BIOASSAY EVALUATION OF A MIXTURE OF HORSEGRAM AND SESAME PROTEINS

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I INTRODUCTION

The high incidences of protein malnutrition and nutritional diseases among weaned infants and young children in India call for effective step to improve the protein content of Indian diet (Gopalan, 1964). The Third World Food Survey of the Food and Agriculture Organisation (FAO, 1963) had revealed that the daily percaput consumption of animal protein in the developing regions is only nine grams while the world picture of protein intake eighty, is depressing. It has been shown that in a large part of the under developed regions, a consumption of legumes ranging from 30 - 70 grams per head daily is the general rule (FAO, 1964., ICMR, 1964). The predominance of cereals and the lack of animal foods make the Indian diet still worse, Milk is almost the sole source of animal protein for a majority of the vegetarian population in India but is available only in a limited quantity of five grams according to an estimation of FAO/WHO (1965). Surveys carried out in India show that there is a shortage of proteins in the diets of major section of the population (Pandit and Someswara Rao, 1961., Subramanyam et al., 1962., Radhakrishna Rao, 1957). So, the locally available legumes, as Aykroyd (1961) points out may offer a practical solution to meet at least partially the protein needs of the growing population. Therefore, it becomes necessary to include low cost and easily available protein rich foods in the diet.

Horsegram was selected for this investigation because of its low cost* and availability compared to other pulses like red gram, bengal gram and green gram which are used commonly in South Indian diets. Horsegram, like the other legumes is deficient in methinnine while sesame is comparatively rich in sulphur containing amino acids (Kuppuswamy et al., 1958., Venkata Rao et al., 1964). So sesame protein was selected for supplementation of horsegram protein in the ratio of 4:1. This ratio was chosen after a comparison of the calculated amino acid composition of the horsegram-sesame mixture (Aykroyd et al., 1966., Orr and Watt, 1957) with the amino acid requirements of the growing rats as proposed by Almquist (1959).

In general, two methods serve as a basis for evaluating the nutritive value of proteins. These are the determination of biological value (BV) (Thomas, 1909) and the Protein Efficiency Ratio (PER) (Osborne, et al., 1919). Little progress has been made in protein evaluation since the introduction of PER, except to point out the short comings and discrepancies in this method (Bender, 1957., Janson, 1962., Forbers, 1956).

Recently Hegsted and Chang (1965) have applied the principles of a quantitative biological assay or bioassay.

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* One kilo gram of horsegram costs sixty four paise, whereas one kilogram of red gram costs one rupee and sixty paise and greengram costs one rupee and forty eight paise as quoted by 'Chinthamani' Coimbatore Cooperative Super Market on 20-2-1967.

The slope ratio technique using gain as the response and nitrogen intake as the dose appears to be a satisfactory index for protein evaluation. The 'RELATIVE GROWTH INDEX' is proposed as a satisfactory measure of nutritive value, this being a slope of the regression between nitrogen intake and body weight gain. An attempt was made in the study to evaluate with skim milk protein as reference, a mixture of horsegram and sesame proteins using the bioassay techinique proposed by Hegsted and Chang (1965).

Measurement of hepatic nitrogen has been found to reflect the protein nutriture of the animal with reference of ingested protein (Henry *et al.*, 1961).

Enzymes being metabolically active proteins their activity might be considered as an index of dietary protein quality. As a considerable part of the nitrogen is retained in the body in the form of enzymes, the determination of enzyme levels seems to offer possibilities in assessing the value of dietary proteins (Mauron, 1966). The activities of various hepatic enzymes have been found to increase, remain constant or decrease when the amount and source of protein intake is changed. (Litwack *et al.*, 1952., Muramatsu and Ashida, 1962., Allison, 1963). According to Ashida (1963) the dynamic equilibrium existing between the anabolism and catabolism of essential constituents within the cell is reflected in the alterations of the activities of certain hepatic enzymes.

Hence, the determination of hepatic nitrigen content and hepatic succinic dehydrogenase have been taken up for the present study.

An attempt was made in the study to evaluate with skim milk protein as reference, a mixture of horse-gram and sesame proteins using the bioassay technique proposed by Hegsted and Chang (1965). Each of these two dietary proteins was fed at four different concentrations. For the horse-gram and sesame mixture the protein concentration chosen were 9, 11, 13 and 15 per cent and for skim milk 7, 9, 11 and 13 per cent. These levels were chosen as "equally effective levels for growth" as proposed by Hegsted and Chang (1965).

The criteria used for the bioassay evaluation are as follows:

1. Growth rate
2. Food and Protein intake
3. Hepatic weight and nitrogen
4. Hepatic succinic dehydrogenase activity
5. Regression of body weight gain on nitrogen and protein intake.

II REVIEW OF LITERATURE

Methods of Protein Evaluation

A number of methods had been developed and improved upon for the determination of the nutritive value of proteins (N.R.C. 1963). Osborne, Mendel and Ferry (1919) developed methods using the growth promoting value of a protein in a diet containing adequate amounts of other nutrients. There are basically two biologic procedures in common use for the evaluation of the nutritive value of proteins. Biological value as determined by the methods of Thomas (1909), and modified by Mitchell (1924) and Mitchell and Carmen (1926) is defined as the "per centage of absorbed nitrogen retained" and is generally considered the method of choice. Justification of the value or correctness of chemical score (Block and Mitchell, 1946) or protein score (FAO, 1957) was infered from the correlation between biological value and the computed values. Biological value can be determined only when the animal is depleted of nitrogen and the intake of protein is low so that the animal is in negative balance. The "nitrogen balance index" of Allison and Co-workers (Allison, 1955) is a modification of this method.

The other method is the protein efficiency ratio originally proposed by Osborne and Co-worker (1919). In this method the gain per gram of protein eaten is computed in young rats fed diets in which protein is limiting usually 9 to 10 per cent of the diet. This method has been criticised (Hegsted and Worcester, 1947). Since PER is highly correlated with weight gain. The PER is thus not characteristic of protein but of the rate of gain in weight of the animals consuming the diet. The recent development of NPU (Miller, 1963) attempts to account atleast in part for the maintenance requirement by the inclusion of a negative control fed no protein.

Cannon et al.(1944) evaluated proteins by their relative ability to promote weight recovery in protein depleted adult rats. Allison (1969) correlated body weight gain and nitrogen intake of adult animals and growing ones. The carcass nitrogen analysis developed by Miller and Bender (1955) improved the accuracy in measuring the utilisation of protein for growth and maintenance.

The methods based on the regeneration of the constituents of blood and liver (Campbell and Kosterlitz, 1948) provide additional information regarding the value of dietary proteins for different physiological functions. Apart from these biological methods determination of availability of amino acids also indicate an estimate of nutritive value of individual proteins (Kuiken and Lyman, 1948).

All the above mentioned methods do not measure exactly the same metabolic function of protein and hence give only a rough estimate of the relative nutritive values of dietary proteins.

Factors influencing the estimate of nutritive value of Proteins.

Many experimental conditions might influence the results of protein evaluation. Some of the major ones are discussed here. The nutritive value of a protein was found to be a function of the level of dietary proteins (Harris and Buress, 1959., Morrison, 1964). If the calorie intake is low, efficiency of utilisation of the dietary proteins would fall because the protein might be used as a source of calories (Swanson, 1959). Species (Arnrich et al., 1951), age (Block and Mitchell, 1946), pregnancy and lactation (Nelson and Evans, 1953) and possibly patterns of eating (Cohn et al., 1962) might influence the amino acid requirements of the experimental animals.

The requirement for maintenance is much lower than that for growth. A diet which is used efficiently during a period of rapid growth may provide more protein than needed for maintenance; hence it may be used less efficiently in adult animals than in the young (Fisher, et al., 1964). There is also evidence that requirements for certain essential amino acids change during maturation or during depletion. Changes of this type could result in a difference in the apparent nutritive value of a protein fed to matured rather than growing subjects (Mitchell, 1959).

It is inevitable that assay methods differ in classifying proteins according to their nutritive value. Despite the vagaries inherent, protein evaluation by various methods had led to classification of many commonly used dietary proteins in the same general order of adequacy. As a result of early experiments, the proteins of milk and egg have frequently been used as standards of excellence (Block and Mitchell, 1946., Rama Rao et al., 1964), while grain and legume proteins had been found to have a lower nutritive value.

Of the many methods of protein evaluation only the Bioassay method would be discussed in great detail.

Bioassay Technique and Protein Evaluation

Bioassays had been developed over the past 30 to 40 years in several branches of Science. They are defined as a determination of the potency of a physical, chemical or biological agent by means of a biological indicator (Bliss, 1952).

The purpose of a biologic assay is to determine the relative potency of some test material compared with a standard. It is important to stress this fact since, otherwise it is known only that the proteins tested have a different nutritive value. It is believed that biological value does this. When the requirement of one protein is known, the requirement for other proteins can be compared (Hegsted, 1957., N.R.C., 1957). It forms the basis of recommended dietary allowances for proteins (N.R.C., 1964). Similar use cannot be made of PER values and the equivalent protein content of food cannot be estimated.

Bioassays are based on the following principles: (1) Potency is a property of the substance under investigation and not of the response. (2) Potency is relative and not absolute. That is, the potency of one preparation (unknown) can be in terms of another (the standard) which elicits a similar biological response. (3) The assayed potency is only an estimate of the true value. A suitable measure of the precision of each estimate must be possible from the data obtained. (4) A sound experimental design is an indispensable requirement (Nutrition Reviews, 1966).

Thus it is essential that the test substance and the standard be studied concurrently in the same species under identical conditions. The type of responses have extra-ordinary variety but nearly all can be separated into two categories (Finney, 1952), the all or none response, such as survival or death and the graded response. The latter is related to some function of dosage and is suitable for measuring responses to increasing dosages.

The application of a standard bioassay procedures for the evaluation of nutritive value of proteins with growing albino rats had been examined by Hegsted and Chang (1965a). The slope ratio technique using nitrogen intake as the measure of dose and body weight as the response appeared to be most satisfactory for the protein evaluation. "The relative growth index" as proposed by Hegsted and Chang (1965b) is the slope of the regression between dose and response expressed as a percentage of slope obtained with a protein of maximal nutritive value.

The Bioassay method of protein evaluation as proposed by Hegsted and Worcester (1966) was as followed. The protein tested were mixed in the diet at varying predetermined or arbitrary levels which are known as equally effective levels for weight gain. Eighteen weanling rats were used for each protein tested at three levels. The diets were fed for three weeks and food consumption and body weight were recorded. Analysis of the data were done. Regression equation of body weight gains on nitrogen or protein intakes were calculated. The slope of the regression for a test protein is expressed as a percentage of slope obtained with a protein of a maximum nutritive value.

Using the bioassay technique and lactalbumin as a standard protein, the relative nutritive values for casein, soy protein and gluten were 70, 34 and 22 respectively. The above data showed that conventional biological values, PER and NPU indices overestimated the nutritive potency of poor quality proteins for growing rats. As an example the biological value of wheat gluten was reported as 40, whereas the relative growth index by the bioassay method gave it a value of about 20. More recent work by Hegsted and Chang (1965) with the same proteins lead to the conclusion that net nitrogen utilization was essentially constant for each of these proteins at all levels of intake upto those which support maximal growth. The authors pointed out that if such indices are valid estimates of nutritive value, the intake of each protein should be convertible to lactalbumin equivalents that would give the quantity of any test protein which would produce the same increase in body weight as a given amount of lactalbumin. When the combined data for net nitrogen utilization were plotted against "lactalbumin nitrogen equivalents" nitrogen utilization appears to be similar for each protein upto levels which closely approach those required for maximum growth.

Thus the results of Hegsted and Chang (1965) lent strong support to the thesis that reliable bioassays are feasible for studies of nutritive value of proteins, and may have a significant advantage over the assays presently employed like PER, NPU etc. The evidence indicated that bioassays have capability for contributing data from a wide range of protein concentrations and adaptability for testing biological potency of proteins in terms of a standard. Although bioassays may contribute limited information as to cause and effect and therefore be of marginal value in determining amino acid content of proteins, they had demonstrated their usefulness as measures of overall biological potency for a variety of substances, such as vitamins, hormones, antibiotics and various chemical agents.

Contrary to the conclusions of Miller and Payne (1961) or Morrison et al. (1963), Hegsted and Chang (1965) were not able to show any decrease in the utilisation of nitrogen as the intake was increased until approximately maximal growth was achieved. In fact, the utilization of wheat gluten appeared to be essentially constant upto the highest level fed, 40 per cent of the diet. The reason for this discrepancy is unknown but it appears possible, at least, that it may relate to the time allowed for the test period. Hegsted and Chang pointed out earlier (1965) that biological values and net protein utilization (NPU) values for low quality proteins appeared to be substantially higher than the values obtained with bioassays.

Protein Efficiency Ratio

This procedure developed by Osborne et al.(1919) is based on the growth promoting value of a protein in a diet containing adequate amounts of other nutrients. The nutritive value is expressed as the Protein Efficiency Ratio (PER) that is, the ratio of the gain in body weight (g) to the protein (g) consumed in a specified period of time. Because of its simplicity, this method has been widely used by different workers for studying the nutritive value and supplementary relations between different proteins. Chapman et al.(1959) and American Association of Official Agricultural Chemists (1960) have described standard procedures for PER using rats aged 21 to 28 days with an experimental period of 28 days.

A number of factors affect the PER values; the most important of these are, the level of protein in the diet (Tasker et al., 1960), age (Block and Mitchell, 1946), Sex (Morrison and Campbell, 1960) and strain of the rats (Jansen, 1962), variation in food intake (Sherwood and Weldon, 1953) and duration of the experimental period (Joseph et al., 1960).

Although attempts have been made to develop other approaches or to make predictions of nutritive value from chemical data, little progress has been made since the introduction of PER by Osborne, Mendel and Ferry (1919) expect to point out the short comings and discrepancies in the data (Nutrition Reviews, 1966). PER index has the following criticisms, (a) the assumptions that the gain in body weight is constant in composition is not always valid; (b) the results vary with the level of proteins in the diet and the period of experiment and (c) in the method no allowance is made for protein requirement for maintenance (Jansen, 1962., Bender, 1956).

It has been suggested that the PER obtained with test proteins should be multiplied by the fraction

$$\frac{2.5}{\text{Determined PER of reference casein}}$$

The purpose of this provision is to reduce the variability among results obtained in various laboratories (Jansen, 1962).

Hepatic Nitrogen

The protein content of liver has been found to be a function of the amount and quality of dietary protein. When rats were fed with a protein free diet, the liver lost 40 per cent of its original protein (Addis et al., 1940). Kosterlitz (1944) found that liver nitrogen in the repletion of depleted rats, expressed in terms of unit body weight, was a function of the amount and kind of dietary protein. Harrison and Long (1945) fasted adult rats for 48 hours and subsequently fed diets containing the test proteins for four days, ad libitum and found out that the regeneration of hepatic proteins were directly proportional to the quantity and quality of dietary proteins consumed.

There are controversial views of using the hepatic nitrogen as a measure of the protein utilization. The results of Guggenheim and Buechler-Czaczkes (1950) do not agree with those of Henry et al. (1953) or Vars and Gurd (1947). They found that regeneration of liver protein was not necessarily related to biological value as determined by growth rate and nitrogen balance. Guggenheim and Buechler-Czaczkes (1950) estimated the biological values of eight proteins by five different methods, namely, protein efficiency ratio, nitrogen balance, liver nitrogen, capacity to regenerate haemoglobin and granulocytes. They observed that though soya bean was well utilised for hepatic protein regeneration, it was poorly utilised for forming haemoglobin and granulocytes. Egg protein ranked high in all the other criteria but not in hepatic protein regeneration. Gelatin was not good in maintaining nitrogen balance and forming haemoglobin but proved superior to the cereal proteins for the regeneration of hepatic protein.

Allison (1955) criticised the hepatic nitrogen method based on the fact that the hepatic nitrogen content might merely indicate the filling up of protein reserves of one portion of body and hence might not reflect the protein nutriture of the other organs.

Babson (1954) found that tumour-bearing rats had high concentrations of liver proteins while they were in a protein depleted stage. Similarly Henry et al.(1961) and Goodlad and Munro (1959) showed that cortisone administration to rats increased the protein content of liver of rats fed either a protein-free diet or one containing 20 per cent Casein. Rosenthal and Allison (1951) found that when the calorie intake was low in dogs, the hepatic protein stores were depleted even when the animal was in positive nitrogen balance. Brown and Allison (1948) found that excess methionine in the diets produced hypertrophy of kidney and liver though nitrogen was being lost from other tissues. Wang and his co-workers (1949) and Williams (1961) found that major changes of liver nitrogen concentration appeared to occur within the first 12 days of protein depletion, but after about 25 days the liver tended to return to the initial chemical composition and cytological structure.

In summary, all the above findings indicate that the hepatic nitrogen measurement might not indicate the protein nutritional status of the whole system but is a measurement of the utilization of dietary protein for a specific physiological function, that is, the capacity of dietary protein to regenerate the hepatic nitrogen.

Hepatic Enzymes

The susceptability of activities of enzymes to dietary protein:

In the experimental animals the enzyme responses to protein depletion varied in different tissues. Wainio *et al.* (1953) found that the activities of cytochrome oxidase, Succinate, cytochrome C reductase and DPNH cytochrome C - reductase in the brain were least affected by protein depletion. But the unit activities of the same enzymes in the kidney, skeletal muscle and spleen were reduced by about 10 to 20 per cent. With the exception of brain, the other organs of protein depleted animals were significantly lower in weight and in total protein content than those of their ad libitum fed controls and hence the 'unit enzyme activity' as well as 'total enzyme activity' in the organs were decreased in protein depletion. There were some enzymes whose total activity decreased but whose unit activity was unchanged in protein depletion. Liver cytochrome oxidase and cathepsin belong to this kind. Srinivasan and Patwardhan (1955) state that severe protein deficiency could radically change the enzyme activity pattern and structure of the liver.

Hepatic enzyme changes related to quantity of dietary Proteins.

Many reports had shown that the activities of various enzymes in animal tissues were changed by dietary alterations (Rowe and Wyngaarden, 1966). In general, liver enzymes were liable to quantity of protein and different enzymes responded to protein depletion in different ways (Muramatsu and Ashida, 1962). There have been few studies however on the changes in the liver enzyme activities of animals receiving diets containing different levels of protein. Lightbody and Kleinman (1939) found that the activity of liver arginase in rats increased with an increase in dietary protein content. Recently, a similar response in hepatic glutamic pyruvic transaminase activities of rats was also reported by Rosen *et al.* (1959). Ross and Batt (1956) indicated that the activity of liver alkaline phosphotase in rats increased when they were fed high protein diets. Litwack and associates (1962) reported that improving the quantity and kind of dietary protein of rats, increased the hepatic xanthine oxidase in rats.

Muramatsu and Ashida (1963) studied the response of several liver enzymes in rats to graded increase in protein concentration. It has been found out that the activity of succinic dehydrogenase dropped markedly in rats receiving low protein diets and it was one of the extremely labile enzymes in protein depletion. (Litwack et al., 1950., Wainio et al., 1953., Miller, 1950).

Hepatic enzyme changes as related to nutritive quality of dietary proteins.

Dietary amino acids greatly influenced hepatic Xanthine Oxidase activity of rats. (Litwack et al., 1953). The rapid loss of this enzyme activity induced by a non-protein dietary regimen proceeded at a greater rate than the loss of flavin adenine dinucleotide coenzyme or other liver proteins (Litwack et al., 1950). Litwack and associates (1954) reported the effects of the quality of dietary proteins on the activity of liver xanthine oxidase in rats. Zigman and Allison (1959) and Allison et al. (1961) have reported that the growth response of rats fed different dietary proteins at graded concentrations were correlated with ribonuclease activity. Hepatic ribonuclease approached a common and relatively high value in rats fed low intake of egg, casein, cotton seed proteins or wheat gluten as source of dietary proteins. As protein intake was increased, the activity dropped, decreasing most rapidly in animals fed egg, less rapidly in animals fed casein and least rapidly in those fed wheat gluten.

Muramatsu and Ashida (1961) found growth rates and activities of hepatic succinic dehydrogenase and xanthine oxidase to be similar. He used egg albumin, fish meal, wheat gluten, soya bean and casein as protein sources. The response curves of hepatic xanthine oxidase or succinic dehydrogenase plotted against the protein levels in diets were similar to curves of growth rates. As the quality of proteins improved, the inflection points of the response curves occured at a lower level of dietary protein.

Nutritive value of Horsegram

Use of locally available vegetable sources such as legumes is being suggested as the immediate solution for solving the problem of protein malnutrition in our country. Horsegram ranks as one of the highest in this respect since it is cheaper than other legumes like red gram, bengal gram and green gram which are commonly used in our country.

Horsegram is a concentrated source of protein containing 22 per cent protein (Aykroyd et al., 1966). Horsegram has a golden brown colour and a very acceptable taste. The different methods of cooking horsegram for human consumption includes roasting and boiling with or without previous soaking. When roasted it has a very pleasant smell and horsegram is used with husk.

Horsegram is deficient in methionine, tryptophan and cystine (Venkat Rao et all., 1964). Methionine seemed to be the limiting amino acid for growing rats fed horsegram as the sole source of protein, since the addition of 0.4 to 0.5 per cent dl-methionine produced marked improvement (Cama and Morton, 1950).

The nutritive value of horsegram is improved by heat (Leitner, 1962). The trypsin inhibitors and growth inhibitors present in horsegram are inactivated by autoclaving the horsegram (Hirwe and Magar, 1953). In the case of rats maintained on raw and autoclaved horsegram diets, there was a noticeable difference in the gain in weight per rat per week. The average figures were 4.4 grams and 10.7 grams respectively. The protein efficiency ratio of raw and autoclaved horsegram diets were 0.7 and 1.7 respectively. There was increase in the liver weight, hepatic nitrogen and per cent of fat in the case of autoclaved horsegram fed rats when compared to raw horsegram fed rats. Thus it can be concluded that the nutritive value of horsegram is improved by autoclaving.

At a 10 per cent level of protein intake, the digestability coefficient of horsegram was found to be 73 and biological value was found to be 66. The moisture and crude protein of powdered housegram was found to be 11.81 and 22.12 respectively (Swaminathan, 1937., Niyogi, 1931).

Nutritive value of sesame

The annual production of sesame in India was 500,000 tons (Aykroyd and Doughty 1964., ICAR, 1961). Scientific and technological research in India had shown that sesame meal with a little care during oil extraction process could be made edible for human population.

Nutritive value of sesame protein:

The sesame meal was found to be a highly concentrated form of protein containing 30 to 48 per cent of protein and 43 to 45 per cent fat (Kuppuswamy et al., 1958). The sesame meal is deficient in lysine which is an essential amino acid (Venkat Rao et al., 1964). When chicks were given sesame meal as protein source, did not grow to the maximum. A 22 per cent protein diet based on sesame meal required fortification to 1.17% lysine for the attainment of maximal growth rate (Brinegar et al., 1950., Edwards et al., 1956). On the other hand sesame protein is richer in sulphur containing amino acid and tryptophan, which are lacking in legumes (Kuppuswamy et al., 1958., Daniel et al., 1965) Natarajan and Cama (1965) found that the digestability of sesame meal ranged from 82 to 89 per cent in rats for the various strains of sesame meals.

The availability of the individual amino acids from the meal ranged from 85 to 90 per cent. The methionine in sesame protein had been found to be completely available for chick growth. Biological values of sesame meals were 51 to 56 in rats as compared to casein having a value of 100.

Protein efficiency ratio ranged from 1.14 to 1.63 when fed at 15 per cent concentration (Natarajan and Cama, 1965). The net protein value ranged from 44 to 47. Trypsin inhibitor activity was absent in sesame meals (Natarajan and Cama, 1965).

Examination of amino acid content of food proteins (Orr and Watt, 1957) suggested that sesame seed could be used effectively to increase the content of sulphur containing amino acids in wheat pea mixture. Supplementation of poor corn diet with protein blends containing sesame flour resulted in doubling the growth rate of rats (Panemangalore, 1965). The sesame blend was found to have a digestability coefficient of 74.7 per cent and resulted in a nitrogen retention of 2.4 grams against the unsupplemented group ~~receiving~~ retaining a nitrogen of 1.46 grams (Guttikar *et al.*, 1965).

Doraiswamy *et al.* (1965) supplemented the poor cereal diets of 5 to 11 years old boys with blend of groundnut, bengal gram and sesame flour fortified with vitamins and minerals. They found that supplementing sesame increased the height, weight and haemoglobin content of boys as compared to unsupplemented group of boys.

Thus the sesame meal had been proved to be useful supplement both by itself or in combination with other oilseed meals or with legumes.

III EXPERIMENTAL PROCEDURE

The experiment was designed to determine the nutritive value of a combination of horsegram and sesame at a ratio of 4:1 by bioassay technique comparing it with a known standard protein, skim milk powder. As suggested by Hegstead and Worcester (1966) the rats were fed with different diets containing arbitrarily selected levels of protein, based upon the estimates of their probable nutritive value.

Selection and grouping of animals

Albino rats of Norwegian strain were selected for the growth study. The weanling rats were 23 days old and weighed between 21 - 36 grams. They were divided into eight groups of littermates. Four groups were fed the vegetable protein mixture and the other four skim milk proteins at different levels which will be described later.

Care of the animals

The rats were housed individually in wire screen bottom cages. The food was given in aluminium dishes. Weighed quantity of each diet was made into a paste and cooked for 15 minutes to minimise spilling. Water was given in aluminium cups. The cooked diet was fed with vitamin mixture (Appendix I), Vitamin B Complex (Appendix II) and cod liver oil daily. The feeding was ad libitum and every day left over food was dried in hot air oven at 80°C and the food consumption was calculated at the end of the experimental period. Weight record of the experimental animals was maintained throughout the study period by weighing the rats on alternate days.

Autopsy

After an experimental period of 28 days the rats were anaesthetised with chloroform. The livers were quickly removed when the hearts were still beating and weighed immediately. They were stored in the freezer at -4°C.

Preparation of the experimental diet

The levels of protein selected were 9, 11, 13 and 15 per cent for the vegetable protein mixture of horse-gram and sesame in a ratio of 4:1 to have a balanced amino acid pattern. Sesame was added to supplement methionine deficit in horsegram. The horsegram was cleaned and powdered with husk and sesame was dehusked by soaking overnight in water and dried. The protein content and fat content of both horsegram and sesame were analysed. Table I gives the analysed values for protein and fat contents of horse-gram and sesame.

TABLE I

PROXIMATE COMPOSITION OF HORSEGRAM AND SESAME

Food stuff	Constituents	Per centage
Horse-gram	Protein	18.4
	Fat	0.5
Sesame	Protein	16.3
	Fat	41.3

Sago powder served as a source of carbohydrate and ground nut oil served as a source of the 10 per cent fat in the diets. The individual components (details of the salt and vitamin mixtures are given in Appendix I, II and III) were mixed by hand so that the diets were free of lumps. ~~Table IV gives the composition of salt mixture.~~

The levels for skim milk diets were 7, 9, 11 and 13 per cent and skim milk powder was used as the source of protein. The protein levels chosen for skim milk powder were lower, to get equal response of body weight gains in both series of the diets using different dietary proteins. Other details were similar to the vegetable protein mixture diets. Table II gives the composition of the experimental and skim milk diets. Two kilo grams of diets were prepared and stored in air tight glass bottles in the refrigerator.

TABLE II

COMPOSITION OF THE EXPERIMENTAL DIET AND SKIM MILK DIET

Constituents	In one kilo gram							
	A g.	B g.	C g.	D g.	E g.	F g.	G g.	H g.
Horsegram powder	327.2	400.00	472.7	545.4	-	-	-	-
Sesame	98.3	120.2	142.0	164.0	-	-	-	-
Skim milk powder	-	-	-	-	184.2	236.8	289.4	342.0
Ground nut oil	54.7	48.1	38.6	29.0	100.0	100.0	100.0	100.0
Salt mixture	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Sago powder	479.8	391.7	306.7	221.6	675.8	623.2	570.6	518.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Protein per cent (by calculations)	9.0	11.0	13.0	15.0	7.0	9.0	11.0	13.0
Protein per cent (by analysis)	9.43	11.1	13.61	15.8	7.9	9.3	11.1	13.6
Fat per cent (by calculation)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00

Analytical procedure

The whole liver was homogenised with 5 times its volume of 0.1 M phosphate buffer (P^H 7.2) and triplicates were taken for the various determinations. Hepatic succinic dehydrogenase activity was measured by using the method of Sreenivasamurthy and Swaminathan (1955). Hepatic nitrogen determinations were made with homogenised liver samples by the micro kjeldahl method (A.O.A.C., 1963).

Statistical Analysis

The regression of grams of gain in body weight to total nitrogen intake or protein intake were calculated for each dietary proteins. The 'relative growth index' which is an expression for the relative nutritive value of dietary protein was calqulated for the horsegram-sesame mixture. This index is the slope of the regression between nitrogen intake and body weight gains expressed as a percentage of the slope obtained with skim milk protein which had been selected as the reference protein.

The residual sum of squares was computed by fitting separately to each protein an equation of the form $y = a + bx$, where 'y' is the response (body weight gain), 'x' is the amount of protein or nitrogen actually consumed by each animal. The regressions were tested for its linearity and standard deviations of the slopes were calculated (Snedecor, 1966).

Mean values for groups are reported with standard error. Analysis of variances with non-orthogonal comparisons (Snedecor, 1956) was carried out in the case of body weight gain, PER, hepatic weight, hepatic nitrogen, protein intake and hepatic SDH activity. Comparisons each with one degree of freedom were used to test for differences in the treatment means. The comparison C is equal to λ isi where λ isi is the co-efficient assigned to a particular treatment. The sum of squares and mean square for the comparisons are equal to $C^2/n\Sigma\lambda_i^2$ (Snedecor, 1956). The 'F' value was obtained by dividing the mean square for the comparison by the error mean square. Only 'F' ratios which had a probability of 0.05 to 0.01 or less of being as large as the calculated value by chance alone were considered to be significant.

IV RESULTS AND DISCUSSION

The aim of the study is to evaluate a vegetable protein mixture of horsegram and sesame by the 'bioassay technique' of Hegsted and Chang (1965). The nutritive value of the mixture of horsegram and sesame at a protein ratio of 4:1 was evaluated in comparison with standard skim milk powder. Eight groups of 23 days old male weanling albino rats were fed the various experimental diets for a period of 28 days. The protein concentrations of the diets in which the mixture of horsegram and sesame formed the protein sources, were 9, 11, 13 and 15 per cent. For skim milk the protein concentrations were 7, 9, 11 and 13 per cent. These levels of protein concentrations were chosen to get equally effective dose levels for the body weight gain response. Feeding was ad libitum. Body growth, PER, hepatic nitrogen and hepatic SDH activities were also determined as indices of protein utilisation.

The coding of diets and experimental groups are given in Table III.

TABLE III

CODING OF DIETS AND GROUPS

Code	Source of Protein	Percentage of Protein	
		By calculation	By analysis
A	Horsegram + sesame	9	9.43
B	"	11	11.10
C	"	13	13.61
D	"	15	15.81
E	Skim milk	7	7.90
F	"	9	9.30
G	"	11	11.18
H	"	13	13.61

Body weight changes

The mean body weight changes of the experimental animals are given in Table IV. The analysis of variance and non-orthogonal comparison are given in Table V.

TABLE IV

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

Group	Source of Protein in the diet	Percentage of protein in the diet	Body Weight		
			Initial g.	Final g.	Increase g.
A	Horsegram + sesame	9	28.7 ± 0.83[a]	42.4 ± 1.41	13.6 ± 0.80
B	-do-	11	27.8 ± 2.01	49.9 ± 0.51	20.0 ± 1.38
C	-do-	13	27.2 ± 0.73	49.7 ± 2.84	22.5 ± 1.87
D	-do-	15	27.2 ± 1.73	64.5 ± 2.88	33.4 ± 2.25
E	Skim milk	7	27.1 ± 1.23	55.1 ± 2.61	27.9 ± 0.78
F	-do-	9	28.1 ± 1.36	68.4 ± 3.94	40.7 ± 0.54
G	-do-	11	28.5 ± 2.30	83.0 ± 4.28	54.6 ± 0.54
H	-do-	13	29.0 ± 1.70	91.4 ± 2.13	62.5 ± 2.25

[a]Mean ± Standard error

TABLE V

ANALYSIS OF VARIANCE FOR WEIGHT GAIN

(NON ORTHOGONAL COMPARISON)

Source of variation	Degrees of freedom	Sum of squares
Total	47	12655.31
Treatment	7	12057.00**
Error	40	598.31
A vs E	1	615.37**
B vs F	1	1293.80**
C vs G	1	3088.02**
D vs H	1	1885.01**
A vs F	1	2203.23**
B vs G	1	5612.41**
C vs H	1	4800.00**
G vs E	1	86.4*
D vs E	1	269.8**
A + B + C + D vs E + F + G + H	2	6931.21**

* significant at five per cent level

** significant at one per cent level

In both the protein groups which were fed graded levels of horsegram - sesame mixture or skim milk powder increased growth rates occurred along with increased protein intakes. As could be seen from Table IV the increases in weights were 13.6 ± 0.80, 20.0 ± 1.38, 22.5 ± 1.87 and 33.4 ± 2.25 for the rats fed 9, 11, 13 and 15 per cent proteins from horsegram and sesame mixture respectively. In animals fed skim milk the increase in weights were 27.9 ± 0.78, 40.7 ± 1.42, 54.6 ± 0.54 and 62.5 ± 2.25 respectively when protein concentration in the diet was increased from 7 to 9, 11, and 13 per cent respectively. Thus the growth rate response for skim milk protein was always significantly greater than that of horsegram-sesame mixture (Table V).

Feeding a diet (E) with 7 per cent protein from skim milk gave a weight increase response even greater than that with 13 per cent horsegram-sesame mixture showing thereby that skim milk is a better quality protein (Diet C). But the growth of 7 per cent skim milk fed animals were lower than those fed 15 per cent protein from horsegram - sesame mixture, thus proving that quantity of protein was limiting at the 7 per cent level for growing rats (Table V).

In the present study the lower growth rates of animals fed horsegram-sesame mixture than that of animals fed skim milk agrees well with the results of Phansalkar and Patwardhan (1956), and Swaminathan (1937), who also observed legumes possess lower growth prompting values than skim milk. As could be observed from the amino acid composition of the diets (Table VI) though sesame supplementation to horsegram protein increased the methionine content, this deficiency was not fully corrected. Moreover the total essential amino acid contents were also limiting in the vegetable protein mixture. These might be the cause for the lower growth rates of the animals fed horsegrams and sesame even at 15 per cent protein level.

TABLE VI

AMINO ACID COMPOSITION OF THE EXPERIMENTAL DIETS USED IN COMPARISON WITH THE AMINO ACID REQUIREMENT OF GROWING RATS BY ALMQUIST (1959)

Diet	Lysine g.	Tryptophan g.	Phenylalanine g.	Methionine + cystine g.	Threonine g.	Leucine g.	Isoleucine g.	Valine g.
Horsegram + Sesame								
9 percent level	0.74	0.10	0.51	0.33	0.33	0.80	0.51	0.53
11 percent level	0.89	0.13	0.63	0.42	0.41	0.98	0.62	0.64
13 percent level	1.06	0.16	0.75	0.50	0.49	1.16	0.73	0.75
15 percent level	1.21	0.19	0.87	0.59	0.57	1.35	0.84	0.87
Requirement of rats (Almquist 1959)	1.00	0.20	0.70	0.50	0.50	0.80	0.50	0.70
Skim milk diet								
7 percent level	0.52	0.11	0.33	0.22	0.29	0.62	0.41	0.44
9 percent level	0.66	0.14	0.42	0.29	0.38	0.81	0.52	0.57
11 percent level	0.81	0.17	0.52	0.35	0.46	1.00	0.64	0.69
13 percent level	0.96	0.20	0.61	0.41	0.54	1.19	0.75	0.82
Requirement of rats (Almquist 1959)	1.00	0.20	0.70	0.50	0.50	0.80	0.50	0.70

Thus, the increase in quantity of protein in the diet resulted in increased gain in weight for both the skim milk and horsegram-sesame mixture proteins. Differences in growth rates of skim milk fed groups and animals fed horsegram-sesame mixture showed that this index is sensitive to differences in quality of protein.

Food and Protein intake

The mean food and protein intakes of rats fed the experimental diets are given in Table VII. The analysis of variance and non-orthogonal comparison are given in Table VIII.

TABLE VII

MEAN FOOD AND PROTEIN INTAKE OF RATS FED DIETS OF VARYING PROTEIN CONTENTS FOR 28 DAYS

Group	Source of protein	Percentage of protein in the diet	Total food intake (g)	Protein intake (g)
A	Horsegram + Sesame	9	133.0 ± 13.21[a]	11.97 ± 0.76
B	-do-	11	164.5 ± 8.25	18.09 ± 1.24
C	-do-	13	148.0 ± 11.29	19.24 ± 0.51
D	-do-	15	160.5 ± 14.39	24.07 ± 1.11
E	Skim milk powder	7	138.4 ± 9.07	9.69 ± 1.77
F	-do-	9	161.3 ± 6.59	14.52 ± 1.58
G	-do-	11	169.3 ± 15.37	18.59 ± 1.30
H	-do-	13	165.2 ± 6.37	21.63 ± 1.89

[a]Mean ± Standard Error

TABLE VIII

ANALYSIS OF VARIANCE FOR PROTEIN INTAKE

(NON-ORTHOGONAL COMPARISON)

Source of Variation	Degrees of freedom	Sum of squares
Total	47	856.09
Treatment	7	848.48**
Error	40	7.61
A vs E	1	15.61
B vs F	1	38.35*
C vs G	1	1.26
D vs H	1	17.86
A vs F	1	19.51
B vs G	1	0.75
C vs H	1	17.86
A + B + C + D vs E + F + G + H	2	30.04*

* significant at five percent level

** significant at one percent level

In general, animals which grew more, ate more food. Increased food intake correlated with the growth rates, as could be seen from Tables VII and VIII. The differences in protein intake among the various dietary groups were mainly due to the differences in the protein concentration of the various diets, and partly due to the differences in food intake.

Protein Efficiency Ratio

Table IX gives the PER values of the various experimental diets.

TABLE IX

THE PROTEIN EFFICIENCY RATIO OF DIETS OF VARYING SOURCES AND CONCENTRATIONS OF PROTEINS AT DIFFERENT LEVELS FED FOR 28 DAYS

Code of the diet	Source of Protein	Percentage of protein in the diet	P. E. R.
A	Horsegram + Sesame	9	1.05 ± 0.091[a]
B	-do-	11	1.10 ± 0.095
C	-do-	13	1.16 ± 0.152
D	-do-	15	1.38 ± 0.101
E	Skim milk	7	2.62 ± 0.081
F	-do-	9	2.82 ± 0.118
G	-do-	11	2.94 ± 0.107
H	-do-	13	2.92 ± 0.057

[a]Mean ± Standard Error

TABLE X

ANALYSIS OF VARIANCE FOR P E R

(NON-ORTHOGONAL COMPARISON)

Source of variation	Degrees of freedom	Sum of squares
Total	47	35.90
Treatment	7	29.29**
Error	40	6.61
A vs E	1	10.50**
B vs F	1	8.80**
C vs G	1	9.51**
D vs H	1	7.08**
A vs F	1	9.39**
B vs G	1	10.15**
C vs H	1	9.29**
A + B + C + D vs E + F + G + H	2	27.85**

* significant at five per cent level

** significant at one per cent level

In general the PER values significantly increased when dietary protein quantity was increased (Table X). The mean PER values increased from 1.05 ± 0.091 to 1.38 ± 0.101 for horsegram-sesame mixture, when protein content of the diets were increased from 9 to 15 per cent (Diet A to D). For skim milk, the PER values increased from 2.62 ± 0.081 to 2.94 ± 0.101 when the protein content was raised from 7 to 11 per cent (Diet E to G). But the PER values remained constant when the protein content of the skim milk diet was further increased to 13 per cent.

So, the differences both in the quality and quantity of proteins affected the PER values. The group fed skim milk had higher PER values than the groups fed the horsegram-sesame mixture and the PER values of 7 per cent skim milk diet (E) (2.62 ± 0.81) was about twice that of the diet containing 15 per cent protein from horsegram-sesame mixture (Diet D) (1.38 ± 0.101).

The increase in PER values with increasing protein concentrations in the present study were similar to several reports (Allison, 1955, 1959). Horsegram proteins had been reported to have a PER value lower than 1.00, while the casein and milk had PER values of nearly 3.00 (Kuppuswamy et al., 1958). But the horsegram-sesame mixture used in the present study seems to have a better PER of 1.38 at

15 per cent level than the unsupplemented horsegram thereby proving that sesame supplementation had improved the methionine content of the legume protein. This is clear from the calculation of amino acid pattern of the diets (Table VI).

The failure of PER values to increase for skim milk diet after a 11 per cent protein concentration in the present study was similar to observations on several good quality animal proteins like egg proteins where a decline was observed when protein concentration was increased above 10 per cent (Allison, 1958,, Jansen, 1962). Jones and Divine (1944) and Hoagland *et al.* (1949) found the PER values of skim milk to decrease as the protein concentration was raised from 8 to 10 and 13 per cent. Tasker *et al.* (1962) observed a lowering of PER value from 3.04 to 2.32 when concentration of skim milk protein was increased from 10 to 15 per cent.

Hegstedd and Worcester (1947) found that weight gain alone was sufficient to evaluate protein quality without the need to consider the protein intake of food intake. This seems to be true from our statistical analysis of the data for weight gain and PER values for the various diets. The significant differences among the various groups seem to be similar for both these responses namely, weight gain and PER values (Tables V and X).

Liver Weight

The mean weights of the fresh livers of the experimental animals are given in Table XI. The non orthogonal comparison between the dietary groups are presented in Table XII.

TABLE XI

MEAN LIVER WEIGHTS AND TOTAL HEPATIC NITROGEN OF RATS FED DIETS OF VARYING PROTEIN CONTENTS

Code	Source of Protein	Percentage of Protein in the diet	Fresh liver weight g.	Heptic Nitrogen per g. of liver mg	Total Hepatic Nitrogen mg
A	Horsegram+ Sesame	9	2.69 ± 0.254[a]	23.90	64.3 ± 7.97
B	-do-	11	3.31 ± 0.291	28.51	95.3 ± 8.96
C	-do-	13	3.83 ± 0.284	35.90	137.8 ±10.56
D	-do-	15	4.09 ± 0.178	37.77	154.5 ±11.21
E	Skim milk	7	3.00 ± 0.308	20.06	60.2 ± 5.25
F	-do-	9	3.75 ± 0.357	30.29	113.6 ±11.90
G	-do-	11	4.82 ± 0.379	36.32	175.1 ±13.50
H	-do-	13	4.81 ± 0.221	45.49	218.8 ±16.21

[a]Mean ± Standard Error

TABLE XII

ANALYSIS OF VARIANCE FOR LIVER WEIGHT

(NON ORTHOGONAL COMPARISON)

Source of Variation	Degrees of freedom	Sum of squares
Total	47	75.04
Treatment	7	13.13
Error	40	61.91
A vs E	1	0.29
B vs F	1	0.56
C vs G	1	2.89
D vs H	1	1.55
A vs F	1	3.38
B vs G	1	6.82*
C vs H	1	2.89
A + B + C + D vs E + F + G + H	2	2.26

* significant at five per cent level

The mean liver weight was the highest in rats fed 13 per cent level of skim milk diet (4.81 ± 0.221 g.) and lowest in 9 per cent level of horsegram-sesame protein mixture which grew least (2.69 ± 0.254 g.) Thus the hepatic weights were a reflection of the final body weights of the animals (Table IV and Table XI). The heaviest livers were of those animals whose body weights were the highest. As the protein concentration of the diets and body weight gains increased, the hepatic weights also increased in the animals fed horsegram-sesame mixture and skim milk.

From the results of the statistical analysis (Table XII), it is clear that none of the mean hepatic weights of the experimental groups were significantly different from the others except between group B and G.

Hepatic Nitrogen

Table XI shows the mean values and standard errors for total hepatic nitrogen in the experimental animals. Table XIII gives the analysis of variance and non-orthogonal comparisons for hepatic nitrogen.

The highest value for hepatic nitrogen was found to occur in animals fed the skim milk at 13 per cent level which was 218.8 ± 16.21 mg. The lowest mean hepatic nitrogen value (60.2 ± 5.25 mg.) was obtained for animals fed 7 per cent skim milk. As the protein concentration was increased from 9 to 11, 13 and 15 per cents and 7 to 9, 11 and 13 per cents, the hepatic nitrogen values increased as could be seen from Table XI and Figure I.

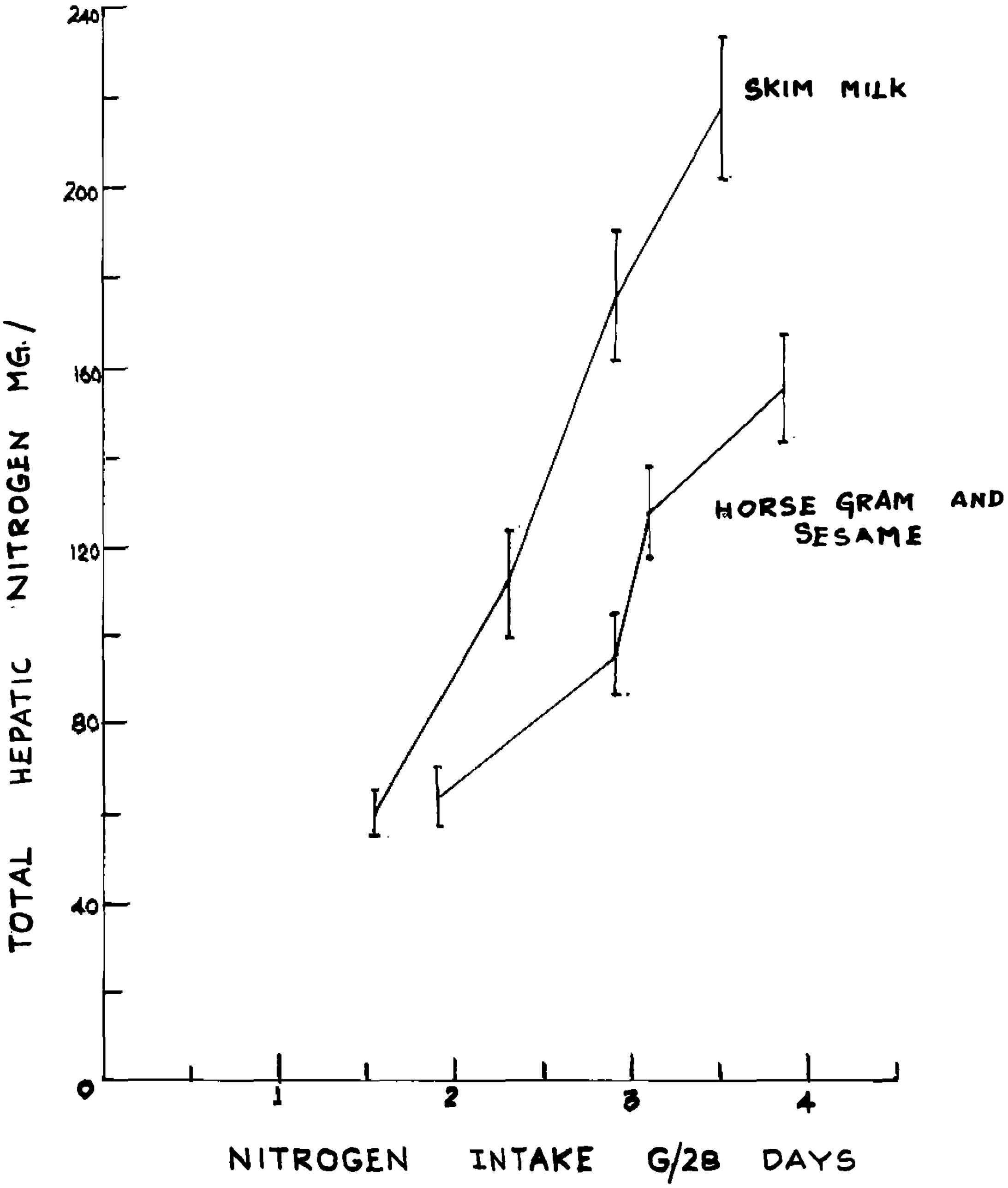
TABLE XIII

ANALYSIS OF VARIANCE FOR HEPATIC NITROGEN

(NON ORTHOGONAL COMPARISON)

Source of Variation	Degrees of freedom	Sum of squares
Total	47	56413.0
Treatment	7	26480.0**
Error	40	29933.0
A vs E	1	52.1
B vs F	1	108.3
C vs G	1	6788.0**
D vs H	1	12403.5**
A vs F	1	7301.3**
B vs G	1	19120.0**
C vs H	1	24824.8**
A + B + C + D vs E + F + G + H	2	5987.5**

** significant at one per cent level



SKIM MILK
HORSE GRAM AND SESAME
TOTAL HEPATIC NITROGEN MG./
NITROGEN INTAKE G/28 DAYS
240
200
160
120
80
40
0
1
2
3
4

The hepatic nitrogen contents of the skim milk fed groups were higher to the group fed the horsegram-sesame mixture for all protein concentration except in the two lower levels. Animals fed either 9 or 11 per cent horsegram-sesame mixture and 7 and 9 per cent skim milk had similar hepatic nitrogen concentrations as per the non orthogonal comparisons (Table XIII).

Thus the picture for hepatic nitrogen concentration is slightly different from that of the growth and PER indices where even the lowest level of skim milk (7 per cent) gave a better response than the vegetable protein mixture. The skim milk proteins which could give a better growth performance than in the animals fed horsegram-sesame mixture, could not fill up the hepatic reserves so very efficiently. Thus the results of protein evaluation based on growth response may not be similar to those based on liver nitrogen concentration. This is in agreement with the findings of Allison (1959) who observed that filling up hepatic nitrogen stores may not fully reflect the protein nutriture of the animal.

Hepatic Succinic Dehydrogenase (SDH) Activity

The activity of hepatic succinic dehydrogenase had been expressed as microgram (mcg.) of triphenyl tetrazolium chloride reduced by g. of wet liver in 30 minutes and presented in Table XIV. The analysis of variance for this data is given in Table XV.

TABLE XIV

THE HEPATIC SUCCINIC DEHYDROGENASE ACTIVITIES OF RATS FED DIETS OF VARYING CONTENTS AND SOURCES OF PROTEINS

Code of the Group	Source of Protein	Percentage of Protein in the diet	Hepatic SDH Activity Mcg. of triphenyl tetrazolium chloride reduced per g./30 minutes
A	Horsegram + Sesame	9	833[a] ± 41.15
B	-do-	11	1064 ± 38.78
C	-do-	13	1354 ± 85.60
D	-do-	15	1683 ± 89.08
E	Skim milk powder	7	954 ± 36.07
F	-do-	9	1182 ± 46.66
G	-do-	11	1564 ± 65.50
H	-do-	13	1862 ± 71.68

[a]Mean ± Standard Error

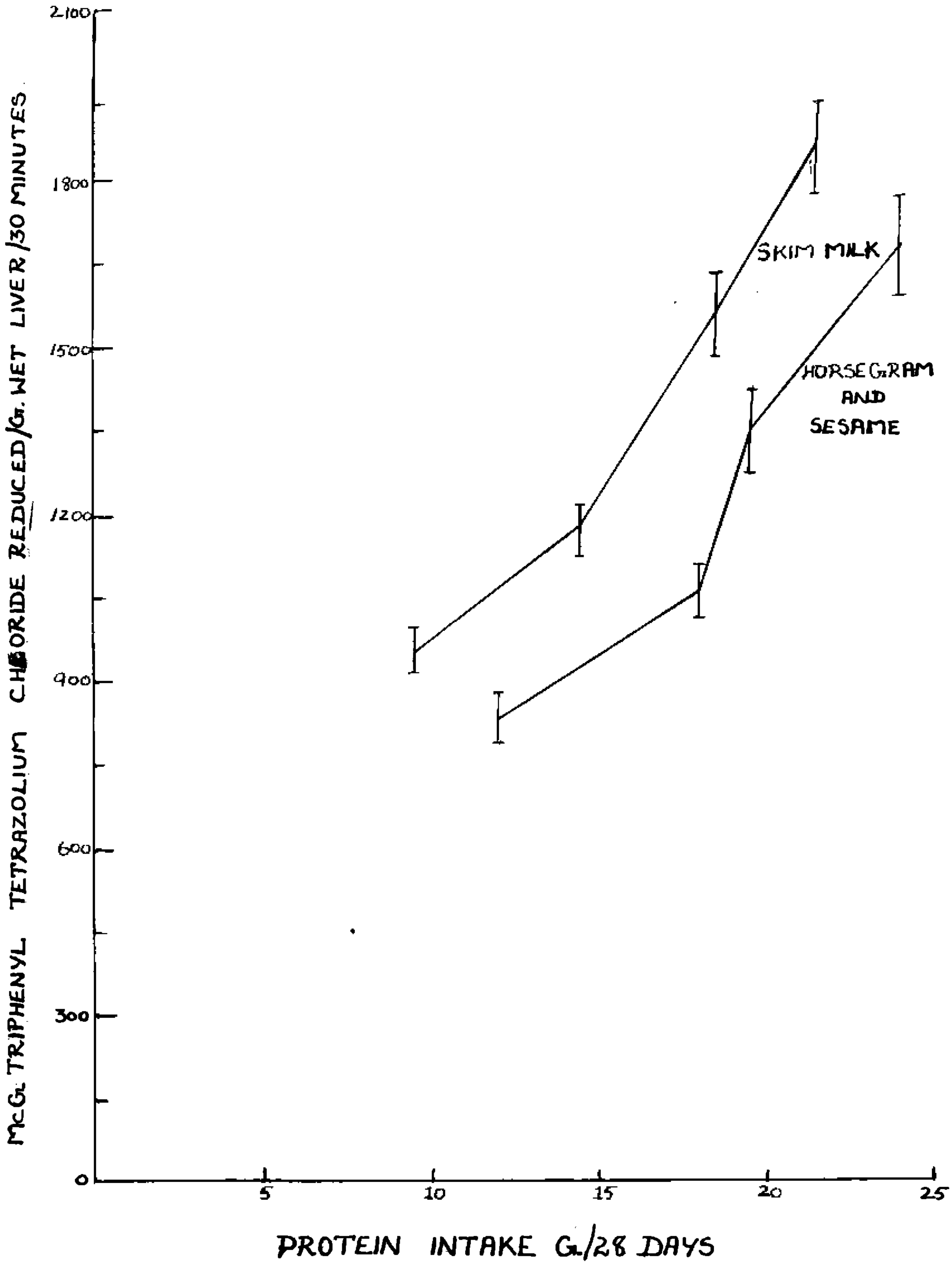
TABLE XV

ANALYSIS OF VARIANCE FOR HEPATIC SDH ACTIVITY

(NON ORTHOGONAL COMPARISON)

Source of Variation	Degrees of freedom	Sum of squares
Total	47	2852949.3
Treatment	7	2784381.3**
Error	40	68563.0
A vs E	1	22082.6**
B vs F	1	20650.6**
C vs G	1	66360.1**
D vs H	1	47882.6**
A vs F	1	182701.5**
B vs G	1	375000.0**
C vs H	1	386688.1**
A + B + C + D vs E * F + G + H	2	147737.0**

** significant at one per cent level


McG. TRIPHENYL TETRAZOLIUM CHLORIDE REDUCED/G. WET LIVER/30 MINUTES
2100
1800
1500
1200
900
600
300
0
5
10
15
20
25
SKIM MILK
HORSEGRAM AND SESAME
PROTEIN INTAKE G./28 DAYS

The highest and lowest hepatic SDH activities were those of the animals fed 13 per cent skim milk (1562 $\pm$ 71.68) and 9 per cent protein from horsegram-sesame mixture (833 $\pm$ 41.15) respectively, which is similar to their growth responses.

In both the experimental protein groups, the hepatic SDH activities increased along with increased protein concentrations (Figure II). Significant differences were observed between the groups of rats fed skim milk protein and horsegram-sesame mixture. All the animals fed skim milk showed higher hepatic SDH activities when compared to the animals fed equally effective doses of horsegram-sesame mixture which showed that the quality of dietary protein influenced the hepatic SDH activities.

Unlike the growth response where the 7 per cent skim milk had a response significantly above that of the 13 per cent horsegram-sesame mixture, the SDH activities were not different for those two groups. So it might be concluded that the hepatic SDH enzyme responses increases proportionally with increasing quantities of protein and quality of proteins.

Muramatsu and Ashida (1962, 1963) observed that the relative values of growth index, protein efficiency ratio and hepatic SDH activities were fairly identical when different dietary proteins were examined. Such a finding agrees with the results of the present study.

Kshirsagar and Patwardhan (1958), Litwack *et al.* (1953), and Muramatsu and Ashida (1962) reported that hepatic SDH response was varied not only due to quality but also to varying level of dietary protein differently and these response curves were correlated with growth rates of animals. Thus as per the above reports and the results of the present study hepatic SDH activity could be used as an index for dietary protein evaluation.

Garcia and Roderuck (1964) found higher total hepatic nitrogen in rats along with higher activities of hepatic SDH. The authors explained such a phenomenon on the basis that enzymes are essentially proteins and changes in the enzyme activities reflect changes in the total hepatic nitrogen. Such a finding seems to be similar to the data presented here.

<u>Regression of gain in body weight to total nitrogen or protein intake</u>

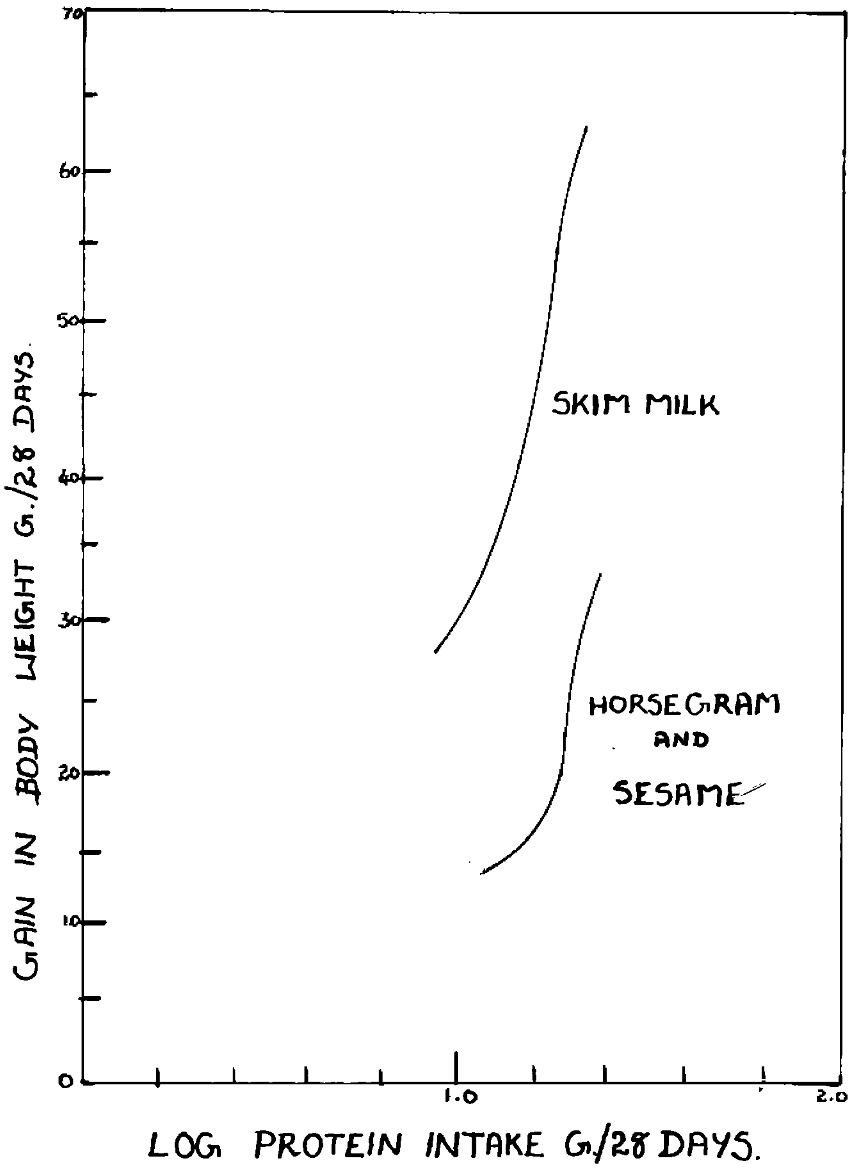
The regression of gain in body weight to total nitrogen or protein intake was calculated for skim milk and horsegram - sesame mixture. The 't' test was used to test for significance, the difference between the slopes of the regression lines which were found to be significantly different (Snedecor, 1956). The relative growth index (Hegsted and Chang, 1965), an expression for the relative nutritive value of dietary protein was calculated for the horsegram-sesame mixture. This index is the slope of the regression between nitrogen intake and body weight gain expressed as per centage of the slope obtained with the reference protein, namely skim milk powder.

TABLE XVI

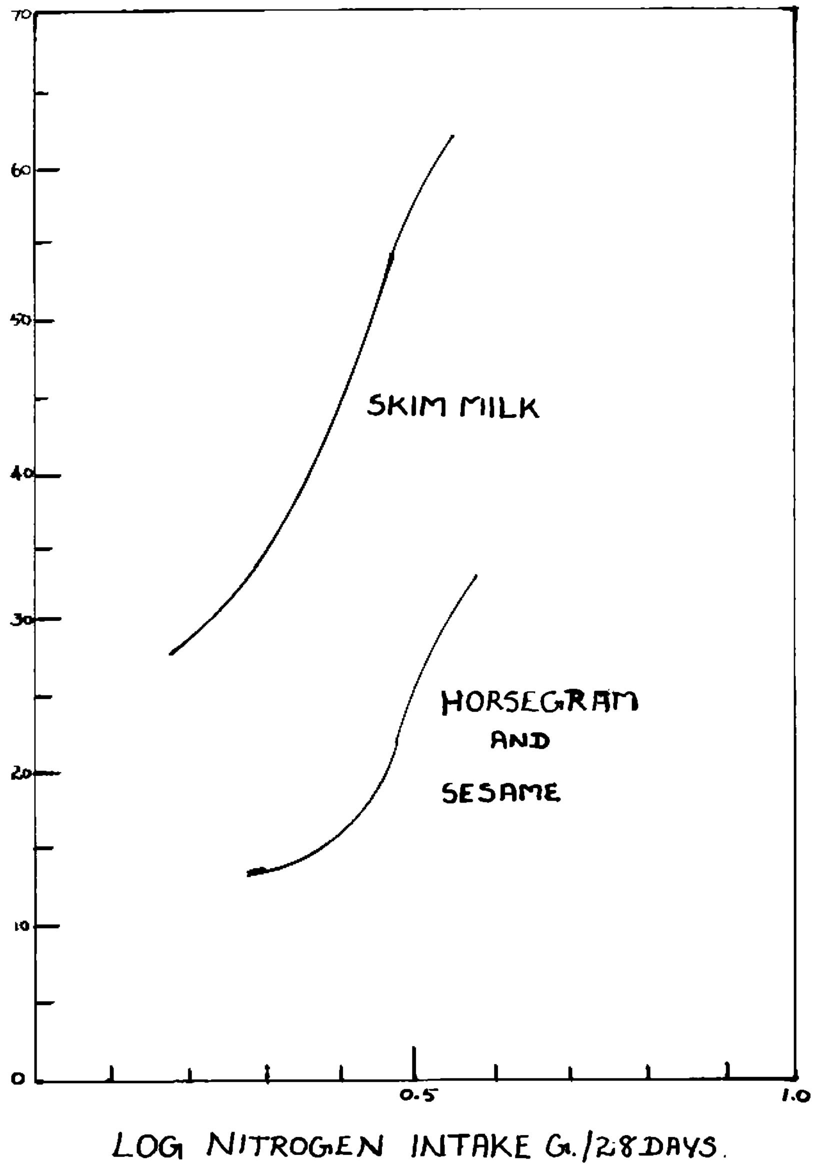
REGRESSION OF GAIN IN BODY WEIGHT ON TOTAL NITROGEN AND PROTEIN INTAKE AND DIETARY PROTEIN LEVEL*

	Regression equations	
	Y = gain in body weight in g/28 days X = total dietary protein or nitrogen intake in g./28 days or dietary protein concentration	
Protein	Regression equation	Correlation coefficient rxy
Horsegram-sesame mixture	1.567X - 6.31 = Y	r = 0.945
Skim milk powder	2.923X - 0.66 = Y	r = 0.999
Nitrogen		
Horsegram-sesame mixture	10.240X - 7.62 = Y	r = 0.995
Skim milk powder	18.840X - 1.99 = Y	r = 0.993
Dietary protein Concentration		
Horsegram-sesame mixture	2.412X - 7.72 = Y	r = 0.850
Skim milk powder	4.530X - 1.14 = Y	r = 0.845

* Calculations given in Appendix VI



GAIN IN BODY WEIGHT G./28 DAYS
70
60
50
40
30
20
10
0
1.0
2.0
SKIM MILK
HORSEGRAM
AND
SESAME
LOG PROTEIN INTAKE G/28 DAYS.



70
60
50
40
30
20
10
0
0.5
1.0
SKIM MILK
HORSEGRAM
AND
SESAME
LOG NITROGEN INTAKE G./28 DAYS.

Suitability of the slope ratio assay

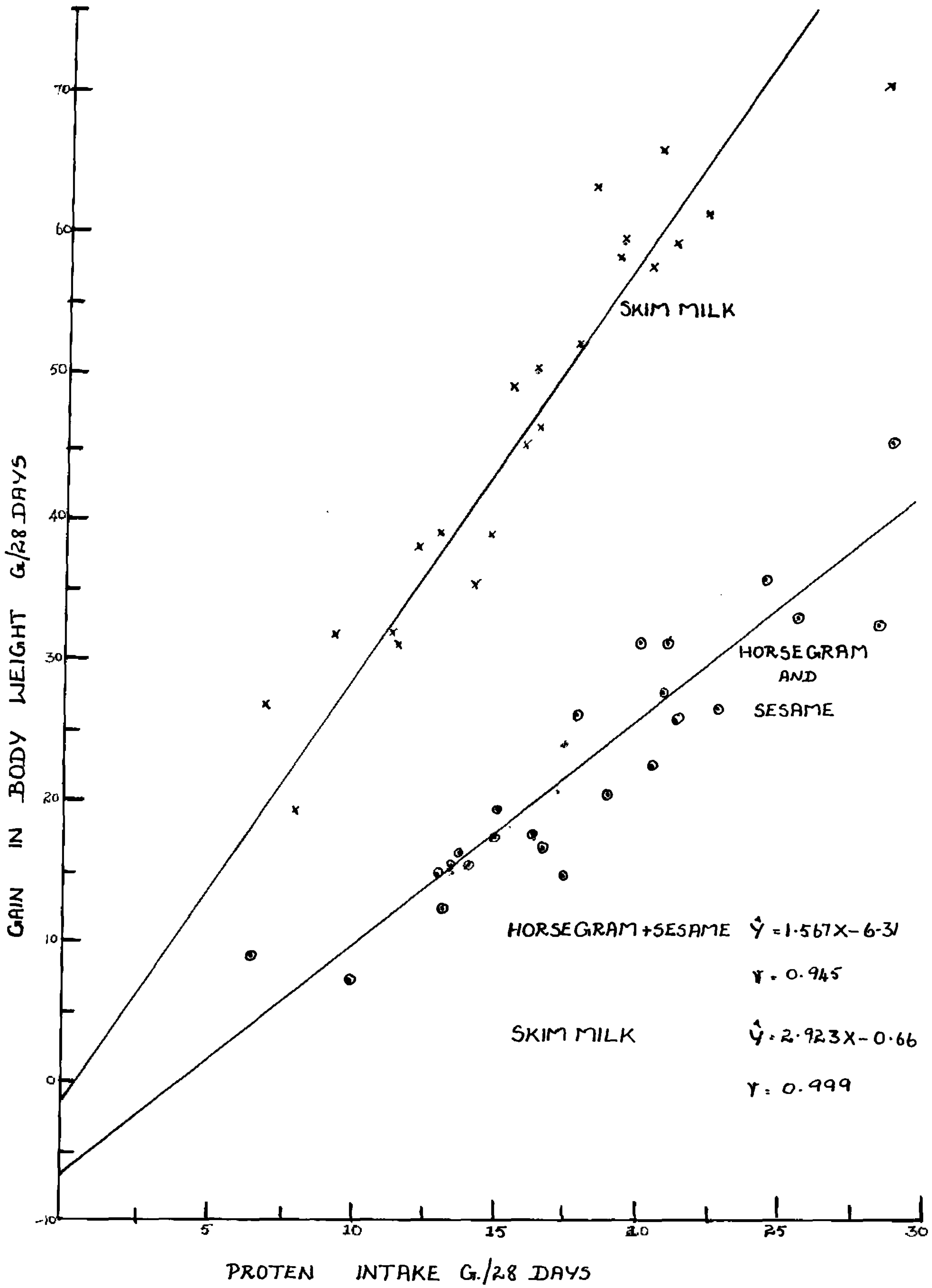
Clarke (1952) had developed a method of analysis for assays in which the response to each preparation was linearly related to the dose. The statistical method is as follows:

> "For each preparation, together with blank or zero dose, the mean responses (Weight gain) are approximately linearly related to the dose (dietary nitrogen). Straight lines intersecting at zero dose are fitted to the mean responses, by estimating the multiple linear regression equation."

The above statistical model was applicable to assays with uniform dose levels for all preparations, equal spacing between consequtive doses of each preparation, and equal replication for all treatments. The present experimental design did not satisfy the above statistical model, because of variations in nitrogen intake between and within the diet groups.

Log dose response assay

The log dose response curves, using body weight gain as the response and log of protein intake or log of nitrogen intake as the measure of dosage were calculated for the data of the present study. Two sigmoidal curves were obtained and there was no linearity in these curves (Figure III). Inspection indicates that the central portions



GAIN IN BODY WEIGHT G./28 DAYS
PROTEN INTAKE G./28 DAYS
SKIM MILK
HORSEGRAM AND SESAME
HORSEGRAM + SESAME Ŷ = 1·567X − 6·31
r = 0·945
SKIM MILK Ŷ = 2·923X − 0·66
r = 0·999

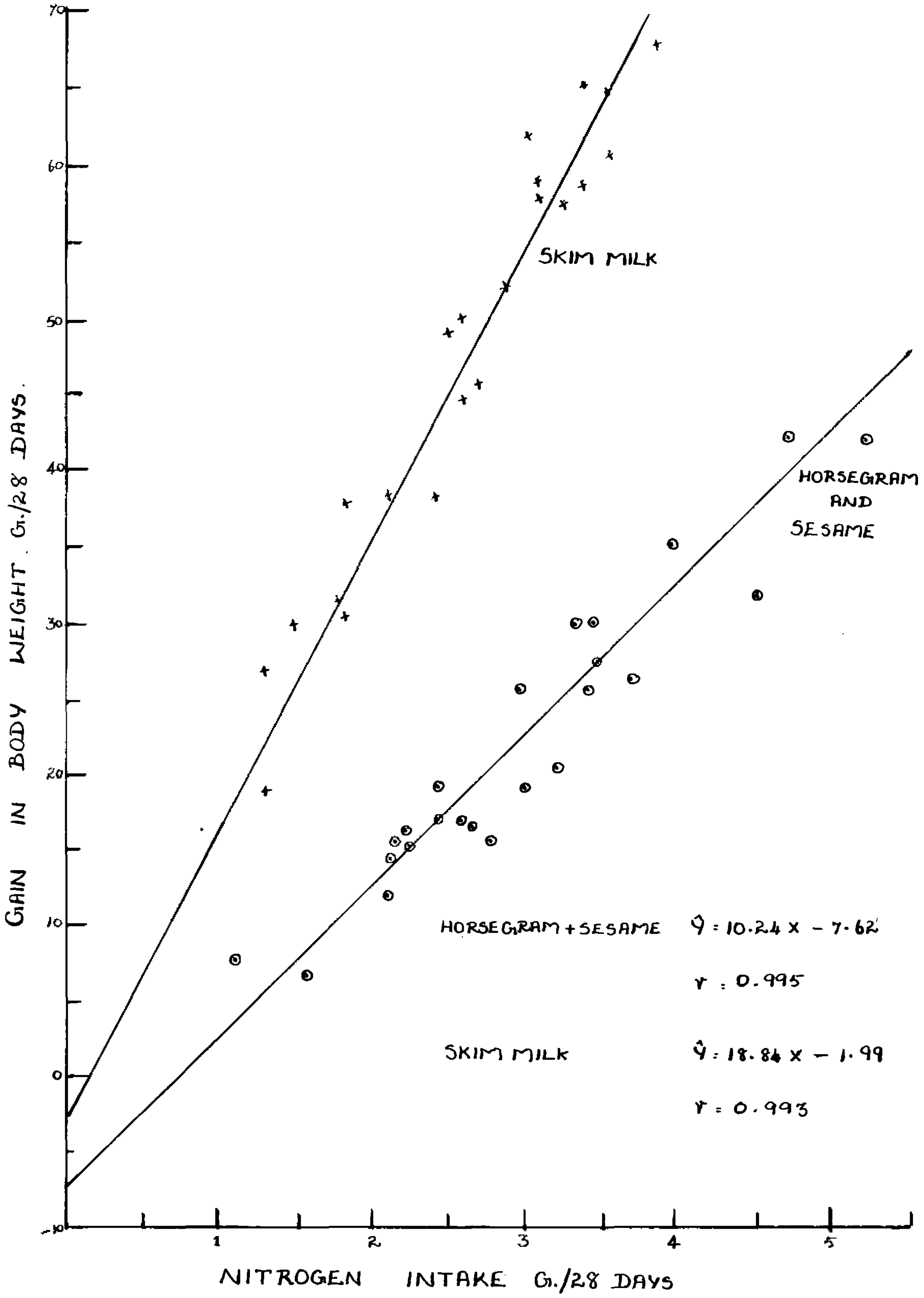
of these curves may be nearly linear and parallel. This finding agrees with the result of Hegsted and Chang (1965) who have expressed that using body weight gain as the response and log of protein or log of nitrogen intake as the measure of dosage, failed to satisfy the requirements of a satisfactory slope ratio assay.

Slope ratio assay

The gain in body weight of each group has been plotted with three metameters of dosage. (1) Dietary protein intake (2) Dietary nitrogen intake (3) Pecentage of dietary protein. (Figures IV, V and VI). The linear regression lines were calculated using the four dietary groups for each protein (Table III).

When the data were plotted against the actual nitrogen intake or protein intake (Figure IV) the fit was better as expected.

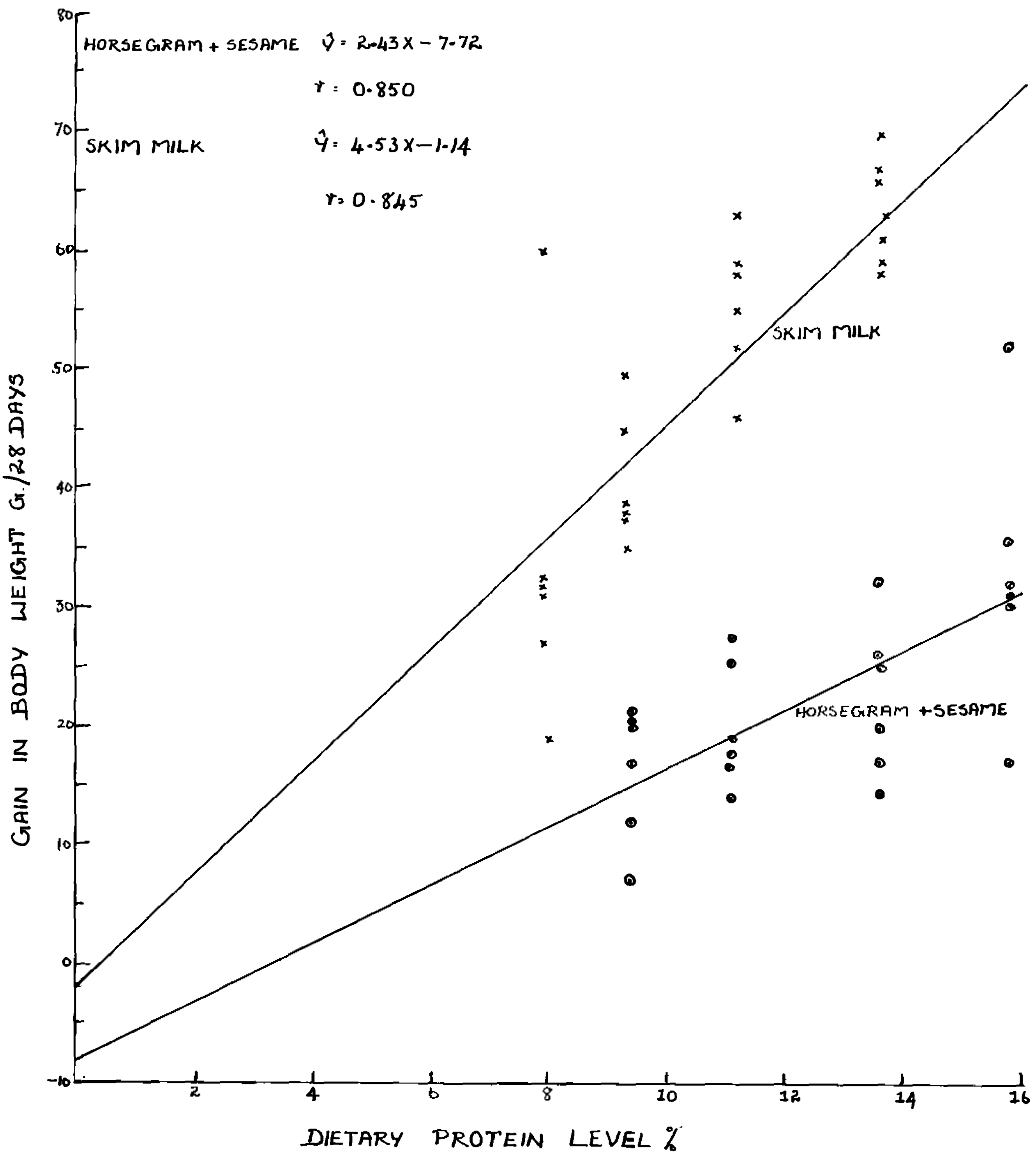
The correlation coefficients reached very high levels ($r = 0.945$ and 0.999 for horsegram-sesame mixture and skim milk respectively for protein and $r = 0.995$ and 0.993 for nitrogen). The two regression lines were having very nearly the same intercepts (- 6 g. and - 3 g.), agreeing close to the observed experimental values and the errors in the slope tended to be smaller ($P > 0.01$). A weight loss of 6 g. are normally encountered in this laboratory for a weanling rat


GAIN IN BODY WEIGHT G./28 DAYS.
NITROGEN INTAKE G./28 DAYS
70
60
50
40
30
20
10
0
-10
1
2
3
4
5
SKIM MILK
HORSEGRAM AND SESAME
HORSEGRAM + SESAME ŷ = 10.24 x - 7.62
r = 0.995
SKIM MILK ŷ = 18.84 x - 1.99
r = 0.993

maintained in a protein free diet for 21 days. According to Finney (1952), for a satisfactory slope ratio assay, the regression lines for the standards and the unknown must intersect at the same point and should correspond to the response of animals given zero dosage. Thus the regression lines of the present assay satisfied this requirement.

When gain in body weight was plotted against per centage of dietary protein, the data appeared to deviate from a linear regression (Figure VI). The correlation coefficient for these regressions were 0.850 and 0.845, although high, were not equal to 0.945 and 0.999 obtained when actual nitrogen or protein intake were used (Figures IV and V).

The standard deviation of the slopes were greater than the previous regressions based on actual nitrogen or protein intake. The intercepts tended to be lower than the experimental values for the zero nitrogen group. Since the measurement of food intake is most time consuming and therefore expensive part of the assay, there would be considerable practial gain if the measurement of food intake could be eliminated without affecting the reproducibility of the assay (Hegsted and Chang, 1965). But our data prove that there is need to measure the actual food and protein intake.


HORSEGRAM + SESAME ŷ = 2.43X − 7.72
r = 0.850
SKIM MILK ŷ = 4.53X − 1.14
r = 0.845
SKIM MILK
HORSEGRAM + SESAME
GAIN IN BODY WEIGHT G./28 DAYS
DIETARY PROTEIN LEVEL %
80
70
60
50
40
30
20
10
0
-10
2
4
6
8
10
12
14
16

Assuming a value of 100 for skim milk, the value for horsegram-sesame mixture was calculated from the slope ratio assay to be 53.6. No report on relative nutritive index is available for horsegram or its mixture for comparison. But while compared to the relative nutritive indices obtained using lactalbumin as a reference protein, for soy beans, casein and wheat gluten, a value of 53.6 for horsegram-sesame mixture is better than what could have been obtained for unsupplemented horsegram or any other legume. As the main purpose of the present study is to evaluate the "bioassay method" of Hegsted and Chang (1965), there were no unsupplemented horsegram dietary groups included in the present study.

In the present study the consistent increase in growth rate of animals fed increasing levels of skim milk protein or horsegram-sesame mixture showed that the relative nutritive value of proteins was essentially constant at varying levels of intake up to the levels used in this study.

So, our results are contrary to the conclusions of Miller and Payne (1961) or Morrison et al. (1963), who by using Net Protein Utilisation (NPU) index observed consistent decreases in the utilisation of nitrogen as the intake of good quality protein was increased until approximately maximal growth was achieved.

Dietary protein levels

The levels of protein chosen are given in Table III. In all adequate bioassays, a standard must be run with the unknown and comparisons between the standard and unknown should be made at equally effective doses. This is emphasized by Hegsted and Chang (1965) because it is rarely done in Protein assays. The above levels were chosen for skim milk and horsegram-sesame mixture by previous exploratory experiments not reported here, for equally effective weight gain response. Our data on the growth ratio also justifies this except that weight gain at 11 per cent skim milk was too high and at 9 per cent horsegram-sesame mixture was too low.

V SUMMARY AND CONCLUSION

The study was planned to investigate the suitability of a bioassay technique for the evaluation of the nutritive quality of a mixture of horsegram and sesame proteins in the proportion of 4 : 1 in order to meet the amino acid requirement of growing rats at 10 per cent concentration (Almquist, 1959) using skim milk as the reference protein. Twenty three days old male rats of Norweigian strain were used for the experiment. Forty-eight rats were divided into eight dietary groups each containing six animals. The animals were fed for 28 days, diets in which four different protein concentrations of either skim milk or horsegram-sesame mixture were incorporated. Feeding was ad libitum. For the horsegram-sesame mixture the protein concentrations chosen were 9, 11, 13 and 15 per cent and for skim milk 7, 9, 11 and 13 per cent. These levels are different because of the differences in the nature of the proteins in terms of growth and other responses. The findings are as follows:

1. The growth rate responses for the skim milk protein diets were always greater than that of horsegram-sesame mixture for all the dietary protein concentrations.

2. The food intake of rats did not vary much except in rats fed 9 per cent horsegram-sesame mixture diet and 7 per cent skim milk diet which consumed less food than others. Therefore the protein intakes of the different dietary groups varied only due to the difference in the concentration of protein of the diet.

3. The mean PER values increased from 1.05 $\pm$ 0.091 to 1.38 $\pm$ 0.101 for horsegram-sesame mixture when protein content of the diets were increased from 9 to 15 per cent. For skim milk the corresponding PER values increased from 2.62 $\pm$ 0.081 to 2.94 $\pm$ 0.101 when the protein content was raised from 7 to 11 per cent. But the PER values remained constant when the protein content of skim milk diet was further increased to 13 per cent.

4. No significant differences in hepatic weights were observed among the eight groups of experimental animals. But there were significant differences inthe total hepatic nitrogen content and hepatic nitrogen

per g. of fresh tissue among the groups fed different levels of protein. The lowest value of 60.2 $\pm$ 5.25 mg. of hepatic nitrogen was found in rats fed 7 per cent skim milk and highest value of the 218.8 $\pm$ 16.2 mg. of nitrogen in 13 per cent level skim milk diet. As the protein concentration was increased from 9 to 15 per cent in the horsegram sesame diets and from 7 to 13 per cent in skim milk diets, the total hepatic nitrogen values also increased from 64.3 $\pm$ 7.97 to 154.5 $\pm$ 11.2 mg. of nitrogen and 60.2 $\pm$ 5.25 to 218.8 $\pm$ 16.2 mg. of nitrogen respectively. Thus the picture of hepatic nitrogen concentration was slightly different from that of growth and PER indices. In the case of PER even the lowest level of 7 per cent skim milk gave a better response than the vegetable protein mixture.

5. The hepatic succinic dehydrogenase (SDH) activity was increased with the increase in the level of protein and quality of protein. At all levels the rats fed skim milk diet had higher hepatic SDH activity than that of rats fed horse gram and sesame mixture. Higher hepatic nitrogen contents were correlated with higher hepatic SDH enzyme activities.

6. The relative nutritive index of horsegram-sesame mixture was found to be 53.6 assuming a value of 100 for skim milk. The "Slope-ratio", bioassay with young

rats for the estimation of the relative nutritive value of a mixture of horsegram and sesame protein in the ratio 4 : 1 had been evaluated.

Analysis of the data and the log dose response curves using body weight gain as the response and log nitrogen intake or percentage of dietary protein as a measure of dosage failed to satisfy the requirements of a satisfactory assay. However calculation of slope ratio using slopes of the regression lines relating actual protein or nitrogen intakes as dose and body weight gain as response provided useful data. The regression lines were linear and indicating that appropriate concentration of various dietary protein could be readily selected for investigation. The best fit of the data was obtained by plotting gain in weight against nitrogen intake. This gave higher correlation co-efficients, small errors on the slopes of the regression lines and intercepts which approached those, found experimentally.

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

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A P P E N D I C E S

APPENDIX I

COMPOSITION OF THE VITAMIN MIXTURE

Constituents	Mg.
Thiamine Hydrochloride	10
Pyridoxine	10
Pantothenic acid	50
Nicotinamide	100
Inositol	400
Choline Chloride	4
Riboflavin (added last)	10
	584

In 100 gms of sucrose.

APPENDIX II

COMPOSITION OF THE DILUTED B-COMPLEX MIXTURE

Constituents	In 100 Ml.
Thiamine mononitrate U.S.P.	4.40 mg.
Riboflavin I.P.	1.47 mg.
Pyridoxine hydrochloride U.S.P.	0.59 mg.
Cyanocobalamin U.S.P.	4.40 mg.
Nicotinic acid amide I.P.	0.20 mg.
D.L. Pante thenyl alcohol	4.40 mg.
Methionine N.F.	102.90 mg.
Choline chloride	29.40 mg.
Sodium glycero phosphate	176.40 mg.
Alcohol 95%	10 ml.
Absolute alchohol	1 ml.

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APPENDIX III

COMPOSITION OF SALT MIXTURE

HOWK OSER SALT MIXTURE

Salts		Amount gms.
Calcium citrate	..	308.2
Ca $(H_2\ PO_4)_2\ H_2O$	..	112.8
H_2 K PO_4	..	218.7
K cl	..	124.7
Na cl	..	77.0
Ca Co_3	..	68.5
3 Mg. Co_3 $(Mg\ OH)_2 \cdot 3H_2O$	..	35.1
Mg SO_4 (Anhydrous)	..	38.2
Fe NH_4 Citrate U.S.P	91.41	
Cu $SO_4 \cdot 5\ H_2O$	5.98	
Na F	0.76	
Mn $SO_4 \cdot 2H_2O$	1.07	16.7
Alum	0.54	
K I	0.24	
Total	100.00	1000.00

APPENDIX IV

CALCULATION OF STANDARD ERROR

The sample standard error is symbolized by $S_{\bar{x}}$ and it is equal to $\frac{S}{\sqrt{n}}$. Where S is standard deviation of the mean and n is the number of observations. Standard error was calculated as follows:

$$\text{Standard error} = S_{\bar{x}} = \frac{S}{\sqrt{n}}$$

$S_{\bar{x}}$

$$S = \sqrt{\frac{\Sigma xi^2 - (\Sigma xi)^2/n}{(n-1)}}$$

For examples:

No.	xi	xi^2
1.	166	27556
2.	153	23409
3.	161	25921
4.	137	18769
5.	175	30625
6.	195	38025
Total	987	164305

Where n = 6

$$\bar{x} = \frac{\Sigma xi}{n} = \frac{987}{6} = 164.5$$

$$(\Sigma xi)^2 = 974169$$

$$\Sigma xi^2 = 164305$$

Using the above formula

$$s^2 = \frac{164305 - \frac{974169}{6}}{(6 - 1)} = \frac{164305 - 162361.5}{5}$$

$$s^2 = 388.7$$

$$s = 19.715$$

$$s_{\bar{x}} = \frac{19.715}{2.449} = 8.255$$

Standard error = 8.255

Based on this the other calculations were done.

APPENDIX V

ANALYSIS OF VARIANCE AND NON ORTHOGONAL COMPARISON

Any observed value is the sum of three parts, (i) an over-all mean, (ii) a treatment deviation and (iii) a random element from a normally distributed population with mean zero and standard deviation σ .

The following table shows a symbolic table of data for p groups with n data in each group.

	Group I	Group II	Group p
	x_1	y_1	p_1
	x_2	y_2	p_2
	n items	n items	n items
Sums	S_1	S_2	S_p
Total number of data	pn = N		

The calculations and analysis of variance are shown in the following table:

Variation due to	Degrees of freedom	Sum of squares	Mean square	Variance ratio (F)
Total	N - 1	(4)	-	-
Among means	p - 1	(5)	(7)	(9)
Within groups	N - p=p(n-1)	(6)	(8)	-

(1) Sum of all data $SX = S_1 + S_2 + \ldots\ldots\ S_p = S$

(2) Sum of squares $SX^2 = X_1^2 + Y_1^2 + \ldots\ldots\ P^2$

(3) Correction term $C = S^2 / N$

(4) Total sum of squares $= SX^2 - C$.

(5) Among means $= (S_1^2 + S_2^2 + \ldots\ldots\ S_p^2) / n - C$

(6) Within groups = Line 4 — Line 5

(7) Among means. Mean square: line 5/(p-1)

(8) Within groups: line 6/(N - p)

(9) Items 7/item 8.

The analysis of variance and non-orthogonal comparisons among the means were done according to Model I. (Snedecor, 1956, 257).

S.No.	Group A		Group B		Group C		Group D		Group E		Group F		Group G		Group H	
	x_i	x_i^2	x_i	x_i^2	x_i	x_i^2	x_i	x_i^2	x_i	x_i^2	x_i	x_i^2	x_i	x_i^2	x_i	x_i^2
1	41	1681	57	3249	80	6400	121	14641	42	1762	80	6400	119	14161	155	24025
2	68	4624	107	11449	131	17161	164	26986	78	6084	126	15876	187	34969	206	42436
3.	53	2809	84	7056	139	19321	166	27556	53	2809	85	7225	198	39204	203	41209
4	51	2601	116	13456	128	16384	143	20449	57	3249	106	11236	150	22500	267	71289
5	81	6561	97	9409	158	24964	168	28224	71	5041	157	24649	198	39204	263	69169
6	92	8461	111	12321	131	17161	165	27225	60	3600	128	16384	199	39601	219	47089
Total	386	26740	572	56940	767	101391	927	146991	361	22547	682	81770	1051	189639	1313	295217

Group A x_i 386., x_i^2 = 26740. Group E x_i = 361., x_i^2 = 22547

Group B x_i 572., x_i^2 = 56940 Group F x_i = 682., x_i^2 = 81770

Group C x_i 767., x_i^2 = 101391 Group G x_i = 1051., x_i^2 = 189639

Group D x_i 927., x_i^2 = 146991 Group H x_i = 1313., x_i^2 = 295217

Source of variation	Degrees of freedom	Sum of squares	Mean ~~Sum of~~ squares ~~degrees of~~ freedom	~~Mean square~~ Variance ratio (F)
Total	47	56413		
Treatment	7	26480	3782.8	5.05
Error	40	29933	748.3	
A vs E	1	52	52.0	0.07
B vs F	1	108.3	108.3	0.14
C vs G	1	6788.0	6788.0	9.13
D vs H	1	12403.5	12403.5	16.58
A vs F	1	7301.3	7301.3	9.75
B vs G	1	19120.0	19120.0	25.55
C vs H	1	24824.8	24824.8	33.17
A+B+C+D vs E+F+G+H	2	11875	5987.5	8.00

Σx_i		Σx_i^2		$(\Sigma x_i)^2$	
	386		26740		148996
	572		56940		327184
	767		101391		588289
	927		146991		859329
	361		22547		130321
	682		81770		465124
	1051		189639		1104601
	1312		295217		1723969
	6059		921235		5347813

$$X = \frac{(6059)^2}{48} = 764822.5$$

$$Y = \frac{5347813}{6} = 891302.3$$

$$Z = 921235.6$$

Treatment = $y - x$ = 891302.3 - 764822.5

= 26480.8

Error = $z - y$ = 921235.6 - 891302.3

= 29933.3

A vs E $\quad \Sigma xi$ = 386, 361, difference 25

$$= \frac{(25)^2}{12} = \frac{625}{12} = 52$$

B vs F $\quad \Sigma xi$ = 682, 572, difference 110

$$= \frac{(110)^2}{12} = \frac{12100}{12} = 108.3$$

C vs G $\quad \Sigma xi$ = 1051, 767, difference 284

$$= \frac{(284)^2}{12} = \frac{81456}{12} = 6788.0$$

D vs H $\quad \Sigma xi$ = 1313, 927, difference 386

$$= \frac{(386)^2}{12} = \frac{148846}{12} = 12403.5$$

A vs F $\quad \Sigma xi$ = 682, 386, difference 296

$$= \frac{(296)^2}{12} = \frac{87616}{12} = 7301$$

B vs G $\quad \Sigma xi$ = 1051, 572, difference 479

$$= \frac{(479)^2}{12} = \frac{229441}{12} = 19120$$

C vs H $\quad \Sigma xi$ = 1313, 767, difference 546

$$= \frac{(546)^2}{12} = \frac{297896}{12} = 24824.8$$

A+B+C+D vs E+F+G+H $\quad \Sigma xi$ = 3407, 2652, difference 755

$$= \frac{(755)^2}{48} = \frac{570025}{48} = 11875$$

A vs E mean square $= \dfrac{52}{748.3} = 0.07$

B vs F mean square $= \dfrac{108.2}{748.3} = 0.14$

C vs G mean square $= \dfrac{6788}{748.3} = 9.12$

D vs H mean square $= \dfrac{12403.5}{748.3} = 16.58$

A vs F mean square $= \dfrac{7301.2}{748.3} = 9.75$

B vs G mean square $= \dfrac{19120}{748.3} = 25.55$

C vs H mean square $= \dfrac{24824.8}{748.3} = 33.17$

A + B + C + D vs E + F + G + H mean square $= \dfrac{11875}{2} = \dfrac{5937.5}{748.3} = 8.00$

Only 'F' ratios which had a probability of 0.05 to 0.01 or less of being as large as the calculated value by chance alone were considered to be significant.

APPENDIX VI

CALCULATION OF LINEAR REGRESSION
(Snedecor, 1956., 123-124)

In the simple linear regression situation it is postulated that the relationship between y and x is of the form

$$\hat{y} - \bar{y} = b\,(X - \bar{x})$$

Where y is the estimated deviation of Y corresponding to any x deviation, b is sample regression coefficient. The doses may be taken as x and the responses as y. Using the formula $\hat{y} - \bar{y} = b\,(X - \bar{x})$ a sum can be worked out in the following way:

	Protein intake Σx	Weight gain Σy	Deviation from means		Squares		Products
			x	y	x^2	y^2	xy
	11.97	13.63	- 6.37	- 8.75	40.5769	76.5625	52.5500
	18.09	20.03	- 0.25	- 2.35	0.0625	5.5225	0.5875
	19.24	22.46	0.90	0.08	0.8100	0.0064	0.0719
	24.07	33.40	5.73	11.02	32.8329	121.4404	63.1600
Total	73.37	89.52	0	0	74.2823	203.5318	116.3694
Mean	18.34	22.38					

Sample regression co-efficient -

$$(b) = \frac{\Sigma xy}{\Sigma x^2} = \frac{116.3694}{74.2823} = 1.567$$

In terms of the original units, the sample regression equation is -

$$y - y = b\ (x - x)$$

$$y - 22.38 = 1.567\ (X - 18.34)$$

$$y = 22.38 + 1.567\ (X - 18.34)$$

$$y = 22.38 + 1.567\ (X - 28.69)$$

$$= 28.69 - 22.38 + 1.567^{X}$$

$$= - 6.31 + 1.567^{X}$$

Based on this the other calculations were done.

APPENDIX VII

CALCULATION OF CORRELATION COEFFICIENTS
(Snedecor, 1956., 167)

Relation between the sample coefficients of correlation and regression

If y were designated as dependent, its regression on x would be $b_1 = \Sigma xy / \Sigma x^2$, but if x were dependent the regression of X on Y would become $b_2 = \Sigma xy / \Sigma y^2$. These expressions differ in their denominations only. If 'r' is rear = 1, the lines are close together. The regression of Y on X is always the line that makes the lesser angle with the vertical axis.

Now it is seen that -

$$b_1 \, b_2 = \frac{\Sigma xy}{\Sigma x^2} \cdot \frac{\Sigma xy}{\Sigma y^2} = \frac{(\Sigma xy)^2}{\Sigma x^2 \Sigma y^2} = r^2$$

Thus,

$$r = \sqrt{b_1 \, b_2} \quad \text{or} \quad \sqrt{\frac{(\Sigma xy)^2}{\Sigma x^2 \Sigma y^2}}$$

For example,

Correlation coefficient for regression of body weight gain on protein intake can be calculated as:

$$\sum xy = 116.3694 \quad \text{(Appendix VI)}$$

$$\sum x^2 = 74.28$$

$$\sum y^2 = 203.5318$$

$$r^2 = \frac{(116.37)^2}{74.28 \times 203.5318} = 0.894$$

$$r = \sqrt{0.894} = 0.945$$

$$r = 0.945$$

In the same way correlation coefficient was calculated for all the other samples.

APPENDIX VIII

BODY WEIGHT CHANGES, FOOD INTAKE, LIVER WEIGHT, HEPATIC NITROGEN AND HEPATIC SDH ACTIVITIES OF RATS FED DIETS OF VARYING PROTEIN CONTENTS OF SKIM MILK AND HORSEGRAM-SESAME MIXTURE FOR 28 DAYS

Group	Rat No.	Body weight Initial	Body weight Final	Daily food intake	Liver weight	Total Hepatic Nitrogen	Hepatic SDH activity Mcg. Ttcl/g per 30 minutes
		g.	g.	g.	g.	mg	
A	A_1	32.5	47.8	151	3.52	41	-
	A_2	33.5	48.8	158	3.12	68	-
	A_3	23.1	30.0	111	2.10	53	-
	A_4	24.5	40.0	75	2.50	51	758
	A_5	34.0	46.1	148	3.00	81	840
	A_6	24.8	41.5	155	1.90	92	900
Mean		28.7	42.4	133	2.69	64	833
B	B_1	27.9	53.6	166	3.70	57	-
	B_2	33.0	49.5	153	3.20	107	-
	B_3	23.5	37.8	161	3.30	84	-
	B_4	25.0	44.0	137	3.00	116	1028
	B_5	33.0	50.2	175	4.00	97	1065
	B_6	24.5	52.0	195	2.10	111	1100
Mean		27.8	47.8	165	3.31	95	1064
C	C_1	28.7	54.5	179	3.81	80	-
	C_2	36.0	56.0	158	4.25	131	-
	C_3	23.4	38.0	103	3.91	139	-
	C_4	23.0	40.0	128	4.25	128	1669
	C_5	27.5	53.1	165	4.26	158	1630
	C_6	24.7	56.5	155	2.50	131	1700
Mean		27.2	49.6	148	3.83	128	1683

Ttcl = Triphenyl tetrazolium chloride

Group	Rat No.	Body weight Initial	Body weight Final	Daily Food Intake	Liver weight	Total Hepatic Nitrogen	Hepatic SDH Activity Mcg. Ttol/g. Pa 30 minutes
		g.	g.	g.	g.	mg.	
D	D_1	28.5	60.0	191	4.00	121	-
	D_2	36.0	68.5	171	3.31	164	-
	D_3	21.0	60.5	101	4.00	166	-
	D_4	24.5	60.0	165	4.50	143	1669
	D_5	29.0	60.0	140	4.45	168	1680
	D_6	24.0	52.7	195	3.30	165	1700
Mean		27.2	60.6	160	4.09	155	1683
E	E_1	28.5	60.0	164	3.80	42	-
	E_2	35.5	66.5	164	3.10	78	-
	E_3	23.0	50.2	114	3.50	55	-
	E_4	24.0	55.0	135	3.00	57	912
	E_5	27.1	55.0	138	1.60	60	950
	E_6	24.6	43.5	115	3.00	71	1000
Mean		27.1	55.0	138	3.00	60	954
F	F_1	34.0	79.0	177	2.70	80	-
	F_2	35.6	85.0	174	4.20	126	-
	F_3	21.3	59.8	146	2.80	85	-
	F_4	24.0	62.0	137	3.80	106	1160
	F_5	28.5	66.9	169	5.00	157	1185
	F_6	24.9	60.0	165	4.00	128	1200
Mean		28.0	68.4	161	3.80	113	1182

contd..

Group	Rat No.	Body weight Initial g	Body weight Final g	Daily food intake g	Liver Weight g	Total Hepatic Nitrogen mg	Hepatic SDH activity Mog.Ttol/g. per 30 minutes
G	G_1	35.2	94.0	197	5.1	119	-
	G_2	35.0	98.0	169	5.2	187	-
	G_3	21.0	71.0	149	4.0	198	-
	G_4	22.5	68.4	152	5.5	150	1515
	G_5	32.5	84.6	164	5.7	198	1578
	G_6	24.5	82.0	185	3.4	199	1600
Mean		28.5	83.0	169	4.8	175	1564
H	H_1	34.0	99.8	164	5.2	155	-
	H_2	31.0	90.0	149	5.2	206	-
	H_3	21.0	79.5	149	4.5	203	-
	H_4	28.9	91.4	165	5.2	218	1825
	H_5	34.5	95.6	174	4.8	267	1860
	H_6	24.3	92.3	190	3.9	263	1900
Mean		28.9	91.4	165	4.8	218	1862