EVALUATION OF THE PROTEIN QUALITY OF INFANT FORMULATIONS DEVELOPED BY A COMMERCIAL FIRM ON ALBINO RATS

BY

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I INTRODUCTION

Hunger still stalks the global horizon and the world food problem will remain one of the most complex and intractable problems during the final quarter of this century (Khanna, 1982). Protein-energy malnutrition, according to Francis (1980) is the commonest nutritional disorder in the Third-world and most frequently occurs in children of weaning age.

Undernutrition is one of the major public health problems of our country (Mathur, 1981). Though clinical forms of protein-energy malnutrition are seen in only 2 to 3 per cent of preschool population, more than 50 percent of the children in this group have body weight for age, less than 75 per cent of well-to-do Indian children.

Rao *et al*., (1980) has reported that malnutrition is a serious social problem in India. Therefore, efforts should be made to irradicate it with the help of low-cost nutrient rich food sources. Green leafy vegetables are the cheapest of all the vegetables within the reach of poor man, being richest in their nutritional value. Murthy (1979) states that in recent years, scientists have been toying with the idea of developing what are called "special protein concentrates". They are trying to extract protein from uncommon, non-traditional sources like, greenleaves, which are not edible otherwise.

Protein-energy malnutrition in children can easily be cured completely by giving them additional foods using common foodstuffs like rice, wheat or jowar, combined with dal and jaggery.

Jelliffee (1976) reported that weaning multimixes, whether domestically prepared or produced commercially at low-cost will be based on the "principles of multimixes", i.e. based on the mutually complementary effect of plant protein mixtures, especially cereals and legumes.

One of the most effective and successful approach to increase the protein food supply, in most of the world, has been to blend legumes and grains to provide the proper amino aicd balance (Altschul, 1978). According to Jeswani (1979), in India several "seed legumes" commonly known as pulses have been traditional supplements to staple cereals. In India, recent interests has centred around proteins from oilseeds, with soyabeans having a special place in the nutritional programmes. Central Food Technological Research Institute (CFTRI) has developed several low-cost nutritious weaning-food supplements based on locally available cereals and legumes to combat malnutrition among infants and children.

Devadas and co-workers (1974) have developed several low-cost locally available vegetable food mixtures and "Kuzhanchai Amudhu" was one such effort and was geared to the economic reach of the rural families. Devadas and co-workers (1977) have also extensively worked on leaf proteins as supplements to the diets of pre-school children. The leaf protein has been found to improve nitrogen retention in infants and on children (Kamalanathan et al., 1969; Devadas, 1978).

Introduction of low-cost commercially manufactured vegetable protein weaning mixtures will further help in irradicating the problem of PEM in developing countries (Aziz, 1980). The commercial manufactures should aim at the usage of low cost protein rich formulations to be incorporated in the weaning infant food mixtures. Modern technological advancement in the field of foods and nutrition has made it possible to manufacture varieties of commercial food mixtures specially designed for infants and pre-schoolers (Gosh and Randhawa, 1981).

On one side, the scientists have evolved several such weaning mixtures, but the commercial firms have not come forward to use their technological know how to popularise these nutritious mixtures. On the other hand, many a commercial firms are today coming out with new food formulations for the infants. There is an urgent need to strike a happy balance in blending both science and technology together and flood the market with nutritious foods within the reach of the common man.

The present study on the evaluation of protein quality of infant formulations developed by a commercial firm was considered to be of worthwhile for information along these lines. It specifically aims at evaluating the protein quality of eight commercially formulated infant weaning mixtures as compared to casein through rat assays.

II REVIEW OF LITERATURE

Literature pertaining to this study on "Evaluation of protein quality of infant formulations developed by a commercial firm" is reviewed under the following heads:

A. Prevalence of protein energy malnutrition in India

B. Strategies to alleviate protein malnutrition

C. Meaning and importance of protein quality

D. Evaluation of protein quality through rat assays

A. Prevalence of Protein Energy Malnutrition in India:

Population explosion is one of the most vitally important cause of protein-energy malnutrition (Singh, 1975). The rapid increase of the worlds population and the consequently the increasing demand on available food sources present a serious problem (Sukhla, 1975, Gour *et al.*, 1977, Das and Banerjee, 1979).

According to the WHO estimates (1981) approximately 125 million babies are born each year. Indeed it is estimated that one-third of the population of the year 2000 is yet to the born. The number of pre-school children (0 to 6 years) is estimated to reach 122 million at the close of this century (Robert *et al.*, 1982).

Possibly as many as 450 million to a billion persons in the world do not receive enough food and most of them live in developing countries. The world population grew by 60 percent from 1950 to 1975 and 80 percent of this growth was in the developing countries (NRC, 1977).

The problem of providing food for future is even more vexing if the runaway of growth in world population is recockened against the lag in food production (Gobber, 1975). Consequently, if nutritional profile of the population is considered the picture is even gloomy.

Protein energy malnutrition is one of the distressing nutritional disorders of childhood in the developing countries (Devadas et al., 1971) and is particularly critical among weaning infants and pre-school children (Mitra, 1972).

In India, the incidence of severe forms of protein-calorie malnutrition (PCM) like 'Kwashiorkor' and "Marasmus" has been estimated to be around 1 to 2 per cent of all children in the age range of 1 to 5 years (Rao, 1971; Gopalan, 1972, Strikantia, 1975; Devadas et al., 1978, Reddy, 1979).

One of the major cause of high infant and young child mortality in our country is calories protein malnutrition (Devadas, 1974). It is estimated that there are about 72 million children in the age group of one to five years (Nagarajan, 1976). Surveys carried out in the different partsof the country indicate that out of seventy five million children in the age group of zero to five years, about fifty per cent suffer from PCM. Forty per cent of all death occur in children below the age of five years (Devadas, 1974, Gopalan, 1976 and Devadas, 1976).

Further according to surveys of protein Advisory Groups (1977) malnutrition is believed to be underlying or associated cause, for a high proportion of deaths of children under five years of age in developing countries. Devadas (1977) states that inadequate intake of calories and proteins, excessive dependence on cereals, starchy foods, low consumption of animal foods and protective foods such as fresh greenleafy vegetables and fruits, wrong methods of food preparation, undesirable customs and taboos are responsible for nutritional dificiencies evidenced among the pre school children.

According to Mathur (1981), undernutrition is one of the major public health problem of our country. Though clinical forms of protein energy malnutrition (PEM) are seen in only 2 to 3 percent of preschool population, more than 50 per cent of the children in this group have body weight for age, less than 75 per cent of well-to-do Indian children. The existence of widespread hunger and malnutrition is perhaps the most obvious and the most disturbing symptom of underdevelopment (PAG, 1977, Devadas et al., 1978, and Boerma, 1977) and is a serious social problem crippling the growing generation (Gopala Rao et al., 1980).

In a clinical survey carried out in Tamilnadu by the Tamil Nadu Nutritional Study (1973). It was revealed that 56.4 per cent had mild protein calorie malnutrition syndrome, while the severe PCM incidence was 0.7 per cent.

According to a report by the United Nation's Children Fund on the "State of the World's children in 1981-82", the lives of another 17 million children born in 1982, are threatened. If adequate care is not taken, 12 million may die before their first birth day and over 5 million before they are five years old (The Hindu, December 19th, 1981).

B. Strategies to Alleviate Protein Malnutrition

The strategies that are being advocated more and more to alleviate protein malnutrition in recent years have been not only the production of more proteins, but also more high quality proteins in foods for children from birth to the age of three or four.

Production and utilization of inexpensive and indigenous protein rich sources in proper combination will help to bridge the protein gap in developing countries like India (NIN, 1972, Sukhatme, 1972, Gupta, 1972 and Gopalan, 1972, 1976).

Several studies have been carried out by various laboratories, nutrition scientists and other agencies to establish strategies to alleviate protein malnutrition with the help of indigenous vegetable protein mixture. Easwaran *et al.*, (1972) evaluated the protein quality of two selected vegetable protein mixtures based on maize, Bengal gram, and groundnut through PER, and nitrogen balance using albinorats.

Devadas *et al.*, (1973) opines that there was highly significant increase in the growth of rats, when low cost balanced foods based on cereals, cotton seed or peanut flour and Bengal gram flour replaced 50 per cent of the rice in the diet. In view of the acute shortage of milk and other protein rich foods of animal origin, there is an urgent need for much larger production of low cost, balanced protein supplements for feeding babies, weaned infants and pre-school children (Swaminathan *et al.*, 1978).

Daniel *et al.*, (1978) reported the effectiveness of a low cost balanced food based on blends of grain or maize, groundnut, Bengal gram, soyabean-flour and sesame flour fortified with limiting amino acids on weaned infants. Gopalan and Srikantia (1975) formulated protein mixtures both with Bengal gram flour, groundnut flour and sesame flour in various proportions as a supplement for Kwashiorkor children.

Food legumes are important and economical sources of proteins and calories as well as certain minerals and vitamins essential to human nutrition. In cassava based diets, legumes provide protein in which the methionine and cystine content are important (Hulse and Rachie, 1977).

Vegetable protein specially legumes offers the most practical means of solving the problem of protein shortage in India for the present (Chandrasekhar and Jayalakshmi, 1978).

Increasing food production and improvement of the quality of food according to Devadas *et al.*, (1978) are long term plan; immediate need must be thus met by improving the existing meal pattern with low cost local food.

Leaf protein is a promising source of food for meeting the growing demands for food proteins in the world (Rao *et al.*, 1972). Judsen and Janson, 1976; Porter *et al.*, 1973), because it is a low cost food and in perennially available (Indian vegetarian congress, 1975, and Kamalanathan *et al.*, 1969). Modern food technology has made possible the production of many high biological value, low cost, protein products from oil seeds up to unicellular proteins (Hidalgo *et al.*, 1972). Jelliffe (1973) reported that vegetable mixtures alone are capable of supplying young children during weaning periods, with diets containing adequate protein, with all essential amino acids and calories. This approach has been advocated at village level as the "principle of multi-mixes" and has been employed in the preparation of low cost transitional foods for young children. Further the author stress his opinion that, weaning multimixes, whether domestically prepared or produced commercially at low cost will be based on the 'principles of multi mixes' i.e. the mutually complementary effect of plant protein mixture, especially cereals and legumes. Mixtures of cowpea with maize were found to give higher protein efficiency ratios than diets containing eighter component alone (Scrimshaw *et al.*, 1976).

Again, feeding trials on weaning foods based on Ragi and green gram have been reported to be beneficial (CFTRI, 1980).

C. Meaning and Importance of Protein Quality

Protein quality is a term used to denote the efficiency with which a protein is utilized for growth and for maintenance. The lower the level at which a dietary protein can support maximum growth or nitrogen balance, the higher in quality that protein is said to be (Altaschul, 1974).

The quality of protein from food depends essentially on its ability to meet the body's requirements for amino acids. Although the chemical composition or amino acid analysis of dietary protein may give an elegant representation of the value of a protein, it is necessary to varify its value by actual biological experiment. Hence, protein-quality has to be measured in the animal or human system directly with respect to growth, tissue repair or maintenance and other biological criteria (Hanson, 1974). The concept of protein quality evaluation has practical application in over coming theproblem of PCM among children (FAO, 1973). The minimal quantity necessary to maintain balance in a normal person varies accordingly to the protein quality. Therefore, the quality of a particular protein has a significant role to play in human nutrition(Hidalgo et al., 1972). A new priority which emphasizes the importance of protein quality and its evaluation is the growing imbalance between world food supplies and population growth (Pellett and Young, 1980). Nearly all the current efforts to produce improved varieties of cerals, (rice, wheat, corn and sorghum) legumes, (common beans, cow peas, grams, pigeon peas, soybeans, and similar edible spices) and root crops (Potatoes and cassave) include evaluation of protein quality (Denish *et al.*, 1980).

The methods available for evaluation of protein quality by rat assays may be divided into the following categories:

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)

NRC(1980) defines PER as the ratio between body weight gain and protein consumed. Protein efficiency ratio represents the simplest methodology (Parihar *et al.*, 1976) and has the advantages of requiring a minimal work in put (Schelling, 1975).

For the determination of the protein quality of a ~~food-stuff~~ the test diets should be formulated at 10 per cent level (NRC, 1963, 1980 and Womack *et al*., 1976). Chapman *et al*., (1959) and America Association of Official Agricultural chemists have described standardised procedures using rats aged 21-28 days with an experimental period of 28 days.

The various factors which affect the PER are:

i. Level of protein (Tasker *et al*., 1960; Swaminathan, 1972)

ii. Age of rat (Chapman *et al*., 1959; Middleton *et al*, 1960).

iii. Variation of food intake (Mitchell and Beadles, 1930, Harte *et al*, 1947, Burt *et al*., 1975).

iv. Length of assay period (Swaminathan, 1937, Chapman *et al*., 1959., Joseph *et al*., 1960, NRC, 1963.

v. Physical environment namely heating and ventilation moisture, odour, dust, light and noise (Porter, 1963).

b. Net Protein Ratio (NPR)

A modification of the PER method, Net Protein Ratio (NPR) was introduced by Bender and Doell (1957). This consists of feeding a group of weanling rats on a diet containing 10 per cent of the test protein and another comparable control group on a non protein diet for a period of 10 days. The NPR is calculated by using the formula:

$$NPR = \frac{\text{Gain in weight(g) of test group + Loss in Weight(g) of non-protein group}}{\text{Protein intake (g)}}$$

c. Gross Protein Value

The gross protein value of a supplementary protein is calculated as the extra growth obtained with supplementary protein and expressed as the percentage of the corresponding figure obtained with casein as test supplement(Rao *et al.*, 1964). The level of protein in the ratio is kept low so that test may be a critical one. A control one. A control group is fed on a diet partially lacking in lysine and based on a cereal providing 8 per cent protein in the diet for a period of 2 weeks while the experimental group receives the same diet supplemented with 3 per cent test protein. The calculated value is then expressed as a percentage of the corresponding figures obtained with casein as the test supplement.

d. Rat Repletion Method

In the rat repletion method developed by Cannon *et al.*, (1934) the nutritive value of different proteins is estimated by studying their relative efficiency to promote weight recovery in protein depleted adult rats. This method have been modified by Venkat Rao *et al.*, (1964) by using protein depleted young rats. In this method young albino rats were depleted of body protein by feeding a protein free synthetic diet for a period of 10 days. The depleted rats were then repleted on the experimental diets containing the different proteins for a period of 10 days, and the increase in weight per gram protein consumed was considered as an index of the nutritive value of protein.

e. Nitrogen Growth Indix

This procedure, developed by Allison *et al.*, (1942) consists of feeding albino rats, diets at different levels of the same protein over a period of 28 days. The nitrogen intakes are plotted against gain in body weight and a curve is thus obtained. Venkat Rao *et al.*, (1964) term the slope of the line relating growth rate to nitrogen intake over a period of 28 days, as the nitrogen growth index.

2. Methods based on Carcass Nitrogen Analysis

a. Nitrogen Retention Method

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

It is actually the percentage of nitrogen, retained in the body and it considers growth as well as nitrogen balance (Shunkers and Mccolum, 1929).

b. Net Protein Utilization

Miller and Bender (1955) developed this method based on the principle of evaluating protein quality by a biological test of nitrogen utilisation. According to this method, one group of rat is fed on non protein diet while the remaining groups are fed proteins at 10 percent level for 10 days. The food intakes of animals are measured. The animals are killed at the end of 10 days and body N determined by Kjeldhal method on a sample of dried and powdered carcass.

NPU = Biological value X Digestibility (NRC, 1963).

3. Methods Based on Nitrogen Balance

a. Nitrogen Balance

The nitrogen balance is simply the relationship between the intake and excretion of nitrogen by an animal (Albanese, 1963). Mitchell (1924) states that the oldest method used to evaluate the nutritive value of dietary protein is to determine the Nitrogen balance i.e. the difference between nitrogen intake and nitrogen excretion. Nitrogen Balance is determined from the equation;

$$B = I - (U + F)$$

where B is the balance, I the Nitrogen intake and U and F, the nitrogen excreted in urine and faeces respectively.

b. Digestibility Co-efficient

A measurement of digestibility Co-efficient of dietary protein is an essential part of the nitrogen balance method. Digestibility coefficient of a protein is defined as:

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

$$= \frac{\text{Nitrogen intake-(faecal nitrogen - endogenous facel nitrogen)}}{\text{Nitrogen intake}}$$

where faecal nitrogen is excreted in the faeces during a period of protein feeding and endogenous nitrogen is the amount of nitrogen excreted in the faeces on a nitrogen free diet.

c. Biological Value

The concept of biological value was first introduced by Thomas in 1909 interms of percent of digestable nitrogen from a test food which was retained by the adult human Mitchell(1924) modified the method for the frowing rat thus including the requirements for both growth and maintenance.

The formulae used to calculate the biological values is as follows

$$B.V = \frac{\text{Food nitrogen utilized}}{\text{Food nitrogen absorbed}} \times 100$$

$$= \frac{\text{Nitrogen intake (faecal nitrogen-endogenous faecal nitrogen - Urinary nitrogen-endogenous urinary nitrogen}}{\text{Nitrogen intake (faecal nitrogen-endogenous faecal nitrogen)}}$$

d. Nitrogen Balance Index

Melnich and Cowgill (1937) studied the minimum amount of dietary protein nitrogen necessary to maintain nitrogen equilibrium by plotting nitrogen intake against nitrogen balance in the region of nitrogen equilibrium, interplating to the point of exact nitrogen equilibrium. Allison et al., (1945) further modified this technique, and showed that it is possible to plot absorbed nitrogen against nitrogen balance to obtain a straight line. The equation to get the balance indexto of nitrogen is as follows:

$K = \frac{B-Bo}{A}$, where B is the nitrogen balance, A is the absorbed nitrogen and Bo is the excretion of nitrogen when a protein free diet is given and K is the slope of the line repr senting the nitrogen balance index of nitrogen absorbed.

4. Methods based on regeneration of blood and liver constituents

Henry *et al.*, (1961) developed a procedure on the basis that the sensitivity with which the nitrogen content of the liver responds to different dietary problems for determining nutritive value of proteins. The concentration of urea in blood was almost univerally proportional to the biological value of the dietary proteins listed and hence proved to be a good criteria for evaluation of protein quality (Prior *et al.*, 1975, Kosterlirtz, 1968).

5. Determination of Availability of Amino Acids

Microbiological methods (Fernell and Rosin, 1956) enzymic methods (Mauron, 1973) and chemical methods are the available methods for determination of availability of amino acids (Venkat Rao *et al.*, 1964).

6. Chemical Scoring Methods

Carpenter and Bjarnason (1973) and NRC (1980) reports that the biological value of many protein foods correlated quite well with the chemical scores of the foods calculated from their amino acid analysis. New principles for scoring protein value from chemical composition have been investigated by regression analysis of data from nitrogen balance studies in man (Kofranyi *et al*., 1973).

a. Amino acid score

Block and Mitchell (1945) express the content of each of the essential amino acidx score can be calculated when both total nitrogen or protein and the concentration of the first limiting amino acid are know (PAG/UN, 1975).

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

b. Essential amino acid index

Essential amino acid inded (EAA inded) is defined as the geometric mean of the ratios of the essential amino acids in a protein to those of standard, usually egg protein (Osser, 1951).

7. Miscellaneous methods

A new methodology, termed the complete protein evaluation (CPE) procedure, has been presented by Shelling (1975). The nutritive value of dietary protein depends upon the pattern and quantity of essential amino acids it presents to the body after absorption from the intestine. Assuming that chemical analysis of dietary protein reveals the pattern by amino acid liberated in and absorbed by the gastro-intestinal tract, an estimate can be made of the nutritive value of protein by comparing the amino acid pattern derived from chemical analysis with that of the reference protein. Block and Mitchell (1946) suggested the use of egg protein as a reference protein, assigned a maximum score of 100 and evolved a chemical scoring method for assessing the relative nutritive value of food protein. Lakin (1973) has reported the evaluation of protein quality by dye binding procedure. Frank et al., (1975) has proposed a simple and practical method, useful, especially for screening purpose, utilizing the phagotophic protoezan, Cilicate Tetrahymena. Histopathological studies have been wisely used to evaluate protein quality based on the changes taking place in the histology of various tissues (Rajan, 1975, Rangaswamy et al., 1979).

III EXPERIMENTAL PROCEDURE

The methodology pertaining to this study on "Evaluation of protein quality of infant formulations developed by a commercial firm" is outlined under the following headings:

A. Selection of the samples

B. Formulation of the diets

C. Selection and grouping of the animals

D. Evaluation of protein quality

A. Selection of the Samples

Seven infant formulations developed by a commercial firm were selected for the protein quality evaluation as there is an increased need for such information with the ever increasing demand of commercial weaning foods (Shukla and Das Gupta, 1981).

B. Formulation of the Diets

The seven selected food formulations were analysed for its nitrogen content using the Microkjeldhal method and fat content by Soxhlet method (Hawk *et al*., 1965). The analysed values for each of the food sample is tabulated in Table I.

TABLE I

PROTEIN AND FAT CONTENT OF THE SEVEN INFANT FORMULATIONS

Code of Infant Formulation	Protein g/100g	Fat (g/100g)
F_{I}	12.2	2
F_{II}	11.8	2
F_{III}	12.0	2
F_{IV}	15.0	2
F_{V}	15.0	2
F_{VI}	15.0	2
F_{VII}	12.9	2

The protein content of the seven diets were maintained at 10 percent level and quantities of each formulation determined by using the analysed protein value. The diets were made isocaloric by adjusting the fat content at 9 percent (ISI, 1979) taking into account the fat content of the protein mixtures. To ensure an adequate supply of vitamins and minerals, a vitamin mixture and mineral mixture were added at 2 percent and 4 per cent levels respectively (NRC, 1980). The composition of the two mixtures are appended (Appendix I). For comparison a casein diet at 10 percent protein level was employed as the eighth diet. The percentage composition of the eight diets thus formulated is given in Table II.

TABLE II

PERCENTAGE COMPOSITION OF THE DIETS

Ingredients	Diets							
	I	II	III	IV	V	VI	VII	VIII
Food mixture	81.96	84.74	83.33	66.66	66.66	66.66	71.51	10.52
Groundnut oil	7.04	7.31	7.33	7.67	7.67	7.67	7.45	0.95
Vitamin mixture	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mixture	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Starch	4.64	1.95	3.33	19.67	19.67	19.67	9.04	82.53
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

C. <u>Selection and Grouping of the Animals</u>

Albino rats from the laboratory stock colony were selected for the different aspects of the study on the following basis:

a. Eighty weanling male rats 21 to 24 days old (NRC, 1980 and Friedman, 1975) weighing between 31.5g and 47.5, withi an average weight of 39.5g., for growth experiment.

b. Fourty eight adult male rats of 100 days old (NRC, 1980 and Friedman, 1975) weighing between 165.0g and 230.5g were selected for the nitrogen balance studies. After feeding the rats a non-protein diet for a period of seven days, depending on the reduction in weight the rats were grouped into eight groups with six rats per group.

D. <u>Evaluation of Protein Quality</u>

1. <u>Growth</u>

Eight groups of selected rats housed individually were placed on the test diets, with water and food given ad libitum (NRC, 1963, 1979, 1980; Rao, 1974; Friedman, 1975). Weighed amounts of the diets were mixed with water, steamed and fed daily. The left over food was collected, dried and weighed. Records of actual food intake were maintained. Rats were weighed individually on every alternate day throughout the experimental period of twnety eight days (Friedman, 1978, Rao, 1974; NRC, 1980).

a. Weight gain and food intake

The quality of protein consumed is reflected by weight gain and food intake (Friedman, 1975; NRC, 1980; Müller and Tobin 1980) and hence this criteria served as a basis for the evaluation of test diets.

b. Protein-efficiency ratio (PER)

From the record of weight gain and protein intake, PER was calculated using the formula given by (NRC, 1980; Pellete and Young, 1980).

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

2. Nitrogen balance

a. Depletion period

A non-protein diet containing 85g starch; 4g mineral mixtures; 2g vitamin mixtures; and 9g groundnut oil, was fed ad libitum during the depletion period of seven days. The first three days were adjustment period and last four days the collection period (Pellette and Young, 1980, Friedman, 1978, NRC, 1980).

During the collection period, conical flasks with funnels were kept under the specially devised metabolic cages to collect the urine. The urine was collected daily in the flask, volume measured and preserved under toluene (NRC, 1980, Brown et al., 1979). For the collection of the faecal samples, a wire gauze was placed on the funnel to separate feaces from urine. The samples were collected daily, bulked for each rat at the end of the collection period. These were dried in the oven, hairs and adhered food particles if any brushed off, weighed and ground in the mortar. Triplicate samples of urine and faeces were taken for nitrogen estimation.

Weight of each rat was recorded on the eighth day of the depletion period, and the reduction in the weight calculated. The rats were divided into eight groups of six according to weight so that the average weight in different groups were the same.

b. Repletion period

The depleted rats underwent an adjustmental period of four days. They were fed with stock diet from the laboratory. After that the rats were fed with the experimental diets for the ensuing seven days. A record of food intake was maintained. Urine and feaces were collected for last four days and preserved. The urine and feaces samples were pooled for each metabolic period and analysed for the nitrogen content in triplicates using the microjkildhal method (Hawk, 1965, Muller and Tobin, 1980). Biological value and digestibilities were calculated using the formula given by NRC (1980).

IV RESULTS AND DISCUSSION

The results of this investigation on the "Evaluation of protein quality of infant formulations developed by a commercial firm" on albino rats are presented and discussed under the indices of:

A. Growth

B. Nitrogen Balance

A. Growth:

The results obtained under the growth indices are discussed in the following order.

1. Weight gain
2. Food and protein intake
3. Protein Efficiency Ratio

1. Weight gain:

The mean weight gain of rats fed with the seven experimental diets and the casein control are presented in Table III. Weight gains of the individual rats are given in Appendix II.

TABLE III

MEAN BODY WEIGHT CHANGES OF RATS FED DIFFERENT EXPERIMENTAL DIET

Diets	WEIGHT (g)		
	Initial	Final	Increments
F I	39.06 ± 3.11	72.86 ± 6.18	33.80 ± 3.91
F II	38.97 ± 3.32	74.76 ± 7.90	35.79 ± 5.22
F III	38.33 ± 4.08	68.48 ± 5.29	30.15 ± 4.21
F IV	37.93 ± 4.70	87.42 ± 10.65	49.49 ± 7.11
F V	38.91 ± 3.77	68.83 ± 6.15	29.92 ± 2.96
F VI	40.47 ± 4.47	84 ± 82 ± 9.44	44.35 ± 7.07
F VII	38.12 ± 2.15	78.88 ± 9.42	40.76 ± 9.42
Cascin control	38.31 ± 2.31	89.79 ± 10.90	51.48 ± 11.58

Summary of Statistical analysis:

Diet : Cascin Control	F IV	F VI	F VII	F II	FI	FIII	FV
Weight gain : 51.48	49.49	44.35	40.76	35.79	33.80	30.15	29.92

Any two values not underscorded are significantly different.

Rats fed on case in control diet registered the highest weight gain (51.48g), while rats fed F IV diet ranked second in weight gain (49.49 g). The lowest increase in weight gain was exhibited by rats fed F v diet (29.92g). Statistical analysis however, indicated that the difference in weight gain exhibited byrats fed casein diet, diets Fiv and FVi were not statistically significant. Similarly, there were no significant difference in the weight increments of rats fed diets FvI and FvII; FvII and FII; FII and FI; and F III and FV. However, the weight increments among the rats fed the other diet comparisions like casein control and all other diets except FIV and FVU; FI and FIII; FI and FVII were significant at one percent level while all other comparisons were significant at five percent level.

The growth pattern (mean increments in weight) of the rats fed the eight diets as depicted in figure I. highlights the quality of protein of the eight food mixtures since weight gain of wealing rats is a sensitive indicator of the adequacy of amino acid supply, on the basis of which growth response, are used to compare proteins (Conolty and Koong, 1977) Miller and Dam, (1972).

2. Food and Protein intake:

In all the eight diets, changes in food intake was linearly related to changes in protein intake, since the level of protein in all the diets were maintained at 10 percent level. The mean total food and protein intake of rats fed the different experimental diets are given in Table IV and individual food and protein intakes are given in Appendix III.

SCALE X AXIS 4 cm = 1 week
Y AXIS 2½ cm = 10 grams

KEY = FI to FVII - EXPERIMENTAL DIETS
FVIII - CASEIN CONTROL DIET

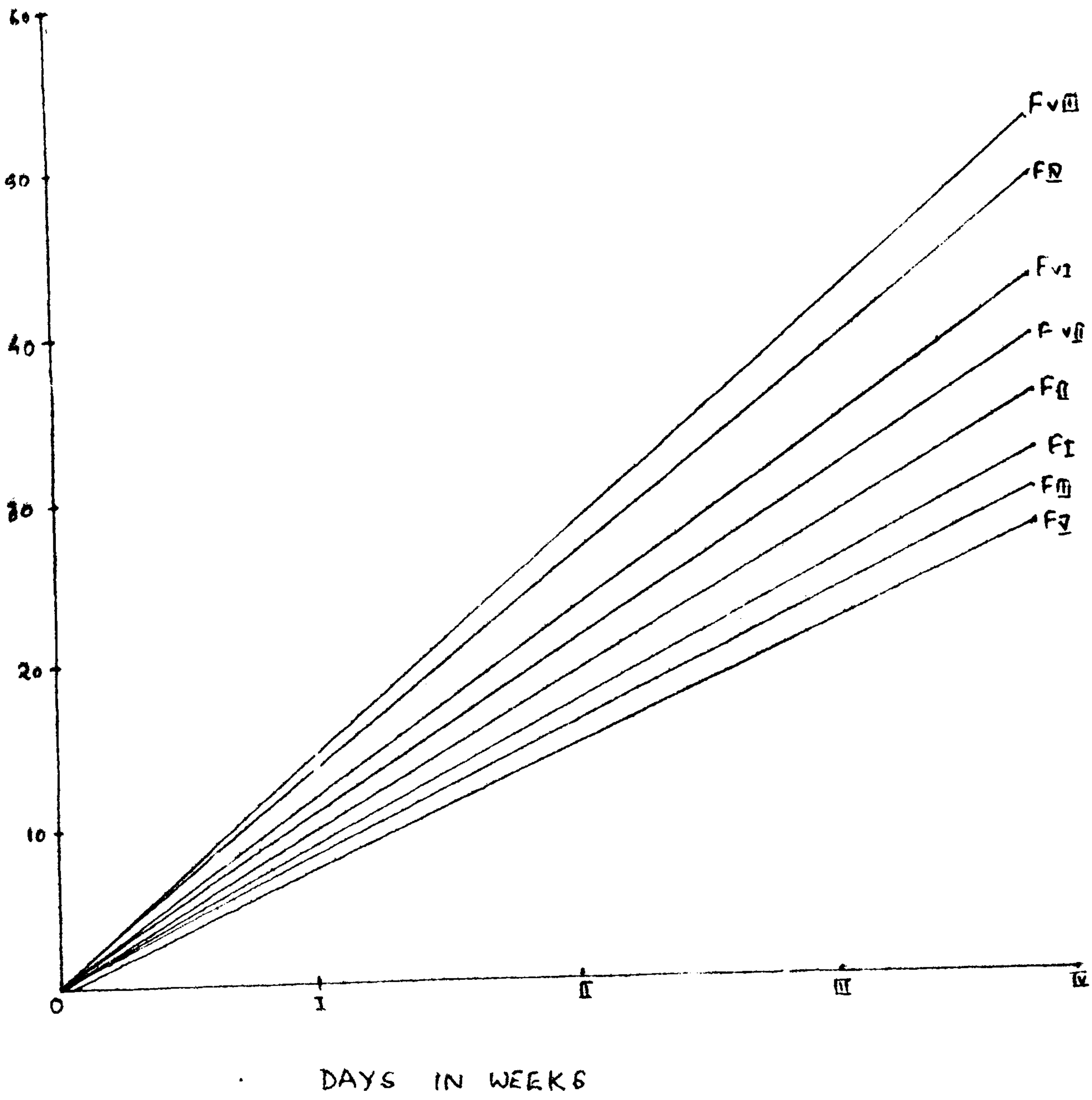

FIGURE 1. GROWTH PATTERN OF RATS FED WITH EXPERIMENTAL DIETS

TABLE IV

MEAN FOOD AND PROTEIN INTAKES OF RATS FED DIFFERENT EXPERIMENTAL DIETS

Diets	Food Intake (g)	Protein Intake (g)
F I	198.16 ± 11.98	19.81 ± 1.19
F II	200.92 ± 15.61	20.09 ± 1.56
F III	195.73 ± 15.40	19.57 ± 1.54
F IV	238.00 ± 11.98	23.90 ± 1.16
F V	181.82 ± 19.28	18.18 ± 1.92
F VI	205.53 ± 39.89	20.66 ± 3.98
F VII	219.88 ± 9.63	21.98 ± 0.96
Cascin control	244.48 ± 3.82	24.44 ± 0.38

Summary of statistical Analysis:

Diet :	Cascin control	F IV	F VII	F VI	F II	F I	F III	F V
Protein Intake:	24.44	23.90	21.98	20.55	20.09	19.81	19.57	18.18

The animals fed casein control diet recorded the highest food and protein intake (244.48 g and 24.44 g respectively) followed by rats fed FIV diet. Rats fed F VII diet ranked third in their food and protein intakes, the remaining diets may be ranked as diet FVI, F II, F I, F III, F V respectively. From the statistical analysis, it was revealed that, the rats fed on F II, F I, F III and F V, diets did not register significantly different food and protein intakes.

Similarly, there were not significant differences in the food and protein intakes of rats fed diets FI and F VII; F II and F VII; F III and F VII; However, the comparisions of diets regarding their food and protein intakes among the rats fed the other diets like case in control and all other diets except F IV and F VI. F I and F III; F I and F VII. were significant at one percent level while all other comparisions were significant at five percent level.

Hurt et al (1974) opine that food intake and subsequent protein intake is a direct indicator of the quality of protein.

When the data on food and protein intakes is considered in relation to the body weight gains, it is observed that the rats fed casein control diet. Which recorded the highest food and protein intake showed the highest weight gain and therats fed diet F V diet which recorded the least food and protein intake showed the least gain in weight. These observations are in tune with earlier observations of Devadas *et al* (1970) and Chandrasekhar *et al* (1976) who opine that the greater the food and protein intakes, greater is the body weight gain.

TABLE V

PROTEIN EFFICIENCY RATIO OF THE EIGHT DIETS

Diets	Protein Intake (g)	Weight gain (g)	Protein Efficiency Ratio (PER)
F I	20.66 ± 3.[illegible]8	33.80 ± 3.91	1.69 ± 0.32
F II	18.18 ± 1.82	35.79 ± 5.22	1.96 ± 0.17
F III	19.18 ± 1.19	30.15 ± 4.21	1.57 ± 0.18
F IV	23.90 ± 1.19	49.49 ± 7.11	2.07 ± 0.24
F V	10.57 ± 1.54	29.92 ± 2.96	1.53 ± 0.08
F VI	21.98 ± 0.96	44.35 ± 7.07	2.01 ± 0.26
F VIII	20.09 ± 1.56	40.56 ± 9.42	2.02 ± 0.04
Cascin control	24.44 ± 0.38	51.48 ± 11.68	2.13 ± 0.44

Summary of statistical Analysis:

Diets:	Cascin Control	F IV	F VII	F VI	F II	F I	F III	F V
PER :	2.13	2.07	2.02	2.01	1.96	1.69	1.57	1.53

Any two values not underscored are significantly different.

3. Protein Efficiency Ratio:

The mean gain in weights protein intakes and protein efficiency ratio (PER) of diets deduced from the data or rats fed the different experimental diets are given in Table V. The details regarding the same for the individual animals is given in Appendix IV.

In the descending order of PER values the diets could be ranked as casein control, F IV, F VII, F VI, F II, F I, F III and F V. Statistically, the PER values between F I, F III and F V diets were not significant while these three diets, were statistically lower (p 0.01) in their PER values when compared with the other diets including casein control.

When the PER values are compared with weight gains, it is evident that casein control diet and F VI diet which ranked to be the highest having statistically insignificant difference between themselves in PER values, also promoted higher weight gains. Again rats fed diet F V, which had lowest PER value had lowest weight gains. Observations in indices based on growth thus helps us to speculate that both diet casein control and F IV showed equal trend with regards to their protein quality. On the other hand diets F I, F III and F V seems to be the lowest as far as protein quality is concerned based on growth indices.

B. Nitrogen Balance

The results obtained from nitrogen balance study on the eight diets are discussed under the following headings:

1. Nitrogen retention
2. Digestibility Co-efficient and Biological value.

TABLE VI

MEAN INTAKE, ABSORPTION, RETENTION OF NITROGEN BY RATS FED WITH EIGHT DIETS

Diets	Nitrogen intake	Nitrogen Absorbed	Nitrogen Retained	Percent Nitrogen Retention
F I	595.72 ± 90.45	551.83	341.49	49.99 ± 9.59
F II	805.62 ± 204.15	619.74	399.84	51.92 ± 11.16
F III	821.62 ± 192.08	568.97	334.25	42.36 ± 16.12
F IV	784.19 ± 197.87	563.62	486.50	63.17 ± 10.57
F V	801.73 ± 102.81	545.65	298.02	38.40 ± 12.14
F VI	749.54 ± 304.54	590.07	419.28	57.05 ± 15.53
F VII	735.18 ± 95.52	625.00	450.55	61.38 ± 13.03
Casein control	587.54 ± 56.11	607.27	463.78	57.95 ± 10.98

Summary of Statistical Analysis :

Diet:	Casein Control	F IV	F VII	F VI	F II	F I	F III	F V
Percent N_2 Retention :	57.95	63.17	61.38	57.05	51.92	49.99	42.36	38.40

(Underscore printed beneath 63.17 – 61.38, and beneath 57.05 through 38.40.)

Any two values not underscored are significantly different

1. Nitrogen Retention:

The mean total intake, excretion, absorption and retention of nitrogen from the eight different diets are presented in Table VI and the details are given in Appendix - VII.

When the nitrogen retention percentage of different diets were compared, diet casein control ranked highest with a retention percent of 67.95%, while Diet F V registered the lowest retention percent 38.40%. There was no statistical significant difference between the diets like F IV, F VII, F VI, FII, F I, F VIII and F V, with regards to their nitrogen retention percent. But when casein control diet was compared to all other diets showed a statistically higher percent nitrogen retention at five percent level of significance, except diet F V. When compared with casein control the difference was significant at one percent level.

2. Digestability co-efficient and Biological value

The digestibility coefficient and biological value calculated from the nitrogen balance data are presented in Table VI and the details pertaining to the individual rats are given in Appendix VIII and IX.

Casein control diet gave the highest digestibility coefficient (92.17) and diet F V gave the lowest digestibility coefficient (69.00). Diet F IV ranked the second, while the digestibility coefficient of the other diets revealed a significantly lower digestibility coefficient values, at five percent level of significance; diet F V, was significantly lower at one percent level when compared with casein control diet.

TABLE VII

DIGESTIBILITY COEFFICIENT AND BIOLOGICAL VALUE OF EIGHT DIETS

Diets	Digestibility coefficient	Biological value
F I	79.83 ± 8.34	62.33 ± 7.52
F II	78.83 ± 8.95	65.38 ± 8.07
F III	71.67 ± 16.47	56.88 ± 13.21
F IV	86.50 ± 9.50	73.50 ± 7.54
F V	69.00 ± 11.03	54.77 ± 11.84
F VI	80.00 ± 12.81	70.54 ± 12.07
F VII	85.50 ± 6.70	71.38 ± 11.52
Cascin control	92.17 ± 10.29	75.04 ± 8.82

Summary of Statistical Analysis:

Diet :	Cascin Control	F IV	F VII	F VI	F I	FII	FIII	F V
D.C	92.17	86.50	85.50	80.00	79.83	78.83	71.67	69.00

Diet :	Cascin Control	F IV	F VII	F VI	F II	F III	F V
B.V :	75.04	73.50	70.54	65.38	62.33	56.88	54.77

Any two values not underscored are significantly different.

Kuppuswamy *et al* (1958) studied the digestability coefficient of 15 species of legumes and found that digestibility coefficient varied from 51 to 93 percent. The results obtain for the present study also showed an range from 69.00 to 92.17 for Digestibility coefficients.

The Biological value probably is the best measure of protein quality and represents the amount of protein absorbed that is retained by the animal organism (Bressani, 1974) Moore, 1967).

In the present study, casein control diet registered the highest Biological value (75.04), while diet F V gave the lowest Biological value (54.77). Diet F IV ranked second (73.50) while the others in the descending order of ranking are diets F VII, F VI, F II, F I, F III, and F V, respectively. Diets F I, F II, F III, F V, F VI, F VII. When compared statistically revealed no significant difference. Casein control and diet F IV when compared to all other diets had biological value significantly higher at five percent level.

Agarwal *et al* (1979) studied the Biological values of 10 different infant food mixtures and found that Biological value varied from 55 to 80 per cent. The results obtained for the present study also showed a range within the range of 54.77 to 75.04.

The Biological value obtained from the experimental diets correlated well with Bark *et al*'s studies (1975) which gives a rnage of 40 to 75 for the Biological value of wheat flour varieties with skim milk.

Phansalkar (1960) reported that, Biological value of cereal proteins vary from 60 to 80 percent and those of legumes between 45 and 74 percent. The present study is in tune with the study by Phansalkar.

When judged from the parameters reported in this study it is evident that among the seven experimental diets, F IV had registered the best protein quality with regards to weight increments, food and protein intakes, protein efficiency ratio, and also resulted in better nitrogen retention percent. The Biologival value and digestibility coefficient registered by F IV diet was the best as compared to other diets. There was also no significant difference in these parameters when compared with casein control. It is evident from the above data that the infant formulation F IV has equal potential with casein in its protein quality. Formulation like F VII may need further enrichment in terms of amino acids but formulative like F I, F III and F V needs greater modification. The fact that F IV has equal potential with casein indicates the possibility of its being put into market with greater benefit to the community.

V SUMMARY AND CONCLUSION

The objective of the present investigation was to evaluate the protein quality of infant formulations developed by a commercial firm on albino rats. Seven infant formulations developed by a commercial firm were selected for the protein quality evaluation. An Eighth diet with casein as the protein source served as a control for comparison. All the eight diets planned were evaluated on groups of-

1. Weanling albino rats over a period of 28 days using the criteria of weight gain, food and protein intake and PER.

2. Adult rats for a period of 14 days using the parameter nitrogen balance to include nitrogen retention, digestibility coefficient and biological value

The results revealed that:

1. The maximum mean weight increment was registered by the rats fed on casein control diet followed by F IV and F VI diet next in order. The minimum mean weight gain was recorded by the rats fed FV diet. Statistically, the weight increments among rats fed casein control and all other diets except FIV and FVI; FI and FIII; FI and F VII, where significant at one percent level, while other comparison like FI Vs FIII, FV and F VII; F VII vs casein control were significant at five percent level. All other diet comparison were statistically not significant.

2. The total food intake ranged from 181.82 (FV) to 244.48 grams (casein control). Subsequently the protein intake ranged from 18.18 to 24.44 grams. Statistically the protein intake of rats feddiets FII, FI, FIII and FIV were not significantly different. Again the comparisons between protein intakes of rats fed FI and FVII; FII and F VII; F III and F VII, were not significant. All other comparisons were statistically significant.

3. The PER value for casein diet were the highest (2.13) and diet F V the lowest (1.53). According to the PER values the diets may be ranked in disording order as F IV (2.07); F VII(2.02); F VI (2.01); F II (1.96); FI (1.69); FIII (1.57). Statistically, the PER values between FI, FIII and F V diets were not significant while these three diets, were statistically lower (p 0.01) in their PER values when compared with the other diets including casein control.

4. The percentage nitrogen retention in adult rats fed with eight diets ranged from 38.40 (FV) to 67.95 (casein control). Statistically the casein control diet showed higher percent nitrogen retention when compared to other diets whereas the nitrogen retention all other diets were not statistically significant. Diet F IV was not statistically significant from casein control.

5. The digestibility coefficient also exhibited similar trends. Highest digestibility coefficient was given by casein control diet (92.17) which was significantly different from all other diet formulations, except FIV diet.

6. The biological value of the diets also exhibited similar trends. The highest biological value (75.04) was obtained for casein control whereas F V diet had the lowest value of 54.77. Statistifically casein control diet was different from all other diets except F IV diet.

The results of this investigation indicates that the infant food formulation F IV had equal potential with regard to its protein quality to that casein diet when weight gains, food and protein intakes, protein efficiency ratio, nitrogen retention, digestibility coefficient and biological value are taken into considerations. With slight modifications may be in its amino acid pattern it would equal casein in protein quality. However, the other formulations required greater modifications and improvement. From the point of view of protein quality, based on the criteria evaluated in this study it may be recommended that formulations FIV can easily be put into marked as an infant food. However, further modification and studies along these lines for other food formulations is recommended.

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A P P E N D I C E S

APPENDIX I

1. Composition of Mineral mixture (g/kg)

Sodium chloride	= 139.3
Potassium Dihydrogen phosphate	= 389.0
Calcium carbonate	= 381.4
Magnesium Sulphate	= 57.3
Ferrous sulphate	= 27.0
Manganous Sulphate	= 4.010
Potassium Iodide	= 0.790
Zinc Sulphate	= 0.543
Copper Sulphate	= 0.477
Cobalt chloride	= 0.030

2. Composition of Vitamin Mixture (Amt/g of corn starch)

Vitamin A	= 1000 I.u
Vitamin D	= 100 I.u
Vitamin E	= 10.I.u
Vitamin K	= 0.5 mg
Thiamine	= 0.5 mg
Riboflavin	= 1.0 mg
Pyridoxin	= 0.4 mg
Pantothenic Acid	= 4.0 mg
Niacin	= 4.0 mg
Choline	= 200 mg
Inositol	= 25 mg
Paba	= 10 mg
Vitamin B_{12}	= 2 mg
Biotin	= 0.02mg
Folic acid	= 0.20mg

APPENDIX - II

WEIGHT GAIN OF THE RATS FED WITH DIFFERENT DIETS

Rat No.	F_1 Initial (g)	F_1 Final (g)	F_1 Increase (g)	F_2 Initial (g)	F_2 Final (g)	F_2 Increase (g)	F_3 Initial (g)	F_3 Final (g)	F_3 Increase (g)	F_4 Initial (g)	F_4 Final (g)	F_4 Increa (g)
1.	39.7	75.5	35.8	37.6	72.0	34.4	36.0	66.5	30.5	45.2	99.5	54.3
2.	41.5	82.0	40.5	42.1	77.3	35.2	37.2	65.5	28.3	42.0	97.8	55.8
3.	35.2	64.2	29.0	34.7	63.4	28.7	34.1	63.0	28.9	39.5	89.0	49.5
4.	38.0	74.0	36.0	39.0	75.2	36.2	38.5	67.2	28.7	35.2	76.3	41.1
5.	40.0	76.2	36.2	38.7	65.5	26.8	40.0	76.5	36.5	32.0	72.6	40.6
6.	36.4	65.0	28.6	45.8	92.0	46.2	32.2	54.3	22.1	36.7	83.5	46.8
7.	45.2	83.2	37.0	35.2	69.2	34.0	47.5	74.3	26.8	31.5	73.9	42.4
8.	35.7	69.5	33.8	34.5	72.5	38.0	41.0	71.0	30.0	34.2	82.5	48.3
9.	40.4	68.5	28.1	42.6	81.5	38.9	36.5	73.6	37.1	38.0	102.7	64.7
10.	37.5	70.5	33.0	39.5	79.0	39.5	40.3	72.9	32.6	45.0	95.4	51.4

WEIGHT GAIN OF THE RATS FED DIFFERENT DIETS

Rat No.	F_5 Initial (g)	F_5 Final (g)	F_5 Increase (g)	F_6 Initial (g)	F_6 Final (g)	F_6 Increase (g)	F_7 Initial (g)	F_7 Final (g)	F_7 Increase (g)	F_8 Initial (g)	F_8 Final (g)	F_8 Increase (g)
1.	42.5	73.6	31.1	45.0	97.8	52.8	39.7	98.5	58.8	38.7	109.5	70.8
2.	40.0	67.5	27.5	47.5	90.1	42.6	40.1	88.2	48.2	38.2	90.2	52.0
3.	39.5	75.2	35.7	41.0	81.4	40.4	38.2	72.9	34.7	39.5	84.7	45.2
4.	40.6	71.0	30.4	39.7	84.5	44.8	36.1	82.2	46.1	36.0	80.5	44.5
5.	39.6	70.5	30.9	38.2	82.4	44.2	35.5	75.4	39.9	33.9	108.6	74.7
6.	35.5	65.7	30.2	33.5	69.7	36.2	41.0	75.5	34.5	35.5	83.0	47.5
7.	31.9	57.2	25.3	40.0	90.8	50.8	40.5	75.7	35.2	40.2	76.7	36.5
8.	34.0	59.5	25.5	33.2	71.4	38.2	39.1	66.6	27.5	39.0	80.5	41.5
9.	40.5	70.9	30.4	41.5	99.5	58.0	36.0	67.5	31.5	41.5	96.0	54.5
10.	45.0	77.2	32.2	45.1	80.6	35.5	35.0	86.3	51.3	40.6	88.2	47.6

SUMMARY OF STATISTICAL ANALYSIS FOR MEN BODY WEIGHT CHANGES

Diet	't' values	Diet	't' values
FI Vs FII	= 0.91 N.S	FIII Vs FIV	= 7.00 **
FI Vs FIII	= 2.10 *	FIII Vs FV	= 0.13 N.S
FI Vs FIV	= 5.78 **	FIII Vs FVI	= 5.16 **
FI Vs FV	= 2.36 *	FIII Vs FVII	= 3.01 **
FI Vs FVI	= 3.90 **	FIII Vs F.C.C.	= 5.14 **
FI Vs FVII	= 2.22 *		
FI Vs F.CC	= 4.92 **	FIV Vs FV	= 7.59 **
		FIV Vs FVI	= 1.25 N.S
FII Vs FIII	= 2.51 *	FIV Vs FVII	= 2.25 **
FII Vs FIV	= 4.64 **	FIV Vs F.C.C	= 0.44 N.S
FII Vs FV	= 2.92 **	FV Vs FVI	= 5.63 **
FII Vs FVI	= 2.91 **	FV Vs FVII	= 3.22 **
FII Vs F.C.C	= 3.67 **	FV VS F.C.C	= 5.35 **
		FVI Vs FVII	= 0.96 N.S
		FVI Vs F.C.C	= 1.56 N.S
		FVII Vs F.C.C.	= 2.18 *

* = Significant at five percent level

** = Significant at one percent level

N.S= Not significant

APPENDIX III

TOTAL FOOD INTAKES OF RATS FED WITH DIFFERENT DIETS FOR 28 DAYS

Rat.No	F_1(g)	F_2(g)	F_3(g)	F_4(g)	F_4(g)	F_5(g)	F_7(g)	F_8(g)
1.	196.4	195.4	204.4	255.5	195.5	230.4	203.6	249.8
2.	192.4	171.3	206.3	238.4	194.6	225.4	214.3	246.7
3.	169.1	155.0	199.5	249.5	214.4	213.1	192.5	238.4
4.	180.8	166.8	184.0	236.4	200.3	224.3	199.4	243.3
5.	288.3	155.1	187.7	222.3	195.4	226.9	199.8	243.4
6.	252.5	204.2	180.9	234.3	189.9	201.8	181.3	242.9
7.	255.4	179.9	185.1	219.5	168.1	228.7	188.8	238.9
8.	158.4	181.2	199.0	232.3	172.1	205.3	179.3	243.7
9.	176.9	215.1	216.9	246.6	216.1	226.5	223.0	248.2
10.	186.1	194.2	217.8	255.1	210.9	216.4	227.2	249.5

SUMMARY OF STATISTICAL ANALYSIS FOR MEAN FOODS AND PROTEIN INTAKES

Diet		't' values	Diet		't' values
FI VS FII	=	0.53 N.S.	FIII VS Fo.c.	=	3.49 **
FI VS FIII	=	0.19 N.S.			
FI VS FIV	=	2.24 *	FIV VS FV	=	2.10 *
FI VS FV	=	0.24 N.S.	FIV VS FVI	=	2.19 *
FI VS FVI	=	2.27 *	FIV VS FVII	=	2.15 *
FI VS FVII	=	0.19 N.S.			
FI VS F o.c.	=	2.24 *	FIV VS F o.c.	=	0.41 N.S.
FII VS FIII	=	0.68 N.S.	FV VS FVI	=	2.15 *
FII VS FIV	=	2.36 *	FV VS FVII	=	2.23 *
FII VS FV	=	0.55 N.S.	FV VS F o.c.	=	2.90 **
FII Vs FVI	=	2.23 *			
FII VS FVII	=	0.73 N.S.			
FII VS F o.c.	=	3.02 **	FVI VS FVII	=	0.97 N.S.
			FVI VS F o.c.	=	2.25 *
FIII VS FIV	=	2.29 *			
FIII Vs FV	=	0.12 N.S.	FVII VS F o.c.	=	2.56 *
FIII VS FVI	=	2.26 *			
FIII VS FVII	=	0.14 N.S.			

* Significant at five per cent level
** = Significant at one per cent level
NS = Not Significant

APPENDIX IV

GAIN IN WEIGHT PROTEIN IN TAKE AND PER OF RATS FED DIFFERENT DIETS

Rat No.	F_1	F_2			F_3			F_4			F_5				
	gain in wt (g)	Protein intake (g)	PER	gain in wt (g)	Prot. intake (g)	Per	Gain in wt. (g)	prote. intake (g)	Per	gain in wt (g)	prote in take (g)	pER	gain in wt. (g)	pro. in take (g)	PER
1.	35.8	19.64	1.32	34.4	19.54	1.76	30.5	20.44	1.49	54.3	25.56	2.12	31.1	19.55	1.59
2.	40.5	19.24	2.10	35.2	17.13	2.05	28.3	20.63	1.37	55.8	23.84	2.34	27.3	19.46	1.41
3.	29.0	16.91	1.71	29.7	15.50	1.85	29.9	19.95	1.45	49.5	24.95	1.98	35.7	21.44	1.67
4.	36.0	18.08	1.99	36.2	16.68	2.17	28.7	18.40	1.56	41.1	23.64	1.74	30.4	20.03	1.52
5.	36.2	20.65	1.26	26.6	15.51	1.73	36.5	18.77	1.94	40.6	22.23	1.83	30.9	19.54	1.58
6.	28.6	25.25	1.13	46.2	20.42	2.26	22.1	18.09	1.22	46.8	23.43	2.00	30.2	18.99	1.59
7.	37.0	26.54	1.39	34.0	17.99	1.89	26.8	18.51	1.45	42.4	21.95	1.93	25.3	16.81	1.51
8.	33.8	15.84	2.13	38.0	18.12	2.10	30.0	19.90	1.51	48.3	23.23	2.07	25.5	17.21	1.48
9.	28.1	17.69	1.59	33.9	21.51	1.81	37.1	21.69	1.71	64.7	24.66	2.62	30.4	21.62	1.41
10.	33.0	18.61	1.77	39.5	19.42	2.03	32.6	21.78	1.50	51.4	25.51	2.01	32.2	21.09	1.53

contd.......

Rat No.	F_6 gain in wt (g)	F_6 protein intake (g)	F_6 PER	F_7 Gain in wt (g)	F_7 protein intake (g)	F_7 PER	F_8 (chain diet) gain in wt (g)	F_8 (chain diet) protein in take (g)	F_8 (chain diet) PER
1.	52.8	23.04	2.29	50.8	20.36	2.89	70.8	24.98	2.83
2.	42.6	22.54	1.89	48.1	21.43	2.24	52.0	24.67	2.11
3.	40.4	21.31	1.90	34.7	19.25	1.80	45.2	23.84	1.90
4.	44.8	22.43	2.00	46.1	19.94	2.31	44.5	24.33	1.82
5.	44.2	22.69	1.95	39.9	19.98	2.00	74.7	24.34	3.07
6.	36.2	20.18	1.79	34.5	18.13	1.90	47.5	24.29	2.13
7.	50.8	22.87	2.22	35.2	18.88	1.86	36.5	23.89	1.64
8.	38.2	20.53	1.86	27.5	17.93	1.53	41.5	24.37	1.86
9.	59.0	22.65	2.56	31.5	22.30	1.41	50.60	24.82	2.44
10.	35.5	21.64	1.64	51.3	22.72	2.26	47.6	24.95	1.76

SUMMARY OF STATISTICAL ANALYSIS FOR PER

Diet		't' values	Diet		't' values
FI VS FII	=	2.15 *	FIII VS FIV	=	3.22 **
FI VS FIII	=	0.96 N.S.	FIII VS FV	=	0.62 N.S.
FI VS FIV	=	2.82 *	FIII VS FVI	=	4.27 **
FI VS FV	=	1.45 N.S.	FIII VS FVII	=	3.04 **
FI VS FVI	=	2.30 *	FIII VS F c.c.	=	3.47 **
FI VS FVII	=	1.09 *	FIV VS FV	=	6.34 **
FI VS F c.c.	=	2.39 *	FIV VS FVI	=	0.51 N.S.
			FIV VS FVII	=	0.32 N.S.
			FIV VS F c.c.	=	0.35 N.S.
FII VS FIII	=	4.83 **	FV VS FVI	=	5.35 **
FII VS FIV	=	1.12 N.S.	FV VS FVII	=	3.52 **
FII VS FV	=	6.35 **	FV VS F c.c.	=	3.94 **
FII VS FVI	=	0.48 N.S.	FVI VS FVII	=	0.06 N.S.
FII VS FVII	=	0.41 N.S.	FVI VS F c.c.	=	0.70 N.S.
FII VS F c.c.	=	1.08 N.S.	FVII VS F c.c.	=	0.55 N.S.

* = Significant at five per cent level
** = Significant at one per cent level
N.S. = Not significant

APPENDIX V

INDIVIDUAL EXCRETION OF URINE AND FAECES DURING DEPLETION AND REPLETION PERIODS

Rat No.	Diet I				Diet II				Diet III				Diet IV			
	Depletion		Repletion		Depletion		Repletion		Depletion		Repletion		Depletion		Repletion	
	Weight of faeces (g)	Volume of urine (ml)	Weight of faeces (g)	Volume of urine (ml)	Weight of faeces (g)	Volume of urine (ml)	Weight of faeces (g)	Volume of urine (ml)	Weight of faeces (g)	Volume of urine (ml)	Weight of faeces (g)	Volume of urine (ml)	Weight of faeces (g)	Volume of urine (ml)	Weight of faeces (g)	Volume of urine (ml)
1.	2.35	13.00	5.71	10.55	2.67	11.20	6.21	11.72	3.22	10.61	6.50	10.62	2.06	10.11	5.66	10.05
2.	2.60	11.76	6.27	11.60	3.22	11.61	7.05	13.77	4.00	7.95	5.51	13.41	3.01	10.06	6.12	10.61
3.	3.01	21.01	5.93	13.26	2.51	12.21	6.51	13.78	4.29	6.99	7.29	13.74	2.51	12.05	6.09	11.21
4.	2.94	12.91	6.05	14.01	2.46	12.05	5.22	14.21	3.67	11.43	6.21	12.12	2.40	13.11	5.68	11.70
5.	3.64	13.21	6.71	12.51	3.70	13.02	12.01	13.80	3.91	10.21	5.92	12.60	3.66	13.69	7.07	12.15
6.	3.20	10.06	7.56	11.90	3.66	10.78	5.66	12.50	3.87	10.09	5.60	11.09	3.78	12.50	6.23	12.09

Rat No.	Diet V				Diet VI				Diet VII				Diet VIII			
	Depletion		Repletion		Depletion		Repletion		Depletion		Repletion		Depletion		Repletion	
	Weight of faeces (g)	volume of urine (ml)	weight of faeces (g)	volume of urine (ml)	weight of faeces (g)	volume of urine (ml)	weight of faeces (g)	volume of urine (ml)	weight of faeces (g)	volume of urine (ml)	weight of faeces (g)	volume of urine (ml)	weight of faeces (g)	volume of urine (ml)	weight of faeces (g)	volume of urine (ml)
1.	2.00	10.19	5.66	10.71	2.56	11.09	6.01	10.91	3.25	9.90	6.29	11.71	3.00	10.06	6.09	11.07
2.	2.81	10.26	5.12	10.29	3.02	11.33	6.72	10.62	4.09	8.51	7.58	12.91	4.52	8.78	7.51	13.21
3.	3.55	10.30	6.02	11.36	2.78	14.06	7.51	10.25	3.77	7.62	6.29	13.21	4.21	9.05	6.00	13.76
4.	3.75	11.06	6.55	11.41	2.61	15.62	7.09	11.71	4.29	1.05	5.82	13.02	3.99	10.12	4.08	13.19
5.	2.89	11.28	5.31	11.06	2.90	15.11	6.29	11.39	3.22	6.79	11.01	12.90	3.67	5.27	5.64	12.09
6.	3.66	12.05	5.62	12.09	3.60	12.56	6.35	12.51	3.61	7.92	6.79	12.20	4.21	10.21	6.42	12.71

APPENDIX VI

EXCRETION OF NITROGEN BY THE INDIVIDUAL RATS

Rat No.	Diet I				Diet II				Diet III				Diet IV			
	Endo N_2	Met N_2	Uri N_2	Fae N_2	Endo N_2	Met N_2	Uri N_2	Fae N_2	Endo N_2	Met N_2	Uri N_2	Fae N_2	Endo N_2	Met N_2	Uri N_2	Fae N_2
1.	50.5	70.9	266.1	240.9	66.5	75.6	306.3	401.7	39.2	60.7	206.7	303.7	36.2	59.2	201.6	316.2
2.	56.0	45.3	249.5	109.6	60.8	49.8	375.2	209.6	40.6	55.2	199.2	271.2	42.1	62.5	290.0	129.6
3.	64.2	39.3	198.2	114.2	75.60	55.8	255.6	118.6	33.8	49.5	260.5	260.5	40.6	67.8	301.6	171.5
4.	70.2	33.6	306.2	100.5	72.0	55.6	259.4	150.2	41.6	38.6	404.2	225.8	33.8	75.2	431.6	109.6
5.	69.0137.5		129.6	87.6	77.5	53-9	266.2	169.5	42.8	29.7	316.7	116.7	29.5	48.9	329.6	119.6
6.	58.3	29.5	265.3	109.6	82.6	60.9	380.6	139.7	38.6	50.6	309.2	146.2	36.1	61.5	326.1	142.9

N_2 Nitrogen
Endo: Endogenous Nitrogen
Met : Metabollic Nitrogen
Urt : Urinary Nitrogen
Fae : Faecal Nitrogen

EXCRETION OF NITROGEN BY THE INDIVIDUAL RATS

Rat Nos	Diet V				Diet VI				Diet VII				Diet VIII			
	End N_2	Met N_2	Urt N_2	Fac N_2	End N_2	Met N_2	Urt N_2	Fac N_2	End N_2	Met N_2	Urt N_2	Fac N_2	End N_2	Met N_2	Urt N_2	Fac N_2
1.	56.5	72.9	239.0	244.6	61.5	75.6	209.7	406.0	28.6	62.5	201.9	329.6	30.1	66.2	203.6	391.3
2.	64.8	42.9	291.6	190.2	60.8	60.5	375.2	391.2	40.2	59.1	190.6	189.2	40.6	52.1	195.2	227.6
3.	72.3	37.5	166.5	114.0	71.6	49.6	306.9	217.2	39.6	48.5	271.5	129.6	39.9	49.6	282.0	128.9
4.	69.4	36.9	330.5	105.6	66.8	59.2	254.8	160.9	42.5	52.6	403.6	225.6	42.1	52.6	404.6	302.1
5.	59.3	33.6	129.6	79.9	70.9	49.6	257.2	158.2	47.0	51.9	329.2	129.6	51.9	48.7	329.8	275.1
6.	62.2	29.5	251.0	112.6	80.6	57.8	261.5	139.6	39.6	48.7	326.5	201.8	61.2	51.2	340.2	291.6

APPENDIX VII

PERCENT NITROGEN RETENTION OF DIFFERENT DIETS PER RAT

Rat No.	Diet I			Diet II			Diet III			Diet IV		
	N_2 Int	N_2 Ret	Percent Ret	N_2Int	N_2Ret	Percent Ret	N_2Int	N_2	Percent Ret	N_2Int	N_2Ret	Percent Rt
1.	757.58	300.00	39.60	1142.74	450.00	39.38	779.38	134.30	17.23	1095.58	470.00	42.90
2.	853.67	350.70	41.08	915.38	400.00	43.70	1171.60	271.40	23.16	614.13	397.00	64.64
3.	620.45	398.00	64.15	568.18	400.25	70.44	970.66	557.70	57.46	714.37	500.00	69.69
4.	704.23	301.78	42.85	657.89	301.78	45.87	646.05	368.20	56.99	526.32	352.00	66.88
5.	565.98	397.00	60.43	626.01	397.00	63.42	633.81	330.70	52.21	978.09	750.00	76.68
6.	581.40	301.45	51.85	923.53	450.00	48.73	728.64	343.20	47.10	776.67	450.00	57.94

N_2 Int = Nitrogen Intake

N_2 Ret = Nitrogen Retained

Percent Ret = Percent Retained

Rat No.	Diet V			Diet VI			Diet VII			Diet VIII		
	N_2 Int	N_2 Ret	Percent Ret	N_2Int	N_2 Ret	Percent Ret	N_2Int	N_2 Ret	Percent Ret	N_2Int	N_2 Ret	Percen Ret
1.	801.67	259.60	32.38	818.49	304.30	33.13	750.00	339.40	45.25	709.65	356.00	50.17
2.	695.92	378.50	54.39	687.59	316.40	46.02	881.14	502.60	57.04	756.63	502.60	66.43
3.	667.30	362.00	54.25	683.15	534.30	78.21	820.56	569.30	72.67	733.49	545.00	74.30
4.	869.84	186.90	21.49	760.25	385.20	50.66	602.89	306.60	50.86	620.78	469.00	75.55
5.	973.54	350.60	36.63	754.51	548.40	72.68	647.13	540.31	83.62	604.47	500.00	82.72

SUMMARY OF STATISTICAL ANALYSIS FOR PERCENT NITROGEN RETENTION

Diet	't' values	Diet	't' values
FI Vs FII	= 0.29 N.S	FIII Vs FVII	= 2.05 N.S
FI Vs FIII	= 0.91 N.S		
FI Vs FIV	= 20.05 N.S	FIII Vs Casein control	= 2.93 *
FI Vs FV	= 1.67 N.S		
FI Vs FVI	= 0.86 N.S	FIV Vs FV	= 2.21 N.S
FI Vs FVII	= 1.57 N.S		
FI Vs Casein control	= 2.75 *	FIV Vs FVI	= 0.73 N.S
		FIV Vs FVII	= 0.24 N.S
FII Vs FIII	= 1.09 N.S	FIV Vs F.C.C	= 0.70 N.S
FII Vs FIV	= 1.63 N.S		
FII Vs FV	= 1.83 N.S	FV Vs FVI	= 2.11 N.S
FI Vs FVI	= 0.60 N.S	FV Vs FVII	= 2.88 *
FII Vs FVII	= 1.23 N.S	FV Vs F.C.C	= 4.03 **
FII Vs F.C.C	= 2.29 *	FVI Vs FVII	= 0.48 N.S
FIII Vs FIV	= 2.10 N.S	FVI Vs C.C	= 1.28 N.S
FIII Vs FV	= 0.44 N.S	FVII Vs F.C.C	= 0.86 N.S
FIII Vs FVI	= 1.47 N.S		

* Significant at five per cent level

** Significant at one percent level

N.S = Not significant . F.C.C = Casein control.

APPENDIX VIII

DIGESTIBILITY COEFFICIENT OF DIFFERENT DIETS PER RAT

Rat No	Diet I			Diet II			Diet III			Diet IV		
	N_2 Int	N_2 Abs	D.C	N_2 Int	N_2 Abs	D.C	N_2 Int	N_2 Bas	D.C	N_2 Int	N_2 Abs	D.C
1.	757.58	500.00	66.00	1142.74	708.65	62.00	779.38	374.10	48.00	1095.58	723.08	66.00
2.	853.67	700.00	82.00	915.38	732.30	80.00	1171.60	585.80	50.00	614.13	565.00	92.00
3.	620.45	546.00	88.00	568.18	500.00	88.00	970.66	737.70	76.00	714.37	621.50	87.00
4.	704.23	500.00	71.00	657.89	500.00	76.00	647.05	555.60	86.00	526.32	500.00	95.00
5.	656.98	565.00	86.00	627.01	557.15	89.0	633.41	519.40	82.00	978.09	880.28	90.00
6.	581.40	500.00	86.00	923.53	720.35	78.0	728.64	641.20	88.00	776.67	691.24	89.00

(Cont.....d)

Diet V			Diet VI			Diet VII			Casein control		
N_2Int	N_2Abs	D.C	N_2Int	N_2 Abs	D.C	N_2Int	N_2 Abs	D.C	N_2Int	N_2Abs	D.C
801.67	432.90	54.00	918.49	486.80	53.00	750.00	555.00	74.00	709.65	546.43	77.00
695.92	528.90	76.00	687.59	543.20	79.00	881.14	696.16	79.00	756.63	696.10	92.00
667.30	593.90	89.00	583.15	628.50	92.00	820.56	730.30	89.00	733.49	667.48	91.00
869.84	548.00	63.00	760.35	646.30	85.00	602.89	542.60	90.00	620.78	554.27	112.00
973.54	632.80	65.00	754.51	618.70	82.00	646.13	600.90	93.00	604.47	556.11	92.00
802.09	537.40	67.00	693.15	616.90	89.00	710.34	625.10	88.00	700.22	623.20	89.00

SUMMARY OF STATISTICAL ANALYSIS FOR D.C

Diet	't' value	Diet	't' value
FI Vs FII	= 0.18 N.S	FIII Vs FVII	= 1.74 N.S
FI Vs FIII	= 0.99 N.S	FIII Vs F.C.C	= 2.36 *
FI Vs FIV	= 1.18 N.S		
FI Vs FV	= 1.75 N.S	FIV Vs FV	= 0.90N.S
FI Vs FVI	= 0.02 N.S	FIV Vs FVI	= 0.91 N.S
FI Vs FVII	= 1.18 N.S	FIV Vs FVII	= 0.19 N.S
FI Vs F.C.C	= 2.23 *	FIV Vs F.C.C	= 0.94 N.S
FII Vs FIII	= 0.85 N.S	FV Vs FVI	= 1.45 N.S
FII Vs FIV	= 1.31 N.S	FV Vs FVII	= 2.85 *
FII Vs FV	= 1.55 N.S	FV Vs F.C.C	= 3.43 * *
FII Vs FVI	= 0.17 N.S	FVI Vs FVII	= 0.85 N.S
FII Vs FVII	= 1.33 N.S	FVI Vs F.C.C	= 1.67 N.S
FII Vs F.C.C	= 2.24 *	FVII Vs F.C.C	= 1.21 N.S
FIII Vs FIV	= 1.74 N.S		
FIII Vs FV	= 0.30 N.S		
FIII Vs FVI	= 0.89 N.S		

F.C.C = Casein control

* Significant at five percent level

N.S = Not significant

** = Significant at one percent level

APPENDIX IX

BIOLOGICAL VALUE OF DIFFERENT DIETS PER RAT

No	Diet I			Diet II			Diet III			Diet IV		
	N_2Int	N_2 Ret	B.V	N_2Abs	N_2Ret	B.V	N_2Abs	N_2 Rat	B.V	N_2Abs	N_2 Rat	B.V
1.	500.00	300.00	60.00	708.65	450.00	63.50	371.3	134.3	35.90	723.08	470.00	65.00
2.	700.00	350.70	50.10	732.30	400.00	54.62	585.8	271.4	46.33	565.00	397.00	70.25
3.	546.00	398.00	73.00	500.00	400.25	80.05	737.7	557.7	75.60	621.50	500.00	80.45
4.	500.00	301.78	60.36	500.00	301.78	60.36	555.6	368.2	66.27	500.00	352.50	75.00
5.	565.00	397.00	70.24	557.15	397.00	71.25	519.4	330.7	63.67	880.28	750.00	85.20
6.	500.00	301.45	60.30	720.35	450.00	62.47	641.2	343.2	53.52	691.24	450.00	65.10

No. 1.	Diet V N_2Abs	N_2Ret	B.V	Diet VI N_2Abs	N_2 Ret	B.V	Diet VII N_2Abs	N_2Ret	B.V	Casein control N_2Abs	N_2Ret	B.V
1.	432.90	259.60	59.97	486.80	304.30	62.51	555.00	339.40	61.15	546.43	356.00	65.15
2.	528.90	378.50	71.65	543.20	316.40	58.25	696.10	502.60	72.20	696.10	502.60	72.20
3.	593.90	362.00	60.95	628.50	534.30	85.01	730.30	596.30	81.65	667.48	545.00	81.65
4.	548.00	186.90	34.11	646.30	385.20	59.60	542.60	306.60	56.60	620.78	469.00	75.55
5.	632.80	350.60	55.40	618.70	548.4	88.64	600.90	540.31	89.91	556.11	500.00	89.91
6.	537.40	250.50	46.61	616.90	427.10	69.23	625.10	418.10	66.88	623.20	410.00	65.79

SUMMARY OF STATISTICAL ANALYSIS FOR B.V

Diet	't' values	Diet	't' values
FI Vs FII	= 0.62 N.S	FIII Vs FVI	= 1.71 N.S
FI Vs FIII	= p.80 N.S	FIII Vs FVII	= 1.85 N.S
FI Vs FIV	= 2.34 *	FII Vs F.C.C	= 2.56 *
FI Vs FV	= 1.20 N.S		
FIV Vs FVI	= 1.29 N.S	FIV Vs FV	= 2.98 *
FI Vs FVII	= 1.47 N.S	FIV Vs FVI	=0.46 N.S
FI Vs F.C.C	= 2. 47 *	FIV Vs FVII	= 0.34 N.S
FII Vs FIII	= 1.23 N.S	FIV Vs F.C.C	= 0.36 N.S
FII Vs FIV	= 1.64 N.S	FV Vs FVI	= 2.08 N.S
FII Vs FV	= 1.65 N.S	FV Vs FVII	= 2.10 N.S
FII Vs FVI	= 0.79 N.S	FV Vs F.C.C	3.08 *
FII Vs FVII	= 0.95 N.S		
FII Vs F.C.C	= 2.23 *	FVI Vs FVII	= 0.11 N.S
		FVI Vs F.C.C	= 0.68 N.S
FIII Vs FIV	= 2.44 *	FVII Vs F.C.C	= 0.57 N.S
FIII Vs FV	= 0.27 N.S		

F.C.C = Casein control

* Significant at five per cent level

N.S. Not significant