All measures of protein quality are a function of the limiting amino-acid, because the usefulness of a protein is limited by the amino-acid in shortest supply. The tests do not, therefore, yield any information about the other amino-acids, i.e. the potential value of the protein if given in combination with other proteins. This point needs emphasis because so often its B.V. or P.E.R. is taken as a complete description of a protein. It is possible to find two proteins with the same B.V. but with different amounts of the non-limiting essential amino-acids.

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Chemical methods of evaluating protein quality

By K. J. CARPENTER, *School of Agriculture, University of Cambridge*

It has been shown experimentally that one protein may be superior to another in one set of circumstances, and yet in another set of circumstances be inferior. This difference arises, of course, from the requirement for protein being really a compound set of requirements for individual amino-acids (which are contributed in different proportions by different foods) and from the practical situation where

the total protein of a diet is derived from several different materials, so that an excess of a particular amino-acid in one ingredient may balance a deficiency in another.

It follows then that we cannot hope to devise an 'ideal' chemical test (or a biological one for that matter) which will rank proteins in their 'correct' nutritional order for all conceivable circumstances. It would be equivalent to evaluating all vitamin concentrates on a single scale regardless of whether they were to be used for treating scurvy or rickets. This situation has been a spur to the development of laboratory methods for the analysis of foods for individual amino-acids, and the results of many thousands of analyses are now recorded in the literature (cf. tabulations by De Man & Zwiep (1955), Harvey (1956) and (U.S.A.) National Research Council: Committee on Feed Composition (1956)).

The chemical elegance of modern techniques for amino-acid analysis must not blind us to their shortcomings when applied to foods with the solution of nutritional problems in view. Firstly, the results give no indication of the availability or digestibility of the amino-acids and, secondly, the time, skill and special apparatus required to carry through these procedures limit their application in circumstances where particular reliance has to be placed on chemical methods. When time and facilities are ample a nutritional decision (for example on a formula to recommend for a particular type of diet) may well be delayed for feeding trials, to confirm expectations based on chemical analyses; but in other circumstances a quick decision is essential, and only information obtainable within a short time is of any value. It is the object of this paper to consider what such situations are likely to be, and how far the range of individual amino-acids likely to be limiting is reduced by such factors as the species to be fed and the other components of the diet so that simple procedures can give worth-while information for the particular conditions in which it is required. A comprehensive review is impossible, and examples will be chosen only to illustrate the various principles employed and omitting problems of ruminant feeding.

Cereal grains

Cereals are the most important source of dietary protein for both man and the non-ruminant farm animals. Each species varies little in protein quality, with the exception of maize. In maize bred to contain a high level of protein, quality is reduced, because the extra protein is largely zein which is of low quality by any criterion. With the common varieties of maize a simple nitrogen analysis will therefore give an indication of the quality as well as the quantity of protein present (Mitchell, Hamilton & Beadles, 1952).

The ordinary baking of cereals for bread probably has little effect on protein quality, but serious damage may occur with imperfect control of some industrial processes for manufacturing breakfast foods (Mitchell & Block, 1946). Chemical methods developed for use with oilseed meals may prove useful indicators of this damage.

Oilseeds

The development of chemical methods for oilseed meals has been intensively studied because of the increasing importance of these meals as sources of supplementary protein for non-ruminants, and of the effect on their nutritive value of the procedures used for extraction of the oil. Producers need quick tests to know whether processing machinery is being operated so as to cause minimum damage, and buyers also need to discriminate between good and bad lots. Fortunately each type of raw oilseed has a very constant amino-acid composition, so that only changes induced by processing have to be considered. In addition, the meals are normally used as supplements to cereal proteins, which greatly narrows the range of essential amino-acids that may be limiting in the complete diet.

Of the commonly used oilseed meals the most profound effect of processing has been found with soya beans. The methionine in underheated material is largely unavailable to poultry, and the availability is increased by heating (cf. review by Rice & Beuk, 1953). The heat treatment required also inactivates most of the enzyme urease that occurs in raw soya beans (Bird, Boucher, Caskey, Hayward & Hunter, 1947). This inactivation has been widely used as a test for inadequate heating; the test material has only to be digested in the presence of urea, which will be hydrolysed to a varying extent to ammonia and CO_2. The production of the former is easily measured; a titrimetric procedure is described by Croston, Smith & Cowan (1955).

The improvement with moderate heating is not thought to be due to any change in the main protein molecules, but to inactivation of other factors present in soya, including a trypsin inhibitor. Further heating of the meal will now begin to reduce its protein quality, in a way that seems common to all protein foods, and to reflect changes in the condition of the main protein molecules.

An obvious change in the physical properties of heated proteins is their reduced solubility in water, but even with optimal heating little soya-bean protein remains soluble in water, or mild acid or alkali (Balloun, Johnson & Arnold, 1953). Greater differences between optimally heated and overheated meals are shown if the test material is shaken successively with water, 5% KCl, 70% ethanol and 0·2% KOH. By this procedure, the range of values for the percentage of the original nitrogen remaining undissolved was from 30 to 60 for a series of commercial soya-bean meals (Evans & St John, 1945). The correlation of these values with results obtained in chick-feeding experiments is shown diagrammatically in Fig. 1. The results suggest that this procedure can give an indication of the nutritive value of a meal, but its use has not been reported by other workers. The same series of meals was also tested for their solubility after digestion with pepsin and dilute acid; but this treatment dissolved from 93 to 95% of all the meals, so that the values could not be used to discriminate between them.

Much better discrimination between samples of different quality has been obtained by measuring the release of individual amino-acids from meals subjected to in vitro digestion by enzyme preparations. Riesen, Clandinin, Elvehjem & Cravens

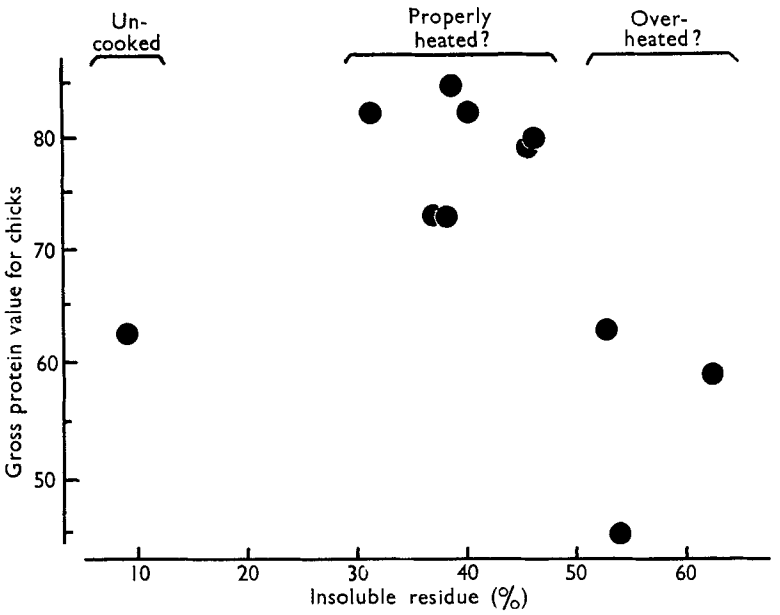

Fig. 1. Chemical measure of solubility and biological 'gross protein value' of soya-bean meals processed in various ways (Evans & St John, 1945). 'Insoluble residue' refers to the percentage of nitrogen in the test materials that remained insoluble after successive digestion with water, 5% KCl, 70% ethanol and 0·2% KOH.

(1947) used a 5-day digestion period in pancreatin, and found that Van Slyke determinations on the extracts showed that 58% of the total $-NH_2$ groups had been released from a good-quality soya-bean meal, and only 39% from an overheated one. Microbiological assays for ten individual amino-acids were also carried out on the digests. Only one-sixth as much lysine was released from the overheated sample as from the other, and only one-third as much arginine; the release of other amino-acids was much less affected (release of cystine was not measured). This result agreed with findings from a chick-feeding test, in which most of the nutritional inferiority of the heated material was made good by the addition of lysine. Further consideration of methods involving enzyme digestion followed by microbiological assay would be inappropriate here. Our present task is to consider what more purely chemical procedures may prove adequate for the objectives described above, and remain rapid and simple.

Other attempts to detect processing damage in soya-bean meals have been based on changes in the general reactivity of the protein molecules. Frölich (1954) obtained results suggesting that heat damage increased the absorptive property of meals for dyes containing a phthalein group. Almquist & Maurer (1953) found, on the other hand, that overheated meals showed a reduced reactivity with formaldehyde; a preliminary comparison of rat-growth results with values from a simple formol-titration procedure showed a promising correlation. The change in formol-titration values is thought to reflect a reduction in the number of reactive $-NH_2$ groups in

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the protein, and particularly of ϵ–NH_2 groups of lysine. A recently published procedure for the more specific estimation of the latter groups in vegetable materials (Bruno & Carpenter, 1957) may therefore prove a useful tool; its principle is further considered on p. 96.

All the methods discussed in connexion with overheated soya-bean meal may apply to the other oilseed meals, which have received less attention. In a study with groundnut meal, Lord & Wakelam (1950) showed that heat damage may be accompanied by a loss of sulphur from the material; simple protein-solubility measurements were again of little value.

The recent striking development of methods for processing cottonseed to yield a high-quality protein concentrate has been accompanied by an intensive search for quality-control procedures. The problem has been bedevilled by the difficulty of disentangling the twin factors of protein damage and of the presence of gossypol in a toxic form, and by the apparent interaction of gossypol with protein during processing. Cottonseed proteins show a considerable range of solubility in 0·02 N-NaOH according to treatment, and Lyman, Chang & Couch (1953) proposed a 'chemical quality index' defined as percentage of nitrogen soluble in 0·02 N-NaOH divided by either percentage of gossypol in the meal or 0·85, whichever is less, which showed a close correlation with chick-growth tests for a series of twenty-two samples. However, further work suggests that the index fails to grade meals prepared by a wider range of procedures (Condon, Jensen, Watts & Pope, 1954; Eagle, Bialek, Davies & Bremer, 1956). There is the complication in making this comparison that the different groups of workers used different types of animal-feeding tests, which might in themselves give different gradings for the same series of products.

Animal proteins

These represent the third important protein source in both human and animal nutrition. Of human foods in this category dried milk is the only material for which regular quality control of manufacture seems important, though the production of dehydrated fish and meat for people in special circumstances is expanding. However, in animal nutrition fish and meat meals are of the greatest importance and are relied on as sources of high-quality protein. They cause special difficulties in chemical evaluation because, in addition to changes induced by differences in processing, the amino-acid composition (and nutritive value) of the raw material may vary considerably. This is particularly true for concentrates prepared from slaughterhouse by-products.

There are, first, certain tests for deducting values for obviously useless nitrogenous components from the crude-protein figure. The insoluble residue from the peptic digestion of meat meals is of little nutritional value and comes from contamination with horn, hoof and wool (Gehrt, Caldwell & Elmslie, 1955). Similarly urea nitrogen can be estimated and deducted from the total nitrogen in meals made from dogfish and sharks (Rhian, Carver, Harrison & Hamm, 1942).

Secondly, tests are made to distinguish large protein molecules from smaller ones and peptide fractions, on the basis that the former are more easily precipitated by

heavy ions as with phosphotungstic acid. The precipitable fraction is traditionally described as 'true' protein. In principle, of course, there is no reason why 'true' protein should be nutritionally superior to more soluble molecules, but in practice, with animal tissue, the latter are largely derived from connective tissue which is inferior in amino-acid composition to the larger muscle proteins. Any violent processing that partially hydrolyses proteins and increases their solubility will also tend to destroy labile amino-acids, so that there is some empirical justification for the distinction. Almquist, Stokstad & Halbrook (1935) suggested the calculation of a 'protein quality index' based on the combined results of four different solubility tests; the formula suggested was that which correlated best with the feeding results for nineteen fish and meat meals that had been studied. Independent studies have shown positive correlations between the index and feeding results with chicks for both fish and meat meals (e.g. that of March, Stupich & Biely, 1949). However, a drawback to the general adoption of the test is the time and skill needed for the laboratory operations (Kokoski, 1947).

Since the most severe effect of processing seems to be a reduction in the nutritional availability of lysine, and the concentration of this amino-acid in animal tissues is a major reason for the high value of protein foods made from them, any chemical procedure that could show that a particular product is rich in available lysine would also indicate that the raw material had been of good quality and the processing well controlled. Experiments with purified proteins suggest that the reduced availability of the lysine is largely due to its ϵ–NH_2 group combining with other active groups under conditions of moist heat to form a linkage that resists hydrolysis by enzymes (Eldred & Rodney, 1946). This hypothesis, that only lysine molecules with reactive ϵ–NH_2 groups are nutritionally available, was the basis for a recently developed procedure in which dinitrofluorobenzene is allowed to react with free –NH_2 groups in intact protein and the dinitrophenyl-lysine released after subsequent acid hydrolysis is measured colorimetrically (Carpenter & Ellinger, 1955; Bruno & Carpenter, 1957). The principle is illustrated in Fig. 2. Results obtained with a series of fish, whale and meat products have shown a close correlation with corresponding results of chick-feeding tests, under conditions that emphasized the lysine contribution of the test materials (Carpenter, Ellinger, Munro & Rolfe, 1957).

The comparative speed of this procedure (it can be completed in an afternoon and a morning with overnight refluxing) and the simplicity of the apparatus involved give it some advantages; but it remains to be seen how far values obtained with it can predict the nutritional worth of materials when some amino-acid other than lysine is the limiting factor. The sulphur amino-acids are the likely alternative limiting factor. It is possible that when raw materials are relatively uniform in composition, processing damage to lysine and cystine (more susceptible than methionine) will prove to be roughly parallel. When the raw material is also variable, the 'available lysine' values must obviously be a less useful guide, though a lower-than-usual figure for a particular product would give grounds for suspicion also as to its contribution of other essential amino-acids.

The Stutzer procedure for detecting decomposition of raw material before

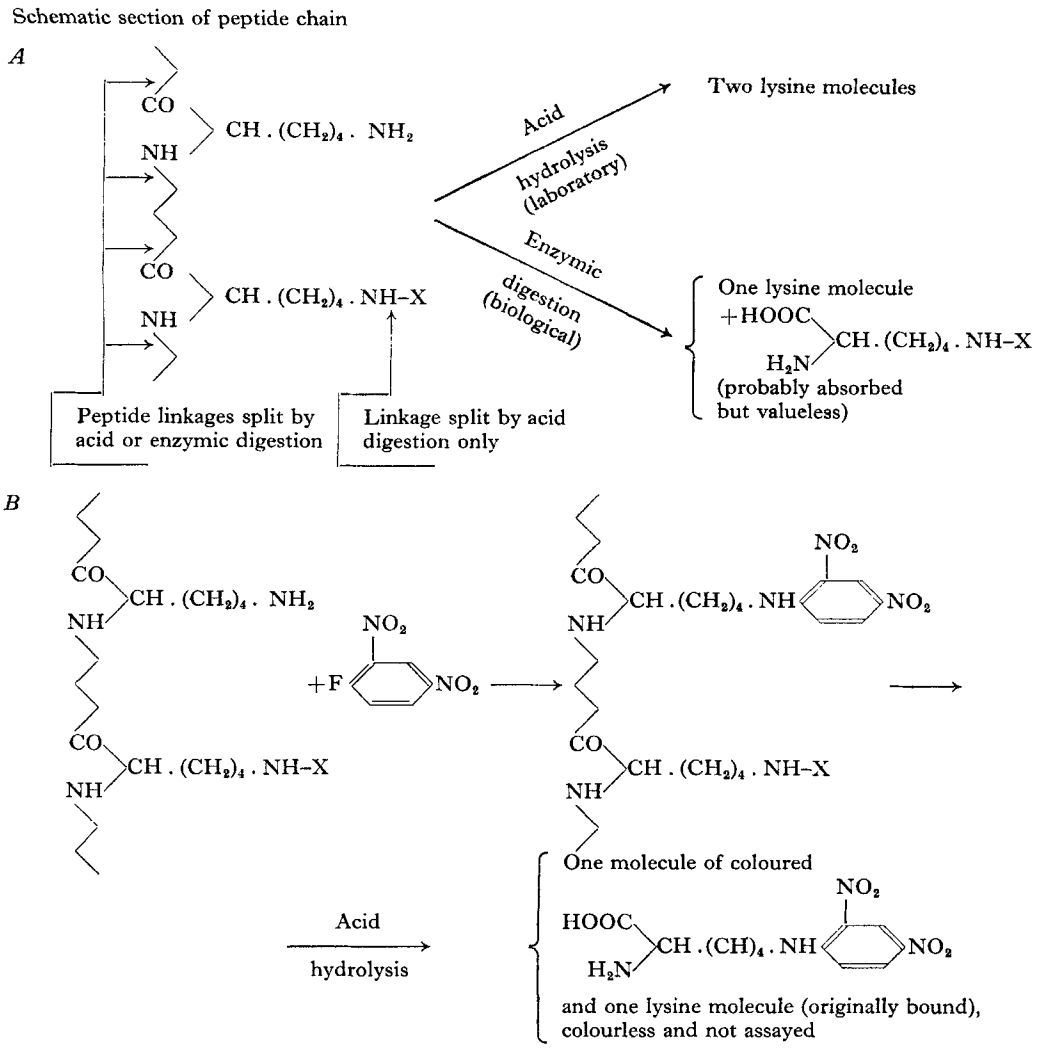

Fig. 2. Schematic diagram to illustrate the theoretical explanation for the difference between results by 'acid hydrolysis' and by 'biological assay' for the lysine content of processed foods, and the use of dinitrofluorobenzene to overcome the discrepancy. A peptide chain is shown with two lysine units, of which one is bound through its ϵ-NH_2 group to a reactive group (X).

A shows the contrasting effects of acid and enzymic digestion.

B shows the reaction of the free ϵ-NH_2 group with dinitrofluorobenzene and the products obtained on subsequent hydrolysis. Only the lysine unit that was originally free yields a coloured compound.

manufacture by measuring H_2S production on digestion with alkali does not, in fact, give results that parallel the findings of feeding tests (Almquist *et al.* 1935).

Ensuring the high quality of dried milk is of great importance. The amino-acid composition of the raw material is very constant, but the presence of carbohydrate and moisture makes the protein very sensitive to damage. Recent work (Mauron, 1956, 1957) has confirmed that considerably more lysine is made unavailable than

is actually destroyed, which can be measured either with in vitro digestion followed by a microbiological assay of a dialysate, or by the dinitrofluorobenzene chemical procedure—the two methods giving parallel results

Mixed diets

It seems generally agreed that solubility studies are of little value without reference to normal figures for the particular class of material being considered (e.g. Evans & St John, 1945). One would, of course, expect this, in view of wide differences in the amino-acid composition. In general, therefore, there seems no purely chemical alternative to the use of in vitro enzymic digestion followed by individual assays for released amino-acids. Even this may give misleading results if only a single enzyme is used, since there is reason to believe that some proteins are relatively more susceptible to attack by one digestive enzyme than by others.

With lysine in particular, the correlation between chemical figures from the dinitrofluorobenzene method and the gross protein values for chicks of all the various materials studied so far is shown in Fig. 3. The range of materials includes the common oilseeds, leaf-protein concentrates, and the usual sources of animal protein. The generally good correlation regardless of the protein source suggests

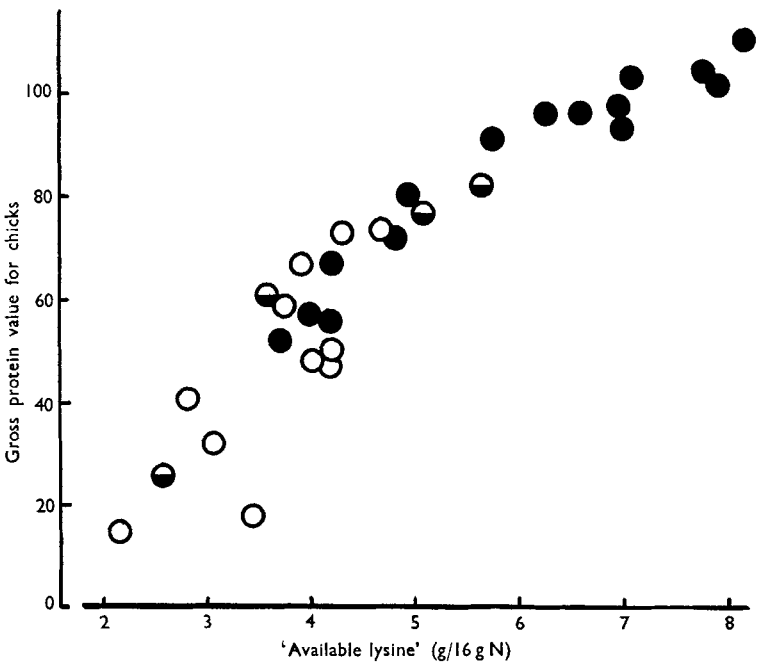

Fig. 3. Chemical values for 'available lysine' and biological 'gross protein value' of test materials. ●, materials of animal origin including fish, whale and milk products (Carpenter *et al.* 1957); ○, leaf products; ◒, cereal and oilseed products. The origin of the vegetable products has been described by Bruno & Carpenter (1957), but the available-lysine values have been raised by 20% in an attempt to compensate for destruction of dinitrophenyl-lysine during hydrolysis in presence of carbohydrate.

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that the method may prove useful with mixed diets of unknown composition. Lysine added as such (i.e. not as a constituent of a peptide chain) would not be measured.

An instance where some such procedure might be of value has been described by Egli (1957). In a study of the preparation of special high-protein biscuits for supplementing deficient African diets it was found that even slight differences in the baking could cause such a profound drop in nutritive value as to make the biscuits useless.

Colour and heat damage

Most feeding-stuffs are bought after simple visual inspection. Undoubtedly, the same raw material subjected for varying periods to a particular cooking process will give successively darker 'browning' that relates to nutritive value (cf. Carpenter *et al.* 1957). But this effect may not apply when the same raw material is treated by different processes such as 'solvent extraction' and 'expeller' processing of oilseeds (Bird *et al.* 1947), or the 'hot-air' and 'steam-jacket' drying of fish meals. Also changes in the raw material may change the colour of the product without necessarily affecting the nutritive value, as, for example, the difference between fish meals made from white fish or from herring. Lastly, browning can be prevented or the colour removed by a bleaching agent without any improvement in nutritive value (Miller, 1956). Measurement of colour, like nearly all the other tests, can only be a guide to 'most materials, most of the time'.

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The importance of protein quality in animal feeding

By GABRIELLE M. ELLINGER, *Rowett Research Institute, Bucksburn, Aberdeenshire*

The importance of protein quality for practical rations becomes evident as soon as an attempt is made to economize and to establish for such rations the lowest level of protein that will give efficient production. Woodman & Evans (1951) demonstrated this point with pigs fed on a simplified barley–fine-bran ration. Supplementation with fish meal at the rate of 4·2% protein gave efficient production that could be attained with groundnut meal only when included at the rate of 9·9% protein. In laying experiments, the difference between fish meal and groundnut meal was reflected in egg production only when the total protein content of the ration was reduced to 11% (Carpenter, Duckworth & Ellinger, 1954*a*). The same proportions of fish meal and groundnut meal in a 14% crude-protein ration gave equal egg production. Consequently, economies that are permissible with a high-quality protein may not be so with a concentrate of a poorer quality.

The principal aim of animal production is the economic and efficient conversion of feeding-stuffs. Financially, the real economies depend mainly on market conditions. Economy in terms of protein—also important to us as importers of a large proportion of concentrates—can be achieved by the correct allocation of available feeding-stuffs and by the conservation of quality during their production. The formulation of economic rations depends on knowledge, on the one hand, of the function of the rations in terms of the animals' requirements and, on the other hand, of the way to use available feeding-stuffs to fulfil these requirements. The poorer-quality concentrates may then be included in successful rations, provided their limitations are recognized and corrected.

Here, the feeding of non-ruminants will be considered and that only under intensive systems of management where requirements must be satisfied entirely by the rations. The utilization of protein by ruminants is, to a large extent, governed by the metabolism of the rumen micro-organisms. Criteria that determine protein quality under those conditions have been discussed by Chalmers & Synge (1954). Should the early weaning of calves become an established practice, many considerations given here may find application in calf rations used during the period before rumen activity becomes fully developed.

Practical rations are generally compounded to contain protein at levels higher than the requirement determined under experimental conditions. This excess is regarded as a margin of safety which compensates for fluctuations in the quantity and