EVALUATION OF THE PROTEIN QUALITY OF SPROUTED HORSE GRAM AND GREEN GRAM IN ALBINO RATS

By

CHITRA, S.

A Thesis Submitted to the University of Madras
in Partial Fulfilment of the Requirements
for the Degree of Master of Science,
April, 1977.

ACKNOWLEDGEMENT

The author wishes to express her heartfelt thanks and sincere gratitude to Dr.(Mrs.) Usha Chandrasekhar, M.Sc., Ph.D (Purdue) Professor of Nutrition, Sri Avinashilingam Home Science College for Women, for her untiring help and guidance in the conduct of the study.

She records her deepfelt gratitude to Dr.(Mrs.)Rajammal P. Devadas, M.A., M.Sc., Ph.D., (Ohio State) Principal, Sri Avinashilingam Home Science College for Women, for her valuable suggestions and continued interest in this investigation.

LIST OF CONTENTS

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXURES

I INTRODUCTION

There is no universal panacea to solve the problems of food and nutrition in any country except through the acts of self-help and self-reliance. Talking in this vein, Beghin (1976) and Devadas (1977) recommend the removal of the constraints and recognition of the problems to make explicit choice of method to attain self-sufficiency as an individualistic pattern for all societies. To Indians, talking of hunger and malnutrition has become the second nature, but the urge should come from within to solve this pathetic situation of hunger and misery of millions of its people.

Out of the 500 million undernourished children in the world, 50 million children are in India (Nayar, 1977) and every year a million more children are victimised as a result of severe malnutrition (Devadas, 1977). The wide prevalence of malnutrition is reflected by the high morbidity and mortality rates, especially among the vulnerable group-the worst sufferers. The general mortality rates of the different age groups in the developing countries are 10 to 40 times those of their counterparts in the affluent areas (Mahler, 1976).

Malnutrition, especially protein energy malnutrition and infections have a synergistic influence on the physical and mental development of children (Cravioto, 1971; Devadas, 1972;

Mc Namara, 1973; Bengoa, 1974; Behar, 1976 and Devadas 1977) and on the health, working capacity and productivity of adults.

This universal problem of malnutrition has been a severe blow to the developing countries where the population on the one hand and food production on the other never come closer. Inadequate intakes of calories and other dietary essentials, along with several other factors worsen the attack of this 'enemy of mankind'. To fight malnutrition, developing countries like India have to give due consideration and attention to the utilization of the existing resources to the utmost. Cereals like rice, jowar and ragi which are the staple, provide proteins to some extent in addition to giving energy, as the average Indian diet rarely contains a protein rich food especially foods of animal origin. Consequently, plant foods have to be depended upon for meeting the protein needs (Borlaug, 1973; Oliveira, 1973; Swaminathan and Jain, 1973; Crisp, 1974; Milner, 1974 and Betschart, 1975).

Legumes often are the only protein component of diets, either alone, or in combination with cereals, and this common man's food has to be fully used in attempting to decrease the protein gap. (Swaminathan, 1973; Berg, 1973; Borlaug, 1973; Oliveira, 1973, Jaffe, 1973 and PAG/UN, 1975). Though many a novel protein food is being developed, exploitation of the

existing conventional, less expensive, widely used sources of food proteins will yield better results in the long run, for the people belonging to the low socio-economic strata of the society who are also invariably undernourished and malnourished, have an easy access to these foods.

Legumes supply not only proteins but also other important nutrients to the diet in addition to providing energy. Legumes have been in use from ancient times and various methods have been used to improve their nutritive value and render them more palatable, digestible and tastier. (Kylen and Mc Cready, 1975) Germination is a very simple, inexpensive method of home-scale processing which increases the nutrient content, improves the availability and digestibility of legumes (Babu, 1976; Subbalakshmi et al 1976 and Ganeshkumar and Venkataram, 1976). This method of processing is being widely used in South India to sprout some of the commonly used legumes like green gram, horse gram, cowpea and chick pea (Protein Foods Association of India, 1973; Sreekantaradhya, et al 1975; Babu, 1976 and Krishnamurthy and Rama Rao, 1976).

With a few exceptions, legumes have been greatly ignored from the point of view of research and related efforts to increase production (FAO/U.N., 1964; Oliveira, 1973; PAG/UN 1973; Milner, 1974 and PAG/UN, 1975). Information regarding

the changes in the nutritive value and availability of proteins of legumes as a result of sprouting is still limited. This investigation is a step towards this end. Horsegram and green gram are two of the most commonly used legumes in South India (Babu, 1976), and horsegram being less expensive, seems to be the favourite of the poorer sections of the population especially in Coimbatore district (Devadas et al 1973). Hence, this investigation has been planned to evaluate the protein quality of sprouted horsegram and green gram supplemented with skim milk at 5 per cent level, alone or with additional supplementation with the limiting sulphur containing amino acids, on albino rats.

II REVIEW OF LITERATURE

The literature pertaining to this study on Evaluation of the protein quality of Sprouted horse gram and green gram is reviewed under the following heads:

A Place of proteins in human dietaries

B Legumes - Conventional sources of food proteins

C Effects of germination on the nutritive value of legumes

D Evaluation of protein quality

A Place of proteins in human dietaries:

Protein is essential for both growth and maintenance of the human body. Protein deficiency results in various clinical and subclinical conditions such as decreased growth rates and poor physical and mental development in children and adolescents, and impaired health, resistance to disease and reduced working efficiency in adults, (FAO/U.N., 1964). Research has suggested that the intellectual development of infants and children is impaired by inadequacy of dietary proteins (Cravioto et al, 1962; 1966; Read, 1968; Monckeberg, 1968; 1969; Cravioto et al 1969; Frisbh, 1970; Cravioto, 1970; Gopalan, 1970; Frisch, 1971; Mc Cance, 1971; Srikantia and Yogananda Sastri, 1971; Cravioto et al 1971; Mc Namara, 1973; Tizard, 1974; Labouisse, 1975 and Behar, 1976).

The FAO (1966) has reported that those countries with lowest per capita daily protein and caloric consumption are also those with the lowest productivity.

The calorie supply available/person/day in India averaged about 2000 during the decade 1963-72 against a requirement of a little over 2200. By contrast the protein supply averaged about 50g/day/person against the average requirement of 30g. and the recommended allowance of 56g. based on ICMR recommendation and those of WHO/FAO, 1965, 1971 (Sukhatme, 1973). For protein, far from the overall gap, there is a large excess. Even so, protein deficiency is widespread because of uneven distribution. An association between protein and calorie intakes is to be expected since the major source of energy, as of proteins, is the staple cereals in the diet at least in the low income groups. So the problem of protein malnutrition is secondary to inadequate calorie intake (Gopalan, 1975).

It will be seen that as long as the caloric intake is limiting, but protein intake is not, there is a loss of body protein. It is not until the caloric intake exceeds the calories needed for maintenance of body that protein is fully utilized (Sukhatme, 1973). As long as the energy intake is below the level for maintenance the body will tend to use even its own tissues to meet the energy needs. A diet

deficit in calories is compensated for, by reduced activity but the body has no comparable mechanism for protein deficiency. (Nutrition and Food Science, 1974). So adults 'adapt' to Protein Energy deficiency by a loss of weight and reduction in voluntary work, children 'adapt' by retarded growth rates as well as by increased activity. Such adaptations are unacceptable as a national policy. Sen Gupta (1974) quoting the work of Scrimshaw says that in some developing countries, people even receiving adequate calories could not discharge full day's work because of insufficient proteins.

Food policies that have the aim of simply increasing the average protein intake per head of the population may be totally ineffective in decreasing the incidence of deficiency, since in practice, the extra protein consumed will not be uniformly distributed. The only policy measures that are likely to be effective are those aimed at improving food distribution relative to needs, as well as ensuring an adequate overall supply of foods (FAO/WHO, 1973 and Nagarajan, 1977).

B <u>Legumes - Conventional sources of food proteins</u>:

By now, it is well accepted that supplying adequate amounts of high quality protein to the population in developing countries is not an easy endeavour (Bressani, 1972). A large number of grain legume and oil-seed legume crops can be

helpful in alleviating the protein crisis of the developing nations. (Patwardhan, 1962; Aykroyd and Doughty, 1964; Berlaug, 1973; Oliveira, 1973; Anonymous, 1973; Swaminathan and Jain, 1973; FAG/UN, 1975). The food legumes are the step children in the family of man's food crops (Roberts, 1972). With a few exceptions they have been largely ignored from the standpoints of research and allied efforts to increase production (FAO/UN, 1964; Oliveira, 1973; FAG/UN, 1973 and Milner, 1974) although the potential use of food legumes as sources of protein to improve human nutrition has long been on the minds of people interested in Foods and Nutrition (Jaffe, 1973).

Legumes form part of the daily diet of India(Yadav and Bharadwaj, 1971; Sundaravalli and Desai, 1973; Protein Foods Association of India, 1973; and Swaminathan and Jain, 1973), and form the only source of protein for about 50 per cent of the rural population of the developing countries lying in the tropical belt (FAO/UN, 1964; INCAP, 1969 and FAG/UN, 1975). Legumes have a high content of protein (20 to 25 per cent) and are nutritionally important among vegetable foods (Aykroyd and Doughty, 1964; Bressani, 1973; Burr, 1974; Adams, 1974; Gritton et al 1975; and Subbulakshmi et al 1976). Food legumes or pulses occupy a unique position in Indian agriculture and no other country in the world has so much area under these crops as does India (Swaminathan, 1971 and Swaminathan and Jain, 1973).

There are about 20 species of food legumes that are used in appreciable amounts in the human diets in one part or another of the world (Roberts, 1972, and Molina et al 1975), depending on the dietary patterns, availability and local traditions (Patwardhan, 1962). Legumes are very adaptable and are found in all agroecological conditions (FAO/UN, 1964).

Dry legumes have been called 'Poor man's meat', a designation of interest and importance from historical and other stand points. In the first place, it implies a meat substitute. But in the developed countries, legumes are now an almost insignificant item in the diet and their consumption is tending to decrease. They can no longer be called 'Poor man's meat' there, as even people in the lowest income groups get real meat. Legumes assume greater importance in countries where starchy roots and fruits replace cereals to a large extent as staple foods (Aykroyd and Doughty, 1964).

Legumes contain 60 per cent of carbohydrates, 20 to 30 per cent protein and 1 to 2 per cent fats. They also contain 0.4 to 0.5 mg/100g. of thiamine, 2.0mg/100g. of riboflavin, negligible amounts of β carotene and ascorbic acid. But they are rich in calcium (100 mg/100g) and iron (7mg/100g). Thus, legumes supply not only proteins but also other essentials. The high protein content is itself a fact

of great significance, but, the contribution which any protein makes to the fulfilment of requirements depends not only on the quantity in which it is present in the diet, but also on its quality which in turn depends on the amino acid composition. The majority of reported Protein Efficiency Ratios for legume proteins are between 0.5 to 1.5 and the Biological Value between 40 to 50.

The value of a protein depends on its content of amino acids, since the amino acids are the units used by the organism for growth and maintenance. Legume proteins are poor sources of sulphur containing amino acids methionine and cystine (Aykroyd and Doughty, 1964; Swaminathan, 1967; Bressani, 1973; Jansen, 1973; Adams, 1974; Miller, 1974; Burr, 1974; Gritton et al 1975 and Liener et al 1977). Many are also somewhat deficient in tryptophan, isoleucine and threonine. Low protein digestibility is a serious nutritional problem in most feed legumes. Legumes contain toxic substances - trypsin inhibitors haemagglutinins, goiterogenic factors, cyanogenic glycosides, lathyric factors and compounds causing favism (Bressani, 1973). One of the best known problems that results from consumption of legume foods is increased flatulence (Bressani, 1973).

Even with all these disadvantages legumes fare well against cereals, because of their high content of lysine which is limiting in cereals (Bressani, 1973; Jansen, 1973). It has been shown that small amounts of legume proteins added to

cereal grains improve the amount of utilizable protein in the mixture by their mutual supplementation (Aykroyd and Doughty, 1964; Bressani et al 1968; Aiyar and Sreenivasan, 1972; Bressani, 1973; Jansen, 1973 and Gritton et al 1975).

Fortification of legume proteins with sulphur containing amino acids and supplementation by means of other proteins have also been suggested as measures of increasing protein utilization from legumes. Studies by Woods et al (1943), Russell et al (1946), Lehrer et al (1947), Jaffe (1949), Bressani, Elias and Valiente (1963), Kakade and Evans (1965), Clark et al (1966), Swaminathan (1967), CFTRI (1970), Bressani (1973), Lachance (1973), Jansen (1973), Oliveira (1973), Burr (1975) and Liener et al (1977) support this statement.

Contribution of legumes to diets in developing countries is limited by their scarcity. In India, diets comprised of 80 per cent ragi, rice or maize and 20 per cent legumes would meet the needs of growing children and expectant or nursing mothers (Swaminathan and Parpia, 1971). Legumes have been used in the preparation of low cost protein foods (Subramanyam et al 1957) weaning foods (Narayana Rao, 1960) and protein foods used in the treatment of Kwashiorkor (Venkatachalam et al 1956).

The Protein Advisory Group recommends urgent research attention to 6 major species of legumes - dry beans, pigeon peas, cow peas, chick peas, broad beans, peas, peanuts and

soy beans. While increased yield and improved consumer acceptance qualities are primary objectives, priorities are also proposed for genetic improvement of various nutritional factors. These include increased protein concentration, higher methionine and cystine levels, augmented lysine where feasible and protein digestibility (Milner, 1974).

India, in common with other developing countries is being successfully persuaded to expand the production of semi-conventional protein in the name of commitment to combat malnutrition. In fact, this effort amounts to a little more than adding to the dietary variety and caters more to the needs of those who already have enough or more to eat (Milner, 1974). Instead, the conventional sources especially legumes, which provide the best means of attacking the protein problem and which have a very great potential for increasing yields and production could be tapped to the maximum (Roberts, 1972).

C <u>Effects of germination on the nutritive value of legumes</u>:

Processing is a pre-requisite for several foodstuffs in order to improve their palatability and digestibility. The methods improved in processing of food vary widely and the nutritive value may be improved or diminished depending on the method adopted (Babu, 1976). Some of the commonly adopted methods are cooking, heating, roasting, dehydrating, autoclaving, germinating, fermenting and soaking.

The ancient practice of sprouting seeds for food use is increasing in popularity (Kylen and Mc Cready, 1975). Legumes, sprouted and unsprouted constitute a major source of food proteins (Patwardhan, 1961). Legumes are often sprouted and consumed. Some of them like chickpea, cowpea and green gram (Protein Foods Association of India, 1973) and bengal gram (Babu, 1976) are consumed after germination which is a very simple process.

Young in 1782 observed that germinated seeds acquired antiscorbutic properties (Chayen, 1953). During sprouting, the ascorbic acid is converted to dehydro-ascorbic acid which possesses full biological activity by the action of ascorbic acid oxidase (Chattopadhyay and Bannerjee, 1952). Thiamine content is not affected but there is an increase in niacin content of 50-100 per cent in 48 to 72 hours and even greater increase in available iron (Banerjee and Banerjee, 1950). The dry seeds have measurable amounts of ascorbic acid (Ahlberg, 1935; Bhagvat and Rao, 1942; Cravioto et al 1945) but the amount is increased by germination (Ahlberg, 1935).

Kylen and McCready (1975) have shown higher protein content of sprouted alfalfa and lentils. This is in support with results of earlier studies by Klain (1955) who reported rise in amino acids in seeds during germination. Shastri et al (1975) found no biogenesis of thiamine but of riboflavin,

the maximum levels at 72 hours of germination in Dolichos Lab lab. A similar pattern has been noted by Nath and Shastri (1966) while germinating mung bean. A fluctuating pattern was obtained for niacin and no significant biogenesis could be seen (Shastri et al 1975) which is different from what has been reported by Burkholder (1943) during germination of oat, wheat, barley and maize.

Subulakshmi et al (1976) have observed a progressive increase in non-protein nitrogen with a decrease in protein nitrogen in horse gram and moth bean on germination. The digestibility was also increased in both the legumes after germination (Ganesh Kumar and Venkataram, 1976). A decrease in starch content was also noted supporting the statement of Hsu et al (1973).

Babu (1976) has reported a marked increase in the folic acid levels of bengal gram and ragi after germination similar to that observed in garden pea by Rose and Cousins (1971).

During germination the seeds undergo pronounced metabolic change and the structural profile of the various metabolic components are altered (Koller et al 1962). Proteins are hydrolysed to form simple peptides and amino acids (Rogers, 1968; Altschul et al 1966 and Mayer et al 1968),

due to protease activities (Danielson, 1951, Varner, 1965 and Beevers, 1968). Changes in trypsin inhibitor and hemagglutinin activities during germination have also been reported (Chattopadhyay and Banerjee, 1953; Kakade, 1966; Simm and Liener, 1970 and Maner and Pond, 1971).

Rats fed cowpeas soaked for 36 hours showed superior weight gain and Protein Efficiency Ratio compared to that of rats fed unsoaked peas (Onajimi et al 1976). This also suggests that soaking has a beneficial effect related to destruction of trypsin inhibitors or synthesis of other factors. Ray (1968) has shown that when raw horse gram was fed to rats at 10 per cent protein level, it induced high mortality indicating that either the raw seeds created an extreme nutritional imbalance or possessed some toxic substance. Autoclaving improved the growth performance remarkably, while germination did not bring any such improvement. But there was no toxic effect and mortality index was reduced appreciably. Ray (1968) also found that the intake of raw horse gram was low, compared to casein and autoclaved horsegram.

D Evaluation of protein quality:

The biological utilization of proteins depends upon many factors such as protein content, protein quality and protein digestibility (Kakade, 1974). The protein nutritive

value or utilization may be defined as the ability of a protein to provide a pattern of amino acids in proper concentration similar to body protein, although a quantitative aspect of protein quality has been only recently reemphasized in evaluating the utilisable protein from various sources (Hegsted, 1969).

The efficiency with which a protein is used for growth or maintenance is a measure of its quality which is predominantly determined by its Amino acid composition (FAO/WHO, 1973). Campbell and McLaughlan (1970) outline the main reasons for developing methods of evaluation of proteins to be i) to provide a procedure for ranking proteins according to their efficiency of utilization under some set of standard conditions and ii) to compare the efficiency of utilization of proteins as sources of nitrogen and amino acids for meeting the amino acid requirements of man and animals.

The methods available for the evaluation of protein quality as suggested by Block and Mitchell (1946) and Venkat Rao et al (1964) are given below:

1 Methods based on growth and body weight changes

2 Methods based on carcass nitrogen analysis

3 Methods based on nitrogen balance

4 Methods based on regeneration of blood and liver constituents

5 Determination of availability of amino acids

6 Chemical scoring methods

7 Miscellaneous methods

1 Methods based on growth and body weight changes:

a) Protein Efficiency Ratio (PER):

It is the simplest method of determining protein quality since it requires no chemical measurements (FAO/WHO, 1973). Protein Efficiency Ratio (PER) is defined as the weight gain of a growing animal per gram of protein consumed (Beaton and McHenry, 1964; Munro and Allison, 1964). Venkat Rao et al (1964) state that many factors are likely to affect the PER values, the most important being the level of protein in the diet, age, sex, strain of rats, variation in food intake and duration of experimental period. However this method has been criticized by Hegsted and Chang (1965, Hegsted (1971) and McLaughlan, and Campbell, (1974) since the values are not directly proportional to the nutritive value of the protein tested.

b) Net Protein Ratio (NPR):

This method introduced by Bender and Doell (1957) is a modification of the PER method. This method makes an allowance for protein requirement for maintenance. The ratio is calculated as

$$NPR = \frac{\text{Gain in Wt. (g) of the test group} + \text{loss in Wt. (g) of the non-protein group}}{\text{Protein intake (g)}}$$

c) Gross Protein Value (GPV):

Venkat Rao et al (1964) report that the Gross Protein Value was first suggested by Heiman et al (1939)and modified by Carpenter et al (1957) later. The gross protein value of a supplementary protein is calculated as the extra growth obtained with the supplementary protein and expressed as the percentage of the corresponding figure obtained with casein as test supplement.

d) Nitrogen Growth Index:

The slope of the line relating growth rate to nitrogen intake oger a period of 28 days has been called Nitrogen Growth Index (Venkat Rao et al 1964).

2 Methods based on carcass nitrogen analysis:

a) Nitrogen Retention Method:

This method introduced by Shankers and McCollum (1929) considered growth as well as nitrogen balance.

$$N_2 \text{ retention percentage} = \frac{\text{Gain in body } N_2 \text{ (g)} \times 100}{N_2 \text{ intake (g)}}$$

It is the percentage of nitrogen retained in the body.

b) Net Protein Utilization (NPU):

This method developed by Miller and Bender (1955) is based on the principle of evaluating protein quality by

a biological test of nitrogen utilisation. NPU is defined as the proportion of intake nitrogen that is retained in the body under specified conditions.

NPU = Biological value X Digestibility

c) Net Dietary Protein Cal % (ND_p Cal %):

This method found useful by Platt in 1961 for evaluation of human diets takes into account the utilizable protein content of a diet in terms of calories.

$$ND_p \text{ Cal\%} = \frac{\text{Retained N X 6.25 X 4 K.Cal}}{\text{Total food intake X K.Cal/g. of food}}$$

3 Methods based on Nitrogen Balance:

a) Nitrogen Balance:

Nitrogen balance is the balance struck between the intake and excretion of nitrogen by an animal.

Biologic value = % of the absorbed nitrogen retained.

(Beaton and McHenry, 1964)

The method of Thomas (1909) modified by Mitchell et al (1945) is used. The following data are required.

Endogenous nitrogen (U_o) = Urinary N when a N free diet is fed
Metabolic nitrogen (F_o) = Fecal N when no N is fed
Dietary nitrogen (D) = N fed during test
Urinary nitrogen ( ) = Urinary N during test
Fecal nitrogen ( ) = Fecal N during test

Absorbed N = $D-(F-F_o)$

Retained N = Absorbed N$-(U-U_o)$

$$\text{Biologic value} = \frac{\text{Retained N}}{\text{Absorbed N}} \text{ X } 100$$

$$= \frac{D-(F-F_o) - (U-U_o)}{D - (F-F_o)} \text{ X } 100$$

b) Digestibility Co-efficient:

Measurement of digestibility co-efficient is an inevitable part of the N balance method.

Digestibility Co-efficient (DC) is the proportion of food nitrogen that is absorbed.

$$DC = \frac{\text{Food N absorbed}}{\text{Food N intake}}$$

c) Nitrogen Balance Index:

The nitrogen balance index is the slope of the line relating nitrogen balance to absorbed nitrogen. In most circumstances this is equivalent to Biologic Value. This method was introduced by Allison and Anderson in 1945.

d) Egg Replacement Value:

Clark et al (1949) define Egg Replacement Value as the extent to which a test protein will give the same Nitrogen Balance as an equal quantity of egg protein.

$$ERV = \frac{\text{N balance (test)} - \text{N balance (egg)}}{\text{N intake}}$$

4 Methods based on regeneration of blood and liver constituents:

Regeneration of specific proteins or proteins of selected organs after depletion may have special merit. Regeneration of plasma proteins (Miller et al 1945), haemoglobin

campbell and Kosterlitz, 1948) liver RNA and activity of liver xanthine oxidase and Succinic dehydrogenase (Muramatsu and Ashida, 1963) are examples.

5 Determination of availability of amino acids:

The suggested methods for the determination of the availability of amino acids are microbiological methods (Fernell and Rosen, 1956 and Orten and Orten, 1957) chemical methods (Venkat Rao et al 1964) enzymatic methods and assays using chick or rat. These methods are not used widely due to inherent short comings (Campbell, 1961).

6 Chemical Scoring Methods:

a) Amino Acid Score:

An amino acid score can be calculated when both total nitrogen or protein and the concentration of the first limiting amino acid are known (FAG/UN, 1975).

The pattern that should be used is the 1973 Joint FAO/WHO provisional reference pattern (FAO/WHO, 1973) which is an estimate of the amino acid requirements of young children.

The quality of a protein may be estimated from its amino acid composition in comparison with the reference pattern amino acids. The content of each of the essential amino acids of a protein is expressed as a percentage of a standard; the lowest percentage is taken as the score. Block and Mitchell, (1946) suggested that since all amino

acids must be present at the site of protein synthesis in adequate amounts for protein synthesis to proceed, an equal percentage deficit of any essential amino acid would limit protein synthesis to a comparable degree.

Generally amino acid scores are calculated as the percentage of adequacy rather than as deficits.

$$\text{AA Score} = \frac{\text{Mg. of AA in 1 g. of test protein}}{\text{Mg. of AA in reference protein}} \times 100$$

b) Essential Amino Acid Index:

Essential Amino Acid Index (EAA Index) is defined as the geometric mean of the ratios of the essential amino acids in a protein to those of a standard (Usually egg protein). Oser (1951) developed this method of computation.

7 Miscellaneous methods:

Longnecker and Hause (1961) and Venkat Rao et al (1964) have explained that the patterns of amino acids in protein markedly influence the level of some free amino acids in the blood. The most limiting amino acids are elevated to a lesser extent and possibly quantitatively related to the amounts needed. Murlin et al (1948)showed a linear relationship between the Biologic value and urinary creatinine of animals fed different ranges of proteins.

Though a wide variety of methods are available, only two methods namely PER and NPU have been recommended as the most suitable methods for the evaluation of the quality of dietary proteins (Food and Nutrition Board, 1963).

III EXPERIMENTAL PROCEDURE

This study aimed at evaluating the protein quality of sprouted horse gram and green gram on albino rats. The procedure included:

A Sprouting of the legumes

B Nutrient analysis

C Formulation of the diets

D Selection and grouping of animals

E Evaluation of protein quality

A Sprouting of the legumes:

Horse gram (Dolichos uniflorus) green gram (Phaseolus aureus) from the local market were cleaned and soaked in pure water for six hours at the end of which they were tied in separate cloths and allowed to sprout. The sprout length was kept constant at 2 to 2.5 cm. for both the legumes and at the end of 30 hours (for green gram) and 36 hours (for horse gram) when the sprout length was equal in both cases, the legumes were taken carefully with the sprouts, dried and powdered. A sprout length of 2 to 2.5 cm. was chosen because it is at this stage that these sprouted legumes are normally consumed.

B Nutrient analysis:

Nutrient analysis was done in horse gram and green gram sprouted for 12 hours, 24 hours, 36 hours, 48 hours and

60 hours respectively. Ascorbic acid was estimated using the dye method, when the sprouted grams were fresh. For all other estimations, the sprouted legumes were dried and powdered. Protein, calcium, iron and phosphorus were estimated in the dried samples using the procedures given in the ICMR Manual (1971).

C Formulation of the diets:

As earlier studies by Ray (1968) have shown that horse gram alone incorporated at 10 per cent level resulted in high mortality, skimmed milk powder was used in this experiment to provide half of the protein (5 per cent). Raw and sprouted green gram and horse gram formed the protein sources for the diets along with skimmed milk powder at 10 per cent level. NRC (1963). Gyorgy (1964) and Srinivas et al (1969) suggest 10 per cent as the ideal level for PER determinations. Refined ground nut oil was added to make the fat content upto 9 per cent after calculating the fat in the food. Vitaminised starch, vitaminised oil and mineral mixture were added according to ISI standard (1974) to ensure adequacy in other nutrients. Corn starch was added to make up the diet composition to 100. Twelve diets were formulated in all, six each with horse gram and green gram respectively. While raw and sprouted horse gram and green gram formed the protein component of four diets along with skimmed milk powder,

supplementation with 0.5 per cent methionine and 0.5 per cent methionine and 0.5 per cent cystine were also done to both raw and sprouted horse gram and green gram in the other eight diets. The percentage composition of the twelve diets is given in Table I.

TABLE I

PERCENTAGE COMPOSITION OF THE TWELVE EXPERIMENTAL DIETS

Foodstuffs	DIETS											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Raw Horse gram	22.7	---	---	---	22.7	22.7	---	---	---	---	---	---
Sprouted Horsegram	---	22.7	---	---	---	---	22.7	22.7	---	---	---	---
Raw Green gram	---	---	20.9	---	---	---	---	---	20.9	20.9	---	---
Sprouted Green gram	---	---	---	20.9	---	---	---	---	---	---	20.9	20.9
Skim milk	13.2	13.2	13.2	13.2	11.9	10.6	11.9	10.6	11.9	10.6	11.9	10.6
Methionine	---	---	---	---	0.5	0.5	0.5	0.5	00.5	0.5	0.5	0.5
Cystine	---	---	---	---	---	0.5	---	0.5	---	0.5	---	0.5
Mineral Mixture	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mixture	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitaminised oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Groundnut oil	8.9	8.9	8.7	8.7	8.9	8.9	8.9	8.9	8.7	8.7	8.7	8.7
Corn starch	49.2	49.2	51.2	51.2	50.0	50.8	50.0	50.8	52.0	52.8	52.0	52.8
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

D Selection and grouping of rats:

Animals from the laboratory stock colony were chosen and grouped thus:

i) 120 weanling male rats 21 to 24 days old, (Scott and Quint, 1946) were chosen and grouped into 12 groups of 10 rats each, the average weight of the rats in a group falling between 44.2 to 44.9 g. ii) The rats were housed in individual cages with provision for water and food ad libitum.

D Evaluation of protein quality:

The rats were placed on the experimental diets ad libitum using the procedures of Devadas and Sutton (1961) NRC (1963) Droubiscos and Bowland (1969). The diets were weighed mixed well with water, steamed and fed daily. The left over food along with spilt food was dried in an oven and weighed, the following day. This weight subtracted from the weight of food given, gave the weight of food actually consumed by the animal.

A record of daily food intake was maintained. The feeding was continued for 28 days (NRC, 1963). The rats were weighed every second day and a record of the weight gain maintained.

i) Weight gain and food intake:

It has been generally accepted that the growth of weanling rats under standardised conditions provides a reliable

measure of the value of dietary proteins (Campbell, 1961). Both weight gain and food intake which reflect the quality of the protein given (Munro and Allison, 1964) served as the basis of evaluation of the diets tested.

ii) PER:

The total intake of protein was calculated for each rat from the total food intake. From the weight gain and intake of protein, the protein Efficiency Ratio was calculated.

$$PER = \frac{\text{Gain in weight (g)}}{\text{Total protein intake (g)}}$$

NRC, (1963) Beaton and McHenry (1964)

iii) Hepatic weight and nitrogen:

Livers contain labile proteins and respond to an increase in nitrogen intake and to improved nutritive value of dietary proteins (Allison et al 1962)

At the conclusion of the experiment the rats were sacrificed, the livers removed, cleaned of blood and weighed. They were then grouped so as to have 3 groups per diet on the basis of weight. The pooled livers were homogenised well and the nitrogen content was estimated taking 0.5g aliquots in triplicates using the Microkjeldahl method (Hawk et al 1965)

iv) Apparent Digestibility:

During the last 4 days of the experiment, faeces was collected giving ferric oxide as the marker. The faecal

matter collected was brushed off from food and hair, weighed and preserved in polythene bags. They were then grouped so as to have 3 groups per diet on the basis of weight. The pooled faecal matter was finely ground, mixed well and the nitrogen content estimated by taking 0.5g. aliquots in triplicates using the microkjeldahl method (Hawk et al 1965).

From the data on faecal nitrogen and nitrogen intake thus obtained, Apparent Digestibility was calculated using the formula,

$$\text{Apparent Digestibility} = \frac{\text{Total nitrogen absorbed}}{\text{Total nitrogen intake}} \times 100$$

IV RESULTS AND DISCUSSION

In this investigation an attempt was made to evaluate the protein quality of two commonly used pulses, namely horse gram and green gram, in the raw and sprouted forms using albino rats. Accordingly, both these legumes were fed at 5 percent protein level, the remaining 5 per cent coming from skimmed milk powder, since as indicated by Ray (1968) incorporation of horse gram alone at 10 per cent level results in high mortality. Sprouting was carried out in such a manner that a sprout length of 2 to 2.5 cm. for both the grams was kept constant, as these represent the stage at which sprouted legumes are normally consumed. In order to further enhance the protein quality supplementation with the most limiting amino acids methionine and cystine (0.5 per cent level) to both the raw and sprouted forms was also investigated. The results obtained on feeding the above diets to a group of young weanling rats over a period of 28 days, on their weight gains, Protein Efficiency Ratio, Liver constituents and Apparent Digestibility are presented and discussed under the following headings:

A Analysis of the nutritive value of the sprouted grams

B Body weight gains of rats

C Food and Protein Intakes

D Hepatic Weights

F Hepatic nitrogen

G Apparent Digestibility

A <u>Analysis of the nutritive value of the sprouted grams</u>:

Both horse gram and green gram were sprouted and at a time interval of 30 to 36 hours, a sprout length of 2 to 2.5 cm. a common edible length was obtained. In order to evaluate the possibility of nutrient changes, two six-hourly intervals below and above 36 hours were chosen and the sprouted grams thus analysed for protein, calcium, iron, phosphorus and ascorbic acid contents. The values obtained are presented in Table II.

TABLE II

PERCENTAGE NUTRIENT CONTENT OF THE SPROUTED GRAMS

Nutrients		Raw	Sprouted for				
			12 hours	24 hours	36 hours	48 hours	60 hours
Protein (g)	Horse gram	22.0	22.5	22.0	22.1	22.4	22.5
	Green gram	24.0	33.9	23.5	24.2	23.8	24.6
Calcium (mg)	Horse gram	287.0	282.4	285.5	288.3	287.5	290.1
	Green gram	124.0	126.0	127.4	124.8	125.0	124.7
Iron (mg)	Horse gram	8.4	8.4	8.6	8.7	8.9	9.2
	Green gram	7.3	7.3	7.8	7.9	8.2	8.4
Phosphorus (mg)	Horse gram	311.0	312.3	313.5	312.8	315.2	312.4
	Green gram	326.0	327.1	328.9	329.1	331.1	335.4
Ascorbic acid (mg)	Horse gram	1.0	11.5	47.9	93.3	129.1	190.8
	Green gram	0.0	25.6	80.4	101.5	147.7	211.3

Sprouting does not seem to have made any appreciable differences in the protein, calcium, iron and phosphorus contents of the legumes when compared with the raw values. On the contrary, the ascorbic acid content seems to have increased considerably with increase in the length of the sprouting period. These results are in tune with the results reported in the literature for other legumes that total protein does not appreciably change on sprouting (Kylen, 1975; Fordham et al 1975 and Subbulakshmi et al 1976).

As far as the ascorbic acid content is concerned, an enormous amount of 200 to 400 per cent increase in legumes like Bengal gram and green gram has been reported by Ahlberg (1935), Bhagvat and Rao (1942), Chattopadhyay and Banerjee, (1952), Dhand (1964), Aykroyd and Doughty (1964), Babu (1976), and Rajalakshmi (1976). Banerjee and Banerjee (1950) Singh and Banerjee (1953) and Rajalakshmi (1976) have reported that although the iron content may not appreciably change, there seems to be a change in the available iron due to sprouting. Though the increase in ascorbic acid increases upto a 72 hour sprouting period, for purposes of human consumption, a sprout length obtained at 36 hours, seems to be more reasonable and at this stage also about 100 per cent increase in ascorbic acid is perceivable. Hence for the purpose of this investigation, which was mainly aimed at studying the protein quality of

sprouted grams, sprouting for a period of 36 hours was chosen to be more appropriate. Though there is no change in the total protein content due to sprouting, possible changes in the availability and improvements in digestibility have been reported by Chattopadhyay and Banerjee (1953), Aykroyd and Doughty (1964) Kakade (1966) Subbulakshmi et al (1976) and Babu (1976) and hence is within the spectrum of the aim of this investigation. Changes in the pattern of amino acids which in turn would lead to improvement in protein quality are also speculated (Klain, 1955) but the analysis of amino acid per se was not within the scope of this investigation.

B Body weight gains of rats:

The mean initial and final weights and the total weight gains of the rats fed the twelve experimental diets, for 28 days are presented in Table III. The individual initial and final weights are given in Annexure II.

TABLE III

MEAN GAIN IN WEIGHT OF RATS FED DIFFERENT DIETS

Diet	Sources of Protein	Initial Weight (g)	Final Weight (g)	Gain in Weight (g)
I	Raw Horse gram + SM	44.36 ± 7.491	76.10 ± 15.060	31.74 ± 7.04
II	Sprouted Horse gram + SM	44.37 ± 7.090	84.45 ± 8.303	40.98 ± 6.28
III	Raw Green gram + SM	44.69 ± 5.845	88.33 ± 11.730	43.64 ± 6.68
IV	Sprouted Green gram + SM	44.34 ± 6.654	92.55 ± 11.390	48.21 ± 7.67
V	Raw Horse gram + SM + 0.5% meth.	44.87 ± 5.595	86.41 ± 7.730	41.54 ± 4.24
VI	Raw Horse gram + SM + 0.5% meth. + 0.5% cys.	44.48 ± 5.793	96.95 ± 15.180	52.47 ± 9.02
VII	Sprouted Horse gram + SM + 0.5% meth.	44.77 ± 3.242	133.26 ± 7.280	88.49 ± 8.46
VIII	Sprouted Horse Gram + SM + 0.5% meth. + 0.5% cys.	44.82 ± 5.673	115.75 ± 9.539	70.73 ± 7.41
IX	Raw Green gram + SM + 0.5% meth.	44.16 ± 4.874	111.04 ± 3.589	66.88 ±10.38
X	Raw Green gram + SM + 0.5% meth. + 0.5% cys.	44.56 ± 3.948	104.78 ± 9.245	60.22 ± 8.34
XI	Sprouted Green gram + SM + 0.5% meth.	44.74 ± 2.017	131.72 ± 7.550	86.98 ± 6.79
XII	Sprouted Green gram + SM + 0.5% meth. + 0.5% cys.	44.77 ± 4.863	102.06 ± 9.905	59.29 ±10.24

Summary of Statistical analysis

Diet	VII	XI	VIII	IX	X	XII	VI	IV	III	V	II	I
gain in weight	88.49	86.98	70.93	66.88	60.22	59.20	52.47	48.54	43.64	41.54	40.98	31.78

Any two values not underscored are significantly different.

The highest increase in weight (88.49g.) was registered by rats fed sprouted horse gram, + skim milk + methionine (Diet VII). The lowest increase was exhibited by the rats fed raw horse gram + skim milk (Diet I) 31.74 g. Statistical analysis indicated that the differences in weight gain of rats fed sprouted horse gram + skim milk + methionine (Diet VII), Sprouted horse gram + skim milk + methionine + cystine (Diet VIII), Raw green gram + skim milk + methionine (Diet IX) and sprouted green gram + skim milk + methienine (Diet XI) were not significant. When weight gains of other diets were considered, statistically pointing out, rats fed Diet VIII (Sprouted horse gram + skim milk + skim milk + methionine), Diet IX (raw green gram + Skim milk + methienine), Diet X (raw green gram + skim milk + methionine + cystine), Diet XII (sprouted green gram + skim milk + methionine + cystine) and Diet VI (raw horse gram + skim milk + methienine + cystine) were not significantly different. Again, the increments in weight between rats fed Diets IX (Raw green gram + skim milk + methionine) Diet X (Raw green gram + skim milk + methienine + cystine), Diet XII (Sprouted green gram + skim milk + methionine + cystine), Diet VI (Raw horse gram + skim milk + methienine + cystine) Diet IV (Sprouted green gram + skim milk) and Diet III (Sprouted green gram + skim milk) were not statistically significant and also those between Diet X (Raw green gram + skim milk + methionine + cystine) Diet XII (Sprouted green gram +

skim milk + methionine + cystine) Diet VI (Raw horse gram + skim milk + methionine + cystine) Diet IV (Sprouted green gram + skim milk) Diet III (Raw green gram + skim milk) Diet V (Raw horse gram + skim milk + methionine) and Diet II (Sprouted horse gram + skim milk).

However, all the diets were significantly superior to Diet I (Raw horse gram + skim milk) at 1 per cent level. This indicates that both Diets VII (Sprouted horse gram + skim milk + methionine) and Diet XI (Sprouted green gram + skim milk + methionine) where sprouted gram was supplemented with methienine alone seem to be having higher growth promoting potential over that of all other diets and between themselves there does not seem to be any significant difference. Addition of cystine does not seem to enhance growth over and above that of supplementation with methionine alone. Raw horse gram as such even with a 5 per cent skim milk supplementation does not support growth in experimental animals and this may be due to the lower digestibility in the raw state or due to the presence of anti nutritional factors like trypsin inhibitors present in it. Ray (1969) studied the effect of raw and autoclaved horse gram in growth promotion with albino rats and found that raw horse gram did not promote weight gain whereas autoclaving facilitated weight gain in albino rats.

In the present investigation also, raw horse gram seems to fall in line with the studies available in the literature (Miyogi et al, 1931, Hirwe and Magar, 1953 and Ray 1968).

Sprouting horse gram seems to have improved weight gain though not considerably, at least on par with raw horse gram supplemented with skim milk and methionine. Raw green gram on the other hand seems to be better than raw horse gram but on par with sprouted horse gram. Sprouting of green gram per se does not seem to improve the growth potential. These clearly indicate that sprouting though seems to improve the weight gain in the case of horse gram, methionine still remains the limiting amino acid, which is evident in the consequently higher weight gain of animals fed sprouted horse gram + skim milk + methionine.

Figure 1 presents the marked differences in weight gains of rats fed the 12 different diets. While all the rats showed very slight improvements in the weight gain during the first week, rats fed Diet VII (Sprouted horse gram + skim milk + methionine)and Diet XI (Sprouted green gram + skim milk + methionine) seem to have shooted up right from the first week and the continuous gains over the next 3 weeks are markedly superior to that of the other diets. Figure 1,2,3 also indicate the growth trend of rats fed some experimental diets which are marked by their growth promoting quality While sprouting of both horse gram and green gram does not seem to have improved the growth potential.

two rats in the sprouted horse gram + skim milk group (Diet XI) have even lesser weight than the weight of one rat in the sprouted horse gram + skim milk + methionine group (Diet VII)

C Food and protein intakes:

The level of protein in all the diets was maintained at 10 per cent level and hence the changes in the protein intake were directly related to changes in food intake. The mean total food and protein intakes of rats fed the different experimental diets are given in Table IV and the individual food and protein intakes are given in Annexure III.

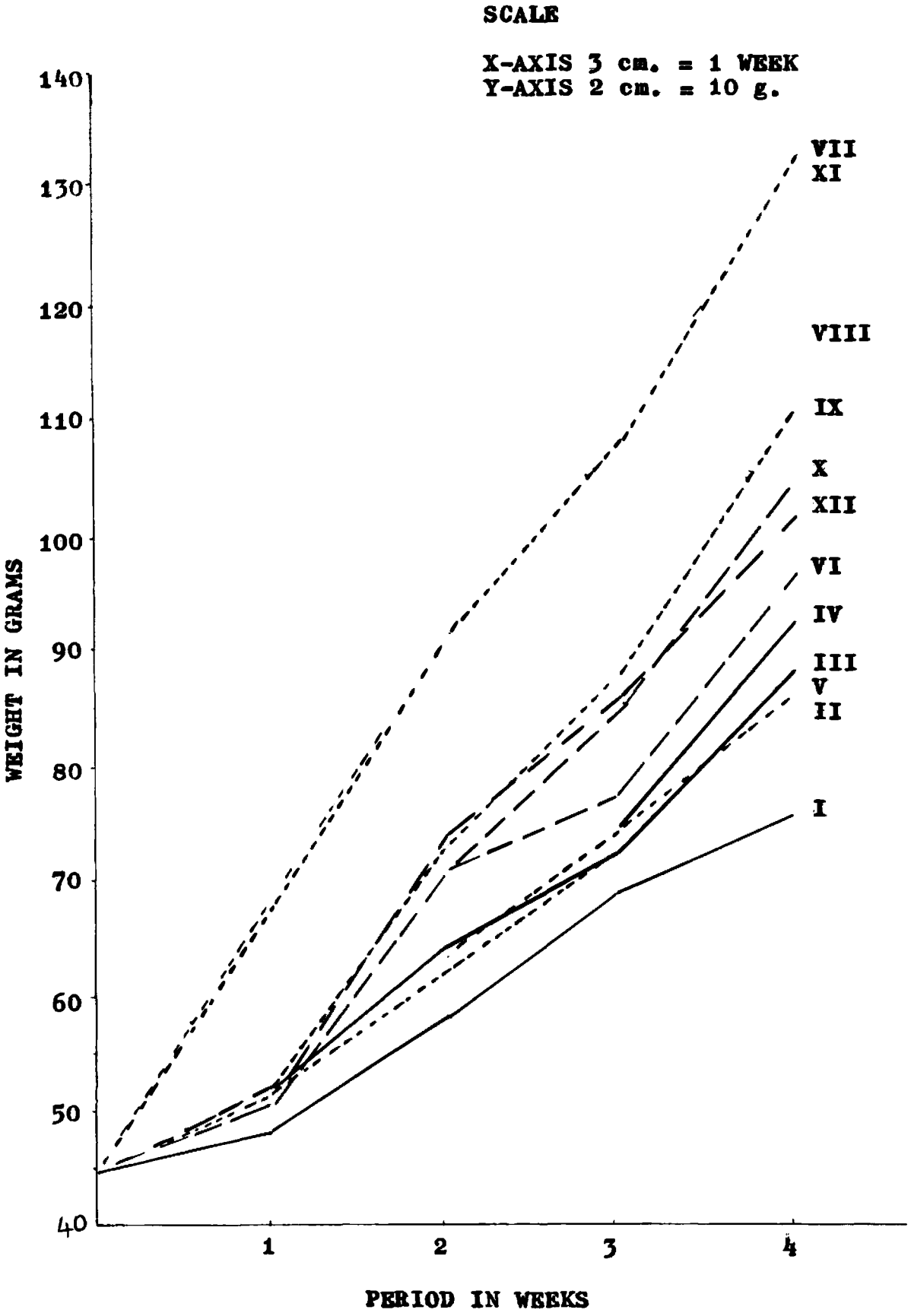


FIG. 1. GROWTH PATTERN OF RATS

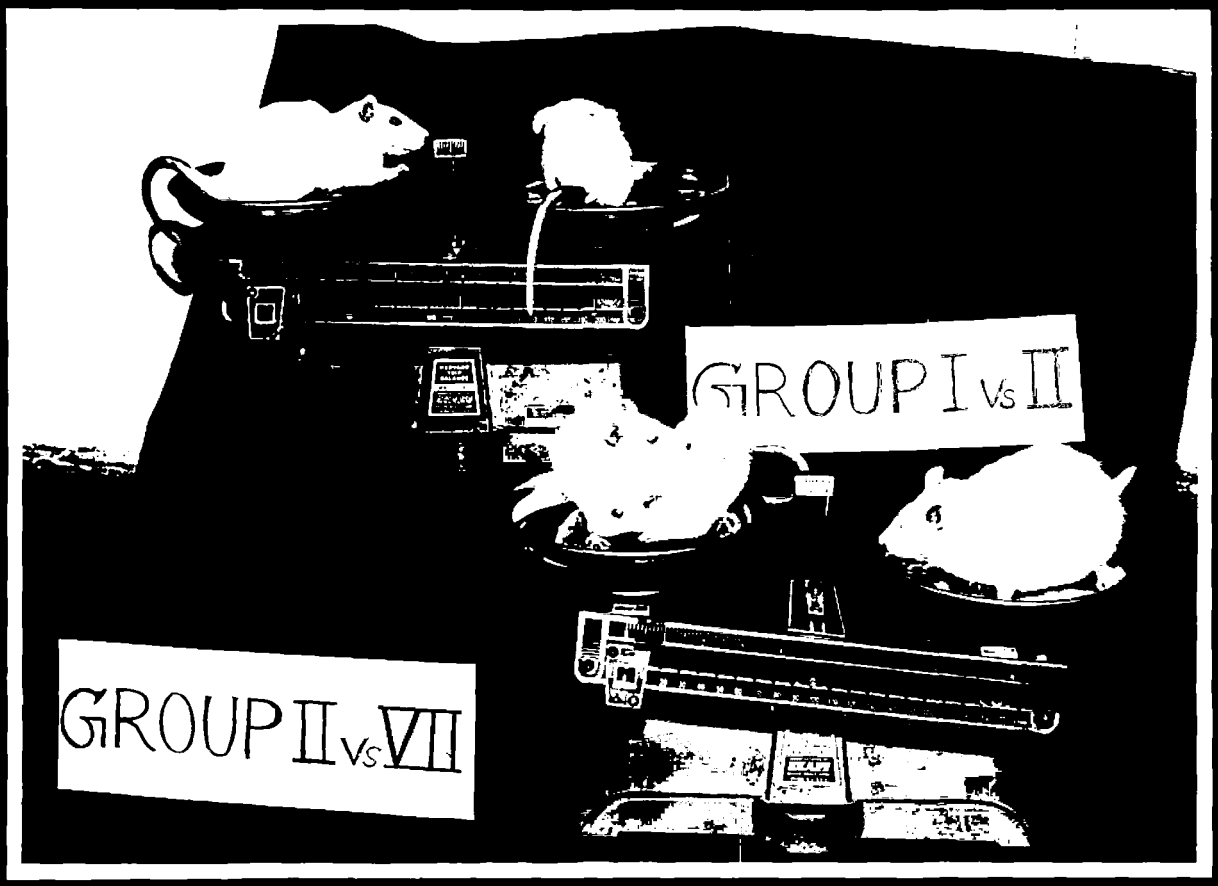


FIG. 2. COMPARISON OF RATS FED HORSE GRAM--RAW, SPROUTED AND SPROUTED PLUS 0.5% METHIONINE

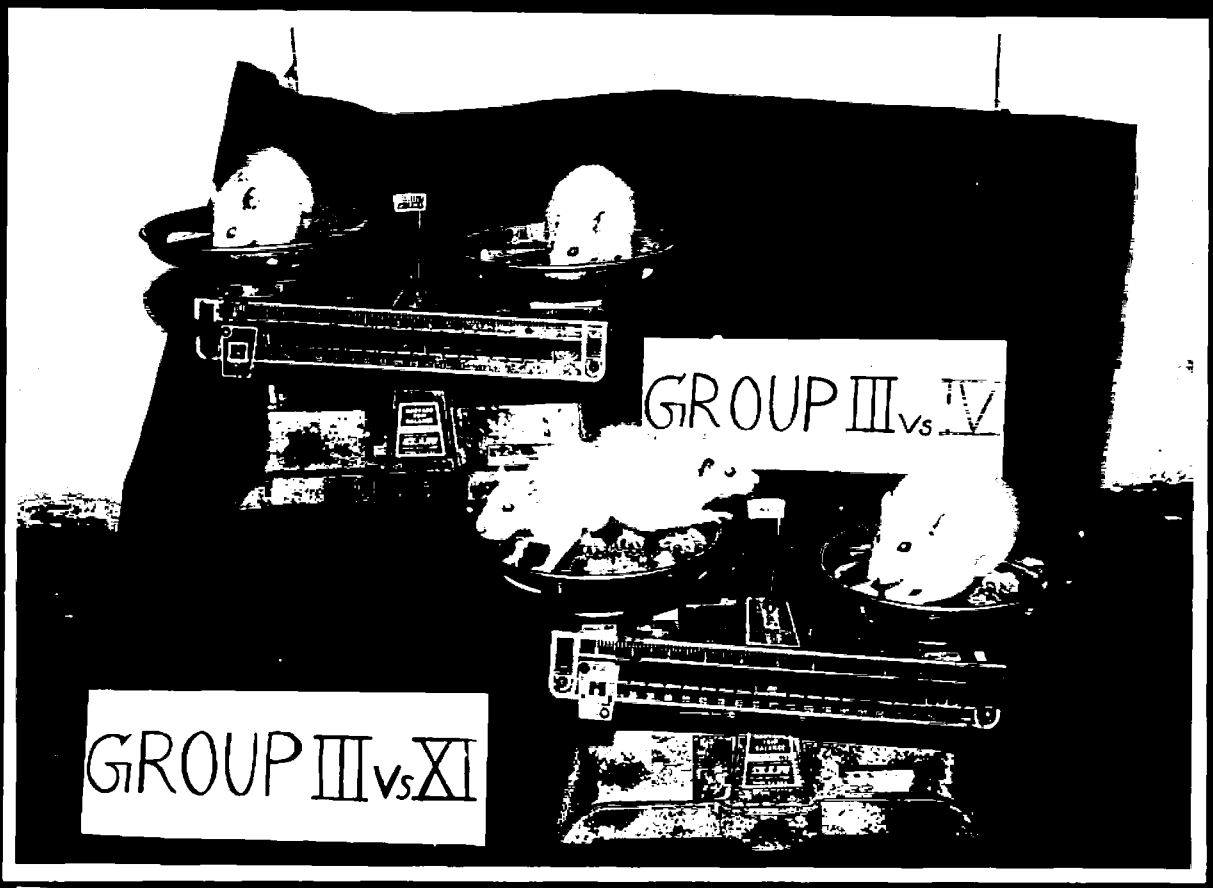


FIG. 3. COMPARISON OF RATS FED GREEN GRAM--RAW, SPROUTED AND RAW PLUS 0.5% METHIONINE

TABLE IV

MEAN FOOD INTAKES AND PROTEIN INTAKES OF RATS FED DIFFERENT DIETS

Diet	Sources of protein	Mean food intake (g)	Mean protein intake (g)
I	Raw Horse gram + SM	187.89 ± 27.08	18.79 ± 2.71
II	Sprouted Horse gram + SM	195.23 ± 21.02	19.52 ± 2.10
III	Raw green gram + SM	207.60 ± 29.31	20.76 ± 2.93
IV	Sprouted Green gram + SM	199.85 ± 27.92	19.99 ± 2.79
V	Raw Horse gram + SM + 0.5% meth.	214.50 ± 11.91	21.45 ± 1.19
VI	Raw Horse gram + SM + 0.5% meth. + 0.5% cys.	212.09 ± 16.41	21.21 ± 1.64
VII	Sprouted Horse gram + SM + 0.5% meth.	297.52 ± 6.52	29.75 ± 0.65
VIII	Sprouted Horse gram + SM + 0.5% meth. + 0.5%cys	228.37 ± 12.01	22.84 ± 1.20
IX	Raw Green gram + SM + 0.5% meth.	229.53 ± 19.14	22.95 ± 1.91
X	Raw Green gram + SM + 0.5% meth. + 0.5% cys.	222.75 ± 19.38	22.28 ± 1.94
XI	Sprouted Green gram + SM + 0.5% meth.	310.08 ± 0.05	31.04 ± 0.81
XII	Sprouted Green + SM + 0.5% meth. + 0.5% cys.	224.14 ± 21.25	22.51 ± 2.13

Summary of Statistical analysis

Diet	XI	VII	IX	VIII	XII	X	VI	V	III	IV	I	II	I
Protein intake	31.04	29.75	22.95	22.84	22.41	22.28	21.45	21.21	20.76	19.99	19.52	18.99	

Any two values not underscored are significantly different.

The animals fed sprouted green gram + skim milk + methionine Diet (XI) recorded the highest food and protein intakes (310.38g. and 31.04g. respectively) followed by rats fed sprouted horse gram + skim milk + methionine (Diet VII) (297.52g. and 29.75 g. respectively). Rats fed Diet IX (raw green gram + skim milk + methionine) ranked third in their food and protein intakes and in the descending order of food and protein intakes, the diets may be ranked as diet VIII, XII, X, V, VI, III, IV, II and I respectively.

The analysis of the significance of difference in protein intake between rats fed the different diets showed that there was practically no difference between rats fed Diet VII (sprouted horse gram + skim milk + methionine) and Diet XI (sprouted green gram + skim milk + methionine) but these values for these diets were significantly superior to all other diets. The difference in the protein intakes of rats fed all other diets were also not statistically significant.

Food intake and subsequent protein intake of rats is a direct indicator of the quality of protein (Munro and Allison, 1964 and Hurt et al, 1974). The higher intake of rats fed Diets VII and XI when compared to those of the other experimental diets might be due to the improvement of the quality of these protein due to supplementation with methionine which is the first limiting amino acid in pulse proteins.

Burr (1974) and de Oliveira (1974) have found in their studies with pulses, specially beans, that supplementation with methionine improved the food intakes of the rats.

A correlation of food intake with body weight gain indicates that greater the feed and protein intake, greater is the body weight gain, for both Diets VII and XI, protein intake as well as weight gain are higher when compared to the other diets. Similar observations have been reported by Devadas et al (1970). Narayanaswamy et al (1974) and Chandrasekhar et al (1976).

D Protein Efficiency Ratio:

The mean gain in weights, protein intakes and the Protein Efficiency Ratio (PER) of rats fed the different experimental diets are given in Table V. The details regarding the same for all the animals are given in Annexure IV.

TABLE V

PROTEIN EFFICIENCY RATIO OF THE TWELVE EXPERIMENTAL DIETS

Diet	Sources of Protein	Weight gain (g)	Protein intake (g)	PER
I	Raw Horse gram + SM	51.74 ± 7.04	18.79 ± 2.71	1.68 ± 0.186
II	Sprouted Horse gram + SM	40.98 ± 6.28	19.52 ± 2.10	2.09 ± 0.138
III	Raw Green gram + SM	43.64 ± 6.68	20.76 ± 2.93	2.10 ± 0.119
IV	Sprouted Horse gram + SM	48.21 ± 7.67	19.99 ± 2.79	2.41 ± 0.175
V	Raw Horse gram + SM + 0.5% meth.	41.54 ± 4.24	21.45 ± 1.19	1.93 ± 0.123
VI	Raw Horse gram + SM + 0.5% meth. + 0.5% cys.	52.47 ± 9.02	21.21 ± 1.64	2.46 ± 0.278
VII	Sprouted Horse Gram + SM + 0.5% meth.	88.49 ± 8.46	29.75 ± 0.65	2.97 ± 0.271
VIII	Sprouted Horse gram + SM + 0.5% meth. + 0.5% cys.	70.93 ± 7.41	22.84 ± 1.20	3.09 ± 0.279
IX	Raw Green gram + SM + 0.5% meth. + 0.5% cys.	66.88 ±10.38	22.95 ± 1.91	2.90 ± 0.250
X	Raw Green gram + SM + 0.5% meth. + 0.5% cys	60.22 ± 8.34	22.28 ± 1.94	2.70 ± 0.187
XI	Sprouted Green gram + SM + 0.5% meth.	86.98 ± 6.79	31.04 ± 0.81	2.80 ± 0.385
XII	Sprouted Green gram + SM + 0.5% meth. + 0.5% cys.	59.29 ±10.24	22.41 ± 2.31	2.62 ± 0.238

Summary of statistical analysis

Diet	VIII	VII	IX	XI	X	XII	VI	IV	III	II	V	I
PER	3.09	2.97	2.90	2.80	2.70	2.62	2.46	2.41	2.10	2.09	1.93	1.68

Any two values not underscored are significantly different.

The highest PER Value of 3.09 was obtained for Diet VIII which had sprouted horse gram + skim milk + methionine + cystine as the protein sources. The PER values obtained for Diets VII, IX, and XI with protein sources sprouted horsegram + skim milk + methionine; raw green gram + skim milk + methionine and sprouted green gram + skim milk + methionine were 2.97, 2.90 and 2.80 respectively and the statistical analysis indicated that the PER values for the above 4 diets (Diets VIII, VII, IX and XI) were not significantly different. The lowest PER value of 1.68 was obtained for raw horse gram + skim milk (Diet I) and this value was significantly lower than all other PER values. Sprouting increased the PER value to 2.09 whereas addition of methionine alone to raw horse gram raised the PER to 1.93. Addition of methionine to sprouted horse gram increased the PER value to 2.97 which was further increased to 3.09 by adding cystine as well. This value is similar to those PER values reported for skim milk 3.08 to 3.26 (Devadas et al 1970).

In the case of green gram, raw green gram had a PER value of 2.10 and sprouting increased it to 2.41. Addition of methionine to either raw or sprouted green gram increased the value of PER to 2.90 and 2.80 respectively which were not significantly different. Addition of cystine along with methionine though increased the value to 2.70 for raw green

gram + skim milk and 2.62 for sprouted green gram + skim milk supplementation with methionine alone seems to be more beneficial. It may however be pointed out that since no statistically significant differences were obtained for Diets VII and VIII (Sprouted horse gram + skim milk + methionine and sprouted horse gram + skim milk + methionine + cystine respectively) supplementation with methionine alone could well help to increase the PER for horse gram.

When PER values are compared with weight gains, it is evident that to some extent the Diets VIII, VII, IX and XI which ranked to be having statistically non significant differences in PER values also promoted weight gains, which were statistically non sighificant. Again rats fed Diet I (raw horse gram + skim milk) which had the lowest PER had also a lower protein intake and subsequent lower weight gain. Studies in the literature by Kakade and Evans (1965), Vijayalakshmi (1969) Aiyar and Sreenivasan (1972) and Onajimi, Pond and Krook (1976) show that addition of methionine or sulphur containing amino acid improves the PER values of legumes in general. It has also been pointed out by Subbulakshmi et al (1976) Babu (1976) and Ganesh kumar and Venkataram (1976) that sprouting of legumes has a beneficial effect in improving the protein quality. The results obtained in the present investigation are in tune with the findings in the literature for other pulses.

The fact that sprouting increases the protein quality may be ascribed to differences in the amino acid profiles which might further be enhanced by the addition of the first limiting amino acid methionine and it is recommended that further analysis of the amino acid profiles of these sprouted grams may prove beneficial. It is also possible to hypothise that in the raw state, both horse gram and green gram, particularly horse gram may have some antinutritional factors intervening with the utilization of the protein or is it due to the bulk or fibre of these legumes in their raw state which might have played an obstructive role. Again, the carbohydrate of these legumes, specially that of horse gram might have a role to play in its interference with the utilisation of protein. These areas need further exploration.

E Hepatic weight:

The mean hepatic weights of the rats fed the different diets along with the statistical appraisal are given in Table VI. The individual values of the same are presented in Annexure V.

TABLE VI

MEAN HEPATIC WEIGHTS OF LIVER SAMPLES OF RATS FED DIFFERENT DIETS

Diets	Sources of protein	Mean Hepatic Weight in g.
I	Raw Horse gram + SM	3.27 ± 0.682
II	Sprouted Horse gram + SM	3.39 ± 0.362
III	Raw green gram + SM	3.20 ± 0.436
IV	Sprouted Green gram + SM	2.43 ± 0.472
V	Raw Horse gram + SM + 0.5% meth.	3.36 ± 0.283
VI	Raw Horse gram + SM + 0.5% meth. + 0.5% cys.	3.67 ± 0.698
VII	Sprouted Horse gram + SM + 0.5% meth.	5.17 ± 0.691
VIII	Sprouted Horse gram + SM + 0.5% meth. + 0.5%cys.	4.73 ± 0.362
IX	Raw Green gram + SM + 0.5% meth	4.25 ± 0.468
X	Raw Green gram + SM + 0.5% meth. + 0.5% cys.	3.93 ± 0.522
XI	Sprouted Green gram + SM + 0.5% meth.	5.16 ± 0.382
XII	Sprouted Green gram + SM + 0.5% meth. + 0.5% cys.	3.86 ± 0.312

Summary of statistical analysis:

Diet	VII	XI	VIII	IX	X	XII	VI	IV	II	V	I	III
Hepatic weights (g)	5.17	5.16	4.73	4.25	3.93	3.86	3.67	3.43	3.39	3.36	3.27	3.20

Any two values not underscored are significantly different.

Maximum liver weight of 5.17g was recorded by rats fed Diet VII, rats fed Diet XI ranked next (5.16 g), Rats fed Diets VIII and IX followed with a mean hepatic weight of 4.73g and 4.25g. respectively. All the other diets ranked in the descending order as follows: 3.93g, 3.86g, 3.67g, 3.43g, 3.39g, 3.36g, 3.27g and 3.20g respectively for Diets X, XII, VI, IV, II, V, I and III. Rats fed Diet VII containing sprouted horse gram + skim milk + methionine had registered significant higher weights which were comparable to that of sprouted horse gram + skim milk + methionine + cystine (Diet VIII) but significantly superior to that of all other diets. In this criteria again, the diets Diet VII, XI, VIII and IX which had been ranking in the foregfront for all other criteria seem to be almost having potency in promoting hepatic weight which are significantly higher than that of rats fed all other diets in this experiment. This again speaks well of the effect of sprouting and addition of methionine in augmenting protein sources.

F <u>Total hepatic nitrogen and nitrogen per gram of the liver sample:</u>

The mean hepatic nitrogen and nitrogen per gram of the liver sample are given in Table VII and the corresponding individual values given in Annexure V.

TABLE VII

MEAN HEPATIC NITROGEN AND NITROGEN/GRAM OF LIVER SAMPLE OF RATS FED DIFFERENT DIETS

Diet	Sources of Protein	Total hepatic nitrogen (mg)	Nitrogen/g. of liver in mg.
I	Raw Horse gram + SM	75.64 ± 4.340	20.79 ± 0.513
II	Sprouted Horse gram + SM	83.24 ± 15.530	22.13 ± 0.375
III	Raw Green gram + SM	83.47 ± 7.027	23.34 ± 0.628
IV	Sprouted Green gram + SM	94.16 ± 6.297	24.00 ± 0.911
V	Raw Horse gram + SM + 0.5% meth.	84.51 ± 20.130	22.68 ± 0.911
VI	Raw Horse gram + SM + 0.5% meth. + 0.5% cys.	97.69 ± 1.609	24.00 ± 0.566
VII	Sprouted Horse gram + SM + 0.5% meth.	157.73 ± 16.300	27.48 ± 0.563
VIII	Sprouted Horse gram + SM + 0.5% meth. + 0.5% cys.	115.04 ± 18.830	21.87 ± 0.382
IX	Raw Green gram + SM + 0.5% meth	613.36 ± 10.860	23.94 ± 0.827
X	Raw Green gram + SM + 0.5% meth. + 0.5% cys.	103.20 ± 8.946	22.63 ± 1.009
XI	Sprouted Green gram + SM + 0.5% meth	181.69 ± 10.590	31.75 ± 0.756
XII	Sprouted Green gram + SM + 0.5% meth. + 0.5% cys.	106.16 ± 16.730	24.81 ± 0.512

Summary of statistical analysis.

Diet	XI	VII	XII	IV	VI	IX	III	V	X	II	VIII	I
Nitrogen/g of liver	31.75	27.48	24.81	24.00	24.00	23.94	23.34	22.68	22.63	22.13	21.87	20.79

Any two values not underscored are significantly different.

The highest hepatic nitrogen was obtained by rats fed Diet XI (181.69mg) and next ranked those fed Diet VII (157.73mg). Next came in the order, Diet VIII (115.04 mg) and Diet IX (115.36 mg). Other diets as far as the total nitrogen is concerned may be ranked as Diets XII, X, VI, V, III, II and I (with mean hepatic nitrogen contents of 106.16mg, 103.20 mg, 97.69mg, 94.16mg, 84.51mg, 83.47mg, 83.24mg, and 75.64mg respectively.

When nitrogen per gram of the liver sample was considered Diet XI (31.75mg) ranked to be the best with significantly higher retention over that of rats fed all other experimental diets. Next ranked Diet VII (with a hepatic nitrogen per gram of liver sample of 27.48 mg.) There was no significant difference between the Diets I, II, IV, VI and IX when nitrogen per gram of the liver sample was considered as also those between rats fed Diets IV, VI, IX, III, V and X and Diets V, X, II and VIII. Diet I however was again the least efficient in promoting nitrogen retention of the liver as well.

Sprouted horse gram and green gram supplemented with skim milk and methionine ranked significantly superior to the other diets in improving the total hepatic nitrogen and this higher hepatic nitrogen is partly due to the higher hepatic weights of the animals fed the same diets. Devadas et al (1970, 1972). Chandrasekhar et al (1975, 1976) have reported

that the liver weights were proportional to the body weights. The results of this study also indicate the same trend on hepatic weight gain on comparison with body weight gains. Addis et al (1940) Henry et al (1958; 1961) and Kawatra et al (1970) have observed linear relationship between hepatic nitrogen and protein quality. The higher the nitrogen retention in the liver, better is the protein quality of the diet. It may be that rats fed sprouted horse gram and green gram when supplemented with methionine which had registered better total hepatic weight and nitrogen as well when compared to the values for all other diets is because this sprouting and supplementation have improved the amino acid pattern of these diets with consequent quality improvement.

6 Apparent digestibility:

The mean Apparent digestibility co-efficients of the 12 experimental diets along with the statistical appraisal are presented in Table VIII and the details of the same given in Annexure VI.

TABLE VIII

MEAN APPARENT DIGESTIBILITY OF THE TWELVE EXPERIMENTAL DIETS

Diet	Sources of Protein	Apparent digestibility
I	Raw Horse gram + SM	60 ± 1.105
II	Sprouted Horse gram + SM	65 ± 0.984
III	Raw Green gram + SM	75 ± 0.290
IV	Sprouted Green gram + SM	78 ± 0.424
V	Raw Horse gram + SM + 0.5% meth.	70 ± 0.000
VI	Raw Horse gram + SM + 0.5% meth. 0.5% cys.	76 ± 0.502
VII	Sprouted Horse gram + SM + 0.5% meth.	81. ± 1.109
VIII	Sprouted Horse gram + SM + 0.5% meth.+0.5% cys.	77 ± 0.771
IX	Raw Green gram + SM + 0.5% meth. + 0.5% cys.	69 ± 0.751
X	Raw Green gram + SM + 0.5% meth. + 0.5% cys.	74 ± 0.580
XI	Sprouted Green gram + SM + 0.5% meth	84 ± 0.534
XII	Sprouted Green gram + SM 0.5% meth + 0.5% cys.	79 ± 0.106

Summary of statistical analysis.

Diet	XI	VII	XII	IV	VIII	VI	III	X	V	IX	II	I
Apparent Digestibility	84	81	79	78	77	76	75	74	70	69	65	60

Any two values not underscored are significantly different.

The values for Apparent digestibility of the diets ranged from 60 to 84 with diet XI (sprouted green gram + skim milk + methionine) having the highest value and Diet I (raw horse gram + skim milk) having the lowest value. The apparent digestibility co-efficient of Diet VII (Sprouted horse gram + Skim milk + methionine) was 81 while those of diets XII and IV were 79 and 78 respectively. Statistical analysis indicated that the Apparent digestibility of the Diets XII and IV, Diets VIII and III, Diets VI and X and Diets X and V were not significantly different while the values for all other diets were significantly different among themselves.

Pulses, in general, in the raw form have been reported to have lower digestibility and Ray (1969, 1970) has specifically pointed out the very low digestibility of horse gram in its raw and autoclaved forms. Burr (1975) points out that their digestibility is somewhat limited since not all their nitrogen is absorbed from the intestine into the blood stream in the raw form. The digestibility values for many of the pulses are rather low in comparison with those for whole wheat (91) and rice (98). These results suggest that a considerable portion of pulse protein is not hydrolysed in the digestive tract into amino acids for absorption into the blood stream. In the present investigation also, raw horse gram + skim milk has been found to have the lowest Apparent

digestibility and that sprouting seems to have improved slightly the values in both horse gram and green gram. As pointed out by Liener (1962), Aykroyd and Doughty (1964), Kakade, Simm and Liener (1970), Maner and Pond (1971) Owusu and Donfeh (1972) Onajemi, Pond and Krook (1976) sprouting has a beneficial effect in improving the Apparent digestibility of pulses and the findings of this study further substantiate the same. Woods et al (1943); Russell et al, (1946); Lehre , (1947); Richardson, (1948); Jaffe, (1949); Bressani et al, (1963); Kakade and Evans, (1965) and Ray, (1969) have emphasized that supplementation with methionine improves protein quality of legumes. The present study also evidences improvements in the Apparent Digestibility values and protein quality when both raw and sprouted grams were supplemented with methionine which are obvious. In Diet XI where sprouted green gram + skim milk + methionine and Diet VII (sprouted horse gram + skim milk + methionine) Apparent digestibility values of 84 and 81 were obtained. These values are similar to those reported for vegetable protein mixtures by several workers, where in a cereal, pulse and oil seed combination has given the Apparent digestibility values of the range 77 to 82 (Chandrasekhar, 1972 and Devadas et al, 1973) This prones one to speculate that sprouting of both horse gram and green gram and supplementation with methionine or other food protein

high in methionine would have a complementary effect on pulse proteins and would help evolve better amino acid profiles in the formulation of low cost locally acceptable foods and recipes.

From the foregoing discussion, it is evident that, when all the indices evaluated in this investigation are considered, sprouting and supplementation with methionine in both horse gram and green gram have a significantly beneficial effect in improving the protein quality of these pulse proteins. This combination of sprouting and methionine supplementation promoted higher weight gains, better nitrogen retention in the liver, registered higher PER values and better Apparent digestibility co-efficients. Raw horse gram + skim milk seem to be the least desirable in its protein quality and even after sprouting the protein quality judged from all parameters does not seem to be enhanced. In spite of having 5 per cent protein from skim milk both in the raw and sprouted forms, horsegram does not seem to have improved in its protein quality and this raises the question of the high fibre content of this pulse and its consequent interferences with digestibility and absorption of protein and also the possibility of the presence of anti-nutritional principles coupled with the possible varieties of carbohydrates in the pulse. These areas provide an interesting opportunity for further investigation.

As far as the green gram is concerned, the raw form, in spite of 5 percent skim milk, does not seem to have any beneficial outcome on protein quality. Sprouting, however though have not had any significant impact on growth promotion and nitrogen retention seems to have improved the digestibility considerably. In general, raw or sprouted green gram supplemented with skim milk and methionine seem to have better potentials when compared to horse gram. Supplementation with cystine does not seem to further enhance the protein quality over and above that of methionine supplementation alone. These results lead one to conclude that sprouting of both horse gram and green gram and supplementation with methionine at 0.5 per cent has scope of improving the protein quality. Further research to strengthen the information on the possible obstacles of protein quality in these legumes in recommended to augment information on processing techniques of such commonly consumed legumes and popularisation of such processing for the better feeding of human population.

V SUMMARY AND CONCLUSION

The aim of this investigation was to evaluate the protein quality of horse gram and green gram in the raw and sprouted forms and by supplementing with sulphur containing amino acids on albino rats. In order to arrive at the time needed for sprouting, horse gram and green gram were sprouted at 12 hourly intervals for 60 hours and the sprouted samples were analysed for protein, calcium, iron phosphorus and ascorbic acid contents. A sprout length of 2 to 2.5 cm at 30 to 36 hour interval was found to be the most acceptable edible length and hence was chosen for protein qulaity evaluation.

Twelve diets were planned at 10 per cent protein level using the 2 legumes both in the raw and sprouted forms. The diets were so formulated that 5 per cent of the protein came from the legume and the other 5 per cent from skim milk. Thus while raw and sprouted horse gram and green gram, along with skim milk as the protein component constituted 4 diets, supplementation with 0.5 per cent methionine and with 0.5 per cent methionine and with 0.5 per cent cystine to both the raw and sprouted horse gram and green gram respectively constituted the other 8 diets. Thus in all 12 diets as given below were formulated:-

Diet I - Raw Horse gram plus skim milk
Diet II - Sprouted Horse gram plus skim milk.

Diet III - Raw Green gram plus skim milk
Diet IV - Sprouted Green gram plus skim milk
Diet V - Raw Horse gram plus skim milk plus 0.5 per cent methionine
Diet VI - Raw Horse gram plus skim milk plus 0.5 per cent methionine plus 0.5 per cent cystine.
Diet VII - Sprouted Horse gram plus skim milk plus 0.5 percent methionine
Diet VIII- Sprouted Horse gram plus skim milk plus 0.5 per cent methionine plus 0.5 per cent cystine
Diet IX - Raw Green gram plus skim milk plus 0.5 per cent methionine
Diet X - Raw Green gram plus skim milk plus 0.5 per cent methionine plus 0.5 per cent cystine
Diet XI - Sprouted Green gram plus skim milk plus 0.5 per cent methionine
Diet XII - Sprouted Green gram plus skim milk plus 0.5 per cent methionine plus 0.5 per cent cystine.

The planned diets were fed to a group of 10 weanling male albino rats each, over a period of 28 days. Weight gain, food and protein intakes, Protein efficienty Ratio, Hepatic weight, Hepatic nitrogen and Apparent digestibility co-efficient were the criteria used for evaluation.

The findings reveal that:

1) There was no difference in the protein, calcium, iron and phosphorus contents of both horse gram and green gram in the sprouted form when compared to that of the raw form. Ascorbic acid content on the other hand increased to an amount of 200 per cent at the end of 60 hours sprouting

2) The mean body weight gains ranged from 37.74g. to 88.49 g. with rats fed Diet VII having the highest

weight gain and those fed Diet I having the least weight gain. The difference in the weight gain of rats fed Diet VII, Diet XI, Diet VIII, and Diet IX; Diet VIII, Diet IX, Diet X, Diet XII, Diet VI, Diet IV, Diet III, and between Diet X, Diet XII, Diet VI, Diet IV, Diet III, Diet V and Diet II were not statistically significant, whereas the weight gain of rats fed all these diets were significantly higher than that of Diet I.

3. The mean food intake of rats fed the different diets ranged from 187.89g (Diet I) to 310.38g (Diet XI) while the protein intake ranged from 18.7g (Diet I) to 31.4 g (Diet XI) The difference in the protein intakes of rats fed Diet XI and VII though not significantly different between each other, were significantly higher than that of all other diets. The protein intake of rats fed the other diets were not significantly different from each other.

4. The highest PER value of 3.09 was registered by Diet VIII which was not significantly different from those values obtained for Diet VII (2.97), Diet IX (2.90) and Diet XI (2.80). The PER values for the other diets in the descending order were 2.70, 2.62, 2.46, 2.41, 2.10, 2.09, 1.93 and 1.68 respectively for Diets X, XII, VI, IV, III, II, V and I. The least PER value was obtained for Diet I (1.68) which was significantly lower than that of all other diets.

5. Significantly higher hepatic weights were registered by rats fed Diet VII (5.17) and Diet XI (5.16g) respectively. Next in the order of hepatic weight came the rats fed Diets VIII and IX having values of 4.73g. and 4.25g. respectively which were not significantly different from each other. The lowest hepatic weight was registered by rats fed Diet III (3.20g). While there was no significant difference between the hepatic weights of rats fed Diets VII, XI and VIII, these were significantly different from those of rats fed all other diets.

6. Total hepatic nitrogen and hepatic nitrogen per gram of the liver sample were higher for animals fed Diet XI (181.69 mg and 31.75 mg respectively) which were significantly higher than those of rats fed all other diets. Rats fed Diet VII with a total hepatic nitrogen content of 157.73 mg. and nitrogen per gram

of the liver sample of 27.48 mg.ranked next and these values were also significantly higher than those of all other diets. Rats fed Diet I, with a nitrogen per gram liver sample, of 29.79mg. ranked last and Diet I was the least efficient in promoting liver nitrogen retention.

7. The highest Apparent digestibility co-efficient was obtained by Diet XI (84) and the lowest Apparent digestibility co-efficient by Diet I (61). The difference in the Apparent digestibility co-efficient of Diet XI (84) was significantly higher than that of all diets with Diet VII (82) coming next, which was also significantly higher than those of all other diets. There was no significant difference between the Digestibility co-efficients of Diets XII and IV; Diets VIII and III, Diets VI and X and Diets X and V while the Apparent digestibility co-efficients of all other diets were significantly different.

The results of this investigation indicate that, judged from the parameters of weight gain, food and protein intakes, Protein Efficiency Ratio, Hepatic weight and nitrogen and Apparent digestibility, Diets VII and XI which had sprouted horse gram and green gram along with skim milk and 0.5 per cent methionine as their protein components had greater potential over that of all other diets. Judged from the indices of this investigation raw horse gram and raw green gram seem to be less effective in growth promotion in spite of skim milk supplementation at 5 per cent level. Sprouting however seems to have improved the Apparent digestibility in both horse gram and green gram, which was considerably improved with green gram. Supplementation with 0.5 per cent methionine in the raw form for horse gram does not seem to be beneficial but in

green gram seems to be tolerable. Supplementation of sprouted horse gram and green gram with 0.5 per cent methionine seems to be the most beneficial, while a further supplementation with 0.5 per cent cystine does not seem to improve the protein quality any further.

These observations suggest that sprouting of grams and supplementation with the first limiting amino acid, methionine improves the quality of these legumes and raise it on par with that of skim milk in its PER values and similar to these of vegetable protein mixtures in their Apparent digestibility. This observation leads us to have a further probe into the potentials of sprouted grams, commonly used for human consumption and opens up new possibilities of utilising such simple home-scale processing techniques in enhancing the nutritive value of common man's food. Further, this also offers scope for complementing methionine rich foods available at low cost to such processed legumes, thereby giving more scope for variety at no additional cost, but enhancing the nutritive value of the common dietaries. These areas need further investigation.

LITERATURE CITED

Adams, M.W. (1974) — "Need for Rapid Assays of Protein quality", J.Agric, Fd. Chem., Vol.22, No.4, pp.564-565.

Addis, T., Lee, D.D., Low, W. and Poo, L.J. (1940) — "The protein content of the organism and tissues at different levels of protein consumption" Journal of Nutrition. Vol.19, pp. 199-205.

Aiyar, A.S and Sreenivasan, A. (1972) — "Protein rich foods from plant sources Nutritional and technological considerations", Proceedings of the First Asian congress of Nutrition, National Institute of Nutrition, Hyderabad, pp.427-453.

Allison, J.B. and Anderson, J.A (1945) — "The relation between absorbed nitrogen balance and biological value of proteins in adult dogs" Journal of Nutrition, Vol.29, p.413.

Altschul, A.M., Yatsu, L.Y., Ory,R.L. and Engleman, E.M(1966) — "Seed Proteins", Annual Review of Plant Physiology, Vol.17, p.118.

Anon (1973) — PAG statement No.22 on "Upgrading human nutrition through the improvement of food legumes" PAG Bulletin 3 (2) p.1.

Anonymous (1974) — "Western hemisphere nutrition congress IV" Nutrition Today, Vol.9, p.28.

Aykroyd, W.R. and Doughty, J. (1964) — "Legumes in human nutrition", Food and Agriculture Organisation, Rome, pp.3-9, 20-21.

Babu, S. (1976) — "Effect of germination on folic acid content of bengal gram and ragi" Indian Journal of Nutrition and Dietetics, Vol.13, No.5, pp.139-141.

Bannerjee, S. and Banerjee, R. (1950) — "Studies on the biosynthesis of niacin. 1) Biosynthesis of niacin by germinating pulses", Indian Journal of Medical Research, Vol.38, pp.153-160.

Banerjee, S., Guha, A.R. and Ghosh, P.K. (1959) "Folic acid metabolism in germinating seeds" Food Research, Vol.24, p.332.

Beaton and Mc Henry. (1964) "Nutrition-a comprehencive treatise", Vol.I, Academic Press, New York, pp.129-137.

Beevers, L. (1968) "Protein degradation and proteolytic in the cotyledons of germinating peaseeds Phyto chemistry, Vol.7, p.1837.

Beghin, I. (1976) "Improving nutrition at the local level", Assignment children, No.35, July-September, pp.9-13.

Behar, M. (1976) "Nutrition and the future of mankind" WHO chronicle, Vol.30, No.4, pp.140-143.

Bender, A.E. and Doell, B.H. (1957) "Biological evaluation of proteins - a new aspect" British Journal of Nutrition, Vol.11, pp.140-148.

Bengoa, J.M. (1974) "The problem of malnutrition" WHO chronicle Vol.28, No.1, pp.3-7.

Bengoa, J.M. (1974) "Hunger and malnutrition", World Health, Feb-March, pp.4-7.

Berg, A. (1973) "The Nutrition Factor-its role in role in national development", The Brookings Institution, Washington.D.C, pp.50-51.

Betschart, A.A. (1975) "Factors influencing the extractability of safflower proteins", Journal of Food Science, Vol.40, No.5, pp.1010-1011.

Brandt, W. (1975) "A new deal for deprived children". Assignment children, No.30, April-June, pp.3-13.

Borlaug, N. (1973) "In: Nutritional improvement of food legumes by breeding PAG/UN. Proceedings of a Symposuim organised by PAG at Rome, 1972 and PAG statement No:22 on Upgrading human nutrition through improvement of food legumes" Ed: Max Milner. PAG, New York, p.8

Bhagvat, K. and Rao, K.K.P.N. (1942) "Vitamin C in germinating grains", Indian Journal of Medical Research, Vol.30, pp.493-504.

Block, R.J. and Mitchell H.H. (1946) "The correlation of amino acid composition, of proteins with their nutritive value, Nutrition, Abstracts Review, Vol.16, No.2, pp.249-278.

Bressani, R. Valiento, A.T. and Tejada, C.D. (1962) "All vegetable protein nutrin for human feeding VI, The value of combinations of line treeated corn and cooked black beans" J. Fd.Sci. Vol.27, p.394.

Bressani, R. Elias, L.G. and Valiente, A.T. (1963) "Effect of cooking and of amino acid supplementation on the nutritive value of black beans", British Journal of Nutrition, Vol.17, pp.69-77.

Bressani, R. and Elias, L.G. (1968) "Processed vegetable protein mixtures for human consumption in developing countries" Advances in Food Research, Vol.16, pp.76-103.

Bressani, R. (1973) "Legumes in human diets and how they might be improved" In: Nutritional improvement of fo food legumes by breeding, PAG Statement No.22, Upgrading human nutrition through improvement of legumes, PAG, New York, pp.15-40.

Burkholder, P.R. (1943) "Vitamins in dehydrated seeds and sprouts" Science, Vol.97, p.562.

Burr, H.K. (1975) "Pulse proteins" In: Protein Nutritional quality of foods and feeds. Vol.1, Part 2, Ed: Friedman, M., Marcel Dekkar Inc., N.Y., pp.119-125.

Campbell, H. and Kesterlitz, H.W. (1948) "The assay of the nutritive value of a protein by its effect of lowering cytoplasm" Journal of Physiology, Vol.107, p.563.

Campbell, J.A. (1961) "Methodology of protein evaluation", Nutrition document. H. 10/Add.27, WHO/FAO/UNICEF/PAG, New York, pp.41-50.

Campbell, J.A and McLaughlan, J.M. (1970) "What are the problems in evaluating protein quality" In: Proteins in human nutrition, Academic Press, New York, pp.15-22.

Chandrasekhar, U. Ammini C.I. and Devadas R.P. (1975) "Evaluation of the protein quality of CSM, Balahar, and LPC on albino rats" Indian Journal of Nutrition and Dietetics, Vol.12, p.1.

Chandrasekar, U. Nandini, S. and Devadas, R.P. (1976) "Protein quality and acceptablity of CARE'S Kerala Indigenous Food", Indian Journalaof Nutrition and Dietetics, Vol.13, p.1.

Chandrasekar, U. and Manjana, K. (1976) "Evaluation of protein quality of mixtures based on sunflower meal through rat assays- I Growth studies", Indian Journal of Nutrition and Dietetics, Vol.13, pp.135-138.

Chayen (1953) "Ascorbic acid and its intracellular location with special reference to plants, The occurrence and function of vitamin C during germination and growth of seedlings, International Review of Cytology, Vol.2, p.118.

Carpenter, K.J. Ellinger, G.M. Henex, M.I. and Delfe, B.J. (1957) "Fish products as protein supplements to cereals", British Journal of Nutrition, British Journal of Nutrition, Vol.11, p.162.

CFTRI (1970) "Annual Report", CFTRI, Mysore, p.45.

Chattopadhyay, H. and Banerjee, S. (1953) "Effect of germination on the biological value of proteins and the trypsin inhibitor of common Indian pulses" Indian Journal of Medical Research, Vol.4, pp.185-189.

Clark, L.C. Ryer, M. and Marlin, J.R. (1949) "Egg biological value and replacement values of some cereal proteins" Journal of Nutrition, Vol.38, pp.405-416.

Clark, H.E., Myers, P., Goyal, K. and Rinehart, J. (1966) "Influence of variable quantities of lysine, tryptophan and isoleucine on nitrogen retention of adult human subjects" American Journal of Clinical Nutrition, Vol.18, No.2, pp.102.

Cravioto, J., Pinero, C., Arroyo, M. and Alcalde, E. (1969) Mental performance of school children who suffered malnutrition in early age, Symposia of Swedish Nutrition Foundation-7, Uppsala, p.25.

Cravioto, J., De Licardie, E.R. and Birch, H.G. (1966) Nutrition, growth and neurointegrative development, An experimental and ecological study", Paediatrics, Vol.38, No.2, Suppl. Part II, p.319.

Cravioto, J. and De Lecardie, E.R. (1971) "The Long term of consequences of protein calorie malnutrition" Nutrition Reviews, May, p.XII.

Cravioto, J., De Licardie, E.R. Pinero, C., Lindero, M. Arroyo, M. and Alcalde, E. (1971) "Mental development and malnutrition neuro integrative development and intelligence in school children recovered from malnutrition in infancy, Proceedings of the Nutrition Society of India, No.10, p.218-219.

Crisp, D.E. (1974) "Protein for the new generation" Nutrition and Food Science, Vol.No.36, pp.9-12.

Daniel, V.A., Desai, B.L.M., Venkat Rao, S., Swaminathan, M. and Parpia, H.A.B. (1969) "Mutual and amino acid supplementation of proteins IV. The nutritive value of the protein blends of wheat and bengal gram fortified with limiting amino acids", Journal of Nutrition and Dietetics Vol.6, p.15.

Devadas, R.P. Vijayalakshmi, V. and Girija Bai, R. (1970) "Evaluation of low-cost stock diets for albino rats", Indian Journal of Nutrition and Dietetics, Vol. 7, pp.85-93.

Devadas, R.P. (1972) "Nutrition in Tamil Nadu", Sangam Sangam Publishers, Madras, p.2.

Devadas, R.P. (1973) Final Technical Report of the PL 480 project for the period 1st July, 1970 to 30th September, p.33.

Devadas, R.P. (1977) "Strategies to overcome malnutrition in India" Abstract of the lecture delivered at Sri Avinashilingam Home Science College Coimbatore (on 26th March 1977). under the programme of national lectures of U.G.C. 1976-77.pp. 1-2.

De Oliveira, B. (1973) "Prospectives on the consumption and utilization of legume seeds as food and feed", In: Nutritional improvement of food legumes by breeding, PAG/UN, Proceedings of a Symposium organised by PAG at Rome, 1972 and PAG statement No.22. on "Upgrading human nutrition through improvement of food legumes" Ed: Max Milner, PAG, New York, pp.305-315.

Dhand, H. (1964) "Nutritive value of some processed foods and food mixtures" M.Sc. (Home) Thesis, M.S. University of Baroda.

Droubiscos, N.J. and Bowland, J.P. (1969) "Biological evaluation of rape seed meal, Soya bean meal and casein fed to the weanling and mature rats", British Journal of Nutrition, Vol.23, p.113.

Easwaran, P.P., Gopinath, S. Jamala, S. and Devadas, R.P. (1972) "Evaluation of the protein quality of two selected vegetable protein mixtures using albino rats" Indian Journal of Nutrition and Dietetics, Vol.9, p.387

Elias, L.C., Conde, A, Munz, A. and Bressani, R. (1973) "Effect of germination and maturation on the nutritive value of common beans".

FAO/UN (1964) "Protein - At the heart of the world food problem" FAO, Rome, pp.1-20.

FAO/WHO (1971) "Food fortification and protein calorie malnutrition", Joint FAO/WHO Expert Committee on technical report series No.477, pp.9-29, 61-63.

FAO/WHO (1973) "Energy and protein requirements" Report of a joint FAO/WHO ad hoc expert committee, WHO technical report series No.522, WHO, Geneva, pp.63-67.

Fernell, W.R. and Rosen, G.D. (1956) "Characteristics of growth of the organism and determination of relative nutritive values of intact proteins". British Journal of Nutrition, Vol.10, pp.143-155.

Food and Nutrition Board, National Research Council, (1974) "General policies in regard to improvement of nutritive quality of foods", Ecology of Food and Nutrition, Vol.3, No. 1, pp.17-18

Ford, J.E. (1964) "A microbiological method for assessing the nutritional value of proteins. Further studies on the measure of available amino acids", British Journal of Nutrition, Vol.18, p.449.

Fordham, F.D., Wells, E.E. and Chow, L.H. (1975) "Sprouting of seeds and nutrient composition of seeds and sprouts", Journal of Food Science, Vol.40, No.3, pp.552-556.

Frisch, R.E. (1971) "Malnutrition and mental development- A critical review", Indian Journal of Nutrition and Dietetics, Vol.8, pp.149-151.

Ganeshkumar, G. and Venkataram, L.V. (1976) "Studies on the in vitro digestibility of starch in some legumes before and after germination", Nutrition Reports International, Vol.13, No.1, pp.115-120.

Goodwin, E. (1962) The Biosynthesis of vitamin and related compounds, Academic Press, New York, p.14.

Gopalan, C., Balasubramaniam, S.C., Rama Sastry, B.V. and Visweshwara Rao, K. (1971) Diet atlas of India, National Institute of Nutrition, Hyderabad, p.37.

Gopalan, C., Swaminathan, M.C., Krishnakumari, V.K., Hanumantha Rao, D. and Vijayaraghavan, K. (1973) "Effect of calorie supplementation on growth of undernourished children" American Journal of clinical Nutrition. Vol.26, pp.563-566.

Gopalan, C. (1975) "The terrible ravages of malnutrition" The UNESCO Courier, May, pp.24-27.

Gopalan, C. Rama sastri, B.V. and Balasubramanian, S.C. (1976) "Nutritive value of Indian Foods, National Institute of Nutrition, Hyderabad, pp.62-63.

Goyco J.A. (1956) "A study of the relation between the liver protein regeneration capacity and the hepatic necrogenic activity of yeast proteins", Journal of Nutrition, Vol.58, p. 299.

Graham, G.C. (1971) "Methionine or dietary protein for infants and small children In: Amino acid fortification of protein foods, Ed. Scrimshaw, N.S and Altschul, A.M., I.T Press.

Gritten, E.T., Pomeranz, Y. and Robbins, G.S. (1975) "Protein content and Amino acid composition of developing peas", Journal of Food Science, Vol.40, No.3, pp.584-588

Gyorgy, P. (1964) "Protein rich foods in calorie-protein malnutrition", American Journal of Clinical Nutrition, Vol.14, p.7.

Hammer, W.C.K. (1976) "Food legislation in International Trade-its significance for developing countries", Food and Nutrition, Vol.33, p.115.

Hegsted, D.M. and Chang, Y. (1965) "Protein utilization in growing rats 1. Relative growth index as a bio assay procedure", Journal of Nutrition, Vol.85, p.159.

Hegsted, D.M. (1969) "Protein enriched cereal foods for world needs", American Association of Cereal chemistry Ed. Milner, M.,St.Paul, Minnesota, p.38.

Henry, K.K. Kosterlitz, H.W. and Quensvilla, K.H. (1961) "A method of determining the nutritive value of a protein by its effect on liver proteins", Nutrition, Vol.7, p.51.

Hirwe, R. and Magar, N.G. (1953) "Effect of autoclaving on the nutritive value of pulses", Indian Journal of Medical Research, Vol.41, p.191.

Horn, M.J. and Warren, H.W. (1961) "Availability of amino acids to micro-organisms III Development of hydrolysates of food with synthetic simulated mixtures", Journal of Nutrition, Vol.74, p.226.

Hsu, S.K., Hadley, W.W. and Hymowitz, T. (1973) "Changes in carbohydrate content of germinating soy bean seeds", Crop science, Vol.13, p.407.

Hurt, H.D., Forysthe, R.H and Krieger, C.H. (1975) "Factors which influence the biological evaluation of protein quality by the PER method" In: protein nutritional quality of foods and feeds, Ed: Friedman,M Marcel Dekker Inc, New york, pp.90-105.

Immink, M.D.C. Viteri, F.E and Torun, B. (1975) "Working capacity and productivity in sugar cane cutters" In: 10th International congress of Nutrition, Kyoto.

INCAP (1969) Evaluacion Nutricional de la Poblacian de centro America y panama, Guatemala Instituto de Nutricion de Cen tro America y panama' (INCAP) Oficina de Investigaciones Internacionalaes de los Institutos Nacionales de salud Ministero de salud Publica y Assistencia Social de Guatemala.

Jaffe, W.G. (1983) "Nutritional aspects of common beans and ether legume seeds as animal and human foods", Proceedings of a meeting held in Ribeirao Preto, p.13.

Jansen, G.R. (1973) "Amino acid supplementation of common beans and ether legumes", In: Nutritional aspects of common beans and ether legume seeds as animal and human foods, Ed. Jaffe W.G., pp.217-230.

Jansen, G.R. (1973) "The amino acid fortification of cereals", In: New protein foods, Vol.I, Altschul, A.M. (ed) academic Press, N.Y., pp.39-120.

Juo, P. and Stotzky, G. (1970) "Changes in the protein spectra of bean seed during germination", Canadian Journal of Botany, Vol.48, p.1347.

Kakade, M.L. (1974) "Biochemical basis for the difference in in plant protein utilization", Journal of Agricultural Food Chemistry, Vol.22, No,4, pp.550-554.

Kakade, M.L., Simm, N and Lenier, J.E. (1970) "Nutritional effects induced in rats by feeding natural and trypsin inhibitors", Journal of Nutrition, Vol.100, p.1003.

Kamalanathan, G. Gita Malinakshi and Devadas, R.P. (1970) "Effect of a blend of protein foods on the nutritional status of preschool children in a rural balwadi" Indian Journal of Nutrition and Dietetics. Vol.7, p.288.

Kawatra, B.L, Garcha, J.L and Wagle, D.S. (1970) "Effect of treatment and additions of methionine and lysine on the nutritive value of guar dhal and its supplemental value to whole wheat flour diet", Indian Journal of Nutrition and Dietetics, Vol.7, pp.145-151.

Kelly, J.F. (1973) "Increasing protein quality and quality", In: Nutritional aspects of common beans and other legume seeds as animal and human foods, Ed. Jaffe, W.G., pp.179-184.

Koller, D., Mayer, A.M., Poljakoff-Mayber and Klein, S. (1962) "Seed germination", Annual Review of Plant Physiology, Vol.13, p.437.

Krishnamurthy, K.S. and Rama Rao, G. (1976) "Studies on the protein of green gram", Indian Food Packer, Vol.30, No.1, pp.28-32.

Kurien, S., Daniel, V.A Venkat Rao, S., Swaminathan, M. and Parpia, H.A.B. (1969) "Effect of calorie restriction on the supplementary value of protein foods to poor vegetable diets based on rice and ragi", Journal of Nutrition and Dietetics, Vol.6, p.III.

Kylen, A.M. and Mc Ready, R.M. (1975) "Nutrients in seeds and sprouts of alfalfa, lentils, mung beans and soy beans", Journal of Food Science, Vol.40, No.5, pp.1008-1009.

Labouisse, H.R. (1975) "Improve the quality of life for each family", Assignment children, No.30, April-June, pp.14-21.

Lachance, P.A. (1973) "The alternatives in the complimentation and nutification of legumes", In: Nutritional improvement of food legumes by breeding, PAG/UN. Proceedings of a symposium organised by PAG at Rome, 1972 and PAG statement No.22 on "Upgrading human nutrition through improvement of food legumes", pp.114-117.

Lachance, P.A. (1974) "New sources of proteins", In: Encylopaedia of Food Technology Ed: Johnson and Peterson, Avi Publishing co, Westport, Connecticut, pp.721-726.

Liener, J.E. Yadav, N.K. and Brown, C. (1977) League for international Food Education-Newsletter. January, p.1.

Lohrer, W.P. Woods, E and Beeson, W.M. (1947) "The value of meat and peas alone or in combination as a source of proteins for growth", Journal of Nutrition, Vol.33, pp. 469-479.

Longnecker, J.B. and Hause, N.L. (1961) "Relationship between plasma amino acid composition of ingested protein. II A shortened procedure to determine plasma amino acid (PAA) ratios", American Journal of Clinical Nutrition, Vol.9, p.356.

Liener, J.E. (1962) "Toxic factors in edible legumes and their elimination", American Journal of Clinical Nutrition, Vol.11, p.28.

Mahler, H. (1974) "Better food for a healthier world", World Health, Feb.-March, p.3.

Mahler, H. (1976) "The challenge of health care-Fresh approaches", Assignment children, No.33, Jan_March, pp.9-16.

Maner, J.H. and Pond, W.G. (1971) "Effects of processing and methionine supplementation on black eyed peas by rats" Journal of Animal Science, Vol.33, p.233

Mecanse, R.A. (1971) "The effect of malnutrition on growth, metabolism and final form", Proceedings of the Nutrition Society of India, No.14, pp.123-128.

Mc Dowall, W.R. (1973) "Petroleum may help solve world protein crisis", Food Industries Journal, Vol.5, No.9, pp.15-16.

Mc Namara, R.S. (1973) "The environmental dilemma", Span, Vol.14, p.30.

Miller, D.S. and Bender, A.E. (1955) "Determination of NPU by a shortened method", British Journal of Nutrition, Vol.9, p.382.

Milner, M. (1974) "Need for improved plant proteins in World Nutrition", Journal of Agricultural Food Chemistry, Vol.22, No.4, pp.550-551.

Mitchell, H.H. and Block, R.J. (1946) "Some relationships between the amino acid contents of protein and their nutritive values for the rat" Journal of Biological Chemistry, Vol.169, p.599.

Mitchell, H.H., Hamilton, T.S., Steggerda, F.R. and Bean, H.W. (1945) "The chemical composition of the adult human body and its bearing on the biochemistry of growth", Journal of Biological chemistry, Vol.158, pp.625-637.

Molina, M.R., Delafuente, G. and Bressani, R. (1975) "Interrelationships between storage soaking time, cooking time, nutritive value and other characteristics of the black bean", Journal of Food Science, Vol.40, No.3, pp.587-591.

Monoekeberg, F. (1957) "Effect of early marasmic malnutrition on subsequent physical and psychological development", In: Malnutrition and Learning Behaviour, Ed. Scrimshaw, N.S. and Gordon, J.E.M.I.T. Press pp.269-278.

Munro, H.N. and Allison, J.D (1964) "Mammalian protein metabolism", Vol.II, Academic Press, New York, pp.95-135.

Muramatsu, K. and Ashida, K. (1962) "Relationship between the nutritive value of the dietary protein and liver Xanthine oxidase activity in young rats", Journal of Agricultural Biological Chemistry, Vol.26, p.25.

Muramatsu, K. and Ashida, K. (1963) "Influence of varying levels of different dietary proteins on growth rate, liver xanthine oxidase and succinic dehydrogenase of young rats", Journal of Nutrition, Vol.79, p.365.

Marlin, J., Szymanski, T.A. and Nasset, E.C. (1948) "Creatinine nitrogen percentage as a choke on the biologic value of proteins", Journal of Nutrition, Vol.30, pp.171-175.

Nagarajan, V. (1977) "Food, agriculture and nutrition situation situation in India", Nutrition, Vol.11, p.5.

Narayana Rao, M., Joseph, K., Swaminathan, M. and Subramaniyan, V. (1960) "Development of precooked balanced proteins foods for weaned infants", Proceedings of the Symposuim on Proteins held in Mysore, August, pp. 1-4.

Nath, M.C and Belkhode, M.L. (1958) "Synthesis of glucose cyclo aceto acetate and biosynthesis of ascorbic acid in germinating mung beans" Proceedings of the Society of Experimental Biological Medicine, Vol.99, p.544.

Nayar, T.K.G. (1977) "National nutrition programme" Foods and Drinks, Vol.1, No.9, p.1.

NIN (1971) "Analysis of foodstuffs - calcium, protein iron and phosphorus," A manual of laboratory techniques, National Institute of Nutrition. (ICMR) Hyderabad, pp.3-12.

Miyogi, S.P., Narayanan, N. and Desai, B.G. (1951) "Studies on the nutritive value of Indian vegetable foodstuff, I Nutritive value of pigeon pea and field pea", Indian Journal of Medical Research, Vol.18, p.1217.

NRC (1968) "Evaluation of protein quality", NRC Committee on protein Malnutrition, NRC Publication No.100, NAS, Washington D.C., pp.25-40.

Nutrition and Food Science (1974) "Is there a protein problem", No.34, pp.22-23.

Orten, A.U. and Orten, J.M. (1943) "The role of dietary proteins on haemoglobin formation", Journal of Nutrition, Vol.38, pp.405-416.

Orten, A.U. and Orten, J.M. (1957) The effect of nitrogen mustards on the incorporation of amino acids into proteins by staphylococcus aureus, Biochemical Journal, Vol.76, p.37.

Onajemi, O., Pond, W.G. and Krook, L. (1976) "Effects of processing an the nutritive value of cowpeas for the growing rat" Nutrition Reports International, Vol.13, No.3, pp.299-306.

Oser, B.L. (1965) "Hawk's Physiological Chemistry, 14th edition, Mc Graw Hill Book Co.,New York, Pp.663-665.

Owusu-Domfeh, K. (1972) "Trypsin inhibitor, activity of cowpea and Babbara beans," Ghana Journal of Agricultural Science, Vol.5, pp.99.

Pai, K.K. (1974) "Nutritional deficiencies-some remedial measures", Proceedings of the Nutrition Society of India, No.17, p.86.

PAG (1975) "PAG Guideline on protein methods for cereal breeders as related to human nutritional requirements", PAG Bulletin, Vol.5, No.2, pp.22-44.

PAG (1975) "News candian global legume survey", PAG Bulletin, Vol.5, No.2, p.21.

Parpia, H.A.B (1971) "Increased production and utilization of legumes for supplementation of human diets", Report of the International conference, M.I.T Boston, Massachusettes, p.103.

Patwardhan, V.N (1962) "Pulses and beans in human nutrition" American Journal of Clinical Nutrition, Vol.11, p.11.

Pekkaren, M (1975) "World food consumption patterns", In: M.Reaboble J. (ed). Man, Food and Nutrition, CRC Press, Cleveland, Ohio, p.176.

Platt, B.S, Miller, D.S and Payne, R.R (1961) "Recent advances in human nutrition with special reference to clinical methods" Ed: J.P Brock, Churchill Ltd. London, p.350.

Protein Foods Association of India (1975) "Food Habit survey in South India, Vol.I Summary and deductions by operations research group, Baroda.

Rajalakshmi, R (1976) "Preschool child malnutrition" Baroda Journal of Nutrition, Vol.3, No.1, pp.4, 40-48.

Ray P.K (1968) "A comparison of growth of rats fed the raw seeds of Dolichos Biflorus (Horse gram)" Science and culture, Vol.34, No.8, p.350.

Ray, P.K (1969) "Nutritive value of horse gram-Effect of feeding raw and treated seed flour on growth of rats", Indian Journal of Nutrition and Dietetics, Vol.6, No.4, pp.328-330.

Ray, P.K (1970) Nutritive value of horse gram (Dolichos Biflorus)digestibility and net protein utilizat on" Indian Journal of Nutrition and Dietetics, Vol.7, pp.71-73.

Read, M.S. (1968) "Malnutrition and mental retardation" CENTO pp.23.

Richardson, L.R. (1954) "Southern peas and other legume seeds as a source of protein for the growth of rats", Journal of Nutrition, Vol.36, pp.451-462.

Roe, J.H., Mills, M.E., Ostertrig, M.S. and Damron, C.M. (1948) "The determination of diketo L-gudonic acid dehydro-ascorbic acid and L-ascorbic acid on some tissue extract by the 2,4 diketo phenyl hydrazine method" Journal of Biological Chemistry, Vol.174, p.201.

Rose, A.J. and Cossins, E.A. (1971) Pteroyl glutamate derived in Pisum Sativum during germination", Biochemical Journal, Vol125, p.17

Rosen, G.D. and Fernell, W.R. (1956) Microbiological evaluation of protein quality with tetrahymena pyriformis in relatively nutritive foodstuffs," British Journal of Nutrition, Vol.10, p.156.

Rosen field, D. (1973) Food production Development, p.57.

Russell, W.C., Taylor, M.W., Mehrhof, T.G. and Hirsch, R.R. (1946) "The nutritive value of the protein of varieties of legumes and the effect of methionine supplementation", Journal of Nutrition, Vol.32, pp.313-325.

Roberts, L.M. (1972) "Prospects for meeting protein needs from conventional food sources", Proceedings of Western Hemisphere Nutrition Congress III. Futura Publishing Co.Inc., New York, pp.33-36.

Sen Gupta, P.M. (1974) "The Protein and energy needs in India and their available supplies", Every man's Science, Vol.9, No.1, pp.14-24.

Shastri, N.V., Dhamankar, V.S. and Akarte, M.R. (1975) "Biosynthesis of some water soluble vitamins during the germination of Dolichos Lab Lab", Indian Journal of Nutrition and Dietetics, Vol.12, No.8, pp.238-242.

Sirinit, K., Soliman, A.G.M., Van Loo, A.T. and King, K.W. (1965) "Nutritional value of Haitian cereal legume blends", Journal of Nutrition, Vol.86, p.415.

Sreekantaradhya, R., Shettar, B.I., Chandrappa, H.M. and Shivashanker, G. (1975) "Genetic variability in horse gram" Mysore Journal of Agricultural Sciences, Vol.9, No.3, p.361.

Srikantia, S.G. and Yogananda Sastri, C. (1971) "Observations on malnutrition and mental development", Proceedings of the Nutrition Society of India, No.14, pp.216-220.

Srikantia, S.G. (1976) "Nutritional problems of rural people and corrective measures", Swasth Hind, Vol.20, No.2, pp.44-46.

Srinivas, H., Tasker, P.K., Rajalakshmi, D., Narayana Rao, M., Rajagopalan, R. and Swaminathan, M. (1966) "Studies on microatomized protein foods based on blends of low fat groundnut, soya and sesame flours and skim milk fortified with vitamins, calcium Salts and limiting amino acids II Amino acid composition and the nutritive value of the protein", Journal of Nutrition and Dietetics, Vol.3, p.42.

Subbulakshmi, G., Ganesh Kumar, K. and Venkataram, L.V. (1976) "Effect of germination on the carbohydrate, protein, trypsin inhibitor, amylase inhibitor and haem-agglutinin in horse gram and moth bean", Nutrition Reports International, Vol.13, No.1, pp.19-28.

Subramaniyam, V., Rama Rao, G., Kuppusamy, S., Narayana Rao, M., and Swaminathan, M. (1957) "Standardization of conditions for the production of Indian multipurpose food", Food science, Vol.6, pp.76-80.

Sukhatme, P.V. (1973) "The protein problem", Every man's science, Vol.8, No.1, pp.14-28.

Sukhatme, P.V. (1975) "Recent Trends in world food availability and their implications". In: Chavez, A., Bourges, H. and Basta, S. (ed) "Foods, for the Expanding world", Proceedings of the 8th International Congress of Nutrition, Vol.3, No.1, p.30.

Sundaravalli, O.E., Desai, B.L.M. and Shurpalikar, K.S. (1973) "Effect of cooking and germination on the flatus inducing capacity of some legumes", In. Nutritional aspects of common beans and other legume seeds as animal and human foods, Ed. Jaffe, W.G, pp.133-134.

Swaminathan, M. (1972) "Evaluation of protein quality", In. Proceedings of the First Asian Congress of Nutrition Ed. Tulpule, P.G. and Jaya Rao, K.S. National Institute of Nutrition, Hyderabad, pp.392-407.

Swaminathan, M.S. (1973) "Malnutrition in a two-tier world", Every man's Science, Vol.8, No.3, pp.101-105.

Swaminathan, M.S. and Jain, H.K. (1973) "Food legumes in Indian Agriculture" In. Nutritional improvement of food legumes by breeding, PAG/UN. Proceedings of a symposium organised by PAG at Rome, 1972 and PAG statement No.22 on "Upgrading human nutrition through improvement of food legumes" Ed: Max Milner, PAG, New York, pp.69-81.

Swaminathan, M.S. (1974) "Food production technology and achievement of social goals" Proceedings of the Nutrition Society of India, No.17, pp.13-21.

Tixard, J. (1974) "Can the brain catch up" World Health, Feb-March, pp.10-14.

Tracy, W. (1975) "S.O.S. children", The Unesco Courier, May, pp.34-36.

UN (1968) "International action to avert the impending protein crisis", U.N.New York, pp.40-42, 75.

Varner, J.E. (1973) "Seed development and germination in plant" In. Plant Biochemistry, Academic Press, New York, pp.763.

Venkat Rao, S., Narayana Rao, M., Swaminathan, M. and Subramaniyam, V. (1964) "Evaluation of the nutritive value of proteins". Indian Journal of Nutrition and Dietetics, Vol.1, pp.42-56.

Venkatachalam, P.S. Srikantia, S.G. Mehta, M. and Gopalan, C. (1966) "Treatment of nutritional oedema with vegetable protein diets". Indian Journal of Medical Research, Vol.44, pp.539-545.

Vijayalakshmi, D. (1969) Nutrition Reports International, Vol.6, No.6, pp.349-355.

Whitehead, R.G. (1964) "Rapid determination of some plasma amino acids in subclinical Kwashiorkor", Lancet, pp.250-252.

Wokes, F. (1968) "The role of plant foods in solving the World food problem", Plant foods for Human nutrition, Vol.1, No.1, pp.23-30.

Yadav, N.R. and Bharadwaj, V.R. (1971) "Biochemical studies on Indian wild legumes", Indian Journal of Nutrition and Dietetics, Vol.8, No.1, pp.1-4.

A N N E X U R E S

ANNEXURE I

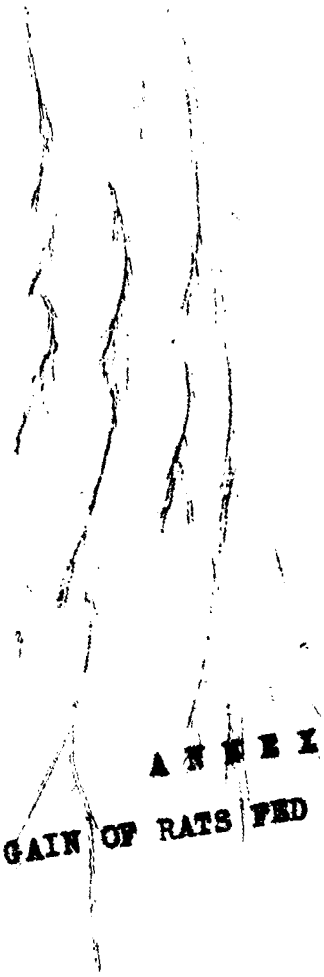
COMPOSITION OF THE MINERAL AND VITAMIN MIXTURES

Composition of the Mineral Mixture (g/kg)

Potassium dihydrogen phosphate	- 389.0
Calcium carbonate	- 391.4
Sodium Chloride	- 139.3
Magnesium sulphate	- 57.3
Ferrous sulphate	- 27.0
Manganous sulphate	- 4.010
Potassium iodide	- 0.790
Zinc sulphate	- 0.549
Copper sulphate	- 0.477
Calcium chloride	- 0.030

Composition of the vitamin mixture (Amt./g of corn starch)

Vitamin A (menadione)	- 0.5 mg
Thiamine	- 0.5 mg
Riboflavin	- 1.0 mg
Pyridoxine	- 0.4 mg
Calcium pantothenate	- 4.0 mg
Niacin	- 4.0 mg
Choline	- 200.0 mg
Inositol	- 25.0 mg
Para amino benzoic acid	- 10.0 mg
Vitamin B_{12}	- 2 mcg
Biotin	- 0.02 mg
Folic acid	- 0.2 mg



A N N E X U R E II

WEIGHT GAIN OF RATS FED DIFFERENT DIETS

WEIGHT GAIN OF RATS FED DIFFERENT DIETS

Group		Rat No. 1	2	3	4	5	6	7	8	9	10
I	Initial weight	28.5	37.5	37.9	43.5	50.8	46.3	44.5	50.4	48.9	55.3
	Final weight	49.2	66.7	71.8	70.3	82.0	88.5	71.2	95.1	75.8	90.4
	Increase	20.7	29.2	33.9	26.8	31.2	42.2	26.7	44.7	26.9	35.1
II	Initial weight	31.5	37.1	38.5	42.3	42.7	46.3	46.4	50.9	51.8	56.2
	Final weight	75.3	75.3	69.3	91.0	84.3	80.5	81.8	99.8	90.6	95.6
	Increase	43.8	38.2	30.8	48.7	41.6	34.2	35.4	48.9	38.8	49.4
III	Initial weight	33.8	37.6	40.2	42.0	45.5	45.5.	49.2	50.4	50.9	51.8
	Final weight	68.3	75.8	81.9	79.7	80.0	97.5	100.6	100.1	99.3	100.1
	Increase	34.5	38.2	41.7	37.7	34.5	52.0	51.4	49.7	48.4	48.3
IV	Initial weight	33.2	38.4	39.6	40.8	43.7	43.9	48.2	46.4	53.1	56.1
	Final weight	74.3	100.8	88.0	78.5	85.5	88.5	103.6	88.3	106.2	111.8
	Increase	41.1	62.4	48.4	37.7	41.8	44.6	55.4	41.9	53.1	55.7
V	Initial weight	32.8	39.7	43.3	45.7	46.0	46.0	44.4	47.3	47.9	55.7
	Final weight	68.5	82.5	90.5	86.3	85.0	82.6	89.8	85.2	96.5	97.2
	Increase	35.7	42.8	47.2	41.3	38.4	36.6	45.4	47.9	48.6	41.5
VI	Initial weight	32.5	40.3	45.0	42.4	42.5	43.3	45.9	47.0	49.2	56.7
	Final weight	78.1	85.3	99.8	90.6	91.2	84.8	92.7	108.0	110.7	128.3
	Increase	45.6	45.0	54.8	48.2	48.7	41.5	46.8	61.0	61.5	71.6
VII	Initial weight	42.1	42.7	42.9	43.4	43.7	45.0	45.7	46.4	47.4	48.4
	Final weight	114.7	89.4	137.1	137.0	147.3	135.2	129.4	131.1	128.7	132.7
	Increase	72.6	96.7	94.2	93.6	103.6	90.2	83.7	84.7	81.3	84.3

Group		Rat No. 1	2	3	4	5	6	7	8	9	10
VIII	Initial weight	34.5	38.4	39.7	43.6	45.4	45.7	49.7	47.6	48.8	54.9
	Final weight	93.6	99.5	120.8	120.4	113.9	121.0	123.7	126.1	121.3	117.2
	Increase	59.1	61.1	81.1	76.8	68.5	75.3	74.0	78.5	72.5	62.4
IX	Initial weight	38.3	38.6	39.8	40.0	45.8	42.0	49.5	46.5	48.1	53.0
	Final weight	106.5	90.0	93.1	125.1	119.2	119.5	106.4	110.0	113.5	127.1
	Increase	68.2	51.4	53.3	85.1	73.4	77.5	56.9	63.5	65.4	74.1
X	Initial weight	41.0	38.7	39.7	40.9	45.7	45.3	46.7	47.4	49.9	50.3
	Final weight	95.6	95.3	91.2	106.8	97.6	104.0	112.6	106.6	130.8	107.3
	Increase	54.6	56.6	51.5	65.9	51.9	58.7	65.9	59.2	80.9	57.0
XI	Initial weight	42.9	42.9	43.0	43.1	43.7	43.9	45.6	45.6	47.6	49.2
	Final weight	134.7	124.2	128.6	133.1	137.9	126.5	134.9	117.3	134.6	145.4
	Increase	91.8	81.3	85.6	90.0	94.2	82.7	89.3	71.7	87.0	96.2
XII	Initial weight	50.7	39.4	39.0	42.0	42.6	44.4	47.0	47.6	43.3	51.7
	Final weight	100.0	94.0	108.9	107.0	98.8	94.4	94.8	98.2	115.7	119.8
	Increase	51.3	56.6	71.9	67.0	58.2	42.0	49.8	52.6	74.4	69.1

SUMMARY OF STATISTICAL ANALYSIS

Diet	't' value	Diet	't' value	Diet	't' value
I Vs II	2.94**	III Vs VIII	2.75*	VI Vs XI	3.05**
I Vs III	3.68**	III Vs IX	1.85 N.S.	VI Vs XII	0.496 N.S.
I Vs IV	4.74**	III Vs X	1.54 N.S.		
I Vs V	1.20 N.S.	III Vs XI	4.56**	VII Vs VIII	1.56 N.S.
I Vs VI	5.55 **	III Vs XII	1.28 N.S.	VII Vs IX	1.61 N.S.
I Vs VII	5.14**	IV Vs V	0.076 N.S.	VII Vs X	2.38 *
I Vs VIII	3.85**	IV Vs VI	0.056 N.S.	VII Vs X	0.139 N.S.
I Vs IX	2.81*	IV Vs VII	3.53**	VII Vs XII	2.19 *
I Vs X	2.61 *				
I Vs XI	5.63*	IV Vs VIII	2.14*		
I Vs XII	2.22*	IV Vs IX	1.44 N.S.	VIII Vs IX	0.32 N.S.
II Vs III	0.029 N.S.	IV Vs X	1.06 N.S.	VIII Vs X	2.40*
		IV Vs XI	3.80**	VIII Vs XI	1.47 N.S.
II Vs IV	0.073 N.S.	IV Vs XII	0.85 N.S.	VIII Vs XII	0.92 N.S.
II Vs V	0.074 N.S.				
II Vs VI	1.90 N.S.	V Vs VI	1.09 N.S.		
II Vs VII	4.52**	V Vs VII	4.96**	IX Vs X	0.50 N.S.
II Vs VIII	3.08**	V Vs VIII	3.44**	IX Vs XI	1.62 N.S.
II Vs IX	2.14*	V Vs IX	2.26*	IX Vs XII	0.52 N.S.

Contd.

Diet	't'	Diet	't' value	Diet	't' value
II Vs X	1.85 N.S.	V Vs X	2.06 N.S.		
II Vs XI	5.00**	V Vs XI	5.66**	X Vs XI	2.48*
				X Vs XII	0.007 N.S.
II Vs XII	1.52 N.S.	V Vs XII	1.60 N.S.		
III Vs IV	0.45 N.S.	VI Vs VII	2.92**	XI Vs XII	2.25 *
III Vs V	0.026 N.S.	VI Vs VIII	1.58 N.S.		
III Vs VI	0.078 N.S.	VI Vs IX	1.05 N.S.		
III Vs VII	4.06**	VI Vs X	0.63 N.S.		

N.S. Not significant

* - Significant at 5% level
** - Significant at 1% level

A N N E X U R E III

TOTAL FOOD AND PROTEIN INTAKE OF RATS FED DIFFERENT DIETS

TOTAL FOOD INTAKE OF RATS FED DIFFERENT DIETS FOR 28 DAYS

Group		Rat No. 1	2	3	4	5	6	7	8	9	10
I	Protein intake	15.46	15.37	18.51	16.33	19.52	22.95	17.28	23.54	18.82	20.11
	Food intake	154.6	153.7	185.1	163.3	195.2	229.5	172.8	235.4	188.2	201.1
II	Protein intake	18.97	18.99	15.83	21.93	20.38	17.54	17.46	22.90	19.54	21.59
	Food intake	189.7	189.9	158.3	219.3	203.8	175.4	174.6	229.0	195.4	215.9
III	Protein intake	16.43	16.64	20.90	19.17	17.80	24.36	24.15	21.62	24.00	22.53
	Food intake	164.3	166.4	209.0	191.7	178.0	243.6	241.5	216.2	240.0	225.3
IV	Protein intake	17.05	25.76	19.22	17.28	19.64	19.04	20.11	16.42	22.65	22.68
	Food intake	170.05	257.6	192.2	172.8	196.4	190.4	201.1	164.2	226.5	226.8
V	Protein intake	19.42	20.80	22.53	21.22	20.65	20.05	21.71	22.01	23.44	22.67
	Food intake	194.2	208.0	225.3	212.2	206.5	200.5	217.1	220.1	234.4	226.7
VI	Protein intake	19.58	20.02	21.68	20.18	21.52	18.93	20.96	21.65	24.66	23.11
	Food intake	195.8	200.2	216.8	201.8	215.2	189.3	208.6	216.5	246.6	231.1
VII	Protein intake	29.28	29.77	30.76	30.17	29.88	29.69	28.17	29.41	29.25	31.15
	Food intake	292.8	297.7	307.6	301.7	298.8	296.8	281.7	294.1	292.5	311-5

Group		Rat No.									
		1	2	3	4	5	6	7	8	9	10
VIII	Protein intake	20.22	21.34	24.00	25.03	22.49	23.77	23.98	23.80	22.27	23.47
	Food intake	202.2	213.4	240.0	250.3	224.9	237.7	239.8	238.0	222.7	234.7
IX	Protein intake	22.48	20.11	19.90	25.75	24.08	23.59	22.24	23.32	23.62	25.44
	Food intake	224.8	201.1	199.0	257.5	240.8	235.9	224.4	223.2	236.2	254.4
I	Protein intake	22.38	20.63	20.03	23.23	20.35	21.74	24.28	20.61	26.33	23.17
	Food intake	223.8	206.3	200.3	232.3	203.5	217.4	242.8	206.1	263.3	231.7
XI	Protein intake	32.48	30.75	30.27	31.04	30.40	30.02	31.69	30.26	31.15	32.32
	Food intake	324.8	307.5	302.7	310.4	304.0	300.2	316.9	302.6	311.5	323.2
XII	Protein intake	21.77	20.51	25.67	24.10	22.20	19.40	20.23	20.61	24.05	25.62
	Foodintake	217.7	205.1	256.7	241.0	222.0	194.0	202.3	206.1	240.5	256.2

SUMMARY OF STATISTICAL ANALYSIS

Diet	't' value	Diet	't' value	Diet	't' value
I Vs II	0.21 N.S.	III Vs IX	0.63 N.S.	VI Vs XI	5.36 **
I Vs III	0.49 N.S.	III Vs X	0.43 N.S.	VI Vs XII	0.44 N.S.
I Vs IV	0.31 N.S.	III Vo XI	3.38 **	VII Vs VIII	15.3 **
I Vs V	0.90 N.S.	III VsXII	0.45 N.S.	VII Vs IX	3.26 **
I Vs VI	0.76 N.S.	IV Vs V	0.43 N.S.	VII Vs X	3.21 **
I Vs VII	3.93 **	IV Vs VI	0.38 N.S.	VII Vs XI	1.23 N.S.
I Vs VIII	1.41 N.S.	IV Vs VII	3.41**	VII Vs XII	3.23**
I Vs IX	1.23 N.S.	IV Vs VIII	0.94 N.S.	VIII Vs IX	0.05 N.S.
I Vs X	1.05 N.S	IV Vs IX	0.93 N.S.	VIII Vs X	0.24 N.S.
I Vs XI	4.34 **	IV Vs X	0.68 N.S.	VIII Vs XI	5.60**
I Vs XII	1.04 N.S.	IV Vs XI	3.80**		
II Vs III	0.34 N.S.	IV Vs XII	0.68 N.S.	VIII Vs XII	0.17 N.S.
II Vs IV	0.13 N.S.	V Vs VI	0.12 N.S.	IX Vs X	0.36 N.S.
II Vs V	0.80 N.S.	V Vs VII	6.10 **	IX Vs XI	3.89**
II Vs VI	0.63 N.S.	V Vs VIII	0.82 N.S.	IX Vs XII	0.19 N.S.
II Vs VII	4.33**	V Vs IX	0.67 N.S.	X Vs XI	4.21 **
II Vs VIII	1.37 N.S.	V Vs X	0.37 N.S.	X Vs XII	0.05 N.S.
II Vs IX	1.21 N.S.	V Vs XI	6.65**	XI Vs XII	3.71 **
II Vs X	0.96 N.S.	V Vs XII	0.39 N.S.		
II Vs XI	5.11**				
II Vs XII	0.96 N.S.	VI Vs VII	4.82**		
III Vs IV	0.19 N.S.	VI Vs VIII	0.80 N.S.		
III Vs V	0.22 N.S.	VI Vs IX	0.69 N.S.		
III Vs VI	0.13 N.S.	VI Vs X	0.42 N.S.		
III Vs VII	2.99 **				
III Vs VIII	0.66 N.S.				

N.S. Not Significant
* - Significant at 5% level
** - Significant at 1% level

A N N E X U R E IV

GAIN IN WEIGHT, PROTEIN INTAKE OF RATS AND PER OF THE DIFFERENT DIETS

GAIN IN WEIGHT, PROTEIN INTAKE AND PER OF RATS FED DIFFERENT RATS

Group		Rat No. 1	2	3	4	5	6	7	8	9	10
I	Weight gain	20.7	29.2	33.9	26.8	31.2	42.2	26.7	44.7	26.9	35.1
	Protein intake	15.46	15.37	18.51	16.33	19.52	22.95	17.28	25.54	18.82	20.11
	PER	1.339	1.900	1.831	1.641	1.599	1.839	1.545	1.899	1.429	1.746
II	Weight gain	45.8	38.2	30.8	48.7	41.6	34.2	35.4	48.9	38.8	49.4
	Protein intake	18.97	18.99	15.83	21.93	20.23	17.54	17.46	22.9	19.54	21.59
	PER	2.508	2.011	1.945	2.221	2.041	1.950	1.971	2.131	1.986	2.299
III	Weight gain	34.5	38.2	41.7	37.7	34.5	52.0	51.4	49.7	48.4	48.3
	Protein intake	16.43	16.64	20.9	19.17	17.80	24.36	24.15	21.62	24.0	22.33
	PER	2.100	2.244	1.995	1.966	1.958	2.134	2.128	2.299	2.017	2.143
IV	Weight gain	41.1	62.4	43.4	37.7	41.8	44.6	55.4	41.9	53.1	55.7
	Protein intake	17.05	25.76	19.22	17.28	19.64	19.04	20.11	16.42	22.65	22.68
	PER	2.411	2.423	2.519	2.179	2.128	2.342	2.755	2.552	2.344	2.457
V	Weight gain	35.7	42.8	47.2	41.3	38.4	36.6	45.4	37.9	48.6	41.5
	Protein intake	19.42	20.80	22.53	21.22	20.65	20.05	21.71	22.01	23.44	22.67
	PER	1.839	2.057	2.094	1.946	1.859	1.825	2.091	1.722	2.073	1.830
VI	Weight gain	45.6	45.0	54.8	48.2	48.7	41.5	46.9	61.0	61.5	71.6
	Protein intake	19.58	20.02	21.68	20.18	21.52	18.83	20.96	21.65	24.66	23.11
	PER	2.329	2.248	2.527	2.389	2.263	2.204	2.243	2.817	2.494	3.098

Group		Rat No. 1	2	3	4	5	6	7	8	9	10
VII	Weight gain	72.6	96.7	94.2	93.6	103.6	90.2	83.7	84.7	81.3	94.3
	Protein intake	29.28	29.77	30.76	30.17	29.89	29.68	28.17	29.41	29.25	31.15
	PER	2.479	3.249	3.063	3.102	3.466	3.039	2.971	2.880	2.780	2.706
VIII	Weight gain	59.1	61.1	81.1	76.8	69.5	75.3	74.0	78.5	72.5	62.4
	Protein intake	20.22	21.34	24.0	23.03	22.49	23.77	23.98	23.80	22.27	23.47
	PER	2.922	2.863	3.302	2.335	3.047	3.168	3.085	3.298	3.255	2.651
IX	Weight gain	68.2	51.4	53.3	85.1	73.4	77.5	56.9	63.5	65.4	74.1
	Protein intake	22.48	20.11	19.9	25.75	24.08	23.59	22.24	22.32	23.62	25.44
	PER	3.035	2.557	2.678	3.305	3.049	3.285	2.558	2.845	2.769	2.914
X	Weight gain	54.6	56.6	51.5	65.9	51.9	58.7	65.9	59.2	80.9	57.0
	Protein intake	22.38	20.63	20.05	23.23	20.35	21.74	24.29	20.61	26.33	23.17
	PER	2.440	2.744	2.571	2.836	2.551	2.700	2.714	2.872	3.072	2.460
XI	Weight gain	91.8	81.3	85.6	90.0	94.2	92.7	89.3	71.7	87.0	96.2
	Protein intake	32.48	30.75	30.27	31.04	30.40	30.02	31.69	30.26	31.15	32.32
	PER	2.926	2.644	2.828	2.898	3.088	2.755	2.818	2.361	2.791	2.977
XII	Weight gain	51.3	56.6	71.9	67.0	58.2	42.0	49.8	52.6	74.4	69.1
	Protein intake	21.77	20.51	25.67	24.1	22.20	19.40	20.23	20.61	24.03	25.62
	PER	2.356	2.759	2.802	2.791	2.621	2.166	2.461	2.552	3.026	2.698

SUMMARY OF STATISTICAL ANALYSIS

Diet	't' Value	Diet	't' value	Diet	't' value	Diet	't' Value
I Vs II	5.25**	III Vs IV	4.51**	V Vs IX	10.39**	X Vs XI	0.72 N.S.
I Vs III	4.38 **	III Vs V	2.79*	V Vs X	10.30**	X Vs XII	0.73 N.S.
I Vs IV	8.69**	III Vs VI	5.65**	V Vs XI	6.37**		
I Vs V	3.38**	III Vs VII	8.94**	V Vs XII	7.71 **		
I Vs VI	6.12**	III Vs VIII	9.87**	VI Vs VII	3.98**	XI Vs XII	1.17 N.S
I Vs VII	11.80**	III Vs IX	8.68**	VI Vs VIII	4.82**		
I Vs VIII	12.70**	III Vs X	8.09**	VI Vs IX	3.52**		
I Vs IX	11.84**	III Vs XI	5.24**	VI Vs X	2.10*		
I Vs X	11.59 **	III Vs XII	5.98**	VI Vs XI	2.14**		
I Vs XI	7.89**	IV Vs V	6.78**	VI Vs XII	1.32 N.S.		
I Vs XII	9.40**	IV Vs VI	0.148 N.S.	VII Vs VIII	1.19 N.S.		
II Vs III	0.17 N.S.	IV Vs VII	1.67 N.S.	VII Vs IX	0.006 N.S.		
II Vs IV	4.46**	IV Vs VIII	2.01 N.S.	VII Vs X	2.52*		
II Vs V	2.49*	IV Vs IX	4.93**	VII Vs XI	1.11 N.S.		
II Vs VI	3.64**	IV Vs X	3.36**	VII Vs XII	2.25*		
II Vs VII	8.68**	IV Vs XI	2.76*	VIII Vs IX	1.55 N.S.		
II Vs VIII	9.69**	IV Vs XII	2.17*	VIII Vs X	2.64*		
II Vs IX	8.57**	V Vs VI	5.22**	VIII Vs XI	1.85 N.S.		
II Vs X	7.93**	V Vs VII	10.50**	VIII Vs XII	3.85 **		
II Vs XI	2.38*	V Vs VIII	11.40**	IX Vs X	1.95 N.S.		
II Vs XII	5.85**			IX Vs XI	0.065 N.S.		
				IX Vs XII	2.40*		

N.S. Not significant
* Significant at 5% level
** significant at 1% level

A N N E X U R E V

TOTAL HEPATIC WEIGHT, TOTAL HEPATIC NITROGEN(MG) AND NITROGEN/GRAM OF LIVER OF RATS FED DIFFERENT DIETS

Diet	Sample No.	Rat No.	Total Hepatic weight in g	Total Hepatic nitrogen in mg	Hepatic nitrogen mg/g
I	1	2,8,9	10.8963	226.6	20.75
	2	3,4,5	10.6696	222.0	20.80
	3	1,6,7,10	11.1650	232.2	20.81
II	4	11,14,20	10.5478	236.2	22.39
	5	15,17,18	11.1877	241.6	21.60
	6	12,13,16,19	11.1189	271.4	22.40
III	7	21,22,24,25	10.7957	259.2	24.00
	8	23,26,27	10.7752	250.0	22.93
	9	28,29,30	10.4314	242.0	23.20
IV	10	31,34,35,36	11.9165	286.0	24.01
	11	32,33,37	11.9855	287.8	24.00
	12	38,39,40	11.3864	273.4	24.01
V	13	41,42,44,48	12.2534	274.4	22.41
	14	43,45,46	10.4915	226.4	21.62
	15	47,49,50	10.8227	259.8	24.00
VI	16	51,54,55,56	12.1974	292.8	24.00
	17	52,57,60	12.3297	295.8	24.00
	18	53,58,59	12.0997	290.6	24.02

Diet	Sample No.	Rat No.	Total Hepatic weight in g	Total Hepatic nitrogen in mg	Hepatic nitrogen mg/g
VII	19	62,66,69,70	18.1609	495.0	27.62
	20	61,63,65	16.7414	468.8	28.00
	21	64,67,68	16.7640	455.8	27.19
VIII	22	72,75,76,80	17.0898	369.0	21.60
	23	73,74,78	15.117	326.4	21.60
	24	71,77,79	15.082	340.0	22.41
IX	25	82,83,85,87	14.7540	354.0	24.00
	26	81,89,90	13.7559	341.2	24.80
	27	84,86,88	14.0079	325.0	23.03
X	28	91,95,96,98	13.9559	312.6	22.40
	29	92,93,99	12.7772	306.8	24.00
	30	94,97,100	12.5634	291.4	21.50
XI	31	101,106,107	16.2886	521.2	32.04
	32	102,103,105,108	19.0131	593.2	31.20
	33	104,109,110	16.2790	520.8	32.00
XII	34	113,117,118,119	14.2970	343.0	24.00
	35	111,112,120	11.9628	306.2	25.63
	36	114,115,116	12.3519	306.2	24.79

SUMMARY OF STATISTICAL ANALYSIS

Diet	't' value	Diet	't' value	Diet	't' value
I Vs II	2.85*	III Vs IV	1.10 N.S.	VI Vs VII	6.21**
I Vs III	4.39**	III Vs V	1.95 N.S.	VI Vs VIII	4.43**
		III Vs VI	1.10		
I Vs IV	4.73**	III Vs VII	6.90 **	VI Vs IX	0.08 N.S.
I Vs V	2.52*	III Vs VIII	2.92*	VI Vs X	1.57 N.S.
I Vs VI	4.73**	III Vs IX	0.92 N.S.	VI Vs XI	11.57 **
I Vs VII	12.14 **	III Vs X	0.91 N.S.	VI Vs XII	1.65 N.S.
I Vs VIII	2.29*	III Vs XI	12.01 **		
I Vs IX	1.64 N.S.	III Vs XII	2.54*	VII Vs VIII	11.68**
I Vs X	2.13*			VII Vs IX	4.98**
I Vs XI	16.61**	IV Vs V	1.76 N.S.	VII Vs X	5.57 *
I Vs XII	7.44**	IV Vs VI	0.00	VII Vs XI	6.37**
II Vs III	6.15**	IV Vs VII	6.21**	VII Vs XII	5.56**
II Vs IV	3.99**	IV Vs VIII	4.43**		
II Vs V	0.79 N.S.	IV Vs IX	0.08 N.S.	VIII Vs IX	3.23**
II Vs VI	3.99**	IV Vs X	1.57 N.S.	VIII Vs X	0.93 N.S.
II Vs VII	11.95**	IV Vs XI	11.57**	VIII Vs XI	16.74**
II Vs VIII	0.68 N.S.	IV Vs XII	1.65 N.S.	VIII Vs XII	6.39**
II Vs IX	2.85*	V Vs VI	1.76 N.S.	IX Vs X	1.35 N.S.
II Vs X	0.43 N.S.	V Vs VII	6.40**	IX Vs XI	9.88**
II Vs XI	16.30**	V Vs VIII	1.27 N.S.	IX Vs XII	1.26 N.S.
II Vs XII	5.82**	V Vs IX	1.47 N.S.	X Vs XI	9.80**
		V Vs X	1.049 N.S.	X Vs XII	2.56*
		V Vs XI	8.13**	XI Vs XII	10.84**
		V Vs XII	2.97*		

N.S. Not significant
* - Significant at 5% level
**- Significant at 1% level

A N N E X U R E VI

DIGESTIBILITY COEFFICIENT OF THE TWELVE EXPERIMENTAL DIETS

Diet	Sample No.	Rat No.	Total faecal weight in g.	Total faecal nitrogen in mg.	Total food nitrogen in mg.	Apparent Digestibility
I	1	1,2,3,9	12,5199	430.6	1114.0	61
	2	4,5,7	11.6257	400.0	1084.0	59
	3	6,8,10	12.1092	416.2	1064.0	61
II	4	11,12,13,14	12.9088	492.0	1400.0	65
	5	15,18,20	13,0116	499.6	1446.0	67
	6.	16,17,19	12.6771	497.0	1390.0	65
	7	21,23,24,26	12.7436	428.0	1804.0	76
III	8	25,27,28	12.7197	427.4	1764.0	76
	9	22,29,30	12.6285	434.4	1779.0	76
	10	32,35,37	14.3254	434.4	2263.0	78
IV	11	31,33,34,36	14.7222	506.2	2396.0	79
	12	38,39,40	13.5878	478.4	1447.0	78
	13	41,42,46,47	15.1266	532.6	1774.0	70
V	14	44,45,50	14.1879	499.4	1650.0	70
	15	43,48,49	14.6967	505.6	1701.0	70
	16	51,52,53,56	16.0745	450.2	1923.0	75
VI	17	54,57,59	15.3819	443.0	1717.0	74
	18	55,58,60	15.6956	451.8	1757.0	74
	19	61,65,69,70	12.1947	524.6	2836.0	81

Diet	Sample No.	Rat No.	Total faecal weighting	Total faecal nitrogen in mg.	Total food nitrogen in mg.	Apparent digestibility
VII	20	62,67,69	14.3934	375.7	2052.0	82
	21	63,64,66	12.7640	397.9	2069.0	81
	22	71,72,78,80	19.7747	676.2	2903.0	77
VIII	23	74,75,79	17.3539	656.8	2770.0	77
	24	73,76,77	17.7250	652.4	2727.0	76
	25	81,82,83,87	16.1716	504.4	1624.0	69
IX	26	84,88,90	16.5675	516.8	1638.0	68
	27	85,86,89	16.4120	498.8	1622.0	69
	28	91,92,94,96	15.4172	481.0	1907.0	75
X	29	93,97,100	15.1619	460.8	1792.0	74
	30	95,98,99	14.6021	455.6	1712.0	73
	31	101,104,107,108	13.2145	374.3	2372.0	84
XI	32	102,106,110	16.7871	354.8	2240.0	84
	33	103,105,109	13.8237	345.8	2246.0	85
	34	111,112,113	14.6907	458.2	2217.0	79
XII	35	115,116,117,118	14.6556	457.6	2243.0	80
	36	114,119,120	14.7742	460.8	2244.0	80

Summary of Statistical Analysis

Diet	't' value	Diet	't' value	Diet	't' Value	Diet	't' value
I vs II	4.31**	III vs VIII	1.26 N.S.	VI vs VII	7.66**	XI Vs XII	12.64**
I vs III	25.66**	III vs IX	12.31**	VI vs VIII	2.92**		
I vs IV	21.42**	III vs X	2.69*	VI vs IX	8.93**		
I vs V	12.05**	III vs XI	13.71**	VI vs X	0.37 N.S.		
I vs VI	16.43**	III vs XII	10.90**	VI vs XI	18.51**		
I vs VII	18.66**			VI vs XII	13.48**		
I vs VIII	16.87**						
I vs IX	8.91**	IV vs V	24.29**				
I vs X	15.52**	IV vs VI	7.95**	VII vs VIII	4.99**		
I vs XI	27.62**	IV vs VII	3.50**	VII vs IX	12.98**		
I vs XII	24.33**	IV vs VIII	2 .82	VII vs X	8.02**		
		IV vs IX	15.41**	VII vs XI	3.45**		

Contd.

Diet	't' value	Diet	't' value	Diet	't' value	Diet	't' value
II Vs III	14.89**	IV Vs X	9.07 **	VII VS XII	2.15*		
II VS IV	17.43**	IV VS XI	12.47**	VII VS IX	10.01**		
II VS V	7.02**	IV VS XII	1.22 N.S.	VII VS X	15.49**		
II VS VI	12.30**	V VS VI	11.82**	VIII VS XI	11.71**		
II VS VII	15.57**	V VS VII	14.19**	VIII VS XII	4.27**		
II VS VIII	13.07**	V VS VII	11.68**				
II VS IX	4.42**	V VS IX	2.07 N.S.				
II VS X	11.26**	V VS X	9.40**	IX VS X	7.98**		
II VS XI	27.21**	V VS XI	34.73**	IX VS XI	23.66**		
II VS XII	14.51**	V VS XII	55.64**	IX VS XII	19.63**		
III VS IV	4.56**			X VS XI	19.02**		
III VS V	22.71**			X VS XII	12.66**		
III VS VI	3.09**						
III VS VII	6.48**						

N.S. Not significant

* Significant at 5% level

** Significant at 1% level