Studies
On some important amino acid composition of a few poultry feeds & poultry products.

Thesis
Submitted to the faculty of Veterinary Science and Animal husbandry, Rajendra Agricultural University, in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (ANIMAL HUSBANDRY)
1971

BY
Bhaba Nand Thakur
B.V. Sc, B.A. H.
Post-Graduate Department of Animal Nutrition,
BIHAR VETERINARY COLLEGE,
PATNA.
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BY
Bhaba Nand Thakur
B.V. Sc & A.H.
Post-Graduate Department of Animal Nutrition, BIHAR VETERINARY COLLEGE, PATNA.
Department of Animal Nutrition, Bihar Veterinary College, PATNA

I certify that this Thesis has been prepared under my supervision by Sri B.N. Thakur, a candidate for the M.Sc. (A.H.) with animal nutrition as major subject, and that it incorporates the results of his independent study.

D. B. Mukherjee 9/3/72
( D.B. Mukherjee )
G.V.Sc., M.Sc., Ph.D., Professor of Animal Nutrition, Bihar Veterinary College, PATNA.
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wishers whose inspiration and encouragement goded me to complete
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Bhaba naid Jekus

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CHAPTER I

INTRODUCTION
INTRODUCTION

No field of agriculture has made more outstanding progress in recent times than in the nutrition of Farm Livestock, and the knowledge continues to grow as the result of investigations in many research institutions throughout the world. This activity is part of the expression of man's concern with the quality of his life. As his economic circumstance improves, there is accompanying intensification in the scale of his livestock production and accompanying increase in feed conversion.

In new developing countries three main factors affect the relationship between the population and available food supply: the expanding health services; the growth of literacy and the efforts to raise the standard of living. To maintain the already insufficient food supply for a rapidly growing population, the present high level of animal protein in the diets of countries with a high standard of living is produced at the expense of many times the energy value in crops on which the stock is fed. High quality animal protein or its equivalent in amino acid composition in plant proteins is essential for a balanced diet. It is however the supply of essential amino acid that is important. Its consumption by adults can be done without any danger to health provided there is sufficient of essential amino acid and Vitamin B₁₂ in the diet; animal protein is a great asset in making of attractive dishes.

Different classes of non ruminant livestock have different requirements for some of these, and in the bird glycine
cannot be synthesized sufficient rapidly. Since the protein keratin of feathers has a large proportion of glycine in the molecule. Glycine followed by methionine, threonine and tryptophane are the most likely essential amino acids to be limiting growth and reproduction through insufficiently in the protein of of the diet. Their deficiency can be made good by adding the limiting amino acids to the rest of the food. In general, animal proteins are much superior to plant proteins as source of essential amino acids; their biological value is greater. Protein is essential for all plant and animal life as component of active protoplasm of each active cells and its nucleus. In plants the protein is largely concentrated in the actively growing portions such as leaves and the reproductive portions e.g. seeds, plants have the ability to synthesize their own proteins from relatively simple compounds such as carbon dioxide, water, nitrates and utilize atmospheric nitrogen in place of nitrates. Plants are therefore the basic source of proteins.

In animal the proteins display a much greater diversity of function than in plants. For example protein is a primary constituents of many structural and protective tissues, such as bones, ligaments, hair, nails and skin, as of the soft tissues which includes the organs and the muscles. Animals lack the ability of the plants to synthesize protein from simple material and must depend upon the plants or other animal as a source of dietary protein.

Since proteins are continually being utilized by the animal, either to build new tissues, as in growth and reproduction
or for repair of worn out tissues, a fairly regular dietary intake of protein is essential. If adequate proteins are lacking in the diet, there is retardation or even permanent cessation of growth or a loss of weight and finally there is a withdrawal of body proteins to maintain the vital functions and requirements of tissues.

In common with the fats and carbohydrates, the proteins contain carbon, hydrogen and oxygen. In addition they contain a large and fairly constant percentage of nitrogen. Most of them also contain sulfur and a few contain phosphorus and iron. They are complex substances, colloidal in nature and of high molecular weight. The range of elementary composition of a more typical proteins is as follows:

<table>
<thead>
<tr>
<th>Element</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>51.0 to 55.0</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>6.5 to 7.3</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>15.5 to 18.0</td>
</tr>
<tr>
<td>Oxygen</td>
<td>21.5 to 23.5</td>
</tr>
<tr>
<td>Sulfur</td>
<td>0.5 to 2.0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.0 to 1.5</td>
</tr>
</tbody>
</table>

(Quoted from Animal Nutrition by Maynard & Loosli, 1969)

The recognition that the nitrogen present in the body had its origin in nitrogen compounds present in the diet dates primarily from the work of Magendie (1816). After it became established that proteins were the nitrogen compounds essentially concerned, Magendie produced the first evidence, that all proteins were not of equal value. In his famous "gelatin report"
published in 1841, he showed that gelatin would not take the place of meat protein in the diet.

The finding of Magendie was taken up in the form of growth and nitrogen balance studies by German, Swiss and Danish Scientist. Hermann (1870) a German physiologist, first gave the satisfactory explanation as to why protein differ in nutritional quality. In 1876 Escher, a Swiss physiologist fed dogs on a purified diet contained gelatin, which caused them to lose weight. The weight was however maintained when tyrosine was added. The amino acids analysis of protein by Abderhalden lead Kauffmann towards a more meaningful studies in 1905, where in he showed that cystine in which gelatin was low was needed along with tyrosine as a supplement.

Next work on proteins was taken up by more exact methods. Willock and Hopkins while using purified diets found that mice getting zein as the sole protein ingredient, died, others receiving casein survived. Zein when supplemented with tryptophane, isolated and identified by Hopkins in 1901 gave the opposite effect. In 1909 Osborne and Mendel in the United States, while doing purified diet experiments using pure proteins found that certain proteins which caused nutritive failure could be rendered efficient by the addition of missing amino acids.

Since the year 1915 further work on the study of proteins were taken up including studies with chickens and pigs. All these study led to realization that the value of a given protein in nutrition is governed by its amino acids make up. The
body is unable to synthesize many of the amino acids which are present in its proteins, and thus the protein in food must be of a nature which will supply them.

The modern advances in the field of amino acid nutrition owes much to the work of W.C. Rose of Illinois University. In the year 1930 he began a brilliant series of studies. By the use of diet designed to be otherwise adequate for the normal growth of rats, in which the sole source of nitrogen was supplied by amino acids, the effect of removal and addition of each of amino acids was studied. Thus Illinois workers were able to classify 10 as essential dietary constituents and others as non-essential. Arginine was found to be a special case, where in growth occurred in its absence but not at the normal rate. This showed that body could synthesize Arginine but not as sufficient as to meet the need of growth. Rose thus classed essential amino acids as those which cannot be synthesized in the body at a rate required for the normal growth.

Rose (1938) conducted his experiment with human being. Applying the nitrogen balance method, using pure amino acids as sole source of nitrogen, the dietary needs of each of the amino acids were determined. It is evident from the above explanation that there are certain quantitative differences as to the need of essential amino acids required by different species and for different function in the same species. There are also quantitative differences per unit body weight or of growth tissue formed. The classification of amino acids as essential and
nonessential is shown in Table 1.1, below:

**TABLE 1.1**

Classification of amino acids with respect to their growth effects in the rat.

<table>
<thead>
<tr>
<th>Essential</th>
<th>Nonessential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine.</td>
<td>Glycine.</td>
</tr>
<tr>
<td>Tryptophan.</td>
<td>Alanine.</td>
</tr>
<tr>
<td>Histidine.</td>
<td>Serine.</td>
</tr>
<tr>
<td>Phenylalanine.</td>
<td>Cystine.</td>
</tr>
<tr>
<td>Leucine.</td>
<td>Tyrosine.</td>
</tr>
<tr>
<td>Iso-leucine.</td>
<td>Aspartic acid.</td>
</tr>
<tr>
<td>Threonine.</td>
<td>Glutamic acid.</td>
</tr>
<tr>
<td>Methionine.</td>
<td>Proline</td>
</tr>
<tr>
<td></td>
<td>Hydroxy proline.</td>
</tr>
<tr>
<td>Arginine.</td>
<td>Citrulline.</td>
</tr>
</tbody>
</table>


The above discussion of essential and nonessential amino acids does not apply for ruminants. The essential amino acids can be produced in the rumen by bacterial synthesis, particularly from non protein sources of nitrogen. Thus the amino acid absorbed by the ruminant may contain sufficiently the required amino acids, independent of its supply in the diet.

The finding that many of the amino acids composing body proteins, must be supplied as such by food proteins, explains as to why different ration of the same protein content differs in protein values in nutrition i.e. they differ in protein
quality. As a matter of fact no ration ever contains only one protein, they contain a mixture of proteins. Different ration differ in the quantitative relation of their amino acids, thus subject to variation in digestion and absorption. The ration which has the highest protein quality is the one which provides all the essential amino acids in proportions needed for the proteins to be formed as well which also supplied a non-specific source of nitrogen to form the nonessential acids.

It is generally accepted that the usefulness of feeds as source of protein depends primarily on two factors, the total concentration of the proteins and the distribution of amino acids making up the proteins. There is ample evidence that imbalance among amino acids of the ration results in inadequate protein nutriton.

The relative usefulness of a particular feed in meeting the animal's protein needs, is often referred to as protein quality.

There are different methods to estimate the biological value of a given protein, but the method envisaged by Thomas and Mitchell is one of the outstanding method. This method was first originated by Karlthomas (1909), who first used the term biological value. The Thomas method was modified by Mitchell and this modified procedure continues to be widely used.

The important fact with this procedure is that it takes into account the metabolic and indogenous nitrogen which is being utilized by the body, although appearing as excretions.
The biological value can also be estimated by nitrogen balance index. The application of this procedure applies to measure the biological value both for growth and maintenance. It estimates the rate of change of nitrogen balance with respect to absorbed nitrogen; the higher the rate, the greater the efficiency of retention and higher the biological value.

Biological value of the proteins of some important human foods are given below:

<table>
<thead>
<tr>
<th>Food</th>
<th>Biological value of proteins.</th>
<th>Food</th>
<th>Biological value of proteins.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole egg.</td>
<td>94</td>
<td>Whole wheat.</td>
<td>67</td>
</tr>
<tr>
<td>Milk.</td>
<td>85</td>
<td>Potato.</td>
<td>67</td>
</tr>
<tr>
<td>Egg white.</td>
<td>83</td>
<td>Rolled oats.</td>
<td>65</td>
</tr>
<tr>
<td>Beef liver.</td>
<td>77</td>
<td>Whole corn.</td>
<td>60</td>
</tr>
<tr>
<td>Beef heart.</td>
<td>74</td>
<td>Wheat flour.</td>
<td>52</td>
</tr>
<tr>
<td>Beef round.</td>
<td>69</td>
<td>Navy bean.</td>
<td>38</td>
</tr>
</tbody>
</table>

(Data from H.H. Mitchell. The protein value of food in nutrition).

The quality of a given protein can be estimated by feeding trials also. The method was originally developed by Mendel, Osborne and Persy (1919) in which the efficiency of a given protein is calculated in terms of gain in body weight against the gram of protein or nitrogen fed. The process as carried out with laboratory animals, where protein sources are to be compared, a nitrogen free otherwise adequate basal diet is fed with graded level of nitrogen to different groups of animals. With
record of growth and feed consumption the comparative value of protein quality is estimated.

The limitation of this method of evaluating protein quality is that the weight gain in animal may be variable. Thus some workers have included slaughter data as check on the results.

Frost, Narsy and Sady (1949) evolved a method of estimating protein quality of a given protein which they named as "Rat repletion Method". Here the relative ability of various protein sources to restore weight in protein depleted adult rat is measured.

Bernard and Oser (1959) gave an another method to estimate the protein quality of various proteins. Their method is based on the integrated amino acid index for predicting the biological value of proteins.

However, there is no perfect single procedure for determining protein efficiency or biological value which holds good for all the purposes.

Rippon (1959) gave a comparative value of various methods for calculating the nutritive value of a protein. The terms essential and nonessential refer to a need in the diet. All the amino acids found in the body protein consists of nonessential amino acids to the extent of 40 percent, therefore the dietary and metabolic sources of this, is of large quantitative importance. They must be available to the tissues to build body protein. Amino groups from various sources can be united to a keto or hydroxy
acid to form the corresponding amino acid. Thus several amino acids are mutually inter-convertable by transamination. Amino acids may also be formed by the union of a keto acid with an amino group provided by ammonium salts, as shown by Sprinson and Rittenberg.

Diet free of the nonessential amino acids, all of them can be formed by uniting an NH$_2$ group arising from dietary or metabolic sources to an appropriate carbon-hydrogen chain, which can arise from the products of carbohydrate and fat metabolism. Various enzymes are involved in these reactions. Thus it is now stated that for the normal protein nutrition of the growing rat there is a need of 10 essential amino acids with a dietary supply of some nitrogen compound or compounds to provide the needed ammonia groups for the synthesis of nonessential amino acids. The amination of carbon-hydrogen chain of an amino acid is not limited to nonessential acids only; in the case of the essential acids, however, the appropriate chain is not available except as it is supplied in food. The above discussion is based primarily on studies with rats, but there is evidence that these findings regarding the utilization of non-specific sources of nitrogen for the formation of nonessential acids apply to chicks and probably to other species.

The earlier work relating to the quantitative determination of amino acid were largely concerned with purified diets. The modern studies have been focussed on the amino acid composition of the ration or foods in order to provide information to the field of nutrition. For the analysis of the feed or proteins the
first step commonly in practice is the hydrolysis to set free
the amino acids. The process is subject to error, because certain
of the acids may be destroyed during hydrolysis, and in the case
of feeds the presence of non-protein substance further compli-
cates the problem. By using acid hydrolysis for certain amino
acids and alkali hydrolysis for others and by adopting other spe-
cific procedures, these complications are generally overcome.

Different procedures are in use for analyzing the hydro-
lysates, but the chromatographic methods are the most widely used.
These methods were thus named because they were first utilized
for the separation of plant pigment. The most commonly used
method involves as in exchange column in which the amino acids
are absorbed from solution on specific resins and then eluted by
a suitable solvent. The other procedure is referred to as paper
chromatography, involved differential absorption on filter paper.

The microbiological methods has also been used for ana-
lyzing the hydrolysates. For example Lactobacillus arabinorus
17-5 requires nine amino acids for its growth. Any one of them
can thus be determined in a protein hydrolysate by adding an ali-
quot of it to the nutrient medium that is complete for this organ-
ism, except for the amino acid in question. The quantitative
metabolic response of the organism as determined by the measurement
of resulting turbidity or by titration of the amino acids produ-
ced, measures the amount of amino acid supplied by the hydrolysates.

As regards to the proteins metabolism the end products
of protein digestion in the digestive tracts of non ruminant are
largely amino acids produced by the action of proteolytic enzymes in the intestine. In ruminants however, microbiological processes in the rumen play a dominant role in protein nutrition.

The digestion of proteins to amino acids by enzymes secreted in the stomach and intestinal tract has long been known. The proteins are digested in the stomach and intestine to the constituents amino acids and then pass through the intestinal walls into the blood stream and are thus distributed throughout the body. Thus the body does not use the proteins of the food directly for body building, but first break them down and from the resulting building stone reconstructs new and different proteins to meet the specific requirement of each living cell.

Nature has imposed a peculiar limitation on the ability of the body to manufacture its own requirement of amino acids. It can produce some, but for others it is entirely, dependent upon the proteins in the food. Each one of the different kinds of proteins which enter into the composition of tissue cells requires for its construction a different but very definite assortment of amino acids. If only one of the amino acids for the synthesis of a given protein is lacking, that particular protein cannot be formed, no matter how surplus other amino acids may be.

Keeping in view the great role of proteins and amino acids in nutrition, the present investigation has been aimed at to estimate five essential amino acids in sal seed, silkworm pupae, rice bran, eggs and in breast muscle tissue of day old chick in order to evaluate their protein quality and also to study
any substantial difference in the pattern of these five amino acids in eggs and day old chick muscles of the same breed.

Sal seed and silk worm pupae once regarded as useless by-product are gaining importance as livestock feed. The tannin present in the sal seed have been found (Murty et al, 1969) to increase the biological value of groundnut cake protein for ruminants by reducing the ruminal ammonia production.

Silk worm pupae which is very rich in animal protein have been successfully utilized (Srivastav et al, 1967) to replace a part of animal protein (fish meal) in poultry ration.

Sal seed and silk worm pupae have so far received very little attention of animal nutritionists and as such an attempt has been made to know some of the essential amino acid content in them in the present investigation. An important fact that has come to light in recent years is that some of the nutrients pass from the breeding hens as "carry over" through the egg yolk into the body of the chick at hatching time for the baby chick to begin life with. An investigation was made on this aspect with reference to some of the essential amino acids of the egg and that of the muscle tissue of the day old chick.
CHAPTER II

REVIEW OF LITERATURE.
REVIEW OF LITERATURE

The field of protein and amino acid nutrition is very vast and every aspect of this is not indicated within the limited scope of this work. As such the review work has been limited to the following aspects of the field:

(i) Essential and nonessential amino acids.
(ii) Nutritive value of proteins,
   (a) Nutritive value of animal protein.
   (b) Nutritive value of vegetable protein.
(iii) Carcass analysis and protein nutrition.
(iv) Amino acid requirement of different species.
(v) Methods for determination of amino acids.
(vi) Amino acid contents in muscle, eggs, rice bran, silkworm pupae and salseeds.

(I). ESSENTIAL AND NONESSENTIAL AMINO ACIDS:

With respect to nutrition, the amino acids are the components which differentiate the different proteins. These structural components in an organism have been categorised into two groups, particularly on the basis of prevalent dietary needs of the species.

Kossel and Kutscher (1900-1901) presented a method for the determination of basic amino acids, in which precipitation of the group followed by subsequent separation of the amino acids were involved. Emil Fisher (1901) described the ester procedure for the isolation of monoamino acids. These two methods were
responsible to a great extent, for accumulation in the subsequent years, of reasonably reliable informations about the composition of the proteins, such as deficiency of lysine in gliadin, inadequacies of lysine and tryptophan in zein.

By 1932, only three amino acids had been shown unequivocally to be indispensable dietary components for the growing rat. They were tryptophan (Willock and Hopkins, 1906–1907), (Osborne and Mendel, 1914), lysine (Osborne and Mendel, 1914), and histidine (Ackroyd and Hopkins, 1916; Rose and Cox, 1924). In addition, evidence had been presented in support of the idea that cystine or methionine or both must also be supplied. Jackson and Block (1931, 1932) and Weichselbaum et al (1932) had made the important observation that a large part of the cystine may be replaced by methionine, but it was not known whether the substitution was possible, because methionine was transormed into cystine or because either of the two amino acids might perform independently certain functions in which both ordinarily took part. Nor it is known whether the reverse replacement of methionine by cystine could occur (cited by Rose, 1957) McCoy et al (1935–1936), Meyer and Rose (1937) with their simultaneous discovery and identification of threonine, made it feasible for the first time to attack the problem of nitrogen requirements of the organism by giving diets containing mixtures of highly purified amino acids in place of proteins.

Rose (1957) while reviewing the past achievements in this field of research, remarked, "once an abundant supply of threonine had been acquired by its isolation from proteins, the
demonstration of the nutritive importance of each amino acid became simple.

During the investigations with the weanling rats by the use of diets designed to be otherwise adequate for the normal growth of rats, in which the sole source of nitrogen was supplied by amino acids, Rose, Osterling and Womack (1948) showed that only ten of the nineteen or twenty amino acids ordinarily found in proteins were necessary for maximum gain of weight. Those amino acids were valine, leucine, iso-leucine, methionine, threonine, lysine, phenyl-alanine, tryptophan, histidine and arginine. The exclusion of any one of them from the diet with the exception of arginine, lead to diminished appetite, loss of weight, profound nutritive failure and eventual death, as they were classified as indespensable ones for rats.

Summarising the earlier statements, Rose (1947) explained that an indespensable dietary component was one which could not be synthesized by the species in question from materials ordinarily available to the cells at a rate required for the optimum growth.

On the basis of a critical study of a large body of data on chick amino acids requirements, an indespensable amino acid was defined by Almquist (1951) as "one which must normally be obtained from the gastrointestinal tract inorder that synthesis of body proteins may take place".

Almquist (1951) further observed that the most recent analysis of body tissue showed the presence of practically all of
the dispensable amino acids to the extent of 40 percent or more of tissue protein. Thus an emphasis, that the "dispensable" amino acids were physiologically indispensable to the formation of the characteristic proteins of the animals.

White et al (1959) explained that the term "essential" and "nonessential" is related only to dietary requirements and had no meaning with respect to the relative importance which the amino acids might have been in metabolism. Those amino acids which were essential in the diet represented compounds whose carbon skeleton could not be readily synthesized by the body.

(II). NUTRITIVE VALUE OF PROTEINS:

Animal Proteins:

Meat has significant importance as a dietary source of protein. It contains vitamins, particularly vitamin B₁₂, minerals and trace elements, a fair amount of fat and both essential and nonessential amino acids within its structural folds. Palatability, better digestibility and utilization are the extra attributes which accounts for its higher nutritive value.

The assay of the nutritive value of the protein is arrived at, by the study of the comparative growth rate, gain in body weight or in body protein per unit of protein ingested, biological values, nitrogen balance and tissue protein formation. Chemical scoring to achieve the essential amino acids index is also applied for such purposes. Nevertheless the value of a given protein varies with the level of total protein intake, the
presence of other types of protein in the diet or the substances in the diet or the substances in the diet which interfere with digestion. The value of a given protein is also related with the caloric intake.

Mendel (1923) first suggested that "the efficiency of a particular protein satisfying the nutritive needs of the body, depends on the indispensable amino acids that it will yield".

Beach, Bernstein and Macy (1941) were first to evaluate amino acid requirement, based on the amino acid analysis of the food intake, essential for normal growth. Beach, Munk and Robinson (1943) also observed that protein structure is similar in animals. Block and Bolling suggested that a perfect protein or amino acid mixture would be, one, which could replace weight for weight, tissue protein or supply new material for growth and lactation, leaving no excess to be utilized for other purposes.

Mitchell and Block (1946) advocated a method on the basis of amino acid assay for estimating the biological value of proteins for growth. The amino acid content of whole egg proteins "almost perfectly utilizable in rodent metabolism" was used as standard of reference.

Almquist (1953) summarising the principles of amino acid balance stated, "when a dietary protein is partly short in one essential amino acid the fraction which contains amino acids in optimum proportions to the deficient amino acid is the only part available for growth. Raising the balance of unbalanced protein, will increase the amount of balanced fraction and hence permit the
increased growth up to the natural limit. If slightly deficient protein is given at a high level, so that maximum growth response is achieved, addition of the deficient amino acid will increase the efficiency of the utilization of feed.

While with the study of premature infants, Ferriera et al. (1953) found that human milk alone was not the best food for rapid growth but the excellent results were found by the addition of one percent of a mixture of amino acids and salts. Sure and his associates (1953) found with weanling rats, that the nutritive value of cereal grains improved with the addition of individual amino acids and concentrate fish solubles. In the same year, struck recorded brown pigmentation of fat and appearance of faecal smell in pork carcasses due to protein deficient ration.

Since the NH₂ group of lysine was supposed to form a nutritionally unavailable complex, when intact protein are damaged during processing, Carpenter and Ellinger (1955) observed that free NH₂ group could be estimated by reaction with dinitro-fluorobenzene and available lysine value could be obtained which may be used for the prediction of the protein quality of the animal product.

Conducting feeding trials with pigs Richter and Oslege (1953) found that a low protein diet had a poor carcass quality, with pale coloured meat that gave water more readily.

Maynard and Loosli (1956) observed on the basis of the studies with rats and much fewer one with chicks and pigs, a comparative biological value of individual foods and combinations.
Animal products as such were superior to the foods of other origin. At the top were eggs and milk followed by liver, kidney and other glandular organs. Muscle meat ranked somewhat lower but was above most seeds and other vegetable products.

Meyer (1957) noted that increasing amount of animal protein in the ration, diminished the feed requirement per kg. gain and also raised the daily gain of weight. In the same year Chrzaszcz could get small differences in the meat quality of Sussex, Rhodeislandred, white leghorn, greenleg chickens and their crosses, when fed on the same rations. However, in crosses, meat had more dry matter particularly in breast muscle.

With single group of adult rats Nasset (1957) compared whole egg with two mixture of amino acids. He found that the amino acid mixture based on the minimum requirement of 9 essential amino acids, was sufficiently low in maintaining nitrogen balance to either egg protein or the amino acid mixture which simulated egg protein. Deshpande, Harper and Ehvenjem (1957) with weanling rats noted maximum growth, when the basal diet was supplemented with intact protein in the form of casein, fibrin and beef. However, the same growth rate was not observed when the protein supplement was replaced by equivalent amount of essential and nonessential amino acids.

Schuphan and Weinmann (1958) estimated the crude protein and 8 essential amino acids in a number of food commodities used in human diets. The biological values expressed as F.A.A. index, were compared with beef and pork, which had a value of 78 and 76.
Values for seven cereals were from 55 in wheat to 73 in rice, for 5 legumes from 49 in unripe peas to 72 in soybeans; for 20 vegetable from 41 in kohlrabi stems to 72 in potatoes.

It has been reported by Watts et al. (1959) that availabilities of the amino acids to human subjects were similar for eggs and pork, except for the tyrosine. The availabilities of the amino acids in the milk and egg diet were similar or slightly higher than egg, except for isoleucine.

Arpae et al. (1960) noted that taste of pigs maintained on animal protein was superior. They also noted that the muscles held more its juice in comparison to those maintained on plant protein.

Bigwood and Martin (1960) found that there was no significant difference among breeds (lactating Jersey and Friesian cow) in the amount of amino acids in the total protein in the milk. In the meat of those breeds about 90% of total nitrogen was protein nitrogen, as compared with 98 percent in milk protein. Kofranyi and Muller-Weker (1960) reported that the biological value of dried milk for human subject was about parallel to that of eggs.

Since collagen is low in several amino acids Easton and Long (1960) stated that the presence of large amount of collagen in bone meal and meat meal might be the cause of reduction in the nutritional value of the total protein of the meal. Their suggestion for the estimation of hydroxyproline, which collagen almost contains, could form the basis of estimation of protein quality of bone meals and meat meals.
Pritchard and Smith (1960) observed that in the fish meal the length of time between death and processing of whale had no effect on the nutritive quality of the meal except the rapid increase of the free fatty acid content in the meal.

Kertész and Csirke (1961) stated that lower cost of vegetable protein could not overcome the lower efficiency of feed conversion. Their findings was based on a group of Hungarian Yorkshire pigs which were fattened from 30 to 150 kg. liveweight on rations with equal quantity either all vegetables or with milk. Pigs getting 15 percent milk protein reached 90 kg. in 61 days and 150 kg. in 83.7 days, sooner than those getting vegetable protein only. To achieve these weight, pigs getting only vegetable protein required 38.9 and 31.6 percent more protein and 13.2 and 18.9 percent more starch equivalent. They considered that milk to supply 10 percent of total protein would be adequate.

Free amino acids (8 essential one) were estimated in blood serum of human being by Matsuno (1961). He estimated both the blood of persons getting only vegetable protein and also of those getting 54 percent of protein from animal origin. He found the values after a meal which revealed the difference in the quality of ingested protein.

Owings and Ballou (1961) studied the effect of protein and amino acid supplementation on the intestinal microflora and plasma amino acids in chicks. Addition of arginine to diets with casein as the source of protein improved weight gain and enhanced efficiency of feed utilization, but no improvement was noted when
it was added to diets with casein and zein. The presence of 1.8 percent casein in the diet, did not affect the concentration of arginine in blood but there were changes in other amino acids. It was suggested that arginine affected the absorption of the amino acids and enabled the chick to utilize them less completely. When lysine was added to the diet the value in the blood was increased, sometimes as much as 100 percent and at the same time arginine was decreased. Either amino acids, or both added to the diet, generally increased microbiological count.

Heller et al (1961) made a study on the availability of lysine from beef and lamb applying heat treatment. His finding was based on a growth experiments with weaning rat. The results showed that 75 percent to 90 percent of lysine was utilized from meats before or after normal cooking at 200°F to 400°F in an electric oven. There was difference found in lysine content between the inner or outer portions of such cooked meats. Autoclaving at 250°F for 16 hours reduced availability of lysine 40 to 50 percent.

Bender (1961) established the nutritive value of proteins by chemical analysis and the relation between the chemical score and biological value of proteins was investigated. He observed that defatted egg protein, which is usually considered to be the "ideal protein" for the rat was obtained by diminishing the concentration of those present in excess in mixture simulating egg protein. The mixture had a net protein utilization (N.P.U.) of 100 when given to rats.

Kik (1962) demonstrated that diet with protein supplied
by chicken meat and rice gave better growth than either at the same protein level in rats. Dark chicken meat had comparatively higher nutritive value than light and both were better than whole egg. Chicken contained much more tryptophan, lysine, threonine, cystine, and methionine than rice and possibly that was mainly responsible for the supplementary effect.

Puchal et al (1962) made a study of growth and feed utilization by young pigs, in an experiment lasting for 28 days. The diet given was of dried skimmed milk and soyabean, fish meal or cotton seed meal, which supplied 20 percent protein. Two separate pens, one pen having 4 pigs were assigned to each treatment. On the 26th day pooled samples of blood were taken from animals in each pens and amino acids with the exception of tryptophan were estimated by ion exchange chromatography in protein free plasma and diets. They noted that growth and efficiency of feed utilization increased and plasma urea decreased in the order - meatmeal-cotton seed meal - fish meal - soyabean meal - dried skimmed milk. The concentration of individual amino acids in the diet varied with particular acid as well as with the type of proteins, but concentration in plasma were in general greater with those protein that promoted best growth.

Cahilly et al (1963) observed the effects of various levels of dietary lysine on muscle protein of growing swine for biological value. They concluded that biological value of the meat for growing rats did not differ between groups.

Dvoro'k and Uognarv'a (1965) reported the mean values
for available lysine in 8 beef cuts which ranged from 6.53 to 9.15 g. per 17 g. nitrogen and in pork cuts from 5.66 to 8.94. They found that such deviation was due to unequal distribution of connective tissue protein. From the ratio of tryptophan to hydroxyproline, it was concluded that the available lysine in raw meat is in direct proportion to the content of connective tissue. Reduction on heat processing depended on the time of heating and the proportion of meat products lost. Smoking at 320°F and treatment with nitrite reduced lysine but glucose or starch had no effect.

Lewis (1966) compared the chemical and biological method of protein evaluation. The amino acid composition of unheated and excessively heated pork protein was determined together with biologically determined net protein utilization values (N.P.U.). Comparison of biologically determined (N.P.U.) and chemically determined protein score of unheated and heat treated pork protein showed that unavailability of essential amino acids, rather than their destruction is responsible for the marked lowering of protein utilization following excessive heat treatment.

Miller, Dawson and Bauer (1966) made a study of "free amino acids content of chicken muscle from broiler and hens". Six broilers on commercial broiler ration and 6 hens on a commercial laying ration, all of the same strain, age and sex, were divided into similar groups. All were killed, scaled, picked, pinned, enerviscerated, washed and placed in slush ice for 18 hours and samples of fat free and connective tissue free muscles were taken
immediately from one group. The samples were ground and depro-teinised with picric acid and the supernatant was passed through a Dowex 2x10 resin bed. The effluent and bed washing were concen-trated and aliquot was treated with sulphite before analysis on a Beckman spinco amino acid analyser. Samples were similarly pre-pared from the second group after one week's refrigeration at 35°F. Except for proline free amino acids increased on refrigeration. Broiler meat generally contained more free amino acids than hen meat and light meat had less free amino acids than dark meat. Except of lysine and histidine, taurine was higher in dark of both hens and broilers. No relation was found between tenderness and either the general pattern of free amino acid concentration or the concentration of any single free amino acid.

Stekar and Muck (1968) studied the effect of protein quality on egg production by hens. Leghorn hens were given for weeks laying feed with 13 percent crude protein or test feed with 2.5 percent, added gelatin or casein to make crude protein content 15.5 percent. All three mixtures supplied sufficient essential amino acids. The basal mixture appeared to supply the needs for egg production, which was not increased by the addition of casein. The addition of gelatin resulted in a fall of egg production. That is taken to indicate that an incomplete protein such as would be supplied by hide, scrapings should not be included in mixtures of laying hens.

Burin (1969) studied the amino acid composition of muscles of pigs of different breeds. He noted that the muscles from 3 and 6 months old pigs contained from 2.53 to 2.61 and 2.73 to
2.86 percent of nitrogen respectively. Lysine, argine glycine, aspartic acid, glutamic acid threonine, alanine, valine, lysine and iso-leucine were high and cysteine, histidine, serine, tyrosine, methionine and phenylalanine were relatively low. In 6 month old pig cysteine, lysine, histidine, arginine, asparagine, glutamic acid alanine, proline, tyrosine and methionine were lower, but phenyl-alanine was high compared with the values at 3 months age.

**Vegetable Proteins:**

Macrander (1935) observed that extracted soyabean meal gave better weight increase and higher egg production than earthnut cake meal, and a mixture of the two was better than either alone. It was claimed that this mixture gives satisfactory results as sole protein supplements for laying hens and even in large amounts has no harmful effect on health or egg production.

Temperton and Dudley (1941) observed that both palm kernel meal and decorticated earthnut meal were palatable to poultry. With the former as a substitute for wheat middlings and latter replacing fish meal and dried skimmed milk, satisfactory egg production was maintained in batteries over a period of 9 months.

Macdonald (1943) noted that the addition of NaCl to cereal ration improved growth and lowered mortality. A cereal rations containing 10.5 percent protein and supplemented with NaCl and green food gave poor growth specially during the first 8 weeks. The inclusion of soyabean meal or earthnut meal to give
a level of 17 percent protein gave increased growth, both being of equal value as protein supplements. It was concluded that although they are less satisfactory than separated milk or meat offal they could be used with fair success when animal protein were expensive or unavailable.

Grau (1946) observed the effects of adding various amino acids, on growth rate and efficiency of gain in rations containing 20 percent protein derived solely from maize gluten meal, cottonseed meal or peanut meal.

The nutritive value of maize gluten meal was improved by the addition of arginine, lysine and tryptophan as was shown by an increase in growth rate from 2 percent to nearly 6 percent per day. The addition of cystine, glycine, methionine, threonine and valine yielded no significant benefit and these amino acids were deemed to be already present in adequate amount. Supplementation of cottonseed meal both with methionine and lysine increased the growth rate from 4 to 7 percent. The methionine deficiency was found to be less marked than that of lysine, a result, which was expected from the amino acid content of the cottonseed protein. Peanut meal proved to be lacking primarily in methionine and to a limited extent in lysine.

Carpenter, Duckworth and Ellinger (1954) observed equal egg production in birds given all vegetable rations during the laying period or from hatching to 18 months of age to that of birds receiving rations containing animal proteins. Early growth is somewhat retarded by the absence of animal protein and was
accelerated by aureomycin supplements, but neither the final weight of the pullet nor their egg production was thereby affected. The limited amount of fresh droppings available to hens kept on wooden floors which were loomed daily provided enough of the animal protein factor to supply their requirements for sustained egg production. When the ration contained 8 percent of cereal protein, supplemented with 3 percent groundnut meal or whitefish meal, groundnut meal proved significantly inferior to fishmeal; when the amounts were larger the supplement were of equal value. A ration containing 11 percent protein, 3 percent of which was provided by whitefish meal proved adequate for good egg production if the birds were kept in laying pens but appeared to be less adequate for birds kept in straw yards.

Fangauf, Vogt and Barlowen (1958) gave rations to day old New Hampshire chicks for 8 weeks consisting of 70 percent cereal, 6 percent codmeal, 2 percent dried yeast, 1 percent mineral mixture and soyabean meal at 15-10-5-0 percent level with groundnut cake at 0-5-10-15 percent level respectively. Vitamin A, D₃ and Riboflavin were also given. They observed that groundnut cake at all 3 levels increased weight gain and feed consumption and at 15 percent level reduced nutrient requirement.

Lamsbury (1962) conducted a trial from day old chicks to 20 weeks of age in 3 groups. They were given an orthodox diet to 8 weeks, then the all vegetable diet. He observed that the weight of the birds at 14 weeks of age on the all vegetable diet was 64 percent of that those given the orthodox diet. But by the end of the trial there was little difference between the groups. Between
8 and 12 weeks when the birds changed from one diet to other suffered a small check in growth, possibly because they found all vegetable diet less palatable. He also studied the egg production of 10 birds from each group for a period of one year. He found that there was no difference between groups. He concluded that expensive animal protein should be reserved for starting and breeding feeds.

Prasad and Mukherjee (1967) made a comparative study of vegetable and animal protein supplementation by double reversal method. Two groups of 24 Rhode Island Red hens in individual battery cages were fed on diets with 18.7 percent protein, one all vegetable and other 5 percent fish meal included at the expense of groundnut cake. After 8 weeks each group was changed to other diet for another 8 weeks. There was no significant difference in egg production, body weight or efficiency of feed conversion. The all vegetable diet had maize 44, wheat-bran 17, groundnut meal 28, and mineral 6 parts by weight.

Rathore (1968) conducted two experiments. In each experiments 50 hens in pens were given a diet based on maize alone or with furazolidone or on grain sorghum alone, with furazolidone or with maize oil. Each diet had 16 percent protein and energy values were similar. In the first experiment percentage egg production for 5 periods of 56 days was 69.99, 72.30, 69.52, and 66.45 respectively and in the second it was 64.39, 67.32, 63.8, 66.76 and 57.11. There was no significant difference among diets on efficiency of feed conversion, mean egg weight, weight of yolk or haugh units.
(III). **Carcass Analysis and Protein Nutrition**

Slaughter experiment deals with the procedure of killing an animal and analysis of certain specific tissues or of the body as a whole. The studies of the proteins and amino acids, required for growth or of the relative value of varying protein sources, it is important to know the specific effect in terms of protein tissue formed.

Munk et al (1945) proposed that amino acids analysis of tissues may afford a measure of amino acids requirements, for growth were different from those for egg production.

Mitchell (1950) suggested an effective method of estimating the requirements of essential amino acids for growing animals. In this way the requirement of one amino acid such as lysine in grams per day was determined and the requirements of other acids was determined with the ratio that exists between lysine and other amino acids in the body of the animal. The ratio of lysine with other acids in the body was made by amino acids assay of entire carcass or approximately by amino acid assay of dominant tissue such as muscle. This method was as good as the current methods of measuring amino acids requirements.

Schweigert and Guthneck (1953) proposed a method to find the utilization of amino acids from foods by the rats. Adult protein depleted rats for 12 to 15 days were used for the study of quantitative utilization of lysine from foods, when graded amounts of L-lysine to a lysine deficient basal ration of purified amino acids or of sesame meal, ammoniumcitrate and certain amino
acids. The weight gain of the depleted animal in 4, 8 and 11
days were proportional to the amount of lysine fed. Food pro-
ducts of known lysine content (estimated microbiologically) were
then added to the basal diet to provide the graded level of L-
lysine, and from the weight gains the percentage of lysine uti-
лизed from the foods was calculated.

William's and Coworkers (1954) estimated the amino acid
requirement for growth, by assay of whole carcass of the rat,
chick, pig at different stages of growth. They found that not
only the patterns of amino acids composition was comparable within
each species at various level of growth but also remarkably simi-
lar among the species. Most of the differences between two sets
of figures they explained as due to use of amino acids of the
food for purposes other than protein formation e.g. methionine
as a methyl donor, or tryptophan for nicotinic acid formation,
or to possible inadequacies of nutritional requirements. The
relatively similar requirements among species and the close agree-
ment of the calculated values as those determined by nutritional
studies were the basis for their support that the carcass analysis
is the valid method for evaluating growth requirements for most,
if not all the "essential" amino acids.

Almquist (1954) stated that when a dietary protein is
partly deficient in one essential amino acids, the fraction which
contains amino acids in optimum proportions to the deficient is
the only part available for growth. Increasing the amount of
unbalanced protein will increase the amount of the balance fraction
and hence permit increased growth rate. Increasing the relative
amount of the deficient amino acids increases the proportion of the protein available for growth, until the whole of the protein is so used or natural limit is reached. If a slightly deficient protein is given at a high level so that maximum growth response is obtained, addition of the deficient amino acids will increase the efficiency of the utilisation of feed.

According to Cardi et al (1960) the effect of protein depletion on the amino acid composition of sarcoplasm proteins of gastrocnemius muscle in rats may cause some change, chiefly the decrease in the relative proportions of isoleucine, leucine, lysine and tryptophan. In their findings, Myosin (alkali soluble) fraction remained devoid of such alterations but distinct loss in the initial weight was noted, particularly, the weight of gastrocnemius muscle lowered down unto 35 percent in 21 days period. They pointed out that protein preferentially broken down in such conditions, were those richest in lysine.

Fry and Stadelman (1960) studied the effect of dietary methionine on methionine and cystine content of poultry meat. A basal ration of maize and fish-n-fifty fortified with vitamins and minerals was given to day old chicken for 12 weeks alone or supplemented with 0.05 or 0.50 percent DL-methionine. Methionine was estimated in muscle after 6, 9, and 12 weeks and liver and heart after 12 weeks. The addition of methionine had no significant effect on the rate of growth or on the methionine and cystine contents of muscles and liver and produced only slight increase in case of heart. Cystine, but not methionine in muscle increased. Further, they investigated the effect of cooking on
the carcass part and observed that the increases occurred in both amino acids due to loss of other nitrogenous components, and thus suggested the stability of the amino acids.

Summer and Fisher (1961) adopted the carcass analysis for the evaluation of protein. In their preliminary experiments, they found the ratio of body water to nitrogen under different feeding conditions remained constant in the chicken. In the final procedure, they used diets with 13 percent protein to quadruplicate groups of 5 chickens, at the same time provided N-free diet to the control group. Such a method was used for obtaining net protein values for the growing chickens and simultaneously, by analysis of the carcasses 5 sources of protein could be evaluated.

Ericson (1961) observed that although the amino acid composition of the body is similar for all species, there are marked differences in the proportion of amino acids required for Nitrogen balance. Enzyme kinetics are used to show that the requirement for one amino acid in relation to another cannot be predicted from a knowledge of the concentration of the acids in the organism. The functions of amino acids other than in protein synthesis are discussed together with inter-relations between individual amino acids and between amino acids and vitamins.

The effect of acute amino acid deficiencies on carcass composition was investigated by Lyman and Wilcox (1963), by group feeding the long Evan rats, while conducting the two separate trials. The animals were forcibly fed with about 10 g. daily in two portions of a synthetic diet in which single amino acid was
replaced by sucrose. Rats on complete diet gained 5 to 8 g. in 10 days but those on diets lacking in histidine, methionine, phenylalanine or threonine lost 10.5, 17.5, 11.0 and 14.0 g. respectively. There was no difference in gross body composition.

In the second trial, rats deprived of isoleucine suffered severest weight loss, 24 g. and in the deprived group where valine, lysine, tryptophan and leucine were absent, the range of this loss was 11 to 14 g.

Lewis et al (1963) conducted an experiment with a group of 10 white leg horn x light sussex cockrels 3 weeks old. They were given 10 weeks diets with crude protein from 16 to 24 percent and arginine from 0.65 to 1.5 percent. After 5 weeks of experiments Nitrogen balance was measured for 3 days. Total amino acids were estimated in all constituents of the diet by ion exchange chromatography. Performance was improved when arginine was added to the diet containing 0.65 percent and there was evidence that 0.8 percent was perhaps marginally inadequate. When the amino acid balance of the diet was upset by giving excess lysine, arginine requirement increased. It is concluded that arginine requirement of chickens is about 0.8 percent in a diet with 21 percent protein.

Holmes et al (1963) studied the amino acid composition of broiler cockrels in relation to the dietary amino acid requirement. They fed a diet with 25 percent and 1370 kcal. metabolisable energy to 16 pairs of Chunky Chick Cockrels from day old for 10 weeks and killed at 1 day and 2, 6, and 10 weeks of age, gutted and analysed the carcass by ion exchange chromatography.
except for tryptophan. According to their statement the composition of the carcasses were constant, with only variation that seemed to be due to increased contribution of feather protein. Only for glycine there was more than 47 percent of intake retained.

In their classical experiments for growth on selected lines of white rocks, Teporie et al (1963) reported the proximate amino acids composition of eggs and chicks as follows:

(i) In proximate composition of eggs, differences between the high weight and low weight lines were significant for fresh and dry weights, and the amounts of proteins, ash and carbohydrate values for the former being greater.

(ii) Ratio of weight of yolk to weight of white was similar except that on percentage basis none was significant.

(iii) On the basis of tabulated findings for amino acids in two separate analysis of eggs and chickens, calculating the efficiencies of conversions, it could be concluded that differences in efficiencies were greatest in high weight line but on subsequent tests with large number of specimens, differences were statistically significant for histidine only.

(iv) Energy utilized by embryo was calculated to be 26.9 and 28.1 Kcal. in respective lines, but with the larger chickens in the high weight line, utilization was more efficient.

Gruhn and Anke (1965) observed that the changes in
crude nutrients, amino acids and minerals in poultry during development from day old chicks to pullet. Composition of plucked carcass and of feathers and down was estimated for sussex X Leghorn chickens at intervals of 2 weeks from hatching to 12 weeks of age. In plucked carcass, they reported, that there were with aging increases in dry matter from 23.7 to 31.0 percent, crude protein from 13.4 to 17.5 percent and ash from 1.8 to 4.9 percent of fresh weight. Fat was least at 14 days of age, 4.7 percent and increased to 7.8 percent. Most amino acids and P, K, Na and Zn in ash were noted as fairly constant but glycine tended to fall and calcium to rise slowly with age.

(IV). AMINO ACID REQUIREMENTS FOR DIFFERENT SPECIES:

Rose and Co-workers made an effort to investigate the amino acid requirement of man. They (1937) observed that the amino acids requirement of human being is similar to that of rats, except for histidine.

Maynard and Loosli stated in his book "Animal Nutrition" (1969), that many data had been secured on the quantitative requirements of amino acids, and this continued to be a very active field of study. The general procedure has been to feed a diet, otherwise adequate, except in the amino acid to be tested and to add a varying levels of it to different groups to arrive at the level which is sufficient to result in satisfactory growth. Weight increase has been the usual criterion, but in some experiments the nitrogen-balance measure has also been employed.
Requirement of essential and nonessential amino acids for human being is given in the table below:

**TABLE NO. 2.1**

Table showing essential and nonessential amino acids in Human Nutrition.

<table>
<thead>
<tr>
<th>Essential Amino acids</th>
<th>Minimum daily requirement*</th>
<th>Recommended daily intake*</th>
<th>Daily intake with self selected diets**</th>
<th>Non-essential amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-tryptophan</td>
<td>0.25 gm.</td>
<td>0.5 gm.</td>
<td>0.92 gm.</td>
<td>Glycine</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td>1.10</td>
<td>2.2</td>
<td>3.66</td>
<td>Alanine</td>
</tr>
<tr>
<td>L-lysine</td>
<td>0.40</td>
<td>1.6</td>
<td>6.35</td>
<td>Serine</td>
</tr>
<tr>
<td>L-threonine</td>
<td>0.50</td>
<td>1.0</td>
<td>2.86</td>
<td>Cystine</td>
</tr>
<tr>
<td>L-valine</td>
<td>0.30</td>
<td>1.6</td>
<td>4.61</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>L-methionine</td>
<td>1.10</td>
<td>2.2</td>
<td>1.89</td>
<td>Aspartic acid</td>
</tr>
<tr>
<td>L-leucine</td>
<td>1.10</td>
<td>2.2</td>
<td>6.00</td>
<td>Glutamic acid</td>
</tr>
<tr>
<td>L-isoleucine</td>
<td>0.70</td>
<td>1.4</td>
<td>4.62</td>
<td>Proline</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hydroxyproline</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Citrulline</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Histidine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Arginine</td>
</tr>
</tbody>
</table>

* Rose's values are calculated for normal males.

** These values were computed from data on self-selected diets of women (Fulrell et al, J. Nutri; 1952, 46, 299).

In evaluation of these, the phenylalanine sparing action of tyrosine and the methionine sparing action of cystine have to be considered.
### Table No. 2.2

Table showing essential amino acid requirements for optimum growth of chickens, turkeys, swine and rats (Percent of diets).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Starting chicks</th>
<th>Starting pouls</th>
<th>Pigs (20 to 35 kg.)</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary proteins</td>
<td>20.0</td>
<td>28.0</td>
<td>16.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.2</td>
<td>1.6</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.0</td>
<td>1.0</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.4</td>
<td>?</td>
<td>0.18</td>
<td>0.30</td>
</tr>
<tr>
<td>Iso-leucine</td>
<td>0.75</td>
<td>0.84</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.4</td>
<td>?</td>
<td>0.60</td>
<td>0.80</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.1</td>
<td>1.5</td>
<td>0.70</td>
<td>0.90</td>
</tr>
<tr>
<td>Methionine*</td>
<td>0.75</td>
<td>0.87</td>
<td>0.50</td>
<td>0.60</td>
</tr>
<tr>
<td>Phenylalanine**</td>
<td>1.3</td>
<td>?</td>
<td>0.50</td>
<td>0.90</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.7</td>
<td>?</td>
<td>0.45</td>
<td>0.50</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.2</td>
<td>0.26</td>
<td>0.13</td>
<td>0.15</td>
</tr>
<tr>
<td>Valine</td>
<td>0.85</td>
<td>?</td>
<td>0.50</td>
<td>0.70</td>
</tr>
</tbody>
</table>

* Cystine can replace 45 percent of the needs for methionine in starting chicks; 40 percent in starting pouls, 40 percent in pigs and 33 to 50 percent in rats.

** Tyrosine can replace 50 percent of the needs for phenylalanine in starting chicks; 33 percent in pigs, and 33 percent in rats.

Cf. Quoted from Maynard and Loosli; Animal nutrition, 6th Ed. 1969, Page 457.
Sym (1938) found that rumen content has got proteolytic action, but it was the finding of Pearson and Smith (1943), which first showed both breakdown and synthesis of protein in the rumen. Locati et al (1949) got the specific evidence that microbial action in the rumen can synthesize from urea, all the 10 amino acids which are essential for the growth of rat. McDonald (1948-1952) established that the breakdown of protein in the rumen was of quantitative importance and demonstrated that the resultant product ammonia re-enters the rumen as salivary urea; thus forms nitrogen cycle.

Annison and Lewis (1959) stated that the requirements of essential amino acids cannot be demonstrated for ruminants. But on the contrary the young sucking ruminant in which the rumen is not properly developed and non-functional, needs several essential amino acids.

Macblin (1955) estimated the leucine requirement of laying hens. Four trials were reported on the production of hens on diets containing 16 percent protein and from 1.0 to 1.6 percent leucine. In the first trial an egg production of 78 percent was maintained for 4 weeks on ration containing 1.0 percent leucine. In the second over, 10 weeks birds on ration containing 1.0 percent leucine laid better than birds on the same ration with a supplement of 0.2 percent leucine. In the third 8 birds were given a low leucine ration (1.0 percent) and a high leucine ration (1.6 percent) for 12 weeks. The superior production on the high leucine ration in the first period was not affected in
the second period on the low leucine ration. It was concluded that leucine requirement of laying hens does not exceed 1 percent of the ration.

Kalin et al (1960) evaluated the protein requirement of growing chick with amino acid mixture. On a diet with different level of amino acids mixture groups of 6 chickens 7 days old gained about 10.5 g. daily from 3.2 to 4.8 percent nitrogen. Higher levels of nitrogen up to 72 percent depressed growth, feed efficiency was highest with 4.8 to 6.4 percent nitrogen. Protein in the carcass at 14 days was almost constant from 16.8 to 17.9 percent.

In an experiment on groups of 5 chickens 9 days old, alanine, serine and aspartic acid were omitted as non essential and maize oil was raised to 15 percent at the expense of maize starch. Best daily gains about 13 g. were obtained with 3.2 and 4.0 percent nitrogen, at which level nitrogen retention was best. Feed efficiency was highest with 4 percent nitrogen. Percentage of fat in carcass was highest at the lowest level of nitrogen. Protein was hardly affected. At levels between 5.6 and 3.2 percent nitrogen the chicken were able to compensate by eating more.

Romanov (1962) investigated the protein requirement for laying hens. Russian white chickens from 2 to 5 months of age were given a diet with 15 percent protein and with 8 percent animal products or entirely of vegetable origin. The pullet were then caged individually and given 1 of 9 diets with 12, 14 and 17 percent protein, each with no animal protein, or different
levels from fish meal alone or with meat and bone meal. With vegetable protein egg production rose with the level of proteins; with 12 percent production was 85.5 percent of that with total protein 17 and animal protein 6 percent, with 1 percent of animal origin, egg production was as good as with 12 or 14 percent total protein as on all diets with 17 percent. With 12 or 14 percent protein production was better when the chicks have been given animal protein during rearing. The number of pullets culled was highest with total protein 17 and animal protein 6 percent. With 12 or 14 percent protein percentages culled increased as the proportion of animal protein fell.

(V). METHODS FOR DETERMINATION OF AMINO ACIDS:

Kossel and Kutscher (1900-1901) presented a method for the determination of basic amino acids in which precipitation of groups followed by subsequent separation of amino acids were involved.

Emil Fisher (1901) described the ester procedure for the isolation of mono amino acids. The more recent methods often do not require preliminary separation of the basic amino acids.

(1) Specific precipitants:

Block and Bolling (1945) noted that most of the data on amino acids composition of proteins were obtained by isolation method. This is based on the precipitation by reagents specific for single amino acids or groups of related amino acids.
Further purification by recrystallization, resulted fractions which after having been characterized as pure by elemental analysis and melting point determination. Thus informations relating to the amounts of amino acids in the original proteins is obtained. However the method involves a laborious processing.

Selim (1965) while making the micro determination of lysine in hydrolysates, dissolved the residues in 0.6 ml. borate buffer, obtained from the hydrolysis of 1 to 2 mg. of protein with 6N HCl. A lysine copper complex was prepared by adding a copper phosphate suspension to the buffered hydrolysates, shaking for 5 minutes and centrifuging. Fluorodinitrobenzene was added and the absorbance of the final yellow solution was measured at 390 m/m with dinitrophenyllysine as standard. Values for lysine in egg albumin, serum albumin, human hair, edestin and wheat-gluten agreed with those obtained by other methods as reported in the literature. Reproducibility and recoveries were good.

(2) Microbiological method:

Hawk, Oser and Summerson (1954) described that the basic principles employed in the microbiological assays to determine the response of bacteria, yeast or moulds to graded increments of the samples and of standard solutions added to media, providing all the nutrients required by micro-organisms, except the amino acids under assay. The graded response may be determined by the increase in the population of the micro-organisms (i.e. turbidometrically) or by the product of their metabolism (acid or CO₂) production. The method is advantageous, less expensive,
simple but has got some limitation also.

Lahiry and Proctor (1956) estimated microbiologically the essential amino acids in fish proteins. They also evaluated the amino acids in fish processed by various methods. In their first experiment 10 essential amino acids were estimated microbiologically in the muscles of haddock, shad and pomfret. The values were comparable to those reported for beef and pork muscles. In another experiment they found that dehydration or canning did not significantly affect the essential amino acid of haddock and shad.

Fry and Stadelman (1960), microbiologically made the assay of amino acids, methionine and cystine in poultry meat. Methionine and cystine were estimated in hydrolysates of chicken meat by incubation with Leucomostoc mesenteroides; turbidimetric titration after incubation for 16 to 20 hours was of doubtful value. Acidimetric titration after 72 hours being preferred. Hydrolysis in sealed tubes with 3N HCl took longer to release cystine than refluxing with 6N HCl, but caused less destruction of amino acids. The most suitable times for hydrolysis in sealed tubes were 4 hours for cystine and 8 hours for methionine; hydrolysis for 6 hours might give nearly maximum values for both.

(5) Enzymatic decarboxylation:

Eicott (1951) stated in this method of amino acid determination, enzyme systems that have the property of specifically decarboxylating one amino acid. An aliquot of a neutralised protein hydrolysate was mixed in a warburg or van Slyke
apparatus and carbon dioxide evolved is estimated by standard techniques. The total amount liberated is equivalent to the amino acid present in the hydrolysate. The method appears to be rapid and capable of accuracy provided the enzyme system is available.

(4). Chromatography:

Upto early nineteenforties it could have been said that the history of chromatography was simple. Mikhail and Tswett invented the method in early nineteen hundreds. It was known that fixed beds of charcoal was used long before this, yet it was generally felt that such applications could not be called chromatographic. However the situation was changed, when in the early nineteenforties more processes, some newly invented, other not, began to be spoken of as chromatographic.

The concept of chromatography is epitomized as a separation process applicable to essentially molecular mixtures, which relies on distribution of the mixture between essentially two dimensional or thin phase and one or more bulk phases which are brought into contact in a differential counter current manner. Chromatography as has been defined by Gordon, Mastin and Syage (1944) is a procedure of analysis by percolation of fluid through a body of comminuted or porous rigid material. They developed a system of partition chromatography which was used by Tristram (1946) for determining the mono amino, mono carboxylic acid contents of several proteins.
(5) **Paper chromatography**:

Consden, Gordon and Martin (1944) used for the first time, partition chromatography on a submicro scale. Cellulose in the form of a strip of filter paper was used as the stationary phase. Majority of the methods which have been devised for paper chromatography are based on the principles "of separation of substances from a mixture by the passage of solvent in a definite direction and selective fixation" Weil (1953). A drop of solution containing mixture of substances is placed on one end of a piece of filter paper. This end is placed in a suitable solvent within a closed container. The solvent passes over the spot where the solution had been applied. Each substance in this mixture will ideally move along with the solvent at a unique rate, so that after some time all components of the mixture will occupy a distinct position somewhere along the path of the solvent.

Rutter (1953) described some new technique in paper chromatography, in which substances under analysis are separated into circular zones. It is said to have the advantages of speed and sharpness of separation, possibility of removing test sectors during development and small and compact apparatus. Methods are given for the location of colourless absorbates, based on changes of temperature at which the paper chars, pH changes, changes in absorptive property of the paper and changes in light transmission and reflection. A application of the technique to the analysis of dyes, biological materials and inorganic substances are described.
Landua et al (1957) illustrated the results of a comprehensive study of the influence of sample pH on the size and position of the spots of a series of amino acids and related substances; the solvent were phenol, 2:4 lutidine and 1 butanol; pH effects for solvents chemically related to these were roughly parallel. The different pH values were obtained by using varying concentrations (10^{-1} N to 10^{-6} N) of HCl or NaOH in the samples. The results apply to single amino acid or to mixture and may be used to select a pH most favourable to separation in 1 dimension or in the first direction of 2 dimensional chromatograms. It was difficult to obtain reproducible Rf values in the second direction of 2 dimensional chromatograms.

Franklin et al (1951) making the study of blood plasma protein mixture by paper chromatography gave an account of the general application of the modified technique to blood plasma. The developing solutions were 0.1 M sucrose and 0.1M NaK tartarate. The developed chromatograms were steaked with a benzidine reagent and photographed, since the colour are rapidly marked by the background colour. The separation of protein was aided by inclusion of surface active reagents in the sample.

Storgards and Linquist (1953) made a chromatographic investigation, concerning the presence of free amino acids in different types of cheese. By means of one dimensional paper chromatography 200 samples of cheese comprising 11 varieties were examined for the presence of the various free amino acids.

Giri, Radhakrishosan and Vaidyanathan (1953) reviewed
previously described method and described in detail the method depending on circular paper chromatography. The amino acids separated with mixture of n-butanol, acetic acid and water (40:10:50) are detected with ninhydrin and eluted with 75% ethanol containing copper sulphate and colour is compared with that by a standard run simultaneously.

Giri and Rao (1953) describing circular paper chromatography, described a new technique for the separation and estimation of overlapping amino acids. Overlapping amino acids are those which do not form discrete bands on circular paper chromatograms developed by mixture of n-butanol, acetic acid and water (40:10:50). These mixed bands are cut out and used as the "wick" by means of which a second chromatograms is irrigated, using as solvents pyridine and water (80:20), pyridine, amylacetate and water (10:10:7) and pyridine isoamyl alcohol and water (10:5:5). By this technique the following groups can be separated and estimated: leucine, isoleucine, valine, methionine, yamino butyric acid tryosine, palamine, proline, glutamic acid, theonine glycine, serine, aspartic acid, arginine, asparagine lysine, histidine. Results were given for the analysis of hydrolysates of casein and edestin.

Wood (1956) applied paper chromatography to the examination of meat extract protein (12 percent) was removed by dialysis and inorganic salts (29 percent) electrolytically. The chief solvent mixture used for chromatography was butanol, acetic acid, water (60:15:25). Glutamic acid, serine, lako conserine,
creatine, methylguanidine, choline were identified; betaine and sugar were not detected.

Kay, Harnis and Enteman (1956) made a quantitative analysis of a mixture of amino acids by paper chromatography. The chromatograms were dried for 20 minutes at 65°C and sprayed with ninhydrin and colour was developed by heating at 65°C for 22 minutes. The absorption of the spots elutes was measured at 575 m\(\mu\) and compared with standard curves. The paper had to be washed and a 1:1 mixture of 95 percent ethanol and 1.2 N acetic acid was found suitable of which the 19 solvents mixtures tried the 2 did not give quantitative results, contained HCl stronger than 0.1 N. Heating between 52°C and 68°C produced the greatest colour development, a maximum within 15 minute at 65°C and without decline within next 15 minutes. The colour development of taurine was 4 times greater, when the ninhydrin spray contained 0.5 percent of NaOH than in its absence. Amino acids added to urine, plasma and liver homogenates were satisfactorily recovered.

George et al (1958) made a rapid quantitative analysis of free amino acids in animal tissues by paper chromatography. Fresh section of frozen muscle, liver, kidney from frog, pigeon and rat were directly applied on circular pieces of whatman No.1 filter paper and chromatograms were run twice with n-butanol, acetic acid and water (40:10:50). The paper was dried at room temperature, sprayed with 0.2 percent ninhydrin in 95 percent acetone and heated for 15 minutes at 65°C. Comparision was made with alcoholic extracts of the same materials and acid hydrolysate of casein and a standard mixture of known amino acids; the
reactions gave all the bands characteristics of the extracts.
No free amino acid was left in tissue residue.

Karapetjan (1960) made a chromatographic study of the
amino acid composition of egg white from hens kept in cages and
in runs. The proteins of egg white and those of yolk were stu-
died by paper chromatography. No difference was found between
eggs from hens kept in batteries and in runs under similar feeding
conditions.

Scharrer and Paker (1960) made a quantitative study of
amino acids in foods and feeding stuffs by paper chromatography.
The estimation of 13 amino acids by descending paper chromato-
graphy was described with buffer at pH 6.2 for lysine, arginine
and phenylalanine, aspartic acid, glutamic acid, serine, cystine,
glycine, threonine and alanine and pH 8.4 for valine, methionine,
isoleucine and leucine. The solvent mixture were 2:4 lutidine
and phosphate buffer (1:1) used for phenylalanine. The spots
revealed with ninhydrin and copper nitrate mixture were cut out
and eluted with methanol. The absorbance of the elute was mea-
sured at 504 m/μ.

Mizell and Simpron (1961) made a chromatographic study
for the separation of amino acids. A mixture of 24 amino acids
were separated by chromatography at 21°C for 30 hours with n-
butanol, acetic acid and water (26:6:25) and then for 20 hours
with n-butanol, methyl ethyl ketone and water (2:2:1) with a
beaker of cyclohexylamine, 1 ml. of every 25 ml. of the second
solvent in the chamber. The latter solvent alone resolved most
amino acids and being dried for 30 minutes at 45°C. The chromatogram was dipped in 0.25 percent (w/v) ninhydrin in acetone containing 7 percent acetic acid (v/v) and finally heated for 15 minutes at 45°C.

Membrev (1961) while applying paper chromatography attempted for large scale estimation of amino acids. Estimation of amino acids was by the method of "Bode" (Nutrition Abstract and Review, 4570 Vol. 25). Preparation and hydrolysis was as described in an earlier paper (Nutritional Abstract Review, 40 Vol. 29). This paper deals only with large scale chromatography of amino acids on paper. The paper was washed in 8 oxyquinoline to remove metal salts. For chromatography of amino acids the solvent was a mixture of n-butanol, acetic acid and water (4:1:1) applied 7 times in 24 hours. The method allowed simultaneous treatment of 12 chromatograms 26x26 cm.

Doboszynski et al. (1964) evaluated the methods of quantitative paper chromatography of essential amino acids. A modification of the method of "Bode" (Nutrition Abstract and Review 4570, Vol. 25) was recommended. The solvent mixture was n-butanol, acetic acid, water (4:1:5). The chromatogram was first treated by an ascending method and then run twice by a descending method. The separated amino acids were revealed by means of ninhydrin treated with Cu (No.3), eluted and estimated photometrically. In this amino acids were separated and estimated on one chromatogram.

Pasieka and Logan (1966) advocated a technique of preparative paper chromatography. By a solvent redevelopment technique
amino acids can be separated from complex biological material in the presence of high concentration of salts. The separation are affected by a 4 or more successive development with solvent stage before the staining of chromatogram or isolation of partic-
cular bands. Results are illustrated with photographs of chromato-
tograms. Thick filter paper sheets up to 4th in length are required for analytical and particularly for preparative chromatograms.

Spinella (1969) made a two dimensional paper chromatographic determination of amino acids in urine. Urine, 2 and 3 ml. was desalted in the compartments of a tobel desalter at pH 5, 7 and 8 respectively. This purified the urine and ensured the recovery of as many amino acids as possible. The portions were combined and amino acids in 125 micro-litre were separated by 2 dimensional ascending chromatography with solvents pyridine, acetone, NH₄OH and water (9:6:1:4) for 1.5 hours and then iso-
propanol, formic acid and water (6:1:1) for 2.5 to 3 hours. The chromatogram was dried, sprayed with ninhydrin reagent and heated at 110°C and 115°C for 1 to 4 minutes. If a sample was quantita-
tively abnormal it was then analysed by amino acid analyzer.

Natzenadel and Lutz (1970) attempted a study of the elution of chromatographic separation of plasma amino acids from blood absorbed on filter paper. The amino acids in a drop of blood dried on filter paper are eluted with a mixture of ethanol and water. One dimensional thin layer chromatography gave good separation of the amino acids and allowed semiquantitative esti-
mation of their concentration. Some values were given for their recovery and a comparison was made between phenylalanine
concentration obtained by this method and by column chromatography.

(b) **Adsorptive techniques:**

For basic amino acids, the technique necessitates an adsorbent mixture and a filter aid.

(c) **Ion exchange methods:**

Ion exchange materials have recently been introduced to amino acids separation. Folin used natural and later, synthetic zeolites to remove bases from biological media and Whitehorn showed that passage of the solution through a column of the zeolite was more effective than simple agitation with it, since the reversal by the liberated sodium ions was prevented. Thus the ion exchange chromatography was originated, but little application was made to amino acid separation. Titaniagel found a limited use but acid washed alumina has been used in a number of different application. The ionizable chloride groups, introduced by washing alumina with HCl, may exchange by the anions of dicarboxylic amino acids in solutions in water or 80 percent alcohol. Glutamic acid may be eluted by acetate buffer and aspartic acid subsequently by caustic soda, a process difficult to understand "Boric alumina", an analogous cation-exchange materials has been applied to the separation of basic amino acids. In the presence of formaldehyde, the neutral amino acids are rendered more acidic and the carboxyl group is strong enough to exchange with anions of acid washed alumina. Lederer and Tehen found that di and tri-peptides, glycine, serine and B-alanine but not L alanine were retained
from mixture with other amino acids in the presence of formalin. Acid peptides are adsorbed from water, glycol peptides from 1 percent formalin and other neutral peptides from 10 percent to renalin, while the adsorption of aromatic neutral peptides on carbon completes the group separation.

Cations exchange resins were originally applied to separate basic from neutral amino acids in analysis, but difficulties were evident as it was not appreciated that neutral acids have distinct affinity for the sulphonc group of resin used. Partridge and westall have investigated the behaviour of Zeokarb-215, a sulphonc acid resin. In this method, the amino acids are adsorbed on the acid resin and in order to avoid the difficulties of selective elution the displacement technique of Tiselius and claesson was employed. A detailed consideration of many of the factors involved, is given. The retention isotherms for single amino acids take the form of the Langmuir equation. Effective separation is achieved if the isoelectric points of the components differ by more than 0.5 to 1.0 pH unit. Use is made of the graphical methods of Tiselius for calculating the concentration of a given displacing agent necessary for a given concentration of issuing amino acids.

The separation of amino acid mixture into groups for subsequent analysis has been achieved by the methods outlined in these two sections. The use of acid aluminas with formaldehyde and the use of hydrogen exchange resin by Block to separate basic from neutral amino acids have been described in principles already
and systems are available by which four groups may be obtained. For analytical purposes, more specific separation may be achieved by partition method, without group separation, but the comparative investigation of the two methods remains to be done.

Robbert and Kolor (1959) made a rapid quantitative determination of arginine, histidine, and lysine by ion exchange chromatography. Arginine, histidine, and lysine were separated from a mixture of 17 amino acids by the method of Thakerman (Nutrition Abstract and Review 4664 Vol. 24). After descending chromatography for 4 hours the amino acids were developed with ninhydrin and 3 spots were measured densitometrically. The concentration were estimated from standard curves prepared by plotting the logarithm of concentration against maximum density. Standard has to be run with each unknown solutions.

Jamallian and Pevet (1968) estimated by ion exchange chromatography the amino acid composition of 26 items common in Middleeast. A preoxidation procedure was used to estimate cystine but was not satisfactory for methionine. The protein quality scores were calculated by two methods and reported together with total essential amino acids in total N.

Seidman et al (1969) made an study of egg yolk by ion exchange chromatography. Egg yolk proteins and lipoproteins were separated by chromatography on a column of carboxymethyl cellulose. Freezing, spray drying and pasteurisation had some effect on chromatographic peaks. There were some differences in peaks between chicken and turkey yolks.
Partition chromatography:

Taking advantage of the gradation of partition coefficients of the acetyl amino acids, Martin and Synge devised a counter current machine for their separation and used it in the analysis of wool hydrolysates. This idea was soon modified to form the well known partition chromatogram and a scheme of analysis for certain acetyl amino acids was devised. This was modified later by Tristram. The method has been used for the analysis of hydrolysates of wool gelatin. The preparation of silicogel of non adsorptive nature necessary for the method has not proved to be straightforward and this is one factor which has tended to prevent its rapid development.

Diatomaceous earth has been used as a substitute for silicogel and has found application in the analogous problem of the separation of the penicillins. Its application to the determination of N-dinitrophenyl derivatives of aminom acid in hydrolysates combines the use of a separation technique with the possibilities of a sensitive absorptiometric method. It has been so applied to the determination of both free amino groups and the individual amino acids in polymyxins. Frenery have described the use of partition columns of silicogel for the separation of the coloured copper complexes of aminom acids.

Starch chromatography:

A development of paper chromatography, which is treated in the next sections, that it chromatography on starch, was introduced in order to increase the scale on which free amino acids may
The advantages of the new volatilization techniques are many.  They allow for the simultaneous analysis of several samples in a single experiment.  By increasing the volatility of the compounds, the extraction process can be accelerated, allowing for faster and more efficient separations.

In addition, these new techniques offer improved precision and accuracy, which is crucial for accurate results.  The ability to analyze multiple samples simultaneously also reduces the risk of contamination, ensuring the reliability of the data.

Moreover, these methods are particularly useful in the field of environmental analysis, where the identification and quantification of volatile organic compounds are essential.  The new volatilization techniques provide a powerful tool for environmental scientists, enabling them to detect and measure trace amounts of pollutants with greater sensitivity and specificity.

Overall, the advancements in volatilization techniques represent a significant step forward in the field of analytical chemistry, offering improved efficiency, accuracy, and versatility for a wide range of applications.
6. **Isotope dilution method**:

Shemin (1945) stated, "this method is based upon the principle that the usual laboratory procedures for isolating amino acids do not separate isotope containing molecules from their normal analogues. If a known amount of an amino acid, that has been labeled by the incorporation of a stable or radioactive isotope element, is added to a protein hydrolysates, and the same amino acid is isolated from the mixture and purified, a determination of the concentration of isotope in the sample will give a direct measure of the amount off the amino acid originally present in the hydrolysate. The decrease in the concentration of the labeled element from the present in the added amino acid to that present in the isolated amino acid indicates the extent to which the labeled amino acid has been diluted".

The technique has so far been applied only with amino acids labeled with $^{15}N$. The measurement of $^{15}N$ requires mass spectrograph, an instrument which is not generally available, although the method has the possibility of being the most accurate and reliable.

(VI). **AMINO ACID IN MUSCLE TISSUE OF CHICK, EGGS, SILKWORM PUPAE, RICE BRAN AND SAFFEREDS**: 

(1) Amino acids in muscles and egg:

A number of muscle fibres make the lean part of meat. These fibres differ in length inm various types of meat and are generally longer in older than in young animals. Principal solids
of meat are muscle proteins, which are usually divided into sarcoplasm (water soluble) fractions, myosins (alkali soluble fractions), and collagen. On hydrolysis distinctions disappear, as the hydrolytic and products are more but the constituent units, the amino acids. The knowledge of the quantitative presence of these units in meat and their availability to the organism, facilitates in the formulation of human diets and an ideal ration for livestock.

Ray Ewing (1951) in his book poultry nutrition gave an account of amino acid composition of poultry products on percentage of crude protein. He enumerated the values for arginine, histidine, lysine, methionine, cystine, phenylalanine, tyrosine, tryptophan, threonine in the egg edible portion and in the chicken muscle. He further mentioned in his book the amino acid content of chicken egg and muscle protein in percent of dry matter and values for arginine, histidine, lysine, phenylalanine, tyrosine, tryptophan, threonine, cystine and methionine are presented in Table No. 2.3.

**TABLE NO. 2.3**

Amino acid content of chicken egg and muscle protein. (values in percent of dry matter)

<table>
<thead>
<tr>
<th></th>
<th>Egg Edible Protein</th>
<th>Egg Membrane</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White*</td>
<td>Yolk*</td>
<td>Total</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.8</td>
<td>6.8</td>
<td>6.4</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.4</td>
<td>1.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>
### Table No. 2.3

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Egg Edible Protein White</th>
<th>Egg Edible Protein Yolk</th>
<th>Egg Edible Protein Total</th>
<th>Egg Membrane**</th>
<th>Muscle*** Light</th>
<th>Muscle*** Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>4.9</td>
<td>5.7</td>
<td>5.2</td>
<td>2.8</td>
<td>8.4</td>
<td>8.4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>6.2</td>
<td>4.4</td>
<td>5.8</td>
<td>2.6</td>
<td>3.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4.5</td>
<td>4.7</td>
<td>4.8</td>
<td>2.0</td>
<td>4.2</td>
<td>4.3</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>2.5</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.0</td>
<td>3.5</td>
<td>3.9</td>
<td>5.2</td>
<td>4.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Cystine</td>
<td>1.9</td>
<td>2.2</td>
<td>2.2</td>
<td>11.2</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>6.6</td>
<td>3.0</td>
<td>5.5</td>
<td>5.4</td>
<td>3.4</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Fat extracted dry weight corrected for ash and moisture and nitrogen content calculated to 16 percent.

* Averages per egg from analysis of mixed white and yolk (separately) one dozen cooked eggs.

** Averages per egg from analysis of mixed whites and yolks of one dozen eggs. Average total weight (including shell) uncooked, 58.7 gm; average weight, yolk + white, 51.2 gm. (Protein, 5.4 gm.)

*** Averages per egg from analysis of composite of membranes from several dozen eggs (protein, 0.12 gm).

**** Beach Munks and Robinson, 1943.


Price et al (1953) and Williams et al (1954) investigated the comparative value for lysine arginine, isoleucine, leucine and phenylalanine content of chickens at different stages
of growth, which are as given in Table 2.4.

### TABLE No. 2.4

Comparative values of lysine, arginine, leucine, isoleucine, phenylalanine in m chickens at different stages of growth.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Day old* gm./16 gm. nitrogen</th>
<th>Week old** (Amino acid content calculated to 16% N.)</th>
<th>4-5 week** old (amino acid content calculated to 16% N.)</th>
<th>10 weeks* old gm/16 gm. nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>7.75</td>
<td>6.8</td>
<td>6.4</td>
<td>7.25</td>
</tr>
<tr>
<td>Arginine</td>
<td>6.69</td>
<td>6.6</td>
<td>6.7</td>
<td>6.63</td>
</tr>
<tr>
<td>Leucine</td>
<td>7.07</td>
<td>7.3</td>
<td>7.1</td>
<td>6.50</td>
</tr>
<tr>
<td>Iso-leucine</td>
<td>4.17</td>
<td>4.2</td>
<td>4.5</td>
<td>4.29</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.39</td>
<td>4.4</td>
<td>4.1</td>
<td>4.78</td>
</tr>
</tbody>
</table>

* The values are from the work of William et al (1954).

** The values are from the work of Price (Jr.) et al (1953).

Scott (1959) analysed meats from 5 Broad breasted turkey toms and 5 hens and tabulated the findings as percentage off proteins for all the essential amino acids and few nonessential ones. The histidine content was noted to be greater than that of lean meat. On comparing the result with value reported for other meats, milk and eggs, he recommended that turkey meat with its high protein content was a rich source of essential amino acids.
Ducay et al (1960) studied the free amino acid content of infertile chicken eggs. Extract of egg white and yolk each contained at least 16 percent amino acids. Total values ranged from 0.14 to 0.54 and 38 to 41 micromoles per ml.

Naidu (1967) stated in his book "poultry keeping" in India based on the report of F.A.O. technical committee on poultry products of the Asia and the far East (1966), the amino acid composition of proteins in egg and chicken meat. The value for arginine, histidine, lysine, tyrosine tryptophan, phenylalanine, cystine, methionine, threonine, serine, leucine, isoleucine and valine were thus innumerable, Table 2.5.

**TABLE NO. 2.5**

Composition of protein in rice, milk, egg and chicken meat.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>% percent</th>
<th>Rice</th>
<th>Cow milk</th>
<th>Hen egg</th>
<th>Chicken meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>1.2</td>
<td>4.2</td>
<td>6.6</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>1.7</td>
<td>2.6</td>
<td>2.4</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>5.2</td>
<td>8.7</td>
<td>7.0</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>5.7</td>
<td>6.0</td>
<td>4.5</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.3</td>
<td>1.5</td>
<td>1.5</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5.0</td>
<td>5.5</td>
<td>6.3</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Cystine</td>
<td>1.3</td>
<td>1.0</td>
<td>2.4</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>3.0</td>
<td>3.2</td>
<td>4.0</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>3.8</td>
<td>4.7</td>
<td>4.3</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td></td>
<td>4.3</td>
<td></td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>8.2</td>
<td>11.2</td>
<td>9.2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.2</td>
<td>7.5</td>
<td>7.7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>6.2</td>
<td>7.0</td>
<td>7.2</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Report of F.A.O. technical Committee on poultry products of Asia and far east. Quoted from Poultry Keeping in India by Naidu (1967).
Eassay and Ritchary (1968) estimated and reported comparative value of amino acid composition of proteins in turkeys, beef pork, milk and eggs. His values for amino acid composition were with respect to arginine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine, which is as shown in Table 2.6.

**TABLE NO. 2.6**

Comparasion of amino acid composition of various animal food (percent of protein).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Turkey</th>
<th>Chicken</th>
<th>Beef</th>
<th>Prok</th>
<th>Milk</th>
<th>Egg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine.</td>
<td>6.6</td>
<td>6.7</td>
<td>6.4</td>
<td>6.7</td>
<td>4.3</td>
<td>6.4</td>
</tr>
<tr>
<td>Cystine.</td>
<td>1.0</td>
<td>1.8</td>
<td>1.3</td>
<td>0.9</td>
<td>1.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Histidine.</td>
<td>3.0</td>
<td>2.0</td>
<td>3.3</td>
<td>2.6</td>
<td>2.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Isoleucine.</td>
<td>5.0</td>
<td>4.1</td>
<td>5.2</td>
<td>3.8</td>
<td>8.5</td>
<td>8.0</td>
</tr>
<tr>
<td>Leucine.</td>
<td>7.6</td>
<td>6.6</td>
<td>7.8</td>
<td>6.8</td>
<td>11.3</td>
<td>9.2</td>
</tr>
<tr>
<td>Lysine.</td>
<td>9.0</td>
<td>7.5</td>
<td>8.6</td>
<td>8.0</td>
<td>7.5</td>
<td>7.2</td>
</tr>
<tr>
<td>Methionine.</td>
<td>2.6</td>
<td>1.8</td>
<td>2.7</td>
<td>1.7</td>
<td>3.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Phenylalanine.</td>
<td>3.7</td>
<td>4.0</td>
<td>3.9</td>
<td>3.6</td>
<td>5.7</td>
<td>4.9</td>
</tr>
<tr>
<td>Threonine.</td>
<td>4.0</td>
<td>4.0</td>
<td>4.5</td>
<td>3.6</td>
<td>4.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Tryptophan.</td>
<td>0.9</td>
<td>0.8</td>
<td>1.0</td>
<td>0.7</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Tyrosine.</td>
<td>1.5</td>
<td>2.5</td>
<td>3.0</td>
<td>2.5</td>
<td>5.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Valine.</td>
<td>5.1</td>
<td>6.7</td>
<td>5.1</td>
<td>5.5</td>
<td>8.4</td>
<td>7.3</td>
</tr>
</tbody>
</table>

AMINO ACID COMPOSITION OF SILKWORM PUPAE, RICE BRAN AND SALSEEDS:

The silkworm pupae and salseeds are relatively new indigenous ingredient to be dealt by nutritionist as a source of feed to the livestock. It has attracted little attention of the nutritionist to explore its nutritive worth for feeding it to livestock. As such the literature on the composition of protein and amino acid is scarcely available for these uncommon food items. However whatsoever information was available on these feed are incorporated here as follows:

Mukherjee (1964) while evaluating the chemical composition of Assam Muga silkworm pupae tabulated the finding as follows:

- Crude protein: 74.36%
- Ash: 6.39%
- Crude fibre: 9.7%
- Calcium: 0.26%
- Phosphorus: 0.86%

(Value quoted from Indian Veterinary Research Vol. 42, No. 5 May, 1965).

Ronda et al (1966) estimated the proximate constituents in 10 samples of different residues from 1963 rice crops of suez in valencie province. Values for crude protein ranged from 6.71 to 18.09 percent and for crude fibre from 0.31 to 18.57 percent.

In seven of the samples after hydrolysis 18 amino acids were estimated (Moore et al (Nutrition Abstr. 36, Vol. 29). In
the same 7 samples protein quality was assessed by the criteria of Block and Mitchell (Nutritional Abst. and Dev. 1947, 16: 249) and of Oser (Nutritional Abst. 2205, Vol. 21).

Subhas Chandran (1969), mentioned in his book "animal nutrition", the chemical composition and the amino acid composition of protein of silkworm pupae based on the findings of food technological research institute, Mysore which is as detailed below:

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>10</td>
</tr>
<tr>
<td>Crude protein</td>
<td>55</td>
</tr>
<tr>
<td>Lipids</td>
<td>25-27</td>
</tr>
<tr>
<td>Ash</td>
<td>5</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>3</td>
</tr>
</tbody>
</table>

The amino acid composition of silkworm pupae protein is in the given Table 2.7.

**TABLE No. 2.7**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Percent of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>5.7</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.0</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.4</td>
</tr>
<tr>
<td>Methionine</td>
<td>4.4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.7</td>
</tr>
<tr>
<td>Valine</td>
<td>3.8</td>
</tr>
</tbody>
</table>
Harris and Crampston (1960) in his book "Applied animal nutrition" reported the quantitative figures of arginine, cystine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine tryptophan and valine in the rice bran protein, which is as shown in Table 2.8.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Percent</th>
<th>As fed</th>
<th>On D.W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>.50</td>
<td>.55</td>
<td></td>
</tr>
<tr>
<td>Cystine</td>
<td>.10</td>
<td>.11</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>.20</td>
<td>.22</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>.40</td>
<td>.44</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>.60</td>
<td>.66</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>.50</td>
<td>.55</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>.40</td>
<td>.44</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>.40</td>
<td>.44</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>.10</td>
<td>.11</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>.60</td>
<td>.66</td>
<td></td>
</tr>
</tbody>
</table>

Cf.: Quoted from "Applied animal nutrition" by Champton and Harris (1969) Page no. 664.
CHAPTER III

MATERIALS AND METHODS

PREPARATION OF STANDARD AMINO ACIDS SOLUTION

The method of Cawtho and Pardee (1952) with some modifications was followed for preparing the standard amino acids solution and their respective application on the chromatograms for developments.

0.1 millimole of each amino acid were weighed, in a
MATERIALS AND METHODS

COLLECTION OF SAMPLES OF DIFFERENT FEEDS UNDER STUDY:

The samples of the different feeds studied in the present investigation were collected in the following manner:

The samples of egg were procured from Central Poultry Farm, Patna. The eggs of the pure breed Black australops were collected from the laying pens. The birds at the poultry farm were maintained under the identical environment.

The day old chicks of the Black australops were also procured from the Central Poultry Farm, Patna from a single hatch.

The sample of rice bran was obtained from a neighbouring village of Bihar Veterinary College, Patna.

The sample of salseed was procured from Ranchi and of silkworm pupae from Government Silk Institute, Nathimgar near Bhagalpur (Bihar).

CHROMATOGRAPHY:

Preparation of standard amino acids solution:

The method of Cowgill and Pardee (1962) with some modifications was followed for preparing the standard amino acids solution and their respective application on the chromatograms for development.

0.2 millimole of each amino acids were weighed, in a
previously weighed clean and dried test tubes. The quantities of each amino acids taken were as follows:-

(i) Lysine monochloride (F. Merck) - 36.53 milli grams.
(ii) Arginine monochloride (F. Merck) - 42.14 , ,
(iii) DL-Phenylalanine (B.D.H.) - 33.04 , ,
(iv) L-Leucine (containing isoleucine) - 26.38 , ,
(B.D.H.).

The quantity weighed both leucine and isoleucine was equivalent to 0.2 millimole of leucine. Leucine and isoleucine have identical molecular weight and as such their locations on the developed chromatograms are close to each other.

To each test tubes containing weighed amounts of amino acids, 10 ml. of 10 percent isopropanol in distilled water containing dilute HCl was added. Presence of HCl in isopropanol solution helps the process of dissolving the amino acids. Test tubes were shaken carefully till the amino acids got dissolved. Further 10 ml. of 10 percent isopropanol was added to each test tubes inorder to make the amino acids solution of required strength. Thus the standard amino acids solution of lysine, arginine, phenylalanine, leucine and isoleucine (one solution) was prepared. 1 micro litre of each of the standard solution contained 0.01 micromole of the respective amino acids except for leucine and isoleucine. The test having standard solution were stoppered and kept for the preparation of standard curves.

The weight of the amino acids contained in standard solutions utilized for the making of standard curves, Table 3.1.
<table>
<thead>
<tr>
<th>Quantity in Molecules</th>
<th>Lysine in Microgram</th>
<th>Arginine in Microgram</th>
<th>Phynylalanine in microgram</th>
<th>Leucine isoleucine in Microgram</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>1.8264</td>
<td>2.1066</td>
<td>1.652</td>
<td>1.319</td>
</tr>
<tr>
<td>0.02</td>
<td>3.6528</td>
<td>4.2132</td>
<td>3.304</td>
<td>2.638</td>
</tr>
<tr>
<td>0.03</td>
<td>5.4792</td>
<td>6.3198</td>
<td>4.956</td>
<td>3.957</td>
</tr>
<tr>
<td>0.04</td>
<td>7.3056</td>
<td>8.4264</td>
<td>6.608</td>
<td>5.276</td>
</tr>
<tr>
<td>0.05</td>
<td>9.1320</td>
<td>10.5330</td>
<td>8.260</td>
<td>6.595</td>
</tr>
<tr>
<td>0.06</td>
<td>10.9584</td>
<td>12.6396</td>
<td>9.912</td>
<td>7.914</td>
</tr>
<tr>
<td>0.07</td>
<td>12.7848</td>
<td>14.7462</td>
<td>11.564</td>
<td>9.233</td>
</tr>
<tr>
<td>0.08</td>
<td>14.6112</td>
<td>16.8528</td>
<td>13.216</td>
<td>10.552</td>
</tr>
<tr>
<td>0.09</td>
<td>16.4376</td>
<td>18.9594</td>
<td>14.868</td>
<td>11.871</td>
</tr>
<tr>
<td>0.10</td>
<td>18.2640</td>
<td>21.0660</td>
<td>16.520</td>
<td>13.190</td>
</tr>
<tr>
<td>0.11</td>
<td>20.0904</td>
<td>23.1726</td>
<td>18.172</td>
<td>14.509</td>
</tr>
<tr>
<td>0.12</td>
<td>21.9168</td>
<td>25.2792</td>
<td>19.824</td>
<td>15.828</td>
</tr>
<tr>
<td>0.13</td>
<td>23.7432</td>
<td>27.3858</td>
<td>21.476</td>
<td>17.147</td>
</tr>
<tr>
<td>0.14</td>
<td>25.5696</td>
<td>29.4924</td>
<td>23.128</td>
<td>18.466</td>
</tr>
<tr>
<td>0.15</td>
<td>27.3960</td>
<td>31.5990</td>
<td>24.780</td>
<td>19.785</td>
</tr>
<tr>
<td>0.16</td>
<td>29.2224</td>
<td>33.7056</td>
<td>26.432</td>
<td>21.104</td>
</tr>
<tr>
<td>0.17</td>
<td>31.0488</td>
<td>35.8122</td>
<td>28.084</td>
<td>22.433</td>
</tr>
<tr>
<td>0.18</td>
<td>32.8752</td>
<td>37.9188</td>
<td>29.736</td>
<td>23.742</td>
</tr>
<tr>
<td>0.19</td>
<td>34.7016</td>
<td>40.0254</td>
<td>31.388</td>
<td>25.061</td>
</tr>
<tr>
<td>0.20</td>
<td>36.5280</td>
<td>42.1320</td>
<td>33.040</td>
<td>26.380</td>
</tr>
</tbody>
</table>
Preparation of reference amino acids mixture:

Since the standard amino acids solutions were prepared for utilizing them in preparing standard curves, 10 ml. from each of the four standard solutions were pooled in a cleaned and dried test tube. It was later used for applying it by the side of the hydrolysates, for the purpose of identifying the resolved amino acids on the chromatogram.

Laboratory for chromatography:

All the chromatographic work were conducted in the laboratory meant solely for chromatographic work and free from contamination by fumes and ammonia. The laboratory room for chromatography was centrally placed in the building and was having minimum of window space, which prevented from radical change in temperature. These conditions facilitated to get the developed chromatogram with uniform spots of amino acids and with straight solvent fronts.

The following equipments and materials were used for the estimation of amino acids in the present investigation:

(1) Two dimension paper chromatography cabinet (sirco make) size 24"x12"x24", having glass and wooden fittings (paraffined), along with 3 solvent troughs, 3 bended glass supports, and 6 antisyphon rods.

(11) A packet of 100 sheets of whatman filter paper No. 1 for chromatography.

(iii) Hair drier, glass atomizer, table lamp.
(iv) Pipettes, micro pipettes, funnels, separating funnels, watch glass and other routine glass wares used in laboratory works.

(v) Table with glass plates.

Absolute cleanliness was maintained during the chromatographic work. The glass wares along with the glass plate, solvent troughs and rods were perfectly washed with hot soap water and then with distilled water. The micropipettes were cleaned with hot soap water, distilled water and finally with acetone. It was an usual practice to see that all the apparatus in use were perfectly cleaned and dried before use. Every care was taken to avoid contamination of the applicances. During the work hands were kept clean and clean rubber hand gloves were also used when necessary, particularly while touching the chromatograms.

The method of Block and Zweig (1958) was adopted, one dimension descending paper chromatography with a single solvent system was employed throughout the work, which comprised of the following main procedures:

(i) Preparation of sample (Hydrolsates).

(ii) Application of sample on the filter paper.

(iii) Development of chromatogram.

(iv) Drying of the chromatogram after development and detection of spots.

(v) Quantitative elution of the ninhydrin treated amino acids.

Preparation of sample Hydrolsates:

The method of Block and Zweig (1958) was followed for
the preparation of hydrolysate from dehydrated, ether extracted samples of eggs, day old chick muscle, salseed, silkworm pupae and the rice bran. 100 mgms. of the sample of each component were weighed accurately in a previously weighed test tubes respectively. 10 ml. of 6N HCl was added in each test tubes and the test tubes were stoppered with rubber cork along with reflex tubes. It was clamped and kept in the boiling water bath for hydrolysis. The hydrolysis was continued for 24 hours spread over three consecutive days. Except for the muscle hydrolysate of day old chick, all other hydrolysates were transferred quantitatively to clean and dry silica basin by repeated washing with 5 ml. of glass distilled water each time. The basin were then kept in steam over for the removal of excess of hydrochloric acid present in the hydrolysate. The basin were kept there till thin films of amino acid hydrochlorides appeared. The basins were now kept inside the desicacor over calcium chloride for 24 hours. After 24 hours the basins were taken out of the desicator and the contents of the basins was dis-solved in 20 ml. of warm glass distilled water. The contents of the basins were thoroughly mixed by gentle shaking and filtered slowly inorder to avoid any loss by spilling. The filter paper over the funnel were washed repeatedly by glass distilled water the volume of water used for washing was 15 ml. The filtrates along with the washing was collected in a cleaned dried silica basin. The basin was again kept in steam oven as earlier and the content was evaporated to dryness. The basin was kept inside the desicacor over calcium chloride for 12 hours inorder to be sure of perfect drying and cooling of
the contents in the basin. Now a known volume of 10 percent isopropanol in glass distilled water was added to respective basins to dissolve the amino acids by gentle and persistent shaking. The contents of the basin were later transferred to cleaned, acetone washed dried test tubes. The test tubes were then shifted for analysis work.

Because of shortage in the material the hydrolysates of day old duck muscles were transferred to cleaned and dried 25 ml. volumetric flask and the volume of the hydrolysates were made 25 ml. by adding glass distilled water. The content of the flasks was mixed thoroughly.

Now 10 ml. from each of the flask was taken in silica basins and the rest of the procedure were as stated above.

Application of the sample (spotting) on the paper:

A sheet of Whatman filter paper No. I was taken and was kept straight over the glass kept on the table. A straight line was drawn 8 cm. below the shorter edge and with a light pencil. Eight pencil points at a distance of 5 cm. apart were marked on the line drawn over the paper and a circle of about 4 mm. diameter was drawn around each pencil points. The circle at both the ends were 2.5 cm. away from the edgem of the paper. There were eight circle in all.

The micro pipettes used for spotting were 100 micro liter in capacity having subdivisions indicating 1 micro liter and was without automatic transferring device prior the commencement
of actual spotting, manipulations with other micropipettes was practiced inorder to acquire proficiency in this delicate procedure.

One cleaned dried micro pipette (0.1 ml) was taken and was dipped in the solution which was to be applied on the paper. The micropipette was allowed to get filled by capillary actions, mouth sucking was avoided because of getting contaminations. When the solution moved to the desired level in the pipette than it was removed from the solution, the outer sides of the pipette was wiped, with the help of dry clean ordinary filter paper. A watch glass was always kept below the filter paper during the spotting process and was moved along the line drawn over the paper inorder to avoid the possible loss of solution to the glass plate. The solutions were applied at the designated points, marked and encircled with soft pencil along with the identifications marks below each one of them. The solutions were applied on the paper by gently touching the paper repeatedly with the pipette containing the solutions. After each such touching the solution was allowed to dry with exposure of ordinary table lamp. Since, spot size, the uniformity of spot and shape of it can affect, the subsequent developed chromatogram. Enough care was taken to each application so that ultimately each spot was small, round and uniform. Average size of the spot was 3.5 to 4 mm. in diameter. Care was taken not to exceed more than eight spots in total in one chromatogram. The spots were then allowed to dry completely. After the spots got dried, the paper was folded sharply along a line 5 cm. from the edge. Such folding kept the distance of the spots
2.5 cm. from the fold. Finally the paper was shifted to the chromatographic cabinet where it was kept in manner described as below:

Both edges near the fold were caught hold by hand protected by clean rubber gloves and the paper was inserted gently in the glass trough which was resting on the upper groove of the cabinet. The paper was fixed in the trough with the help of syphon rod and a glass support which had bended ends. The paper was thus in the hanging position and the antisyphon rod was in a position to control the excess flow of the solvent. A beaker containing the solvent was kept in side the chromatographic chamber inorder to saturate the air with the solvent vapour. After the chromatographic paper was hung in the trough, the chromatographic chamber was closed and tightened by means of screws fitted to it. The solvent was now added in the trough containing the chromatographic paper through the holes in the upper lid of the chamber. The two third of the trough was filled with the solvent and some more was poured when necessary.

Preparation of solvent:

Partridge's solvent (n-butanol : Glacial acetic acid : water = 40 : 10 : 50) was the only solvent used throughout the chromatographic work. This solvent has the following advantages:

(i) It has appreciable resolving power for all the L-amino acids commonly occurring in protein hydrolysates.

(ii) It has nontoxic properties and can be handled conveniently.
(iii) This solvent system does not destroy the amino acids while drying the developed chromatograms.

(iv) In this solvent system the chromatographic paper do not need lengthy pre-equilibration time.

(v) It is easily available and simple to prepare.

In a one litre volumetric flask 400 ml. of n-butyl alcohol was taken and to it 100 ml. of glacial acetic acid was added. The flask was now shaken for a while and then glass distilled water was gradually added up to the mark. The contents was thoroughly mixed by vigorously shaking the flask. During this procedure butanol and acetic acid got saturated with water and their saturation point was ascertained by the absence of bubble at the junction of the two layers of solvent. Now the two layers of the solvent was separated by separating funnel. The contents of the lower layer in the separating funnel was collected in a clean and dry bottle and stoppered. 50 ml. of this solution in a beaker was always kept in the chromatographic chamber. The upper layer in the separating funnel containing butanol, acetic acid mixture was also collected in a separate clean and dry bottle, stoppered and kept for future use as solvent.

Development of the chromatogram:

During the development of the chromatogram, it was checked whether the trough contained sufficient quantity of the solvent. After 20-22 hours when the solvent front travelled a distance of 44 to 46 cm. approximately 5 to 3 cm. away from the other edge, further development was stopped. The developed chro-
matogram was taken out from the solvent trough and kept for drying in the room at the room temperature.

**Locating the spot on the chromatogram:**

Methods of Giri et al. (1952) were applied for drying and development of the chromatogram and the same was also applied during the subsequent elution of the ninhydrin spots for quantitative estimation.

Plastic clips were used for hanging the developed chromatograms. When the developed chromatogram became completely dry, it was sprayed with 0.5 percent ninhydrin in 95 percent acetone. The spraying was performed with the glass atomizer, the following techniques was applied during the spraying operations:

The glass container, with the ninhydrin reagent was caught hold with left hand kept in slightly titled position at a distance of 15 to 20 inches from the developed chromatogram. With the gentle manipulation of rubber bulb, thin spray of the ninhydrin reagent spread out which fall on the paper. The glass atomizer with the ninhydrin reagent was slowly moved to the left on the same line till it reached the other corner. The same procedure was repeated continuously till the entire paper was sprayed with the reagent. Spraying was carefully done, so that it did not overflood the chromatogram, which may result in the formation of diffused and ill-defined spots. The entire process of spraying the developed chromatogram with ninhydrin reagent took about 10-15 minutes and 30 ml. of the reagent was needed. After the spraying
treatment of the developed chromatogram, the paper was folded and placed in hot air oven at 60°C while putting the paper inside the hot air oven, care was taken so that the chromatogram did not touch the side of the oven. To prevent this clean white paper was placed inside hot air oven before placing the chromatogram there. This was solely done to prevent any possible contamination of the ninhydrin spots. The paper was kept inside the hot air oven for 30 minutes. The chromatogram was then taken out of the hot air oven. The resolution of the sample was examined and the ninhydrin spot was located against the corresponding known reference amino acids.

**Elution of the ninhydrin spots:**

Distinct zones of ninhydrin spots were formed at different distances depending upon the amino acids. The ninhydrin spots on identification against reference amino acids in the same chromatogram were cut and kept in separate cleaned and dried test tubes, marked with glass pencil for identification, while cutting the spots on the paper clean and dry scissors was used and the cut paper was kept in the test tubes with the help of clean forceps. A portion of the paper from the original application of the sample and some from the randomly selected portion of the paper were cut and kept in a separate clean and dry test tube for Blank determination.

To each test tube, containing paper cuts, 4 ml. of 75 percent ethyl alcohol containing 0.2 milligram of Copper Sulphate was added. The test tubes were vigorously shaken, till the
ninhydrin colour was properly eluted. The optical density of the coloured solution was estimated using klett summerson photoelectric colorimeter applying green filter (540 mμ). Colorimetric readings were noted and averaged. For the standard amino acids solutions these were plotted on the graph against the respective quantities of amino acids originally taken for application on paper, the graph was drawn. The colorimetric readings obtained from the unknown mixture or hydrolysates were utilized for evaluation of quantities of the respective amino acids with the help of the respective standards curves.

**Estimation of moisture, dry matter, and fat content:**

The moisture, dry matter and ether extract of the respective samples were estimated as recommended in A.O.A.C. method with some slight modifications.

**Estimation of total nitrogen in dehydrated, ether extracted samples was done in the following manner:**

**Digestion:**

Micro-kjeldahl digestion by the Arnold Curning method was followed. 100 mg. of the sample of eggs, rice bran, salseed silk worm pupae were weighed and transferred to the micro-kjeldahl digestion flask. To each flask 3 ml. of conc. sulphuric acid 10 drops of 5 percent copper sulphate solution, 1 gm. of potassium sulphate were added. The digestion of the sample was continued till the mixture in the digestion flask became clear. 1 ml. of conc. H2SO4 was further added in each test tubes and the digestion
was further continued for about an hour inorder to be sure of complete digestion. After the digestion was complete, the micro-digestion flask was cooled and the contents was transferred to a clean and dry 50 ml. volumetric flask. The volume of the digest was finally made 50 ml. by adding glass distilled water to the digest. The contents of the volumetric flask was then roughly mixed by shaking the flask: 5 ml. of the diluted digest was utilised for the estimation of ammonia, which was done by distillation and titration as described below:

N.B.:— For shortage of material the determination of total nitrogen in the pooled muscle samples of day old chick, 10 ml. of left portion of the sample hydrolysates were taken in the micro-Kjeldahl flask.

**Distillation of the ammonia and titration for the estimation of nitrogen**

For the distillation of ammonia and determination of nitrogen Meeker and Wagner (1940) method was followed. Here the ammonia is absorbed in the boric acid solution. 4 percent boric acid solution was used for this purpose. On distillation the solution of boric acid atonce turns alkaline to methyl red due to presence of ammonia. To get back the original colour of the solution, standard sulphuric acid was added, drop by drop from the micro-burette which amounted to the ammonia distilled in the boric acid solution.

20 ml. of 4 percent boric acid solution containing Meekar's indicator, comprising methyl red and methylene blue was
taken in 100 ml. conical flask. The conical flask containing the boric acid solution were marked at two levels, one at 50 ml. level and another at 80 ml. level. The tip of the condenser was kept dipped in the boric acid with a view to prevent the possible loss of ammonia to outside. 5 ml. of diluted digest of the samples were put in inner chamber of the markham’s still and to it saturated solution of sodium hydroxide was added, till solution in the inner chamber of the markham’s still turned black. The steam was allowed to pass in the inner chamber and the distillate was collected in receiving flask containing 4 percent boric acid solution. When the volume of the distillate reached the 50 ml. level in the flask, the distillation was discontinued. The tip of condensor was washed with glass distilled water and the washing was collected in the receiver flask. The flask was then removed and was kept for titration against N/35 $\text{H}_2\text{SO}_4$.

After the distillation was complete the inner chamber of the markham’s still was cleaned by washing it repeatedly with distilled water and fluid inside the chamber was removed by back suction. Before starting the distillation of second sample, blank distillation was done for a while inorder to remove any trace of ammonia present in the distillation plant.

Titration was performed in a microburette having a graduation of 0.05 ml. The end point of the titration was confirmed by matching with control flask containing 20 ml. of 4 percent boric acid with indicator, diluted to 80 ml. The volume in the experimental flask was also made 80 ml. by diluting it with glass distilled water inorder to match the volume of the control flask.
Now the volume of the N/35 $\text{H}_2\text{SO}_4$ used for bringing back the colour of the content in the experimental flask to the level of the control flask was noted. The calculated value of the nitrogen was noted and thus the crude protein content of the samples was ascertained with the help of calculation.

REAGENTS USED

The following reagents were used in the present investigation:

(a) Reagents used for the estimation of amino acids:

1. 10 percent isopropanol in distilled water (containing dilute HCl).
   10 ml. isopropanol, 1 ml. $4\text{N HCl}$, 89 ml. distilled water (when it was used for hydrolysates HCl was being omitted, and distilled water was taken in 90 ml. quantity).

2. 6 N HCl.

3. Partridge's solvent.
   $n$-n-butanol: Glacial acetic acid: water (40:10:50).

4. Ninhydrin solution (for colour development).
   0.5 gm. ninhydrin ($C_6H_4\text{CO. CO. CO. H}_2\text{O}$) was dissolved in 100 ml. of acetone (Analar).

5. 75 percent ethyl alcohol (containing 0.2 mg. of $C_4\text{SO}_4\cdot 5\text{H}_2\text{O}$ in 4 ml.).
   50 mg. $C_4\text{SO}_4\cdot 5\text{H}_2\text{O}$, was dissolved in 210 ml. of distilled water. To this solution 790 ml. of ethyl alcohol was added to make it one litre.
(b) Reagents used for estimation of total nitrogen:

1. Copper sulphate 5 percent solution.
2. Boric acid 4 percent solution.
   40 g. boric acid dissolved in hot distilled water
   and volume made upto 1 litre.
3. Meeker's indicator.
   25 mg. methylene blue and 100 mg. of methyl red in
   100 ml. of absolute alcohol. 2 ml. of this indicator
   was added to 1 litre of boric acid 4 percent solution.
4. Standard sulphuric acid.
   N/35 H₂SO₄
5. Strong alkali.
   60 g. sodium hydroxide pellets in 80 ml. distilled
   water.

METHODS FOR CALCULATIONS

During the various quantitative estimations of the
samples under study, calculations were made as follows:

1. Dry matter, moisture and fat content:
   (a) Dry matter percent.
   \[
   \text{Weight of D.M.} \times 100 = \text{D.M. percent.}
   \text{Weight of fresh sample}
   \]
   (b) Moisture percent.
   \[
   \frac{\text{Weight of sample} - \text{weight of D.M.} \times 100}{\text{Weight of sample}} = \text{Moisture percent of fresh muscle tissue.}
   \]
(c) Fat percent (in D.M.).

\[
\text{Weight of ether extract} \times \frac{100}{\text{Weight of D.M.}} = \text{Fat percent of D.M.}
\]

2. Estimation of total nitrogen in dehydrated, ether extracted samples:

Since, 50 ml. of digest was prepared from 100 mg. of dehydrated ether extract samples.

Therefore, 5 ml. of it contained 10 mg. of the samples - A. Since, 1 ml. of N/35 H₂SO₄ is equivalent to 0.4 mg. of nitrogen.

So \( A = \text{ml. of N/35 H}_2\text{SO}_4 \times 0.4 \text{ mg.} \) ........... B

or 100 mg. of dehydrated either extract sample contains = B \times 10

mg. of Nitrogen .......

or 100 g. of sample contains \( C \) grams of Nitrogen.

or 100 g. of sample contains \( C \times 6.25 \text{ gms. of crude protein. In case of muscle samples, due to shortage of the material, the hydrolysates prepared from 100 mg. of dehydrated ether extracted samples, were diluted to 25 ml. and 10 ml. of which was utilized for estimation of nitrogen. 50 ml. of digest was prepared from the 10 ml. of the hydrolysates, which contains only 40 mg. of the dehydrated other extracted sample. So 5 ml. of this digest used for the estimation of Nitrogen contained only 4 mg. of the sample. The rest of the calculation for determination of N₂ is the same as adopted with other samples.}

3. Amino acid content in dehydrated, ether extracted sample:

First, the amount of amino acid was estimated in 10 microlitres of hydrolysates and then from this estimated amount the amino acid present in 2 ml. of hydrolysates was calculated (stan-
-dard curves and Table 3.1 were used during such calculations). Since 2 ml. of hydrolysates was prepared from 100 mg. of dehydrated, ether extracted samples.

Therefore, 1 ml. of it represents 50 mg. of the sample or 10 micro litres of it represents 0.5 mg. of the sample. When G represented the total amount of amino acid present in 10 micro litres of hydrolysates and I, the amount of amino acid in 2 ml. of the hydrolysates.

Then I = G X 200 microgram (i.e. amount present in 2 ml. hydrolysate or 100 mg. of the sample).

\[
\frac{G \times 200}{1000} \text{ mg.}
\]

or \[
\frac{G \times 100}{1000} \text{ mg. of amino acid present in 1 ml. of the hydrolysates or 50 mg. of the sample.}
\]

\[
\frac{G \times 200 \times 10 \times 100}{1000} \text{ mg. = Total amount of amino acid in 100 gm. of dehydrated, ether extracted sample.}
\]

\[
\frac{G \times 200 \times 100}{1000 \times 1000} \text{ mg. = Grams of amino acid in 100 g. of dehydrated, ether extracted sample \ldots \ldots \ldots \ldots \ldots R.}
\]

Since the salseed is having very low percentage of protein, the hydrolysates of salseed was dissolved only in 0.5 ml. of isopropanol, 10 micro litre of which was used for spotting and subsequent amino acid estimation by the above formula.

The mode of calculation of amino acids in muscle tissue were also the same, except that in this case hydrolysates was having only 40 mg. of dehydrated, ether extract muscle sample, in 25 ml. instead of 100 mg. as with other samples.
4. Various ways of representing the amount of amino acid present in the sample:

During the present work of the determination of amino acids in different samples, the values were represented as follows:

(i) Grams of amino acid per 100 gram of dehydrated ether extracted samples.

\[ R \] - represents this value.

(ii) Grams of amino acid per 16 gms. of nitrogen in the dehydrated ether extracted samples.

\[
\text{Grams of amino acid in 100 gms. of dehydrated, ether extracted samples} \times 16 = \frac{R \times 16}{C} \text{ gms.}
\]

Total nitrogen in gms. in 100 gms. of dehydrated ether extracted samples.

(iii) Amino acid content in grams of Nitrogen per 100 g. of dehydrated, ether extracted samples.

Calculation were made on molar basis. The number of atoms of nitrogen in the amino acid was multiplied by 14 and the product was divided by the molecular weight of amino acid in question. Then the value was expressed in grams.

Amino acid nitrogen for Arginine = \[
\frac{4 \times 14}{210.66} = \ldots \ldots P \text{ gm.}
\]

The total quantity of amino acid nitrogen was calculated by multiplying \( P \) with the amount of amino acid estimated in the hydrolysates. For example :-

Amino acid nitrogen in estimated Arginine of muscle tissue

\[
\frac{4 \times 14}{210.66} \times 6.38 = 1.69 = Q.
\]

(iv) Amino acid content in gram of Nitrogen per 100 gram of nitrogen of dehydrated ether extracted samples.
Amino acid Nitrogen
Total nitrogen in 100 g. of
dehydrated ether extracted
samples.

(v) Amino acid in grams per 100 grams nitrogen.

\[
\text{Amino acid in g. in 100 g. of}
\text{dehydrated ether extracted samples} = \frac{R}{C} \times 100
\]

\[
\text{Amount of nitrogen in 100 g. of}
\text{dehydrated ether extracted samples}
\]

**STATISTICAL METHOD**

The different observations in the present study were subjected to statistical analysis. For this purpose calculation of standard error and non-paired t test was performed.

(i) **Calculation of standard error:**

The following formula was used to calculate the standard error of the different observations (Snedecor, 1967):

\[
S.E. = \sqrt{\frac{x^2 - \frac{(x)^2}{n}}}{n(n-1)}
\]

Where \(x^2 = \text{Crude sum of square}\)

\[
\frac{(x)^2}{n} = \text{Correction factor.}
\]

\(n = \text{Total number of observation.}\)

(ii) **t Test:**

In order to see the statistical difference between two samples, the data of the present study were subjected to non-paired t test as described by Snedecor (1967). The following formula
was used.

\[ t = \frac{\bar{X}_1 - \bar{X}_2}{\text{S.E. of mean difference}} \text{ with } (2n-2) \text{ d.f.} \]

\[ = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{2s^2}{n}}} \]

Where \( \bar{X}_1 \) = Mean of sample I.
\( \bar{X}_2 \) = Mean of sample II.
\( s^2 \) = pooled sample variance.

'F' test: In order to perform non-paired 't' test the \( s_1^2 \) (variance of feed I) and \( s_2^2 \) (variance of feed II) were subjected to 'F' test. To see the significant difference between \( s_1^2 \) and \( s_2^2 \), when \( F \), was non significant the pooled \( s^2 \) (variance) was obtained to calculate t value. The following formula was used to calculate 'F' (Snedecor, 1967):

\[ F = \frac{s_1^2}{s_2^2} \text{ with } (n_1 - 1, n_2 - 1) \text{ d.f.} \]

where \( s_1^2 \geq s_2^2 \)

\[ F = \frac{s_2^2}{s_1^2} = \text{ with } (n_1 - 1, n_2 - 1) \text{ d.f.} \]

where \( s_2^2 \geq s_1^2 \)

**Pooled variance:** The following formula was to estimate the pooled variance:

\[ s^2 = \frac{s_1^2(n_1-1) + s_2^2(n_2-1)}{2n-2} \]

Where \( s_1^2 \) = variance of sample I.
\( s_2^2 \) = variance of sample II.

**********
CHAPTER IV

RESULTS AND DISCUSSION.

It is well established that in the field of pharmaceutical chemistry the choice of the developing solvent plays an important role in the uninterrupted efficiency of the process of resolutions and the isolation of products. This work was based on the findings of previous works.

Nelson (1949) preferred the use of butanol-acetic acid and water mixture as solvent. He stated that when the samples were separated, the developing solvent in the moving phase and the distribution of the solute and the solvent placed in the chromatogram were different. Different mixtures of butanol and water were used as developing solvents. Fisk et al. (1950) appreciated the use of butanol-ethyl acetate mixture as a solvent. Lee and Chung (1953) used lamellar solutions with water. Black and Peek (1953) also recommended 10% of the solvents of acetic acid for the analysis of amino acids on paper.

Nagan and Nagar (1954), while doing dissolving water chromatography used butanol, acetic acid, and water (1:1:1 v/v) for the separation and extraction of lignins, lignocelluloses, aldehydes, vanillic, conidendric, dihydroxy, tyrosine, tryptophan, proline, alanine, and glycine. Butanol, acetic acid, and water
RESULTS AND DISCUSSION

Choice of solvent:

It is a well established fact in the field of chromatography that choice of the developing solvent plays an important role in the ultimate efficiency of the process of resolutions and the solvent chosen in this work was based on the findings of previous works.

Woiwood (1949) preferred the use of Butanol:Acetic Acid:Water mixture as solvent. He stated that when the two layers separated, the upper layer served as the moving phase and an aliquot of lower layer was placed in the chromatogram cabinet. Redfield and Barron (1952) observed that all common amino acids could be separated with different aliphatic alcohols and water mixture, as the developing solvent. Kowkabany (1952) explained that slower moving solvents produced more sound and less diffused spots. Giri et al (1952) appreciated the use of butanol, acetic acid, water mixture, as a solvent. Levy and Chung (1953) used the same mixture with success. Block and Zweig (1958) also recommended it, as one of the solvents of choice, for the analysis of amino acids on paper.

Gangal and Magar (1964), while doing descending paper chromatography used butanol, acetic acid and water (4:1:5) v/v for the separation and estimation of leucine, isoleucine, phenylalanine, valine, methionine, threonine, tyrosine, tryptophan, proline, alanine and glutamic acid. Butanol acetic acid and water
(40:14:15) v/v after two runs was used for separation and identification of lysine, asparagine and histidine. Butanol acetic acid and water (250:60:350) v/v after two runs was used for separation and identification of cystine, lysine, histidine, arginine, hydroxyproline, alanine and tyrosine.

In view of other's work, it was considered proper to use partridge's solvent throughout the chromatographic work. The advantages narrated earlier, made it more alluring for the purpose.

**Fixation of amino acid location on a chromatogram:**

The specific distance travelled by the particular amino acid during the solvent development, indicates its resolution from the mixture of amino acids or hydrolysates applied on the chromatographic filter paper. Different amino acid occupies different position on the chromatogram. Their locations on the paper along with the distance travelled by the solvent front, assists in calculating their Rf. value. Determination of the Rf. value have been appreciated as the guideline for identification of the amino acids. However Zimmerman (1953) put forward a number of facts that may cause variation in Rf. value. Block and Zweig (1958) stated that such calculated value must not be believed entirely. They further recommended that while applying the aliquot of a sample on the chromatographic paper, a good number of spots of reference standard amino acids mixture may be applied. The application should be such as to facilitate identity of unknown. They also advocated that such application on the same chromatogram cancelled the probable variation.
Williams and Harding (1958) reported that width of the paper may affect the Rf. value. The Rf. values of lysine, alanine, tyrosine, glutamic acid and theonine obtained by ascending chromatography with collidine or isobutyric acid saturated with water as solvent, increased significantly when the width of the paper was reduced.

In a given apparatus and solvent the following factors may influence the Rf. values.

(i) **The temperature**:

As the temperature rises, the viscosity of the solvent will decrease thus increasing the rate of travel of the solvent. Further the solubility of the individual substances will vary in the warmer solvent, with the result that Rf. values will vary in different ways.

**The grade of paper**:

At least six grade of paper are in common use and these possess quite different running properties. Thus solvent travel faster along thick than thin paper.

(ii) **Existence of poly ionic forms**:

Certain compounds particularly the basic amino acids can exist in a number of ionic forms, each having its own Rf. value. Thus on running a chromatogram in a neutral solvent a long streak and not a discrete spot is obtained. This can be overcome by using a solvent of sufficiently high or low
pH such that one of the ionic species predominates to the exclusion of the other. For example arginine, histidine and lysine streak in aqueous phenol but run as discrete spots in phenol ammonia or butanol acetic acid, although with much altered Rf. values.

(iii) **Labile compounds:**

Compounds such as adrenaline and sulphur amino acids decompose in many solvents during the actual flow and again result in a streak. This can sometimes however be overcome by conversion into a more stable form prior to chromatography.

(iv) **Substances with same Rf. values:**

When two substances have the same or very similar Rf. values they often affect the rate of travel of each other one pushing the other head. This is particularly because when these substance are present in large amount. Thus in certain abnormal urines the urea pushes tryptophan ahead and it appears as a spot on the top of urea in chromatograms run in butanol acetic acid.

(v) **The salt effect:**

Possibly the most important factor responsible for the production of poor chromatograms, is the inorganic salts always present in biological material. This effect is probably due to the fact that salt form strong hydrophyllic centres which extract from the solvent to such an extent
that pools of water form on paper. Further solvent has thin flow around these pools before moving normally along the rest of the paper.

(vi) **Large molecules**

Protein and plant pigments are two examples of large molecules which adversely affect normal chromatographic separation. Protein and pigments act probably in the same way as salts.

(vii) **Homologous series**

Most of the solvent used in chromatography are rich in organic liquids and therefore an increase of CH₂ unit renders the resultant compounds more soluble in the solvent. Thus the Rf. values of homologous series usually lie on a straight curve when plotted against CH₂ in a series of compounds, but as the Rf. values approach 100, the straight line curves off and tends to become asymptotic with the front.

(viii) **Length of solvent flow**

As solvent composition is continuously changing its flow that Rf. value determined after short runs will be different from those obtained after long runs. Further as corollary to the above, the distance of the origin from the liquid solvent surface will affect Rf. values.

In all some nine or 10 factors cause Rf. values to vary
both in the same and in different apparatus. Some factors may be standardized such as same apparatus, type of solvent, grade of paper etc. Other factors may be eliminated such as inorganic salts, large molecules.

Therefore, in the beginning, it seemed to be a necessity to have an idea of the approximate location of each amino acid of the standard solution. When the solvent front is not allowed to travel off the paper, the Rf. values is defined as follows:

\[
R_f = \frac{\text{Distance substance has travelled from the origin}}{\text{Distance solvent front has travelled from the origin}}
\]

This value which is the physical constant of the substance concerned, should therefore be reproducible. Here the above values were calculated by the formula given above.

Two chromatographic filter papers were taken. The standard amino acid solution of arginine, lysine, phenylalanine and leucine-isoleucine were applied on one of the chromatographic paper in quantities of 5 and 10 micro litres. Duplicate spotting of the same quantity were also made on another chromatographic paper. The first chromatographic paper was developed only once but the another one was developed for a second time for better separation. The rest of the procedure were similar in both the cases.

The solvent front and the various ninhydrin spots were measured (distance from the point of application to the ninhydrin spots). Rf. values were calculated. The calculated values are represented in Table 4.1.
Apprently the movement of lysine, arginine, phenylalanine, leucine and isoleucine were in the descending order. The Rf. value for the respective amino acid was different. For leucine and isoleucine, due to incomplete resolutions same values were taken for both.

The spot of lysine and arginine was distant from each other whereas the distance between the spots of phenylalanine and leucine isoleucine was relatively less.

**TABLE NO. 4.1**

Table showing the distances of solvent fronts, amino acids, in a single run and in redeveloped chromatograms.

<table>
<thead>
<tr>
<th>Solvent development</th>
<th>Description (in micro litres)</th>
<th>Lysine</th>
<th>Arginine</th>
<th>Phenylalanine</th>
<th>Leucine</th>
<th>Isoleucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Run 'A'</td>
<td>Distance travelled by solvent fronts in cm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>........................................... a.</td>
<td>40 cm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room temp. 30°C</td>
<td>Distance travelled by amino acid in cm.</td>
<td>4.5</td>
<td>4.5</td>
<td>7.4</td>
<td>7.4</td>
<td>26.0</td>
</tr>
<tr>
<td></td>
<td>........................................... b.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rf. = $\frac{b}{a}$</td>
<td>.11</td>
<td>.11</td>
<td>.18</td>
<td>.18</td>
<td>.65</td>
</tr>
<tr>
<td>Redeveloped 'B'</td>
<td>Distance travelled by solvent front in cm.</td>
<td>7.7</td>
<td>7</td>
<td>8.5</td>
<td>8.5</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>........................................... a.</td>
<td>46 cm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distance travelled by amino acid in cm... b.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>*Rf. = $\frac{b}{a}$</td>
<td>0.15</td>
<td>0.15</td>
<td>0.18</td>
<td>0.18</td>
<td>0.67</td>
</tr>
</tbody>
</table>
**The distance of the solvent front in chromatogram in second run (46 cm.) was taken for calculation of Rf. values.**

**Preparation of standard curve:**

During the preparation of standard curves, all the steps as described in the chapter 'Materials and Methods' were strictly followed. Solvent re-development techniques was however adopted, when necessary for better separation of amino acids.

A standard amino acid solution for arginine was taken and an aliquot of 5, 10, 15, 20, 25 and 30 microlitres were applied on chromatographic paper. Duplicate applications of these quantities were also spotted on separate sheet of paper. Utmost care was taken while applying different volumes of the solution. The chromatograms were developed with partridge's solvent, for 20 hours and then redeveloped after drying for 16 hours. Colour development with 0.5% ninhydrin in 95 percent acetone produced regular, uniform ninhydrin spots. The ninhydrin spots were then cut and eluted. The optical density of the eluates were measured and the corresponding colorimetric readings were noted. The colorimetric readings of the different eluates obtained from the second chromatogram were also noted. The average values were thus calculated. These values were plotted on the graph paper against the corresponding quantities of amino acid applied on the chromatographic paper. A straight line was drawn connecting the different points which consequently served as standard curve.

The same procedure was repeated for lysine, phenylalanine,
Fig. 7 Standard Curve for Arginine

1 Small div = 0.025 Micromole

Amino Acids (in Micromole)
and leucine (containing isoleucine). The four standard curves were thus drawn and were used as reference during estimation of amino acids in sample hydrolysates.

The standard curve for arginine, lysine, phenylalanine and leucine-isoleucine has been represented graphically in figure 1, 2, 3 and 4, respectively.

**Determination of optimum concentration of hydrolysate for use in chromatographic work:**

Earlier experience have revealed that while making spotting on the chromatographic paper, it is necessary to apply a minimum volume of hydrolysates containing the optimum amount of amino acid. Therefore in the present investigation an aliquot of 10 microlitres were always taken from the hydrolysates of different samples. During chromatographic analysis, optimum concentration of the amino acids in the mixture is essential for the better resolutions of different amino acid and it was also observed during the preparation of standard curves that there existed a correlation between the amounts of amino acids and the corresponding sizes of ninhydrin spots. As such due care was taken for the determination of their optimum concentration for analysis work.

Three hydrolysates were prepared taking 100 mg. of dehydrated ether extracted whole egg every time. The hydrolysates were dissolved in 1 ml., 2 ml., and 4 ml. of 10 percent isopropanol in glass distilled water. Hydrolysates were then transferred into three clean and dry test tubes. The three test tubes were marked as A., B., C., respectively. Test tube A. contained 1 ml., B. 2 ml.
FIG. 9: STANDARD CURVE FOR LYSINE.

1 SMALL DIVISION = 0.0025 MICROMOLE AMINO ACIDS (IN MICROMOLE)
and C. 4 ml., respectively. An aliquot of 10 microlitre was taken from test tube A. and was applied on chromatographic paper in duplicate. 10 microlitre of the reference amino acid mixture was also taken and was spotted by the side of hydrolysates. In the like manner the spotting of the rest of the hydrolysates was made on the same chromatographic paper. A fixed volume of 10 microlitre of hydrolysates and the same volume of reference amino acid mixture was used every time. The chromatogram was redeveloped and colour was developed after ninhydrin spray. After the colour development the chromatogram was kept in a hanging position and the resolution of the different amino acid was examined. The spots formed from the aliquot of reference amino acid mixture, served in identifying the resolved amino acid from the hydrolysates. Following were the observation with three hydrolysates of varying concentration.

(A) **Lysine and arginine and phenylalanine:**

*Ninhydrin spots were bigger than other hydrolysates but due to trailing appeared not to be fully separated.*

**Leucine and isoleucine:**

*A big, single spot was noticed without showing any resolution.*

(B) **Lysine, arginine and phenylalanine:**

*Ninhydrin spots were of medium size and had regular shapes. Trailing was absent.*
Leucine-iso-leucine:

A clear spot was noticed, without any sign of separation.

(c) Separation was not clear, due to presence of diffused and irregular spots:

After the proper observation, the different ninhydrin spots for lysine, arginine, phenylalanine and leucine-iso-leucine formed by hydrolysate B. and C. were cut and kept in clean dry separate, previously labeled test tubes. The separation was incomplete with respect to hydrolysate A, so the ninhydrin spots in this case was not taken into consideration. Elution was made in the manner as described earlier, optical density of the elutriates were determined by Klett-summersion photo electric colorimeter using green filter (540 m/u). The values of the estimated amino acids were as tabulated below:

**TABLE NO. 4.2**

Different concentration of amino acids in whole egg hydrolysates.

<table>
<thead>
<tr>
<th>Hydrolysates</th>
<th>Observations</th>
<th>Lysine</th>
<th>Arginine</th>
<th>Phenylalanine</th>
<th>Leucine-iso-leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>mg./ml.</td>
<td>mg./ml.</td>
<td>mg./ml.</td>
<td>mg./ml.</td>
</tr>
<tr>
<td>A.</td>
<td>II</td>
<td>Not estimated.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.</td>
<td>I</td>
<td>6.09</td>
<td>6.40</td>
<td>5.12</td>
<td>8.04</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>6.02</td>
<td>6.52</td>
<td>5.35</td>
<td>8.09</td>
</tr>
<tr>
<td>C.</td>
<td>I</td>
<td>3.10</td>
<td>3.25</td>
<td>2.75</td>
<td>5.25</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2.98</td>
<td>3.00</td>
<td>3.10</td>
<td>5.00</td>
</tr>
</tbody>
</table>
It is evident from the above table that for the hydrolysates B., the estimated values for lysine, arginine, phenylalanine, and leucine-isoleucine are in close agreement between the duplicates. The values obtained for the hydrolysates C., indicated some variation in all observation.

Recovery test:

Olcott (1951) stated that one of the unresolved problems in analytical chemistry concerns with the extent of destruction of amino acid during hydrolysis. However, he further stated interaction of amino acids during hydrolysis had not been appreciated so far. In the present work a recovery test was conducted in duplicate with added arginine in dehydrated ether extracted whole egg sample, inorder to estimate any possible loss of amino acid during hydrolytic process.

In two separate test tubes 100 mg. of dehydrated ether extracted whole egg sample were taken. In test tube B. 1 ml. of standard arginine solution was added. The test tube B. now contained the whole egg sample plus 2.10 mg. of the added arginine. These were hydrolyzed and chromatographed in the usual way (described in the chapter 'Materials and Methods').

The value of arginine in test 'A' and in test tube B with added arginine were calculated which is shown in the table below:
<table>
<thead>
<tr>
<th>Name of sample</th>
<th>Lysine</th>
<th>Phenylalanine</th>
<th>Leucine</th>
<th>Isoleucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>7.61</td>
<td>6.45</td>
<td>9.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.44</td>
<td>5.93</td>
<td>9.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.34</td>
<td>6.52</td>
<td>9.83</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>7.46</td>
<td>6.30</td>
<td>9.86</td>
<td>0.019</td>
</tr>
<tr>
<td>Muscle</td>
<td>8.94</td>
<td>5.01</td>
<td>12.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.80</td>
<td>4.42</td>
<td>12.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.22</td>
<td>6.14</td>
<td>12.62</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>8.98</td>
<td>5.19</td>
<td>12.36</td>
<td>0.17</td>
</tr>
<tr>
<td>Silkworm pupae</td>
<td>6.58</td>
<td>3.43</td>
<td>6.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.17</td>
<td>2.66</td>
<td>6.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.46</td>
<td>3.21</td>
<td>6.60</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>6.49</td>
<td>3.13</td>
<td>6.63</td>
<td>0.065</td>
</tr>
<tr>
<td>Ricebran</td>
<td>3.76</td>
<td>4.44</td>
<td>8.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.69</td>
<td>3.07</td>
<td>6.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.37</td>
<td>3.55</td>
<td>7.33</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>3.27</td>
<td>3.67</td>
<td>7.53</td>
<td>0.33</td>
</tr>
<tr>
<td>Salseed</td>
<td>2.75</td>
<td>5.06</td>
<td>3.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.43</td>
<td>4.26</td>
<td>2.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.69</td>
<td>5.65</td>
<td>3.65</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>2.62</td>
<td>4.99</td>
<td>3.03</td>
<td>0.44</td>
</tr>
</tbody>
</table>
Table showing the percentage recovery of arginine.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Amount of arginine I</th>
<th>Amount of arginine II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of amino acid in test tube 'A'.</td>
<td>6.10</td>
<td>6.35</td>
</tr>
<tr>
<td>Amount of arginine added.</td>
<td>2.10</td>
<td>2.10</td>
</tr>
<tr>
<td>Total amount of arginine 'B'.</td>
<td>8.20</td>
<td>8.45</td>
</tr>
<tr>
<td>Amount of arginine recovered from 'B'.</td>
<td>7.76</td>
<td>8.03</td>
</tr>
<tr>
<td>Percentage of recovery.</td>
<td>94.63</td>
<td>95.02</td>
</tr>
<tr>
<td>Average percentage of recovery.</td>
<td>94.84</td>
<td></td>
</tr>
</tbody>
</table>

The findings of recovery test is satisfactory and the possibility of getting a reliable results from the hydrolysates is evident from the good agreement of the duplicate.

Block and Zweig (1958) found that the elution technique when adopted during chromatographic work for substances, which are clearly separated is liable to a variation of 5 percent. The loss of added arginine in recovery test might be due to breakdown of some amino acid during hydrolysis or due to elution technique as stated by Block and Zweig (1958).

Estimation of amino acid in eggs, muscle tissue of day old quail, silkworm pupae, rice bran and salsed:

The process of hydrolysis adopted for the estimation of
amino acids in the present investigation has been described in the chapter 'Materials and Methods'.

100 mg. of dehydrated, ether extracted samples of whole egg were obtained from three fresh eggs and were hydrolysed separately in the usual way. The hydrolysate were dissolved in 2 ml. of 10 percent isopropanol in glass distilled water.

Taking 10 microlitre in duplicate, each hydrolysate was spotted on chromatographic filter paper. 10 ml. of reference x amino acid mixture was also applied on wither side of the chromatogram. The chromatogram was developed in solvent (Butanol - Acetic and water) for 20 hours. After redevelopment of the chromatogram ninhydrin was sprayed. The ninhydrin spots of the different hydrolysates were compared with the corresponding ninhydrin spots of the reference amino acid mixture. The resolution of the different hydrolysates were thus identified. The spots of lysine, arginine and phenylalanine were cut separately and the spot of leucine-isoleucine was cut together because of non-separation of these amino acids. Each ninhydrin spots were then eluted in 4 ml. of 75 percent ethyl alcohol containing 2 mg. of coppersulphate. The optical density of each eluate was determined in Klett summerson photoelectric calorimeter using green filter (540 m/u). The duplicate reading of each amino acid under study were taken and values were averaged. From the reading thus obtained the actual amount of lysine, arginine, phenylalanine and leucine-isoleucine content of the whole egg hydrolysates was ascertained with the help of respective standard curves. These values were later expressed in different standard forms by various standard methods.
in use expressing the amino acid composition of proteins (Table 4.5).

Same procedure was adopted for the estimation of lysine, arginine, phenylalanine and leucine-isoleucine content in the hydrolysates obtained from the samples of muscle tissue of day old chick of the same hatch, silkworm pupae, ricebran and salseeds. The only exception in the procedure was that in the case of salseed because of very low protein content, the hydrolysates were dissolved only in 0.5 ml. of 10 percent isopropanol. The volume of the hydrolysates used for spotting during chromatography was however the same.

The estimated value of lysine, arginine, phenylalanine and leucine-isoleucine of different feed stuffs under taken in the present investigation have been presented in Table 4.4. The chemical composition of the different feed stuffs observed in the present study are also incorporated in the same table (4.4).

**Amino acids in egg, muscle tissue of day old chick, silkworm pupae, ricebran and salseed:**

Stein (1946) emphasized the necessity of two kinds of analytical techniques for amino acids, "primary standard" and "routine". Primary method need not necessary be a convincing or rapid method nor adaptable to handling of small quantities of protein. O'Dcott (1951) pointed to 'isotope dilution method' which closely approached to the criteria of primary standards. The rest of the methods classified as 'routine' may have some inherent experimental errors. Most of the estimated value for amino acids
Fig. 5. Histogram showing Arginine, Lysine, Phenylalanine and Leucine-Isoleucine content in egg and muscle tissue of day old chick.

One small division = 0.1 gram of amino acid.
were obtained from microbiological assay, which too have certain limitations viz. changes in micro-organisms, synthetic potentials of the organism, presence of inhibitors in the hydrolysates. Estimated values for amino acid obtained by micro-biological assay have been usually taken as standard for comparison of the values obtained by other methods.

The composition of several amino acid present in egg, muscle tissue of day old chick, silkworm pupae, ricebran, and salseed studied in this work is shown in Table 4.4.

**Amino acid content in egg and muscle tissue of day old chick:**

**Arginine:**

It is obvious from the Table 4.4 that the estimated value of arginine in 100 gms. of dehydrated ether extracted samples are almost similar in three eggs. The values are 6.40, 6.10 and 6.52 grams and the average value is 6.34 grams.

The arginine content in egg as reported by F.A.O. technical committee on poultry products for Asia and far East expressed as percent of protein is 6.6 table 2.5. Fassary et al (1968) Table 2.6, indicated a value of 6.4 (percent of protein) for egg. In the present study the average arginine value in egg expressed as percent of protein is 7.7.

The arginine content in 100 grams of dehydrated ether extracted muscle tissue of day old chick in three samples are 6.31, 6.00 and 6.84 grams and average being 6.38 grams.
FIG-6. HISTOGRAM SHOWING ARGinine, LYSINE, PHENYLALANINE AND LEUCINE-ISOLEUCINE CONTENT IN SILK WORM PUPAE, RICE BRAN AND SALSEED.

- **Silk Worm Pupae**
- **Rice Bran**
- **Salseed**

One small division = 0.1 gram of amino acid.
William (1954) reported that the arginine content in the ether extracted muscle tissue of day old chick in grams per 16 grams of nitrogen as 6.69 Table 2.4. The average value of arginine in the ether extracted sample of muscle tissue of day old chick in present study is 6.81 grams per 16 grams of nitrogen. The present value and the value furnished by William as stated above is in fair agreement.

**Lysine:**

The estimated value of lysine is 100 grams of dehydrated ether extracted sample of egg in the present study are 6.31, 6.09 and 6.02 grams respectively. The average value is 6.14 grams.

According to the report of P.A.O. technical committee (1967) on poultry products for Asia and far East table 2.5 and Essary et al (1968) table 2.6, the value of lysine in egg expressed as percent of protein are 7.0 and 7.2 respectively. In the present study the lysine content in egg calculated as percent of protein is 7.48, which agrees fairly with the findings of above workers.

The lysine content in 100 grams of dehydrated ether extracted muscle tissue of day old chick in the present study are 8.39, 8.21 and 8.67 respectively in the three samples. The average value for lysine is 8.42 grams.

According to William et al (1954) table 2.4, lysine content in ether extracted muscle tissue of day old chick is 7.75 grams per 16 grams of nitrogen. The average lysine content in
muscle tissue of day old chick in the present investigation is 8.98 grams per 16 grams of nitrogen table 4.4. Thus the present value appears slightly higher than the value reported by above worker. This minor variation in the lysine content of day old chick muscle may be due to breed difference or due to methods of estimation adopted by various workers.

Phenylalanine:

The estimated value of phenylalanine in 100 grams of dehydrated ether extracted samples of egg are 5.12, 4.85 and 5.35 grams in three samples respectively. The average value for phenylalanine is 5.10 grams.

The value of phenylalanine in egg expressed as percent of protein is 6.3 according to a report of F.A.O. technical committee (1967) on poultry products for Asia and far East table 2.5. Eassary et al (1968) table 2.6 observed that the phenylalanine content of egg was 4.9 calculated as percent of protein. In the present study the value of phenylalanine in egg expressed as percent of protein is 6.69, which agrees fairly with the F.A.O. report stated above. However the findings of Eassary (1968) is comparatively less than the present investigation.

The phenylalanine content in 100 grams of dehydrated ether extracted muscle tissue of day old chick as studied in the present investigation are 4.70, 4.13 and 5.78 grams respectively in three samples. The average value being 4.87 grams.

The phenylalanine content in ether extracted sample of
day old chick in grams per 16 grams of nitrogen reported by William (1954) table 2.4 is 4.17. The phenylalanine content in muscle tissue in the present study in grams per 16 grams of nitrogen is 5.19. The present value thus appears slightly higher than the value reported by above worker. The probable reasons for the difference in the value of phenylalanine may be due to different breed or different analytical procedures adopted by various workers.

**Leucine-isoleucine:**

It has been stated earlier that because of non-clear separation of these two amino acid, the value of leucine-isoleucine has been considered together. While comparing the value of leucine-isoleucine in the present investigation with the findings of other worker, the cumulative value of the two amino acids have always been considered.

The leucine-isoleucine content in 100 grams of dehydrated ether extracted samples of egg in the present analysis are 8.22, 8.04 and 8.09 grams respectively in three samples. The average value being 8.11 grams. The leucine-isoleucine value expressed as percent of protein is 9.8 in the present study.

The value of leucine-isoleucine content of egg as has been reported by P.A.O. technical committee on poultry products for Asia and far East (1967) is 18.7, expressed as percent of protein (Table 2.5). Essary et al (1968) table 2.6 observed that the leucine-isoleucine content of egg was 17.2 calculated as percent of protein.
The leucine-isoleucine content in 100 grams of dehydrated ether extracted samples of muscle tissue of day old chick in the present investigation are 11.67, 11.22, and 11.87 grams respectively in three samples. The average value being 11.58 grams.

The value for leucine-isoleucine in ether extracted muscle tissue of day old chick in grams per 16 grams of nitrogen is 11.24 according to William (1954) table 2.4. The leucine-isoleucine content in the muscle tissue of day old chick in the present study in grams per 16 grams of nitrogen is 12.36. Thus the value observed in the present study indicates non-significant difference with the value furnished by William.

The value of leucine-isoleucine in egg in the present study is very low than the findings of other worker, which is difficult to explain. However the close agreement observed in three samples of egg offers confidence on our finding. The close agreement in value for leucine-isoleucine between present finding and others, observed in case of chick muscle and rice bran makes the situation more difficult to explain.

**TABLE NO. 4.6**

Comparison of arginine, lysine, phenylalanine and leucine-isoleucine in dehydrated ether extracted samples of egg and muscle tissue of day old chick.

<table>
<thead>
<tr>
<th>Name of the amino acid</th>
<th>No. of observation</th>
<th>Average &amp; S.E.</th>
<th>Difference</th>
<th>'t' value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Egg</td>
<td>Muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>3</td>
<td>3</td>
<td>6.34 ± 0.04</td>
<td>6.38 ± 0.02</td>
</tr>
</tbody>
</table>
Cont'd Table No. 4.6

<table>
<thead>
<tr>
<th>Name of the amino acid</th>
<th>No. of observation</th>
<th>Average &amp; S.E.</th>
<th>Differ.</th>
<th>t (value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Egg</td>
<td>Muscle</td>
<td>Egg</td>
<td>Muscle</td>
</tr>
<tr>
<td>Lysine.</td>
<td>3</td>
<td>3</td>
<td>6.14 ± 0.02</td>
<td>8.42 ± 0.42</td>
</tr>
<tr>
<td>Phenylalanine.</td>
<td>3</td>
<td>3</td>
<td>5.10 ± 0.35</td>
<td>4.87 ± 0.48</td>
</tr>
<tr>
<td>Leucine-isoleucine.</td>
<td>3</td>
<td>3</td>
<td>8.11 ± 0.005</td>
<td>11.58 ± 0.60</td>
</tr>
</tbody>
</table>

** denotes significant at 1% level.

The arginine, lysine, phenylalanine and leucine-isoleucine in egg and muscle tissue of day old chick as shown in the above table, on statistical analysis reveals that there is significant difference in the pattern of amino acid lysine and leucine-isoleucine between egg and day old chick. No such difference is observed in case of arginine and phenylalanine. On close examination of the observations of the egg samples, it appears that egg has a slightly higher amount of arginine and phenylalanine than that of day old chick, which however appears non-significant when subjected to statistical analysis as stated above. Even for leucine-isoleucine the high significant value in muscles of day old chick, observed in the present study may not be of real consequence because of appreciably lower value for eggs obtained in the present study.

**Silkworm pupae:**

Protein is one of the main ingredients in poultry ration
The requirement of protein in ideal chick mash is about 20 percent. This is usually met by incorporating various feed stuffs from both vegetable as well as animal protein. Usual animal protein sources for poultry feeds are fish meal, meat meal and milk products, out of which fish meal is most commonly used.

The literature on amino acid composition of silkworm pupae is very meagre, all available literature is confined to its usefulness as a source of protein only. Naidu (1959) states that powdered silkworm pupae after it is sundried and separated from fibrous cocoons would make a good protein supplement for poultry mash. Desai et al (1960) studied the requirement of animal protein in poultry feeding in relation to liver residue and blood meal as compared to fish meal.

The present production of fish meal in this country is very much below its actual requirement. As a result, in recent years attempts are being made to utilize the byproducts of organized industries as possible source of animal protein supplements.

Large quantity of silkworm is available as by product of the silk industry in different parts of the country. Recent investigation carried out at central food technological research institute, Mysore has indicated that by suitable pretreatment of the outercoat, the silkworm pupae could be economically exploited for the preparation of silkworm pupae meal.

In the present investigation the "tassar" silkworm pupae was studied for its amino acid composition with reference to amino
acid arginine, lysine, phenylalanine and leucine-isoleucine.

Before taking up the actual work of amino acid determination, the
chemical composition was also studied inorder to ascertain the
crude protein content of the pupae. The chemical composition of
silkworm pupae as studied in the present investigation is as
follows:

**Percentage chemical composition of silkworm pupae.**

<table>
<thead>
<tr>
<th>Composition</th>
<th>No. of observation</th>
<th>Average and S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>3</td>
<td>21.80 ± 0.47</td>
</tr>
<tr>
<td>Dry matter</td>
<td>3</td>
<td>78.20 ± 0.47</td>
</tr>
<tr>
<td>Ether extract of dehydrated sample.</td>
<td>3</td>
<td>31.23 ± 0.48</td>
</tr>
<tr>
<td>Crude protein content of dehydrated ether extracted sample.</td>
<td>3</td>
<td>71.43 ± 0.47</td>
</tr>
</tbody>
</table>

The average amino acid composition of the dehydrated ether extracted sample of silkworm pupae with reference to arginine, lysine, phenylalanine and leucine-isoleucine is indicated in the table 4.7.

**TABLE NO. 4.7**

<table>
<thead>
<tr>
<th>Name of amino acid</th>
<th>No. of observation</th>
<th>Average and S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>3</td>
<td>3.53 ± 0.48</td>
</tr>
</tbody>
</table>
### Cont'd Table No. 4.7

<table>
<thead>
<tr>
<th>Name of amino acid</th>
<th>No. of observation</th>
<th>Average and S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>3</td>
<td>$4.59 \pm 0.028$</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3</td>
<td>$2.33 \pm 0.60$</td>
</tr>
<tr>
<td>Leucine–isoleucine</td>
<td>3</td>
<td>$4.67 \pm 0.07$</td>
</tr>
</tbody>
</table>

While comparing the value of lysine, phenylalanine and leucine–isoleucine in the present study with the values given by Subhas Chandran (1969), in his book on animal nutrition, it is observed that the present values are slightly lower than the value furnished by Subhaschandran (Table 2.7). As the arginine content of silkworm pupae was not available from the literature, the value for arginine in the present investigation could not be compared with findings of other workers.

A little lower value for the lysine, phenylalanine, leucine–isoleucine in the present investigation may be because of difference in the species of silkworm pupae. As it has been observed that silkworm pupae has got different level of protein percentage. Further the variation may be due to different analytical techniques adopted by various workers.

The present study also confirms the views expressed by previous worker that silkworm pupae can best be utilized as a source of animal protein in poultry feeding. As it is evident from the present finding that silkworm pupae can serve as a good source of essential amino acids like arginine, lysine, phenylalanine, leucine–isoleucine and thus it may help in meeting the
present pressure on fishmeal.

**TABLE NO. 4.8**

Table showing arginine, lysine, phenylalanine and leucine-isoleucine in silkworm pupae and fishmeal expressed as percent of protein.

<table>
<thead>
<tr>
<th>Name of amino acid</th>
<th>Silkworm pupae</th>
<th>Fishmeal*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>4.93**</td>
<td>6.7</td>
</tr>
<tr>
<td>Lysine</td>
<td>6.42</td>
<td>8.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.26</td>
<td>3.7</td>
</tr>
<tr>
<td>Leucine-isoleucine</td>
<td>6.53</td>
<td>15.7</td>
</tr>
</tbody>
</table>

* value for fishmeal has been quoted from "applied animal nutrition" by Crampton and Harris (1969).

** value for silkworm value has been furnished from present investigation.

Ricebran:

Ricebran consists of ricebran and germ removed in milling rice for human foods. It should contain only such quantities of hulls fragments as are unavailable in regular milling process. It is similar to oats and wheat in protein content and decidedly lower than wheatbran and middlings. Ricebran is known to supply protein of considerable better quality than corn.

Ricebran is fed chiefly to dairy cows, when usually not forming more than one third the concentrate mixture for cows. It
has been approximately equal to wheat bran and worth about 75 to 80 percent as much as ground corn or milo bran in its food value for dairy animals.

Rice bran has given good results in swine feeding as well, when forming not more than 25 to 30 percent of the ration. When thus fed it has been usually worth as much as corn and sometimes even more.

Rice bran is largely used in poultry feeding in place of wheat bran and wheat middlings.

The average amino acid content of rice bran protein investigated in the present study is given in Table 4.7.

**TABLE NO. 4.7**

Percentage of amino acid content with respect of arginine, lysine, phenylalanine and leucine-isoleucine in dehydrated ether extracted sample of rice bran.

<table>
<thead>
<tr>
<th>Name of amino acid</th>
<th>No. of observation</th>
<th>Average and S.P.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>3</td>
<td>0.65 ± 0.0084</td>
</tr>
<tr>
<td>Lysine</td>
<td>3</td>
<td>0.52 ± 0.06</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3</td>
<td>0.59 ± 0.2</td>
</tr>
<tr>
<td>Leucine-isoleucine</td>
<td>3</td>
<td>1.21 ± 0.01</td>
</tr>
</tbody>
</table>

The amino acid content of dehydrated, ether extracted sample of rice bran with respect of arginine, lysine, phenylalanine and leucine-isoleucine in the present finding fairly agree with
the value of these amino acid as quoted by Crampton and Harris in their book "applied animal nutrition" (6th Ed., 1969) Table 2.8.

It is evident from the table (4.9) that ricebran is rather a poor source for essential amino acid like arginine, lysine and phenylalanine in livestock feeding. The ricebran samples used in the present work did not seem to contain significant amount of husk as evidenced by visual examination and a high average protein content of 15.98 percent.

**Salseed**

Salseed is abundantly available in the forest area of Bihar, Orissa, Assam and other parts of India. Salseed, so far has been a waste and was not given any importance as livestock feed. Recently attention has been paid to explore its nutritive worth as a subsidiary feed to livestock. Very few works on chemical composition of salseed is available. Murty et al (1969) indicates that salseed because of high percent of tanmin increases the biological value of groundnut cake protein for ruminants by reducing ruminal ammonia production. Literature on amino acid composition of salseed is not available.

As little work is available on the chemical composition of salseed, it is felt worthwhile to cite the chemical composition of salseed as observed in the present study.
### Percentage chemical composition of salseed

<table>
<thead>
<tr>
<th>Composition</th>
<th>No. of Observation</th>
<th>Average and S.F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>3</td>
<td>9.16 ± 0.56</td>
</tr>
<tr>
<td>Dry matter</td>
<td>3</td>
<td>90.83 ± 0.22</td>
</tr>
<tr>
<td>Ether extract</td>
<td>3</td>
<td>15.19 ± 0.23</td>
</tr>
<tr>
<td>Crude protein content of dehydrated ether</td>
<td>3</td>
<td>11.25 ± 0.14</td>
</tr>
<tr>
<td>extracted sample</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The average amino acid content of dehydrated, ether extracted sample of salseed estimated in the present study is as given in the Table 4.10.

### TABLE No. 4.10

Percentage amino acid content of arginine, lysine, phenylalanine and leucine–isoleucine in dehydrated ether extracted sample of salseed.

<table>
<thead>
<tr>
<th>Name of amino acid</th>
<th>No. of Observation</th>
<th>Average and S. F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>3</td>
<td>0.26 ± 0.057</td>
</tr>
<tr>
<td>Lysine</td>
<td>3</td>
<td>0.29 ± 0.013</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3</td>
<td>0.56 ± 0.016</td>
</tr>
<tr>
<td>Leucine–isoleucine</td>
<td>3</td>
<td>0.34 ± 0.016</td>
</tr>
</tbody>
</table>

It is observed from the estimated chemical composition
of salseed that it has a low percentage of protein, but it is equally rich in energy due to high ether extract content. Salseed, can thus be utilized to some extent for replacing a part of cereals like maize in poultry ration and thereby a comparatively cheap ration can evolved.

The amino acid content of salseed could not be obtained from the available literature and as such no comparison can be made.

Comparing the values for these essential amino acids obtained in the present study (Table 4.11) it is observed that salseed is a more poor source of essential amino acid like arginine, lysine, phenylalanine and leucine-isoleucine than ricebran. In order to overcome the deficiency of these essential amino acids, salseed and ricebran should be fed to livestock in combination with other protein supplements.

**TABLE NO. 4.11**

Table showing arginine, lysine, phenylalanine and leucine-isoleucine in ricebran, wheatbran, yellow maize and salseed expressed as percent of protein.

<table>
<thead>
<tr>
<th>Name of the amino acid</th>
<th>Ricebran</th>
<th>Wheatbran</th>
<th>Yellow maize</th>
<th>Salseed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>4.06</td>
<td>6.2</td>
<td>3.6</td>
<td>2.3</td>
</tr>
<tr>
<td>Lysine</td>
<td>3.25</td>
<td>3.7</td>
<td>2.4</td>
<td>2.5</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.62</td>
<td>3.1</td>
<td>4.0</td>
<td>4.9</td>
</tr>
<tr>
<td>Leucine-isoleucine</td>
<td>7.57</td>
<td>9.3</td>
<td>11.7</td>
<td>3.02</td>
</tr>
</tbody>
</table>
SUMMARY

Five essential amino acids e.g. arginine, lysine, phenylalanine and leucine–isoleucine were estimated quantitatively in Ricebran, silkworm pupae, salseed and a comparative study of these acids in eggs and day old chick of the same breed were also made by descending paper chromatography.

Resolution of the constituents amino acids were satisfactory and the five amino acids studied were easily located. Solvent redevelopment technique showed a better resolution.

Standard curves for the individual amino acids were prepared using 0.05 to 0.3 micromoles of each standard amino acid for the purpose.

Rf. values of the amino acid under study were determined.

The method of Block and Zweig was adopted for hydrolysis. The Chromatographic procedure envisized by Block and Zweig (1958) with some modification was employed during chromatographic work.

A recovery test with added arginine in egg hydrolysates was performed and a recovery of 94.84 percent was obtained.

Three separate hydrolysates were prepared from three different samples of egg, muscle tissue of day old chick, silkworm pupae, ricebran and salseed and chromatographed in duplicate.

The substances studied in this work could be serially arranged on the basis of their amino acid content determined as
(i) The value of wheatbran and maize has been quoted in the above table from "Applied animal nutrition" by Crampton and Harris (1969).

(ii) The value for ricebran and salseed has been furnished from the present investigation.
CHAPTER V.

SUMMARY.
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Five essential amino acids e.g. arginine, lysine, phenylalanine and leucine-isoleucine were estimated quantitatively in rice bran, silkworm pupae, salseed and a comparative study of these acids in eggs and day old chick of the same breed were also made by descending paper chromatography.

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The substances studied in this work could be serially arranged on the basis of their amino acid content determined as
muscle and egg, silkworm pupae, rice bran and salseed. The average arginine content in grams per 16 grams of Nitrogen in dehydrated ether extracted samples of muscle tissue of day old chick, egg, silkworm pupae, rice bran and salseed were 6.81, 7.70, 4.93, 4.08 and 2.36. The average value for lysine 8.98, 7.46, 6.40, 3.27 and 2.62. The phenylalanine content were 5.19, 6.38, 3.10, 3.67 and 4.99, likewise the value for leucine-isoleucine were found to be 12.36, 9.86, 6.63, 7.53 and 3.03 respectively.

On statistical analysis no significant difference was observed in arginine and phenylalanine content of eggs and day old chick. A significant difference, however in their lysine and leucine-isoleucine content was observed with a value being higher in muscles.
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