COMPARISON OF THE EFFICIENCY OF DIETS COMPOSED OF A VEGETABLE PROTEIN MIXTURE VERSUS A VEGETABLE PROTEIN PLUS FISH PROTEIN CONCENTRATE AS DEMONSTRATED BY FEEDING ALBINO RATS

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Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
July, 1970

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ACKNOWLEDGMENTS

The author is grateful and thankful to the Almighty God whose invisible direction lead her to complete this study.

A deep appreciation and grateful acknowledgment is extended to the author's adviser, Dr. Helen F. Barbour, for her advice, guidance, encouragement, and enthusiasm during each and every phase of this research.

Gratitude is also expressed to Dr. Claypool for assistance in the statistical analysis of the data. Appreciation is extended to Miss Mary Leidigh and Dr. Elizabeth C. Hillier for their advice and comments as members of the graduate committee.

A special appreciation and thanks is given to the author's parents and brothers and sisters for their understanding, patience and encouraging letters during her stay in the United States.

The author wishes to express sincere appreciation and thankfulness to her American parents, Mr. and Mrs. Kermit Kuelzer, and their family for their loving care and affection in this foreign country.

Thankfulness is expressed for her friends whose encouragement helped her to complete this thesis.

CHAPTER I

INTRODUCTION

Malnutrition is a major contributing factor to the high mortality and morbidity in early childhood in developing countries. The predominant form which malnutrition takes under these circumstances is what is now commonly called protein-calorie malnutrition. This is the most serious and wide spread nutritional disorder known to medical and nutritional science (45). In common with other developing nations of the world, the children of Pakistan suffer from protein calorie malnutrition which causes a wasting away of the vitality of the people of this nation (2).

It has been calculated that a minimum of 63.5 grams of protein should be the intake of protein per adult person and 1.5 grams per kilogram for children per day. According to the nutrition survey in East Pakistan (49) the present intake is 57.5 grams without considering the "waste factor" in the household which may be about 10 to 15 percent. Most of the protein that the people consume is cereal protein and therefore is of low biological value. The contribution of a high quality protein from an animal source is meager. The intake is below the minimum allowance in 60 percent of the households in East Pakistan. This undernutrition especially affects the vulnerable groups of the population, namely the pre-school children and the expectant and nursing mothers (49).

According to the 1960 census there are 9.26 million children under five years of age in East Pakistan. The vast majority of child deaths are due to infectious diseases which precipitate fetal protein malnutrition in children where protein nutritional status is already precarious. For those who survive this malnutrition there is permanent retardation of mental and physical growth (2).

The effect of protein-calorie malnutrition, which certainly afflicts over 80 percent of the rural population of Pakistan, is far-reaching in consequences. It limits the potential capability of the child to mature so that he can contribute to the society he lives in (3).

Dr. M. S. Swaminathan, Director of Indian Agricultural Research Institute, New Delhi, has stated that, "If the challenge of malnutrition and protein hunger is not tackled immediately, India may have to face a large scale intellectual dwarfing in the next two decades" (33,ix). The same situation is true for Pakistan.

The poverty of the Pakistani diet in protein is obvious from the fact that the bulk of their food is cereal which is a poor source of protein qualitatively and quantitatively. 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 (3).

Ignorance is the ally of hunger. Together with poverty, which it often accompanies, it is basically responsible for virtually every case of malnutrition. In countries where food supplies are inadequate, existing resources are generally badly utilized. Pakistan produces varieties of legumes, cereals and seeds which are cheap sources of protein with a wide variety of amino acids. However, people fail to satisfy their protein needs from these sources because they do not know how to choose a mixture which has high biological value.

The author intends to formulate vegetable protein mixtures using some of the typical legumes and seeds available in Pakistan and fortify them with an animal protein. The diets will be planned to include the essential amino acids in sufficient amounts to maintain health and growth of white rats. It is believed that weanling male rats fed these mixtures for four to six weeks of time will permit the demonstration of their growth and maintenance qualities.

Assumptions

1. No single vegetable protein has all the essential amino acids needed to promote growth and maintain body structure.
2. All dietary essentials for the rat can be obtained in sufficient amount from a mixture of vegetable protein.
3. Inadequate protein quality and quantity causes failure in proper growth, development and reproduction.
4. Fish or fish protein concentrate is known to be a source of complete protein which will support growth.
5. It is possible to use a combination of animal and vegetable proteins so that they compliment each other in relation to amino acid content.
6. Vegetable proteins are widely available and less expensive than animal proteins in Pakistan.

Hypotheses

This study is concerned with the testing of the following hypotheses:

1. Combination of fish protein concentrate and legumes can be made in proportions that will support life and optimum growth of white rats.
2. A combination of legumes and seeds can be compounded which will contain a balanced - essential amino acid content that will support life and optimum growth of white rats.
3. Sufficient calories to spare the protein can be obtained per day by the addition of vegetable oil to the diet of the rats.

CHAPTER II

REVIEW OF LITERATURE

Protein and Amino Acids

Modern investigations have revealed that proteins are composed of at least twenty-two amino acids. During the process of digestion the amino acids are liberated, and pass unchanged into the portal circulation. They are distributed throughout the organism, and serve as the substrates for the formation of many characteristic tissue proteins. Other non-protein nitrogenous components of the cells are also formed from this amino acid. Thus the amino acids occupy a unique position in nutrition (60).

Amino acids that the body cannot synthesize in adequate amounts are called essential or indispensable because they must be supplied by the diet in proper proportions and amounts to meet the requirements for maintenance and growth of tissue. Non-essential or dispensable amino acids are those the body can synthesize in sufficient amounts to meet its needs if the total amount of nitrogen supplied by protein is adequate. Various amino acids which are considered as essential or non-essential (47, p. 37) are as follows:

Essential	Non-Essential
Isoleucine	Alanine
Leucine	Asparagine
Lysine	Aspartic acid
Methionine	Cysteine
Phenylalanine	Cystine
Threonine	Glutamic acid
Valine	Glutamine
Arginine*	Glycine
Histidine‡	Hydroggoseiproline
	Proline
	Serine
	Tyrosine

According to Rose eight amino acids are essential for maintenance of nitrogen equilibrium in man. The food eaten must contain these amino acids because they cannot be manufactured in the body rapidly enough to supply the need for them (61).

Quality of Protein

The effects of protein on the maintenance and growth of animals are determined by the amounts of each of the eight to ten essential amino acids which are present in the specific protein eaten. Osborne and Mendel in their pioneer work with rats showed that individual

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*Arginine can be synthesized by animal organisms. But the rate of synthesis is limited. A dietary supply may be necessary for maximum growth. †Histidine is required for growth, but is not needed for maintenance by the adult human.

proteins differ in their ability to maintain life and support the growth of animals. Casein, when fed at a level of eight percent of the total calories, both maintained life and supported growth and hence was classified as a complete protein. Gliadin, since it maintained life but did not support growth, was called a partially incomplete protein. Incomplete proteins such as zein neither maintained life nor supported growth (47).

The requirement for protein is influenced by its quality, which is largely determined by the amino acids it contains. Two factors have to be distinguished, namely the proportion of essential to non-essential amino acids and secondly the relative amounts of the essential amino acids. Amino acids have many complex interrelationships. They share certain metabolic pathways and compete with one another for transport. An unbalanced pattern may have an adverse effect on nutrition even if the quantity of essential amino acids is sufficient for a normal individual (53).

Protein Requirement

Many factors have an effect on the protein requirement of an individual. Body size, the quality of protein fed, the adequacy of caloric intake, the previous state of nutrition, the efficiency of digestion and the special physiologic need such as during growth, pregnancy, or recovery from illness are some factors which determine the protein requirement of an individual.

The protein requirement during the whole life span is not the same. Given below are the Recommended Daily Dietary Allowances for protein for a standard man and woman in the United States of America

(18, index).

Age and Sex	Weight (kg)	Height (cm)	Protein (gm)
Male:			
18-22	67	175	60
22-35	70	175	65
35-55	70	173	65
55-75	70	171	65
Female:			
18-22	58	163	55
22-35	58	163	55
35-55	58	160	55
55-75	58	157	55

The Recommended Dietary Allowances of protein for adults in the United States of America has been 0.9 gm of protein per kilogram per day.

The Committee on Amino Acids for Food and Nutrition Board and the FAO Committee of Protein Requirements (54) established the minimum need for adults to be between 0.3 and 0.35 grams per kilogram per day when the diet contained protein of maximal nutritive value.

During pregnancy and lactation the protein allowance for the woman should be increased by 20 grams and 40 grams over the basic level respectively (57). Given below are the amounts of protein recommended during pregnancy and lactation (18, index).

Age of Woman	Weight (kg)	Height (cm)	Protein (gm)
Pregnancy		157	65
Lactation			75

The greatest amount of protein per unit of body weight is needed during childhood. Between the ages of one and 12 years the allowances gradually decrease from about 2 grams to 1.7 grams per kilogram body weight.

The Recommended Daily Dietary Allowances for protein set by the Food and Nutrition Board (18, index) for infants and children of the United States is as follows:

Age and Sex	Weight (kg)	Height (cm)	Protein (gm)
Infant (Up to 1 year)	9		Kilogram X 1.8
1-2 years	12	81	25
2-3 years	14	91	25
3-4 years	16	100	30
4-6 years	19	110	30
6-8 years	23	121	35
8-10 years	28	131	40
Male:			
10-12	35	140	45
12-14	43	151	50
14-18	59	170	60
Female:			
10-12	35	142	50
12-14	44	154	50
14-16	52	157	55
16-18	54	160	55

The Protein Committee of Pakistan has recommended 62.2 grams of protein as average daily per capita allowance of protein for normal individuals (54, p. 38).

Factors Affecting Protein Utilization

Non-protein calories play a part in the establishment of the nutritional status. Swanson (71) found that the total energy balance and the nitrogen balance are interwoven. An increase in the protein supply to the undernourished will not be effective if the energy control of the diet is restricted. The influence of calorie intake on protein metabolism can be observed by the rapid development of negative nitrogen balance when the energy content of the diet is below requirement. The lower the intake of protein and calories, the greater is the need for including high quality protein in each meal if nitrogen is to be well utilized (10).

The time factor influences nitrogen utilization. Distribution of amino acids among the daily meals is a factor to consider in meeting 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 (64).

Geiger (19) showed that in laboratory animals both maintenance and growth require that all of the essential and non-essential amino acids be present simultaneously as well as in adequate amounts.

Minerals and vitamins which are needed for normal growth and metabolism can affect protein utilization. It is thought that potassium is necessary for effective utilization of amino acids, Enough niacin and phosphorous is also needed (53).

Amino Acid Imbalance

Amino acid imbalance is the term commonly used to designate a relative deficiency of an essential amino acid resulting from an excess of one or more amino acids in the medium. When the excess is large and the disturbance is severe it is called amino acid toxicity (63).

Harper (29, p. 405) describes an amino acid imbalance as "any change in the proportion of amino acids in a diet that results in an adverse effect which can be prevented by supplementing the diet with a relatively small amount of the most limiting amino acid or acids." In a series of experiments using growth of rats as a criterion of amino acid imbalance Harper (29) found that the magnitude of growth depression was influenced by the level and kind of protein in the original diet as well as the lack of specific amino acids. He stated that it is not known at what degree of imbalance it becomes impossible to satisfy requirements of an animal by increasing the level of protein in the diet, that is, the stage at which amino acid supplementation become mandatory.

The possible effects of amino acid imbalance must be taken into account in any evaluation of essential amino acid patterns of foods and in planning supplementation.

The condition termed "amino acid imbalance" can be created by small supplements of individual amino acids. Rats fed the imbalanced diets grow less rapidly than those fed the control diets (58, 59). To prevent the retardation of growth the concentration of limiting amino acid in the imbalanced diet must be increased. This suggests that a mixture of amino acids causing an imbalance reduces the efficiency of utilization of growth-limiting amino acids.

When the imbalanced diet is fed to the animal the severity of adverse effect on food intake and growth increased with increasing concentration of imbalancing amino acid mixtures added to the diets (28).

A study was undertaken in an effort to determine more precisely the influence of the severity of an amino acid imbalanced diet and the importance of the quantity of the limiting amino acid on the choice of the rats for or against the imbalanced diets. When rats were allowed to choose between an imbalanced diet and a protein-free diet, they selected the latter almost exclusively (27).

On the other hand human beings cannot select a balanced mixture. Six healthy young men were given a choice between balanced and deficient amino acid mixtures containing seven grams of nitrogen per day with a formula diet adequate in all other nutrients to see if they would select the balanced diet. The result of the study revealed that human subjects showed no evidence of being able to choose a properly balanced amino acid mixture. The selection was made by appearance, taste, and smell of the food (9).

The environment had some affect on the acceptance of an imbalanced diet by the rats. Experiments showed that adaptation of rats to imbalanced diets was more rapid in cold environments than at room temperature. Weight gain of the animals fed the imbalanced diet in the cold were higher than the animals fed this diet in a warmer environment (26).

Effect of Heat on Protiens

When food products are heated, their proteins are known to undergo certain changes in nutritive value. The digestibility may be improved or depressed and the biological value may be similarly changed. The result of feeding experiments with rats shows that the nutritive value of cereal proteins may be greatly impaired by the application of heat with no demonstrated alteration in their content of the essential amino acids (6).

The improvement in the digestibility and biological value of certain legume proteins by heat is a striking phenomenon.

Several investigators have found that heat in the preparation of certain foods does alter the nutritive value of the protein. When casein, meat, liver, kidney, cereals, and fish meal are heated to high temperatures, there is a definite decrease in their nutritional value. This is not so with the soybean. There is much evidence that there is definite improvement in growth of animals when they are changed from a raw soybean diet to a heat-treated soybean diet (30).

Optimal heat processing such as steaming or mild-autoclaving has increased the availability of the amino acids, methionine and cystine (16).

The poor nutritive value of raw legumes are well known to nutritionists and has been attributed, at least in part, to the presence of trypsin inhibitors and a deficiency of sulfur-containing amino acids (41).

In one study the availability of cystine in the presence of trypsin inhibitors was studied by using both heated and unheated trypsin inhibitor (72). Feeding experiments with chicks showed that the availability of cystine increased in the heated trypsin inhibitor mixture. The mixture was heated at 120 degrees for two hours (72, p. 35).

Protein Calorie Malnutrition

The term "protein-calorie malnutrition" has been proposed, and is now widely used, to specify the childhood nutritional disorders that primarily involve protein and calories (50). Workers engaged in the study of protein-calorie malnutrition all the world over have now agreed that two distinct clinical forms of protein-calorie malnutrition, namely marasmus and kwashiorkor can be recognized. It has been generally assumed on the basis of experimental studies that kwashiorkor is the end result of a situation wherein the diet is adequate in calories and poor in proteins, while marasmus is brought about by diets that are inadequate in calories (22, 70).

In broad terms, four principal clinical syndromes have been described for protein-calorie malnutrition: kwashiorkor, incomplete or mild kwashiorkor, nutritional marasmus, and nutritional dwarfing. Because a combination of clinical features are common, the term marasmic kwashiorkor is often appropriate (45).

The clinical picture of protein-calorie malnutrition is characterized by edema, lesions of the skin, hair changes, apathy, enlarged fatty liver, and low serium protein. There may also be manifestations of various vitamin deficiencies (57, 76).

In one study the plasma amino acid patterns of children with kwashiorkor or marasmus were studied using unidimensional paper chromatography (56). The amino nitrogen in the plasma of kwashiorkor children was found to be low and after treatment increased significantly. In marasmus the levels were higher than in kwashiorkor and did not change significantly after treatment. The proportion of plasma essential amino acids was low in kwashiorkor and there was no increase after treatment. In marasmus there was consistent increase in the proportion of essential amino acids on treatment.

Intake of protein below the recommended allowance is found in many parts of the world. Protein malnutrition is the most widespread, serious nutritional problem in the underdeveloped areas, particularly serious in its effect on infant and children (38).

A recent survey of East Pakistan showed that protein malnutrition is the most wide spread nutritional problem in terms of prevalence and effects on the population (49). Studies on the albumin concentrations of blood plasma, which reflect the nutritional status in respect to protein intake, revealed that low concentrations are observed in almost half of the rural and one-third of the urban population (54). As deficiencies are almost always associated with other maladies, their ill effect on health are varied and serious, including impaired growth, subnormal existence and death. Nearly 26 percent of all live-born children die before their fifth birthday. Of such children, 17 percent die within the first year and 9 percent between one and four years of age. Even those children who survive fail to grow in a normal way physically or mentally. The overall effect of protein deficiencies along with those of other food factors, ultimately give rise to several nutritional disorders like anemia, goiter, rickets, and beri beri manifested clinically through syndromes of visible lesions.

New Sources of Food Protein

Providing adequate food to the ever increasing population of the world is a great problem of the day. Nutritionists always try to make the best use of the available food stuffs. Constant search for a better use of different foods led them to develop some new sources of protein.

Protein foods may be plant or animal in origin. Since animal protein originates through the conversion of plants, plant protein must be considered to be of primary importance. Measures aimed at increasing the supply and utilization of plant protein will ultimately benefit the human population of the world. The primary plant protein sources available for feeding consist of seeds, leaves and tubers. Seeds, in general, contain relatively large amounts of protein and constitute the most important kind of protein food for man and for non-ruminant livestock (77).

The more exacting requirements of man and non-ruminant farm animals with respect to protein led to the idea that individual feedstuffs may be fractionated in some way. These fractions provide products of superior quality for classes of people requiring them and leave a residue which is suitable for ruminant feeding. Food which contains mixed protein can be treated chemically to separate high quality proteins from poorer ones. Separation, accomplished easily by means of extraction with aqueous salt solution, leaves a residue deficient in amino acids but suitable for ruminant feeding (77).

Fish can contribute largely to the world's protein supply. Most species of fish are edible and are enjoyed as human food. Conversion of fish into food additives such as fish protein concentrate is very common today. Some 40 percent or more of the world's fish catch is presently converted directly into fish protein concentrate. Fish meal is made by the relatively simple process of cooking, pressing out most of the oil and about half of the water and drying the press cake. The product contains 65-70 percent protein (42).

Most of the attempts to manufacture tasteless fish protein concentrate and to introduce it into some staple food such as bread have in fact used fish meal, rather than fish as the raw material. The problem with fish meal is to extract both water-soluble flavors and lipid materials (42).

Another new source of protein is leaf protein. Here protein is separated from fibers mechanically. The machine crushes the leaves and weeds and extracts protein from them. The extracted protein is then processed into a food having a cheese-like texture (40). The amino acid composition of protein samples are similar in regard to source of leaves in relation to different species, ages and cultural backgrounds. In spite of their uniformity in amino acid composition, leaf protein preparations are not all equally good as protein supplements (52).

Ideally, leaf protein would be made from leaves that are by products of some other crop. In the temperate zone the obvious sources are pea and bean hulls from canneries, and tops from early potatoes and sugar-beets. The leaves should contain a large amount of water. One thousand pounds of these leaves would yield 150 pounds of dry matter, in turn yielding 30 pounds of protein (40, 52).

It is known that the percentage of extractable protein in a leaf tends to diminish as the crop matures although the total amount of protein per hectare is still increasing. It is expected that during the next few years measurements will be made of the yield of extractable protein from many different crops growing in different climates and harvested at different ages after different fertilizer treatments. Once the idea of using leaf protein has been accepted in any region, there will be no lack of research problems for agronomists and plant physiologists (52).

Leaf protein in its dark green form is acceptable in West Africa and South West India.

With the advancement of modern science the researchers have found another source of protein from organisms grown on hydrocarbon residue, Yeast produced by fermentation on either pure in-paraffins or heavy gas oil is a good source of protein. Because of the absence of oxygen in these substances, it is necessary to supply oxygen as well as a nitrogen source and an aqueous solution of the minerals required for yeast growth (65).

The world's present need for protein is so large that no single novel source could meet it for at least a decade (52). All the proteins discussed above are likely to be needed. Research on all of them should be intensified. There is not a best source for a particular country. For people living beside an underexploited ocean, it would be fish; in a tropical rain forest, leaves; in arable savannah, soya; and where there is abundant molasses, yeast. But, however attractive the local best source may seem in theory, it should never be allowed to dominate the scene so that people become heavily dependent on a single protein source. A mixed diet should always be preferable.

Protein-Rich Food of Vegetable Origin

For immediate relief of the problem of protein-calorie malnutrition, several kinds of protein-rich foods have been devised and tested. In these mixtures the limiting amino acids in a vegetable protein needed for growth is made up by another one of the vegetable proteins used. Thus the mixture furnishes all of the essential amino acids necessary for growth (43).

One of the most successful of these vegetable protein products is called Incaprina, named for the organization that developed it, the Institute of Nutrition of Central American and Panama-INCAP. Incaprina is a mixture compounded mainly of cornmeal, yeast, and cottonseed or soybean flour (75). The composition of Incaprina Vegetable Mixture 8 is as follows (12, p. 31):

Dried Corn Masa	50 percent
Sesame Meal	35 percent
Cottonseed press cake	9 percent
Torula Yeast	3 percent
Kikuyls Leafmeal	3 percent

The mixture had a protein content of 25.1 percent.

L'aubina is produced in Lebanon and consists of locally grown chick peas, and boiled wheat mixed with dried skim milk from America (75).

A feeding experiment was continued in Beirut with L'aubina. To their delight the doctors found that the diet wiped out all signs of malnutrition (75).

INCAP Vegetable Mixture 8, was palatable, and gave good growth and efficiency of feed utilization in rat trials. Neither the addition of 0.45 percent of free lysine nor the substitution of skim milk for part of the corn improved the growth or feed efficiency of rats fed the mixture. Addition of lysine did improve growth and feed utilization of the mixture by chicks. When the mixture was diluted with cornstarch to feed rats at a 15 percent protein level, added lysine improved growth and feed efficiency on the basis of the studies. INCAP Vegetable Mixture 8 was recommended for clinical feeding trials in children (7).

Early in 1965, a vegetable protein mixture was prepared by Juggenheim and Szmeleman from food available in Middle Eastern countries (24). It consisted of 47 percent autoclaved chick peas, 35 percent defatted sesame flour, and 18 percent meat processed, low-fat soybean flour. The protein content of that particular mixture was 37.8 percent, and its biological value and protein efficiency ratio in young rats was proven to be satisfactory.

A protein-rich mixture prepared from chick peas, sesame flour, and low-fat soybean flour was evaluated in infants in a controlled metabolic study (24). One group of infants was fed a suspension containing 10 grams of vegetable protein mixture and 5 grams of sugar (100 milliliters water). Three grams of starch, as corn flour, were added to each 100 milliliters of vegetable protein mixture. Eight infants were fed homogenized, bottled, pasteurized cow's milk with 5 grams sugar added to each 100 milliliters. Semolina, 7.5 grams, and sugar, 5 grams per 100 milliliters of milk were fed to eight infants. One group was fed with Incaprina. For the last group vegetable protein was supplemented with amino acid L-lysine or L-methionine.

Nitrogen retentions and weight gains in infants fed the vegetable-protein mixture compared favorably with those of infants fed a cow's milk diet. Supplementation of vegetable protein with amino acids did not increase nitrogen retention. Weight gains and nitrogen retentions in infants fed Incaprina were similar to those of infants on the vegetable protein mixture. When sufficient calorie intake in the form of added starch, was provided, weight gain in infants fed a vegetable protein mixture was similar to that in infants fed cow's milk. It can be concluded, that vegetable protein mixtures compare quite favorably with cow's milk when sufficient calories are present in the diet (24).

Many protein-rich foods of vegetable origin have been used successfully in the treatment of kwashiorkor. Cottonseed protein has been processed at the Regional Research Laboratory, Hyderabad. This protein has been used as a food in the treatment of children suffering from kwashiorkor (62).

In this particular experiment four mixtures were prepared in which the major portion of the protein was derived from the cottonseed flour. Fifty-two children suffering from kwashiorkor were studied as patients. They had an age range from 18 to 48 months and their body weight was 5.2 to 11.7 kilograms. All children were grossly underweight, edematous and had varying degrees of hypoalbuminemia.

Samples of blood were obtained at the time of admission and on the tenth or thirtieth days after treatment. Clinical and biochemical responses were poor with the cottonseed flour diet. Edema cleared up very slowly. The mental condition improved only slowly. In some of the children, the clinical condition showed no improvement. They were taken off of the diet after 10 to 14 days.

When lysine supplemented cottonseed flour was provided, the rate of response was slightly faster than above (62).

Children treated with the diets where 25 percent of cottonseed protein was replaced with milk protein showed a better response. When children were treated with solvent-extracted cottonseed flour, of low gossypol content, good clinical responses result.

The result of the experiments reviewed above show that the children suffering from kwashiorkor had an unsatisfactory response to the diets which contain cottonseed as a main source of protein. Vegetable protein mixtures with cottonseed flour as an ingredient should be used only for children without obvious clinical manifestations of deficiency.

One study was made to see the efficiency of combinations of vegetables as a source of protein in the diet and to determine the extent to which such diets could provide for growth and maintenance in rats (14). Male, young albino rats of six weeks of age were used. The diet was composed of vegetables commonly consumed in the southern region of the United States. Abilities of field peas and peanuts to improve responses to this food were also evaluated.

Results showed that though the young animal was unable to grow, maintenance of body weight occurred after a weight plateau was reached. Addition of field peas or peanuts to the basal diet was effective in stimulating nitrogen retention in young rats (14).

Forced feeding of young rats of the rations containing peanuts, resulted in loss of hair from the lateral region of the body. This was due to unintensified amino acid imbalance in this diet.

Amino Acid Fortification

In the developed countries crystalline amino acids are available at lower costs. This suggests the fortification of food with limiting amino acids. Different quantities and proportions of these amino acids are necessary to devise a mixture which is complete and balanced (31).

In one study (55), protein food based on oil seed meals, was powdered finely in a microatomizer. It was dissolved in hot water to yield a milk-like emulsion that was highly acceptable and could be readily fed to children suffering from kwashiorkor.

Microatomized protein food was prepared by mixing low fat, edible peanut, soya, and sesame flours which were finely powdered in a microatomizer. A second mixture was prepared by mixing the oil seed flour and skim-milk powder in required proportions and fortifying them with vitamins A, D, thiamine, riboflavin, and calcium phosphate (55). Children with moderate to severe kwashiorkor, admitted to the pediatric ward of the Krishnarajendra Hospital, Mysore, India served as subjects for the study. Results showed that the initial anorexia was easily overcome by the diet. The edema began to decrease after a few days of treatment and disappeared completely as treatment was continued.

Microatomized protein foods based on blends of oil seed meal and skim milk powder, are readily acceptable to children suffering from kwashiorkor and are quite effective in initiating cure. Vegetable proteins, in general, lack one or more of the essential amino acids and possess nutritive value lower than that of animal protein. If the pattern of amino acids in vegetable protein does not deviate too much from the optimum, feeding at higher levels of protein intake can overcome the deficiency in indispensable amino acids.

In another experiment a mixture was developed with different amino acids. This mixture was tasted and approved as acceptable by adult humans in a balance study. Two mixtures were prepared. Amino acids were provided, in part, as white wheat flour and in part as purified amino acids. Both the mixtures contained limited amounts of all essential amino acids. These mixtures maintained nitrogen equilibrium in adult human subjects (4).

From the experiment stated above it is recognized that relatively small quantities of eight essential amino acids will maintain nitrogen equilibrium in men and women. Nitrogen retention improves steadily as the quantities of essential amino acids increase simultaneously. Quantities of individual amino acids are of primary importance in determing man's ability to utilize dietary protein effectively. The value of supplementary nitrogen depends on the amount and proportions of essential amino acids that are present (4, 15).

This author does not recommend the use of crystaline amino acids for Pakistan which is still a developing country. Synthetic amino acids are not available in great amounts, and in most cases the cost is high. It is wise to find out how to devise and prepare mixtures from the commonly and locally available food sources.

Fish Protein Concentrate as a Supplement

Fish protein concentrate is a food supplement consisting primarily of a dried and processed food protein and of the naturally associated vitamins and inorganic minerals (25).

Fish protein concentrate when added to cereal and legume proteins, brings an improvement in protein quality because of the supplementary effects of the required amino acids in fish flour. Stillings (67), for example, showed that a mixture of wheat flour and fish flour had a nutritional value apparently higher than that of wheat flour alone. A mixture of ten percent fish flour and 90 percent wheat flour had a protein efficiency ratio of 3.0 as compared to 1.0 for wheat flour alone.

Metta (46), studied the nutritional value and acceptability of an odorless, defatted fish flour as a possible supplement for East Indian diets. The diet containing fish flour was compared with a diet containing whole egg protein. Equal amounts of both the diets were given to five male rats for 20 days. The mean daily growth of the animals consuming fish flour protein was 91 percent of the mean daily growth of the animals consuming whole egg protein.

In one experiment animal feeding tests were conducted to determine if the nutritive quality of the protein of four vegetable supplements, used to improve deficient diets, could be improved by addition of high quality fish protein concentrate. Supplements tested were (1) multipurpose food prepared by General Mills, Inc. for Meals for Millions, (2) multipurpose food, (3) nutrobiscuite both formulated by technologists at the Central Food Technological Research Institute, India, and (4) Incaprina formulated by investigators at the Institute of Nutrition for Central America and Panama. Groups of male weanling rats were fed diets containing a total of ten percent protein, eight percent from the vegetable supplement alone, or with one-fifth or two-fifths of protein from fish protein concentrate. In all cases the addition of one of the levels of fish protein concentrate to the vegetable protein resulted in significantly improved growth suggesting a better amino acid balance of the dietary protein. Amino acid analysis of protein in each supplement and in fish protein concentrate supports this conclusion (68).

In another study, chemical composition, biological quality of the protein, and toxicity were determined for fish protein concentrate and sunflour press cake. Fish-protein concentrate enriched food improved the biological quality of cereal foods when fed as the only source of protein. Fish-protein concentrate produced normal growth in infants. Mixtures containing fish flour and sunflour press cake showed a high net protein utilization in rats, and produced normal growth in children when they supplied 70 percent of the total daily nitrogen (78).

From the above results it can be concluded that fish-protein concentrate is a new food source that can be used to meet the protein needs of children. It has high protein content, good biological value and absence of toxicity. Its high available lysine makes it valuable for improving biological value of poorer proteins, especially those of cereals.

Besides improving the protein of the diet, fish flour also increases the mineral content, especially in rice diet (46).

Vegetable puree and cereal dishes enriched with fish-protein concentrate produced in Morocco were fed to a test group of infants for a six months period. A three percent concentration of fish-protein concentrate in the food as consumed represented the maximum acceptance level. This percentage of fish-protein concentrate in two meals provided just over ten grams of this concentrate daily per infant. Together with the vegetable protein derived from the local cereal and vegetable weanling dishes, this yielded the equivalent of 13.2 grams of reference protein which more than fulfilled the FAO standard of 12.6 grams recommended for the weanling-age group. Weight and length gains in this test group of infants compared with a control group did not show any statistical difference (20).

Pakistan has a vast potential for increasing her catches of marine and fresh-water fish. This potential must, therefore, be tapped to raise the supplies of fish intended for consumption as such, as well as in the form of products like fish-protein concentrates. The Protein Committee of the National Science Council has recommended this (54).

Factories should be established in both provinces to prepare fish-protein concentrate of good quality, with a process like that of the Brue of commercial fisheries of the United States.

To popularize the consumption of fish-protein concentrate, investigation should be organized (a) to find out safe and convenient methods of using the concentrate in various foods acceptable to the common man, and (b) to establish an organized system of marketing fish-protein concentrate and its products among the masses at low prices (54).

Legumes in Human Nutrition

The word legume is derived from the Latin legumen, which meant any leguminous plant. It is cultivated throughout the world, in both the tropics and temperate zones.

Dry legumes have been called the "poor man's meat," a designation of interest and importance from historical and other standpoints (13, p. 9). The legumes, in dry form yield almost as many calories per unit of weight as cereals. They have a high protein content, ranging from 17 to 25 percent. This amount of protein is about double that of the cereals in general, and slightly higher than that of meat, fish, and eggs. The soybean and groundnut are exceptional in containing about 38 and 25 percent respectively of protein.

Most of the legumes commonly eaten by man contain little fat. They are fairly good sources of thiamine, niacin, calcium and iron. Dry legumes are almost devoid of ascorbic acid, but they synthesize ascorbic acid when they sprout (13). Legumes as a group are one of the important human foods and one of rather special interest from the standpoint of nutrition.

From the nutritional standpoint, the salient characteristic of legumes is their high protein content. But proteins differ in their capacity to fulfill protein needs, according to their amino acid composition. So combinations of several legumes can make a mixture which is high in protein quantity as well as quality.

The first worker to use a legume to treat protein deficiency was Dean (13, p. 81) who gave soybeans mixed with banana to children with kwashiorkor in Kampala, in Uganda.

In India, children with kwashiorkor were given chick-peas in the form of flour made from grains allowed to germinate for two days. The flour was mixed with banana, unrefined sugar and water, and autoclaved, giving a product resembling steamed cake. Taken in amounts providing about 60 grams of protein daily, this preparation produced a good clinical response, comparable to that seen in similar children receiving skim milk powder (21). These experiments do not, however, cast doubt on the conclusion that legumes in general enhance the nutritive value of diets.

Pulses and grain occupy a prominent place in the dietary patterns throughout Pakistan. Several varieties of pulses, peas, and legumes are eaten after cooking alone or with meat. These are also mixed with rice, or wheat to make bread, or ground to a powder, for curry broth or roasted to be eaten as such (54). Even though pulses form an integral part of the average man's diet in Pakistan, very few attempts have been made to form a mixture with several varieties. On the other hand mixing of various protein foods, each containing some of the essential amino acids, may in additive fashion, supply all the essential amino acids. Total quantity of poor quality protein ingested must necessarily be much larger than the amount of good quality protein (38).

In her study of plant protein mixtures Shakil (66) tried several combinations of pulses and legumes available in Pakistan in order to find one which could meet the daily requirements of an adult man. She recommended the following vegetable mixture (66 p. 8) as a satisfactory one to meet the requirement of essential amino acids:

Peanuts	75 grams
Mung	125 grams
Gram	100 grams
Sesame seed	40 grams
Either Whole Wheat or Rice	75 grams
	415 grams

This researcher analyzed her mixture for methionine content with paper chromatography. The same mixture can be tried to determine the biological value of the protein through the growth of white rats.

Jan (34) prepared four diet mixtures and evaluated their value by rat feeding experiments. Grains and legumes used in her study include whole wheat flours, white rice, cotton seed, ground mung bean, sunflour seed and pumpkin seeds. The mixtures did not permit animals to perform satisfactorily. The reason for this was the low methiomine content of the diet mixture.

The Rat in Laboratory Experiments

The albino rat is one of the important experimental laboratory animals which has contributed much in the knowledge of science of nutrition. Optimum conditions for the housing, maintenance, breeding, and survival of laboratory animals have been the subject of numerous authoritative works some of which have been sponsored by scientific organizations dedicated to this purpose (51).

Rats are omnivorous. They are selected for experiments because they have similar nutritional requirement to man. They are economical to buy and to feed and require little space to live. Their life cycle is about thirty times the rate of the human species.

White rats are a good choice for longitudinal studies. They multiply rapidly and have large litters which permit a high degree of experimental control. The female is ready for breeding 100 days after birth and the gestation period is 21 to 22 days which makes it possible to study many generations in a short time period.

The life span of a white rat compares to that of man as follows (32):

Human Age in Years	2½	3	3½	4	4½	5	5½	6	6½	30	96
Rat Age in Weeks	4	5	6	7	8	9	10	11	12	52	156

Weanling rats, twenty-one days or older, and still in the rapid growth period are used for short-term feeding experiments which are desirable to observe growth effects.

The known dietary requirements for the rats (23, p. 98) are:

Calcium:	40-50 mg.
Phosphorus:	0.5 percent
Potassium:	males 15.0 mg; females 8.0 mg.
Sodium:	0.5 percent
Chlorine:	5 mg.
Iron:	0.25 mg; 5 mg. for reproduction and lactation
Copper:	0.005 mg.
Iodine:	1-2 mcg.
Magnesium:	4 mg. per kilogram of body weight
Zinc:	40 mcg.
Cobalt:	0-4 mcg.
Aluminum:	1 mcg.
Arsenic:	2 mcg.
Boron:	0.8 mcg.
Bromine:	5 parts in 10 million in diet
Fluorine:	1 part in 10 million in diet
Protein:	25 to 30 percent
Fatty Acids:	25 mg. methyl linoleate
Vitamin A:	4 mcg. per kilogram of body weight
Thiamine:	10 mcg., 120 mcg. for lactation
Choline:	1 mg. to prevent renal lesions; 2-3 mg. to prevent fatty liver; 15 mg. for lactation

Vitamin D:	none if Ca:P ratio is between 1:1 to 2:1
Vitamin E:	1 mg.; 3 mg. for gestation
Vitamin K:	not required

Visual comparison of the experimental rat to the control animals is an important part of the animal feeding demonstration. The characteristics of the well-nourished and poorly nourished rat (5, p. 2) as follows:

THE WELL-NOURISHED RAT	THE POORLY-NOURISHED RAT
Clean smooth, glossy hair; smooth tail, free from roughness; bright pink eyes; pinkish nose, ears, feet, and tail; clean and tidy habits; quick, alert movements; good muscle control; easily handled; good natured; firm nails.	Shaggy, dull and thin fur; rough, dry, scaly ears, feet and tail; eyes not clear; pinched look in face; whiskers not long and sharp, possibly dirty; restless, irritable and cross; breathing difficulty, susceptible to "sniffles;" possible soft nails.

From a long series of rat feeding experiments, it has been found that both the amount of food and its composition influence well being of the rats, the kinds of diseases they contract, the severity of their ailments, and especially, their longevity.

Essential Amino Acid Requirements of the Growing Rat

Several attempts have been made so far to evaluate the nutritive value of proteins from their amino acid content. Block and Mitchell (6) used the amino acid composition of whole egg protein as a standard for evaluating the chemical score of protein based on the most limiting amino acid and obtained a correlation of 0.86.

Later on Johnson and others (35, 36) thought that the essential amino acid requirement would serve best as a standard for calculating of the amino acid index. The minimum requirements for the essential amino acids by the weanling rat for maximum growth have been determined, using casein amino acid diet at the ten percent protein level. The following requirement index is proposed as a chemical estimate of the nutritive value of protein (36, p. 88).

PATTERN OF AMINO ACIDS REQUIRED BY
GROWING RAT

Amino Acid	Percent of Diet	
L-Histidine	0.25	
L-Lysine	0.90	
L-Tryptophan	0.11	
L-Isoleucine	0.55	
L-Valine	0.55	
L-Leucine	0.70	
L-Threonine	0.50	
L-Methionine	0.16)	) 0.50
L-Cystine	0.34)	
L-Phenylalanine	0.42)	) 0.72
L-Tyrosine	0.30)	

Non-essential amino acid mixtures were added to make a total conventional protein content of ten percent.

The above reference pattern served as the minimal essential amino acid requirement of growing rats. The growth on this minimal amino acid diet was compared with whole egg protein diet. The growth rate in both the groups were equal at about five grams per day, and the efficiency of protein utilitization ratio was the same in both the groups.

This particular requirement index can be used as a standard for protein requirements of rats (36).

Effect of Fat on the Growth Rate of the Rat

The amount of fat in the diet also affects the growth of rats. In one study two diets were prepared for rats. The diets were modifications of relatively simple diets composed chiefly of semipurified components. In one group of diets, protein and fat were varied by replacing 20 to 25 percent of the semipurified diets with egg, milk, beef and peanut butter. In a second group of diets, the source and level of protein remained constant but the kind and level of fat varied. The fats were hydrogenated vegetable oil, lard and butter; the levels were eight to 16 percent (1).

In general, growth was good on all of the experimental diets under investigation. No evidence of dietary deficiency was apparent. Young rats tended to grow more rapidly when the levels of fat were 17 to 19 percent than when the diet contain nine percent fat or less. With some diets the growth rate was associated with calorie intake; with others, such as those containing high levels of egg, milk, beef or peanut butter, it appeared to be associated with efficiency of utilization (1).

Protein Efficiency Rating

Protein efficiency ratio is a measure of weight gain of a growing animal divided by protein intake:

$$PER = \frac{\text{Weight Gain (gm)}}{\text{Protein Intake (gm)}}$$

The PER was used as early as 1917 by Osborne and Mendel in their studies establishing differences in protein quality. It has most often been applied to studies on laboratory rats. It is the simplest method for evaluating protein quality since it requires only an accurate measure of dietary intake and weight gain. However, the method requires strict adherence to certain conditions: the calorie intake must be adequate. The protein must be fed at an adequate but not excessive level. Because of a higher level of dietary protein, weight gain does not increase proportionally with protein intake (8).

The experimental period in this method is usually about four weeks. Certainly the method affords good comparison of biological values between various proteins or between the proteins of various mixed foods and feeds (74).

The greatest source of error in the PER method lies in the use of weight gain per se as criterion of protein value. Weight gain cannot be assumed to represent proportional gain in body protein under all conditions.

Food Habits

Food habits are the way people have learned to select and consume food as a result of social pressure and cultural tradition. In general, they are formed early in life and are influenced by all the forces which mold an individual's personality and his behavior (48).

Food is always defined culturally, because each group in their own evolution, sets up a complex pattern of standarized behaviors. Food habits vary from one cultural group to another. Individuals within a culture respond to the approved behavioral pressures by selecting, consuming, and using those foods which are available. Dr. May says (43,

p. 87):

> It is true, therefore, that food habits of a group are the product of the group's present environment and past history. Those food habits and customs which have become meaningful to the group are carefully held and not quickly changed. It follows, therefore, that any one who would change a food habit must first understand the deeper meanings of the particular habit to the people.

There is considerable symbolism associated with particular items in the diet. In the words of Mead (17, p. 336):

> In most societies, food is the focus of emotional associations, a channel for interpersonal relations, for the communication of love or discrimination or disapproval; it usually has a symbolic reference.

Food habits become deeply imbedded in personalities as people are raised in a particular pattern. It is one of the basic media through which attitudes and sentiments are communicated to the child. The family meal situation is one of the important events in producing morale or a sense of unity. Certain foods, eaten early in life, become associated with these family sentiments.

Introducing change in food habits requires an understanding of the symbolic aspects of the different meals. Change can be easily introduced in relation to the foods which have less sentimental connection (17).

Techniques of changing behavior were studied in two experiments dealing with changes in food habits. Individual instruction and group decision, and lecture and group-decision methods were compared. The first experiment was concerned with infant feeding, the second with increasing milk consumption in the family. The subjects were mothers and housewives of low-socioeconomic status. Results showed that the group decision method was significantly more effective in leading mothers and housewives to action than were either individual instruction or lecture (37).

Selling new food products or introducing change in food habits are seldom easy. Introduction of any new food product should be developed in such a way that it is acceptable to the local population and it should fit well into existing or already changing eating habits. Raw material should be supplied in large amounts so that it is cheap. Production method and quality control should be sound. Sensible price structure should be established which will result in a selling price which the consumer can afford. This should also offer a fair profit to the producer (44).

CHAPTER III

METHOD OF PROCEDURE

Extensive literature was reviewed to learn the possibility of making vegetable protein mixtures which would provide growth and maintain the life of animals. Some of the grains and legumes used in this study include rice, lentils, mung beans and sesame seeds. In one ot the experimental diets, fish protein concentrate was used as a source of animal protein. Grains and pulses were selected on the basis of their cost, availability and acceptibility by the common Pakistani people. Three diet mixtures were prepared and fed to 18 rats for a period of six weeks. Out of the three diet mixtures one was used as a control diet while the other two were used as Experimental Diets I and II.

Determination of Composition of Diets

The control diet was taken originally from Lamb's Manual for Nutrition Courses in which cornstarch formed the major source of cereal (39, p. 19). Its composition was changed some to equalize the protein and calorie content with Experimental Diets I and II. The protein level in all of the diets was lowered to approximately 18.5 percent since many investigators have used from 10 to 18 percent protein (7, 35,36). After selecting the particular grain and legumes to make into the two experimental diets, the combination of different ingredients in two groups were calculated several times in different combinations.

Attempts were made to get the combination in such proportion that its essential amino acid composition was as close as possible to the following:

	% of Diet
L - Histidine	0.25
L - Lysine	0.90
L - Tryptophan	0.11
L - Isoleucine	0.55
L - Valine	0.55
L - Leucine	0.70

This pattern given above is considered as the minimum amino acid requirement of rats for maximum growth (36, p. 88). It was realized that it is difficult to get the ratio of amino acids in exactly the same amount as the standard ratio for rat growth. Finally, two ratios were chosen which were closest to the standard amino acid pattern selected. The amino acid composition of the three diets used in this study are given in Table I.

In Experimental Diet I fish-protein concentrate was added which made it quite easy to balance the amino acids. Methionine and lysine were the two most limiting amino acids in Experimental Diet II which was composed of vegetable proteins only. Methionine and lysine were adjusted by varying the amounts of sesame seeds and mung beans.

Providing sufficient calories was another problem while working with these diet mixtures. Especially in Experimental Diet I, the calorie content was low at the beginning. The amount of cottonseed oil was increased to make the calorie content the desired level. This made the diet extremely oily. No vitamins were added in both the

TABLE 1

AMOUNT OF ESSENTIAL AMINO ACIDS[1] IN 100 GRAMS DIETS

CONTROL DIET

	HISTI	LYSIN	TRYP	ISO	VALIN	LEU	THREO	METH	CYS	PHE	TYRO
Casein - 12%	.364	.967	.161	.791	.892	1.213	.516	.372	.046	.640	.702
Brewers Yeast 20%	.204	.539	.116	.392	.445	.527	.384	.035	.099	.310	.310
TOTAL FOR CONTROL	.568	1.506	.277	1.183	1.337	1.740	.900	.407	.145	.950	1.012
EXPERIMENTAL DIET 1											
Fish Protein Conc.[2] 12%	.148	.849	.086	.487	.450	.712	.504	.232	—	.317	—
Lentil - 30%	.174	.452	.064	.394	.408	.528	.268	.054	.061	.331	.199
Rice - 41%	.043	.101	.027	.119	.179	.220	.100	.046	.034	.128	.116
TOTAL FOR DIET 1	.365	1.402	.177	1.000	1.036	1.460	.872	.332	.095	.776	.315
EXPERIMENTAL DIET 2											
Mung Bean - 26%	.139	.427	.046	.346	.370	.564	.196	.068	.039	.299	.100
Lentil - 30%	.174	.452	.064	.394	.408	.528	.268	.054	.061	.331	.199
Rice - 16%	.017	.039	.010	.047	.070	.087	.039	.018	.013	.050	.046
Sesame Seeds - 20%	.070	.092	.011	.151	.141	.267	.112	.101	.078	.232	.151
TOTAL FOR DIET 2	.400	1.010	.131	.938	.989	1.446	.615	.241	.191	.912	.496

[1]The amino acid content of the diet ingredients was calculated using Amino Acid Content of Foods (73)

[2]Fish protein concentrate was obtained from Viobin Corporation, Monticello, Illinois 61856. It contained 85% protein on a dry basis.

experimental diets. Salt mixture W formulated per Wesson modification of Osborne and Mendals Salt Mixture was added to all the diets.[1]

The composition of the three diets in respect to the percentage of protein, fat and total calories is given in Table 2.

Preparation of Mixtures

Ingredients like casein, cornstarch, Brewers Yeast and salt mixture, which were already finely ground were carefully weighed. Lentil and mung bean were heated at 120 degrees for two hours to prevent the action of trypsin inhibition (72, p. 35). The ingredients such as rice, lentils, mung beans and sesame seeds were ground to a fine consistency by using a Waring blender. All the ingredients were weighed. The dry ingredients of each diet were mixed thoroughly in large crockery bowls with the help of a pastry fork. Oil was then added and was mixed very completely with the total ingredients until the mixture was homogeneous. The diet mixtures were stored in labeled glass jars and were placed in a freezer at once.

Sampling and Care of Rats

Weanling albino male rats weighing 50 to 60 grams were used.[2] Each rat was confined to an individual wire-mesh cage with removable

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[1]Salt mixture W was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio 44128.

[2]Animals were obtained from The Holtzman Company, 421 Holtzman Road, Madison, Wisconsin.

TABLE 2

COMPOSITION OF THE CONTROL AND EXPERIMENTAL DIETS[3]

INGREDIENT	AMOUNT %	PROTEIN %	FAT %	CALORIES
Control				
Casein	12	12	--	48
Cornstarch	50	--	--	200
Brewers Yeast	20	7.6	--	56.60
Cottonseed Oil	12	--	12	108.00
Cod Liver Oil	2	--	--	--
Salt Mixture	4	--	--	--
TOTAL	100	19.6	12	412.60
Experimental Diet I				
Fish Protein Concentrate	12	9.36	--	37.44
Lentil	30	7.41	--	102
Rice	41	2.74	--	148
Cottonseed Oil	14	--	14	126
Salt Mixture	3	--	--	--
TOTAL	100	19.5	14	414.27
Experimental Diet II				
Mung Bean	26	6.29	--	88.40
Lentil	30	7.41	--	102.00
Sesame Seed	20	3.64	10.6	116.40
Rice	16	1.07	--	58.08
Oil	5	--	5.0	45.00
Salt Mixture	3	--	--	--
TOTAL	100	18.41	15.6	409.88

[3]The protein, fat and calorie content of each diet mixture was calculated using Bowes and Church Food Values of Portions Commonly Used (11).

bottom trays. Paper was changed and the cages washed once per week with strong detergent, dipped in chlorox water and rinsed in clear water. The animals were born on February 16, 1970, were received in the laboratory on March 11, 1970 and feeding was started the same day.

Animals were randomly selected for each diet group for the experiment. Each rat was taken randomly from the total group, weighed and placed in one of the 18 cages which were previously numbered from one through 18. Numbers one through 18 were written on small pieces of paper, placed in a container and shaken well. One number was drawn at a time. The rat corresponding to this number was placed on the Control Diet. A second number was drawn, and the rat corresponding to this number was placed on Experimental Diet I. A third number was drawn and this rat was placed on Experimental Diet II. This procedure was repeated until all 18 animals were assigned to the three diet groups. Their initial weight was recorded in the laboratory record book.

All the 18 rats were marked by using ear notches and/or color as follows:

Rat Number 1 - No notches
Rat Number 2 - Right ear one notch
Rat Number 3 - Left ear one notch
Rat Number 4 - Right ear two notches
Rat Number 5 - Left ear two notches
Rat Number 6 - Right and left ear one notch each
Rat Number 7 - Right ear one notch, left ear two notches
Rat Number 8 - Left ear one notch, right ear two notches
Rat Number 9 - Right ear two notches, left ear two notches
Rat Number 10 - Right ear one notch, red head
Rat Number 11 - Right ear one notch, red tail
Rat Number 12 - Left ear one notch, green head
Rat Number 13 - Left ear one notch, green tail
Rat Number 14 - Right ear two notches, red head
Rat Number 15 - Left ear two notches, green head
Rat Number 16 - One notch right and left ear, red head
Rat Number 17 - Green head
Rat Number 18 - Red head

Rats number one through six served as controls, rats seven through 12 were in Experimental Diet I and rats 13 through 18 were in Experimental Diet II. Three cages were placed together on the carrier shelves in the laboratory.

Feeding Procedures

From the beginning of the experiment 15 grams of food was given daily to each rat in each of the three diet groups. These amounts were increased according to the consumption of the animals. All animals were fed ad libitum. Any food not eaten was carefully weighed and the total food eaten for the day was calculated. The amount eaten was recorded in a permanent record book which was ruled as follows:

Rat Number:
Diet:
Marking of Rat:

Date	Food Given gms.	Food Left gms	Food Eaten gms	Wt. of Rat gms	Remarks

Plenty of distilled water was given in the feeding bottles every day. The rats were weighed at the beginning of the experiment and each successive Tuesday and Friday.

The temperature of the room was maintained at 72^{o} to 74^{o} F. The room was cleaned every day.

At the end of the experiment an autopsy was made on one animal from each group to see if there was any internal change in organs. The overall length from tip of nose to tip of tail was also recorded.

ANALYSIS OF DATA

A nonparametric statistical analysis was used to evaluate the data. The Kruskal-Wallis one-way analysis of variance by ranks was used to test the null hypothesis that the "k" samples came from the population or from identical populations with respect to averages. Seigel (69, p. 184-185) outlines the analysis as follows:

> In the computation of the Kruskal-Wallis test, each of the "N" observations are replaced by ranks. That is, all of the scores from all of the "k" samples combined are ranked in a single series. ...When this has been done, the sum of the ranks in each sample (column) is found. The Kruskal-Wallis test determines whether these sums of ranks are so disparate that they are not likely to have come from samples which were drawn from the same population.

It can be shown that if the "k" samples actually are from the same population or from identical populations, that is if H_o is true, then H . . .is distributed as chi square with df=k-1, provided that the size of the samples are not too small. The formula used in this analysis is given in Siegel (69, p. 185) and is as follows:

$$H = \frac{12}{N(N+1)} \sum_{j=1}^{k} \frac{R_j^2}{n_j} - 3(N+1)$$

Where k = number of samples
n_j = number of cases jth sample
$N = \Sigma N_j$, the number of cases in all samples combined
R_j = sum of ranks in jth sample (column)

$\sum_{j=1}^{k}$ directs one to sum over the k samples (columns)

is distributed approximately as chi square with df = k-1 for sample sizes (njs) sufficiently large.

> When there are more than 5 cases in the various groups, that is $n_j>5$, the probability associated with the occurrence under H_o of values as large as an observed H may be determined by reference to Table C of the Appendix (69, p. 249).

If ties occur between two or more scores the value of H is somewhat influenced by these ties. One may correct for ties in computing H by using the formula for H and then dividing by (69, p. 188):

$$1 = \frac{\Sigma T}{N^3 - N}$$

Where $T = t^3 - t$ (when t is the number of tied observations in a tied group of scores).

N = number of observations in all k samples together, that is $N = n_j$.

ΣT directs one to sum all groups of ties.

Thus a general expression for H corrected for ties is:

$$H = \frac{\frac{12}{N(N+1)} \sum_{j=1}^{k} \frac{R_j^2}{n_j} - 3(N+1)}{1 - \frac{\Sigma T}{N^3 - N}}$$

The effect of correcting for ties is to increase the value of H and thus to make the result more significant than it would have been if uncorrected.

By interpolation in the table of χ^2 ordinates having two degrees of freedom the significance of the H value can be determined (69, p. 249).

CHAPTER IV

RESULTS AND DISCUSSION

At the end of six weeks of feeding, three rats, one from each group, were sacrificed and examined internally. Comparison of the lungs, liver, kidney and intestinal tract revealed no deviation from normal. Presented below is the final weight and overall length of each sacrificed animal from the three groups:

	Final Weight	Overall Length
Control	215	14 1/2 inches
Experimental Diet I	198	14 1/4 inches
Experimental Diet II	219	13 3/4 inches

Animal number ten in Experiment Diet I was ill from the onset of the experiment and was eliminated early in the study.

The calculated protein efficiency ratios for each animal and an average for each experimental group is presented in Table 3.

The protein efficiency ratios presented in Table 3 were ranked in preparation for analysis of the data as follows:

TABLE 3

PROTEIN EFFICIENCY RATIO OF EACH ANIMAL
FOR THE SIX WEEKS OF FEEDING

Rat Number	Total Weight Gain in gm	Total Protein Consumed	Protein Efficiency Ratio
	Control Diet		
1	177 gm	103.50 gm	1.71
2	161 gm	88.78 gm	1.81
3	206 gm	108.30 gm	1.90
4	190 gm	104.52 gm	1.81
5	156 gm	104.70 gm	1.48
6	235 gm	124.40 gm	1.88
			AVERAGE = 1.76
	Experimental Diet I		
7	119 gm	80.73 gm	1.47
8	115 gm	74.02 gm	1.55
9	203 gm	116.90 gm	1.73
11	142 gm	91.29 gm	1.55
12	94 gm	74.50 gm	1.26
			AVERAGE = 1.51
	Experimental Diet II		
13	110 gm	69.00 gm	1.59
14	145 gm	97.83 gm	1.48
15	112 gm	74.46 gm	1.50
16	115 gm	77.80 gm	1.47
17	158 gm	92.16 gm	1.71
18	163 gm	90.04 gm	1.81
			AVERAGE = 1.59

$n_1 = 6$ PER	Rank	$n_2 = 5$ PER	Rank	$n_3 = 6$ PER	Rank $N = 17$
1.71	10.5	1.47	2.5	1.59	9.0
1.81	14.0	1.55	7.5	1.48	4.5
1.90	17.0	1.73	12.0	1.50	6.0
1.81	14.0	1.55	7.5	1.47	2.5
1.48	4.5	1.26	1.0	1.71	10.5
1.88	16.0			1.81	14.0
	$76=R_1$		$30.5=R_2$		$46.5=R_3$

Substituting the various figures in the formula for H it is:

$$H = \frac{12}{17\ (18)} \left(\frac{(76)^2}{6} + \frac{(30.5)^2}{5} + \frac{(46.5)^2}{6} \right) - 3\ (18)$$

$$= \frac{12}{17\ (18)}\ (1509.09 - 3\ (18)$$

$$= 59.18 - 54 = 5.18$$

To correct for ties the value for H′ was determined by the following calculation:

$$H' = \frac{5.18}{1 - \frac{48}{17^3 - 17}} \qquad \Sigma T = 4\ (8-2) + 1\ (27-3)$$

$$= 24 + 24 = 48$$

$$= \frac{5.18}{.9902} = 5.23$$

Going to the table for χ^2 ordinates having two degrees of freedom the probability of obtaining a t value of $H > 5.18$ (or $H' > 5.23$) lies between 0.07 and 0.08. That is, based on the results of this experiment one would not reject the null hypothesis that the three populations of PER's are identical in favor of the alternative that the populations differ in some respect for any significance level less than about 0.08.

Since the statistical calculations do not show any significant differences in the three diets, the three general hypothesis must be rejected. However, comparison of the feeding charts shows that the daily food intake among the three groups were not the same. The Experimental Diet I was the least acceptable. This might be due to the oily appearance of the diet, the flavor of fish protein or lack of some B vitamins since no vitamin concentrate was added. If equal amounts of food had been eaten daily by all the groups this might make the PER values high enough to make them significant.

A second factor which could have affected the results is the number of rats in the different groups. Only six rats were used for each group. In Experimental Diet I one rat was eliminated because of illness of unknown origin. Seventeen rats, that composed the total population in the experiment, is a small group which was not large enough to prove any statistical significance. Increasing by two to three times the number of animals in each group might make the results significant.

A lower percentage of protein in the diets may be a factor which influenced the results. The three diets had a protein content at the 18 to 19 percent level, whereas Griffith and Farris (23) recommended a 25 percent level of protein for good growth. But there is evidence in which the experimenter used only a 10 percent level of protein and obtained good growth (7,35,36). Since there is a scarcity of protein food in Pakistan the higher level did not seem to be advisable in this experiment. However, if a 25 percent level of protein is fed it might produce PER values of significance without changing the sources of protein used in the diet.

CHAPTER V

SUMMARY AND RECOMMENDATIONS

The aim of this study was to compare the efficiency of a vegetable-protein mixture and a vegetable-protein mixture fortified with fish protein concentrate. The two experimental diet mixtures were composed of legumes, seeds and cereals that are available in Pakistan. Seventeen rats were fed the diets for a period of six weeks.

The visual observation of the rats during the experimental period did not show much difference among the animals receiving Experimental Diets I and II. The Control Diet was readily acceptable. The rats in this group ate more food daily and gained more weight per week than the experimental animals. Experimental Diet I was the least acceptable of the three diet mixtures. Rats in this group gained the least weight. The rats in Experimental Diet II showed fairly good growth. The overall condition of the rats in this group was superior to the animals on Experimental Diet I. Statistical calculation did not show a significant difference in the protein efficiency ratios among the three diets.

Protein efficiency ratio differences might have been observed if the rats in all three groups had consumed the same amount of their diets daily. Use of a considerably larger sample of animals in each group might have shown significant differences among the three mixtures.

Since the two diet mixtures were good enough to support life and growth of white rats, they can be recommended for human feeding if the total protein content is increased to 25 percent or more. Vitamins which the Pakistani people get through leafy green vegetables may make these mixtures more efficient.

Experiments with such a small number of animals are not valid enough to prove the efficiency of these diets. Since it is not possible for one person to handle a large number of rats, continued research in the area is highly recommended. The author thinks that it will be wise to try this same combination of ingredients but to increase the percent of protein fed. Care should be taken to maintain the same ratio of essential amino acids as in this study. It is also suggested that the composition of food in Experimental Diet I be changed to lessen the oily appearance of this diet in an effort to increase acceptability. Vitamins may be added to both experimental diet mixtures in natural form by using green leafy vegetables available in Pakistan.

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APPENDIX

TABLE 4

SIX WEEKS RECORD OF FEEDING THE CONTROL DIET
(Animal No. 1)

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
No Notches	1970 March	grams	grams	grams	grams	
	11	59	15	11.7	3.3	Normal and Active
	12		15	7.4	7.6	Normal and Active
	13	64	15	6.3	8.7	Normal and Active
	14		30	11.6	18.4	Normal and Active
	16		15	3.9	11.1	Normal and Active
	17	92	15	2.8	12.2	Normal and Active
	18		15	3.8	11.2	Normal and Active
	19		15	2.7	12.3	Normal and Active
	20	114	15	3.6	11.4	Normal and Active
	21		30	7.0	23.0	Normal and Active
	23		15	1.2	13.8	Normal and Active
	24	136	15	2.1	12.9	Normal and Active
	25		15	0	15.0	Normal and Active
	26		18	8.1	9.9	Normal and Active
	27	147	18	6.1	11.9	Normal and Active
	28		36	11.0	25.0	Normal and Active
	30		18	2.2	15.8	Normal and Active
	31	164	18	6.3	11.7	Normal and Active

TABLE 4 (Continued) - Animal No. 1

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
No Notches	1970 April	grams	grams	grams	grams	
	1		18	2.1	15.9	Normal and Active
	2		18	7.3	10.7	Normal and Active
	3	170	18	3.6	14.4	Normal and Active
	4		36	8.5	27.5	Normal and Active
	6		18	6.4	11.6	Normal and Active
	7	186	18	6.4	11.6	Normal and Active
	8		18	7.0	11.0	Normal and Active
	9		18	4.2	13.8	Normal and Active
	10	192	18	1.5	16.5	Normal and Active
	11		36	12.4	23.6	Normal and Active
	13		18	4.1	13.9	Normal and Active
	14	202	18	5.1	12.9	Normal and Active
	15		18	5.0	13.0	Normal and Active
	16		18	1.9	16.1	Normal and Active
	17	217	21	4.5	16.5	Normal and Active
	18		42	17.2	24.8	Normal and Active
	20	227	21	8.2	12.8	Normal and Active
	21		21	8.9	12.1	Normal and Active
	22		21	6.6	14.4	Normal and Active
	23	236				Terminated Weight

TABLE 4. (Continued) - Animal No. 2

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Right	1970 March	grams	grams	grams	grams	
	11	56	15	8.9	6.1	Normal and Active
	12		15	8.0	7.0	Normal and Active
	13	68	15	5.2	9.8	Normal and Active
	14		30	11.3	18.7	Normal and Active
	16		15	4.6	10.4	Normal and Active
	17	92	15	4.3	10.7	Normal and Active
	18		15	5.3	9.7	Normal and Active
	19		15	5.6	9.4	Normal and Active
	20	106	15	4.3	10.7	Normal and Active
	21		30	10.5	19.5	
	23		15	2.3	12.7	Turned food upside down
	24	129	15	6.0	9.0	
	25		15	3.2	11.8	Normal and Active
	26		18	8.2	9.8	Normal and Active
	27	138	18	7.3	10.7	Normal and Active
	28		36	16.7	19.3	Normal and Active
	30		18	6.3	11.7	Normal and Active
	31	146	18	5.7	12.3	Normal and Active

TABLE 4 (Continued) - Animal No. 2

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Right	1970 April	grams	grams	grams	grams	
	1		18	7.9	10.1	Normal and Active
	2		18	10.1	7.9	Normal and Active
	3	150	18	10.5	7.5	Normal and Active
	4		36	19.7	16.3	Normal and Active
	6		18	7.5	10.5	Normal and Active
	7	161	18	9.0	9.0	Normal and Active
	8		18	9.1	8.9	Normal and Active
	9		18	4.0	14.0	Normal and Active
	10	166	18	1.7	16.3	Normal and Active
	11		36	12.2	23.8	Normal and Active
	13		18	6.2	11.8	Normal and Active
	14	193	18	6.2	11.8	Normal and Active
	15		18	5.4	12.6	Normal and Active
	16		18	5.0	13.0	Normal and Active
	17	198	21	11.7	9.3	Normal and Active
	18		42	16.2	25.8	Normal and Active
	20		21	9.4	11.6	Normal and Active
	21	217	21	8.5	12.5	Normal and Active
	22		21	9.7	11.3	Normal and Active
	23	217				Terminated Weight

TABLE 4 (Continued) - Animal No. 2

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Left	1970 March	grams	grams	grams	grams	
	11	55	15	8.6	6.4	Normal and Active
	12		15	6.9	8.1	Normal and Active
	13	68	15	4.3	10.7	Normal and Active
	14		30	10.3	19.7	Normal and Active
	16		15	5.0	10.0	Normal and Active
	17	94	15	3.8	11.2	Normal and Active
	18		15	5.3	9.7	Normal and Active
	19		15	4.1	10.9	Normal and Active
	20	112	15	3.9	11.1	Normal and Active
	21		30	9.2	20.8	Normal and Active
	23		15	5.8	9.2	Normal and Active
	24	126	15	5.2	9.8	Normal and Active
	25		15	4.3	10.7	Normal and Active
	26		18	10.5	7.5	Normal and Active
	27	130	18	6.2	11.8	Normal and Active
	28		36	8.5	27.5	Normal and Active
	30		18	3.9	14.1	Normal and Active
	31	161	18	2.8	15.2	Normal and Active

TABLE 4 (Continued) - Animal No. 3

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Left	1970 April	grams	grams	grams	grams	
	1		18	4.5	13.5	Dump the Food Jar
	2		18	4.8	13.2	
	3	173	18	.4	17.6	Normal and Active
	4		36	5.3	30.7	Normal and Active
	6		18	5.9	12.1	Normal and Active
	7	197	18	2.8	15.2	Normal and Active
	8		18	5.1	12.9	Normal and Active
	9		18	2.6	15.4	Normal and Active
	10	210	18	2.9	15.1	Normal and Active
	11		36	7.7	28.3	Normal and Active
	13		18	.7	17.3	Normal and Active
	14	232	21	6.0	15.0	Normal and Active
	15		21	6.4	14.6	Normal and Active
	16		21	12.2	8.8	Normal and Active
	17	240	24	4.9	19.1	Normal and Active
	18		42	14.2	27.8	Normal and Active
	20		21	4.4	16.6	Normal and Active
	21	248	21	5.5	15.5	Normal and Active
	22		21	1.4	19.6	Normal and Active
	23	261				Terminated Weight

TABLE 4 (Continued) - Animal No. 4

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
2-Right	1970 March	grams	grams	grams	grams	
	11	56	15	7.3	7.7	Normal and Active
	12		15	6.1	8.9	Normal and Active
	13	74	15	6.1	8.9	Normal and Active
	14		30	10.8	19.2	Normal and Active
	16		15	6.0	9.0	Normal and Active
	17	97	15	4.8	10.2	Normal and Active
	18		15	5.8	9.2	Normal and Active
	19		15	.2	14.8	Normal and Active
	20	116	15	1.1	13.9	Normal and Active
	21		30	6.2	23.8	Normal and Active
	23		15	3.8	11.2	Normal and Active
	24	136	15	4.9	10.1	Normal and Active
	25		15	.1	14.9	Normal and Active
	26		18	.1	17.9	Normal and Active
	27	157	18	2.3	15.7	Normal and Active
	28		36	9.6	26.4	Normal and Active
	30		18	3.6	14.4	Normal and Active
	31	171	18	7.4	10.6	Normal and Active

TABLE 4 (Continued) - Animal No. 4

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
2-Right	1970 April	grams	grams	grams	grams	
	1		18	1.1	16.9	Normal and Active
	2		18	5.9	12.1	Normal and Active
	3	181	18	8.5	9.5	Normal and Active
	4		36	4.4	31.6	Normal and Active
	6		18	9.2	8.8	Normal and Active
	7	201	18	2.7	15.3	Normal and Active
	8		18	5.1	12.9	Normal and Active
	9		18	.4	17.6	Normal and Active
	10	219	21	6.7	14.3	Normal and Active
	11		42	12.4	29.6	Normal and Active
	13		21	10.6	10.4	Normal and Active
	14	230	21	8.5	12.5	Normal and Active
	15		21	9.1	11.9	Normal and Active
	16		21	5.7	15.3	Normal and Active
	17	240	21	7.6	13.4	Normal and Active
	18		42	22.2	19.8	Normal and Active
	20		21	9.1	11.9	Normal and Active
	21	242	21	8.6	12.4	Normal and Active
	22		21	10.7	10.3	Normal and Active
	23	246				Terminated Weight

TABLE 4 (Continued) - Animal No. 5

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
2-Left	1970 March	grams	grams	grams	grams	
	11	59	15	8.8	6.2	Normal and Active
	12		15	5.4	9.6	Normal and Active
	13	74	15	5.2	9.8	Normal and Active
	14		30	9.6	20.4	Normal and Active
	16		15	3.9	11.1	Normal and Active
	17	98	15	3.3	11.7	Normal and Active
	18		15	4.0	11.0	Normal and Active
	19		15	3.4	11.6	Normal and Active
	20	115	15	1.0	14.0	Normal and Active
	21		30	7.5	22.5	Normal and Active
	23		15	0	15.0	Normal and Active
	24	143	18	5.0	13.0	Normal and Active
	25		18	4.9	13.1	Normal and Active
	26		18	5.9	12.1	Normal and Active
	27	143	18	6.0	12.0	Normal and Active
	28		36	10.5	25.5	Normal and Active
	30		18	4.1	13.9	Normal and Active
	31	157	18	4.3	13.7	Normal and Active

TABLE 4 (Continued) - Animal No. 5

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
2-Left	1970 April	gram	gram	gram	gram	
	1		18	3.1	14.9	Dump the Food Jar
	2		18	9.1	8.9	Normal and Active
	3	162	18	2.4	15.6	Normal and Active
	4		36	11.4	24.6	Normal and Active
	6		18	6.3	11.7	Normal and Active
	7	174	18	5.4	11.6	Normal and Active
	8		18	6.6	11.4	Normal and Active
	9		18	4.4	13.6	Normal and Active
	10	181	18	4.2	13.8	Normal and Active
	11		36	10.8	25.2	Normal and Active
	13		18	4.9	13.1	Normal and Active
	14	192	18	5.2	12.8	Normal and Active
	15		18	4.7	13.1	Normal and Active
	16		18	2.7	15.3	Normal and Active
	17	204	21	6.8	14.2	Normal and Active
	18		42	16.9	25.1	Right side is out
						of fur; everything
						else is OK
	20		21	4.6	6.4	A big patch of fur
	21	213	21	6.6	14.4	missing; it increases
	22		21	9.7	11.3	little; it seems patch
						will develop on other
						side too
	23	215				Terminated Weight

TABLE 4 (Continued) - Animal No. 6

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Right	1970	grams	grams	grams	grams	
1-Left	March					
	11	58	15	7.5	7.5	Normal and Active
	12		15	7.3	7.7	Normal and Active
	13	69	15	6.1	8.9	Normal and Active
	14		30	9.9	20.1	Normal and Active
	16		15	6.2	8.8	Normal and Active
	17	94	15	2.7	12.3	Normal and Active
	18		15	4.7	10.3	Normal and Active
	19		15	2.1	12.9	Normal and Active
	20	114	15	2.0	13.0	Normal and Active
	21		30	4.2	25.8	Normal and Active
	23		15	.1	14.9	Normal and Active
	24	146	18	.7	17.3	Normal and Active
	25		18	2.4	15.6	Normal and Active
	26		18	1.3	16.7	Normal and Active
	27	167	18	4.0	14.0	Normal and Active
	28		36	5.8	30.2	Normal and Active
	30		18	2.4	15.6	Normal and Active
	31	192	18	0.2	17.8	Normal and Active

TABLE 4 (Continued) - Animal No. 6

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Right	1970	grams	grams	grams	grams	
1-Left	April					
	1		21	2.0	19.0	Normal and Active
	2		21	8.1	12.9	Normal and Active
	3	199	21	6.6	14.4	Normal and Active
	4		42	11.6	30.4	Normal and Active
	6		21	6.6	14.4	Normal and Active
	7	218	21	5.4	15.6	Normal and Active
	8		21	.9	20.1	Normal and Active
	9		21	7.1	13.9	Normal and Active
	10	235	21	6.2	14.8	Normal and Active
	11		42	11.9	30.1	Normal and Active
	13		21	3.4	17.6	Normal and Active
	14	254	21	2.7	18.3	Normal and Active
	15		21	2.9	18.1	Normal and Active
	16		21	.7	20.3	Normal and Active
	17	268	24	6.5	17.3	Normal and Active
	18		48	12.5	35.5	Normal and Active
	20		21	3.4	17.6	Normal and Active
	21	288	21	2.7	18.3	Normal and Active
	22		21	4.5	16.5	Normal and Active
	23	293				Terminated Weight

TABLE 5

SIX WEEKS RECORD OF FEEDING THE EXPERIMENTAL DIET I
(Animal No. 7)

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Right	1970					
2-Left	March	grams	grams	grams	grams	
	11	58	15	7.6	7.4	Normal and Active
	12		15	9.2	5.8	Normal and Active
	13	65	15	9.5	5.5	Normal and Active
	14		30	19.8	10.2	Normal and Active
	16		15	11.7	3.3	Rough fur with
	17	72	15	8.6	6.4	slight yellow
	18		15	9.2	5.8	color
	19		15	7.6	7.4	Rough fur with
	20	78	15	8.2	6.8	slight yellow
	21		30	13.9	16.1	color
	23		15	5.6	9.4	
	24	99	15	6.2	8.8	
	25		15	8.0	7.0	
	26		15	6.1	8.9	Fur looks much improved
	27	106	15	7.8	7.2	
	28		30	12.1	17.9	
	30		15	4.0	11.0	Fur looks normal
	31		15	6.3	8.7	

TABLE 5 (Continued) - Animal No. 7

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Right	1970					
2-Left	April	grams	grams	grams	grams	
	1		15	6.1	8.9	Normal and Active
	2		15	4.5	10.5	Normal and Active
	3	120	15	2.5	12.5	Normal and Active
	4		30	12.9	17.1	Normal and Active
	6		15	5.0	10.0	Normal and Active
	7	130	18	8.2	9.8	Normal and Active
	8		18	7.5	10.5	Normal and Active
	9		18	9.2	8.8	Normal and Active
	10	134	18	7.7	10.3	Normal and Active
	11		36	7.4	28.6	
	13		18	4.5	13.5	Normal and Active
	14	146	21	8.4	12.6	Normal and Active
	15		21	10.8	10.2	Normal and Active
	16		21	9.4	11.6	Normal and Active
	17	154	21	11.7	9.3	Normal and Active
	18		42	15.6	26.4	Normal and Active
	20		21	3.9	17.1	Normal and Active
	21	170	21	5.1	15.9	Normal and Active
	22		21	4.2	16.8	Normal and Active
	23	177				Terminated Weight

TABLE 5 (Continued) - Animal No. 8

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Left	1970					
2-Right	March	grams	grams	grams	grams	
	11	58	15	7.5	7.5	Normal and Active
	12		15	9.5	5.5	Normal and Active
	13	63	15	9.3	5.7	Normal and Active
	14		30	18.6	11.4	Normal and Active
	16		15	10.7	4.3	
	17	74	15	8.4	6.6	Slight rough fur
	18		15	9.1	5.9	
	19		15	8.1	6.9	Seems better fur today
	20	82	15	6.8	8.2	
	21		30	13.2	16.8	
	23		15	7.2	7.8	Improved
	24	97	15	5.9	9.1	
	25		15	4.0	11.0	
	26		15	5.3	9.7	
	27	111	15	4.5	10.5	
	28		30	23.1	6.9	
	30		15	7.2	7.8	
	31	115	15	4.3	10.7	Fur looks normal

TABLE 5 (Continued) - Animal No. 8

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Left	1970					
2-Right	April	grams	grams	grams	grams	
	1		15	7.2	7.8	
	2		15	3.1	11.9	
	3	122	15	8.5	6.5	Normal and Active
	4		30	12.8	17.2	Normal and Active
	6		15	2.9	12.1	Normal and Active
	7	132	18	10.0	8.0	Normal and Active
	8		18	7.8	10.2	Normal and Active
	9		18	5.2	12.8	Normal and Active
	10	136	18	8.4	9.6	Normal and Active
	11		36	16.7	19.3	Normal and Active
	13		18	8.8	9.2	Normal and Active
	14	144	21	9.9	11.1	Normal and Active
	15		21	9.2	11.8	Normal and Active
	16		21	12.8	8.2	Normal and Active
	17	154	21	10.7	10.3	Normal and Active
	18		42	18.9	23.1	Normal and Active
	20		21	8.9	12.1	Normal and Active
	21	165	21	11.8	9.2	Normal and Active
	22		21	4.1	16.9	Normal and Active
	23	173				Terminated Weight

TABLE 5 (Continued) - Animal No. 9

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
2-Right	1970					
2-Left	March	grams	grams	grams	grams	
	11	57	15	5.9	9.1	Normal
	12		15	5.4	9.6	
	13	72	15	6.9	8.1	
	14		30	13.7	16.3	
	16		15	6.3	8.7	
	17	92	15	7.7	7.3	Normal
	18		15	4.7	10.3	
	19		15	6.9	8.1	Normal and Active
	20	106	15	1.5	13.5	Normal and Active
	21		30	6.0	24.0	Normal and Active
	23		15	1.3	13.7	Normal and Active
	24	132	15	3.3	11.7	Normal and Active
	25		15	.3	14.7	Normal and Active
	26		15	.3	14.7	Normal and Active
	27	154	18	1.4	16.6	Normal and Active
	28		36	7.7	28.3	Normal and Active
	30		18	2.7	15.3	Normal and Active
	31	178	18	3.8	14.2	Normal and Active

TABLE 5 (Continued) - Animal No. 9

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
2-Right	1970					
2-Left	April	grams	grams	grams	grams	
	1		18	0.9	17.1	Normal and Active
	2		18	1.0	17.0	Normal and Active
	3	187	21	1.7	19.3	Normal and Active
	4		42	7.0	35.0	Normal and Active
	6		21	5.6	15.4	Normal and Active
	7	202	21	3.5	17.5	Normal and Active
	8		21	3.4	17.6	Normal and Active
	9		21	3.2	17.8	Normal and Active
	10	213	24	5.7	18.3	Normal and Active
	11		48	23.5	24.5	Dump food jar
	13		24	4.0	20.0	
	14	226	27	8.7	18.3	
	15		27	14.7	12.3	
	16		27	11.0	16.0	
	17	234	27	12.7	14.3	
	18		54	28.2	25.8	
	20		27	9.3	17.7	
	21	250	27	12.4	14.6	
	22		27	10.2	16.8	
	23	260				Terminated Weight

TABLE 5 (Continued) - Animal No. 11

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Right Red Tail	1970 March	grams	grams	grams	grams	
	11	56	15	7.1	7.9	Normal
	12		15	8.0	7.0	
	13	66	15	8.9	6.1	
	14		30	16.4	13.6	Mucus in the right eye
	16		15	8.1	6.9	Right eye better
	17	83	15	7.1	7.9	
	18		15	6.9	8.1	Eye OK
	19		15	7.0	8.0	
	20	92	15	2.9	12.1	Normal and Active
	21		30	8.9	21.1	Normal and Active
	23		15	3.0	12.0	Normal and Active
	24	124	15	5.4	9.6	Normal and Active
	25		15	1.5	13.5	Normal and Active
	26		15	3.3	11.7	Normal and Active
	27	141	15	4.3	10.7	Normal and Active
	28		30	4.2	25.8	Normal and Active
	30		15	3.8	11.2	Normal and Active
	31		15	2.6	12.4	Normal and Active

TABLE 5 (Continued) - Animal No. 11

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Right	1970					
Red Tail	April	grams	grams	grams	grams	
	1		15	1.2	13.8	Normal and Active
	2		15	1.1	13.9	Normal and Active
	3	163	18	5.9	12.1	Normal
	4		36	9.4	26.6	
	6		15	1.2	13.8	
	7	165	18	6.6	11.4	
	8		18	6.8	11.2	
	9		18	7.2	10.8	
	10	165	18	9.9	8.1	No gain, OK
	11		36	11.8	24.2	
	13		18	8.7	9.3	
	14	174	21	7.7	13.3	
	15		21	12.2	8.8	
	16		21	10.9	10.1	
	17	176	21	9.0	12.0	
	18		42	17.1	24.9	
	20		21	9.4	11.6	
	21	181	21	8.2	12.8	Little mucus right eye
	22		21	7.1	13.9	Eyes OK
	23	198				Terminated Weight

TABLE 5 (Continued) - Animal No. 12

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Left Green Head	1970 March	grams	grams	grams	grams	
	11	56	15	7.1	7.9	Normal and Active
	12		15	7.6	7.4	Normal and Active
	13	66	15	8.1	6.9	
	14		30	15.0	15.0	
	16		15	8.4	6.6	
	17	80	15	9.4	5.6	Very slight rough fur
	18		15	9.3	5.7	
	19		15	8.4	6.6	
	20	84	15	6.8	8.2	
	21		30	14.5	15.5	Normal fur
	23		15	9.0	6.0	Normal
	24	95	15	8.3	6.7	Normal
	25		15	7.7	7.3	Normal
	26		15	8.2	6.8	Normal
	27	102	15	5.3	9.7	Normal
	28		30	12.9	17.1	Normal
	30		15	6.6	8.4	
	31	114	15	3.9	11.1	Normal

TABLE 5 (Continued) - Animal No. 12

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Left	1970					
Green Head	April	grams	grams	grams	grams	
	1		15	6.5	8.5	
	2		15	6.1	8.9	
	3	118	15	4.7	10.3	
	4		30	17.8	12.2	
	6		15	6.4	8.6	
	7	116	18	6.0	12.0	Weight loss
	8	123	18	7.0	11.0	Weight gain
	9		18	8.9	9.1	Dump food jar, OK
	10	123	18	13.4	4.6	
	11	122	36	12.2	23.8	Normal
	13		18	10.8	7.2	
	14	127	21	9.0	12.0	Normal
	15		21	10.9	10.1	Normal
	16		21	9.5	11.5	Normal
	17	138	21	9.0	12.0	Normal
	18		42	17.2	24.8	Normal
	20		21	6.6	14.4	Normal
	21	146	21	12.3	8.7	Normal
	22		21	7.1	13.9	Normal
	23	150				Terminated Weight

TABLE 6

SIX WEEKS RECORD OF FEEDING THE EXPERIMENTAL DIET II
(Animal No. 13)

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Left Green Tail	1970 March	grams	grams	grams	grams	
	11	56	15	9.4	5.6	Normal and Active
	12		15	5.9	9.1	Normal and Active
	13	63	15	9.5	5.5	Normal and Active
	14		30	18.2	11.8	Normal and Active
	16		15	9.9	5.1	Normal and Active
	17	74	15	8.9	6.1	Normal and Active
	18		15	8.3	6.7	Normal and Active
	19		15	7.1	7.9	Normal and Active
	20	79	15	8.4	6.6	Normal and Active
	21		30	19.5	10.5	Normal and Active
	23		15	9.0	6.0	Normal and Active
	24	83	15	10.1	4.9	Normal and Active
	25		15	10.4	4.6	Normal and Active
	26		15	7.0	8.0	Normal and Active
	27	83	15	8.7	6.3	Normal and Active
	28		30	15.0	15.0	Normal and Active
	30		15	6.1	8.9	Normal and Active
	31	95	15	9.1	5.9	Normal and Active

TABLE 6 (Continued) - Animal No. 13

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Left	1970	grams	grams	grams	grams	
Green Tail	April					
	1		15	5.1	9.9	Normal and Active
	2		15	8.0	7.0	Normal and Active
	3	99	15	5.0	10.0	Normal
	4		30	11.5	18.5	Normal
	6		15	1.2	13.8	Normal
	7	116	18	10.9	7.1	Normal
	8		18	4.5	13.5	Normal
	9		18	7.1	10.9	Normal
	10	121	18	8.8	9.2	Normal
	11		36	8.9	27.1	Normal
	13		18	12.7	5.3	Normal
	14	128	18	8.0	10.0	Normal
	15		18	7.2	10.8	Normal
	16		21	4.6	16.4	Normal
	17	139	21	9.7	11.3	Normal
	18		42	16.7	25.3	Normal
	20		21	14.4	6.6	Dump the food jar
	21	152	21	5.7	15.3	
	22		21	8.7	12.3	
	23	166				Terminated Weight

TABLE 6 (Continued) - Animal No. 14

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
2-Right Red Head	1970 March	grams	grams	grams	grams	
	11	60	15	7.9	7.1	Normal and Active
	12		15	7.5	7.5	Normal and Active
	13	70	15	6.8	8.2	Normal and Active
	14		30	11.5	18.5	Normal and Active
	16		15	5.9	9.1	Normal and Active
	17	88	15	5.5	9.5	Normal and Active
	18		15	6.3	8.7	Normal and Active
	19		15	5.8	9.2	Normal and Active
	20	97	15	5.3	9.7	Normal and Active
	21		30	7.1	22.9	Normal and Active
	23		15	3.2	11.8	Normal and Active
	24	118	15	3.9	11.1	Normal and Active
	25		15	2.9	12.1	Normal and Active
	26		15	2.2	12.8	Normal and Active
	27	128	15	3.2	11.8	Normal and Active
	28		30	1.4	28.6	Normal and Active
	30		15	1.7	13.3	Normal and Active
	31	143	15	1.2	13.8	Normal and Active

TABLE 6 (Continued) - Animal No. 14)

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
2-Right	1970	grams	grams	grams	grams	
Red Head	April					
	1		15	2.2	12.8	Normal and Active
	2		15	1.5	13.5	Normal and Active
	3	150	15	0.7	14.3	Normal and Active
	4		30	3.0	27.0	Normal and Active
	6		15	1.9	13.1	Normal
	7	165	18	1.7	16.3	Normal
	8		18	3.0	15.0	Normal
	9		18	4.6	13.4	Normal
	10	176	18	4.0	14.0	Normal
	11		36	11.4	24.6	Normal
	13		18	4.9	13.1	Normal
	14	192	18	6.4	11.6	Normal
	15		18	1.9	16.1	Normal
	16		21	6.6	14.4	Normal
	17	198	21	4.6	16.4	Normal
	18		42	16.5	25.5	Normal
	20		21	3.9	17.1	Normal
	21	202	21	9.1	11.9	Normal
	22		21	5.4	15.6	Normal
	23	205				Terminated Weight

TABLE 6 (Continued) - Animal No. 15

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
2-Left Green Head	1970 March	grams	grams	grams	grams	
	11	58	15	8.1	6.9	Normal and Active
	12		15	9.3	5.7	Normal and Active
	13	64	15	6.9	8.1	Normal and Active
	14		30	18.2	11.8	Normal and Active
	16		15	7.5	7.5	Normal and Active
	17	78	15	8.9	6.1	Normal and Active
	18		15	6.6	8.5	Normal and Active
	19		15	6.1	8.9	Normal and Active
	20	92	15	3.8	11.2	Normal and Active
	21		30	12.7	17.3	Normal and Active
	23		15	6.3	8.7	Normal and Active
	24	103	15	7.1	7.9	Normal and Active
	25		15	6.3	8.7	Normal and Active
	26		15	5.1	9.9	Normal and Active
	27	110	15	4.3	10.7	Normal and Active
	28		30	10.7	19.3	Normal and Active
	30		15	5.5	9.5	Normal and Active
	31	118	15	6.2	8.8	Normal and Active

TABLE 6 (Continued) - Animal No. 15

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
2-Left Green Head	1970 April	grams	grams	grams	grams	
	1		15	6.8	8.2	Normal and Active
	2		15	4.4	10.6	Normal and Active
	3	123	15	5.7	9.3	Normal
	4		30	9.5	20.5	Normal
	6		15	4.7	10.3	Normal
	7	132	18	8.3	9.7	Normal
	8		18	9.2	8.8	Normal
	9		18	8.4	9.6	Normal
	10	133	18	8.4	9.6	Little Gain, OK
	11		36	17.0	19.0	Normal
	13		18	9.2	8.8	Normal
	14	142	18	6.5	11.5	Normal
	15		18	6.1	11.9	Normal
	16		21	10.7	10.3	Normal
	17	150	21	11.2	9.8	Normal
	18		42	19.4	22.6	Normal
	20		21	7.7	13.3	Normal
	21	163	21	7.7	13.3	Normal
	22		21	9.0	12.0	Normal
	23	170				Terminated Weight

TABLE 6 (Continued) - Animal No. 16

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Left	1970	grams	grams	grams	grams	
1-Right	March					
Red Head						
	11	58	15	6.7	8.3	Normal and Active
	12		15	9.7	5.3	Normal and Active
	13	68	15	9.8	5.2	Normal and Active
	14		30	14.7	15.3	Normal and Active
	16		15	7.9	7.1	Normal and Active
	17	78	15	8.4	6.6	Normal and Active
	18		15	7.1	7.9	Normal and Active
	19		15	6.2	8.8	Normal and Active
	20	83	15	9.7	5.3	Normal and Active
	21		30	15.8	14.2	Normal and Active
	23		15	8.6	6.4	Normal and Active
	24	93	15	6.5	8.5	Normal and Active
	25		15	7.3	7.7	Normal and Active
	26		15	3.4	11.6	Normal and Active
	27	104	15	6.6	8.4	Normal and Active
	28		30	16.2	13.8	Normal and Active
	30		15	3.0	12.0	Normal and Active
	31	112	15	9.3	5.7	Normal and Active

TABLE 6 (Continued) - Animal No. 16

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
1-Left	1970	grams	grams	grams	grams	
1-Right	April					
Red Head						
	1		15	3.7	11.3	Normal and Active
	2		15	6.8	8.2	Normal and Active
	3	113	15	3.2	11.8	Normal and Active
	4		30	13.1	16.9	Normal and Active
	6		15	1.1	13.9	Normal and Active
	7	124	18	5.3	12.7	Normal and Active
	8		18	6.7	11.3	Normal and Active
	9		18	6.2	11.8	Normal and Active
	10	131	18	2.8	15.2	Normal and Active
	11		36	11.8	24.2	Normal and Active
	13		18	5.9	12.1	Normal and Active
	14	148	18	4.2	13.8	Normal and Active
	15		18	6.9	11.1	Normal and Active
	16		21	9.8	11.2	Normal and Active
	17	154	21	7.2	13.8	Normal and Active
	18		42	14.5	27.5	Normal and Active
	20		21	8.4	12.6	Normal and Active
	21	166	21	6.7	14.3	Normal and Active
	22		21	10.2	10.8	Normal and Active
	23	173				Terminated Weight

TABLE 6 (Continued) - Animal No. 17

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
Green Head	1970 March	grams	grams	grams	grams	
	11	59	15	8.1	6.9	Normal
	12		15	6.2	8.8	Normal
	13	69	15	7.8	7.2	Normal
	14		30	12.3	17.7	Normal
	16		15	7.0	8.0	Normal
	17	84	15	9.9	5.1	Normal
	18		15	4.1	10.9	Normal
	19		15	4.7	10.3	Normal
	20	94	15	4.9	10.1	Normal
	21		30	9.3	20.7	Normal
	23		15	4.4	10.6	Normal
	24	110	15	2.4	12.6	Normal
	25		15	2.9	12.1	Normal
	26		15	5.5	9.5	Normal
	27	120	15	3.6	11.4	Normal
	28		30	2.7	27.3	Normal
	30		15	2.8	12.2	Normal
	31	133	15	4.9	10.1	Normal

TABLE 6 (Continued) - Animal No. 17

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
Green Head	1970 April	grams	grams	grams	grams	
	1		15	.5	14.5	Normal
	2		15	2.0	13.0	Normal
	3	144	15	.2	14.8	Normal
	4		36	8.8	27.2	Normal
	6		18	5.4	12.6	Normal
	7	163	18	6.8	11.2	Normal
	8		18	2.2	15.8	Normal
	9		18	12.1	5.9	Dump Food Jar
	10	167	21	6.9	14.1	Normal
	11		36	28.6	7.4	Dump Again
	13		21	1.8	19.2	Normal
	14	172	18	11.7	6.3	Dump Food
	15		18	1.7	16.3	Normal
	16		21	5.2	15.8	Normal
	17	186	21	3.4	17.6	Normal
	18		42	16.2	25.8	Normal
	20		21	2.1	18.9	
	21	209	21	6.3	14.7	
	22		21	3.0	18.0	
	23	217				Terminated Weight

TABLE 6 (Continued) - Animal No. 18

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
Red Head	1970 March	grams	grams	grams	grams	
	11	56	15	9.3	5.7	Normal and Active
	12		15	9.6	5.4	Normal and Active
	13	63	15	9.0	6.0	Normal and Active
	14		30	15.0	15.0	Normal and Active
	16		15	8.8	6.2	Normal and Active
	17	76	15	8.4	6.6	Normal and Active
	18		15	8.7	6.3	Normal and Active
	19		15	6.7	8.3	Normal and Active
	20	84	15	5.7	9.3	Normal and Active
	21		30	12.3	17.7	Normal and Active
	23		15	6.1	8.9	Normal and Active
	24	105	15	3.1	11.9	Normal and Active
	25		15	4.9	10.1	Normal and Active
	26		15	1.9	13.1	Normal and Active
	27	120	15	2.4	12.6	Normal and Active
	28		30	7.8	22.2	Normal and Active
	30		15	1.3	13.7	Normal and Active
	31	136	15	2.4	12.6	Normal and Active

TABLE 6 (Continued) - Animal No. 18

Ear Marks	Date	Weight Gain	Food Given	Food Left Over	Food Eaten	Remarks
Red Head	1970 April	grams	grams	grams	grams	
	1		15	1.7	13.3	Normal and Active
	2		15	4.9	10.1	Normal and Active
	3	142	15	6.5	8.5	Normal and Active
	4		30	3.7	26.3	Normal and Active
	6		15	1.7	13.3	Normal
	7		18	5.1	12.9	Normal
	8		18	4.9	13.1	Normal
	9		18	4.2	13.8	Normal
	10		18	6.4	11.6	Normal
	11		36	10.7	25.3	Normal
	13		18	1.7	16.3	Normal
	14	180	18	3.6	14.4	Normal
	15		18	4.0	14.0	Normal
	16		21	9.0	12.0	Normal
	17	189	21	3.5	17.5	Normal
	18		42	14.9	27.1	Normal
	20		21	3.9	17.1	Normal
	21	208	21	5.2	15.8	Normal
	22		21	5.9	15.1	Normal
	23	219				Terminated Weight

VITA

Meherun Nessa

Candidate for the Degree of

Master of Science

Thesis: COMPARISON OF THE EFFICIENCY OF DIETS COMPOSED OF A VEGETABLE PROTEIN MIXTURE VERSUS A VEGETABLE PROTEIN PLUS FISH PROTEIN CONCENTRATE AS DEMONSTRATED BY FEEDING ALBINO RATS

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