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Measures of Protein Quality-
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Measures of Protein Quality-

It is generally accepted that usefulness of feeds as sources of protein depends primarily on two factors, the total concentration of the protein (N×6.25) and the distribution of the amino acids making up the proteins. Protein quality is the kind and quantity of essential amino acids and non-specific amino nitrogen that arrive at the individual cell which determines the protein value of a feedstuff whether for ruminants or for nonruminants. Different approaches to the evaluation of protein sources are necessary for nonruminants and ruminants because considerable degradation of feed proteins and microbial synthesis of protein occurs in the rumen.

Evaluation of Protein of Feeds: Common Methods

1. Crude protein: Johann Kjeldahl of Denmark developed the method of nitrogen estimation in 1883. In calculating the protein content, it has been assumed that all N is present as protein and all food proteins contain 16% N. But both these assumptions are unsound. About 95% of N of most mature seeds is present as true protein, while leaves and stems contain 80 to 90 and 60%, respectively as true protein. The remaining occurs as amides, amino acids, glycosides, alkaloids, ammonium salts and compound lipids. Similarly different food proteins have different N contents and therefore different factors.

2. True protein: Stutzer's reagent (alkaline copper sulphate) is used to precipitate the true protein and determination of its N by the Kjeldahl method and multiply with 6.25 gives the true protein content of the feed.

3. Digestible crude protein- is a measure that has been widely used to evaluate the protein for ruminants inspite of its drawbacks. The digestibility figures routinely calculated are apparently digestible protein, which are generally lower than the true values. But since the loss of metabolic faecal nitrogen is inevitable, the apparent digestibility values are considered to be a more realistic measure of nutritive value. DCP is not employed with nonruminants because proteins can have the same digestibility and yet still differ in their value to the animal. That is, the proteins are said to differ in protein quality.

Measures of Protein Quality for Monogastric Animals-

Estimation of CP and DCP are not enough since the efficiency of the absorbed protein varies considerably from one source to another and hence biological methods have been devised. These include weight gain methods, N balance experiments and body N retention method.

1. Weight Gain Methods-

i. **Protein efficiency ratio (PER)**: It is defined as the weight gain per unit weight of protein consumed. Osborne, Mendel and Ferry in 1919 developed this method. This was carried out with rats to compare specific proteins or protein sources. A nitrogen free, otherwise adequate basal diet is used to which the protein sources to be compared are included for different groups of young animals and records for growth and feed consumption are maintained.

$$\text{PER} = \frac{Gain\ in\ weight}{Protein\ \ intake}$$

Limitations: It is a tedious method. This method can not assess the digestibility of protein. It cannot measure how much of the protein fed is used for maintenance and how much for tissue formation. Further, the weight gain may be due to bone or fat formation and so the protein content of the gain may be variable.

ii. Net protein retention (NPR): - It is a modification of the PER method. It makes an allowance for maintenance. It is claimed to give more accurate results than the PER method.

$$\text{NPR} = \frac{Weight\ gain\ of\ TPG - Weight\ loss\ of\ NPG}{Protein\ intake}$$

Where TPG= Group fed on test protein.

NPG= Group fed on non-protein (protein free) diet.

iii. Gross protein value (GPV):- It refers to a measurement with chicks fed a depletion diet containing 8% protein for 2 weeks, after which they are divided into three groups. The live weight gain of chicks receiving a basal diet containing 80 g CP/kg are compared with those of chicks receiving the basal diet plus 30 g CP / kg of a test protein, and of others receiving the basal diet plus 30 g CP/kg of casein.

$$\text{GPV} = \frac{g\ increased\ weight/gtestprotein}{g\ increased\ weight\ gain/gcasein}$$

The extra live weight gain per unit of supplementary test protein, stated as a proportion of the extra live weight gain per unit of supplementary casein, is the gross protein value of the test protein.

2. **Nitrogen Balance Experiments-**

i. Biological value (BV): Karl Thomas has first used the term BV in the year 1909. It is defined as the proportion of the nitrogen absorbed which is retained by the animal.

$$\%\ \text{BV} = \frac{N\ intake - (FN + UN) \times 100}{NI - FN}$$

This is called apparent BV. It measures the BV of protein for growth purpose only. A more useful measure is one that takes account of maintenance as well. Karl Thomas method was modified by Mitchell (1924). This is called the Thomas-Mitchell formula. Crampton and Harris (1968) called it as true BV.

$$\%\ \text{B.V.} = \frac{\text{NI- (FN- MFN)-(UN-EUN)}}{\text{NI- (FN- MFN)}}$$

MFN or FN(m) = Metabolic faecal nitrogen. It consists of 'spent digestive enzymes, abraded mucosa and bacterial N.

UN= Total urinary nitrogen.

EUN or (Une)= Endogenous urinary nitrogen. It is analogous to the energy equivalent of basal metabolism. The EUN refers to the nitrogen resulting from normal catabolism of nitrogen constituents in the body that are regularly voided in the urine.

Determination of BV: Conditions to be fulfilled-

1. Protein under test should form greater part of the dietary protein of the experimental animal.
2. Protein intake must be sufficient to allow adequate N retention, but should not be in excess of that required for maximum retention. TBV data for individual feeds will change according to the rations in which they are used. In order to compare the TBV for different feeds TBV must be determined at the same protein levels of intake and such rations are adjusted to 10% CP.
3. Sufficient non-nitrogenous nutrients must be given to prevent catabolism of protein to provide energy.
4. The diet must also contain other nutrients.

Factors that influence the effective TBV of a protein include age and class of the animal and amino acid composition of the protein. Proteins with all the essential amino acids in right amount and proportion have higher BV. Animal proteins have higher BV compared to plant proteins. Deficiency or excess of any one of the amino acids lowers the BV.

Nitrogen balance index:- It is applicable to studies of BV for maintenance and some growth. A linear relationship exists between N intake and N balance in the region of negative and low-positive balance. This is represented by the following equation.

$$Y=bx-a$$

where Y = N balance mg N per basal

x= N absorbed KJ

a= N loss at zero intake mg N/basal KJ.

Since farm animals are fed for productive purposes, BVs for the combined function of maintenance and production are ones of practical importance.

ii. Net protein utilization (NPU):- The usefulness of a protein to an animal will depend upon its digestibility as well as its BV. NPU is the product of these two values and is the proportion of N intake which is retained. The product of NPU and the percentage CP is the net protein value (NPV) of the feed and is a measure of protein actually utilizable by the animal.

iii. Protein replacement value (PRV): Murlin (1938) devised this measurement to overcome the limitations of the Thomas Mitchell biological value. Replacement value of food protein is a measure of the retention of the total nitrogen intake rather than the digestible nitrogen. It compares the extent to which a test protein will give the same N balance as an equal amount of standard protein.

$$PRV=100-\left(\frac{NB1-NB2\times100}{NI}\right)$$

NB_1 is Where NB_1 is the N balance of animals fed standard protein, NB_2 the N balance of animals fed the test protein and NI as N intake.

- Weight gain methods are less accurate than nitrogen balance methods. The use of young animals and standard conditions, however, has given high correlations between the growth and nitrogen balance methods.

3. Body Nitrogen Retention Method-

Miller and Bender (1955) devised this method. This is based on a comparison of the body N content resulting from a test protein with that resulting over the same period on a nitrogen-free diet. The value is computed as follows.

$$\text{Body N retention} = \frac{\text{Body N content with test protein} - \text{Body N content with N-free diet}}{\text{N I}}$$

The formula measures efficiency for growth. At the close of the experiment animals are killed, the body water determined and the N calculated from body water or actual analysis of the carcasses for nitrogen.

- The efficiency of utilization of dietary protein may be estimated from the difference between nitrogen intake and excretion, or from the increase of body proteins.
- Biological methods require a regular supply of standard young animals (rats, chicks, etc.) and are expensive and require considerable technical resources. Hence alternative methods were developed.

4. Estimation of Protein Quality from Amino Acid Composition-

To overcome the economical limitations of biological methods, chemical methods have been proposed. A comparison of the quantitative distribution of the essential amino acids in a feed with the relative amounts needed by the body per unit of feed provides a method of estimating protein quality.

i. **Chemical score**: R.J. Black and H.H. Mitchell (1946) devised this. In this concept the quality of a protein is decided by that constituent indispensable amino acid which is in greatest deficit when compared with a standard. The standard generally used is egg protein, but now many workers use a defined amino acid mixture recommended by FAO. For example, amino acid levels in % egg protein (standard) and % wheat protein (test protein) are given. Then amino acid deficiencies are calculated. The amino acid present in the greatest deficit is considered as the limiting amino acid and the complement of its percentage deficit is the chemical score for that protein. Between egg protein and wheat protein, calculation revealed lysine was the most limiting amino acid (-63% deficit) and hence chemical score for wheat is 100-63 = 37.

Chemical score appears to be a useful measurement for separating proteins into categories of usefulness. With protein that is deficient in several amino acids, correction of one deficiency still leaves a combination that is biologically imperfect.

i**i. The essential amino acid index (EAAI):** B.L.Oser (1951) devised this measure based on the contribution the protein makes to all essential amino acids rather than to the one in greatest deficit. It may be defined as the geometric mean of the ten egg ratios found by comparing the content of ten EAAs in a feed protein with that found in whole egg protein. Algebraically the index is expressed as

$$EAAI = 10\sqrt{\frac{100a}{ae} \times \frac{100\ b}{be} \times \frac{100\ c}{ce} \times \ldots \frac{100\ j}{je}}$$

Where a, b, c, ...j = % concentrations of the indispensable amino acids in a feed protein.

a_e b_e c_e .....j_e = % concentrations of the same amino acids in egg protein.

It has the disadvantage that proteins of very different amino acid composition may have the same or a very similar index.

Both the chemical score and EAAI are based upon gross amino acid composition. A more logical approach, hence, would be to use figures for the amino acids available to the animal. The principal reason for the reduction of amino acid availability is heat damage occuring during processing or storage of feedstuffs due to maillard reaction.

Maillard Reaction - The reaction between sugars and amines is known as Maillard reaction (after the French chemist who studied it). Louis Camille Maillard (1878- 1936) observed the reaction in 1912. The brown color is caused by the formation of melanoidins. Maillard reaction is one of the nonenzymatic browning reactions. The reaction is also termed as "Sugar-amine reaction". In this reaction, free amino groups of the peptide chain, most usually the ϵ amino group from lysine, react with the aldehyde group of reducing sugars such as glucose or lactose to yield an amino-sugar complex that is no longer available to the animal. As a result of the complexing, trypsin can no longer cleave the peptide bond and lysine is not available.

5. Estimation of the Availability of Amino Acids-

i. **Biological Assay of Available Amino Acids**: The available amino acid content of a food protein may be assayed by measuring the live weight gain, feed conversion efficiency or N retention of animals given the intact protein as a supplement to a diet deficient only in the amino acid under investigation. **Chick is the usual experimental animal and the response to the test material is compared with responses obtained with supplements of pure amino acids**. The method has been used successfully for lysine, methionine and cystine. Disadvantages are time consuming, needs technical expertise, require regular supply of animals and the major problem is constructing diets deficient in specific amino acids but adequate in other respects.

ii. Microbiological methods: Certain microorganisms such as **Streptococcus zymogenes**, a bacterium and **Tetrahymena pyriformis**, a protozoan have amino group requirements similar to those of higher animals. Hence these microorganisms have been used for evaluation of feed proteins. The methods are based on measuring the growth of the microorganisms in culture media which include the protein under test. **Streptococcus zymogens is used mainly for the determination of available methionine while Tetrahymena pyriformis is used for lysine.**

iii. Chemical methods: It would be ideal if simple chemical procedures could be used to determine the availability of amino acids, provided the results correlated well with those of accepted biological methods.

a. FDNB-reactive lysine method: One of the most promising chemical methods used is to test the availability of lysine, the amino acid most likely to be limiting in diets high in cereal grains. The method is based on the idea that the lysine that is unbound has free epsilon (e)-amino group, and that is the available lysine. The free e-amino groups of the lysine in the protein will react with the chemical called **fluro-2,4-dinitrobenzene (FDNB) to produce a coloured derivative (DNP-lysine), the depth of the colour indicating the availability of the lysine.** This method was originally proposed by Dr. K.J. Carpenter.

b. Dye-binding method: Dye binding methods have been used for protein in foods such as cereals and milk. These methods have been used for measuring total basic amino acids and reactive lysine. The latter requires **blocking the e-amino group to prevent its reaction with the dye. The dye 'orange G' has been used along with 2, 4, 6-trinitrobenzene sulphonic acid and propionic anhydride as blocking agents.** It has proved effective for estimating the lysine content of cereals but less so for fish and meat meals.

Amino acid digestibility: It is defined as the difference between the amount of amino acids in the diet and in ileal digesta or in faeces, divided by the amount in the diet. Amino acid digestibility should not be confused with amino acid availability.

Ileal Digestibility of Protein-

Digestibility coefficients based on collection and analysis of digesta from the terminal ileum (last part of the small intestine) are generally considered to give a more accurate measure of the nitrogen absorbed than do those based on the more usual total faecal collection method. Bacteria of the large intestine in poultry, pig, dog and cat change the amino acid composition of undigested food residues. These bacteria both add and consume amino acids, so that the mixture of amino acids in the faeces contains both undigested food amino acids and amino acids of bacterial origin. This makes accurate determination of nutrient digestion from 'total tract' collections impossible. Ileal collection eliminates the large intestine as a source of errors, and the method is justified since absorption from the large intestine makes little or no contribution to the protein status of the animal. True digestibility of protein may be calculated by using N^{15} labelled dietary protein or by using homoarginine. However, these most favoured techniques also show average differences of about 5% in the true digestibility values derived from them.

Bioavailability of protein sources and other nutrients should focus only on digestion up to the end of small intestine, which would exclude the effects of hindgut fermentation. This measurement requires ileally cannulated animals and caecectomised roosters.

Ileal Digestibility and Faecal Digestibility

T-cannula were fixed at the distal ileum to piglets weaned at 21 days of age. An experiment was conducted to determine the ileal and faecal digestibilities of three protein sources by incorporating them in starch based semi-purified experimental feeds.

Crude protein and amino acid digestibilities in the first feed were determined by the direct method. The digestibilities in the experimental diets containing extruded soybean or full-fat canola were determined by difference.

- **The faecal digestibilities of CP and amino acids in SBM, extruded soybeans and full-fat canola were higher than the corresponding ileal digestibilities**. This difference may have been due to higher amino acid degradation in the faecal samples.
- The digestibilities of protein and most of the amino acids were significantly higher in soybean meal than they were in extruded soybean and in full-fat canola. The lower digestibilities may have been due to the trypsin inhibitor in the extruded soybean and tannins and pectins in the full-fat canola.

Ideal Protein Concept-

- In 1940s the ideal protein concept was introduced in the name of reference protein for evaluating protein quality using BV, Chemical score, etc. Later researchers more importantly H.H. Mitchell and R.J. Block reported that amino acids composition of high quality protein had the same with amino acids composition of body protein and this was the amino acids requirements of growing animal.
- Almquist in 1947 stated, that the "requirement of any indispensable amino acid for any rate of growth has a fixed proportion to the others in the diet".
- The concept of an "Ideal Protein" was originally proposed by Mitchell (1964) to try to match exactly the amino acid requirements of the animal for growth and maintenance thus avoiding under- and over-supplying amino acids.
- In 1981 ARC reviewed the idea of ideal protein and published the first estimate of an ideal protein requirement for growing pigs. The ideal protein concept was corrected, modified and applied in feeding systems of USA, UK, Australia, New Zealand, etc.
- The benefit of an ideal protein concept in diet formulation is to set all EAA requirements on the basis of lysine. Lysine was chosen as the standard because it is particularly well studied and it is not used extensively for purposes other than protein synthesis. **So ideal protein concept is a statement of EAA requirements in a proportional relationship to the requirement for lysine**. Thus, the dietary percentage of lysine is set and the concentration of other EAAs is determined as a percentage of the lysine concentration according to the ideal amino acid balance. In the latest edition of Nutrient Requirement of Swine (NRC 1998) the amino acid requirements relative to lysine are presented for various growth stages of pigs.
- For poultry, evaluation of protein sources is based upon their contents of the three major **limiting amino acids, lysine, methionine and tryptophan**. It is generally assumed that diets adequate in these acids will automatically provide sufficient amounts of the other amino acids.

Measures of Protein Quality for Ruminants-

"Feed the rumen microbes and they in turn feed the host animal". This is what that has been said on feeding of ruminants. The quality of dietary protein is not important in

ruminants in case of low producing animals since all the essential amino acids are synthesized by the rumen microorganisms. However, protein quality is important for high producing animals and a small portion of 'bypass' protein is advocated to meet their requirement for higher growth and milk production.

1. **Crude Protein**: Since CP contains variable amounts of nonprotein nitrogen (NPN) compounds, true protein had come into use, but this was unsatisfactory since no allowance was made for the nutritive value of NPN fraction (crude protein-true protein).
2. **Protein equivalent**: In an attempt to have NPN fraction half the nutritive value of true protein a concept of protein equivalent (PE) was introduced in 1925.

$$PE = \frac{\%DCP + \%DTP}{2}$$

This means the PE is the arithmatic mean of the percentage of DCP and DTP

3. **DCP:** For many years there has been considerable dissatisfaction with the use of DCP for evaluating feed proteins. This is due to the extensive degradative and synthetic activities of the microorganisms of the rumen.
4. **Biological value** does not apply to ruminants because of the ability of the rumen microorganisms to synthesize amino acid from a variety of N compounds.
5. **Several new systems** have been proposed in lieu of DCP system. These are the 'metabolizable protein' system used in the USA (Burroughs et at., 1975), 'rumen degradable and undegradable protein' system in the UK (ARC, 1980), true protein digested in the small intestine (PDI) system in the France and a similar system in the Germany.

The fundamental principle underlying these new systems is that N requirements of a ruminant animal is most logically considered in two parts: a requirement for N by rumen microorganisms and a requirement for protein by host ruminant animal.

Orskov (1982) reported that the nutritional relevance of DCP for ruminants had ceased to exist and the use of DCP was rejected because-

- Dietary proteins are largely degraded in the rumen
- The extent to which degraded N is utilized by rumen microbes is related to the amount of energy fermented, and
- Faecal excretion could be altered by manipulating the site of fermentation between the rumen and caecum.
- Because of its easy calculation, DCP is a measure widely used to evaluate proteins for ruminants. While it was accepted that dietary protein largely degraded, Orskov (1970) reported that microbial protein alone could not sustain high productivity in young early-weaned ruminants or in lactating ruminants.
- Furthermore, it was soon found that the extent of degradation of dietary proteins varied greatly.

Special facts-

ARC (1980) adopted microbial protein yield as **30 g N/kg** digestible OM. It can be estimated using rumen cannulated animals with the help of markers such as bacterial marker (α, τ-diaminopimelic acid DAPA), protozoal marker (2, aminoethyl-phosphonic acid AEPA; phosphatidylcholine). Isotopes such as ^{35}S, ^{14}C, ^{32}P and stable ^{15}N are also used.

Protein flow to the small intestine can be determined with animals equipped with either simple or re-entrant cannulae to the abomasum or duodenum.

Rumen degradability (N/CP) of feedstuffs is estimated by in sacco/in situ technique to know the rumen degradable protein (RDP) and undegradable dietary protein (UDP). Depending on the nature of the dietary N, microbial N contribute from less than 50% to over 90% of non-ammonia N (NAN) reaching the duodenum.

Cornell Net Carbohydrate and Protein System (CNCPS)The most widely used and **sophisticated multi-chemical approach** for quantifying N fractions in feedstuffs is the protein fractionation scheme used in the CNCPS

Characterization of Proteins in Feeds

The first goal in characterizing feed CP is to obtain reasonably accurate estimates of ruminally degraded protein (RDP) and ruminally undegraded protein (RUP). These two fractions of feed CP have separate and distinct functions in ruminant diets. Ruminally degraded CP is required for ruminal fermentation because it provides the mixture of peptides, free AA, and ammonia required for microbial growth, activity, and synthesis of microbial protein. In contrast, RUP provides a direct source of digestible AA to the animal.

Several methods have been evaluated to partition feed crude protein (CP) into rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) and to estimate the intestinal digestibility of RUP. These methods include in vivo, in situ (using nylon or dacron polyester bags with a 40- to 60 µm pore size), and a variety of in vitro enzymatic methods [(ruminal in vitro methods that involve incubations with mixed ruminal microorganisms (i.e., ruminal digesta) and nonruminal in vitro methods that involve incubations with cell-free enzymes], in vitro chemical methods and in vitro multi-chemical methods.

Methods for Estimating RUP Digestibility-

Methods employed to protect protein from rumen microbial degradation should increase the efficiency of protein utilization in the animal. That is rumen-undegraded protein should be digestible and absorable subsequently in the gastrointestinal tract. Accurate estimate of the intestinal digestibility coefficients of the RUP for each feedstuff is fundamental to balancing diets for RUP. Several methods have been used to obtain estimates of RUP digestibility. These include in vivo procedures, in vitro techniques, nonruminant animal bioassays, the in situ mobile nylon bag technique, and the use of ADICP.

The most widely reported approach is the mobile bag technique-

This approach consists of placing small amounts of washed ruminally undegraded feed residues in bags, pre-incubating them in a pepsin/HCL solution for 1 to 3 h, and then inserting them into the duodenum of cannulated ruminants. The bags are recovered from the faeces, washed thoroughly to remove endogenous and other contaminating protein, and analyzed for

protein content. Research has shown good correlation between estimates of RUP digestibility with this method and in vivo- derived estimates. Precision-fed roosters are valid models for the evaluation of small intestinal digestibility of protein in ruminants in the absence of animals fitted with duodenal and ileal cannulae. The use of caecectomized birds removes most of the fermentative capacity of the avian gastrointestinal tract, thereby allowing the rooster to simulate more closely only the small intestinal portion of the bovine gastrointestinal tract. Use of 'the precision-fed caecectomized rooster bioassay' offers promise as a method for assessing the availability of amino acids reaching the small intestine of cattle. Rumen-undegraded residues are crop- intubated to 4 caecectomized roosters and total excreta are collected for 48 h. Rumen-undegraded residues and excreta are analysed for amino acids. Basal endogenous amino acid loss estimates are estimated from fasted birds and are used to calculate standardized digestibility of RUP-amino acids.

Balanced Ration and its Characteristics

Ration: A ration is the feed offered for a given animal during a day of 24 hours. The feed may be given at a time or in proportions at intervals.

Balanced ration: A balanced ration is one that furnishes nutrients in such proportions and amounts that it will properly nourish a given animal for 24 hours (Morrison, 1956). In addition, the required nutrients must be contained in the amount of dry matter (DM) the animal is able to consume in the 24 hr. period; otherwise the ration can not be considered balanced.

Desirable Characteristics of a Ration

1. The ration should have highly digestible feed ingredients. For example, feather meal contains 87% CP but its digestibility is as low as 15-20%. Therefore it is not the amount which is present in the feed is important but how much is digested by the animal, i.e., DCP and TDN. The ration should be balanced.
2. The feed must be palatable. Evil smelling, musty, mouldy feed should not be given. If unpalatable, improve the palatability by the addition of salt and molasses.
3. Variety of feeds in the ration makes it more palatable. A balanced combination of proteins, vitamins and other nutrients are furnished by incorporating many feeds in a ration.
4. The ration should contain enough of mineral matter. This is especially important in case of milch animals since each litre of milk had more than 0.7% ash.

5. The ration should be fairly laxative; otherwise the animal may suffer from constipation. Hence succulent green fodders should be included in the ration.

6. Green succulent fodders have cooling effect. They aid the appetite and keep the animal in good condition. They are bulky, easily digestible, rich source of carotene, other vitamins and minerals. Leguminous green fodders are rich in proteins and calcium.

7. The ration should be fairly bulky to satisfy the hunger. If it is too bulky the animal will fail to get all its nutrient requirements.

8. Avoid sudden change in the diet; it may cause tympanitis, impaction, etc. All changes of food must be gradual and slow.

9. Maintain regularity in feeding. The time of feeding should be evenly distributed so that the animals are not kept too long without feed.

10. Feed should be properly prepared to render it more digestible and palatable. e.g. grinding of grains, chaffing of coarse fodders, moistening of dry fodders, soaking of cotton seed and other cakes before feeding.

11. Economy in labour and cost: The cost of feed and labour charges should be minimized to make rearing of livestock profitable.