Ecuador, Peru, Uruguay, Venezuela, Brazil, Ethiopia, Libya, Burma, Iran, Jordan, Korea, Lebanon, Pakistan, Philippine Islands, Taiwan, Thailand, Turkey, Viet Nam and Malaya have well-defined nutritional problems (35).

The diets of the developed countries derive proportionally less energy from carbohydrates and more from animal protein and fat than the Pakistani diets in which animal protein contributes only 1.7 percent of the total calories and oils and fats about 4.2 percent. In

Pakistan, the average consumption of protein is 8 grams per capita per day while in the United Kingdom and United States of America it is 49 and 66 grams per capita per day respectively (3).

Estimation of the Food and Agriculture Organization in relation to the intake of protein and calories in some of the developing countries, including Pakistan, by the Food Balance Sheet Method shows that average diets are insufficient in quantity and defective in quality. The details about different foods in the Food Balance Sheet prepared by the Food and Agriculture Organization is given here (5, 57):

PAKISTAN: TOTAL DAILY FOOD SUPPLIES/PER HEAD

Food	Quantity in Grams	Cals./Day	Protein (Grams)
Cereals	412.2	1468	32.1
Sugar	41.5	148	0.3
Pulses & Nuts	24.4	84	5.4
Vegetables	48.8	11	0.7
Fruit	90.9	52	0.5
Meat	2.6	24	1.7
Fish	8.5	5	0.7
Eggs	0.9	2	0.1
Milk (liquid)	159.2	125	5.8
Oils & Fats	9.6	85	-
Total		2004	47.3

The total food supplies provided about 2004 calories per head daily, 73 percent of the total calories were obtained from cereals, 7.4 percent from sugar and 4.2 percent from pulses. Meat, fish and eggs together provided only 1.5 percent and oils and fats 4.2 percent.

It can be easily concluded that in general the Pakistani diet is short of calories and protein, especially animal protein and fat (5).

Need for Protein Supplementation

Proteins of vegetable origin are used in feeding more than two-thirds of the world's human population and almost all of its domesticated animals. It is evident that there is a vast potential for the amino acid supplementation of vegetable protein.

There is sufficient evidence that lysine supplementation of wheat protein results in a product of increased biological value. Added lysine and threonine will improve rice. Salmon (34) has shown that high protein corn is improved by the addition of lysine and tryptophan but to improve low protein corn, lysine, tryptophan, isoleucine, threonine and valine are required.

Hegsted and coworkers (16) found that supplementation of the vegetable diets of young adult females receiving 25 grams of protein per day with lysine and methionine improved the nitrogen retention following a depletion period.

The early work of Geiger (13) with animals has demonstrated the importance of providing all of the essential amino acids at the same time in proper amounts to effect additional protein utilization.

The studies of Leverton and others (23) have extended this concept to human diets. Scrimshaw and associates (37) have made a significant contribution in the development and evaluation of low cost high protein mixtures for human consumption.

Kik (20) has observed that the protein of whole and milled rice can be effectively supplemented by those of whole milk solids, lactalbumin and defatted whole egg.

Near East people follow essentially a cereal diet. This cereal diet however is usually supplemented with liberal amounts of legumes. It is suggested that such legume-cereal mixtures supply a mixed protein which is often of superior nutritive value. From numerous studies (1) it was concluded that among the single legumes the highest value is that shown by chick peas. It is significant that this is the most widely used among the legumes in the Near East. The addition of parboiled wheat to any of the single legumes yields a mixture with increased nutritive value in respect to the protein fraction. The chick peas and parboiled wheat are two food materials that frequently appear together at the same meal, although they are not combined usually in a prepared mixture. The protein efficiency ratio of this chick peas-parboiled wheat mixture is high.

In Uganda, Dean (9) fed a biscuit of 20 percent protein consisting of whole ground peanuts (which supply 60 percent of the total protein) as well as corn meal sugar and a little dry skim milk to infants and children as young as 6 months and compared their growth and weight gains with another group fed on a diet containing primarily milk. The group receiving the biscuits and particularly the youngest infants showed gain in weight about equal to those on the milk diet.

Vegetable Protein Mixtures

Most of the international nutrition and health conferences in the last decade, under the guidance of the World Health Organization and the Food and Agriculture Organization have stressed the need for developing new sources of protein-rich foods for human feeding. In 1955 at Princeton a Protein Advisory Group was formed to develop new protein sources. Projects were sponsored by National Research Council in Mexico, Guatemala, South Africa, French West Africa, Nigeria, Belgium, Congo, Uganda, India, Japan, the United States of America, The United Kingdom, Taiwan and Brazil. A rapid progress is expected in this field with so much interest.

In making vegetable mixtures it is important to consider agriculture, economic and cultural factors simultaneously with nutritional value and palatability. It is practical to develop a combination of plant proteins, locally available, which are suitable for this purpose, and it is believed that it should be something which is done regionally.

For the preparation of such plant protein mixtures it should be kept in mind (33):

1. that the materials be locally available
2. that they be cheap
3. the product be easy to transport and to store
4. that it be safe
5. that it be acceptable, and
6. that it be effective.

Five large groups of vegetable products which can be used for this purpose are:

1. legumes
2. oil seeds
3. nuts
4. Palm kernels, and
5. leaf proteins.

Legume seeds form a vast group with a protein content of around 25 percent. Legumes such as the soy bean have received the greatest attention. Cooked soybean meal contains approximately 44 percent protein of a quality superior to most vegetable protein. Its strong, slightly bitter flavor has limited its acceptability for some people. Legumes include the grain and dals so commonly used in Pakistan.

Another group of important protein sources is that of oil seeds such as cotton, sesame, mustard, poppy, linseed, sunflower, rape, hemp and several others. Cotton seed flour has a biological value of almost 65 percent. It has a very favorable amino acid pattern for complementing cereal protein.

Sesame has a good flavor which makes it very palatable. It has a mixture value similar to cohon seed.

The nuts are not low enough in cost and not abundant enough in quantity to offer important practical advangages for large scale protein supplementation of cereal diets. Leaf protein quality is not better than that of the legumes. It is relatively deficient in methionine and cystine. Many other plant materials may be used as sources of protein concentrates. For example the by-product of the cereal industry such as the germ of the seeds. Maize germ has been used in South Africa to enrich vegetable mixtures (36).

Soy bean products are popular among Japanese as a source of protein for weanling infants. Soybean is given in the form of vatto (soybean curd) and kinako (toasted soybean flour) which proved to be quite acceptable and growth promoting for them (24).

Incaparina mixture, used in extensive trials with rats and chicks, showed that the mixture sustained good growth although it was improved by the addition of lysine. This mixture has the following formula (36, 13):

	%
Dried maize maser	50
Sesame flour	35
Cotton seed flour	9
Torula yeast	3
Kikyum grass leaf meal	3

It has 25.1 percent protein, 13.7 percent fat and 503 calories per 100 grams. Compared with the Food and Agriculture Organization reference protein, the limiting amino acids in decreasing order were lysine, tryptophan and methionine. However this formula proved to be very expensive due to the low supply and high cost of ingredients. So another mixture called 9A was prepared which was based on cotton seed flour as the principle concentrated protein source. Later another mixture called 9B was prepared which had uncooked maize and sorghum. The composition of Incaparina vegetable mixture 9B is as follows (36, 15):

Contents	Per 100 grams	Nutrient	Per 100 gms.
Moisture	7.6	Thiamine	2.1 mgs
Protein	27.5	Riboflavin	1.1 mgs
Ether Extract	4.2	Nicotinic Acid	7.8 mgs
Ash	3.5	Vit A. acetate	450 I.U.
Crude fiber	2.4	Calcium	0.5 mgs
Carbohydrate	54.8	Phosphorus	0.8 mgs
Calories	370.9	Iron	6.2 mgs

The essential amino acid composition of Incaparina Vegetable Mixture No. 9B is given below (36, 16):

Amino Acid	Grms./100 gms	% F.A.O. Ref. Pattern
Arginine	2.3	
Histidine	1.0	
Isoleucine	1.12	94
Leucine	2.08	154
Lysine	1.53	129
Phenylalanine	1.52	192
Total sulphur amino acid	0.92	77
Threonine	0.87	110
Tryptophan	0.24	61
Valine	1.14	96

In Central America, Incaparina is most commonly used as an "atole," a thin gruel made by adding one glass of water for each 25 grams of mixture and cooling it for 15 minutes. It is flavored to taste with sugar, vanilla and cinnamon and served cold or hot. Incaparina can also be substituted for two-thirds of the flour in most non-bread recipes calling for wheat flour and can be made into puddings as well as used for enriching soups. It has a high acceptability for mass feeding.

The concentrated protein sources which have received the most attention in this regard are the common beans, cow peas, Bengal gram, soy bean flour, peanut flour, cotton seed flour and copra proteins (28).

There are a number of factors which have to be considered before a mixture can be claimed satisfactory. These factors are discussed in the following paragraphs (33).

Mixed proteins should contain enough of each essential amino acid to supply the daily needs of the individual when it is added to the usual protein consumed. The amino acids of the combined food should have an adequately balanced pattern.

Some vegetable proteins have been shown in the past to contain various toxic principles which make them unsatisfactory for human use. For the protein to be accepted as safe, it must be shown to have no toxic substances and to develop none after processing or storage. Storage is one of the most difficult problems connected with the use of protein mixtures.

Most people cannot afford expensive food items. To keep down the cost and for ease of acceptance, locally available ingredients need to be used if at all possible.

CHAPTER III

METHOD OF PROCEDURE

Some of the grains and legumes used in this study include whole wheat flours, white rice in the form of flour, cotton seed as flour and finally - ground mung beans. Sunflower seeds and pumpkin seeds, shelled and finely ground. Dried skimmed milk solids and dried whole fish flour were also used as sources of protein. The reason for including the animal protein was to compare the growth effect of these mixtures with those containing only vegetable protein.

Before the author started formulating the diet mixtures, she made a thorough review of all the pulses and grains that are grown in Pakistan, are cheap, widely available and are also accessible in the United States of America.

Determination of Composition of Diets

After selecting the particular grains to make into four separate diet mixtures, the combination of different ingredients in the four groups were calculated many times in different combinations so as to get the best ratio of the ingredients from the point of view of palatability and, more important than that, the content of the essential amino acids for the rat and their ratio to each other. It was realized that it is difficult to get a combination of all these essential amino acids in desirable ratios when vegetable protein alone is used.

Out of these four diet mixtures one was used as a Control Diet while the other three were used as Experimental Diets I, II and III.

The total protein content and the amino acid value of each individual ingredient to be used in the diets were calculated. The content of the diets used was determined by comparing a mixture of vegetable proteins or animal (whole fish flour) and vegetable protein with the essential amino acid needs of the rat per day.

While calculating these diets there were some important considerations to be kept in mind such as the calcium phosphorus ratio, the need for cod liver oil, the ratio of three essential amino acids and the presence of all the essential amino acids in their required amounts.

There has to be a certain calcium phosphorus ratio in order for the body to utilize these two important minerals. A good ratio of calcium to phosphorus is suggested to be 1 to 2 : 1. In all the three experimental diets it was found that either the amount of calcium was too low or the amount of phosphorus was too high creating an imbalance between the two minerals. In order to correct the imbalance calcium carbonate was added to all the three experimental diets making a proper ratio of 1 to 2 : 1 (calcium to phosphorus).

For the very reason that we could obtain the proper ratio in calcium to phosphorus no cod liver oil was added as it is needed only when there is an imbalance of the two minerals.

While trying to get sufficient amounts of all the essential amino acids in the diet mixtures, the author encountered another very important problem. This was the problem of obtaining a certain ratio (1:3:3) between three of the essential amino acids; namely, tryptpphan, lysine and methionine.

Certain changes were made at times in the amounts of different ingredients of all the diet mixtures. This was necessary to correct the ratio of the essential amino acids or to obtain a sufficient amount of an essential amino acid per day.

Upon calculation one of the essential amino acids, methionine, was discovered to be low in Experimental Diets I and III. But with a good ratio of cystine which can be converted into methionine two of the diets did not appear to create a big problem.

To get enough protein in the diets was quite a problem, especially with diets containing exclusively vegetable protein. But with shifting the kind and amounts of different ingredients in the mixtures it was possible to have the required amount of protein per day.

Providing sufficient calories was another problem while working with these diet mixtures. Expecially in Experimental Diet I the amount of calories were not enough in the beginning but with increasing and decreasing of certain ingredients it was possible to work out quite well. In Diet I the amount of cotton seed oil added was about 30 percent of the total diet. This increase in the amount of fat was to provide enough calories per day. The high content of oil made the diet very rich in fat.

From some previous studies (14) it was decided that a minimum of ten grams of the diet per animal should be allowed for one day. After formulating the diet mixtures it was decided to add 5 grams of raw carrots to each of the four diets making the total amount offered to 15 grams per animal per day and to furnish bulk. The feeding of the carrots was not continued because it causes diarrhea in the animals.

Composition and Preparation of the Diet Mixtures

The composition of the four diets in respect to the different kind and amount of ingredients used, the percentage of protein and fat, total calories, and the calcium-phosphorus ratio are given in Table I.

Ingredients like wheat flour, fish flour and cotton seed flour, which were already fine and did not require any grinding, were carefully weighed. For other ingredients like rice, mung beans, pumpkin seeds and sunflower seeds a waring blender was used to grind the separate ingredients to a very fine consistency. A fine sieve was used to separate the kernels from the hull of the sunflower and pumpkin seeds. Only the kernels were used in the diets.

After making all pieces of the ingredients in more or less uniform consistency they were weighed. The ingredients of each diet were thoroughly mixed in big bowls with the help of a large

TABLE I

COMPOSITION OF THE EXPERIMENTAL DIETS

Ingredients	Amt.	Cals/10 gms.	Prot.	Fat	Ca/P Ratio
Control	%		%	%	
Dried skimmed milk	60	53.7	25.8	21.1	1.01:1.0
Wheat flour (whole)	25				
Cotton seed oil	15				
Experimental Diet I					
Cotton seed flour	20	53.8	23.1	71.5	1.04:1.0
Mung beans	10				
Wheat flour (whole)	20				
Cotton seed oil	30				
Calcium carbonate	134.4 mg.				
Pumpkin seeds	20				
Experimental Diet II					
Fish flour (whole)	20	53.4	27.3	60.0	1.15:1.0
Pumpkin seeds	20				
Wheat flour (whole)	40				
Cotton seed oil	20				
Calcium carbonate	65 mg.				
Experimental Diet III					
White rice flour	10	48.1	34.9	30.0	1.10:1.0
Cotton seed flour	50				
Sunflower seeds	20				
Cotton seed oil	20				
Calcium carbonate	144 mg.				

The essential amino acids content of 10 grams each of the four diet mixtures is given in Table II.

TABLE II

THE ESSENTIAL AMINO ACID CONTENT OF FOUR DIET MIXTURES

Diets	Tryptophan Gms.	Threonine Gms	Isoleucine Gms.	Leucine Gms.	Lysine Gms.	Methionine Gms.
Control	0.0341	0.1096	0.1533	0.476	0.172	0.058
Expt. Diet I	0.0310	0.0739	0.1167	0.178	0.1152	0.0366
Expt. Diet II	0.0324	0.1149	0.1440	0.208	0.1904	0.0600
Expt. Diet III	0.0375	0.1040	0.1220	0.265	0.154	0.442
Daily Requirement of Rats	0.020	0.060	0.050	0.090	0.100	0.060

Table II (continued)

Diets	Cystine Gms.	Phenylalanine Gms.	Tyrosine Gms.	Valine Gms.	Arginine Gms.	Histidine Gms.
Control	0.0466	0.0947	0.0889	0.0965	0.1257	0.0491
Expt. Diet I	0.0442	0.1070	0.0427	0.1021	0.2300	0.0553
Expt. Diet II	0.0368	0.1180	0.0198	0.1364	0.2257	0.0508
Expt. Diet III	0.0603	0.1582	0.0184	0.1585	0.3352	0.0791
Daily Requirement of Rats	0.000	0.070	0.000	0.070	0.020	0.040

In the same way 10 grams of each of the four diet mixtures were calculated for the other essential nutrients needed by the rat per day as presented in Table III.

TABLE III

THE ESSENTIAL NUTRIENT CONTENT OF FOUR MIXTURES

Diets	Prot. Gms.	Fat Gms.	Fiber Gms.	Calc. mg.	Vit. A I. U.	Thiamin mg.
Control	2.546	2.103	0.790	81.250	600.180	3.320
Expt. Diet I	2.314	7.153	0.178	9.552	603.400	3.315
Expt. Diet II	2.732	6.035	0.164	96.71	601.400	0.084
Expt. Diet III	3.439	3.028	1.055	24.62	603.000	3.676
Daily Require-ments of Rats	2.580	0.450	0.050	63.010	3.320	0.040

Table II (Continued)

Diets	Riboflavin Mg.	Niacine Mg.	Iron Mg.	Nacl. Gms.	Phosphorus Mg.	Calories
Control	3.013	0.165	0.135	33.530	70.250	53.740
Expt. Diet I	3.079	3.359	1.064	1.490	54.484	53.840
Expt. Diet II	3.212	0.289	0.121	5.070	111.610	53.440
Expt. Diet III	3.132	0.941	0.964	2.769	87.51	48.140
Daily Requirement of Rats	0.100	0.230	0.310	0.110	53.00	49.150

fork. Then each diet mixture was stored in labeled bottles in a freezer.

Sampling and Care of Rats

Weanling albino male rats weighing 45 to 50 grams were used. These animals were obtained from the Holtzman Company and originally came from the Sprague Dawley strain. Each rat was confined to an individual wire mesh cage with removable bottom trays. Paper was changed in the tray daily and cages were scrubbed weekly with a strong detergent and water solution. The temperature of the room was maintained between 77° F. and 80° F. throughout the length of the experiment.

The experimental animals were received on Friday afternoon. They were fed a stock diet for two days to stabilize them following shipment. On Monday animals were randomely selected for each diet group for the experiment. One rat was placed in each of 16 cages which were previously numbered from one through 16. Numbers one through 16 were written on small pieces of paper, placed in a container and shaken well. Four numbers were drawn. Rats corresponding to these numbers were placed on the first shelf of the rack. Four more numbers were drawn from the container and rats in cages which corresponded to these numbers were placed on the second shelf. This procedure was repeated until all 16 animals were grouped in four groups of four rats each.

Feeding Procedure

The four rats selected for the Control Diet were placed on the top shelf. Four rats selected for each of the Experimental Diets I, II and III were placed on the three remaining shelves so that animals on the same diet were placed on the same shelf.

Rats in all four groups were marked by putting notches on their ears to distinguish them from each other.

The rats were weighed in the beginning of the experiment and each successive Monday and Friday.

To eliminate any possible environmental effect cages were rotated weekly one position to the right and one shelf down. The extreme right hand cages were placed in the extreme left position on the racks and bottom cages were moved to the top shelf. During the four weeks of the experiment every animal occupied a space on each shelf level for one week and was in each of the four possible horizontal positions for one week.

The food for rats on the Control Diet and Experimental Diets I, II and III were weighed daily. Any food not eaten was carefully weighed back. If evidence of spilled food was found the amount was estimated and added to the uneaten food weight.

Animals were fed ad libitum by increasing the amount fed above the amount eaten the previous day to be sure some excess remained. An unlimited amount of distilled water in glass drinking tubes was provided to the rats. On Saturday a double amount of food was given and no feeding was done on Sunday. Crockery feeding cups were used for all four groups.

All animals were fed during a four week period. At the end of this time the animals were given to the Psychology Department for further experimental use.

CHAPTER IV

RESULTS AND DISCUSSION

Experimental Diets I, II and III were fed to the animals for four weeks and their growth was compared with the animals in the Control Group. The Control Group was fed all the nutrients known to be needed for growth by the white rats (14).

Control Diet

On this diet the growth attained was below the growth of the standard curve although Griffith and Farris advocated 25 percent of the total diet as protein which was the approximate amount used in this study. More recent authors (27, 6) advocate 10 percent of the diet as protein.

A control diet containing 12 percent of good quality protein (milk) was fed to animals of the same age and strain by a coworker[1]. The results of the growth of her control animals are presented in Table B in the Appendix.

In this study in which vegetable proteins composed all or a major portion of the experimental diets it was believed to be necessary to feed 25 percent of protein in order to provide the essential

[1]Mrs. Evelyn Williams, graduate student in human nutrition at Oklahoma State University.

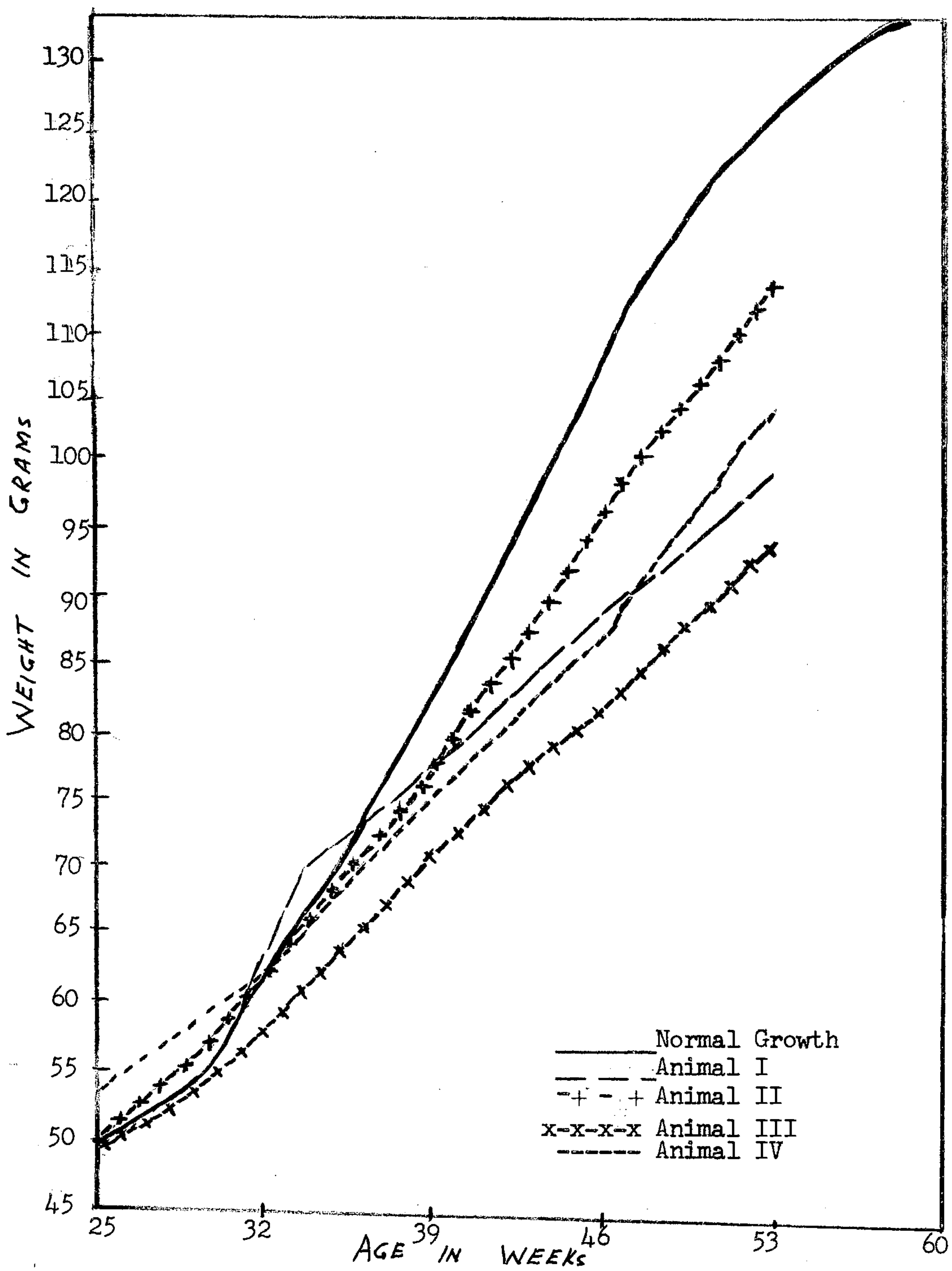

Figure 1. Growth of Weanling Rats on Control Diet

amino acids. Even when this much protein was fed it was possible to get the daily requirement of the rat for methionine in one diet (Expt. Diet II) only. However if the amount of cystine is considered, all of the diets would more closely approach the daily requirement of methionine.

The Control Diet in this study contained 60 percent of dried skimmed milk solids and 25 percent of total protein. Detailed records of food intake, weight gain and observations made during the four weeks of feeding for all the four rats in this group are presented in Table A in the appendix.

The animals in the Control Group were very well-nourished. Their coats were smooth and glossy. Their feet, tails and noses were pink in color and their eyes were a brighter pink. They were playful and active.

Experimental Diet I

This diet contained exclusively protein from vegetable sources, providing 23.1 percent protein to the rats in this group.

As can be seen from the growth curve of these animals, presented in Figure II, the growth of all the four anumals was much lower than the standard growth curve. From the very start of the feeding experiment no one of the four animals ever reached the standard growth curve throughout the four weeks. Two of the animals died before the termination of the experiment for unknown reasons.

Experimental Diet I was a high fat diet providing 71.5 percent fat from cotton seed oil, pumpkin seeds and mung beans. It was found

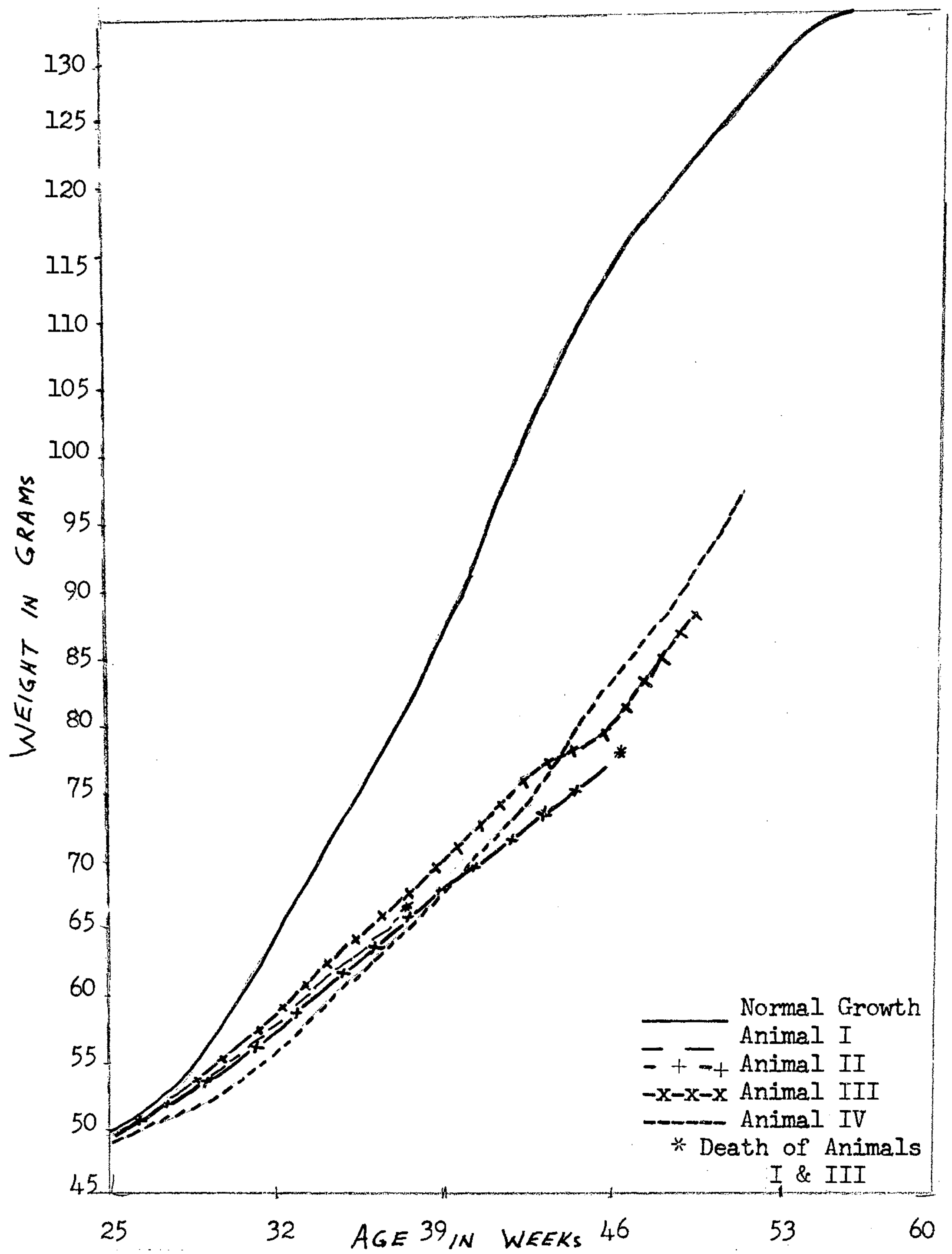

Figure 2. Growth of Weanling Rats on Experimental Diet I.

that this group of rats did not prosper on this diet. It may be possible that the high fat content in the diet caused some toxicity in the animals.

In Experimental Diet I an essential amino acid, methionine was very deficient, about 0.037 grams as compared to the daily requirement of 0.06 grams for the rat. This amount was the lowest among all the four diets and probably accounts for the poor growth of the animals.

There was an imbalance in the ratio of calcium to phosphorus in this diet, which was corrected by adding calcium carbonate.

Detailed records of food intake, weight gain, and observations made during the four weeks of feeding for all the animals in this group are presented in Table C in the appendix.

The animals in this group were small and stunted in growth and their coats had a dull,yellowish color. They seemed to be irritable at times or sat quietly in a corner of their cage.

Experimental Diet II

This diet contained a part of animal protein in the form of whole fish flour (20 percent of the diet). The rest of the protein was from good sources of plant protein food furnishing a total of 27 percent protein.

As far as the total growth attained on this diet was concerned, it can be observed from the growth curve on the next page that the highest growth attained among all the four groups was on this experimental diet--better growth than on the control diet.

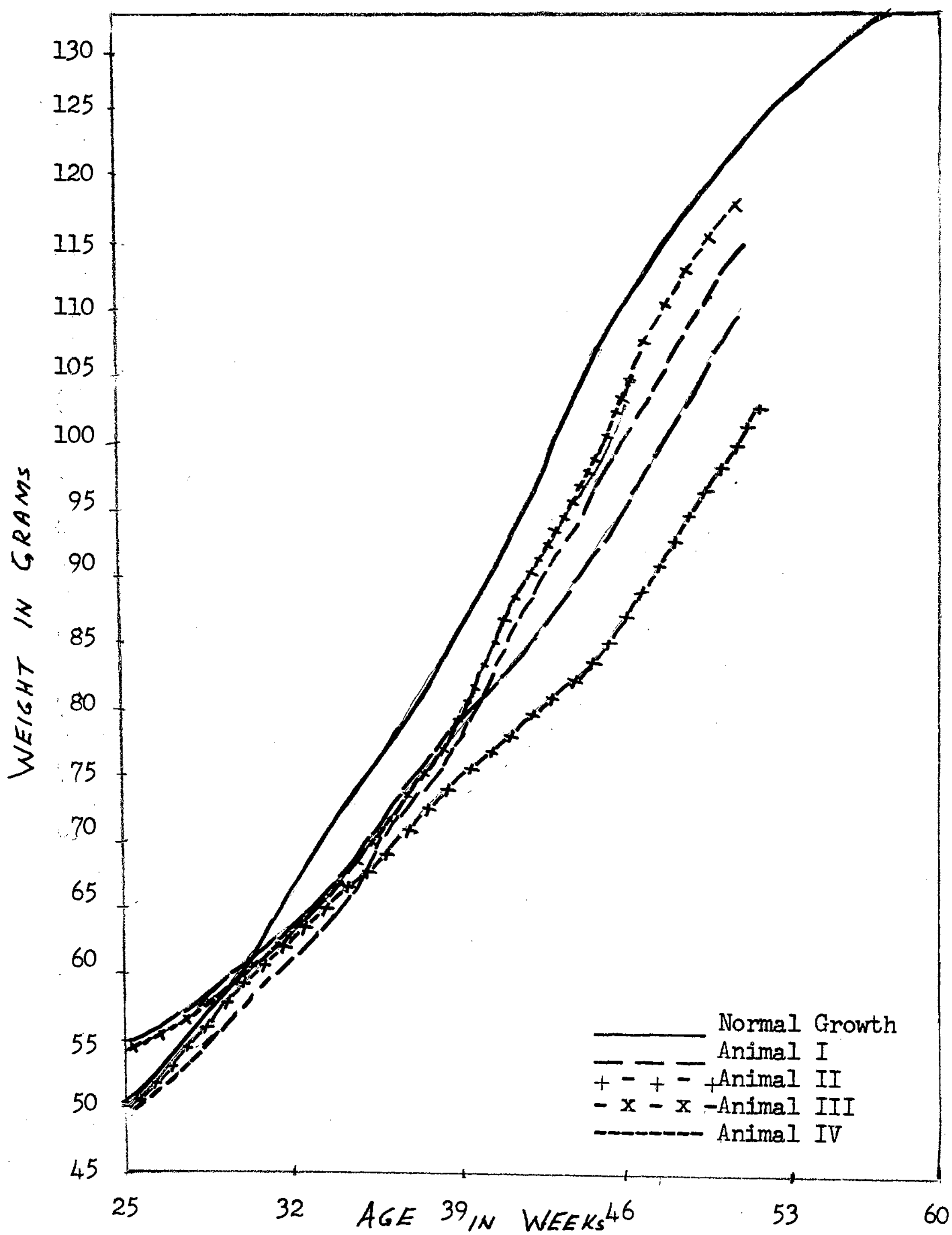

Figure 3. Growth of Weanling Rats on Experimental Diet II.

Although none of the four rats reached the normal growth curve at the end of the experiment, only one of the rats was below the normal growth curve at the beginning of the feeding.

It is surprising to note that although this diet contained about 60 percent fat, still the animals did quite well on this mixture. It would be hard to suspect any toxicity from the fat used here. Their good growth was attributed to the presence of some animal protein which supplemented the vegetable protein mixture in relation to essential amino acids.

Another very good aspect of this diet which contributed to the prosperity of the animals was that this was the only experimental diet which had the required amount of one of the essential amino acids, methionine, which was found very deficient in some of the other diets.

The rats on this diet were vigorous and energetic in their movements. They had strong muscles, and appeared to be very well nourished. Their coats were extremely smooth and glossy, their eyes, tail, feet and nose were pink in color. They were active and playful.

Detailed records of food intake, weight gain and observations made during the four weeks of feeding for all the four animals in this group are presented in Table D in the appendix.

Experimental Diet III

The protein in this diet came exclusively from plant sources. Still it provided the highest content of protein, about 35 percent, among all the four groups.

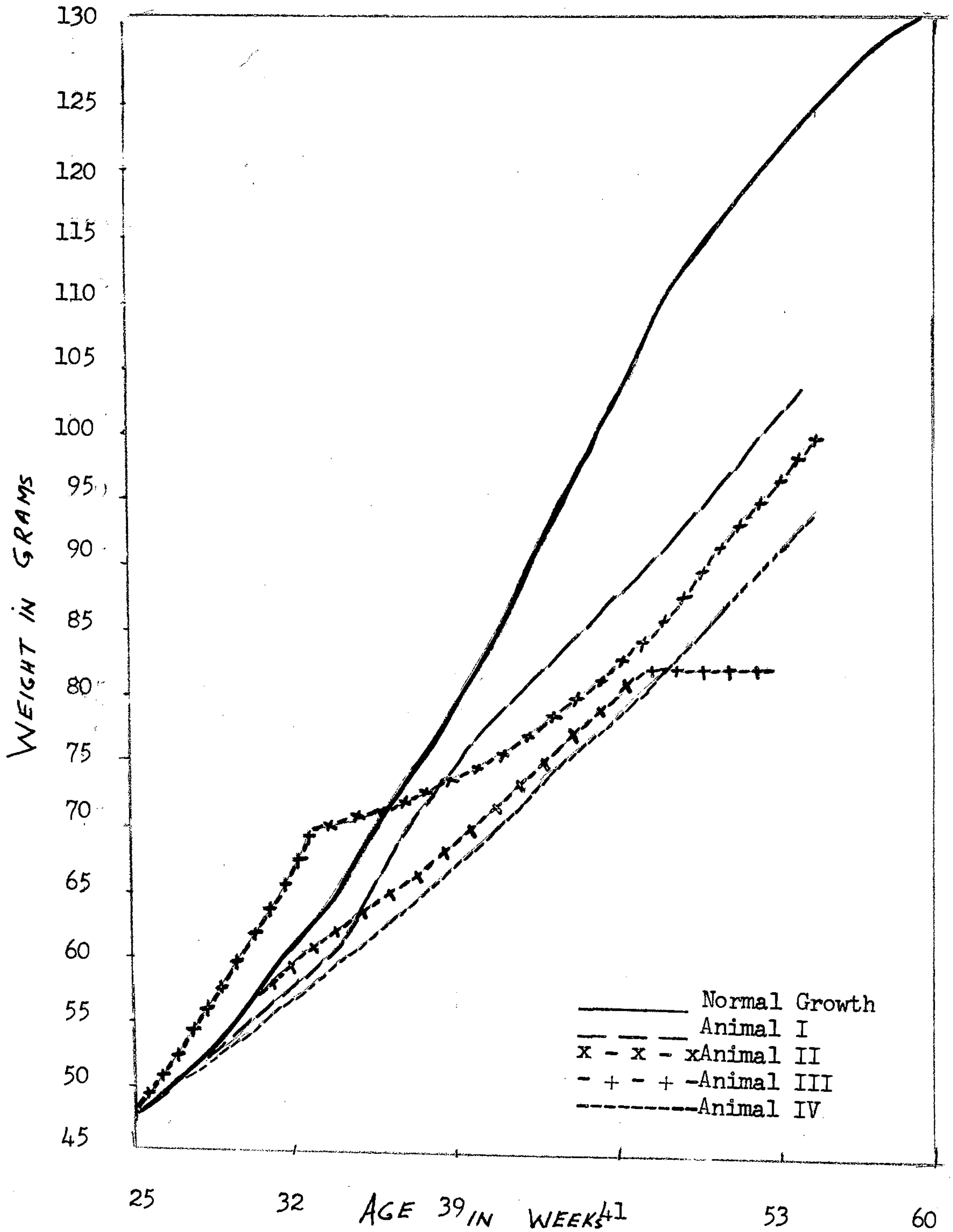

Figure 4. Growth of Weanling Rats on Experimental Diet III.

One difference in the composition of this diet was that, unlike the three diets which contained whole wheat flour as one of the grains, it contained white rice flour instead.

On this diet the growth attained was below the normal growth curve. Only one of the four animals reached the normal growth curve during the first week of feeding but then dropped down again.

This was the second lowest diet as far as the amount of one of the essential amino acids, methionine, was concerned. The amount was only 0.044 grams as compared to the 0.06 grams, required daily by the rat.

Calcium carbonate was added to correct the calcium-phosphorus ratio imbalance to within a 1 to 2:1 limit.

The daily calories in this diet (48.1) were slightly beneath the amount calculated to meet the requirement of 49.1 calories as recommended (14).

The animals in this group appeared to be small, fragile to touch and irritable. They had dull, yellowish coats. The appearance of these animals was not healthy as compared to the other groups with rats in the same weight range as these animals.

Detailed records of food intake, weight gain and observations made during the four weeks of feeding for all the four rats in this group are presented in Table E in the appendix.

In order to compare the average weekly weight gains of four animals in each group, Table IV is presented.

The average weekly food intake of four animals in each of four groups is presented in Table V.

TABLE IV

AVERAGE WEEKLY WEIGHT GAIN OF FOUR ANIMALS
IN EACH OF FOUR GROUPS

Week	Control Group	Expt. Diet I	Expt. Diet II	Expt. Diet III
1	5.6	4.7*	8.4	6.7
2	5.8	5.6	8.2	6.4
3	6.6	6.4*	6.6	4.6
4	6.5	5.3	8.9	5.7
Total	24.5	10.9	32.1	23.4
Average of				
4 Animals	6.12	5.45	8.02	5.85

*These animals died before the termination of the experiment and were not used in the average weight gain in Experimental Diet I.

TABLE V

AVERAGE WEEKLY FOOD INTAKE OF FOUR ANIMALS
IN EACH OF FOUR GROUPS

Weeks	Control Group	Expt. Diet I	Expt. Diet II	Expt. Diet III
	Gms	Gms	Gms	Gms
1	55.12	52.50	58.50	56.70
2	55.75	44.25*	50.85	38.62
3	64.25	49.15*	66.75	32.20
4	65.37	47.25	68.15	34.12
Total	240.49	193.15	244.25	161.64
Average for				
4 Animals	60.12	48.28	61.06	40.41

*In Experimental Diet I the average food intake for the second week was based on three animals and that for the third week was based on two rats because two animals died.

Protein Efficiency Ratio

Protein Efficiency Ratio (26) is the gain in body weight of a growing animal divided by its protein intake. It is a measure to evaluate the quality of protein when determined under specific conditions. The caloric intake must be adequate and the protein must be fed at a level which will promote growth and maintenance of the animals.

It has been observed that when the animals are fed at surfeit levels, weight will no longer increase with protein intake and the ratio will fall.

This measure of determining the protein quality of a food was devised by Osborne and Mendel in 1915 (25).

The protein efficiency ratio of the four diets used in this study is calculated[1] by applying the following formula:

$$PER = \frac{\text{Amount of protein (Gms consumed)}}{\text{Increase in the weight gained}}$$

In Table VI the protein Efficiency Ratios of the four diets are given.

The quality of a protein is determined by the presence of all the eight essential amino acids in it. Even if one of the essential amino acids is absent, or low in amount, the growing rats will fail to prosper. Moreover there are certain recommended daily allowances for these essential amino acids which must be present for the maximum maintenance and growth of the animal body.

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[1]See appendix B for detailed calculation of these ratios.

TABLE VI

PROTEIN EFFICIENCY RATIO OF FOUR DIETS

Diets	Protein Efficiency Ratio (PER)
Control Diet	1.01
Expt. Diet I	1.17
Expt. Diet II	1.03
Expt. Diet III	1.42

It can be seen that the lower the Protein Efficiency Ratio the more desirable the diet becomes in terms of animal growth.

In the formulation of these diets, great effort was made to provide all the essential amino acids in amounts required by a growing rat. Only one of the eight essential amino acids, methionine, was low in Experimental Diets I and III. This is true when vegetable proteins are used to supplement each other. Only a small amount of animal protein would have provided the methionine necessary to bring the daily requirement to the desirable levels in Diets I and III.

For the full utilization of a protein it is essential that there should be enough calories in the diet to provide energy needed. Otherwise part of the protein content would be used for providing energy rather than maintenance and growth. While formulating these diets it was realized that there was a shortage of calories in two diets (Expt. Diet I and II).

Cotton seed oil was added in necessary amounts to meet the requirements for calories in these diets. The reasons for oil as a supplier of calories were:

1. It is a very concentrated source of calories so it would have little effect on the total volume of the diet.
2. In Pakistan people eat a high content of vegetable fat and it is easily available to them.

Great care was taken in providing the desirable calcium to phosphorus ratio (1 to 2:1) in all the four diets. In the three experimental diets there was an imbalance between the two minerals which was corrected by adding calcium carbonate.

Since an acceptable ratio of calcium to phosphorus could be obtained in these diets no cod liver oil was added.

When all the essential amino acids are present, there has to be a certain ratio between three of the essential amino acids namely tryptophan, lysine and methionine. The ratio between these amino acids should be 1:3:3 respectively. If there is an imbalance between these three amino acids then there will be less, or an incomplete, utilization of the other essential amino acids.

CHAPTER V

SUMMARY AND RECOMMENDATIONS

The four diet mixtures used in this study did not permit animals to perform satisfactorily. The main reason for this was the low methionine content of some of these diet mixtures. Therefore before these mixtures can be recommended for human consumption it is necessary to increase the methionine content by substituting some of the ingredients with some other native ingredients which have a higher content of methionine. Vegetable protein sources often are too bulky when enough is eaten to be adequate in essential amino acids.

To increase the vitamin A content and make it more palatable for human use the addition of carrots or lettuce might be permitted.

After these necessary alterations are made in th experimental diet mixtures they can be used in many different ways for human consumption.

The use of vegetable protein mixtures plus some protein-rich concentrates of high biological value should be encouraged because the population of Pakistan consists of large numbers of infants, growing children, expectant and nursing mothers (50 percent of the total population in East Pakistan as revealed by the East Pakistan Nutrition Survey of 1964). This segment of the population needs a higher intake of good quality protein than any other group.

Unfortunately in Pakistan very little alterations are made in the pattern of diet when it is fed to children, pregnant women and nursing mothers. Especially the protein value of the diet is inadequate to meet their requirements.

Protein values may be reduced when caloric intakes are not sufficient as part of the protein will be used for calories. In the case of the majority of the people in Pakistan they do not have enough food to provide even their caloric requirements. The four diet mixtures provide enough calories to meet the caloric needs of the people.

An effort should be made to incorporate these diet mixtures into dishes prepared for old people as very little attention is given to their diets. These diet mixtures can be an ideal basis in the preparation of diets and meals for especially the vulnerable sections of the people, if a small amount of animal protein can be used with them (curd, dried milk and fish or fish flour).

In a developing country like Pakistan where the protein supplies may be below 60 grams daily and those of animal protein less than 10 grams per day (18), great attention should be given to the use of such protein mixtures. An effort should be made to popularize their use in the Pakistan dietary pattern.

These vegetable mixtures could be taken by a commercial concern as a basis for preparation of packaged food mixes on a large scale. Such products might be made available to sell in open markets.

It was recommended by the Pakistan Education Commission Report (1959) that every educational institution should have a school lunch program. Protein mixtures like these could be used as a basic food in lunch programs, especially since the ingredients in these mixtures are available and are quite cheap. With the addition of only a small amount of animal protein they would meet the requirements for essential amino acids.

Foods prepared from these four diet mixtures such as biscuits, Roti (bread), and a beverage may be good items in a school lunch program.

These diet mixtures can be used in dishes for patients in hospitals who are allergic to certain animal protein or for some other reason cannot consume it. The medical authorities can recommend these diet mixtures for patients coming from low socio-economic levels because they can not afford to consume animal proteins which are quite expensive.

Maternal and child welfare centers may be a good media for popularizing these diet mixtures among the expectant and nursing mothers who need a good amount of protein.

The Home Economics Institutions should include these diet mixtures in the nutrition and meal planning classes to encourage the students to use them in a way which is palatable. In this manner they can transmit this knowledge in their homes.

BIBLIOGRAPHY

1. Adolph, W. H., Shames, E. A., and Halaby, S. H.: The nutritive value of legume protein and legume wheat mixed protein in Near East diet. Food Research 20:131, 1955.

2. Ali, S. M.: Problems and Some Solutions: Pakistan Proc. Nutr. Soc. London, Mar., 1961.

3. Ali, S. M.: Effect of diet on health and longevity. Reprinted from Pakistan J. Sci. 19: 6, 1962.

4. Ali, S. M.: Deficiency diseases prevalent in Pakistan. Unpublished M.S. Thesis. Lahore: University of the Punjab Library, 1962.

5. Ali, S. M.: An experimental study of the nutritional properties of Pakistani diets with some practical applications. Unpublished PhD. Dissertation. London: University of London Library, 1962.

6. Altschul, A. M.: Proteins. Their chemistry and politics. New York: Basic Book Inc., 1965.

7. Brock, J. F.: Dietary proteins. Recent advances in Human Nutrition. London: J. & A. Churchil Ltd., 1961.

8. Chaney, M. S.: Nutrition. 6th ed. Boston: Houghton Mifflin Co., 1960.

9. Dean, R. E.: Processed plant protein food stuffs. N. Y. Acad. Sci., 1958.

10. Evaluation of protein nutrition. Natl. Acad. Sci. - Natl. Research Council Pub. 711., 1959.

11. FAO International Conference on Fish in Nutrition. Washington, D. C.: U. S. Department of the Interior Fish and Wild Life Service, Bureau of Commercial Fisheries. Sept. 19-27, 1961.

12. Food and Nutrition Bd.: Recommended Dietary Allowances, Revised 1963 Natl. Acad. Sci.--Natl. Research Council Pub., 1146. 1964.

13. Geiger, E.: The role of time factor in protein synthesis. Science 11:594, 1950.

14. Griffith, J. A. and Farris, J. E.: The rat in laboratory investigation. Philadelphia: J. B. Lippincott Co., 1942.

15. Hansen, J. D.: Protein malnutrition and its prevention and treatment. In Brock, J. F. ed.: Recent Advances in Human Nutrition. London: J. & A. Churchil Ltd., 1961.

16. Hegsted, D. M.: Role of wheat and wheat products in human nutrition. Physiol. Rev. 34:221, 1954.

17. Hopkins, F. G.: Feeding experiments illustrating the importance of accessory factors in normal dietaries. J. Physiol. 5: 629, 1912.

18. Joint FAO/Who Expert Committee on Nutrition. Third Report. 1953, Rome.

19. Karlson, P.: Introduction to Modern Biochemistry. 2nd ed. New York: Academic Press, 1965.

20. Kik, M.C.: Nutritional improvement of rice diets and effect of rice on nutritional value of other food stuffs. J. Am. Dietet. A. 32:647, 1965.

21. Leverton, R. M.: Amino Acids Food. USDA Year Book. Washington, D. C. Govt. Printg. Office., 1959.

22. Leverton, R. M.: Protein Food. USDA Year Book. Washington, D. C. Govt. Printg. Office., 1959.

23. Leverton, R. M. and Gram, M. R.: Nitrogen excretion of women related to the distribution of animal protein in daily meals. J. Nutr. 39: 59, 1949.

24. Mutto, S., Takabashi, E., Hara, M., and Konuma, Y.: Soybean products as protein sources for weanling infants. J. Am. Dietet. A. 43:5, 1963.

25. Osborne, T. B., and Mendel, L. B.: The comparative nutritive value of certain protein in growth and the problem of protein minimum. J. Biol. Chem. 20:351, 1915.

26. Protein Requirements. Joint FAO/WHO Group Report. WHO Tech. Report Series No. 301, 1965., Rome.

27. Rama Rao, P. B., Norton, H. W., and Johnson, B. C.: Amino acid requirement as pattern for protein supplementation. J. Nutr. 82:88, 1964.

28. Register, U. P.: Are non-flesh proteins adequate. The Herald of Health, 11:17, 1963.

29. Rose, W. C.: The nutritive significance of amino acids. Physiol. Rev. 18:109, 1938.

30. Rose, W. C.: The amino acid requirement of adult man. Nutr. Abstr. and Rev. 27:631, 1957.

31. Rose, W. C., Wixom, R. L., Lockhart, H. B., and Lambert, G. F.: The amino acid requirement of man XV. The valine requirement; Summary and final observation. J. Biol. Chem. 217: 987, 1955

32. Rose, W. C.: Adult protein requirements Panel VI Protein and amino acids in nutrition. Washington, D. C.: Fifth International Congress on Nutrition, 1960.

33. Sai, F. T.: Introducing new foods against protein deficiency. Nutr. Rev. 18:353, 1960.

34. Salmon, W.D.: Imbalance of amino acids in nutrition. Am. J. Clin. Nutr. 6:487, 1958.

35. Schaefer, A. E.: Nutritional deficiencies in developing countries. J. Am. Dietet. A. 42:295, 1963.

36. Scrimshaw, N. S. and Bressani, R.: Vegetable protein mixtures for human consumption. Panel II. Protein and amino acids in nutrition. Washington, D. C.: Fifth International Congress on Nutrition., 1960.

37. Scrimshaw, W. S., Bressani, R., Wilson, D. L. Chung, N., and Behar, N.: Supplementation of cereal protein with amino acids VI. Effect of amino acids supplementation of rolled oats as measured by nitrogen retention in young children. J. Nutr. 81:399., 1963.

38. Swanson, P.: Influence of non-protein calories on protein metabolism. Fed. Proc. 10:660, 1951.

39. Watt, B. K. and Merrill, A. L., Composition of foods raw, processed, prepared. USDA Agric. Hand Book No. 8, 1963.

40. Willcock, E. G., and Hopkins, F. G.: The importance of individual amino acids in metabolism. J. Physiol. 35:88, 1905.

A P P E N D I X

APPENDIX A

TABLE A

FOUR WEEKS RECORD OF FEEDING THE CONTROL DIET
Control Diet (No. 1 Animal)

Ear Mark	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
	1966	grams	grams	grams	grams	
	Feb.					
None	14	47.3	15	6	8	Active. 1 gram wasted
	15		15	7	8	
	16		15	4	10	1 gram wasted
	17		15	6	9	
	18	53.8	15	9	6	
	19		20	10	10	
	21	68.3	15	9.5	5.5	Lazy
	22		15	10.5	4.5	
	23		15	8.3	6.7	
	24		15	11.5	3.5	Not eating enough
	25	73.2	15	8.0	7.0	
	26		20	2.0	18.0	Sneezing
	28		15	5.8	9.2	
	March					
	1		15	7	8	Not enough water taken
	2		15	6.5	8.5	
	3		15	6	9	Becoming eating more
	4	84.9	15	3	12	
	5		25	7	18	Quite active and play-
	7	90.8	15	5.5	9.5	ful
	8		15	6	9	Becoming irritable at
	9		15	6.5	8.5	times
	10		15	6	9	Sneezing
	11	95.9	15	6	9	
	12		25	4	19	Ate very well
	13	101.7	15	7	8	
	14		15	8	7	

Control Diet (No. 2 Animal)

Table A (continued)

Ear Mark	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
	1966	grams	grams	grams	grams	
	Feb.					
Rt-1	14	49.5	15	5	10	
	15		15	5	10	Ate good
	16		15	5	10	Happy and playful
	17		15	6	9	
	18	56.9	15	10	5	Not enough water
	19		20	9	8	
	21	60.8	15	10	5	Did not eat good
	22		15	10	5	
	23		15	9.5	5.5	Diarrhea
	24		15	11.5	3.5	Seems to have no appetite
	25	66.6	15	9.5	5.5	
	26		20	9.7	5.3	Slow in his action
	28	73.3	15	9.5	5.5	
	March					
	1		15	9.5	5.5	Diarrhea
	2		15	9	6	
	3		15	7.5	7.5	Becoming active
	4	78.0	15	7.5	7.5	
	5		25	9	16	Ate good
	7	82.6	15	5.5	9.5	
	8		15	8	7	
	9		15	6	9	Ate good. Rather quick
	11		15	4.5	10.5	in his actions
	12		25	16	9	
	13	96.0	15	5	10	
	14		15	4	10	1 gram wasted

Control Diet (No. 3 Animal)

Table A (continued)

Ear Mark	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
	1966 Feb.	grams	grams	grams	grams	
Left No. 1	14	51.1	15	4	11	Ate quite good
	15		15	5	10	
	16		15	3	10	Active
	17		15	2	13	Ate very good
	18	64.3	15	9	6	Appetite is
	19		20	9.5	4.5	Decreasing. Ate very little
	21	65.8	15	5	10	
	22		15	9	6	
	23		15	7	8	Getting to like the food
	24		15	8	7	
	25	72.1	15	7	8	
	26		20	1	19	Ate almost all of the food
	28	80.0	15	5	10	
	March					
	1		15	6.5	8.5	
	2		15	5.5	9.5	Active and playful
	3		15	2.5	12.5	Ate good
	4	88.0	15	4	11	
	5		25	3.6	21.4	Ate quite good
	7	96.2	15	2.5	12.5	Seemed to have good apetite
	8		15	4	11	
	9		15	4	11	Happy and active
	10		15	4	11	
	11	105.2	15	5.5	9.5	Appearance is good
	12		25	7.0	18.0	
	13	114.2	15	6	8	1 gram wasted
	14		15	7	8	

Control Diet (No. 4 Animal)

Table A (continued)

Ear Mark	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
	1966	grams	grams	grams	grams	
	Feb.					
	14	53.6	15	6	9	Ate good
	15		15	4	11	
	16		15	3	12	Has a good appetite
	17		15	2	13	Drank all his water
	18	58.1	15	6	9	
	19		20	5	9	Active and playful
	21	65.0	15	7	8	
	22		15	9	6	
	23		15	5	10	Ate good
	24		15	7.5	7.5	
	25	70.6	15	3.5	11.5	
	26		15			Ate almost all of his food
	28	77.0	20	1	19	
	March					
	1		15	2.5	12.5	
	2		15	8	7	
	3		15	5.5	9.5	Good appearance
	4	83.0	15	3	12	
	5		25	15	23.5	Ate well
	7	88.9	15	2	13	Ate well
	8		15	5	10	Drank all water and the water tube was empty
	9		15	4	11	
	10		15	4	11	
	11	97.3	15	4.5	10.5	Happy and active
	12		25	9	16.0	
	13	105.3	15	4	10	1 gram wasted
	14		15	5	10	

Results of feeding approximately a 12 percent protein diet to control animals in the feeding experiment of a colleague.

TABLE B

WEEKLY FOOD EATEN AND WEIGHT GAINED BY RATS IN THE CONTROL GROUP I
Control Group I

Date	Rat No. 1		Rat No. 2		Rat No. 3		Rat No. 4		Rat No. 5		Rat No. 6	
	Food Eaten	Wt. Gained	Food Eaten	Wt. Gained	Food Eaten	Wt. Gained	Food Eaten	Wt. Gained	Food Eaten	Wt. Gained	Food Eaten	Wt. Gained
April	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm
12-18	67	27.5	56.5	20.5	50.4	16.5	48.5	20.0	60.5	28.0	59.5	22.0
19-25	79	27.5	73.5	32.0	66.5	24.5	68.5	19.5	74.0	26.5	81.0	35.5
26-May 2	69	19.0	92.5	33.5	80.0	28.5	87.5	23.5	92.5	24.5	99.0	-7.5
May												
3- 9	95.5	34.5	95.0	24.5	100.0	29.0	93.0	29.0	74.0	20.5	81.5	48.0
10-16	96.0	16.5	95.5	25.5	112.5	31.5	101.0	29.0	98.0	35.5	96.0	30.0
17-23	101	20.0	111.5	22.0	104.0	22.0	sacrificed		sacrificed		97.5	16.0
24-30	105.5	24.0	111.0	31.0	115.5	30.0					114.5	23.0
31-Jn.6	107	16.0	108.0	15.0	126.0	27.0					97.0	17.0
June												
7-13	115	11.0	65.0	8.0	124.0	12.0					122.0	20.0
14-20	101	15.0	died	died	113.0	17.0					95.0	18.0
Total	1043	211.0	808.5	212.0	1001.9	237.0	398.5	121	399.0	134.5		
Av.	104.3	21.1	89.83	23.55	100.19	23.70	79.7	24.2	79.8	26.9		

TABLE C

FOUR WEEKS RECORD OF FEEDING THE EXPERIMENTAL DIET I
(Animal No. 1)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
2-Rt.	1966 Feb.	grams	grams	grams	grams	
	14	48.6	15	2	13	Ate good, very playful
	15		15	4	11	Ate well
	16		15	6	9	Ate well. Happy and active
	17		15	7	8	
	18	56.0	15	3	7	5 grams of food wasted
	19		20	10	6	4 grams of food wasted
	21	59.0	15	5	6	4 grams of food wasted
	22		15	11	4	The animal seems to be irritable
	23		15	11	4	Did not eat well
	24		15	12	3	Drank little water
	25	64.1	15	11	3	Did not eat well
	26		20	11.5	5.5	
	28	69.0	15	11	4	Lazy and slow in his movements
	March					
	1		15	12.2	2.8	Ate very little
	2		15	12.0	2	Ate very little, 2 grams wasted
	3		15	12.7	2.3	Ate very little
	4	76.0	15	13	1	Ate anything hardly, 1 gram wasted
	5		20	11.5	8.5	Suddenly ate well
	7	78.3	15	10.5	8.0	
	8		15	9	5	Did not eat well
	9		15	5.5	9.5	Ate well
	10	Died				Blood on his nose and mouth

Experimental Diet I (Animal No. 2)

Table C (continued)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
	1966	grams	grams	grams	grams	
1 left, 1st	Feb. 14	49.3	15	5	10	Very active and playful
	15		15	6	9	Ate good
	16		15	4	8	3 grams of food wasted
	17		15	6	7	2 grams of food wasted
	18	57.4	15	5	9	1 gram of food wasted
	19		20	3	10	Ate not well, 5 grams of food wasted
	21	61.5	15	6	5	5 grams of food wasted
	22		15	5	10	Seemed developing appetite for the food
	23		15	4	8	3 grams of food wasted
	24		15	5	6	4 grams of food wasted
	25	66.2	15	5	4	6 grams of food wasted
	26		20	1.5	13.5	5 grams of food wasted
	28	72.0	15	4	5	6 grams of food wasted
	March 1		15	10	5	Did not eat well
	2		15	9	6	Did not drink enough water
	3		15	7	8	Ate well
	4	75.3	15	7	8	Ate well
	5		20	12	8	Did not eat well at weekend
	7	79.8	15	5.5	4.5	
	8		15	6.5	8.5	
	9		15	6	7	2 grams of food wasted

Experimental Diet I (Animal No. 2)

Table C (continued)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
	1966	grams	grams	grams	grams	
1 left,	March					
1st	10		15	12	3	Did not eat well
	11	86.3	15	7.5	7.5	
	12		20	9	10	1 gram wasted
	13	94.8	15	8	7	
	14		15	5	10	Ate Well

Experimental Diet I (Animal 3)

Table C (continued)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
1 left,	1966	grams	grams	grams	grams	
2-rt.	Feb.					
	14	49.7	15	2	13	Ate well
	15		15	4	11	Quiet and lazy looking
	16		15	8	7	Sneezing
	17		15	8	7	
	18	55.2	15	9	6	
	19		20	14	6	Did not eat good and drank little water
	21	59.4	15	10	5	
	22		15	12	3	Did not eat well
	23		15	9	5	Not active and playful
	24		15	13	2	Ate very little
	25	65.3	15	11.5	3.5	Seems very weak and drowsy
	26		20	15	5	Sneezing
	28	69.0	15	14.5	0.5	Ate very little (.05 grams)
	March					
	1		15	15	-	Did not eat at all
	2		10	Died		Blood on his nose and mouth

Experimental Diet I (Animal No. 4)

Table C (continued)

Ear Mark	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
	1966	grams	grams	grams	grams	
2 left	Feb.					
1 rt.	14	48.3	15	4	11	1 gram of food wasted
	15		15	2	12	The animal seems to be active and playful
	16		15	2	12	1 gram of food wasted
	17		15	7	8	Appetite is decreasing
	18	53.4	15	5	9	1 gram of food wasted
	19		20	3	10	7 grams of food wasted
	21	57.9	15	5	7	3 grams of food wasted
	22		15	8.5	6.5	His food intake is decreasing
	23		15	4	6	5 grams of food wasted
	24		15	4	4	7 grams of food wasted
	25	62.2	15	2	6	7 grams of food wasted
	26		20	7.5	12.5	
	28	68.0	15	2.5	9.5	3 grams of food wasted
	March					
	1		15	7.3	6.3	About 2 grams of food wasted
	2		15	2	13	Active and playful animal
	3		15	2.5	9.5	3 grams of food wasted
	4	76.0	15	4	9	2 grams of food wasted
	5		25	14	11	Not drinking enough water
	7	80.1	15	8	6	1 gram of food wasted
	8		15	7	7	1 gram of food wasted

Experimental Diet I (Animal 4)

Table C (continued)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
	1966 March	grams	grams	grams	grams	
	9		15	7.5	7.5	
	10		15	10	5	
	11	91.8	15	6.5	8.5	He is active and seems to be happy
	12		20	10	10	
	13	100.6	15	9	6	
	14		15	9	6	

TABLE D

FOUR WEEKS RECORD OF FEEDING EXPERIMENTAL DIET II
(Animal No. 1)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
2 left	1966	grams	grams	grams	grams	
2 rt.	Feb.					
	14	54.1	15	5	10	Very playful and active animal
	15		15	5	9	Eating well
	16		15	3	12	Eating well
	17		15	3.5	11.5	Seems quite happy and responsive
	18	60.5	15	6	9	
	19		20	2.5	12.5	5 grams of food wasted
	21	66.0	15	5	10	Eating well
	22		15	1	14	Ate almost all of his food and drank all his water
	23		15	5	10	
	24		15	5.5	9.5	Playful and active
	25	74.0	15	7	8	
	26		20	-	20	Ate all of his food None left over
	28	80.0	15	3	12	Very responsive
	March					
	1		15	3	12	Drank all the water Gave extra water
	2		15	4	11	
	3		15	3	12	Has a very good appearance
	4	85.5	15	2.5	12.5	Has a very shinging and white coat
	5		25	-	25	Ate all of his food Could have eaten more
	7	92.6	15	1.5	13.5	
	8		15	4	11	Playful and seemed to be coming out of the cage
	9		15	4	11	Ate well
	10		15	3	12	Ate well

Experimental Diet II (Animal No. 1)

Table D (continued)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
2 left	1966	grams	grams	grams	grams	
2 rt.	March					
	11	103.2	15	2	13	Drank all his water
	12		25	4	21	Ate good
	13	112.3	15	4	11	
	14		15			

Experimental Diet II (Animal No. 2)

Table D (continued)

Ear Mark	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
3 left	1966	grams	grams	grams	grams	
	Feb.					
	14	53.6	15	4	10	1 gram of food wasted
	15		15	3	12	Ate well
	16		15	3	12	Ate well
	17		15	5	10	Drank good amt. of water
	18	61.0	15	8	7	
	19		20	8	7	
	21	65.0	15	12	3	Suddenly his appetite decreased. Ate very little
	22		15	9	6	Ate better than yesterday
	23		15	8.5	6.5	
	24		15	9.5	4.5	1 gram of food wasted
	25	71.2	15	10	5	Seemed developing a taste for the food
	26		20	5	15	Ate quite well
	28	80.0	15	8	7	Ate well
	March					
	1		15	8	7	Animal is playful and active
	2		15	6.5	8.5	
	3		15	7.5	7.5	Seemed to be very responsive
	4	90.6	15	7	8	
	5		25	9	16	Ate very well
	7	98.7	15	6.5	8.5	Ate well
	8		15	6.5	8.5	Has a very healthy appearance
	9		15	7	8	
	10		15	5	10	Ate very well
	11	111.5	15	7.5	7.5	Playful, seemed to be happy
	12		25	13	12	
	13	119.6	15	5	10	
	14		15			

Experimental Diet II (Animal No. 3)

Table D (continued)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
3 right	1966	grams	grams	grams	grams	grams
	Feb.					
	14	51.3	15	4	10	Ate well
	15		15	6	9	The animal is quite responsive
	16		15	8	7	The appetite seemed to be decreasing
	17		15	8	7	Drank very little water
	18	58.5	15	9	5	1 gram of food wasted
	19		20	6	9	5 grams of food wasted
	21	63.0	15	10	5	Ate not very well
	22		15	9	6	Did not drink enough water
	23		15	11	4	Did not eat well
	24		15	7	8	
	25	68.5	15	9	6	
	26		20	4.5	12.5	3 grams of food wasted
	28	73.7	15	9.5	5.5	Seemed to have very little appetite
	March					
	1		15	8.5	6.5	Has quite a healthy appearance
	2		15	7	8	
	3		15	10	5	Active in his actions
	4	82.0	15	5	10	Ate well
	5		20	16.5	3.5	Did not eat well (His water bubble fell in the cage and the water drained off-no water. This could be a reason for his not eating well

Experimental Diet II (Animal No. 3)

Table D (continued)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
3 right	1966 March	grams	grams	grams	grams	
	7	83.7	15	9	6	Playful and active
	8		15	7	8	
	9		15	8	7	
	10		15	8	7	
	11	96.6	15	5.5	9.5	Ate well
	12		25	12	13	Quite responsive and happy animal
	13	103.7	15	5	10	
	14					

Experimental Diet II (Animal No. 4)

Table D (continued)

Ear Mark	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
3 left	1966	grams	grams	grams	grams	
1 rt.	Feb.					
	14	49.9	15	6	9	Seemed to be a happy animal
	15		15	6	9	Ate well
	16		15	6	9	Ate well
	17		15	9	6	Ate well-quite active and playful
	18	58.8	15	7	8	Ate well, drank enough water
	19		20	2	14	4 grams of food wasted
	21	61.0	15	8	5	2 grams of food wasted
	22		15	5	10	Ate well
	23		15	6	9	Seemed to be responsive
	24		15	3	10	Ate very well. 2 grams food wasted
	25	72.6	15	6.5	8	Little food wasted
	26		20	2.5	17.0	Ate very well
	28	76.4	15	3	12	Ate well
	March					
	1		15	4.5	10.5	Very active and responsive
	2		15	3	12	
	3		15	2	13	
	4	91.0	15	5	10	
	5		25	6	17	2 grams of food wasted
	7	97.3	15	2	13	Ate well
	8		15	0	15	Ate all of his food
	9		20	6	14	Ate well
	10		20	10	10	The animal has very good appearance
	11	107.4	15	1	14	Ate almost all his food

Experimental Diet II (Animal No. 4)

Table D (continued)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
3 left	1966					
1 rt.	March					
	12		25	5	20	Ate well
	13	116.2	15	3	12	
	14					

TABLE E

FOUR WEEKS RECORD OF FEEDING EXPERIMENT DIET III
(Animal 1)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
3 rt. 1 left	1966 Feb.	grams	grams	grams	grams	
	14	49.0	15	4	11	1 gram of food wasted
	15		15	5	10	Ate well
	16		15	6	9	The animal is active in his actions
	17		15	6	9	Ate well
	18	57.0	15	8	6	1 gram of food wasted
	19		20	5	12	3 grams of food wasted
	21	69.0	15	6	7	2 grams of food wasted
	22		15	9	5	
	23		15	7	7	Ate well
	24		15	10.5	4.5	Did not eat enough
	25	75.2	15	7	6	2 grams of food wasted
	26		20	9	9	2 grams of food wasted
	28	77.8	15	9	5	1 gram of food wasted
	March 1		15	13	2	His appetite seemed to be decreasing
	2		15	10	3	Ate very little. 2 grams of food wasted
	3		15	7.5	4	About 4 grams of food wasted
	4	86.0	15	7	8	
	5		20	4	10	Ate well. He is active and responsive in his actions
	7	90.2	15	8	7	
	8		15	9.5	4.5	Did not eat well

Experimental Diet III (Animal No. 1)

Table E (continued)

Ear Marks	Date	Wt. Gain	Food Given	Food Left	Food Eaten	Remarks
3 rt.	1966	grams	grams	grams	grams	
1 left	March					
	9		15	4.5	10.5	Ate well
	10		15	6	7	2 grams of food wasted
	11	97.3	15	7	8	
	12		15	4	9	2 grams of food wasted
	13	104.0	15	6	9	
	14		15			

Experimental Diet III (Animal 2)

Table E (continued)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
	1966	grams	grams	grams	grams	
	Feb.					
	14	48.5	15	3	12	Ate very well
	15		15	6	9	Quite an active animal
	16		15	6	9	Ate well
	17		15	9	5	Did not eat well
	18	59.6	15	5	8	2 grams wasted
	19		20	6.2	13.8	
	21	69.5	15	10	5	Did not eat well
	22		15	10	5	He is quiet and sitting in a corner
	23		15	7.5	6.5	1 gram wasted
	24		15	8.5	6.5	
	25	72.4	15	8.5	6.5	
	26		20	9.0	9	2 grams of food wasted
	28	75.0	15	8	7	
	March					
	1		15	9.5	5	Did not eat well seemed to be losing appetite
	2		15	10	5	
	3		15	10.5	2.5	Ate very little
	4	81.0	15	9.3	5.7	Very fragile to touch and quite little
	5		20	7.5	7.5	
	7	84.2	15	5.5	9.5	Ate well
	8		15	11	4	Did not eat well
	9		15	9	6	
	10		15	9.5	5.5	
	11		15	10.5	4.5	Did not eat well
	12		20	10	10	
	13	100.5	15	8	6	1 gram of food wasted

Experimental Diet III (Animal 3)

Table E (continued)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
3 rt. 1 left	1966 Feb.	grams	grams	grams	grams	
	14	49.0	15	4	11	Ate very well
	15		15	5	10	He seems to be happy and active
	16		15	6	9	Ate well
	17		15	6	9	Ate well
	18	57.0	15	6	6	3 grams of food wasted
	19		20	5	12	3 grams of food wasted
	21	69.0	15	6	7	2 grams of food wasted
	22		15	9	6	Sneezing some times
	23		15	7	7	Taking very little water
	24		15	10.5	4.5	Very inactive
	25	75.2	15	7	6	2 grams of food wasted
	26		20	9	9	2 grams of food wasted
	28	77.8	15	9	5	1 gram of food wasted
	March					
	1		15	13	2	Ate very little
	2		15	10	3	Inactive
	3		15	7.5	4	About 4 grams of food wasted
	4	86.0	15	7	8	Very fragile to touch and light in weight
	5		20	4	10	1 gram of food wasted
	7	90.2	15	8	7	Sneezing
	8		15	9.5	4.5	
	9		15	4.5	10.5	
	10		15	8	7	
	11	97.3	15	7	8	
	12		15	4	9	
	13	104.0				

Experimental Diet III (Animal 4)

Table E (continued)

Ear Marks	Date	Wt. Gain	Food Given	Food Left Over	Food Eaten	Remarks
Left 2	1966	grams	grams	grams	grams	
rt. 3	Feb.					
	14	48.5	15	3	12	
	15		15	6	9	
	16		15	6	9	
	17		15	9	5	
	18	59.6	15	5	8	
	19		20	6.2	13.8	
	21	69.9	15	10	5	
	22		15	10	5	
	23		15	8.5	6.5	
	24		15	8.5	6.5	
	25	72.4	15	8.5	6.5	
	26		20	9.0	9.0	
	28	75.0	15	8	7	
	March					
	1		15	9.5	5	
	2		15	10	5	
	3		15	10.5	2.5	
	4	81.0	15	9.3	5.7	
	5		20	7.5	7.5	
	7	84.2	15	5.5	9.5	
	8		15	11	4	
	9		15	9	6	
	10		15	9.5	5.5	
	11	93.5	15	10.5	4.5	
	12		20	10	10	
	13	100.6				

APPENDIX B

APPENDIX B

CALCULATION OF PROTEIN EFFICIENCY RATIO (PER) FOR EACH DIET GROUP IN THE EXPERIMENT

$$\text{PER} = \frac{\text{weight of protein consumed in grams}}{\text{weight of the increase in the body weight}}$$

Control Diet

The Protein Efficiency Ratio of this diet in relation to the feeding of four animals is as follows:

1. $\text{PER} = \frac{11.4}{13.85} = 0.82$

2. $\text{PER} = \frac{16}{17} = 0.94$

3. $\text{PER} = \frac{11.3}{11.62} = 0.97$

4. $\text{PER} = \frac{17}{12.92} = 1.32$

$\text{Average} = \frac{4.05}{4} = 1.01$

Experimental Diet I

The Protein Efficiency Ratio of this diet in relation to the feeding of two animals is as follows:

1. $\text{PER} = \frac{19.9}{11.4} = 1.74$

2. $PER = \frac{8.53}{13.9} = 0.60$

$Average = \frac{2.34}{2} = 1.17$

Experimental Diet II

The Protein Efficiency Ratio of this diet in relation to the feeding of four animals is as follows:

1. $PER = \frac{17.9}{14.55} = 1.23$

2. $PER = \frac{13.8}{16.5} = 0.83$

3. $PER = \frac{12.64}{13.32} = 0.94$

4. $PER = \frac{18.5}{16.53} = 1.11$

$Average = \frac{4.11}{4} = 1.03$

Experimental Diet III

The Protein Efficiency Ratio of this diet in relation to the feeding of four animals is as follows:

1. $PER = \frac{11.73}{11.22} = 1.45$

2. $PER = \frac{14.2}{10.52} = 1.34$

3. $PER = \frac{11.05}{8.42} = 1.31$

3. $$\text{PER} = \frac{14.2}{9.0} = 1.57$$

$$\text{Average} = \frac{5.67}{4} = 1.42$$

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION
1100 ROBERT E. LEE BOULEVARD
NEW ORLEANS, LOUISIANA 70119

REPLY TO:
P. O. BOX 19687
NEW ORLEANS, LA. 70119

November 24, 1965

AIR MAIL

Miss Jameela Jan
Food and Nutrition Department
Oklahoma State University
Stillwater, Oklahoma 74075

Dear Miss Jan:

In accordance with your request by telephone today, we are forwarding to you by parcel post five pounds of cottonseed flour for your use in protein studies. This cottonseed flour is designated sample number 79434 from our pilot plant run number 68.

We would appreciate it if you will let us have your evaluation of this meal. Should you need any further information please do not hesitate to contact us.

Sincerely yours,

Henry L. E. Vix
Chief, Engineering and Development
Laboratory

Separate Cover:
Cottonseed Flour

VITA

Jameela Jan

Candidate for the Degree of

Master of Science

Thesis: BIOLOGICAL VALUE OF VEGETABLE PROTEINS AVAILABLE IN PAKISTAN AS DETERMINED BY RAT FEEDING EXPERIMENT

Major Field: Food, Nutrition and Institution Administration

Biographical:

Personal Data: Born at Peshawar, West Pakistan, October 21, 1942, the daughter of Dr. and Mrs. Jan Mohammad Khan.

Education: Attended Presentation Convent High School in Peshawar, West Pakistan; received high school certificate from the University of Peshawar, West Pakistan, in 1958; received Bachelor of Science degree from the University of Peshawar, West Pakistan in 1962; received the Master of Science degree from the University of Punjab with a major in Home Economics Education in 1964; completed requirements for Master of Science degree, with a major in Food, Nutrition and Institution Administration at Oklahoma State University in July, 1966.