MATERIALS AND METHODS

Nine varieties of groundnut viz., Punjab Groundnut No. 1, 145/12-P, 142/16, 321/2, 69/9, 5/10, 511/28, A 20 and A 23 were selected from among several varieties on the basis of their high oil content and yield per acre, for studying the amino acid composition and the nutritive value of defatted meals. Representative samples of these varieties were grown under identical conditions at the Experimental Farm of the College of Agriculture, Ludhiana.

The origin of these varieties is shown below:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Variety</th>
<th>Origin</th>
<th>Habit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>321/2</td>
<td>$D_3 \times A.H. 477$</td>
<td>Spreading</td>
</tr>
<tr>
<td>2.</td>
<td>511/28</td>
<td>$D_3 \times A.H. 477$</td>
<td>Spreading</td>
</tr>
<tr>
<td>3.</td>
<td>145/12-P</td>
<td>$A_1 - 1 \times D_3$</td>
<td>Spreading</td>
</tr>
<tr>
<td>4.</td>
<td>142/16</td>
<td>$A_1 - 1 \times D_3$</td>
<td>Spreading</td>
</tr>
<tr>
<td>5.</td>
<td>69/9</td>
<td>$A_1 - 1 \times D_3$</td>
<td>Spreading</td>
</tr>
<tr>
<td>6.</td>
<td>Punjab Groundnut No. 1</td>
<td>Single plant selection from local collection</td>
<td>Spreading</td>
</tr>
<tr>
<td>7.</td>
<td>5/10</td>
<td>P.G.No.1 x A.H. 259</td>
<td>Spreading</td>
</tr>
<tr>
<td>8.</td>
<td>A 20</td>
<td>Natal common x Aut(P2-1)</td>
<td>Erect</td>
</tr>
<tr>
<td>9.</td>
<td>A 23</td>
<td>Philippine Pink x Natal Common (P2 - 2-4)</td>
<td>Erect</td>
</tr>
</tbody>
</table>

$D_3 = $ Virginia runner

A.H. 477 = (Arachis hypogaea) from Madras

$A_1-1 = $ Mutant from variety Akola from Madhya Pradesh, India.

A.H. 259 = (Arachis hypogaea) from Madras

Aut = South African variety
The first seven varieties were taken from the varietal trial No. 1 while the remaining two were selected from the varietal trial No. 2. Particulars of the field experiments designed to test the varieties are summarised in Table 5.

**Table 5**

**Particulars of Varietal Trials**

<table>
<thead>
<tr>
<th>Crop and Year</th>
<th>Experiment No.</th>
<th>Number of Varieties</th>
<th>Replicates</th>
<th>Date of Sowing</th>
<th>Date of Harvesting</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960</td>
<td>1</td>
<td>10</td>
<td>6</td>
<td>29th June, 1960</td>
<td>1st Dec., 1960</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>32</td>
<td>6</td>
<td>30th June, 1960</td>
<td>28th Nov., 1960</td>
</tr>
<tr>
<td>1961</td>
<td>1</td>
<td>10</td>
<td>6</td>
<td>10th July, 1961</td>
<td>1st Dec., 1961</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>32</td>
<td>6</td>
<td>3rd July, 1961</td>
<td>1st Dec., 1961</td>
</tr>
</tbody>
</table>

**Design**
- Randomized block

**Plot size**
- 1/80th acre

**Soil**
- Sandy loam

**Fertilizer**
- 25 lbs. Nitrogen per acre

The crop was grown under rainfed conditions. Total rainfall during the crop period was 15.6 inches in 1960 and 12.9 inches in 1961.

Ammonium sulphate was applied as a top dressing. Samples of nine varieties were collected for the purpose of these investigations from four out of six replicates, in each case.

Kernels were removed from the pods and shrivelled immature seeds were rejected. In all, 35 samples each year from these nine varieties representing four replications of each variety were used in the investigations.
Preparation of samples: Weighed aliquots of the kernels, in triplicates, were ground in a pestle and mortar and defatted with petroleum ether (40° - 60°C) in a soxhlet apparatus. The defatted samples were ground finely to pass through 40 mesh sieve and preserved in air tight containers for use as required.

For the determination of nutritive value of the samples only composite sample of each variety was used.

After preliminary trials with various techniques of amino acid determination, the ion-exchange chromatographic method of Moore, Spackman and Stein (1958) was adopted because of its accuracy and reproducibility. Table 6 indicates various procedures which were used to determine 19 amino acids in different samples of groundnut.
Table 6

Procedures followed for amino acid determination

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Neutral and Acidic</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid, threonine,</td>
<td>Ion-exchange chromatographic technique. Moore, Spackman and Stein (1958). Column dimensions: 0.9 x 166 cms.</td>
</tr>
<tr>
<td>serine, glutamic acid,</td>
<td></td>
</tr>
<tr>
<td>proline, glycine, alanine,</td>
<td></td>
</tr>
<tr>
<td>valine, isoleucine, leucine,</td>
<td></td>
</tr>
<tr>
<td>tyrosine and phenylalanine.</td>
<td></td>
</tr>
<tr>
<td>2. Basic</td>
<td></td>
</tr>
<tr>
<td>Lysine, histidine, arginine</td>
<td>Ion-exchange chromatographic technique. Moore et al. (1958). Column dimensions: 0.9 x 30 cms.</td>
</tr>
<tr>
<td>and ammonia.</td>
<td></td>
</tr>
<tr>
<td>4. Methionine</td>
<td>Chemical.</td>
</tr>
<tr>
<td>5. Tryptophan</td>
<td>Steers and Sevag (1949)</td>
</tr>
</tbody>
</table>

Ion-exchange chromatographic method

Operational details of the method of Moore et al (1958) as followed in the present investigations, are described below:

Reagents: Analytical grade chemicals were used.

(a) Buffers: The composition of different buffer solutions is shown in Table 7.
Composition of the various buffers used for eluting the amino acids

<table>
<thead>
<tr>
<th>pH</th>
<th>Sodium concentration</th>
<th>Citric Acid H₂O₂ g.</th>
<th>NaOH g.</th>
<th>HCl Conc. ml.</th>
<th>Final Volume litres</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.20 ± 0.03</td>
<td>0.20 N</td>
<td>105.0</td>
<td>42.0</td>
<td>80.0</td>
<td>5.0</td>
</tr>
<tr>
<td>3.25 ± 0.01</td>
<td>0.20 N</td>
<td>84.0</td>
<td>33.0</td>
<td>42.6</td>
<td>4.0</td>
</tr>
<tr>
<td>4.25 ± 0.02</td>
<td>0.02 N</td>
<td>84.0</td>
<td>33.0</td>
<td>18.8</td>
<td>4.0</td>
</tr>
<tr>
<td>5.28 ± 0.02</td>
<td>0.35 N</td>
<td>98.2</td>
<td>57.6</td>
<td>27.2</td>
<td>4.0</td>
</tr>
</tbody>
</table>

The pH of the buffers were checked with Beckman pH meter and adjusted, if necessary, with concentrated hydrochloric acid or with 50 per cent sodium hydroxide.

(b) Hydrindantin: To 20 grams of ninhydrin dissolved in 500 ml. distilled water at 90°C was added with stirring a solution of 20 grams of ascorbic acid in 100 ml. of distilled water at 40°C. Crystallization of hydrindantin (reduced form of ninhydrin) started immediately and was allowed to proceed for 30 minutes without further heating. During the next hour the solution was cooled to room temperature, under running tap water. The crystals of hydrindantin were filtered off, washed five to seven times with distilled water and then dried over P₂O₅ in a vacuum desiccator, protected from light, for 24 hours. It was then stored in a dark coloured stoppered bottle.

(c) Sodium Acetate buffer pH 5.5 (Moore and Stein, 1954): 544 grams of sodium acetate were dissolved in 700 ml. of distilled water by warming on a water-bath. The solution was cooled to room temperature and 100 ml. of glacial acetic acid were added to it
and the volume made up to one litre. The solution was filtered and its pH was checked with Beckman pH meter and adjusted to pH 5.5 by the addition of 50 per cent sodium hydroxide or glacial acetic acid, as desired.

(d) Methyl cellosolve (Ethylene glycol monomethyl ether): Methyl Cellosolve was redistilled in all glass pyrex distillation apparatus. It was tested for the presence of peroxides by adding 2 ml. of methyl cellosolve to one ml. of freshly prepared 4 per cent aqueous potassium iodide. A colourless to light straw yellow colour indicated the absence of peroxide.

(e) Colour developing reagent: 20 grams of ninhydrin and 3 grams of hydridantin were dissolved in 750 ml. of methyl cellosolve. 250 ml. of sodium acetate buffer of pH 5.5 was then added to it. This reagent is unstable and is kept in a dark coloured stoppered flask flushed with nitrogen gas. The reagent was prepared daily for use.

Hydrolysis of the Samples

250 mg. of defatted samples of each variety were hydrolysed with 200 ml. of constant boiling hydrochloric acid (redistilled in all glass pyrex apparatus) for 24 hours under reflux. The excess acid was removed by evaporation to near dryness followed by further evaporation of three small volumes of added distilled water. The humin was removed by filtration, washed well with distilled water and volume made to 100 ml. Unless immediately used, the hydrolysates were frozen and stored.
were transferred to a jar containing 10 litres of distilled water. The mixture was stirred and the resin present in the foam was transferred to a beaker, treated with little of acetone and then added back to the main suspension. After thorough distribution of the resin in water, it was allowed to settle for about six hours. The supernatant which contained very fine particles of resin was decanted off. The resin was resuspended and resettled three to four times, then passed through a standard 300 mesh sieve with the help of distilled water. The fraction that passed through the sieve was transferred to a large Buchner funnel (with a filter paper pad on it) and washed slowly with 2 litres of 4 N HCl followed by 500 ml. of distilled water. The moist resin was again suspended in one litre of 2 N NaOH, heated on a steam bath for one hour, transferred to a large Buchner funnel having a filter paper pad on it and washed well with distilled water until neutral to litmus paper. The resin was taken up in 0.2 N NaOH solution so that the volume of supernatant was twice that of resin sediment.

Preparation of Column for Neutral and Acidic Amino Acids

The column was prepared in five to six sections, using the resin slurry prepared according to the
method described earlier. To start with, the stop cock of the column was closed and as soon as the slurry was poured in, the outlet was opened. When about 2 cms. of resin bed was formed due to gravity settlement air pressure of 5 lbs. psi was applied at the top of the column from an air-compressor. After the resin had settled to a constant height, the supernatant was withdrawn by suction and then the next section was poured on the top of the firm surface. More slurry was then added and operation repeated so that the final height of the resin was built up to about 155 cms. to allow for compacting caused during the operation of the column. The column was equilibrated with 150 ml. boiled buffer of pH 3.25 after which it was ready for use.

Operation of the column: Water at 50°C ± 0.1°C from a constant temperature water-bath was circulated in the outer jacket of the column. After about one hour, one ml. of hydrolysate solution which represented 2.5 mg. protein, was introduced at the top of the column and an air pressure of 6 lbs. psi was applied until the solution entered the top layers of the resin in the column. The air pressure was removed followed by three successive additions of 0.5 ml. sodium citrate buffer (pH 3.25) with a view to washing the hydrolysate adhering to the sides of the column. 250 ml. separating funnel containing the buffer (pH 3.25) was mounted on the top of the column and air pressure of 6 lbs. psi was continuously applied to maintain the flow of the effluent at the rate of 12 ml. per hour. The effluent was collected in 2 ml. fractions by means of a syphon (2 ± 0.02 ml.). Approximately 220-240 ml. of the effluent was collected before introducing the new buffer of
pH 4.25. At this stage valine emerged first with the new buffer. The change was made at an effluent volume 2.15 times that at which the aspartic acid peak had emerged. In all about 240-250 fractions (480-500 ml. effluent) were collected.

To regenerate the column for next test, the basic amino acids retained by the resin were first eluted with 100 ml. of 0.2 N NaOH at the room temperature, using air pressure of 6 lbs. psi. The column was repoured and equilibrated with 150 ml. of sodium citrate buffer (pH 3.25) each time a new sample was assayed. This was necessary because of considerable packing of the resin and for obtaining reproducible results.

Analysis of the effluent fractions: To each 2 ml. fraction of the effluent was added one ml. of ninhydrin-hydramide reagent, and the tubes were kept in boiling water-bath for exactly fifteen minutes for developing colour in them. The tubes were then cooled to about 20°C so as to avoid fading of colour, specially during the summer months when the room temperatures are high. The volume of the coloured media was made to 10 ml. in each case with 50 per cent alcohol. With a set of 24 tubes, a three point cure of L-leucine, after reaction with ninhydrin-hydramide reagent and appropriate dilution, was plotted for comparison of the intensity of colours in the unknown solutions in the tubes. The intensity of colour (absorbance) was measured in the Klett-Summerson photo-electric colorimeter using 520 nm. filter except in the case of proline where 440 nm. filter was used.
Recovery experiments using mixture of pure amino acids and then mixture of amino acids along with unknown samples after hydrolysis were also run.

**Preparation of Column for Basic Amino Acids.**

Thick walled chromatographic glass tube 30 cms. in length above the sintered plate fitted with water-jacket was used for the preparation of the chromatographic column. The column was poured with a suspension of Amberlite IR - 120 (finer than 300 mesh) in 0.35 N sodium hydroxide. The resin was poured in three sections of about 6 cms. each. The remaining procedure was the same as described in the preparation of 166 cms. column with the exception that an air-pressure of 2 lbs. psi was applied each time the resin slurry was poured. The column was equilibrated with about 30 ml. of sodium citrate buffer (pH 5.28) before it was used.

**Operation of the column:** The column was mounted on a mechanical fraction collector and water from a constant temperature water bath at 50\° ± 1\°C was circulated in the outer jacket. After about an hour, one ml. of aliquot of the hydrolysate representing 2.5 mg. of protein was introduced at the top of the column and air pressure of 2 lbs. psi was applied until the solution penetrated into the top portion of the resin, followed by three successive washings each time with 0.5 ml. sodium citrate buffer (pH 5.28) to ensure complete entrance of the hydrolysate into the column. Elution was carried out with sodium citrate buffer (pH 5.28) at 50\° ± 1\°C using a continuous air pressure of 2 lbs. psi. The rate of the effluent was maintained at about 24 ml. per hour. In
earlier attempts using column of wider diameter than the prescribed 0.9 cm. internal diameter, as many as 120, 2 ml. fractions had to be collected for efficient separation of various basic amino acids and ammonia. However, when column of 0.9 cm. internal diameter was used, it was found that about seventy, 2 ml. fractions were sufficient for complete separation of the basic amino acids and ammonia.

Regeneration of the column was carried out at room temperature by passing 10 ml. of 0.35 N NaOH through the column under an air pressure of 2 lbs. psi followed by 100 ml. of sodium citrate buffer (pH 5.28) when the column was ready for reuse. Repouring of the column was done after every two runs of the sample. Colour in each of the seventy fractions was developed by using one ml. ninhydrin hydrindantin reagent as previously described.

**Cysteine and Cystine**

The ion-exchange chromatographic method of Schram et al. (1954) as modified by Vervaek (1960) was used for determining cysteine and cystine. The modification consisted in using Amberlite IR-120 in place of Dowex X-8 used originally by Schram et al. This modification enabled the determination of these amino acids in about 3 hours as compared with 24 hours required when Dowex X-8 was used. Both the amino acids were first oxidized to cysteic acid using performic acid as follows:

**Performic acid**: Prepared by the addition of one volume of 30 per cent hydrogen peroxide to 9 volumes of 88 per cent formic acid. The solution was allowed to stand for one hour at room temperature to permit maximum formation of performic
acid.

50 ml. of this reagent, previously cooled to 0°C, were added to each of the 250 mg. aliquot of defatted test samples and the oxidation was allowed to continue for sixteen hours in a refrigerator maintained at 4°C. The excess reagent was removed under reduced pressure at 40°C and the residue was immediately suspended in 200 ml. constant boiling hydrochloric acid and refluxed for 24 hours. Excess hydrochloric acid was removed as described earlier. The residue was taken up in sodium citrate buffer (pH 2.2) with which the volume was made to 50 ml. and then filtered. One ml. aliquot was taken for analysis.

Pure samples of cysteine and cystine (1-2 mg.) were oxidized with a 25 ml. of performic acid but for 4 hours at 0°C. The excess of reagent was removed under reduced pressure at 40°C. The syrupy residue was taken up in water and volume made to 10 ml. with distilled water. One ml. aliquot was introduced at the top of the column.

Preparation of Column: The column was set up in 4-5 sections with the resin slurry in 0.2 N sodium hydroxide in which the volume of the supernatant solution was twice that of settled resin. The column was equilibrated with 100 ml. sodium citrate buffer (pH 3.25). The column was operated at 50°C ± 1°C with a continuous air pressure of 6 lbs. psi, at a flow rate of about 12 ml. per hour. Elution was carried out with sodium citrate buffer (pH 3.25). About twenty, 2 ml. fractions were collected and the colour developed with ninhydrin-hydriodinantin reagent and absorbance measured in each case, as described previously.
Expression of Results

Leucine equivalent x Molecular weight in micromoles
in micromoles

= of amino acid

Colour yield

(Moore and Stein, 1951).

Methionine

In the determination of methionine by ion-exchange chromatographic method of Moore, Spackman and Stein (1958) use of thiourea as an antioxidant is essential. As it was not possible to obtain this reagent in the country, the method of Horns, Jones and Blum (1946) described below was used for its determination.

Reagents: 1. 5 N sodium hydroxide
2. 10 per cent sodium nitroprusside (freshly prepared)
3. 3 per cent glycine
4. 85 per cent phosphoric acid

Procedure: To 2 ml. of acid hydrolysate (containing approximately 200 mg. of protein) in a test tube was added 3 ml. of water, one ml. of 5 N NaOH and 0.1 ml. of freshly prepared 10 per cent solution of sodium nitroprusside. The mixture was shaken for 10 minutes. 2 ml. of 3 per cent glycine solution was then added and again shaken for 10 minutes. Then 2 ml. of 85 per cent phosphoric acid was added slowly drop by drop with constant shaking. The absorbance was measured in the Klett-Summerson photo-electric colorimeter, using 540 μm filter.

Recoveries of added DL-methionine in the
test samples were also studied by subjecting them to hydrolysis with constant boiling hydrochloric acid.

Standard Curve: A standard curve of methionine was plotted by using standard solutions containing 200 to 1000 microgram per ml. of methionine. Higher concentrations of methionine were selected as the intensity of colour below 200 microgram was very low.

Tryptophan

500 mg. of each sample were hydrolysed with 5 N sodium hydroxide for 5 hours under reflux as suggested by Block and Bolling (1951). The details of the method are given below:

Reagents: 1. 5 per cent p-dimethyl aminobenzaldehyde in 10 per cent sulphuric acid.
2. 2 per cent sodium nitrate solution
3. Fuming hydrochloric acid
4. 17.5 per cent hydrochloric acid

Procedure: To one ml. of alkaline hydrolysate in a test tube was added 0.1 ml. of p-dimethyl aminobenzaldehyde solution, followed by 0.04 ml. of sodium nitrate solution and mixed well. Then 5 ml. of fuming hydrochloric acid were added and the contents heated for one minute in a 50°C water-bath, and allowed to stand at room temperature for 15 minutes. The solution was diluted to 10 ml. with 17.5 per cent hydrochloric acid and allowed to stand for additional 15 minutes. The intensity of the blue colour was measured in the Klett Summerson photo-electric colorimeter using 560 mm filter.

During the summer months, when room
temperatures were high (37°-40°C) it was observed that the
colour was fully developed. However, during winter months
when the room temperatures were low (10°-15°C) it was
necessary to carry out the reaction in a 40°C hot water-bath
for 15 minutes before the addition of 17.5 per cent
hydrochloric acid.

Recoveries of L-tryptophan were also studied
by adding known amounts of this amino acid to the test sample
and then subjecting them to hydrolysis with 5 N sodium
hydroxide, as in the case of samples of groundnut.

Standard Curve: A standard curve of L-tryptophan was
prepared from solutions containing 10 to 50 microgram per ml.
of L-tryptophan in 1 per cent casein hydrolysate.

B. Effect of Variety on the Nutritive Value of Groundnut Protein

The nutritional quality of groundnut proteins
as influenced by variety was evaluated using albino rats as
the test animals. Experiments were designed to determine
various quality characteristics of the protein such as
Biological Value (BV) and digestibility coefficient (DC)
in accordance with the balance-sheet method of Mitchell
(1923-24).

Preparation of diets: Composite samples of each variety
were deoiled by means of a hand press and analysed for
nitrogen and oil contents. The residual oil in the meals
ranged from 11.6 to 15.6 per cent. Calculated amounts of the
respective meals were used for the preparation of diets as shown
in Table 8.
Feeding of diets: The experimental diets were fed ad lib. Unconsumed residues were collected daily, oven dried at 105°C for eight hours, weighed and the amount of diet consumed was calculated for each rat.

Distilled water was provided for drinking, in 150 ml. volumetric flasks. A jet was inserted into the neck of the flask to allow easy licking of water.

All the ten groups were first fed low nitrogen diet No. XI containing 4 per cent egg powder for a period of eleven days. The various groups were then assigned the respective experimental diets and the reference diet for 31 days after which low nitrogen diet having 4 per cent egg powder was fed again to each group for a period of eleven days more.

Collection of faeces and urine: Arrangement for the collection of urine and faeces separately is shown in Fig. 1. The faeces after passing through the screen rolled over the big funnel, striking the bulb and then going into the receptacle. The urine, likewise, after flowing along the funnel walls trickled on to the bulb and flowed into a small bottle placed underneath the bulb. A small metallic screen was interposed between the glass funnel and the beaker to avoid spilling of extra feed or faeces.

During the first phase of the experiment when low nitrogen diet was fed for eleven days, no faeces or urine were collected for the first three days treating it as non-experimental period. The urine and faeces were collected only during the remaining 8 days. In the second phase of the experiment when experimental and reference egg diets were fed
for 31 days, no collection was made in the first three days. Urine and faeces were collected for the rest of the twenty eight days. The diets contained approximately 10 per cent protein on dry basis and were prepared by intimately mixing the various ingredients. The diet No. X based on egg powder served as reference standard while diet No. XI which contained 4 per cent egg powder was used for determining the endogenous excretions of nitrogen. The diets were adequate with respect to vitamin and mineral requirements and were analysed for moisture and protein contents by the A.O.A.C methods.

Egg powder was prepared in the laboratory by boiling whole eggs followed by removal of shell and washing the edible portion with acetone before drying and powdering.

Albino rats of practically the same age group around 30 days old with average group weights ranging from 40 - 50 grams were distributed into randomized groups on the basis of initial weights of the animals. There were equal number of male and females in each group and were housed in individual metabolism cages of the following description.

Metabolism cages: The dimensions of the metabolism cages were: height 8 inches and diameter 8 inches. The cages were placed on tripod stands about 10 inches high and a little more than 8 inches in diameter so that glass funnel of 8 inches diameter could exactly be fitted under the cage. The outer-edges of the cage were within the periphery of the glass funnel so that no faeces or urine could fall outside. The bottom of the cage was made of wire net. A metallic tube of about 14 inches diameter was fitted near the bottom of
the cage and it was bent at an angle of 75°. A round screw clamp holding a 100 ml. polyethylene beaker was fixed with this tube. The metallic tube was perforated on the upperside for the aeration of the chamber. The diet was easily accessible to the rat and at the same time this arrangement minimized spilling.

In the third phase of the experiment when all the groups were changed to feeding with low protein diet for eleven days, the urine and faeces were collected for the latter eight days only as during the first phase of feeding with a similar diet. The urine and faeces were preserved with sulphuric acid and absolute alcohol respectively, and analysed for nitrogen content by Kjeldahl method.

The nitrogen excretion of animals fed low nitrogen egg diet furnished the amount of endogenous excretion of nitrogen. From the intake, absorption and excretion of nitrogen, the Biological value and Digestibility coefficient were calculated as follows:

Biological value of protein (B.V.) =

\[
\frac{\text{Nitrogen intake} - (\text{Urinary } N - \text{Endogenous } N) - (\text{Faecal } N - \text{Metabolic } N)}{\text{Nitrogen intake}} \times 100
\]

Digestibility Coefficient (DC) =

\[
\frac{\text{Nitrogen intake} - (\text{Faecal } N - \text{Metabolic } N)}{\text{Nitrogen intake}} \times 100
\]

Where Nitrogen intake = Nitrogen in the diet actually taken by the animal.

Urinary N = Nitrogen in the urine during the experimental period.

Faecal N = Nitrogen in the faeces during the experimental period.

Endogenous N = The urinary nitrogen due to catabolism of the body, determined by feeding non-protein diet or nitrogen free diet.

Metabolic N = The nitrogen in the faeces due to catabolism of the body, determined by feeding non-protein diet or nitrogen free diet.
Determination of Protein Efficiency Ratio (PER)

The rat growth method described by Campbell (1961) was used for determining the protein efficiency ratio of the groundnut varieties at a 10 per cent level of protein in the diets. The diets were adequate in respect of vitamin and mineral requirements of the growing rats. The composition of the test and standard diets is given in Table 8.

Four randomized groups of ten weanling male albino rats 21-23 days old, were fed ad lib, for a period of four weeks, during which weekly increases in weight and total food consumption was recorded. The initial mean group weight ranged from 24.0 to 24.5 gms. The animals were housed in individual cages with wire screen bottoms.

The PER was calculated as follows:

\[
\text{PER} = \frac{\text{Gain in wt. in g.}}{\text{Protein consumed, g.}}
\]