Standardization of Analytical Methodology for Feeds

Proceedings of a workshop held in Ottawa, Canada, 12–14 March 1979

Editors: W.J. Pigden, C.C. Balch, and Michael Graham

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Foreword

In preparing rations for farm livestock, a knowledge of the amount and availability of the various nutrients supplied by the available feedstuffs is essential. Lack of reasonably accurate information can result in inefficient feeding systems and enormous wastage of feed resources.

Although feed analysts and nutritionists have numerous analytical methods to measure nutrient values, they apply these according to their variable backgrounds and experience. Some of the methods used are highly standardized and widely accepted but others have been developed and used within comparatively few laboratories. This has resulted in a great lack of standardization in the choice of methods. As a result many unnecessary or inappropriate analyses are done, and of even greater concern, feed wastage often occurs because of faulty information. In particular, there has been a lack of advice on which combinations of the available analytical fractions most adequately reflect the energy value of feedstuffs.

The need for a better standardization of analytical methodology was recognized by Commission VI (Nutrition of Animals) of the International Union of Nutritional Sciences (IUNS), which established a Committee on "Problems of Standardization and Nutritive Value." This Committee, in conjunction with the International Development Research Centre (IDRC), sponsored a small workshop held in Ottawa, Canada, from 12 to 14 March 1979 to discuss standardization. It was attended by 24 scientists from 12 countries.

It was the third IDRC workshop to consider problems of standardizing nutritional techniques. The first was cosponsored with the United Nations Protein Advisory Group (UNPAG) and held at CIMMYT in Mexico in May 1974. Its proceedings were published as "The PAG Guideline (No. 16) on Protein Methods for Cereal Breeders as Related to Human Nutrition Requirements." In July 1976 a similar meeting cosponsored by IUNS, the International Union of Food Science and Technology (IUFOST), and IDRC, in conjunction with the International Centre for Tropical Agriculture (CIAT), the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and the International Centre for Agricultural Research in Dry Areas (ICARDA), was held. The results of this meeting were published as IDRC-TS7e, Nutritional Standards and Methods of Evaluation for Food Legume Breeders.

In both of these earlier workshops, an effort was made to clarify guidelines in a field where there was considerable confusion and where a large number of different techniques were being employed. An identical situation appeared to exist with respect to techniques for feedstuff analysis, a field in which confidence in the value of some traditional analytical fractions has declined in recent years and where various new techniques have been put forward. The confusion arising from this situation has led to considerable problems, particularly in developing countries, where sophisticated analytical and feed evaluation techniques are often not practical and where the bulk of the feed for ruminant stock consists of

tropical forage and browse plants. The aim once again was to put forward guidelines intended to help practical analysts.

In the third workshop, whose proceedings are reported here, primary emphasis was placed on suggesting the analytical methods that best reflect the energy content of forages for ruminants, but at the same time, some attention was given to techniques applicable to mixed feeds. Methodology for poultry and pigs was given only limited attention. Participants were asked to devote particular effort to relating their papers and discussion to the problems confronted by analysts in developing as well as developed countries.

It is hoped that the papers contributed by the many outstanding international authorities, the discussions, and the recommendations will facilitate the adoption of new technology, guide the development and equipment of new laboratories, and promote better standardization of techniques at national and international levels.

The organizing committee is grateful to IDRC and IUNS for providing financial support for the meeting, and to IDRC for funding this publication and hosting the Workshop. We are also happy to acknowledge the support given by NIRD and by the Research Branch of Agriculture Canada to permit two of us (C.C.B. and W.J.P.) to devote so much time to the preparation and report of this meeting. Particular thanks are also due to the rapporteurs, A. Adegbola, C.C. Balch, R.F. Barnes, L.E. Harris, and W.J. Pigden, and to Paul Stinson of IDRC who acted as secretary to the organizing committee and was responsible for all the administrative arrangements relating to the meeting.

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Summary and Recommendations

The discussions focused principally on the methodology for the prediction of the digestible energy value of feedstuffs, measurement of energy intake, and definition of the most suitable expression of energy values. It was considered that the ability to predict feed energy values was especially vital in the planning of economic animal production at the farm, regional, and international level. Analyses for other nutrients, such as nitrogen and minerals, were considered only to a very limited extent insofar as they impinge on energy utilization. Also, because several different units are being used to express the energy value of feed it is important to determine how confusion can be prevented in the interchange of data.

The summary and recommendations are organized into three main sections: (1) energy units and evaluation of energy with animals; (2) analytical methodology and prediction of energy, digestibility, and intake; and (3) consequences of replacing long-established methodology, and guidelines for use of newer methods.

The purposes for which energy evaluation and predictions are done have an extremely important bearing on selection of appropriate methods. Guidelines are provided for matching objectives with appropriate methodology and implications for change are discussed.

Energy Units and Evaluation of Energy with Animals

Several systems are used, and are likely to continue to be used, to evaluate the energy content of feeds in ways that allow prediction of the response of the animals receiving these feeds.

The systems in use for determination of the energy value of feedstuffs with animals can be considered to consist of three stages: (1) determination of digestible energy (DE);[1] (2) determination of the metabolizable energy (ME) either directly or by calculation from the DE value; and (3) determination of net energy (NE) either directly or by calculation from DE or ME using appropriate factors. Metabolizable energy is usually expressed as the energy requirement for maintaining a given level of production. In systems for poultry and in some systems for pigs, the value employed in the second stage is *metabolizable energy*. In systems for ruminants, and in other systems for pigs, account is taken of various biological factors in order to base the third stage on the *net energy* supplied either by the individual feeds or by the diet as a whole.

The problems of evaluating the energy value of forages for ruminants were given special attention, but at many stages of the discussion the paramount

[1]DE can be satisfactorily estimated for many feeds, especially forages, at lower cost than with a bomb calorimeter. Percentage digestible organic matter in dry matter (DOM), percentage digestible dry matter (DDM) of forages (if the ash content of the latter is not excessive), and digestible nutrients can be readily converted to DE values with small errors (see Heaney and Pigden 1963).

importance of knowing the animal's feed intake was stressed. This problem is all the more difficult in situations in which animals receive feed ad libitum.

The meeting considered that provision of reliable metabolizable energy values for individual feeds and for compounded feeds is of critical importance in feed evaluation. For basic experimental purposes the direct determination of metabolizable energy requires measurement of the energy content of feces, of expired methane, and of urine. Estimates of metabolizable energy can, however, be derived from digestible energy, organic matter (DOM), or dry matter (DDM) by the use of conversion factors, which are sufficiently accurate for advisory purposes and for much applied experimentation.

It is therefore *recommended* that methods of feed evaluation should include:

(1) Measurement of the digestibility of the feed energy (DE) or related nutrients such as DOM or DDM of the feed with species appropriate to the use of the feed, e.g. monogastric or ruminant.

(2) Measurement of ad libitum food intake. It is also important to note the method by which voluntary intakes are determined — a level of 90% acceptance, with 24 h access to the feed, should be the aim.

(3) Digestibility measurements carried out at the maintenance level. These should, as a general rule, be the basis for the determination of digestible energy and the calculation, by the use of appropriate factors, of metabolizable energy. Adjustments should be made at a later stage for the consequences of biological factors such as level of feeding. For nonruminants, digestibility is hardly depressed by level of feeding, accordingly measurements need not be made at maintenance.

It was recognized that with poor forages, such as the grazing found in many parts of the arid tropics, voluntary intake may not satisfy the maintenance requirement for long periods of the year. Moreover, selection of the palatable and more nutritious parts of the herbage, or browse, presents additional problems in determining digestibility or intake. Under such conditions it was considered much more important to measure total intake than to attempt to determine digestibility or carry out a detailed analysis of the feed. When the material can be harvested for controlled intake studies it is important to offer at least twice the amount consumed to allow the animal to select the more nutritious portions. When the material is grazed, the most common situation, the energy intake can be estimated with equations derived from the maintenance and production requirements of the particular class of grazing animal (see Logan and Pigden 1969).

Information about digestibility and intake may be used (Moe, p. 25) as the starting point for the development of net energy values of feeds, or of diets, for use in applied feeding situations, or for maintenance (NE_m), fattening (NE_f), or lactation (NE_l). Such derived values have relevance, however, only to biological conditions prevailing, or assumed, in their determination. For the storage and dissemination of data about nutritive value, for example in data banks, metabolizable energy is therefore more appropriate than NE; this is especially relevant to exchanges of information at the international level. By adopting this policy, information can be used irrespective of the units to which it may be converted for local application.

Sufficient information to provide ME values and energy requirements of the accuracy required for the recommendation of feed allowances in many practical systems can therefore be obtained simply from the determination of digestible energy and certain chemical attributes (recommendation 10). It is therefore, *recommended* that:

(4) Metabolizable energy should be the basic unit used for the storage and exchange of information about the energy value of feeds. In reporting results they should be expressed as either DE or ME, or both, but where either value is estimated from other determinations the fact should be clearly stated, along with the method used.

There is a lack of information on the digestible energy contents of many tropical feeds and, in fact, for these feeds there are also very few determined metabolizable energy values. As a consequence, equations for predicting metabolizable energy from digestible organic matter or analytical fractions are unreliable for tropical feeds. It was therefore *recommended* that:

(5) Laboratories in tropical countries be encouraged to measure the digestibility of local feedstuffs primarily using sheep as the pilot ruminant, but also using cattle or other native livestock where sheep appear inappropriate.

(6) There is a need for one or two centres of excellence to make direct measurements of ME and to determine appropriate values for the factors relating the utilization of ME for NE with the feeds, animals, and environment of tropical countries. Such centres could also help many other laboratories by distributing standard samples of known composition and ME.

By-product feeds tend to be highly variable in quality and are often not fed as the sole feed to livestock. Many tropical by-products are, in fact, very low in nutritive value, e.g. bagasse, rice hulls, and straws. There may be associative effects between the digestibility of by-products and other feeds in the ration. It is *recommended* accordingly that:

(7) The digestibility of ingredients of low digestibility should be determined by including varying proportions of the test ingredient in mixed diets.

Several new systems have been introduced to predict the response of ruminants to given inputs of nitrogen; these take account of modern knowledge of the fate of nitrogen in the rumen. These systems relate the response to nitrogen to the energy in the diet, the proportion of that energy digested in the rumen and the amount of bacterial protein synthesized, as well as to the degradability of dietary protein in the rumen. It is *recommended* that:

(8) The analysis of feedstuffs should include DM and total ash so that organic matter, the starting point of these systems, can be calculated. For the time being nitrogen content should be reported as crude protein, but it is likely that eventually a value will be required for the degradability in the rumen; there is at present no general agreement on a method of measuring degradability. There may also be occasions when it will be informative to determine the N content of some of the recommended analytical fractions (see recommendation 10).

(9) Attention should be given to methods for predicting the portion of the truly digestible carbohydrates of feed that will be fermented in the rumen so that the yield of microbial protein in the rumen can be accurately calculated.

A simple, inexpensive method for measuring the ME content of feedstuffs for poultry is needed. A new bioassay for determining the true metabolizable energy (TME) has been developed (Sibbald, p. 38) and has been endorsed by several organizations. The participants agreed that the method has excellent potential, that it could be applied in some situations, and that further developmental work should be encouraged.

Some reservations were voiced that the use of TME would create confusion by introducing into the world literature a new ME term differing from the current expressions of apparent metabolizable energy (AME) and (AME_n).

Additional information is needed on the ME requirements of poultry and the ME content of corresponding feedstuffs for the tropics.

Analytical Methodology and Prediction of Energy, Digestibility, and Intake

Particular emphasis was given to measuring and predicting by laboratory techniques the energy content of forages for ruminants. Forages present the major challenge because of their high content of lignocellulose and the great variability in chemical composition of lignocellulose between species, stages of maturity, parts of the plant, etc. Hence chemical characterization of the fibrous components as related to feeding value is very difficult.

In the past, great reliance was placed on proximate principles analyses, and especially on crude fibre (CF), for predicting feed energy value. These are now considered inadequate predictions. Many alternatives have been proposed but the new detergent methods, acid detergent fibre (ADF), neutral detergent fibre (NDF), and lignin are considered the most promising and have found widest acceptance. Also, in vitro rumen has proved to be a powerful tool for predicting energy digestibility, especially for forages; it has greatly reduced the need for CF analyses or other chemical procedures for some purposes.

Although the neutral detergent methods collectively, and their individual determinations when appropriately matched to objectives, are much more powerful tools for estimating energy digestibility than CF, in practice it is frequently not practical to recommend all three methods (ADF, NDF, and lignin) because of the cost. The investigator must then consider which of the detergent methods, sometimes in association with the in vitro rumen, will give the most cost-efficient procedure. It is *recommended* that:

(10) In the analysis of forages and lignocellulosic wastes, crude fibre should be replaced by one or more of the following as appropriate: neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin (Van Soest and Robertson, p. 49).

Where crude fibre cannot be immediately superseded by ADF or NDF it is advisable to determine fibre by both methods to gradually develop an adequate analytical data base that will allow discontinuance of crude fibre as soon as possible.

Concentrates and mixed concentrate-forage diets present additional problems and challenges. Here the neutral detergent methods and the in vitro rumen are much less applicable than for fibrous feeds. Neutral detergent fibre is much superior to CF for determining the significant fibre content of concentrates for many purposes, but the method is not yet perfected. Some further technique development and collaborative laboratory tests are needed. It was recognized too that CF is firmly embedded in the fabric of feedstuff laws and regulations, in many regression equations still in use, and in historical data. Thus it will continue to be used for some time, especially in quality control and regulatory aspects of concentrates for trading in commerce, nationally and internationally. It is *recommended* that:

(11) Crude fibre determination should be carried out only in situations where it is required by feedstuff regulations or in limited specialized local situations where other analyses are inappropriate.

The in vitro rumen technique was developed as a rapid method for estimating the *relative* energy digestibility (usually as organic or dry matter digestibility) of feeds high in lignocellulose, especially forages (Marten and Barnes, p. 61). It predicts the in vivo energy digestibility of such feeds with the lowest standard error of any laboratory technique now available. Although relatively simple and inexpensive, the animals require skilled attention daily, especially in hot climates, to ensure that the fistula remains healthy, and careful

attention to certain details is critical to success. A source of uniform, high quality, rumen inoculum is essential. The donor animals must be regularly maintained on a diet containing a high proportion of good quality forage with little or no concentrates and enough nitrogen, and fed at about maintenance level. Equally important is the inclusion of "standard" reference forage samples of known in vivo digestibility in each fermentation batch. This enables the investigator to determine whether the fermentations are within acceptable limits. It also allows the application of correction factors to predict in vivo energy digestibility. It is therefore *recommended* that:

(12) An in vitro rumen digestibility technique be adopted as the method of choice where the energy digestibility of large numbers of small forage samples is to be estimated with the least error. The method should preferably be based on the digestibility of organic matter or dry matter.

The in vitro enzyme digestion technique is very similar to the in vitro rumen method except that enzymes (mainly "cellulases") replace the rumen inoculum (Marten and Barnes, p. 61). This eliminates the need to maintain animals as inoculum sources. However, digestion of the fibrous components is much less with some enzymes than by rumen microbes in vitro or in vivo; for the interpretation of the results, prediction equations based on correlations between in vivo and in vitro methods are essential. Variation in the activity of different sources of "cellulase" is a major problem limiting the adoption of this method. Thus, it is *recommended* that:

(13) Further collaborative work be carried out on the enzyme method for predicting in vivo energy digestibility. While this approach shows excellent potential it cannot yet be recommended generally until cellulases from various sources have been investigated and further comparisons completed on the relative merits of pretreatment with acid pepsin versus neutral detergent.

The nylon bag technique consists of suspending a quantity of ground forage or cellulose in a fine mesh bag in the rumen of a fistulated ruminant and measuring the losses (digestion) of dry matter occuring in a given period. The method is poorly standardized in that various pore sizes of cloth, fineness of grinding of test samples, sample size, and other factors influence the results. It is less accurate and useful than the in vitro systems for most applications. This technique is *not recommended* as a substitute for the in vitro systems for forage evaluation. It is however useful for particular applications such as evaluating the effect of supplementation on the digestion of cellulose in the rumen using a standard forage or cellulose source. It has the advantage of extreme simplicity of equipment but requires animals fitted with rumen fistulas.

Standard reference samples of forages, crop residues, industry by-products, and other feedstuffs that have accurate and precise agronomic chemical and in vivo energy digestibility data are essential for the development and calibration of in vitro rumen, in vitro enzyme, and chemical techniques. It is essential to have samples of forages with known in vivo digestibility, measured with animals fed at a level where there are no feed residues. The animals used should be similar to those to which the estimated digestibilities will be applied. By including these samples in in vitro runs the investigator can convert the dry or organic matter disappearance in vitro to estimated in vivo digestibilities. It is *recommended* that:

(14) Emphasis be placed on continued development of standard sample reference libraries at key locations throughout the world.

Standard samples for many tropical species are available from Dr D.J. Minson, Division of Tropical Crops and Pastures, CSIRO, St. Lucia, Brisbane,

Australia. Samples of temperate reference forages are available through Dr W.C. Templeton Jr., Director, US Regional Pasture Research Laboratory, University Park, PA, USA or Dr Ralph McQueen, Research Station, Research Branch, Agriculture Canada, P.O. Box 20280, Fredericton, New Brunswick, Canada.

An infrared reflectance spectroscopy technique, which measures many properties related to forage quality, is under development in a number of laboratories using expensive and sophisticated equipment. Research is needed on the reflectance properties of chemical constituents such as protein nitrogen, artifact lignin, minerals, and other chemical components. It is also important to optimize the specific wavelength selection technique as well as the development of prediction equations. More information is needed on the effects of plant species, stage of plant development and harvest, growth environment, method of preservation, and sample preparation on spectral properties.

The methodology has considerable promise for use as a rapid analytical tool in plant breeding and management studies, in management systems where quick turnaround time is required (such as hay marketing), and in forage and feedstuff testing to estimate chemical composition for use in balanced livestock rations for optimum nutrition. However, it cannot as yet be recommended as a standard technique and is to be avoided by those laboratories with limited resources. It is *recommended* that:

(15) Further research and development be carried out by those laboratories already equipped and with adequate resources to pursue such investigations.

Consequences of Replacing Long-Established Methodology, and Guidelines for Use of Newer Methods

A major problem for the investigator is matching the appropriate analytical method to a specific work objective. In the case of feeds high in lignocellulose a useful concept is to consider the process in the form of a pyramid with several layers. The base represents large numbers of samples to be screened by simple chemical analyses, e.g. neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, and in vitro tests. Out of the base population should be selected the most promising treatments for feeding trials (digestibility and intake) with small ruminants — sheep in most instances. This would represent the second layer. From this a further and more restricted group (pyramid apex) of the best feeds should be selected for testing in actual milk or meat production trials using large ruminants.

To provide somewhat more specific guidelines, broad "purpose" categories were defined with corresponding changes in methodology. These are: (1) Research and Development; (2) Quality Control in the Feed Industry and Trade and Legal Aspects; (3) Advisory Services; and (4) Feed Resource Inventory.

(1) Research and Development

(a) Forage Selection and Breeding. Total yield of digestible energy per hectare and its distribution during the growing season is probably the most important nutritional characteristic of any variety or selection. This energy must be of high digestibility if high animal production is to be achieved. Initial screening should be done by the in vitro rumen. NDF will give an estimate of intake but the error will be large. Promising selections can be followed up for increasing complexity of evaluation as required. Analyses for protein, and in most instances phosphorus, are also important, especially in the tropics. Proximate principles analyses should not be used.

(b) Development of New Feeds from Wastes. Many of these feeds are high in lignocellulose and require specialized treatment (heat, alkali, etc.) to increase the availability of the energy. The in vitro rumen technique is a powerful tool for initial evaluation and screening. A combination of this with the detergent methods of fibre analysis frequently gives the most useful information.

(c) Effect of Supplemental Feeding. Improving the utilization of existing high lignocellulose feeds can be tested by evaluating the extent of fibre breakdown with detergent methods or by in vitro rumen. However, frequently the major effect is reflected in increased intake, which is best measured by feeding trials with ruminants.

Adoption of the recommended analytical procedures would result in major changes in methodology in the research and development sector. It should be recognized that many laboratories are already using these techniques, and that they can make an especially important contribution in the analysis of tropical forages.

(2) Quality Control in the Feed Industry and Trade and Legal Aspects

For the control of quality of raw materials and of finished products the feedstuff industry carries out a wide range of analyses on very large numbers of samples. These are mainly concentrates rather than forages, although in some instances commercial companies in developed countries analyze forages as a service to assist customers in developing balanced rations. Adoption of the recommended procedures would probably lead to few problems within feedstuff companies. However, because many feedstuff regulations call for statements on the moisture, crude protein, crude fibre, and lipid content of products, there will be a continuing need for these determinations while the present regulations remain in force. New feedstuff regulations, for example in the EEC, require ever-increasing lists of analyses. It follows that the trade is anxious to use methods that are rapid and readily automated.

As a measure of the soluble readily digested carohydrates the nitrogen free extract (NFE) is employed by analysts to a varying degree. Many feedstuff companies compensate for the inadequacies of NFE by determining starch and sugar contents to separate the digestible from the poorly digested fraction.

Of major concern is the need for an improved procedure for estimating the "effective" influence of the plant fibre (which varies widely in chemical composition and physical properties from many sources) on digestibility and intake. Although NDF is capable of supplying much of this information, with or without ADF, it is not as yet adequately standardized.

Forages, apart from alfalfa and some hays, are not traded in international commerce to any great extent. However, substantial quantities are traded within some developed countries, and new USA grading standards for hay have been proposed (Rohweder et al. 1978). These proposals are forward-looking and provide a clear example of the potential application of the detergent methods. The procedure is based on a relative feeding value (RFV) determined for each hay and compared to standard values. The RFV is calculated from a combination of estimated dry matter intake (predicted from NDF intake) and dry matter digestibility (predicted from ADF) to provide an estimate of digestible dry matter intake (DDMI). In terms of cost, accuracy of prediction, and turnover time, the procedure clearly has merit although it is still in an evolutionary state.

The Association of Official Analytical Chemists (AOAC) develops and adopts new methods and makes available proven procedures that can be followed by analysts. The ADF procedure has already been adopted by AOAC

and if the recommended procedures are widely accepted it is *recommended* that:

(16) Steps be taken to encourage AOAC acceptance of NDF as soon as the method is perfected. This determination is likely to be especially meaningful for mixed feeds, and adoption of the improved procedures is likely to lead to a need to test and accept other methods, e.g. lignin. Research institutions should also be encouraged to provide additional technique development and to participate in collaborative testing to expedite this development.

(3) Advisory Services

In recent years government and commercial feed analysis laboratories have been developed to provide analytical information for feed formulation purposes.

The in vitro rumen provides the most accurate prediction of energy digestibility but is often considered too slow. The ADF, or even crude fibre, is faster, more convenient, and less costly. NDF can be used to provide some estimate of intake. In most instances protein and possibly phosphorus are also required. Some of the more sophisticated services provide in vivo digestibility using sheep.

Crude fibre will continue to be used, but the aim should be to phase it out in favour of the recommended methods.

(4) Feed Resource Inventory

Many laboratories that are doing extensive proximate principles analyses, including crude fibre, should substitute the recommended methods, i.e. detergent and in vitro. New laboratories should not be equipped for the old methods. This is particularly true where tropical forages are to be analyzed. The in vitro systems should be used much more extensively and more reliance should be placed on in vivo data obtained with small pilot ruminants, e.g. sheep. Proximate principles analyses, especially crude fibre, should be phased out as soon as possible.

One of the main advantages of adopting a given series of analytical procedures would be to facilitate the exchange of information between laboratories at any level, including international exchanges. For successful exchange of information, however, it is also essential that the feeds analyzed be accurately described and sampled. The International Network of Feeds Information Centres (INFIC) is dedicated to the collection, storage, and dissemination of numerical data about the composition and nutritive value, along with factual information about the utilization, of feeds from all parts of the world. An international nomenclature has been developed by INFIC. This enables feeds to be accurately named and described in any one of five interchangeable languages; local names can also be recorded. It is *recommended* that:

(17) In reporting information about feeds the INFIC international nomenclature be used. INFIC collecting centres can utilize published data, but detailed analyses should be submitted to an appropriate centre on standard source forms.

(18) Laboratories providing analytical data for data banks should be encouraged to participate in tests of the accuracy of the methods employed; samples of known composition and nutritive value are available for this purpose.

Evaluation of the Energy Value of Feeds: Overall Appreciation

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The conversion of gross energy (GE) into metabolizable energy (ME) is discussed, with attention being paid to the digestion process and to the production of CH_4 and fermentation heat and to urinary energy losses. On average, for ruminants only about 90% of the ME of a ration is energy in a chemical form. For monogastrics with hindgut fermentation, especially when CH_4 is neglected, part of the "ME" consists of CH_4, heat, and VFA. ME utilization is discussed, for both maintenance and production, with the main emphasis being placed on the energetic efficiency of the biochemical conversions for each of the chemical components of ME. ME content is found to be the major factor influencing the value of the feedstuff for maintenance; whereas, the origin of the ME influences this value by about 20% in nonruminating animals and by less in the case of ruminants.

For nonruminants, feed evaluation according to net-energy-fattening (NEF) approximates the true feeding value of the feedstuff for both maintenance and production fairly closely, and some improvements are suggested to further improve the predictive equation. Some of the new systems for the evaluation of energy for beef and dairy cattle are also discussed. The net-energy-lactation (NEL) system for dairy cattle is well-based and is easy to use in practice, but the new systems for beef cattle still require additional work.

For feed evaluations in warm countries, it is of foremost importance to have information on the composition and digestibility of the feedstuffs. In most cases DE or ME will be sufficiently precise; however, for nonruminants a slightly improved NEF system and for ruminants a NEL system might be preferred. These systems are said to be more precise, and they are well-suited to tropical countries where maintenance metabolism accounts for a great proportion of total metabolism. They require little additional analytical information, and they are no more difficult to use.

For their maintenance and production, farm animals require sufficient quantities of (chemical) energy, amino acids, vitamins, and minerals at the tissue level. The size of the requirement for the first two is much larger than for the latter two; therefore, we shall only pay attention to energy and amino acids. As well, shortages of vitamins or minerals can easily be corrected by supplying the animals with an additional small amount of a vitamin and/or mineral mixture.

A high concentration in a ration of substances supplying energy and amino acids at the tissue level is not a guarantee that the animal's requirements will be met: for that purpose, sufficient quantities of the ration must be ingested. This means that attention should also be paid to the palatability and ingestibility of rations.

Rations are composed of one or more feedstuffs; thus to provide the animal with the required energy and amino acids at the tissue level we need information on the energy, protein, and ingestibility aspects of the separate feedstuffs. It is, therefore, necessary to study how to predict these values.

Before doing so, we must discuss a number of complications. Low ingestibility does not make a feedstuff useless; it only restricts its use in large quantities in rations where a high energy and/or protein intake is needed. It is clear that the ingestibility value of a feedstuff is not constant for all cases; it depends on the purpose for which the feedstuff is to be used. The same holds true for its protein value. Protein can be used by animals both as an amino acid and as an energy source. We might express this more clearly by saying that protein has an energy value as well as an amino acid value. It exerts its first task in all cases, its second only if there is need for amino acids. Also, those amino acids that at the tissue level are in excess of needs are used only as an energy source. Practice is taking account of this by demanding

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that rations contain a sufficient amount of energy as well as a sufficient amount of protein or N: the protein of the feedstuffs is evaluated for both energy and protein.

In warmer and more humid environments, ingestibility and protein aspects are further complicated. The high heat load may lower the animal's appetite, especially for those feedstuffs that result in a relatively high heat production on ingestion. In such environments, farm animals often have higher loads of intestinal and other parasites, which may also decrease their appetite and increase their amino acid needs. In ruminants, many of the rations grazed or fed are often low in N and considerably lignified. Low N levels result in slow microbial digestion in the rumen and reduced feed intake. Furthermore, more lignified feeds are eaten in smaller quantities and produce per kilogram less chemical energy at the tissue level, and per joule of energy more heat. Finally in warmer environments, feeds occasionally contain weeds that are toxic or that depress intake. Tannin levels of feeds can be high, which might result in lower efficiencies of N utilization.

Feed Intake

For monogastrics as well as ruminants, it is a general rule that energy requirement determines intake. There are many examples of high-producing animals eating more than non- or lower-producing ones. However, there are quite a number of exceptions where other factors overrule this general rule: high heat loads decrease feed intake (the high heat load can be due to the environment or to too high metabolic heat production per kilogram of ingested feed); voluminous feeds with low nutrient density cannot be eaten in great quantities; very fat animals have a lower intake due to reduced intestinal capacity, and maybe also due to metabolic feedback; and in ruminants low intakes of N, and also of S or P, may lower intake. On the other hand, feeds of high digestibility and high nutrient concentrations are often eaten in greater quantities than needed.

In ruminants, some of these feeds under certain circumstances may lower feed intake: for example, concentrate rations rich in easily fermentable carbohydrates when eaten in great quantities over short periods may upset rumen fermentation. In such cases, rumen pH may become low and considerably reduce the speed of microbial conversions. The lower emptying rate of the forestomachs, and possibly the low pH or the fermentation products per se, often lead to feed intake reduction, and in severe cases to "off-feed." Part of the reduction in forage intake after increasing the supply of concentrates is due to this phenomenon. Changes in microflora also occur.

Low or high intake of feed is disadvantageous to the farmer when it results in either not meeting the animal's requirements or in exceeding them. In the first case, production might be low and/or the animal's condition might deteriorate. The second case leads to unnecessary feed losses as the direct conversion of feed is physiologically more efficient than production via reserve tissues, i.e. first a conversion into reserve tissue followed by a utilization of these tissues. Nevertheless, for economic or feed availability reasons, it sometimes is necessary to make use of the animal's ability to deposit or utilize reserves.

N Metabolism

Essentially, N requirements in monogastrics consist of a need for essential and nonessential amino acids at the tissue level. Usually in a feed a few essential amino acids are limiting, and these determine the rate of production at a given feed intake level. Nonessential amino acids, although in theory synthesized by the animal, are needed when the deamination of the surplus nonlimiting amino acids and the sources for ammonia in the ration do not deliver sufficient ammonia for their synthesis from this ammonia and suitable N-free intermediates.

In monogastrics the amount of apparently digestible amino acids in most cases gives a good indication of the quantities of the various amino acids absorbed in the blood. Lysine is often the first limiting amino acid, and its apparent digestibility is usually equal to or slightly lower than that of crude protein. Therefore, lysine concentration and apparent digestibility of crude protein give quite a bit of information about the feed's N value. However, in monogastrics with a higher degree of microbial activity in the hindgut, as is the case in older pigs and in horses, apparent digestibility is not a good indicator of absorbed essential amino acids, especially lysine. The microbes in the hindgut modify the amino acid pattern in a way that is not yet well understood.

In ruminants, N metabolism is far more complicated because the proteins and amino acids that enter the small intestine are from undegraded feed protein, microbial protein, and endogenous protein. Most of these proteins (after hydrolysis) and amino acids are absorbed in the blood. Various systems exist for predicting the total amount of absorbed amino acids; unfortunately they differ considerably due to a lack of precise information. The pattern of absorbed amino acids seems to match the required pattern for maintenance and production fairly well. Thus, in ruminants it

is the total amount of absorbed amino acids (absorbed "protein") that is important rather than a given limiting amino acid in this "protein." Unfortunately, there is still a lack of precise quantitative information on the requirements for absorbed "protein" for maintenance and for milk production.

Energy Metabolism

From Gross Energy to Metabolizable Energy

The gross energy (GE) of feedstuffs, as well as of materials like feces, urine, milk, eggs, animal tissue, etc., can be measured with high precision with a bomb calorimeter. In fact, GE is due to the chemical constituents of these materials, especially carbohydrates, proteins, fats, etc.

Part of the feed is not digested. The degree of digestion, by the digestive enzymes of the animal and by the enzymes of the microbes it hosts in the gastrointestinal tract, depends on many factors of feed as well as of animal origin. Feed and animal factors, moreover, show interaction.

Feed factors determining the degree of digestion by animal and microbial enzymes are: species; growth conditions and way of conservation of the plants from which the feed originates; the part of the plant that is used as feed and its treatment prior to feeding; and its composition (protein, fat, carbohydrates-starch, sugar, cellulose, etc., degree of incrustation, tannins, etc.).

Animal factors include: animal species (symbiosis with microbes prior to or after the true stomach or not at all — in view of the host's lack of cellulase); rate of flow of digesta (in view of time available for digestion); and conditions for microbial activity and growth in forestomachs or hindgut.

It is nearly impossible to predict with a reasonable degree of precision the degree of digestion of a feedstuff by a given type of animal at a level of intake sufficient for maintenance when no in vivo digestibility results are available for that or a related feedstuff and for that or a related type of animal. For monogastrics, feeding level has little influence on this degree of digestion. For ruminants, it does. At a higher feeding level the rate of passage increases so that a shorter time is available for fermentation, which results in lower cellulose digestion. Moreover, the high feeding level is often achieved by feeding more concentrates that on the one hand may lead to lower rumen fluid pH and thus lower cellulose digestion, but which may also allow more undegraded starch and protein to pass on to the duodenum for digestion by the animal's enzymes. Whether or not rumen fluid pH decreases markedly and over a longer period after a meal also depends on the animal (speed of eating, ability to produce saliva to buffer microbial acid production); therefore, the effects may differ considerably.

Some endogenous substances (digestion fluids, gastrointestinal wall abrasions) mix with the feed digesta in the gastrointestinal tract during the digestion process and are not completely reabsorbed into the blood or lymph. The part lost with the feces is usually called the metabolic fecal fraction. Its size is related to the amount of ingested dry matter, but other factors also play a part. The fraction is mainly composed of protein and fat. Not all endogenous substances not reabsorbed in blood or lymph as amino acids, fats, and long-chain fatty acids are voided with the feces as the metabolic fecal fraction. Part of the endogenous material is converted into volatile fatty acids (VFA), methane (CH_4), ammonia (NH_3), CO_2, H_2O, and heat, and is for a large part absorbed in the blood in these forms. The greater the microbial activity in the large intestine the more such conversions occur. Part of the feed-digesta undergoes a similar fermentation. Thus, in animals with an intensive fermentation in the hindgut it is nearly impossible to distinguish between fecal matter originating from feed and fecal matter of metabolic origin. Furthermore in such an animal the apparently digested material contains digested feed as well as VFA, CH_4, NH_3, and heat.

In ruminants, most of the microbiological degradation of the feed, its fermentation, takes place prior to the true stomach. Fermentation in the forestomachs is usually far more active than in the hindgut because the feed contains more and easier degradable nutrients than the digesta entering the hindgut.

This fermentation also results in VFA, CH_4, NH_3, CO_2, H_2O, and heat, which are absorbed or eructated, and microbial matter. In the hindgut a second fermentation may take place, and its extent depends on the degradability for microbes of the remaining digesta. Due to the fermentation, the apparently digested matter of ruminants contains far less nonfermented digested feed and far more VFA, CH_4, and heat than in the case of those monogastric animals, which have little fermentation in the hindgut. Besides this, the apparently digested material of ruminants also contains amino acids, fat and higher fatty acids, and sometimes monosaccharides, all resulting from the digestion of microbial matter.

Some research workers prefer to work with truly rather than apparently digested matter. Physiologically they are right; the truly digested

material is the part of the feed that is actually absorbed into the blood. However, the process of digestion itself requires metabolic substances resulting in metabolic fecal losses. Apparently digested matter clearly is closer to the net result of the whole process than truly digested matter. Also for practical reasons, among others, the difficult separation of feed residues from endogenous material in the feces, especially from animals having considerable fermentation, apparent digestion has to be preferred.

Gross energy minus fecal energy gives (apparently) digested energy (DE). From the above discussion on digestion it is clear that the chemical and physical composition of DE may vary considerably. In ruminants, methane losses are 5–12% of GE, but are included in DE although worthless for the animal. So DE is sometimes corrected for methane energy. Very rarely is the DE also corrected for the energy of the heat resulting from fermentation, some 3–8% of GE, because it is impossible to measure this amount with reasonable precision. Methane energy losses in nonruminants are usually neglected. Indeed these losses are less than 1% of GE in poultry, veal calves fed only an artificial milk, and pigs up to 100 kg on rations without forages, but are up to 2% of GE in sows and up to 6% in horses.

Subtraction of both urinary energy and fecal and methane energy from GE gives metabolizable energy (ME), which equals ingested energy minus all energy losses other than heat. Urine contains detoxication products like urea, uric acid, hippuric acid, etc. In monogastrics, urinary energy loss is from 2 to 6% of GE, mainly depending on the excess of N that must be excreted. In ruminants, urinary energy loss is slightly higher, and the higher the content of forages in the ration the higher the loss. Because the N of absorbed amino acids is either deposited in products like tissue, milk, eggs, and hair or excreted as urea or uric acid with the urine, for the same amount of absorbed amino acids higher urinary energy losses due to N excretion occur when N retention and N production in milk and eggs are zero.

In monogastrics, the ME content of a ration is hardly influenced by feeding level. In ruminants, it is influenced to a measurable extent, although less than DE because at a higher rate of passage microbial cellulose digestion and microbial methane production decrease. This gives some compensation, but it is an incomplete one. When the feeding level is mainly increased by adding concentrates, how the concentrate affects the rumen fluid pH determines the magnitude of the decrease in the ME of the concentrate. There is a lack of information on this point, which is so important for high production levels.

Because of what has been said about urinary energy, somewhat higher ME values are found for the same ration at the same feeding level when N is deposited in the tissues or in milk or eggs than when all the N is excreted with the urine. For this reason, ME values are sometimes standardized by correcting them to the situation of zero N retention and production, or to a 30% retention and production of all the N (N corrected ME).

In ruminants part of the DE is heat; therefore the same holds true for ME. On average only about 90% of the ME of a ration is energy in a chemical form.

The Utilization of Metabolizable Energy

Maintenance

Animals use absorbed nutrients for maintenance and production. The maintenance process mainly needs ATP (for blood circulation, respiration, muscle tonus, some work, maintaining concentrations, transport, etc.) and a small quantity of chemical compounds to replace worn tissues and for the synthesis of the necessary enzymes and hormones. As to the latter aspect it concerns only a small net supply of the building blocks of these compounds because the degradation products of the worn tissues, enzymes, and hormones can partially be reutilized. The actual linking of the building blocks, and any conversions required prior to linking, again mainly require ATP. It is therefore fairly safe to assume that maintenance needs for energy consist of a need for ATP.

Because of the biochemical pathways used in the animal, ME consisting of glucose is the most valuable for the synthesis of ATP from ADP, fat-ME some 5% less, and amino acid-ME some 10–20% less. For monogastrics with fermentation in the hindgut, part of the carbohydrate and protein is fermented, which results in an absorption of VFA, CH_4, NH_3, and heat in the blood rather than monosaccharides and amino acids. It is clear that ME containing such fermentation products is less valuable for maintenance, especially because CH_4 production is often neglected (i.e. assumed not to be present). The energy in the methane and heat (at normal environmental temperatures) is of no value to the animal, and the VFA are about 10–20% less valuable as a source for ATP production than glucose-ME.

Poultry, due to their short gastrointestinal tract and because their diet is low in cellulose, have very little fermentation and produce hardly any methane. Nonruminating veal calves fed only liquid milk replacers also show hardly any meth-

ane production. Their ME can therefore be considered to be absorbed monosaccharides, amino acids, and fats. Pigs, especially when fed higher levels of byproducts or roughages, can show fermentation, with methane energy productions of less than 0.5% of GE for pigs weighing less than 50 kg and fed concentrated diets, and up to 2% for other diets when the pigs weigh 100 kg and more. Compared to pigs without fermentation, the ME of these animals, especially the 100-kg pigs, has a slightly lower value for maintenance.

It will be clear that information on the composition of the ME is of importance for a correct evaluation of the feeding value of feedstuffs for maintenance. In experiments with monogastrics with no or little fermentation most of the above-mentioned differences in the relative value of ME from different sources for maintenance have been demonstrated. Information with regard to monogastrics with considerable hindgut fermentation is still limited.

In ruminants with an active rumen fermentation the ME is some 10% less valuable than monogastric-ME as an ATP source because about 10% is lost as (fermentation) heat. Secondly, VFA and amino acids are absorbed from the gastrointestinal tract rather than glucose and fat, again making ruminant-ME less valuable as an ATP source than monogastric-ME. Because VFA and amino acids on a ME basis have about the same potential for ATP synthesis, one would not expect much difference in the value of ME originating from different feeds for maintenance. Nevertheless, in balance and other trials a small influence was found: the lower the metabolizability, q (equal to 100 ME/GE) the more ME was needed for the same purpose — about 0.5% more if q decreased by one unit. Part of this may be due to a shift in the composition of the absorbed VFA towards more acetic acid, which is a slightly poorer source of ATP than the other VFA's. A second consideration is the higher eating and digestion costs of the feed, which becomes more voluminous and more difficult to ingest and digest as its q value decreases.

For maintenance with regard to monogastrics, knowledge is needed of the ME-content of the feed, but it is also of value to have some knowledge of the separate ME-contributions due to glucose, fat, amino acids, and the rate of fermentation (from CH_4 production). For ruminants, knowing the ME content and q is sufficient. In both cases it should be stressed that it is the ME content that largely determines the maintenance value of the feed; whereas, the origin of the ME influences this value by only 20% or less in monogastrics, and even less in ruminants.

Production

Monogastrics and not yet Ruminating Ruminants

The utilization of ME for production not only differs with the origin of the ME but also with the product it is used for, and with the animal's species and physiological state.

Energetically, *fat production* from fat-ME has a high efficiency of utilization, about 90%. However, it cannot be accurately predicted how much of the fat-ME will be used for fat synthesis, i.e. direct incorporation, and how much will be broken down to acetyl CoA for later use in fat synthesis or in maintenance. Direct incorporation is more efficient than incorporation after prior partial breakdown. In general, there appears to be a fair preference for direct incorporation of absorbed fat into body or egg fat, but this is certainly not an absolute preference. Incorporation as such also depends on the fatty acid composition of the fat.

Fat-energy production from glucose-ME has a 75–80% efficiency, from amino acid-ME it is 10–20% less. For VFA-ME the efficiency is somewhat lower than that of glucose-ME. When VFA are present, it means that there was hindgut fermentation; thus some of the ME consists of VFA, CH_4, and heat (which we will call rest-ME) rather than glucose, amino acids, and fat. For fattening, this rest-ME is utilized 20–30% less efficiently than glucose.

These statements as to the utilization of fat-, glucose-, and amino acid-ME for fat production have been generally proven in experiments with monogastric animals. There is little experimental evidence of the efficiency of the utilization of the rest-ME in animals with hindgut fermentation. The size of rest-ME in monogastrics is usually small; furthermore, efficiencies derived above from biochemical considerations, in view of our knowledge obtained from energy utilization studies in the ruminant, will be close to the actual ones. The ME contribution of those feedstuffs that are high in cellulose, and that by monogastrics can only be digested to a large extent by hindgut fermentation, will consist mainly of rest-ME.

Protein deposition in eggs, milk (sows), and meat (chickens, pigs) needs building blocks, an amino acid mixture matching the amino acid pattern of the protein to be synthesized, and ATP to link the amino acids. Theoretically some 5 moles of ATP are needed for the peptide linkages of 100 g protein (2385 kJ). The 5 moles of ATP require about 400 kJ ME, so to produce 100 g protein some 2400 kJ ME of "building block"-

ME and 400 kJ ME as energy for linking are needed. This means that the theoretical efficiency of utilization is about 85%. Proof of this efficiency figure in experiments with animals is very difficult to obtain. In fact, all our attempts to derive this figure, even in the case of experiments specially planned for it, have left us with imprecise estimates. The cause of this is quite clear: energetically, protein deposition even in a rapidly growing animal or productive laying hen is only a small part of total energy metabolism. In growing chickens, pigs, and veal calves production metabolism changes with ages from 60 to 40% of total metabolism, while the protein energy percentage of the deposition of energy decreases from 60 to 20%. Total metabolism of a laying hen is seldom twice maintenance, while only 40% of the egg-energy is protein-energy. Thus, for deriving the actual ME quantity required for protein deposition, the ME needed for total metabolism, for maintenance, and for fat deposition must be known separately and very precisely (the quantity looked for being the difference of two or three large figures).

There is still another problem: precise maintenance estimates of young animals are lacking. Most of the data used for this purpose are derived from mature animals and are corrected for the difference in (metabolic) weight. It is well-known that young animals are more physically active than older ones, and that they are more easily excited, but it is not known to what extent this affects maintenance needs.

Regression calculations, using the model:

$$\text{total ME} = a \times RE_p + b \times RE_f + c \times W^{3/4}$$

in which REp and RE_f are retained protein and fat, $W^{3/4}$ is metabolic weight, and a, b, and c are constants, are often used to derive the efficiency of the utilization of ME for protein deposition in growing animals. Properly said, the model is incorrect for this purpose because c, related to maintenance, probably is not a constant because it changes with age. Moreover, the model is nearly always used on data for which it is not well suited: results with animals of uniform potential for protein deposition fed ad libitum or nearly so rather than results with animals showing considerable variation at all ages in protein and fat deposition due to differences in genotype and in feeding level. Even the best experiment for this purpose, that of Pullar and Webster (1977), was not completely free from bias. Nonetheless, calculations from this kind of research show a fair amount of agreement with regard to the values found for a and b: the value of b being only slightly above the one expected from biochemical considerations and experimentation with mature monogastrics. From the value of a, however, efficiencies of utilization between 50 and 70% can be derived for protein deposition, i.e. much lower than the theoretical 85%. It is not very probable that the biases of the regression are responsible for this because the low estimates of protein efficiency are so consistent. These efficiencies are therefore probably low, especially for growing animals. It has been suggested that this might be due to a higher rate of protein turnover during rapid growth. Isotope studies have indeed shown this for small animals, but unfortunately there is little evidence for large animals. As a consequence of an increase of this rate with the rate of growth, ME is needed not only for actual protein deposition but also for the higher turnover of existing protein. This is because protein synthesis requires ATP; whereas, its degradation to amino acids does not yield ATP. Such an effect could easily explain the low efficiency values. It is a great pity that we know so little about the rate of turnover of protein under various conditions, especially in farm animals. Its measurement requires isotope studies, which are not always easy to interpret; whereas, the other available technique of 3-methylhistidine excretion, which does not make use of isotopes, also has its drawbacks.

Some of the estimates of ME-requirements for *egg-protein* synthesis are not far below the theoretical estimate of 85%; some, however, are. The lack of agreement in the results of the few studies with layers is due to the above-mentioned relatively low amount of protein production and to insufficient information on the hen's maintenance needs.

Reliable studies, from which the ME required for *milk-protein* synthesis in sows may be derived, still are lacking. Sow's milk contains about 60% fat-, 25% protein-, and 15% lactose-energy. For the efficiency of *milk-fat* synthesis from ME we can use the same arguments as body-fat synthesis. The same arguments apply to milk protein, except possibly for the rate of whole body protein turnover. So far, an increased rate of whole body protein turnover has only been found during rapid growth. Clear evidence that lactation does not influence this rate of turnover is however lacking. During rapid growth an increased rate of protein synthesis can be understood to have some purpose, during lactation such a purpose, except for udder tissue, is difficult to imagine. Synthesis of *milk-lactose* from glucose is biochemically a simple process, probably requiring little additional energy. Synthesis from (glucogenic) amino acids is less efficient because it requires urea formation and excretion as well as glucogenesis. Milk-energy synthesis therefore will probably be

slightly more efficient energetically than fat synthesis from carbohydrates.

With regard to feed evaluation, the value of ME of different origins for production in nonruminating animals may be summarized as follows. The main kind of chemical energy synthesized is fat energy. This is formed most efficiently from absorbed fat, some 10–20% less efficiently from carbohydrates, and 20–30% less efficiently from amino acid- and rest-ME. Except for the fat, the relative contributions of the different kinds of ME are of the same order as for maintenance. These considerations led the research workers of the Oskar Kellner Institute in Rostock to use Net Energy Fattening (NEF), i.e. the value of a feedstuff for tissue fat deposition by a mature monogastric, as the criterion of feed evaluation for both production and maintenance. They admit, however, that feed fat does not fit completely in this theory as it is preferentially incorporated in body or egg fat with high efficiency. For a correct evaluation of the carbohydrates of the feed, NEF should not be predicted from digestible crude protein, digestible crude fat, digestible crude fibre, and digestible N-free extract. The latter two fractions do not partition the carbohydrates into valuable and less valuable sources of energy for production and maintenance. Instead, they could better be replaced by: (1) total starch plus sugar, being highly digestible; and (2) the digestible remainder, equal to digestible organic matter minus the sum of digestible protein, digestible crude fat, starch, and sugar. Some experimental evidence for this has already been found.

With regard to protein and lactose production, correct feed evaluation is more difficult. Protein synthesis, especially at high protein turnover rates, needs ATP, which is synthesized with the same efficiency as maintenance ATP from the various ME-sources. Lactose synthesis from glucose-ME is also more efficient than from protein- and especially rest-ME. Thus, the various nutrients rank in about the same order for lactose synthesis as for fat synthesis and maintenance. Therefore, the concept of the Rostock group appears well-founded. However, NEF underestimates the value of protein-ME for protein deposition: it assumes all absorbed protein to be deaminated, resulting in an energy reduction of some 20%; whereas, such an energy loss does not take place at all for the retained or deposited protein. Increasing the NEF-value for protein-ME according to the percentage of feed protein incorporated as protein in tissues, eggs, or milk seems a suitable correction.

Net energy fattening will also evaluate the feedstuffs correctly when physical work is part of production, because this, like maintenance, mainly means an ATP requirement. The same holds true for animals that must walk over long distances or are under some stress because these also usually result in a greater need for ATP.

Ruminants with Active Rumen Fermentation

The main kinds of production to be treated in this section are lactation, growth, and pregnancy. Cow's milk with 4% fat contains 50% fat-, 25% protein-, and 25% lactose-energy. Production of about 12 kg of milk requires as much ME as is needed for the maintenance of a 550-kg cow. Thus to produce 36 kg of milk such a cow has to absorb four times as much ME as for maintenance alone.

Growing cattle or sheep do not reach such high production levels; therefore, ME requirements of twice maintenance are close to the upper limit. A considerable part of the energy deposition is fat-energy, (the more so the more mature the animal and the higher its feeding level). In well-fed, early-maturing, beef cattle protein-energy deposition decreases from about 35% at 200 kg to about 15% at 500 kg, but in late-maturing breeds the decrease is much slower.

Even at the end of pregnancy, daily energy deposition (e.g. 6000 kJ for a cow) is small, but it requires, relative to other productions, an unusually large amount of ME because the efficiency of utilization is only 10–25%. Therefore, the total feed requirement near parturition is 2.0–1.5 times the ME needed for maintenance. The low efficiency suggests that the main need during pregnancy is an ATP supply, maybe because synthesis of fetal tissues is difficult and / or because the changed endocrinological state of the mother may increase her maintenance needs. Thus, with regard to feed evaluation, the value of ME from different sources, relative to each other is very probably the same for pregnancy as for maintenance.

Biochemically seen, lactose and fat synthesis in ruminants give special complications. Usually little glucose is absorbed from the gastrointestinal tract; therefore, glucose for *lactose synthesis* must be synthesized from propionic acid and glycogenic amino acids. This conversion, especially from glycogenic amino acids, requires energy because of the necessary NH_3 excretion. Only a small quantity of the ingested carbohydrates fermented in the forestomachs becomes propionic acid, so the supply of this precursor is not great. Also, the supply of the other precursor, protein, is small because the protein content of the rations is usually low; whereas, at higher protein levels, rumen microorganisms often degrade more protein to VFA and ammonia than they synthesize.

Like lactose synthesis, *fat synthesis* also requires gluconeogenesis, first a small quantity as a precursor for the necessary glycerol; and second for the NADPH supply. It still is not clear if all the NADPH is synthesized from glucose via the pentosephosphate pathway or if other pathways like an extramitochondrial NADH/NADPH exchange at the isocitrate or other steps of the citric-acid cycle also result in a substantial NADPH supply.

It is clear that especially in high-yielding dairy cattle, glucose supply for lactose and fat synthesis may be low; therefore the energetically less efficient pathway of gluconeogenesis from protein might have to be followed. On the other hand, absorption of glucose from the small intestine at high levels of intake and rapid passage rates of ingesta, may have a compensatory effect. In sheep, indeed, such changes with feeding level have often been demonstrated. There is much less evidence with cattle, however, and so far it suggests a smaller compensatory effect than in sheep.

Most results of balance trials suggest that the utilization of ME for milk production is not influenced as much by the origin of the ME as the utilization for body-fat synthesis, the main energy synthesis of growing ruminants. Within the range of the rations studied, which in the case of the dairy cow with a reasonable rate of production cannot be wide, the effect of the ME's quality (q, excess protein) on the efficiency of converting ME into milk energy is small and of the same size as for maintenance. It should be mentioned that not all studies show precisely the same effect of q on this efficiency, but in all cases it is low: at Beltsville half and at Rostock about twice the size as in Wageningen, where an increase of q by one unit improved ME utilization by 0.4%. The differences probably are not statistically significant. All studies show nearly the same small negative effect for ME resulting from protein in excess of protein needs, i.e. protein not deposited as milk or tissue protein. Dairy rations, however, seldom contain large protein excesses, so in practical feed evaluation the lower value of excess protein could be neglected.

On the other hand, many studies tend to show a much greater influence of ME quality on the efficiency of body-fat deposition. However, the evidence is still not quite clear. Although test rations with much greater variation in quality have been used than in the case of dairy cattle, the results regarding the efficiency of utilization of the ME for body-fat synthesis (k_f) are not clearcut. This is mainly caused by the fact that the maximum feeding level in beef cattle is low, not more than 2.5 times maintenance for high-quality rations and hardly above 1 times maintenance for low-quality rations. Even in the first case, accidental experimental errors decrease the precision of the measured k_f markedly, and in the second case the decrease in precision is of course enormous.

The results suggest a greater decrease in k_f when q is lowered by one unit at low levels of q than at high levels. This would agree with biochemical expectation. Rations with low q usually result in a lower percentage of propionic acid in the VFA, which might cause too little gluconeogenesis from propionic acid for NADPH synthesis and lead to less efficient production of NADPH.

During lactation there is a much greater need for glucose than during fattening; and at high milk yields, much higher feed intake levels are also needed. This is the reason why nearly all dairy rations have high q values. It appears logical that at such a q level the tendency is for k_f to be slightly lower than k_l, the efficiency of the utilization of the ME for milk-energy production. At this level, there probably is no shortage of glucose, so that milk- and body-fat synthesis will have the same efficiency. Lactose synthesis from propionic acid and milk-protein synthesis from amino acids, however, have a higher energetic efficiency than fat synthesis, thus k_l will exceed k_f. One would expect that at lower q in dairy cattle glucose shortage would occur sooner during lactation than during fattening and that k_l would decrease more markedly than k_f. As stated earlier, this appears not to be so. While trying to find an explanation for this discrepancy we have to keep in mind that it is difficult to work with such rations for dairy cattle and still maintain milk yields at a sufficient size.

Although clearly our biochemical understanding of the relationship of ME quality with k_l and with k_f is incomplete, at the present time the following procedures for feed evaluation appear fairly correct. For rations of not too extreme composition, with sufficient physical structure, and without large protein excesses, the *same* (small) effect of q on k_m and k_l may be assumed to exist (where k_m=efficiency of the utilization of ME for maintenance). This allows us to express both milk energy production and maintenance in net-energy-lactation (NEL). Because k_l averages 0.60, and taking into account the small effect of q, NEL can be computed as:

$$NEL = 0.60\ (1 + a\ (q - \bar{q})\ ME$$

in which $\bar{q} = 57$. In this equation, according to the Wageningen studies, a might be 0.004, but it

would be lower according to the Beltsville work and higher for that of Rostock. Because the equation was derived using the actual ME value found in the experiments, i.e. at the actual feeding level, ME-values corrected for feeding level have to be used rather than maintenance-ME values.

For feed evaluation for *beef cattle* the situation is more complicated. The first simplification is neglecting protein deposition, i.e. assuming that energy deposition during growth is mainly deposition of fat-energy. For animals weighing 200 kg or more, fat deposition indeed is the main energy deposition. Further, we do not have for such animals (which probably do not have increased protein turnover rates as they are no longer in their youth) any proof that ME-composition has a different effect on the efficiencies of ME-utilization for fat or protein energy deposition.

The second assumption is to use two different values for the effect of q on k_m: the first being small or even zero; the second being higher as has been found from work with mature cattle and sheep. As discussed above, our knowledge of the precise effect of q on k_f unfortunately is still limited. In this way, for any animal production level, equal to net energy for maintenance plus energy deposition divided by net energy for maintenance, the effect of q on the utilization of total ME can be derived. Such an approach clearly leads for the same ration to a greater influence of q the higher the energy retention of the animal. This means that the same ration or feedstuff may have several feeding values depending on the rate of daily gain (in lactating cattle this is not the case because the effect of q on k_l and on k_m is about the same). With the help of a computer, or of tables and graphs for a given animal production level, the correct feeding value of all feedstuffs available can be calculated to compose a least-cost ration. Most beef cattle are fattened either intensively with high quality feed or at a moderate rate on rations with much roughage. Therefore, for an easier comparison of the value of feedstuffs, the average animal production level at each of these two fattening intensities can be fixed and used to compute only two net energy values for maintenance and fattening (one applying to the high, the other to the low intensity.) This would simplify feed evaluation considerably because it permits listing of the two values for all feedstuffs. The precision gained by working with the actual animal production level (APL) rather than with one or two fixed APL's seems small compared to the large source of uncertainty due to insufficient knowledge of the effect of ration composition on k_l.

Feed Evaluation Systems for Countries with a Warm Climate

For various reasons, e.g. the high heat production of animals (especially at higher production levels of milk, meat, eggs), higher level of disease, periods with feed shortage, suboptimal management etc., production levels in countries with a warm climate are moderate to low, often even very low. As a consequence, maintenance metabolism accounts for a greater portion of an animal's total metabolism. Therefore, it seems important, while selecting suitable feed evaluation systems, to give priority to those in which the evaluation for maintenance is done as correctly as possible. Fortunately, as we have seen, feed evaluation for maintenance gives fewer problems than for production. This applies to monogastric as well as ruminating farm animals. For both groups of animals, evaluation on the basis of ME, either actually measured or predicted from digestible components or from the feedstuff's name and, possibly, composition, comes close to an evaluation for maintenance. Moreover, it is, in the case of monogastrics and lactating ruminants, also a fairly good basis for production. Thus measured or predicted ME might be a suitable basis for feed evaluation for most farm animals in countries with a warm climate.

However, in my opinion, without losing the advantage of the simplicity of such a ME system, one could do better, with regard to precision as well as to flexibility. For monogastrics we have seen that the composition of the ME, especially its protein content and its rest-carbohydrate content, influences to some degree the efficiency of utilization of the ME. The NEF equation of the Rostock group, corrected as suggested in the section on "monogastrics and not yet ruminating ruminants" takes such influences into account, while hardly increasing the necessary analytical information on the feedstuffs. Also for ruminants, the type of ME, e.g. its property q, influences its utilization for maintenance and production. So here too, a unit like NEL as described in the section on "ruminants with active rumen fermentation," even for beef cattle, would give a higher degree of precision without additional analytical work. For both groups of animals, feed evaluation in the way suggested would be more flexible, i.e. it would be easy to change one of the factors of the NEF or NEL equations slightly, when new experimental evidence made this desirable.

Even so, such energetic feeding values do not tell everything. They provide information on the energy aspect, which is very important but is not the only consideration.

In all cases, information on digestibility forms the basis of the proposed systems of energetic feed evaluation. It is this information that mainly determines the feed's value, and unfortunately for many plant products in warm countries this information is poor or imprecise. The ability to predict the digestibility of feeds is a must for animal husbandry in these countries, especially as this property also influences ingestibility.

Survey of Feed Evaluation Systems

Feed evaluation for poultry enjoys the greatest uniformity. Nearly everywhere, except in East Germany, ME is the unit on which feed evaluation is based. However, some research workers are of the opinion that the evaluation can be improved. They say that not only the amount but also the origin of the ME should be taken into account because protein-ME, fat-ME, and starch-, sugar-, and rest-carbohydrate-ME do not have the same net-energy value for the animal.

For pigs, several countries use TDN, DE-, or ME- systems, whereas the NEF-system from the Oskar Kellner Institute at Rostock is used in East Germany, and a modification of it is used in the Netherlands. Here too in those countries that use TDN, DE, or ME, attempts are being made to improve the systems for the same reasons mentioned for poultry. In the Netherlands, new research work is due to begin to try and make the present NEF system more applicable to fast-growing pigs fed rations with a greater proportion of by-products.

Until 1960 in Europe, NE systems (starch value, feed unit) were used for ruminants, while in most other parts of the world TDN was the preferred system. Two developments have changed this pattern: (1) energy metabolism was looked at in a factorial way, i.e. the utilization of the feed was studied separately for each of the various purposes like maintenance, lactation, etc.; and (2) biochemistry was used to improve the understanding of energy conversions. This led in 1965 to the new ARC system for ruminants, which was completely factorial and therefore had a very logical construction. Due to insufficient information it was weakly based as to its details and for use in practice it was too complicated. Intensive research work combined with a similar factorial and biochemical approach led in East Germany to the NEF system, at present the system practiced in that country. This system has not found much acceptance elsewhere because it is based largely on work with mature male animals. Work with dairy cattle in Beltsville and the Netherlands has led to systems, based on NEL, that have similar main principles but differ in some details. They are used to some extent in the United States and in the Netherlands, Belgium, France, and Switzerland; West Germany is to introduce a NEL system in 1980. The U.K. extension service introduced a considerably simplified NEL system in 1975. The effect of feeding level and of q on ME utilization for maintenance and lactation was neglected and k_l was put at 0.60; in other words, all ME was assumed to have the same NEL irrespective of origin. It is being used in practice to a considerable extent. Also, the 1965 ARC system for beef cattle was given a more practicable form and introduced with some success in the U.K. by the extension service. Similar systems, even more simplified, were recently introduced into practice in France, Switzerland, and the Netherlands.

Problems of Standardization of Units to Describe the Energy Value of Feedstuffs

P. W. Moe[1]

The most important considerations in selecting units to describe the energy values of individual feeds are whether they are reproducible and whether they account for the major proportion of variation in energy value. Digestible (DE) or metabolizable energy (ME) values as measured at maintenance intake are the most suitable units for compiling data on a large number of feeds from many sources. Additional data on chemical composition are needed to estimate efficiency of use of DE or ME in the producing animal and to estimate change in DE and ME value with change in intake level.

Net energy (NE) values may be derived from tabulated DE, ME, and/or chemical composition for specified feeding conditions. NE values derived in this manner are useful in applied feeding systems, but are not suitable for compilation in large data banks.

Nearly all energy units are based on digestible energy (DE), metabolizable energy (ME), or some form of net energy (NE). These terms are thoroughly discussed in the preceding paper by van Es (1979); therefore, I will only comment on the use of these energy units to develop specific recommendations for feeding animals in practice.

Digestible Energy

Because only the measurement of energy intake and feces energy is required, DE has been measured for many feeds, usually at maintenance intake. Measurement of DE alone accounts for a very large proportion of the variation in efficiency of use of ingested feeds. Limitations to the use of DE include variation in DE value with plane of nutrition and variation in use of DE from different diets.

Metabolizable Energy

Measurement of ME requires the measurement of energy losses in feces, urine, and methane. Because methane is a gaseous loss, its measurement requires relatively expensive apparatus. Therefore, most tabulated ME values have been calculated from DE using an estimate of methane loss. Directly measured ME values have been effectively used in research to characterize energy requirements and efficiency of energy use. Relatively few data, however, are available from directly measured ME values of a large number of feeds. Limitations to use of ME include variation in ME value with plane of nutrition and variation in use of ME from different diets. The latter is of considerably greater importance with growing than with lactating ruminants.

Net Energy

Net energy is defined as the energy recovered in the animal product. NE systems or variations in the form of feed-unit systems have become quite popular because of the relative certainty with which NE requirements for production can be described. NE values of feeds are easy to describe in theoretical terms but very difficult to measure. Measurement of NE whether by calorimetry or slaughter balance is generally by one of three methods: (1) by difference between measurements at two levels of production; (2) by difference between a measured point and another point that is an assumed value chosen to represent either maintenance or fasting; and (3) by regression analysis of measurements at several levels of production. By any of these methods, the magnitude of NE values for production is greatly influenced by the magnitude of the maintenance values that are either estimated or assumed. Correct use of NE values, therefore, requires an exact description of all of the assumptions involved in their derivation.

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Source of Data on Feeds

All of the information obtained about a specific feed falls into two distinct categories. The first consists of direct determinations by chemical analyses. These analyses are repeatable measurements of specific components or classes of components by rigidly defined methodology. Determination of the amount of specific minerals, amino acids, total nitrogen, cell walls, lignin, ash, etc., are examples. Procedures, of course, must be adequately defined or referenced to ensure uniformity of procedures, but in general, the measurements are repeatable for a particular sample material. The important point is that the results are influenced only by the sample material itself and not by how it is used or incorporated into animal diets.

The second category of measurement is biological in nature and is intended to provide a measure of how well a particular feed is used by animals. This introduces a totally new source of error in the measurements, i.e. biological variation. Measurements of digestible, metabolizable, or net energy values are such measurements. These measurements, while influenced by the nature of the test feed, are also influenced by normal biological variation of animals, the design of the experiment, and the interpretation of the data. The measurement of net energy value, for example, includes a sizeable element of "interpretation."

Application of Feed Composition Data

A second consideration in the suitability of attributes of feeds is the question of how the information will be used. I will identify two extremes, although a number of intermediate applications also exist.

The first extreme is the feed composition data bank, especially that maintained on an international basis. The objective of the data bank is to provide a very accessible source of valuable information on a wide variety of feeds. Because the data are used for a wide variety of situations in widely varying geographic locations with differing climatic and management factors, it is essential that the data be as objective as possible. Data derived chemically certainly fit this requirement; data derived biologically, however, are more subjective in nature because they may be influenced by management or interpretation. Some biological measure is necessary, however, as chemical measures alone do not adequately describe the potential biologically available energy in a feedstuff. The question which arises then is: which of the biological measures are suitable for incorporation in data banks? Digestibility as measured at a maintenance intake is undoubtedly the most effective measure for this purpose because it directly accounts for the largest source of variation in feed value. Digestibility is also a useful predictor of efficiency of use of digested energy and is therefore useful for estimating ME or NE values in feeding systems. It is my belief that net energy values are not suited to compilation in data banks because they cannot be measured independent of some assumptions. The assumptions may refer to net energy values of some diet components or net energy requirements for maintenance, for production, or for both. Net energy value may be derived by difference, which may yield a totally different value related to the last increment of feed consumed.

At the opposite end of the spectrum from data banks is the use of information about feeds in the development of practical feeding recommendations. Feeding recommendations to be useful must be as simple as possible for convenience but not so simplified that important sources of variation are ignored. The scope of coverage becomes an important consideration. In the United States, for example, the National Research Council prepares a series of publications on the nutrient requirements of each species of animal and poultry. These are intended for use throughout the country, and are therefore generally broad in application. Each individual state in the United States has extension personnel who are more familiar with specific feeding situations and problems in their geographic location. These groups, as well as private feed companies or nutrition consultants, frequently develop more specific recommendations for their own use. Requirements may be adjusted to include local environmental effects, or the composition and energy value of important feeds may be adjusted for specific management situations or local climatic effects.

For immediate application at the farm level, simplicity is often a very important factor in gaining acceptance of feeding systems. Although the increasing sophistication of managers at the farm level decreases the need for simplicity, it remains an important factor. In this situation, net energy systems have proven very useful. The success of a particular system depends, however, not on whether it is a net energy system or some other system, but whether or not the important variables have been adequately built into the system to account for animal performance. In net energy systems, the burden falls most heavily on the values selected to represent the net energy value of individual feeds. The description of require-

ments is relatively easy. The description of energy values of feeds is much more difficult.

Feeding Systems

Feeding systems introduced in recent years include an ME system in Great Britain (ARC 1965, MAFF 1977) and NE systems in the United States (NRC 1978), the Netherlands (van Es 1978), France (Vermorel 1978), and Switzerland (Bikel and Landis 1978). These are only examples of many others that have appeared recently. All of these systems have in common the consideration of recent knowledge on the amount of energy required by ruminants for specific physiological functions and the variation that occurs in the ME value of feeds and the efficiency with which ME is used by the animal. Several of the new NE systems use a form of "feed unit" to express the requirements and the energy value of feeds. At first glance, it may appear that these systems revert back to the old feed unit systems in which productive values were directly measured against a standard feed. There is a major difference in the newer systems, however, which should not be overlooked. All of these systems, including those using "feed unit" terminology have defined the feed unit in terms of energy contained in the product formed. They are thus defined in precise energy terms on an absolute basis and not simply on a relative basis in comparison with the reference standard feed. The system described by van Es (1978), for example, defines one "feed-unit-lactation" (VEM) as 1.650 kcal NE_l. Similarly, Vermorel (1978) defines one "unite fourragere lait" (UFL) as 1.730 Mcal NE_l. The systems adopted in the United States and Switzerland use NE_l directly instead of feed units and are expressed as megajoules (Bickel and Landis 1978) or megacalories (NRC 1978). Thus, all of these systems express energy requirements either directly as NE_l or are defined in terms of NE_l.

Differences Among Energy Systems

Although much of the current discussion is about the differences among units for expressing the energy value of feeds and the energy requirements of cattle, a more pertinent consideration in judging the relative worth of one system against another is the manner and extent to which important variables are identified and incorporated either in the establishment of energy requirements or in setting the energy values of individual feeds. In discussions of feeding systems among scientists in the field, there is usually universal agreement on the major factors that can influence the use of energy by animals. There is less agreement on the question of how important each factor is. This is explained in part by personal preference for systems that are extremely broad in application and therefore account for all known sources of variation, or a preference for a system that is narrowly defined for a specific situation. Differences of opinion and preferences have resulted in substantial differences in the way various net energy systems are used. The fact that differences exist does not mean that one system is necessarily better than another. It does mean, however, that values from one system may not be interchangeable with values from another system. It is this point that forms the basis of my concern that net energy values not be tabulated in a central data bank and treated as though they were feed attributes in the same sense that the content of crude protein or cell walls or digestibility is used to identify the attributes of a specific feed.

The following are known to influence the amount of feed required to promote a unit of measurable production such as milk production or body weight gain: (1) reduction in DE value of diet at high intake; (2) reduction in ME value of diet at high intake; (3) variation in efficiency of ME for production; (4) change in distribution of energy between milk and body fat; (5) change in milk composition; (6) change in caloric value of weight gain; and (7) change in ratio between protein and fat in body gain.

All of these effects contribute variation that must be accounted for in either energy requirements or in feed values. Regardless of the energy unit used (DE, ME, or NE) it is possible to incorporate these effects into either the listing of requirements or the energy value of specific feeds. With NE systems, essentially all of these effects need to be incorporated into the NE value of feeds for a specific application. With ME systems, many of these can be incorporated into the listing of requirements. Discussions of the superiority of one system versus another are, therefore, less productive than discussions of ways to improve each of the systems.

Before all of these effects can be used effectively, additional information is needed. With dairy cattle, we cannot yet predict very accurately the rate at which the ME value of feeds declines at high intakes. With growing cattle, clarification is needed of the interactions among weight gain, body composition, and efficiency of ME used for body gain. Research now in progress at many locations will be useful in resolving these problems.

Recommendations:

1. The most useful, informative, and least likely to be misused measure of the biological availability of energy for ruminants is the digestibility of that feed by sheep at a maintenance intake. For very low quality forages and for corn or sorghum grains that have not been ground or steam processed, separate determinations are needed for cattle or buffaloes.
2. Additional chemically derived attributes are needed to allow prediction of ME, rate of change of ME with increased intake, and efficiency with which ME is used for a specific productive process.
3. The feed attributes described above may be effectively used to develop NE values of feeds for use in an applied feeding situation. NE values so derived, however, should not be accumulated in massive data banks and treated as fixed attributes of those feeds.

Application of Practical Rationing Systems

G. Alderman[1]

Consideration is given to the problems and questions practical rationing systems must handle. The need for sound data on both feed and animal requirements is stressed, and the importance of voluntary food intake in pastoral production systems is emphasized.

The interrelationships between various indices of food energy supply to ruminants are reviewed and attention is drawn to the dominant role of digestibility measurements in explaining variations in nutritive value. The utility of fodder units for developing countries is considered.

A number of factors requiring consideration when developing a practical rationing system are listed: (1) nature of livestock system involved; (2) degree of control over feed inputs; (3) voluntary intake of feeds by livestock in the system; (4) degree of knowledge of personnel running livestock systems; (5) ease of measurement or prediction of chosen unit; (6) energy requirements of native livestock; and (7) required accuracy of the system. These factors are considered in relation to the specific problems of developing countries.

The use of chemical analysis to predict nutritive value of feeds is reviewed. The use of the Van Soest fibre analysis scheme is recommended, and the possibility of combining this with the use of cellulase enzyme is examined. The reliability of the in vitro digestibility technique of Tilley and Terry is acknowledged, but the special facilities required to maintain this technique at a laboratory may limit its application.

It is useful to determine what questions and problems a practical rationing system is expected to answer. In the author's experience the questions often take the generalized format of the following: (1) Why are the animals not performing well under the feeding system and management on the farm? (2) How can the animals' performance be improved? (3) What is the value (nutritional and/or economic) of this feed? As the farmer develops his experience and understanding, additional questions can be added: (4) What ration should be fed to achieve a particular level of animal production? (5) Can the ration be made cheaper to increase the profitability of the enterprise?

To answer such questions, a professional nutritionist or livestock adviser requires three types of information:

Information about feeds — Three basic parameters are required: dry matter content; energy content in a standard unit; and the crude protein content. Information about major and trace elements need only be checked for an indication of marked deficiencies such as phosphorus, sodium, copper, etc. Most of the information required can be obtained either from standard tables of feed composition or by chemical analysis of a sample of the feed in question. Chemical analyses, however, cannot give a measure of energy content directly, and yet this is commonly the most important parameter to consider.

Information about animal requirements — A matching set of data in the same or relevant units is needed for the breed, sex, age, weight, and performance level of the livestock concerned, together with climatic effects (temperature, humidity, wind, rain/snow) where these are relevant. Where these effects are small they can often be ignored in the light of the overall errors of the situation under study. Energy requirements of livestock are now well documented for much of the developed world, but are rather less well known for developing countries.

Voluntary feed intake — Too little attention has been paid to voluntary intake of the feed by the animal. In many pastoral situations the feed availability and the stocking density imposed by the farmer effectively impose a limit on feed intake, but measurements of intakes under such conditions present considerable difficulties. When livestock are housed, feed availability and duration, stocking density, trough space, etc. can also impose limitations on intake.

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Even when management limitations are not restrictive, particular feeds have their own intrinsic voluntary feed intakes. The effects of chemical composition and physical form upon intake are now well understood, although explanations of the effect of ensilage upon intake are not entirely satisfactory.

A Model Relating Animal Requirements to Feed Data

The simplest form of model is a set of linear equations of the form $y = ax$, where: y = amount of nutrient supplied; a = weight of feed concerned; and x = nutritive value per unit weight of feed. Such a computation is executed for each feed and each nutrient value being considered, summed, and adjusted so that $\Sigma y \nless z$, where z is the nutrient requirement of the animal.

Many existing feeding systems use such a system, particularly for major and trace elements, but more complex computations, particularly for energy and protein, have been embodied in recently proposed feeding systems (ARC 1965; Bickel and Landis 1978; Blaxter 1974; DLVB 1971; Graham et al. 1976; INRA 1978, p. 69; MAFF 1976; NRC 1970; van Es 1978). These complexities arise from several factors. Plane of nutrition correction factors are involved in ARC (1965) and also in the proposals of Blaxter (1974). Net energy calculations for growing animals are solved by difference approaches in INRA (1978), MAFF (1976), NRC (1970), and van Es (1978). This is dealt with later in the section on net energies of feeds.

In the case of protein requirements, the French proposals (INRA 1978) and ARC proposals (Roy et al. 1977), while introducing more complex concepts than digestible crude protein, retain the usual linear rules for ration formulation, but with extra constraints applied with respect to deficiencies or surpluses arising in the calculations.

Computer programs have also been compiled (Graham et al. 1976) that handle both energy and protein in a much more complex fashion, and are not confined to linear relationships. Although these may come into use in countries with technically advanced livestock industries and their supporting nutritionists, it is suggested that these developments have little immediate relevance to the problems of developing countries.

Energy Measurements on Animals and Feeds

The basal unit of an energy system should describe the nutritive potential of feeds as extreme as cereal straw or maize grain in a wide range of diets fed to cattle or sheep from submaintenance to levels of four times maintenance. Substitution of one food by another in the ratio indicated by this unit should result in little change in animal performance. Using this unit as a building block, the practical feeding system must enable the nutritionist or livestock farmer to: formulate diets to give specified levels of performance; predict animal performance from a specified energy intake; and indicate relative values of feeds in different situations. An important property of an energy unit is that it should be additive, i.e. the energy supplied by a diet should be the sum of the energy values assigned to the components of the diet. Energy measures in feeds occur in a well understood and structured sequence: gross energy or energy value (CE or EV); digested energy (DE); metabolizable energy (ME); net energy (NE). Various ratios are also used: DE/GE = digestibility of energy; ME/GE = metabolizability of energy (q); and NE/ME = efficiency of utilization of ME (k).

The work of Blaxter (1974) and Moe and Tyrell (1974) has shown that the values for the various efficiencies, k_m, k_f, and k_l can be calculated from either a knowledge of ME/GE, the closely related term M/D, or particular chemical components such as crude fibre. Processing of feeds by grinding and/or pelleting can influence k_f values (Blaxter 1974), but complete diets for ruminants are not commonly fed in this form, so that the effects are diluted, depending on level of inclusion in the diet. How then can values for GE, DE, and ME be easily measured or predicted?

Gross Energy, Energy Value

The determination of the gross energy value of a feed, although fairly straightforward with modern adiabatic calorimeters, is a skilled operation if reliable results are to be achieved. It is fortunate that given the results of a Weende proximate analysis, GE can be predicted with reasonable accuracy, using the equation published by the Rostock group (DLVB 1971):

$$GE(MJ/kgDM) = 0.0226CP + 0.0407EE + 0.0192CF + 0.0177NFE \quad (RSD \pm 0.2)$$

where: CP is crude protein; EE is ether extract; CF is crude fibre; and NFE is N-free extract (with all values expressed as g/kg DM).

The size of the coefficients indicates that the major influences upon the GE value are crude protein and ether extract contents; whereas, variations in the proportions of crude fibre and NFE produce smaller effects. It should not be overlooked that total ash, although not in the equation, has an effect on the magnitude of NFE

because the latter is calculated by difference from the total organic matter content.

The gross energy values of many common forages do not vary widely (mean value 18.5 MJ/kg DM) because ether extract and total ash show little variation, and protein levels (particularly for grasses) do not show great variation either. In many practical feeding situations it is reasonable to assume a mean value for GE, unless the results of chemical analyses reveal high values for protein, ether extract, or total ash. If the latter is detected, the Rostock equation can be used with confidence to calculate an estimated GE for the forage.

In the light of alternative schemes of forage analysis (Van Soest and Wine 1967), it would be useful to derive a comparable prediction equation based on protein, lipids, ash, and cell walls.

Digested Energy

When measured in vivo using wether sheep, measurement of the GE of both feces and feed are required. Because of the difficulties of GE measurement, a simpler form of trial is often run, which determines the digestibility of the dry matter, or better, digestibility of the organic matter, by merely measuring ash content of feed and feces. Digestibility is subject to marked plane of nutrition effects; therefore, the convention has been established of making all measurements at maintenance to reduce errors.

Digestibility is a concept that farmers can easily visualize because they are only too familiar with the quantities of forage eaten and feces voided by animals in their care. Expressed in percentage terms, the scale of values is easily learned and readily understood. Unfortunately, three different ways of calculating digestibility exist: digestible dry matter (DMD); digestibility of the organic matter (OMD); and digestible organic matter in dry matter (DOMD). These three ways can differ in magnitude by up to 7 units. U.K. extension services have standardized on DOMD, because this is the only parameter that can be applied directly to kilograms of dry matter eaten to give kilograms of DOM consumed. The latter value can be converted to digested energy reasonably accurately by assuming 1 kg DOM to yield 19 MJ of DE. Terry et al. (1974) showed that increased precision is achieved by using the crude protein content of the forage:

GE of DOM = 0.0124 CP + 17.3 (MJ/kg DM)

A number of chemical and microbiological analytical techniques have been established that give high degrees of correlation with in vivo digestibility measurements. These are dealt with later. It is sufficient to note here the wide range of proposed techniques available, most of which were designed to predict the digestibility of either the organic matter or energy of forages. Digestibility coefficients can vary from 0.35 to 0.9; therefore, this parameter is of paramount importance in defining the nutritive value of livestock feeds.

Metabolizable Energy

In addition to the measurement of fecal losses, methane and urine losses now have to be included. Measurement of the latter can be achieved with some additional effort, but methane measurements require sophisticated gas measuring equipment and some type of respiration chamber, which rules it out as a routine method for widespread use. Methane losses can be calculated from the chemical composition of the feed (DAFS 1976), but an alternative approach is to use the observations of Armstrong (1964) that ME/DE averages 0.81. Work by Wainman (DAFS 1976) at the Rowett Feed Evaluation Unit has confirmed the validity of this mean value for common forages and cereals. More importantly, the same centre found that out of 160 test diets examined for ME content, only in three cases was there evidence of nonlinearity of substitution between feeds on the basis of their ME content.

Digestible organic matter, digested energy, and metabolizable energy can therefore be related reasonably accurately to one another for general purposes:

$$\text{DOMD\%} \times 0.19 = \text{DE (MJ/kgDM)}$$

and because ME = 0.81 DE

$$\text{DOMD\%} \times 0.15 = \text{ME (MJ/kgDM)}$$

Therefore measurement or prediction of digestibility will facilitate the calculation of these related parameters. The choice of parameter for use in the feeding system can therefore be based on other considerations if desired.

Net Energy

Older feeding systems such as Starch Equivalent and the related Fodder Unit systems were essentially net energy for fattening systems, i.e. based on the function:

$$\text{ME} \times k_f = \text{NE}_f$$

where k_f was calculated from crude fibre content. There are in fact normally four net energies, each calculated by the use of an appropriate k factor for maintenance, pregnancy, lactation, and gain. Three of these efficiency factors have been shown to be correlated with q, (ME/GE) (Blaxter 1974; van Es 1975):

$$k_m = 0.30\ q + 54.6$$
$$k_f = 0.81\ q + 33.0$$
$$k_l = 0.24\ q + 46.3$$

The widely differing coefficients for q show the impossibility of relating NE_f measurements in any simple fashion to NE_m and NE_l and perhaps explain the replacement of the SE and related systems by those that take these different functions into account. On the other hand, a net energy for lactation system, van Es (1978), can be used to measure requirements for maintenance with considerable precision, given appropriate adjustments of requirements for maintenance. A common net energy value for growing animals is not possible, and approaches have used two net energies NE_m and NE_g (NRC 1970), a range of NE_{mp} values calculated for each animal production level (MAFF 1976), or two selected NE_{mp} values for low and high rates of gain (INRA 1978; van Es 1978; Bickel and Landis 1978).

Fodder Units

Net energies expressed in appropriate scientific units (calories or joules) are not easy concepts to teach to nonscientifically trained personnel and other ways of expressing the relative net energy values of feeds have been sought. Kellner's starch equivalent is the best known example, expressing the NE_f of foods as a ratio of 100 parts of pure starch. A similar approach, the fodder unit, using 100 parts of barley (or oats) as the base unit is in widespread use in Europe. Revised fodder unit systems using the latest net energy values are being introduced in Holland (van Es 1978) and France (INRA 1978). These use three net energies, NE_l, and NE_{mp} at two levels of liveweight gain as the base unit, and retain barley as the reference feed. The amount of food required to replace another for a particular food is therefore easy to calculate and the units used can be those used locally for weight.

The derivation of a fodder unit value for a feed from a simple digestibility trial requires a number of calculation steps. If these are strictly laid down with no room for subjective selection of coefficients in the process, then nutritionists and advisers should be able to derive their own values. The weakness of the Kellner system, which led to a great deal of confusion, was the existence of correction factors that had to be selected by the worker. Discount factors (Van Soest 1973) appear to run exactly the same risk.

A weakness of fodder unit systems is that, because they are based on fresh weights of feed, the calculation of rations can be executed without reference to the dry matter content of the feed and hence the total dry matter intake that consumption of the ration will involve. This can easily lead to the formulation of rations or to calculations of pasture intakes that cannot be consumed, and hence to disappointing animal performance when tested by experiment.

In summary, for the highest precision in expressing relative values of feeds, net energy values are preferred, but are difficult to measure directly. Digestibility and digested energy are easy to measure, but do not define relative feed values precisely, particularly for growing cattle and sheep. Calculation of net energy values from digestibility measurements can be made with a degree of precision that is superior to those predicted from chemical composition.

Considerations Relevant to the Formulation of Feeding Systems

A number of factors, which interact with the choice of units for energy discussed above, come into play when considering how best to construct practical rationing systems for particular countries.

Nature of Livestock System Involved

Depending on climate, soil type, and crops, the ruminant livestock system may be completely pastoral, or only have a short winter period when cattle and sheep must be fed on stored feeds. Such systems are widespread in tropical or semitropical countries. In addition, in contrast to many Western world situations, unpredictable and severe droughts can occur. In pastoral situations, knowledge of the relative food value of the forage on offer may be of secondary importance to information about the voluntary intake of the forage under the conditions imposed.

The development of intensive livestock units, particularly for dairying, but also for beef and lamb production, is also taking place in developing countries and these require all the knowledge and skills developed in the West for their successful establishment and maintenance. Trained personnel can be regarded as vital to their success.

Degree of Control over Feed Inputs

In a completely pastoral situation, control over feed inputs is difficult and often achieved second hand. Without some monitoring of animal performance, there is a lack of sensitive feedback to allow for adjustments of stocking density or the timely introduction of supplements.

Under intensive conditions, where feeding is normally ad libitum and may be on a group basis, good data about the food intake of a group are

not commonly available because the false assumption is often made that ad libitum feeding means that the animals' requirements are being met. A high degree of control over feed inputs can be exercised if desired, but to operate this efficiently, knowledge of voluntary intake of forages under the conditions imposed, and of substitution rates with other foods such as cereals and by-products, is necessary.

Voluntary Intake of Feeds

Depending on the livestock system involved, the achieved voluntary intake of a forage or mixed ration may be the dominant factor controlling animal performance, rather than the energy value per unit weight of feed offered. The information required falls into two main categories: (1) voluntary intake under stall feeding conditions, which can be regarded as the normal maximum achievable and has been well documented by the French workers (INRA 1978) in their 'fill unit' system; and (2) voluntary intake under the grazing system imposed, which is characterized chiefly by the crop available per unit area and the stocking density (Baker and le Du 1977). Under extensive situations, calculations that the feed available per head per day are adequate, may be overridden by the low bite size achieved, and the number of bites actually made in 24 h (Shacon and Stobbs 1976). Nature and amount of the crop on offer may also reduce bite size and number of bites (Stobbs 1973a, b).

Under grazing conditions of an extensive nature, and particularly under drought conditions, the use of feed supplements is not additive because the animals relax their grazing efforts, and the recorded improvements in animal performance are smaller than expected.

Degree of Knowledge of Personnel

If the livestock farmer is untrained in livestock production other than on the basis of experience in feeding and handling stock, then the basal knowledge available is limited by familiarity with the feeds grown, or which can be purchased, and the weight units commonly in use. In this situation the fodder unit approach has obvious attractions, as it describes relative food values in terms of a common food in use by the farmer. However, the base unit chosen, and the methods of calculating relative values, might need to be altered depending on circumstances.

It is easy to teach the concept of digestibility because it is easily visualized by livestock farmers. A scale of relative values based upon digestibility would meet a great many of their needs, particularly as considerable precision is not called for. Whether the base unit will be maize, sorghum, rice, or a common forage is a matter for local choice and decision, which will depend on the crops and feeds in the country concerned. If a degree of scientific training in animal production can be envisaged, particularly on experimental farms or on intensive units, then a wider range of options and units can be entertained. The need to measure the feed value of locally grown crops using simple digestibility trials may also be a possibility.

It is very important to identify the problems confronting the farmer and to choose a system that facilitates the giving of answers in familiar and immediately usable terms. The best unit might be less than the most precise or scientifically pleasing.

Ease of Measurement or Prediction of Chosen Unit

While tables of feed composition are now international in their scope because of the use of computer data banks under the aegis of INFIC (Harris and Kearl 1977), the rate of introduction of new crops and hybrids by plant breeders calls for a continual reassessment of the basic feed values of new introductions. Digestibility trials carried out in vivo call for less sophisticated facilities than do in vitro techniques because the latter require rumen fistulated animals to be maintained in good health.

Chemical analysis, as a method of predicting feed value, calls for much simpler facilities, but the prerequisite is that appropriate regression equations developed on a data base of sufficient in vivo measurements on the relative feeds already exist, or can be developed fairly rapidly at a centre in the country concerned.

Energy Requirements of Native Livestock

To operate a feeding system in a developing country, one of the major needs that may be most difficult to meet is the obtaining of reasonable estimates of the energy needs of the livestock on the farms (or due to be introduced) under the prevailing climatic and housing conditions. The energy costs of grazing at low stocking densities and in drought conditions, and the effects of under feeding on weight loss and subsequent repletion may be needed. Direct measurement of these parameters normally requires sophisticated facilities, or extrapolation from other breeds in other situations. Weaknesses in this area can easily bring a system into disrepute, despite accurate work on feed evaluation. Nevertheless controlled

feeding experiments using feeds of determined feed value can give reasonable estimates of maintenance needs and of liveweight gain. The net energy of milk is easily calculated from its chemical composition, and pregnancy needs are normally related to size of offspring at birth. Work of this nature, however, calls for trained nutritionists with well-equipped feeding trial facilities.

Required Accuracy of the System

The prime requirement of a feeding system is that recommendations derived from its use should be realistic in practice. Further, subsequent changes in a feeding regime should turn out as predicted. The use of a safety factor in recommendations has the distinct advantage of increasing the number of favourable outcomes given the random variation in the estimates of feed value and energy requirements. The accuracy required is dependent on the extent to which there is any recording of animal performance, i.e. liveweights at intervals, or milk recording. Under the high degree of control exercised in intensive livestock units, greater precision may be called for and a safety factor discarded as, overall, it only raises feed costs.

In general, an overall accuracy of ±10% on the relevant production parameter can be regarded as quite satisfactory, e.g. 20 ± 2 kg milk/day or 1.0 ± 0.1 kg/day liveweight gain. This represents an accuracy of better than ±5% on total energy input. Digestibility determinations at maintenance (using four wether sheep) can be run to an accuracy of ±2%, but predictions of digestibility from chemical analysis usually have coefficients of variation of 5–10%, depending on the complexity of the technique used.

The calculated performance of a ration composed of several foods contains errors from many sources, both feed and animal, but given random occurrence, some errors may cancel out, so that the final outcome is often better than might have been expected.

Specific Problems of Developing Countries

The first problems to be solved are quite fundamental: (1) Why is a practical feeding system needed? and (2) Who will be using it? The answers to the first question can range from: (a) to plan the national economy in the livestock area; (b) to develop new systems of livestock production in the country; or (c) to improve existing systems of livestock production. These answers in themselves determine the answers to the second question: if (a) then a few expert nutritionists in the capital city will be using the system, working one hopes with economists; if (b) then teams of animal production and nutritional scientists operating at well-equipped experimental centres are necessary; and if (c) then the existing corps of livestock farmers are the target, and this carries with it the presumption that there is, or will be, an extension service with trained personnel capable of using the feeding system to obtain improvements. In a developing country, some or all of these objectives may exist, and a feeding system that will satisfy all is difficult to formulate.

The next problems are really stocktaking ones: (1) What data on the nutritive value and intake characteristics of local crops and feeds are available? (2) What data on the feed requirements of local breeds of livestock are available? and these lead on to (3) How can the existing data be supplemented and improved? To sum up this far, there is a need for a range of skills in animal production personnel and suitable experimental facilities to act as a base for production of new data on indigenous feeds and livestock.

Given the existence of at least a nucleus of trained personnel then a basal requirement is to record and document existing systems of production, identify crops and feeds in use, and use chemical analysis to obtain their main characteristics. The existing INFIC data banks should be used to confirm likely nutritive values available from measurements made elsewhere.

In predominantly pastoral situations, the chief role of a feeding system may in fact be to back calculate the likely feed intake under the conditions imposed. Because measurements of nutritive value of the forage should be possible, and the level of animal production can be recorded, likely energy intake can be indicated. The interpretation of grazing supplementation experiments is much improved by the use of this technique, as the interaction of supplements on voluntary intake of forage should be made fairly clear.

The Use of Chemical Analysis to Predict Nutritive Value of Feeds

The major variation in the energy values of feeds undoubtedly occurs in the digestible energy value. Variations in gross energy, urine and methane losses, and ultimate net energy value are much smaller in magnitude, and as has been argued earlier, fairly strict relationships have been defined relating these to chemical composition or to the digestible energy measurement itself.

The ability to predict digestibility of feeds is therefore the major objective of much research work. The use of single chemical estimates (such

as acid detergent fibre, ADF) can achieve a reasonable degree of precision, but variation in both the amount and degree of lignification of the cell walls in various feeds and forages means that similar numerical values for ADF do not represent similar levels of digestibility of cell walls.

For this reason the approach of Van Soest (1969) in attempting to predict the digestibility of cell walls from lignin measurements had much to commend it, but the increased precision obtained was disappointing when applied within a particular population of forages, such as grass hay. The use of the cellulase enzyme technique (Hartley et al. 1974) to study the digestibility of cell walls as such, unobscured by the digestibility of cell contents, seems very promising and has the advantage of being a purely chemical procedure, although a two-stage one. There is the hope that this approach might be capable of predicting the digestibility of an unknown forage with reasonable precision, without the need for the establishment of individual regression equations. Satisfactorily high degrees of correlation have also been found between cellulase digested organic matter and in vivo digestibility by introducing a preliminary pepsin digestion stage (Jones and Haywood 1975). Simpler chemical techniques such as acid detergent fibre (ADF) and a modification of it, MADF (Clancy and Wilson 1966) have been adopted (MAFF 1976) for use in extension work in animal production in the U.K.

The in vitro digestibility technique itself (Tilley and Terry 1963) can hardly be called an analytical fraction measurement, but it has shown itself capable of giving accurate values on a wide range of feeds from rice bran and straws (both untreated and alkali-treated) to the common cereal sources, provided standard samples are run in each batch. This sort of facility can be maintained at a research facility inquiring into the use of new feeds, but because of the need for fistulated animals, is not capable of operation routinely in feed evaluation laboratories. However, the technique of den Braver (1977) who uses freeze-dried rumen liquor might be worth further consideration. Nevertheless, large volumes of data have now been collected on the digestibility of feeds using this technique. Care is needed in using the data because of the unit confusion referred to, and because standard samples have not always been used to calibrate the method.

Because another aspect of nutritive value, voluntary intake, can also be predicted from measurements of cell wall content by the neutral detergent fibre technique (NDF) (originated by Van Soest 1965), this parameter now plays a central role in feed evaluation. Variations in protein, oil, and ash content can all affect the gross energy of the feed organic matter, but the amount that will be digested is influenced very largely by the amount and nature of the cell walls in which they are contained, and this can be estimated by using the NDF technique followed by a cellulase assay of the cell wall preparation produced. This technique has already been used on compound feeds in Denmark (Israelsen et al. 1978) with considerable success, although only related to corrected in vitro digestibility measurements and not to in vivo results.

Recommendations

1. The major descriptive unit used in evaluating forages and feeds in developing countries should be digestibility expressed either as % digestible organic matter in dry matter (DOMD %) or digestibility of the gross energy (DE/GE).
2. A fodder unit system, using the common cereal grain of the country as the base unit, and using local units of weight, should be calculated, using digestible energy to establish relativities.
3. Energy requirements of native livestock should be estimated (or approximated) either by extrapolation from data on other livestock in other countries, or from feeding trials using feeds of determined digestibility.
4. Forages and feeds native to the country should be identified, subjected to extensive chemical analysis, and checked against data available from INFIC centres.
5. The Van Soest (1967) scheme of forage analysis, combined with cellulase enzyme assays, offers the best and most logical method of describing important characteristics of feeds of all types. Crude protein, oil, and total ash measurements are still essential, particularly in estimating any of the energy parameters.
6. If increased precision is required for intensive livestock units, appropriate net energy values can be calculated from digestible energy values by means of published relationships.

Feed Evaluation Systems for the Tropics of Latin America

O. Paladines[1]

Cattle production in the tropics is usually done totally under grazing; therefore, special consideration must be given to the feed evaluation systems used under these conditions. The specific effects that variable maintenance costs and extreme variability of the ingested diet have on evaluation systems are discussed.

Cattle production in the tropics, by virtue of the appropriate climate of the region, is done totally under grazing. Only specialized dairy cows receive concentrated supplements, and then only in very limited quantities because an inherent low level of production per animal is accepted and expected. Moreover, hay and/or silage feeding is largely unused because of wet weather interference and because low nutritive value of the

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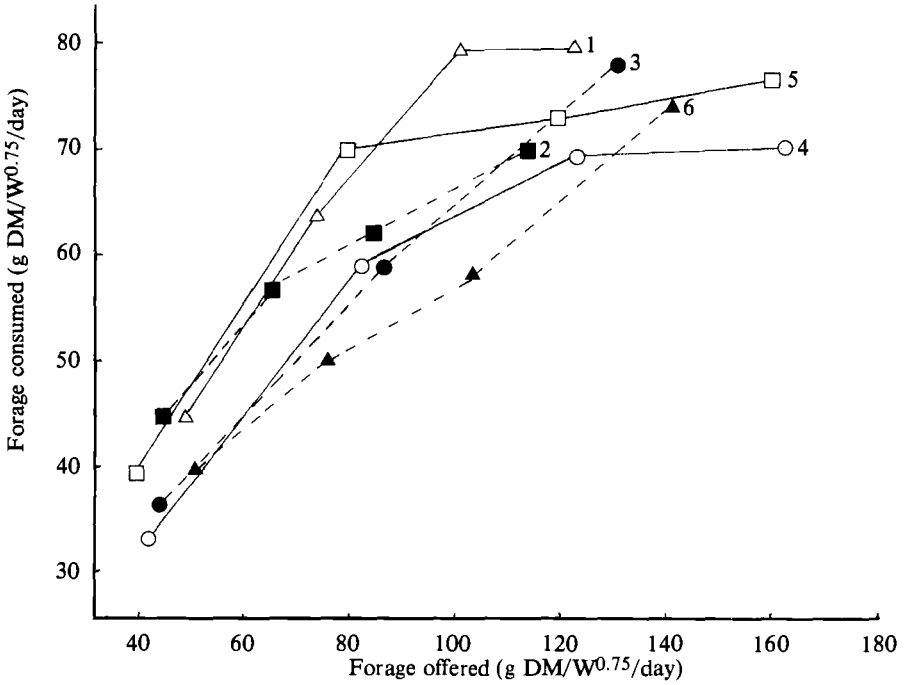

Fig. 1. Relationship between forage offered and forage consumed by crated wethers fed fresh, unchopped tropical legumes and one tropical grass: (1) Stylosanthes guianensis (90 days growth); (2) S. guianensis (150 days growth); (3) Desmodium distrotum; (4) D. ovalifolium (50 days growth); (5) D. ovalifolium (145 days growth); and (6) Hyparrhenia rufa (from CIAT, unpublished data).

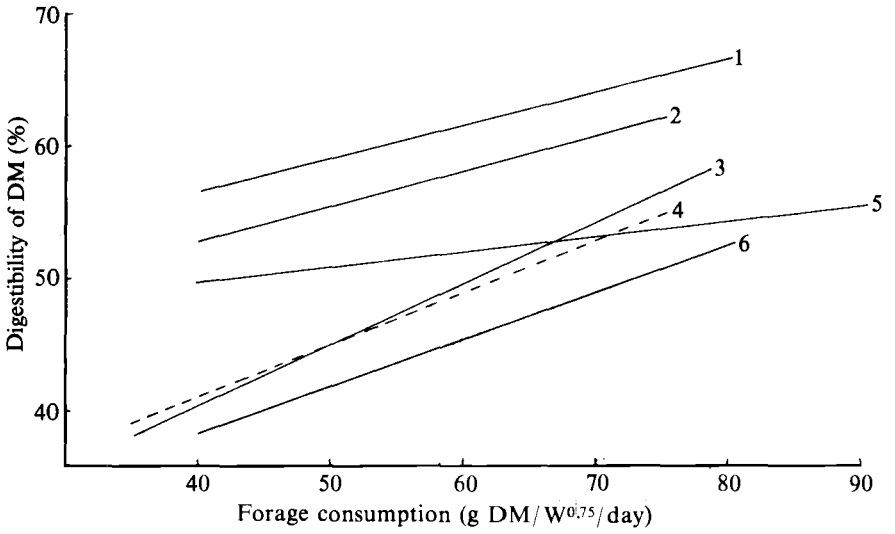

Fig. 2. Relationship between forage consumption and dry matter digestibility in several tropical legumes and one tropical grass: (1) Stylosanthes guianensis (90 days growth); (2) S. guianensis (150 days growth); (3) Desmodium distortum; (4) D. ovalifolium (50 days growth); (5) D. ovalifolium (145 days growth); and (6) Hyparrhenia rufa (from CIAT, unpublished data).

forages at the time of potential conservation makes this practice uneconomical.

Feeding standards must, therefore, be adapted to the conditions under which the animal is grazing, which primarily affects maintenance costs. These maintenance costs are influenced by: (1) variable day-night temperatures; (2) variable energy costs of harvesting the daily diet — harvesting costs are influenced by: density of sward and more precisely by leaf density in the upper strata of the sward; distance walked while harvesting; animal size (cost of movement); stocking rate or more appropriately by grazing pressure; and (3) times of low forage availability that often cause (at times severe) weight losses.

In addition to the above-mentioned effects of changing maintenance costs, feeding standards must also take into account the extreme variability of ingested diet, which varies both in quality and quantity between and within days.

The nutritive value of leaves and stems of many tropical grasses and practically all tropical legumes is different. Animals will select under grazing, and also when stall-fed green-unchopped forage, leaves over stems and terminal portions of the stems over basal parts. By selecting for leaves and young shoots, animals select for a diet of higher digestibility and also for higher intake of dry matter and digestible dry matter.

In vivo digestibilities determined in the conventional way, at a predetermined level of refusal, are of little use because they imply a condition of feeding that can hardly be repeated under grazing. The effect that increasing the level of green, unchopped forage on offer has on dry matter intake and digestibility of several tropical forages is shown in Fig. 1 and 2.

For the conditions found the tropics, then, even the more precise methods of chemical and biological analysis of feeds and those methods that more closely predict potential animal performance will be of little value in determining feeding standards if the effects that forage availability, density of leaves in the upper strata, and animal selectivity for plants and plant parts have on the quality and quantity of the animal diet are not taken into account. Furthermore, all these factors interact with the environment (temperature and precipitation) and with the type of animal and its physiological state to alter maintenance requirements and to affect the efficiency of utilization of the ingested energy.

Finally, it should be clear that it is impossible to obtain a representative sample of the grazing animal's diet. The closest approach to it can be found in samples obtained with esophageal-fistulated animals, but even then, the continuing large variability in quality of the diet (not to mention quantity) makes this technique unreliable for prediction purposes.

A New Technique for Estimating the ME Content of Feeds For Poultry

I.R. Sibbald[1]

The terminology related to the available energy in feedstuffs is defined and described. A bioassay for true metabolizable energy (TME) is described in detail and its advantages over more conventional bioassays for apparent metabolizable energy are outlined. A plea is made for the adoption of a single, standardized, bioassay for available energy in poultry feedstuffs. A brief comment is made on the limitations of indirect assay procedures.

Knowledge of the available energy content of feedstuffs is essential if the most economical poultry diets are to be formulated. Birds tend to eat to satisfy their energy requirements; consequently, nutrients should be included in diets in proportion to available energy. Failure to do this can result in wasted nutrients and impaired productivity.

Although much has been written about energy in poultry nutrition, there are gaps in our knowledge and problems to solve. There are many feedstuffs of regional interest that require evaluation. Such feedstuffs will be better utilized when their available energy values are known. More information is needed about variability among different lots of feedstuffs and about the control of such variability through processing. The energy requirements of birds in the tropics need further study. However, the most important problem is the lack of standardization in the methodology for measuring available energy.

This paper describes a new method for measuring the available energy content of poultry feedstuffs. The assay is simple, rapid, and relatively inexpensive, and it yields data that are additive, reproducible, and more accurate than those obtained by more conventional procedures.

Terminology and Definitions

Poultry nutritionists have adopted metabolizable energy as the measure of available energy in feedstuffs. Unfortunately, metabolizable energy is a generic rather than a specific term. To avoid confusion it is appropriate to present some definitions of terminology.

GE_f is the gross energy of the feed.

FE is the gross energy of the feces.

FE_f is the fecal energy of feed origin, derived from unabsorbed feed residues.

FE_m is the fecal energy of metabolic origin, derived from abraded intestinal mucosa, bile, digestive fluids, etc.

UE is the gross energy of the urine.

UE_f is the urinary energy of feed origin, derived from absorbed, nonmetabolized feed.

UE_e is the urinary energy of endogenous origin, derived from the products of tissue catabolism.

$FE = FE_f + FE_m$

$UE = UE_f + UE_e$

AME (apparent metabolizable energy) is the difference between the gross energy of the feed and the gross energy of the feces + urine. In poultry the energy lost as gases of fermentation is negligible and ignored.

$$AME = GE_f - (FE + UE)$$

AME_n (apparent metabolizable energy corrected to nitrogen equilibrium) is similar to AME but a correction is made because nitrogen retained (NR) in the body, if catabolized, would yield energy containing compounds. The constant (K) in the following equation is usually 8.22 or 8.73 kcal/g. AME_n is the most widely used form of metabolizable energy.

$$AME_n = AME - (NR \times K)$$

TME (true metabolizable energy) is the difference between the gross energy of the feed and the gross energy of feed origin in the feces + urine. It differs from AME inasmuch as the metabolic and endogenous energy losses, which are a body

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maintenance cost, are not charged against the feed.

$$TME = GE_f - (FE_f + UE_f)$$

or

$$TME = GE_f - (FE - FE_m) - (UE - UE_e)$$

TME_n (true metabolizable energy corrected to nitrogen equilibrium) bears the same relationship to TME as AME_n does to AME.

$$TME_n = TME - (NR \times K)$$

Historical

During the course of an experiment designed to measure variation in AME values it was observed that the AME value of a feed, measured with an individual bird, varied from day to day in a saw-tooth manner. That is, one day the value was high and the next day low. The most likely explanation of this phenomenon was that the feed intake of the bird fluctuated from day to day. However, apart from a theoretical paper by Guillaume and Summers (1970) there was no evidence of a relationship between feed intake and AME.

An experiment was conducted in which starved, adult cockerels were allowed to eat various amounts of wheat. The excreta voided during the subsequent 24 h was collected quantitatively, frozen, freeze-dried, weighed, ground, and, together with a sample of the wheat, assayed for gross energy content. As wheat intake increased there was a linear increase in the energy voided as excreta (Fig. 1). The intercept of the regression line (8.5 kcal) was an estimate of the $FE_m + UE_e$ per bird. The slope of the line (0.709 kcal/g) was an estimate of the $FE_f + UE_f$ voided per gram of wheat consumed. The gross energy value of the wheat was 3.88 kcal/g and the TME value 3.17 kcal/g (3.88 - 0.709). The TME value was independent of variations in wheat intake but when the AME values were calculated they were found to increase in a curvilinear manner with intake (Fig. 2). The cause of the curvilinearity was that the $FE_m + UE_e$ was charged against an increasing energy input. Subsequent experiments with other feedstuffs have confirmed these relationships.

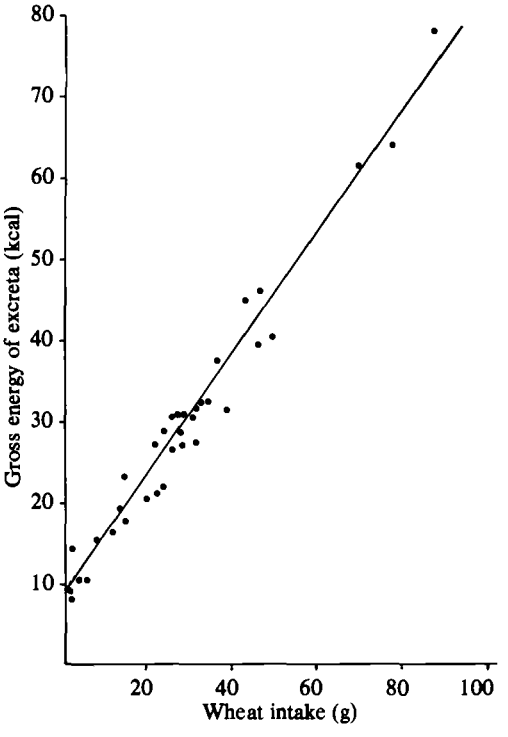

Fig. 1. The relationship between wheat consumption and the gross energy voided as excreta (y_e = 8.5 + 0.709 x; r = 0.991 at 46 degrees of freedom) (from Sibbald 1975).

The TME Bioassay

By assuming that there is a linear relationship between feed intake and excreta energy output it was possible to formulate a simple, rapid bioassay for TME (Sibbald 1976a). The assay involves the following steps:

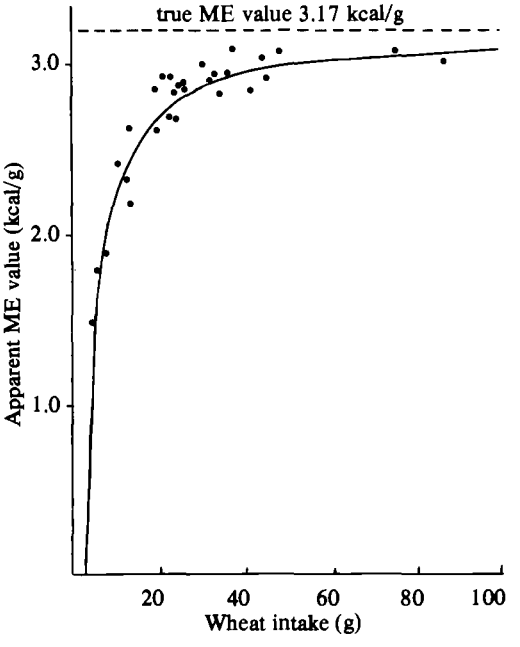

Fig. 2. The effect of level of intake on the apparent ME value of wheat (y=3.17x-8.5)/x) (from Sibbald 1975).

(1) Birds are starved to empty their alimentary canals of feed residues.

(2) A bird is selected, force-fed a known weight of the feedstuff under test, placed in a wire cage over an excreta collection tray, and the time is recorded.

(3) A similar bird is selected and placed in a cage over a tray at a known time but is not fed.

(4) Exactly 24 h after putting the birds in the cages their excreta is collected quantitatively, frozen, dried, and weighed.

(5) Samples of the feedstuff and excreta are ground and assayed for gross energy content.

(6) TME is calculated using the formula.

$$\text{TME (kcal/g)} = \frac{(F_i \times GE_f) - (Y_f - Ye)}{F_i}$$

where: F_i is the feed input (g); GE_f is the gross energy of the feedstuff (kcal/g); Y_f is the energy excreted by the fed bird; and Y_e is the energy excreted by the unfed bird.

The assay has been performed with adult cockerels, laying hens, meat-type hens, turkeys and ducks, and with egg- and meat-type chicks of several ages. However, for routine assay work the adult, single comb, White Leghorn cockerel is preferred. It tends to maintain a steady state, has good livability, and has sufficient feed capacity to minimize experimental errors. Meat-type males become heavy and obese and have higher mortality. Laying birds are not satisfactory because the starvation period, followed by suboptimal feed input, often causes the production of soft-shelled eggs that break and contaminate the excreta. Chicks and growing birds have less feed capacity than mature birds and must be replaced after each assay if several experiments are to be made with birds in a uniform physiological state. In addition, young chicks lose down, which contaminates the excreta

Birds should be housed in individual cages so that they can be handled with a minimum of disturbance. It is desirable to locate the cages in a windowless room so that day-length can be controlled and wide fluctuations in temperature can be avoided. Between assays the birds are fed a maintenance diet ad libitum and fresh water is available at all times, including the starvation and excreta collection periods. Adult cockerels have been used for as many as 30 assays, spaced 14 days apart, without any adverse effects. After an assay the birds are returned to the maintenance regime for a minimum of one day; a longer rest period is preferred to permit return to normal body weight (Sibbald 1978a).

The first step in the assay involves starving the birds to empty their alimentary canals of feed residues. A starvation period of 24 h is adequate but it may be extended to 96 h without altering the data obtained (Sibbald 1976b).

Birds selected for the assay must be healthy and clean. Any excreta adhering to the feet or to feathers around the vent must be removed. The birds must not be in a heavy moult because the feathers and scale make quantitative excreta collection difficult. Body weights should fall within a narrow range because it is assumed that the $FE_m + UE_e$ excretion of negative control birds is the same as that of fed birds. Only a small amount of the variability of $FE_m + UE_e$ can be explained by differences in body weight but it is desirable to minimize experimental variation.

The usual procedure is to assay several feedstuffs simultaneously. This minimizes the amount of work because only one negative control bird is required in each replication. Thus, in planning an assay the number of birds required is: (number of test materials + 1) times the number of replications.

The feed is prepared in advance of the assay. Proper sampling is very important because of the small amount of feed used. The feed is weighed to within 0.01 g and is stored in plastic containers until used. Gross energy and dry matter measurements are made at the time the feed containers are prepared. This ensures accurate knowledge of the dry matter and gross energy inputs and avoids errors that could occur due to fluctuations in moisture content between feed weighing and analysis. Pellets and crumbles are easier to feed than is ground feed. The latter may adhere to the outside of the force-feeding device unless particular care is taken during the feeding operation. Very finely ground feedstuffs may form a lump in the crop, which is slow to disintegrate and which causes delayed passage through the alimentary canal.

Many feedstuffs are assayed as single ingredients but a reference diet and a mixture of the reference diet and test material may be used. The latter introduces an extra treatment but it simplifies the feeding of dusty or greasy materials. It is usual to assay fats in conjunction with a basal diet.

Birds are force-fed because this ensures that the amount and time of the feed entering the bird is controlled. A funnel, which has a stem 40 cm long and 1.3 cm in external diameter, is pushed down the esophagus until it enters the crop. The previously weighed feed is poured from its plastic container into the funnel and pushed into the crop with a plunger. It is important that the feed is placed in the crop and not in the esophagus, as the

latter increases the incidence of regurgitation. If the end of the funnel is in the esophagus the funnel will be pushed out as feed is pushed in. After feeding, the funnel is removed with a rotary motion and pressure is applied to the wall of the esophagus to remove any adhering feed particles. With a 3-man team force-feeding takes less than 1 min per bird: one man catches and delivers the bird to be fed and places the fed bird in the appropriate cage; a second receives the bird inserts the funnel and pours the feed into the funnel; and a third pushes the feed down the funnel and maintains records of bird, feed type and weight, and time of completion.

The amount of feed input depends upon the size of the bird and the form, availability, and nature of the feedstuff being assayed. The greater the input, the smaller the effect of experimental errors; however, as feed input rises the incidence of regurgitation increases. For adult Leghorn cockerels weighing 2–3 kg, the optimum input is 30–40 g of pellets or 25–30 g of ground feed (Sibbald 1977a).

The excreta collection trays must have smooth surfaces and should extend beyond the cage in all directions. Plastic cafeteria trays are satisfactory and inexpensive.

The usual excreta collection period is 24 h. But, whatever time is selected it is essential that it be constant for all birds in an assay; hence, the importance of recording the times when birds are fed. Recently, it was found that the residues of some feedstuffs did not clear the alimentary canal within 24 h. Materials with slow rates of passage include dehydrated alfalfa, peanut skins, and some meat and fish meals (Sibbald 1979a). The problem can be overcome by extending the excreta collection period. If TME values obtained with a 24-h collection period are erratic, and if there is no evidence of regurgitation, it is possible that an extension of the excreta collection period will provide more uniform data.

The collection and processing of excreta are relatively simple but they must be done carefully to minimize experimental errors. Holding birds for force feeding can loosen feathers and scale, which fall onto the excreta collection trays. By blowing them off the trays about an hour after feeding a considerable amount of contamination is avoided. When collecting excreta, care must be taken to include any which adheres to the cage floor, and feathers, mixed with excreta, must be washed before being discarded. It is very important that each tray be examined for regurgitated feed. If regurgitation occurs, data from the bird must be discarded. Freeze-drying is the preferred method of removing water from excreta, but it involves a large capital cost. In a recent experiment it was found that oven-drying at 65, 80, or 95°C was just as satisfactory as freeze-drying (Table 1). The dry excreta is weighed, ground, and assayed for gross energy. Dry excreta tends to pick up atmospheric moisture; therefore, if time permits, the excreta should be equilibrated with the atmosphere for 2–3 days before further processing. Excreta can be ground with a mortar and pestle, which is easier to clean than most mills.

The calculation of TME values is straightforward. It is common practice to use a mean value for the $FE_m + UE_e$ to reduce data variability. If a TME value is large, relative to replicate determinations, it is probable that there was an incomplete collection of feed residues. There are three possible reasons: regurgitation beyond the collection tray; excretion beyond the tray; and incomplete passage of residues during the collection period. An extremely low TME value is usually due to regurgitated feed being mixed with the excreta. Regurgitation should not be a major problem once the art of force feeding is mastered.

Table 1. The gross energy output of negative control birds and the true metabolizable energy values of two feedstuffs (from Sibbald 1978d).

Drying method	Energy output of negative controls (kcal/bird)	TME (kcal/g)	
		Wheat	Laying diet
Freeze dried	11.1±0.8	3.71±0.06	3.37±0.05
Oven (65 °C)	10.0±1.1	3.82±0.04	3.45±0.06
Oven (80 °C)	10.3±0.6	3.71±0.06	3.50±0.11
Oven (95 °C)	11.1±0.6	3.70±0.03	3.46±0.05

Advantages of the Assay

The TME bioassay, with adult Leghorn cockerels, has several distinct advantages over the conventional chick assays developed for the measurement of AME and AME_n. The birds can be used for a large number of assays before being replaced and can be maintained in simple wire cages. By maintaining a flock of birds it is possible to initiate and complete an assay in a short period of time. The labour requirement is relatively small. One technician can make 6 replicate determinations on each of 15 feedstuffs in 10 working days provided a little supplementary help is available for force feeding. In an emergency, a sample can be assayed within 35 h provided 24-h notice of the time of arrival is given to permit starvation of the birds prior to sample receipt.

The small amount of test material required for a TME assay is an attractive feature. A 200-g sample permits six replicate determinations plus associated chemical and physical analyses. The small sample size places greater emphasis on the need for a proper sampling procedure but it has the advantage that materials in limited supply, such as new cultivars of grains, can be assayed. Of greater importance is the feasibility of shipping samples over relatively long distances to a central quality control laboratory.

Although the TME assay is simpler, faster, and less expensive than AME assays, its main advantage resides in the quality of the data it yields. The correction for $FE_m + UE_e$ makes TME values independent of variations in feed intake (Fig. 2). It may be argued that the $FE_m + UE_e$ excretion of a negative control bird differs from that of a fed bird. There is some validity to the argument because it was shown that $FE_m + UE_e$ excretion decreased with the duration of starvation (Sibbald 1976b); however, the error is probably small and is outweighed by the benefits resulting from the correction.

In AME bioassays a high level of test material is included in the assay diet to minimize the effects of experimental errors. If the test material is unpalatable, voluntary feed intake decreases and a low incorrect AME value is obtained. MacAuliffe and McGinnis (1971) found a marked decrease in the AME value of rye as the amount in the assay diet increased. The palatability problem can be overcome by feeding the test material at a practical level in combination with other ingredients that mask its low acceptability. However, this is self-defeating because data obtained with low levels of inclusion are highly variable.

It is known that AME_n values vary according to the type of bird used in their derivation. Work with the TME assay is less extensive but it appears that values obtained with adult cockerels can be used in the formulation of diets for laying hens, broiler hens, turkeys, and chicks (Sibbald 1976a, c; 1978b). Although additional work is required, it seems probable that differences in feed intake relative to $FE_m + UE_e$ output contribute to between bird-type variation in AME data.

There are several examples of AME values of feedstuffs varying according to the compositions of the diets with which they are fed. The TME values of five feedstuffs and of 10 diets prepared from them were measured. The observed values of the diets did not differ from those calculated using the values for the component parts (Sibbald 1977b). This evidence of additivity is important in selecting an available energy assay system. It should be noted that the TME values of fats are not additive because of interactions with other dietary components (Sibbald 1978c; Sibbald and Kramer 1977, 1978), but this is a problem that also affects AME data. There is no apparent solution to the problem.

An important feature of the TME assay is the reproducibility of data between laboratories. In a collaborative study conducted by the Animal Nutrition Research Council, 17 mean AME_n values for a sample of yellow corn ranged from 3.08 to 4.03 kcal/g DM; whereas, 9 mean TME values ranged from 3.98 to 4.15 kcal/g DM (Table 2). By definition, TME values should be greater than AME_n values. The lower variability associated with the TME assay is persuasive to its adoption particularly when it is noted that five of the laboratories had no prior experience with the TME assay.

Although not directly relevant it is of interest that the basic methodology of the TME bioassay has been successfully applied to the measurement of bioavailable amino acids (Likuski and Dorrell

Table 2. A comparative study of the AME_n and TME bioassays — energy values are expressed as kcal/g DM (from Sibbald et al. 1979).

	Corn		Alfalfa	
	AME_n	TME	AME_n	TME
n	17	9	17	7
Mean	3.53±0.07	4.10±0.02	1.52±0.09	1.67±0.05
Low	3.08	3.98	1.06	1.45
High	4.03	4.15	2.63	1.94

1979; Sibbald 1979c,d). It is in fact possible to measure both TME and true available amino acids using a single set of feed and excreta samples. This additional flexibility of the assay should be of interest to those establishing quality control laboratories, particularly where operating funds are limited.

The major objection to the adoption of TME as the available energy standard in poultry nutrition is that most energy requirement data are expressed in terms of AME_n. This should not be a major stumbling block and indeed some feed manufacturers have already adopted the TME system. A temporary solution is the use of a factor to convert AME requirement data to TME values. This has been discussed at length by Sibbald (1977c).

The feed industry and regulatory bodies will decide which measurement of metabolizable energy will be adopted. There is need for additional work on the TME system, but it appears to be more attractive than conventional systems in terms of cost, data quality, reproducibility, and flexibility.

Indirect Assays for Metabolizable Energy

There have been several attempts to predict the metabolizable energy values of poultry feeds from physical and chemical data. Few of the published prediction equations can explain more than 80% of the variability in ME values when tested on independent data. This is not surprising because although chemical techniques can yield accurate quantitative data on absolute amounts of nutrients in feedstuffs they are, as yet, unable to measure those portions that can be digested and absorbed. In addition, they fail to take account of interactions between nutrients and between nutrients and other dietary components.

Major attractions of the indirect assay were the speed with which it could be executed and the relatively low cost. The TME bioassay has reduced the time and cost differentials between bio- and indirect-assays. A simple, rapid, accurate, indirect assay would be extremely valuable, but its development seems unlikely because of the complexity of feedstuffs and the variation in nutrient availabilities.

Sheep as Pilot Animals

D.P. Heaney[1]

In vivo digestibility-intake trials are expensive, time consuming, and require relatively large amounts of feed. Although there have been attempts to develop techniques to use small animals as pilot animals for evaluation of ruminant feeds, none have achieved widespread acceptance. Therefore, the sheep has become, in effect, a pilot ruminant because the extensive data obtained with sheep are extrapolated to cattle. Such extrapolations should be made with caution because important differences can, and do, occur. Cattle digest roughages more efficiently than do sheep and the differences between values determined with cattle, compared to those obtained with sheep, increase as digestibility decreases. Although insufficient data are available to adequately compare voluntary intakes between cattle and sheep, most experienced investigators feel there can be significant differences. Available evidence indicates that relative differences in feed value between herbages are similar for sheep and cattle although absolute values may differ for most feeds. However, such correlations become unreliable for low quality feeds (e.g. digestibilities below 45–50%). Specific suggestions for digestibility and intake assay procedures are discussed.

In most cases the energy from a given ration that is available for the metabolic processes of the animal it is fed to (available energy) is primarily responsible for establishing the level of production that ration will support. Or, conversely, the animal feeder first considers the available energy required for the particular level of production desired (maintenance of breeding animals, gestation, lactation, growth, finishing for slaughter, etc.), then balances the other nutrients, with supplements where necessary, to the required available energy level. In practical feeding operations, rations are developed on the basis of the best estimates, or predictions, that are available for the nutritive value of the feeds to be used and for those that might be required as supplements.

The available energy value of a roughage is usually very closely related to its overall feeding value, so much so that the two terms are often used interchangeably. Roughage available energy is also, however, the most difficult to estimate or predict. Although there has been considerable work undertaken, and progress is being made, there is not yet a laboratory analytical technique that will estimate the available energy value of a roughage satisfactorily over a wide range of conditions and substrates. Therefore animal feeding data are still required for refined estimates of the available energy value of roughages. It has been established that determinations of digestibility and voluntary intake provide estimates of available energy value that are reasonably reliable and go a long way toward bridging the gap between analytical laboratory estimates and actual performance.

This paper will be limited to discussions pertaining to estimating the available energy of "roughages" for ruminants. For the purpose of this paper, roughages will mean feedstuffs derived from the vegetative and more fibrous portion of plants including forages, herbage, waste products high in cellulose, etc.

Principles and Application of a Pilot Ruminant

Feeding experiments with large ruminants are very expensive because of the high cost of facilities and animals, and the relatively large amounts of feed required. The need for a pilot animal for roughage evaluation work is self-evident. Such a pilot animal should be small, so that costs are minimized, and should provide data that can be reliably extrapolated to domesticated ruminants. Although there have been attempts to develop techniques to use meadow voles, rabbits, insects, etc. as pilot animals, none of these attempts have achieved widespread acceptance. Therefore, most of the work is carried out with sheep. In such work, the sheep is the primary animal insofar as

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there are large sheep populations in many parts of the world and the estimates of feeding value obtained with sheep are directly applicable to those populations. However, the sheep can also be considered a pilot ruminant because only limited data are obtained with cattle per se. Instead, values obtained with sheep are extrapolated to cattle.

Before considering the reliability of the sheep as a pilot ruminant for cattle, a very brief synopsis of some basic principles is offered. First, the importance of the feeding value of a roughage decreases as the proportion of concentrate in the ration increases. In high concentrate diets, the value of a roughage is often due to its fibre content, which is required to maintain rumen function, rather than to its nutritive value. Secondly, voluntary intake is a very important index of roughage feeding value in feeding regimes where roughage is fed to appetite, but it is not, of course, of any importance when roughages are fed at levels below appetite (e.g. maintenance feeding, feeding a fixed quantity of roughage with concentrates, etc.). Thirdly, although for a given herbage species there is often a good correlation between digestibility and voluntary intake, the interrelationships break down when comparing different species, or mixtures of herbage species. It has been amply demonstrated (Heaney 1970) that either digestibility or intake can be highly misleading if used alone and must, therefore, be considered together when comparing herbage species (or evaluating mixtures). Lastly, it must be remembered that even in vivo measurements of digestibility and intake are not constants in themselves, but can vary with changes in the physical form (e.g. grinding and pelleting) of the roughage, physiological state of the animal, etc.

Application of Sheep Data to Cattle

Because both sheep and cattle are ruminants, both are highly refined domestic species, and generally speaking they are fed similar feeds, feed value estimates are often freely interchanged between the two species. Such extrapolations should be made with caution, however, because important differences can, and do, occur.

Digestibility

On the average, cattle tend to digest roughages more efficiently than do sheep (Playne 1978). For high quality roughages, having dry matter digestibilities exceeding 55–60%, the differences are small (usually 1–3 percentage points) and can probably be safely ignored for practical purposes. As digestibilities decrease, however, the differences between values determined with sheep, compared to those of cattle, increase. For low quality roughages cattle digestibilities can be significantly higher than those obtained with sheep. In addition, the spread of points also becomes greater at low digestibilities and the correlation between cattle digestibility values and sheep digestibility values diminishes. Thus, the available evidence indicates that there are absolute differences between sheep and cattle digestibility values that increase as digestibility decreases. However, relative differences in digestibility between herbages are reasonably consistent regardless of whether they are determined with sheep or with cattle except for mature, low quality materials of low digestibility (less than 45%). In these latter cases the correlation between sheep and cattle data becomes unreliable.

Intake

The use of the measured voluntary, or ad libitum, intake of a herbage as one of the parameters characterizing its feeding value is a comparatively recent innovation. To date there have not been enough comparative studies to provide sufficient data to adequately evaluate comparative intakes between sheep and cattle. Most experienced investigators feel there can be significant differences. Generally, sheep tend to be more sensitive than cattle and more likely to have lower intakes of feeds such as poor quality silages. On the other hand, there appears to be good agreement between sheep and cattle for both acceptability and intake of the grasses and legumes usually used for pasture and/or conserved forage.

In summary, available evidence to date indicates that feeding value estimates obtained with sheep can be applied to cattle and used with confidence provided reasonable precautions are observed. Relative differences in feed value between herbages are similar for the two animal species even though absolute values may differ. The likelihood of erroneous conclusions resulting from an extrapolation of sheep data to cattle are minimal for most herbages. The possible exception to this generalization is for very low-quality, high cellulose materials such as cereal straws, etc. For these types of low-quality herbages the correlation between sheep and cattle data diminishes to the point where extrapolation can be misleading. There is a need for further research in this area.

Measurement of Intake and Digestibility

Determination of apparent digestibility is based on the principle that the difference between the

quantity of a nutrient consumed and the quantity voided in the feces represents the amount apparently digested and absorbed during passage through the digestive tract. Similarly, measurement of voluntary intake is based on the premise that intake measurements can be accurate and repeatable for a given herbage. To obtain accurate and repeatable values, relatively standard techniques have evolved. For this discussion, the technique for indoor trials conducted in digestion or metabolism stalls will be considered. Design of the digestion stall, per se, will not be discussed. Many variations of plans exist for stalls that suitably restrain sheep, so that they can be individually fed, and have suitable equipment for collection of uncontaminated feces and/or urine (e.g. Cammell 1977). Such equipment is equally effective for housing sheep for determinations of voluntary intake. In practice, it is often standard procedure to measure the digestibility and intake together, either simultaneously or sequentially, for the two determinations. Each requires a suitable preliminary period followed by a suitable measurement period, both of which must be carried out under standardized, controlled conditions. When proper criteria are met, digestibility and intake determinations are, for all practical purposes, biological assays. That is, the resulting values are unique and repeatable, provided the proper procedures and conditions are followed. They are not affected by a change in animals, subsequent periods (i.e. time), time of the year, etc.

Digestion Trials

The basic principle underlying the digestibility determination is quite simple; namely, the difference between what is fed and what is voided in the feces represents what is digested. There is, however, a mixing of subsequent feedings in the digestive tract so that the feces collected do not derive precisely from the feed that was carefully weighed and sampled. Particularly in the ruminant, each feeding is thoroughly mixed with varying proportions of several earlier feeds and the feces voided on a given day derive from feed that was ingested over several days. It is imperative, therefore, that stabilized conditions be established so that meaningful averages are measured. As a result, digestion trials consist of a preliminary period and a measurement period.

Preliminary Period

The preliminary period must be sufficiently long to accomplish three objectives. First, residues from the previous feedstuff must be given time to be expelled from the digestive tract; second, the rumen microflora must be given time to adjust to the new feed; and third, the animal must become accustomed to consuming the new feed. The recommended length of this period depends, to some extent, upon the nature of the feed being assayed and the experimental regime. Most studies have shown that 7–10 days are sufficient when ordinary forages are fed, there is not a major change in feed type from that preceding the trial, and where a constant level of feeding below the ad libitum level is used. Although 7–10 days does not completely clear the digestive tract of previous feeds, and there may not have been a complete readaptation of the microflora, errors caused by these factors will be minute and, usually, undetectable. Longer preliminary periods are desirable when "problem" feeds (such as silages, straws, alkali-processed materials, ground and pelleted forages, etc.) are assayed or when the feed to be assayed is drastically different from the previous feed. In these latter cases the preliminary period should be at least 14–21 days.

Measurement Period

The measurement period must be long enough to ensure that the collected feces accurately represent the residues from the feed that was fed. "Endpoint" errors, caused by the length of time between the last defecation and the start or finish of the measurement period, decrease in direct proportion to the length of the measurement period. At least 7 days are required to minimize such endpoint errors when digestibility is determined at constant daily feed intake. If digestibility is to be determined at an ad libitum level of feeding, the measurement period must be extended to at least 10–14 days because the endpoint errors are further affected by the unavoidable daily fluctuations in food intake. Any feed that is uneaten will usually have a different composition from that which was offered. Therefore, such uneaten feed must be weighed, sampled, and analyzed to accurately correct the feed offered.

Intake Trials

The productive value of livestock rations depends on their ability to provide available nutrients to the animal. For monogastric animals consuming high energy, concentrate feeds, the homeostatic mechanisms tending to regulate intake to meet the animal's energy needs are of major importance. The high roughage diets fed to ruminants do not, however, contain sufficient available energy to meet the animal's needs (except for those few high-quality forages having digestibili-

ties above 65–68%). For roughages, therefore, voluntary intake has come to be recognized as one of the inherent factors related to quality and is, in itself, an indication of roughage feeding value. Although the use of the measured intake of roughage as one of the important parameters characterizing its feeding value is a comparatively recent innovation, assay procedures have been developed that are adequate to provide satisfactory measurements. Precise knowledge about the effects of some variables has not yet been determined but, generally speaking, when there is doubt, possible errors can be minimized by providing safety factors in the form of longer trials.

Preliminary Period

A preliminary period is required for intake measurements for essentially the same reason as for digestibility measurements, but the need to achieve a relatively stabilized intake of a newly introduced roughage is almost always the most important factor determining minimum length of the preliminary period. Characteristically, relatively wide fluctuations in daily feed consumption are encountered when a new roughage is first introduced to a ruminant. Under a regulated feeding regime these fluctuations can be reduced to a minimum, but *not* eliminated, and can be stabilized at a reasonably constant level so that an accurate measurement of intake can be undertaken. For common forages a preliminary period of at least 10 days is required (Blaxter et al. 1961). For "problem" feeds such as silages, straws, processed low-quality feeds, etc., longer periods are required because it takes longer for a stabilized intake to be reached. Usually, however, 2–3 weeks is sufficient. In addition, experience has shown that there can be serious carry-over effects (Heaney and Pigden 1972) whereby the intake of a subsequent roughage can be significantly affected by the previous "problem" roughage. At present there is little precise information regarding the length of period of this type of compensatory intake, but a minimum of 3 weeks and preferably 4 weeks is recommended as a preliminary period whenever this type of problem is suspected.

Measurement Period

Most investigators agree that, provided the preliminary period has been sufficient and effective, a measurement period of at least 7 days is adequate to minimize errors due to the residual daily fluctuations in consumption.

Level of Feeding

Because the objective is to measure true ad libitum intake, each animal must have access to feed at all times and the feed allowance must be sufficient to provide some feed weighback every day. Conversely, the actual amount of weighback should be controlled so that the opportunity for selection is minimized. Otherwise, the higher quality portions will be consumed, the lower quality ones rejected, and the resulting measurement values will be in error insofar as they will measure the feed that was selected and not the entire feed that was intended to be assayed. Only when selection is impossible, e.g. pelleted feeds, can the aforementioned principles be disregarded. Available evidence (Heaney 1973) indicates that an average weighback of up to 15% causes only negligible errors in measured intake. Conversely, if weighbacks are kept at an average of only 1–2%, the intake values tend to be depressed. Therefore, it is usually recommended that weighbacks should be about 10%. The inevitable daily fluctuations in consumption make it difficult to maintain a precise level of weighback but experienced feeders have no difficulty keeping daily weighbacks within a range of 5–15%. Feeding at this level minimizes any errors due to selection but assures that true voluntary intake is measured. If digestibility is to be measured at an ad libitum level of feeding it is even more important to control daily weighbacks because the evidence shows that digestibility is affected much more by selection than is intake.

Number of Animals

The precision of any biological assay procedure is a function of many factors including analytical errors, techniques to ensure collection of representative samples, and inherent animal to animal variability. For intake and/or digestibility assays, inherent animal to animal variability is usually the major factor determining the ultimate precision that can be attained although in some cases obtaining representative samples for analysis can also be a major problem (e.g. Cammell 1977). The standard deviation of an individual dry matter digestibility coefficient is usually between 1.0 and 1.3 percentage units (Forbes et al. 1946; Raymond et al. 1953). Statistically, using three sheep per digestibility measurement will give an 80% chance, at the 5% level of significance, of detecting digestibility differences of 3–4%, and four to six sheep should reliably detect differences of 3% (Fig. 1). Increasing the number of sheep further results in only minimal increases in precision.

Intake measurements, on the other hand, are less precise (Heaney et al. 1968). The standard deviation of an individual measurement is usually

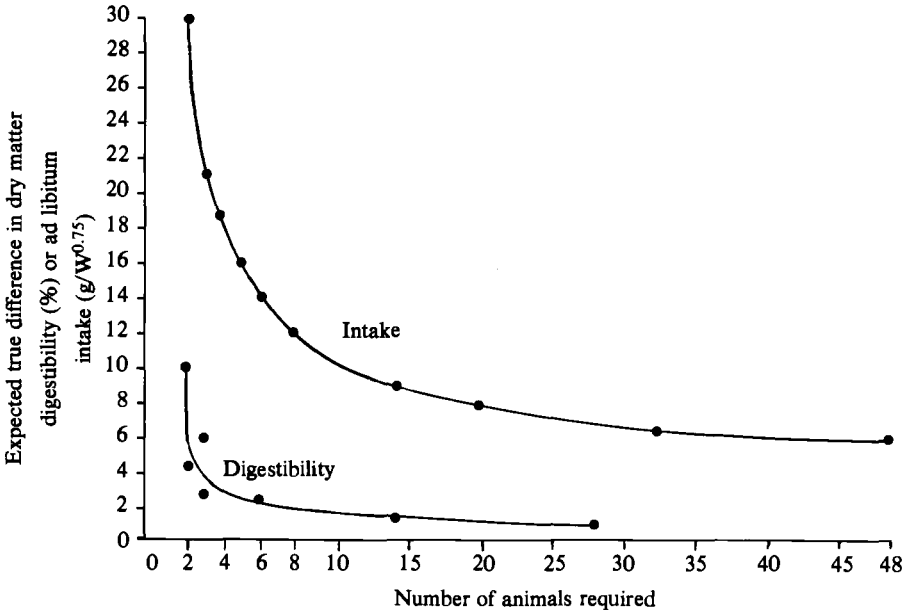

Fig. 1. Number of animals required to obtain an 80% chance that the digestibility or intake difference will be significant at the 5% level (intake from Heaney et al. 1968; digestibility from Raymond et al. 1953).

between 7 and 10 intake units (g consumed/$W^{0.75}$/day). Statistically, about 12 sheep are required to detect differences of 10 intake units (i.e. 80% chance at 5% significance). Increasing the number of sheep above 15 results in only marginal increases in precision, but decreasing the number below 10 rapidly decreases the precision of intake measurements (Fig. 1).

Considering these statistical implications, it is usually recommended that a minimum of three sheep be used if only digestibility is to be measured and six sheep be used if intake, or both intake and digestibility in the same trial, is to be measured. In practice most investigators have found these minimum numbers will allow detection of digestibility differences of 2–3 percentage units and intake differences of 6–10 intake units in most cases. It must be recognized, however, that there will be instances when such differences will not be significant when the suggested minimum numbers are used. Nevertheless, the above recommended minimum numbers are a reasonable compromise between cost and precision.

Systems of Analysis for Evaluating Fibrous Feeds

P.J. Van Soest and J.B. Robertson[1]

Crude fibre has been and remains a common means of evaluating fibrous feeds. It is, however, grossly misleading and involves large errors on the basis of nutritional and biochemical criteria. In searching for a replacement there is a conflict among criteria that evaluate the analytical parameters as indicators of nutritive value. The favoured criterion is that which evaluates on the basis of recovery of refractory and indigestible residues. Another important consideration is economics of the methodology, which needs to be competitive with proximate analysis in terms of laboratory cost and technician time.

At the present time two systems are in use: the detergent system of fibre analysis and that developed by Southgate in England. The Southgate methodology is used mainly in human nutrition. There are a number of important and somewhat divergent modifications of the detergent system in use.

The establishment of any one methodology as a standard system of analysis will require some refinement and further collaborative study. The lead in this direction has been taken by the human nutritionists: International Agency for Research on Cancer (IARC) of the European Economic Community. This group has sponsored a committee on fibre methodology, which has conducted one collaborative study. Another committee sponsored by the American Association of Cereal Chemists has adopted a modification of neutral-detergent fibre.

The object of laboratory feed analysis is to derive compositional information from which estimates of animal responses to dietary inputs can be made. The purpose of this paper is to review systems of analysis for fibre, which has served as a negative index of energy availability in feeds and forages. Recently, a new dimension has been added with the suggestion that human diets in the developed countries are fibre-deficient. This has put emphasis on the positive aspects of dietary fibre quality that parallels the effort to utilize fibrous waste as ruminant feed.

The need for better methods of fibre analysis stems from the deficiencies of crude fibre analysis and associated proximate analysis. The weaknesses of the crude fibre method have been known for a long time but have only recently become recognized as a real concern in monogastric and human nutrition. Most of the effort to improve fibre analysis has been in the ruminant field.

The replacement of crude fibre with a more accurate and scientific method is a problem involving perhaps more politics and economics than chemistry. Crude fibre is the legal method in the United States and many other countries, and legislative action may be required. At the same time an adequate system of feed analysis must not only satisfy scientific criteria but also be sufficiently convenient and economical so as to be competitive with the proximate system of analysis including crude fibre. This latter objective has been a difficult one in view of the problem that true dietary fibre cannot be adequately described by any one single procedure.

There are at present two other international committees charged with developing and recommending a suitable technique for evaluating and characterizing human and animal diets. The Medical Research Committee of the European Economic Community (EEC) in conjunction with the International Agency for Research on Cancer (IRAC) of the World Health Organisation sponsors a fibre work group. This EEC–IRAC fibre group has had two meetings, one in Lyon, France in December 1977 and the other at Cambridge, England in December 1978. It has conducted one collaborative study on dietary fibre methods. The other group is the International Organization for Standardisation (ISO) with headquarters in Amsterdam, The Netherlands. In addition, there is a fibre committee of

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the American Association of Cereal Chemists (AACC), which has adopted an amylase modification of the neutral-detergent method (Schaller 1976), and a nonnutritive residues referee of the Association of Official Analytical Chemists (AOAC).

The EEC-IRAC fibre committee has so far taken the lead in fibre studies and has made the recommendation that crude fibre be dropped even if no other alternatives are immediately available. It feels that crude fibre values are so misleading that they are of little use in human nutritional studies. The group has not recommended any one procedure although many of the members are using some modification of the detergent system (Goering and Van Soest 1970) or the Southgate (1969a,b) methodologies.

Basic Principles

Laboratory analysis of forages and feedstuffs is necessary to have a conveniently quick answer to feed quality. However, chemical analysis also provides some understanding of the nature and effects of a feed. Laboratory evaluation of forage is essentially aimed at obtaining analytical data that predict the extent of biological degradation under specified conditions. The biochemical problem becomes that of assaying the limiting factors in the substrate; hence, there is emphasis on lignins and other components of the fibrous cell wall of plants that provide resistance to digestion.

Table 1. Correlations of various forage components with in vivo voluntary intake and digestibility for 187 forages of diverse species (from Van Soest and Mertens 1977).

	Intake	Digestibility
Digestibility (in vivo)	+0.61	—
Digestibility (in vitro)[a]	+0.47	+0.80
Lignin	-0.08	-0.61
Acid-detergent fibre	-0.61	-0.75
Crude protein	+0.56	+0.44
Cellulose	-0.75	-0.56
Cell wall	-0.76	-0.45
Hemicelluloses	-0.58	-0.12

[a]Two stage procedure of Tilley-Terry as modified by Goering and Van Soest (1970).

The applied objective is to be able to formulate diets from compositional information that will elicit predictable animal responses. This desire leads to the net energy concept of feed evaluation. However, net energy is a complex of digestibility, intake, and efficiency phenomena that is in turn dependent on a variety of factors. Nutritional parameters of feed quality are variably correlated with digestibility or intake in ruminants (Table 1). Cell wall is better related to intake than digestibility even though it recovers the undigestible fractions in the feces. In the case of monogastrics, cell wall is probably a better indicator of digestibility. Fibre quality is an important variable and evaluation will require analysis for components including lignin, cellulose, and hemicelluloses. Although these components are important in themselves, they cannot be individually a substitute for a total fibre analysis. This is a relevant criticism of those who wish to replace crude fibre with cellulose.

The basic requirement for a feed fraction to have an effect on digestibility is for its content to be correlated with its digestible quantity in the diet. This is the so-called Lucas test and is an essential component of modeling studies. Further, for any component to have a consistent effect upon digestibility it must have a true causative effect. Lignin is such a component, but its effect is limited to the plant cell wall, which is in turn another variable. These points are the foundation of the summative equation for estimating digestibility (Van Soest and Jones 1968).

Crude fibre and cellulose are not such prime factors but are associated secondarily with digestibility through association with plant age or maturity. Cellulose is correlated to digestibility only to the extent that it is correlated to lignin. This association fails in many cases, particularly in tropical forages and aftermath cuttings of temperate forage (Van Soest et al. 1978). This lack of association is perhaps the single most unsatisfactory aspect of the use of the proximate system in evaluating forage from developing countries.

Definition of Fibre

The recent interest in the role of fibre in human nutrition has led to the advancement of the concept that total dietary fibre is the polymeric substances from plants that are resistant to mammalian digestive enzymes. This definition contains more than lignin, cellulose, and hemicelluloses and includes pectins, gums, galactans, etc., which are relatively soluble materials. These are for the most part completely degraded by rumen bacteria and the bacteria of the lower tract of

nonruminants, and therefore do not contribute to the true indigestible fecal fraction. They will, however, act as a substrate for the intestinal microflora and affect the quantity of microbial products voided. The EEC–IARC committee has recommended determination of the insoluble (neutral-detergent residue) and soluble components separately as these may have varying effects upon digestion. The insoluble undigested fibre is the principal fraction promoting passage of food residues in man (Van Soest et al. 1978).

The work on fibre in humans has provided a model for application to other nonruminant species where work with fibrous diets is less advanced. Here it is important to state the principle that the recommendation of a world committee must consider an overall view of the role of fibre in animal nutrition in regard to its methological choices.

Categories of organic residues from foods relative to the dietary fibre definition include the following:

(1) Matter that is available but which escapes through fast passage and slow rate of digestion to the lower digestive tract. Further competition between bacterial fermentation rate and passage determines the amount that may escape and be excreted in the feces.

(2) Matter unavailable to mammalian digestive enzymes but which is potentially fermentable may be lost to the feces through competition between passage and fermentation rates. These fractions are recovered in neutral-detergent fibre.

(3) Unavailable and unfermentable matter that is affected only by passage rate and excreted in the feces. Ruminant studies show that obliately unfermentable cellulose and hemicellulose is about 2.5 times the amount of dietary lignin (Smith et al. 1972; Mertens 1973). These carbohydrates will become available to fermentation if the lignin-carbohydrate bond is broken by chemical pretreatment of the dietary fibres.

(4) Microbial matter not originally present in the diet, but generated through microbial action on dietary residues and endogenous secretions. Gross microbial composition includes major amounts of protein and lipid, which may represent the main sources of increased excretion of fecal dry matter when fibre sources are fed. About 30% of the microbial dry matter is composed of cell wall or capsular matter that is resistant to digestion by mammalian enzymes. It is composed of muramic acid complexes including glucosamine and diaminopimelic acid in the polymer (Mason 1969).

Enzymatic methods of analysis will include this fourth fraction as a part of the fecal fibre excretion and may fail to distinguish it from genuine fibre fractions that survive from the diet. The microbial cell wall is soluble in neutral and acid detergent. The detergent methods are specific for plant cell wall and thus are useful for making the separation of microbial and plant-derived residues.

Criteria For Evaluating Analytical Systems

Two contrasting and conflicting sets of criteria have been used to evaluate analytic procedures for their relevance to nutritive evaluation. One is the recovery of unavailable residues in the fibre residue and the other is the degree of correlation of digestibility with the measured parameters. Ironically, the residue that recovers the indigestible fractions is the neutral-detergent fibre, which is poorly correlated with digestibility in ruminants (Table 1). Cell wall content of the diet correlates highest with forage intake of ruminants. Plant cell wall is a better estimate of indigestibility of nonruminant diets (Henry 1976).

The degree of correlation is generally an unsatisfactory criterion, although it reflects the practical desire of obtaining the most accurate estimates of nutritive value from composition. The unsatisfactory aspect arises because most standardized feeds and forages (through digestion trials) are unrepresentative of the environmental and physiological factors affecting plant composition (Van Soest et al. 1978). Any standard set developed on the basis of cutting dates at a university farm are apt to reflect only that environment and practice, which are often ideal relative to that which may be sampled in the adjacent countryside. In a statistical sense such a standard would reflect no variation in quality due to soil and climate as well as that due to variable practices in management. The lack of this variation would in turn bias prediction equations relative to field samples. It would be better to develop a standard set for calibration by random selection and evaluation of farmers' products.

If the objective is to obtain the most accurate estimate of digestibility, more than one analysis becomes essential. The effects of lignification are restricted to the plant cell wall allowing a model to be constructed that assumes non-cell-wall (100-NDF) is completely digestible; whereas, the digestibility of the cell wall itself is estimated by its lignin content. This system, the summative equation (Goering and Van Soest 1970), will satisfactorily estimate digestibility of mixed forage populations from diverse environments (standard error of about 3.6). It will not improve the evaluation of first-cut single species relative to in-

dividual analytical parameters. The summative system requires determination of cell wall (NDF), ADF, and lignin. A silica or insoluble ash correction may also be needed. For a single measurement of digestibility in mixed populations the Tiley-Terry in vitro rumen procedure or a modification of it remains the most accurate. The limit of practical prediction is a standard error of about 3–5, which is the practical level of animal variability under producing conditions. The animal error is variable and depends in part on level of intake and the specific diet (Van Soest 1973b).

The reliability of regression systems depends on whether the standard forages, upon which the equation is founded, reflect the balance of species and environmental interactions characteristic of the forages to be tested. Strict standards must be kept regarding the forage populations used to test the system. There should not be less than 20 forages of determined animal digestibility. Legumes and grasses should be equally represented and several species of each included. Reference forages should come from localities similar to those of the forages to be tested. Aftermath cuttings should be included as well as first cuttings with age of plant. The reliability of the regression system should be tested on a population other than that from which the equation was derived, but from similar localities. This allows the possibility of ascertaining two types of error: the standard error of an estimate; and the bias of systems to over or underestimate the correct value.

When prediction systems are tested against a properly selected group of forages, more realistic estimates of the predictive errors are obtained (Table 2). Systems based on crude fibre and protein involve large errors because of the failure to assess the effect of environment. Protein is associated positively with digestibility through its decline with age of the plant, but nitrogen fertilization increases crude protein content without greatly altering digestibility. Equations based on proximate analyses, while using a large data base (Schneider et al. 1952), suffer from the limitations of the proximate system and the historical nature of much of the data.

Table 2. Predictive errors associated with systems to estimate digestibility from composition. Evaluation is with a balanced group of legumes and grasses of varying geographic origin (Van Soest and Jones 1968; Van Soest 1973).

Method of estimation	Value predicted	Bias[a]	S.D.[b]
		units of digestibility	
Crude fibre	DDM[c]	—	11.0
Acid-detergent fibre	DDM	—	9.0
Equations based on crude fibre and protein[d]			
Legume and grass	TDN	+4.0	7.7
Mixed	TDN	+3.3	8.0
Summative equation[e]			
Unmodified	DDM	−1.0	6.1
With silica correction	DDM	+4.5	3.8
In vitro rumen digestibility[f]	DDM	+2.5	3.7
True digestibility[g]	DDM	+0.7	2.8

[a]Mean difference between predicted and observed values.
[b]Standard deviation from regression.
[c]Digestibility of dry matter.
[d]Equations of Adams et al. 1964 based on the system of Axellson.
[e]Equation of Goering and Van Soest 1970.
[f]Tilley-Terry procedure.
[g]Modification of Tilley-Terry according to Goering and Van Soest (1970).

Critique of Laboratory Methods

Analytical parameters for forage and feedstuffs are of unequal value in providing useful dietary information and there is no such thing as a best method because most nutritive aspects of quality are complex. For example, a method that estimates digestibility may be unsatisfactory for evaluating intake or efficiency. In most forages, digestibility is a function of both plant cell wall and lignification. However, in some species, other factors have a role (tannins, silica, etc.). An individual analysis will be unsatisfactory if substantial feed variation is due to another unassayed factor. Therefore, an adequate system of analyses must attempt to assay the relevant limiting factors in feeds and forages. The following critique is developed from that point of view as well as some practical sense of laboratory economy and utility.

Laboratory analyses can be divided into several categories: those that determine chemical entities; in vitro estimations of quality; and empirical tests. In the first category are analyses for specific feed entities such as lignin, cell wall, cellulose, etc., and in the second, enzymatic techniques. The empirical tests include crude fibre and the various dry matter solubility measurements. The first two categories satisfy some biochemical standards and provide the most valuable information in the form of actual composition or biodegradability. The third category is the least useful because the results can only be correlated with nutritive quality and the statistical associations are dependent

Table 3. The percentages of original feed lignin, pentosans, and cellulose dissolved in the crude fibre determination (from summary by Van Soest 1977).

	Lignin	Pentosans	Cellulose
Legumes			
Range	8–62	21–86	12–30
Average	30	63	28
Grasses			
Range	53–90	64–89	5–29
Average	82	76	21
Other[a]			
Range	—	43–84	7–32
Average	52	64	22

[a]Gymnosperms and angiosperms exclusive of legumes and grasses.

on time and environmental interactions that may not be reproducible. Further, methods must be considered for their utility. There is a conflict of interest in efforts to modify methods according to ideal criteria because modifications almost invariably increase the length and complexity of a procedure.

Crude Fibre and the Proximate Analysis

The crude fibre method is of uncertain origin (Tyler 1975) and has been in use for at least 150 years. The earliest published analysis that is extant was done on Indian corn by John Gorham of Harvard in 1820 (Gorham 1820). Many authors attribute crude fibre to the German chemist H. Einhof. However, recent historical research (Tyler 1975) does not support this, and Einhof's published values, obtained by a maceration procedure (Einhof 1806) correspond to modern cell wall values (Van Soest 1977). Cell wall values, which represent the sum of lignin, cellulose, and hemicelluloses are higher than crude fibre in varying degrees depending on the food source.

The error in crude fibre arises from the sequential extraction with hot dilute acid followed by hot dilute alkali. In this extraction sequence, 50–90% of the lignin, 0–50% of the cellulose, and upwards to 85% of the hemicelluloses are dissolved (Table 3). The error through these losses is variable depending upon the proportions of lignin, cellulose, and hemicelluloses in the fibre and can be as high as 700%. In the case of wheat bran, the most common source of fibre in human food, the true fibre value is about four times that indicated by the crude fibre value.

For over a century we have known about the losses resulting from the crude fibre method and its failure to recover lignin and other genuine components of fibre (Henneberg and Stohmann 1864; Van Soest 1975). Nevertheless, the Wiley Committee of the AOAC was instrumental in obtaining the approval of crude fibre as a legal official method in 1887 (AOAC 1887). Since that time the main effort of the AOAC has been to ensure analytical reproducibility within and among laboratories. Over the past 50 years there have been a number of attempts to develop improved fibre methods, none of which thus far has managed to dislodge crude fibre. This effort has been dominated, but not exclusively, by the fields of ruminant nutrition and grassland husbandry, where fibre utilization has been a main objective of research on forage quality (Raymond 1969).

The methodology itself must be directed towards two separate goals that are not entirely compatible: for research purposes, one needs a detailed system of structural analysis that is definitive in characterizing individual plant fibre sources; and for surveys or quality control work, the methods must be rapid and convenient even though some detail may be sacrificed. Whatever system is adopted, if it is to be competitive with the crude fibre method it must permit the handling of large numbers of samples, yet at the same time, yield more than a single measurement. Fibres are variable in their composition and properties, and it is not possible to describe the characteristics and amount of fibre with a single value.

Nitrogen-free extract (NFE)

The greatest and most fundamental error in the proximate system of analysis is the division of the carbohydrates between NFE and crude fibre. All attempts to unseat and replace crude fibre have attacked in one way or another the problem of carbohydrate fractionation and analysis. The AOAC recommended as far back as 1940 that reporting of NFE be discontinued. The NFE contains the cumulative errors of all the other determinations, the largest of them being due to the solubility and loss of much lignin and hemicelluloses in the preparation and determination of crude fibre. Even cellulose is not wholly recovered and the behaviour of different plant materials is quite variable (Table 3).

Generally, the net solubility of lignin in grasses is greater than that in legumes. The error caused by the inclusion of cell wall fractions in the NFE is lowest in the case of concentrate foods where about three-quarters of the NFE is starch and soluble carbohydrates. In alfalfa, available carbohydrate and organic acids are about 50% of the NFE; whereas, in mature grasses and straws, very

Table 4. Comparison of relative digestibilities of crude fibre and NFE with the proportion of cell wall components (Van Soest 1975).

	Cases where dig. CF≥NFE		Average composition			
	No. of samples	%	No. of samples	Cell wall	Hemicellulose	Lignin
Concentrate (total)						
whole seeds	55	11	5	21	12	2.0
oil meals	24	10	5	33	9	5.7
brans	4	0	3	43	28	2.0
by-products	36	6	6	48	21	3.3
hulls	9	44	4	79	21	11.3
Forage (total)						
temperate legume	104	9	39	43	10	8.5
tropical legume	18	28	—	—	—	—
nongrass nonlegume	49	18	—	—	—	—
annual grasses	71	34	5	59	23	6.2
temperate grass	117	62	26	59	26	4.2
tropical grass	266	74	51	69	28	7.6
straws	9	100	5	74	21	10.4

little of the NFE is available carbohydrate. The effect of this error is to cause the apparent digestibilities of NFE to be less than those of crude fibre in a significant number of cases (Table 4). The presence of a prominent metabolic fraction in fecal NFE contributes greatly to this effect. The proportion of cases where digestibility of crude fibre equals or exceeds digestibility of NFE is about 30% for all feedstuffs, but is notably greater in the forages that contain more hemicelluloses and lignin. The error is largest in tropical grasses and straws.

A further problem with NFE lies in the use of a factor (6.25) to convert nitrogen into an estimate of protein content. True protein forms only about 70–80% of feed nitrogen and only a very little of feces nitrogen so that the application of the 6.25 factor to all feed and feces nitrogen constitutes an error that is reflected mainly in the NFE. The magnitude of this error depends on the nitrogen content of the nonprotein nitrogen compounds and their deviation from the 6.25 ratio. This error is most serious in fecal analysis where little or no true protein at all is ordinarily found and the main nitrogenous constituents are microbial cell walls, which contain 7% nitrogen. Most feces yield considerable NFE upon analysis and calculation but do not ordinarily contain any water-soluble carbohydrates. Insoluble starch is the only nonstructural carbohydrate likely to appear in feces, and then only at high intakes does it appear in substantial amounts.

The Detergent System

The system of analysis employing detergents was originally developed to solve analytical problems relative to ruminant diets, more specifically forages. Since that time (early 1960's) applications have been made to many animal species. The objective of the analysis is the fractionation of foods of plant origin relative to their nutritive availability and fibre content. The extention of the system to a general treatment of herbivorous diets for both ruminants and nonruminants leads to a new set of problems that are presently being faced.

The truly indigestible components of feed are recovered in the neutral-detergent residue (NDF), while acid-detergent divides these into fractions soluble and insoluble in 1 *N* acid. The acid-sol-

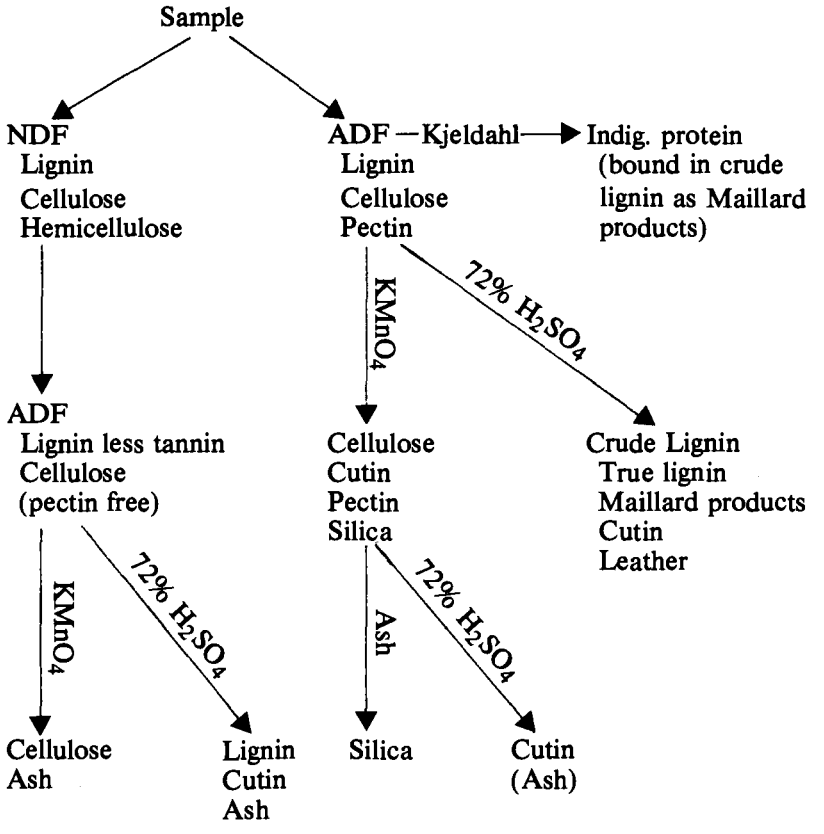

Fig. 1. Sequences of analytical treatments of feed samples subjected to the detergent system: pretreatment with neutral detergent dissolves tannins, pectins, and opaline silica that would otherwise contribute to acid-detergent fibre; permanganate removes tannins but not cutin.

Table 5. Interferences in the estimation of hemicellulose as the difference between neutral-detergent fibre (NDF) and acid-detergent fibre (ADF).

Fraction	Recovery in NDF	Recovery in ADF	Influence on hemicellulose estimate	Reference
Cell wall protein	Recovered	Largely dissolved	Increase	Keys et al. (1969)
Biogenic silica	Considerable solution	Quantitative recovery	Decrease	Van Soest and Jones (1968)
Pectin	Dissolved	Partial precipitation	Decrease	Bailey and Ulyatt (1970)
Tannin	Dissolved	Precipitation as protein complex	Decrease	Robbins et al. (1975)

uble fraction includes primarily the hemicelluloses and cell wall proteins, while the residue (ADF) recovers cellulose and the least digestible noncarbohydrate fractions. Acid-detergent has the advantage of removing substances that interfere with the estimation of the refractory components so that the ADF residue is useful for the sequential estimations of lignin, cutin, cellulose, indigestible nitrogen, and silica (Fig. 1). Silica, in contrast with neutral-detergent, is quantitatively recovered in the acid-detergent residue (Van Soest and Wine 1968).

Plant cell wall as measured by neutral-detergent fibre has proven to be the most fundamental feed characteristic determining feed value. However, it gives quite a poor relationship with digestibility because of the highly variable digestibility of plant cell walls. It follows then that the problem of digestibility prediction is that of estimating cell wall digestibility.

Acid-detergent fibre is widely used as a quick method for determining fibre in feeds, often substituting for crude fibre, but used much on the same basis as proximate analysis. Nitrogen-free extract calculations based on ADF have appeared in the literature although such use has no scientific validity, the hemicelluloses, metabolic fecal matter, and available carbohydrates having been confounded. The use of ADF as a predictor of digestibility is not founded on any solid theoretical basis other than statistical association.

The intended purpose of ADF is as a preparative residue for the determination of cellulose, lignin, Maillard products, and biogenic silica. The Maillard products are formed by complexing of protein and carbohydrate upon heating or drying feeds. The heat damaged protein is totally unavailable through digestion and is recoverable in the fibre, specifically in the lignin fraction. The estimation of heat damage and unavailable nitrogen is rapidly assayed by preparation of acid-detergent fibre and, sequentially, its nitrogen content (Goering et al. 1972).

Interferences in estimating hemicellulose by difference

Neutral-detergent dissolves pectin, tannins, and a variable amount of silica; whereas, acid-detergent recovers silica, the tannin-protein complexes, and pectin partially. Acid-detergent residues are usually lower in protein (nitrogen) than neutral-detergent residues. The influence of these effects on the estimate of hemicelluloses by difference is shown in Table 5. Some of these errors are partly self-canceling when used statistically. Generally, acidic polysaccharides are more likely to be insoluble in acid detergent through precipitation as the quaternary ammonium detergent salts. Pectic acids from legumes, citrus, etc. tend to precipitate giving high values for acid-detergent fibre. The pectins of other plants, e.g. *Brassica*, remain soluble, however (Bailey et al. 1978), and they have recommended that for many purposes where purity of the acid-detergent fibre is sought, neutral-detergent extraction should precede acid-detergent. Preextraction will remove the interferences of pectin, tannins, and silica, although in the case of silica its quantitative measurement will be lost. However, the amount of silica solubilized by neutral-detergent is an estimate of opaline silica. Similarly, estimates of the tannin content can be obtained by the comparison of acid-detergent fibre prepared with and without preextraction with neutral-detergent. A reverse situation exists with regard to cell wall proteins that are soluble in acid-detergent but not in neutral-detergent.

Sequential extraction allows the possibility of alternate routes of analysis which, if performed in parallel, offer the possibility of further differential analysis (Fig. 1). For example, the removal of tannin-protein complexes with neutral-detergent allows parallel lignin analysis to become a means whereby the tannin content can be estimated from the difference obtained between a direct route and that following a preextraction step. Preextraction will allow a more accurate estimate of hemicelluloses, and by difference with the direct, an estimate of interferences that may include pectins, alginates, tannins, etc. A suggested route of analytical sequence is shown in Fig. 2.

The analyses for lignin with permanganate or 24 *N* sulfuric acid (Klason lignin) offers the possibility of separation of the plant cuticle (resistant to $KMnO_4$ oxidation) from the phenolic matter that is oxidizable in permanganate. Preparation of cellulose by oxidative means allows a cuticular fraction to contaminate the cellulose residue.

These problems illustrate the difficulty of designing a single system of analysis for all conditions, and a necessary result is that a single analytical protocol cannot satisfy all conditions. An outline of possible sequences of analysis is shown in Fig. 1. Tannin–protein complexes contaminate the crude lignin fraction unless preextraction by neutral-detergent is done. The alternative lignin procedures by permanganate and sulfuric acid do not measure entirely the same fraction. Cutin is resistant to permanganate oxidation and is also insoluble in 72% H_2SO_4. Its estimation is, therefore, accomplished by the appropriate sequence.

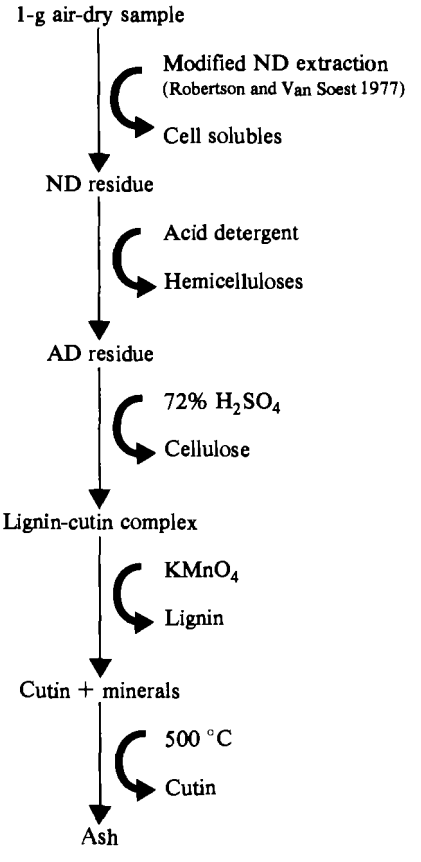

Fig. 2. Flow diagram for sequential analysis.

Procedural Modifications to the Detergent System

A number of procedural alterations have developed out of attempts to overcome certain analytical differences; in particular, the contamination of fibre residues with protein and starch, interferences in the hemicellulose estimate, and difficulties in filtration and handling-related problems in the analysis of certain foods. Some modifications have been made by other groups, including the modification of neutral-detergent fibre by Schaller (1978) and the system devised by Fonnesbeck (1976).

Elimination of decalin

Originally added to overcome foaming problems, this reagent was omitted as a result of the collaborative study on acid-detergent fibre and lignin, where it appeared that decalin increased the fibre yield and contributed to difficult filtering (Van Soest 1973 a,b). Decalin has also been omitted from the preparation of neutral-detergent fibre.

Lipid interference

Fats and oils do not interfer at low levels as long as the detergent can form a stable emulsion. However, at higher levels of lipid (>10%) a separate phase can form. As the detergents (both cetyltrimethylammonium bromide and sodium lauryl sulfate) are soluble in the lipid phase, increased values of fibre can be obtained due to inadequate amounts of detergent in the water phase.

An additional problem of high lipid materials is their greasy character and the resultant difficulty in grinding a dry sample. To solve both of these problems, we have employed the treatment of fresh sample with 4 volumes of acetone or ethanol to prepare a material that can be ground and is sufficiently low in lipid content to avoid interference in the detergent analysis. It is important that this step not involve the use of heat because this will affect the nitrogen content of the fibre.

Protein interference

Protein may cause variation in the analysis of detergent fibres when the protein content is very high such that the sample exceeds the capacity of the detergent to form soluble complexes. This can occur on analysis of samples in excess of 30% protein. It becomes desirable to use a digestion with a protease in this instance. Specific addition of a protease is not ordinarily necessary, because the bacterial amylase employed in one modification of NDF (Robertson and Van Soest 1977) has significant proteolytic activity.

Not all protein or other nitrogen can be removed from vegetable fibres by proteases. Indeed the resistant fraction is more or less recoverable in feces and appears to be indigestible. This nitrogen, which is recoverable in acid-detergent fibre and lignin, is comprised of several fractions, one of which is indigenous to the mature plant, another due to Maillard reactions and heat damage to protein in cooking and baking as well as the tannin-protein complexes already mentioned. It is because the Maillard products are so easily formed as an artifact in sample preparation that drying procedures should be kept below 65 °C.

Sodium Sulfite

This reagent was used to reduce the protein content of neutral-detergent fibre. Sulfite reduces fibre nitrogen content through its ability to cleave disulfide linkages in proteins. This capacity allows it to be a very effective means of eliminating keratinaceous tissues from animal-

derived foods and of such excretions in fecal analysis (Van Soest 1968). However, sulfite unfortunately attacks lignin and causes a significant loss.

Another means of reducing nitrogen content of fibre is through the use of detergent-stable proteases. However, the enzymes will not degrade resistant keratinized animal tissue. There appears at the present moment no satisfactory solution to the analytical problem of separating animal keratin from plant lignin, which is a particular problem of analysis of diets of mixed animal and plant origin. The present consensus is to omit the use of sulfite except as required in specific instances.

Starch interference

One of the main problems of filtering neutral-detergent fibre is starch that tends to form viscous solutions in hot neutral-detergent. The difficulty in filtration is aggravated by cooling during slow filtrations which increases viscosity. Direct NDF preparations in cereals and cereal products often show positive tests to starch (iodine test) indicating that the fibre values are elevated by this contamination.

Two procedures utilizing amylases have evolved to overcome the starch problem. One developed by Schaller (1976) was a hog-pancreas enzyme, while that of Robertson and Van Soest (1977) was an amylase derived from *Bacillus subtilis*. The Schaller procedure requires separate treatment with the enzyme at pH 4.5 and filtration after detergent extraction.

The shorter procedure of Robertson and Van Soest takes advantage of the compatibility and stability of the enzyme in hot neutral-detergent, allowing a more rapid and convenient procedure. The detergent reagent (actually the EDTA in it) inactivates the α1-6 activity but does not restrict the solution of starch. Problems with the method are the need for repeated treatments of the residue to remove starch in some instances and the resistances of modified starches to the enzyme.

Comparison of the two methods in the collaborative work of Schaller shows essentially identical results for most food samples but slightly higher results on foods containing modified starch with the Robertson and Van Soest modification (Schaller 1978).

Modified acid-detergent fibre

The MADF was developed in Ireland using forage standards that had been dried postfeeding at a high temperature in the process of sample preparation. The investigators found that increasing the acid strength and prolonging the boiling time improved the relationship with digestibility of these forages. Subsequent study in Britain using other standards more carefully prepared have not borne out the original observation (Alderman, personal communication). The MADF procedure includes oven-drying at 95 °C as a preliminary step. Unfortunately, this treatment sacrifices the use of acid-detergent as a means of assaying for heat damage and unavailable protein, which is one of the more valuable applications of ADF (Goering et al. 1972).

The Fonnesbeck System

This system of feed analysis (Fonnesbeck 1976) is very similar to the detergent system and is essentially derived from it. Determination of plant cell wall is conducted at pH 3.5 with sodium lauryl sulfate, having been preceded by a pepsin digestion. This is done to reduce the nitrogen content of the fibre and to eliminate starch interference. The analysis proceeds sequentially, hemicellulose being extracted with 4% H_2SO_4 then the lignocellulose residue being treated with 72% H_2SO_4 to remove cellulose and isolate lignin. Lignin is determined as loss in weight on ashing, the residue being acid insoluble ash.

Critique

The procedure sacrifices some of the speed of the detergent system in order to obtain purer fibre fractions. It is not certain that removal of nitrogen from cell walls is a benefit as it appears that this entity is real and associated with insoluble protein and the fraction promoting maximal protein output from the rumen (Pichard and Van Soest 1977). The sequence does not provide the alternatives of the detergent system where tannins, cutin, and Maillard products can be fractionated out of the crude lignin. Results from this procedure have not been compared with other modifications of the detergent system.

The Southgate System

This system (Southgate 1969a,b) was developed for human foodstuffs low in dietary fibre. In this method (Fig. 3) the dietary fibre is fractionated into lignin, cellulosic polysaccharides, and noncellulosic polysaccharides. The latter fraction may be subdivided into water soluble and water insoluble noncellulosic polysaccharides. The polysaccharides are estimated as their constituent simple sugars by chemical rather than gravimetric means with the choice of spectroscopy, gas-liquid chromatography, or high performance liquid chromatography depending on the degree of sophistication desired.

About 5 g of the sample is extracted with 85% methanol to remove the free sugars, then about

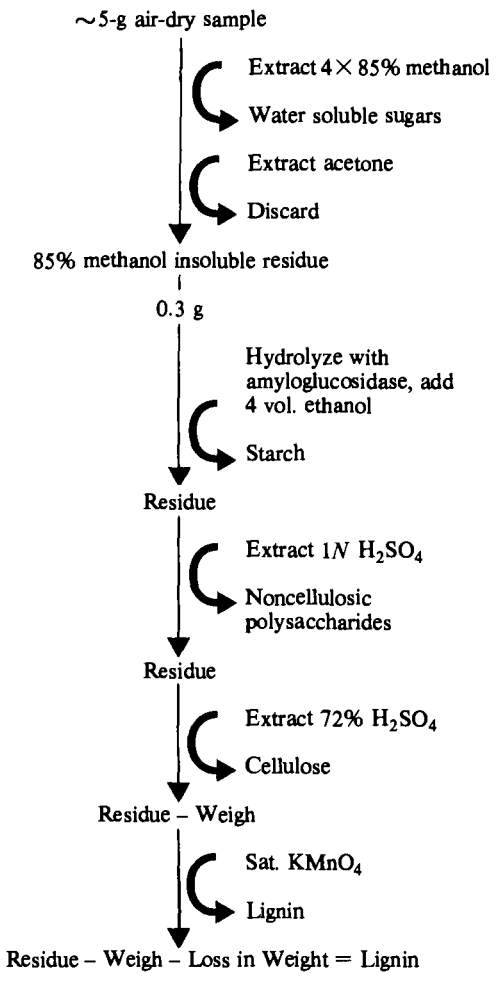

Fig. 3. Flow diagram for sequential analysis in Southgate's system.

0.3 g of the methanol extracted residue is incubated with amyloglucosidase to remove the starch. After removal of the starch the residue is sequentially extracted with 1 N H_2SO_4 to remove the noncellulosic polysaccharides and 72% H_2SO_4 to hydrolyze the cellulose. The residue is then weighed, oxidized with saturated $KMnO_4$, and reweighed to give an estimate of the lignin. The sugars in the various extracts are then measured and the dietary fibre estimated by summation of the noncellulosic polysaccharide, cellulosic polysaccharide, and lignin fractions.

Critique

This system has the benefit of recovering the pectins in the noncellulosic polysaccharide fraction. However, the system does not lend itself to rapid analysis and the precision of the chemical methods may not justify the time and labour required. Although the analytical methods, especially GLC and HPLC are very precise, the extractions are not definitive in their fractionation of the carbohydrate. However, where sugar analysis is required, Southgate's system is probably the method of choice unless more exacting extractions can be justified. McConnell and Eastwood (1974) have reported that the ADF procedure is as precise as the Southgate methods for cellulose and lignin, and perhaps preferable if preceded by a ND extraction because no artifact lignin is produced. An integration of the Van Soest and Southgate methodology may be the route to a rapid, precise system of analysis.

Recommendations and Future Needs

While the desirability for a uniform system of feed analysis is great, the complexity of different purposes and applications may preclude recommendation of a single system at the present time. There are at the present time a number of groups working toward standardization of food and feed analysis and a political problem exists in coordinating the efforts of diverse groups in different places. Coordination is essential if a unified system is to emerge.

Dietary fibre has been defined as the plant polymeric substances resistant to animal digestive enzymes (Van Soest 1978) a definition endorsed by the EEC-IARC working committee in dietary fibre. This group has recommended the discontinuance of crude fibre. The AOAC recommended the discontinuance of nitrogen free extract (NFE) in 1940.

Another suggestion for the replacement of crude fibre is the determination of cellulose (ISO). This suggestion is inadequate because cellulose represents only a portion of the total fibre and is a variable portion of it. The need is for an account of unavailable residues.

The two most commonly used systems in present use are the detergent system of Van Soest and the system devised by Southgate in England. These systems while different in approach give similar results for many feeds and foods (McConnel and Eastwood 1974). The principal difference between the two systems is in regard to the soluble components that are resistant to mammalian digestive enzymes. The EEC-IARC Committee has recognized the problem of soluble substances including pectin and gums and points to the need for method development.

Further definition and refinement of procedures should make careful consideration of the problem of laboratory economy. The needs of detailed research differ from those of the quality control laboratory for broad surveys. Here the need arises for two compatible systems: a rapid one for surveys; and a second for compositional detail. A detailed system of analysis will preferably entail component analysis of cell wall constituents via suitable chromatographic and identification procedures.

The systems in question are available in modifications that allow applications to almost all kinds of feedstuffs. Because data derived by these procedures are far superior to that obtained by proximate analysis, it is recommended that where choice is possible the newer methods should replace crude fibre. The attitude of the EEC-IARC Committee is that crude fibre be abandoned even if an alternative analysis is unavailable. Crude fibre should be deleted from existing tables of composition because its use has been mischievous and misleading in human nutrition.

Prediction of Energy Digestibility of Forages with In Vitro Rumen Fermentation and Fungal Enzyme Systems

Gordon C. Marten[1] and Robert F. Barnes[2]

Two-stage in vitro rumen fermentation (acid-pepsin or neutral-detergent second stage) has become the method of choice for estimating relative energy digestibility of all types of forages. This is true even though numerous sources of variation must be controlled by the individual laboratory to ensure accurate and repeatable in vitro values.

We present two specific recommended in vitro rumen fermentation procedures that were developed collaboratively by members of a North Central Regional Research Committee (NC-64). One of these procedures (A) is a modification of the Tilley and Terry (1963) procedure, principally in that it employs a smaller sample (250 mg rather than 500 mg) and has the option of addition of urea to the bicarbonate buffer to aid digestion of samples having large amounts of soluble carbohydrates. The second procedure (B) is strikingly modified in that it employs direct acidification, without centrifugation, at the end of stage one; this is made possible by substitution of a phosphate buffer (with or without urea) for the original bicarbonate buffer. Both procedures include the optional use of 24-h instead of the usual 48-h second stages (acid-pepsin); this option facilitates routine analysis of large numbers of samples by allowing completion of one or two in vitro runs within a normal work week.

The difficulty of standardizing in vitro rumen fermentation techniques among laboratories, and the expense associated with routine analyses, have resulted in limited application of these techniques for testing of farmers' samples.

Nylon bag in vivo rumen fermentation methods are useful in research programs that require an assessment of the influence of rumen conditions on digestion of limited numbers of samples. Although they have been successfully used for mass screening of forage samples, they have been largely supplanted by the easier-to-standardize in vitro methods.

Recent evidence suggests that fungal cellulase digestibility methods may be able to satisfactorily predict the in vivo digestibility of most forages (after pretreatment with either acid-pepsin or neutral detergent). Cellulase techniques are more convenient than in vitro rumen techniques in that they do not require a source of rumen fluid, and they may be more precise. However, the cellulase techniques appear to be more sensitive to forage species variation, and commercially available cellulases vary considerably in their digestive capacity. More studies are needed to confirm the merit of the cellulase procedures successfully used by several laboratories.

Establishment of In Vitro Rumen Fermentation as an Elite Technique for Forage Quality Evaluation

Pioneering research describing the use of rumen fluid in an "artificial rumen" technique (later called in vitro rumen fermentation) was conducted by Clark and Mott at Purdue University, and reported by Clark (1958). Pigden (Pigden and Bell 1955) was a Canadian pioneer of in vitro rumen fermentation research.

Most in vitro rumen fermentation methods used for forage evaluation are not designed to completely duplicate all rumen conditions, but rather to provide a final result that predicts in vivo parameters. Pigden (1969) outlined two variations of early in vitro rumen fermentation systems: (1) A one-stage digestion in rumen fluid followed by a cellulose determination of the residue to provide digestible cellulose values correlated

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with in vivo available energy; and (2) A short one-stage digestion followed by a cellulose determination on the residue to provide digestible cellulose values correlated with nutritive value index (an estimate of total digestible energy intake per unit of metabolic size of an animal; Donefer et al. 1960).

Although digestible cellulose was often estimated in initial in vitro rumen fermentation research, the almost universal acceptance of the Tilley and Terry (1963) two-stage procedure (or a vast array of modifications thereof) obviated use of cellulose measurements. This technique attempts to measure not only the digestible fibrous fraction of herbage, but the digestible soluble fraction as well. The second stage involves the solubilization of the residue from the first stage by acid-pepsin. The acid-pepsin acts to simulate the in vivo breakdown of feed and microbial protein by the digestive enzymes of the ruminant abomasum.

Also, addition of the second stage (pepsin digestion) to in vitro systems sometimes eliminates the need for development of regressions to predict dry matter loss in vivo from dry matter loss in vitro, in that the two stage in vitro values are often close to in vivo values. Pigden (1969) called the basis for this the "digestion ceiling" concept, because digestion of a forage normally proceeds in vivo until the available cellulose and hemicelluloses of the cell wall constituents are essentially exhausted (their availability being largely determined by the type and extent of lignification).

Van Soest et al. (1966) proposed the use of neutral-detergent solution (neutral-detergent fibre assay) as a substitute for acid-pepsin in the second stage of the Tilley and Terry (1963) procedure. The neutral-detergent solution solubilizes more total dry matter than does acid-pepsin because neutral-detergent solubilizes bacterial cell walls and other endogenous products in addition to protein. Therefore, the Van Soest modification predicts true digestibility rather than apparent digestibility.

While early reports (Simpkins and Baumgardt 1962; Pigden 1969) indicated that two-stage in vitro techniques were unsuitable for estimating in vivo digestibility of silages, Schmid et al. (1975) found that a modification of the Tilley and Terry method (which incorporates urea in the buffer as a nitrogen source) was the best of numerous biological and chemical methods for estimating in vivo digestibility of 51 corn and sorghum silages. Correlations between in vitro and in vivo digestible dry matter of $r = 0.83$ for corn silages and $r = 0.91$ for sorghum silages were obtained. Dowman and Collins (1977) also reported that the Tilley and Terry (1963) procedure accurately predicted the in vivo digestibility of perennial grass silages.

Barnes (1973) presented 16 sets of correlation coefficients and standard errors of estimate between in vitro rumen fermentation and in vivo measurements of digestibility or nutritive value index. In 12 of these cases, correlations (r) between in vitro and in vivo measurements were 0.87 or higher, and they were never lower than 0.71. Standard errors of estimate ($s_{y \cdot x}$) were commonly less than 3.0.

Two-stage in vitro rumen fermentations have become universally recognized as methods of choice to predict digestibility of all types of forages. They are often the standards of excellence against which other procedures are compared for estimating forage digestibility (Terry et al. 1978; McLeod and Minson 1979).

Sources of Variation in Digestibility Estimates via In Vitro Rumen Fermentation Methods

Barnes (1973) reviewed the voluminous literature concerning development, modification, and application of in vitro rumen fermentation methods for estimating forage quality. A brief summary of his systematic review and of more recent reports of the many sources of variation associated with in vitro fermentation systems follows.

Fermentation Vessel

Fermentation vessels that have been primarily used include various vented glass and plastic containers. However, sealed culture vessels have been successfully used because the end products of fermentation apparently do not deter microbial action. Sayre and Van Soest (1972) found that 122 × 28 mm glass centrifuge tubes provided lower in vitro dry matter digestibility (IVDMD) values than did 125 ml Erlenmeyer flasks or 200 × 25 mm glass screwcapped tubes, most likely because they had difficulty providing sufficient agitation of the centrifuge tubes during fermentation (some particles adhered to the rubber stopper during shaking). They also reported fermentation vessel × sample size interactions, and fermentation vessel × forage species interactions. Moore and Mott (1976) reported that polyethylene centrifuge tubes gave higher IVDMD values than did polycarbonate tubes; forage particles formed dense mats that were raised above the level of the media by entrapped gas only in the polycarbonate tubes. They also found that whereas vacuum infiltration of water into samples before inoculation (used by Minson and McLeod

(1972) to reduce floating in polycarbonate tubes) increased IVDMD with both types of tubes, omission of vacuum infiltration gave satisfactory and repeatable results in polyethylene tubes.

Buffer-Nutrient Solution

The buffer-nutrient solution controls pH and supplies nutrients for the rumen microorganisms during fermentation. Buffer-nutrients may include carbohydrates, N, and minerals, but the majority of in vitro procedures rely on "artificial sheep saliva" (McDougall 1948) as the primary buffer. Supplementation of the buffer with N (urea or ammonium sulfate) is recommended for feedstuffs having large quantities of available carbohydrate (Schmid et al. 1969). Nelson et al. (1972) found that rumen fluid efficiency was affected by the diet of the donor animal (legume hay, grass hay, or maize silage) unless both urea and glucose were added to the inocula. These additives had less effect on IVDMD values and on standard deviations when the diet of the donor, the substrate, or both contained a relatively high percentage of crude protein. We have found no need to supplement the buffer-nutrient solution with available energy for assay of any forage (our donor animals receive high-quality alfalfa hay as their primary ration).

Inoculum Source, Processing, and Amount Used

The inoculum represents the greatest source of uncontrolled variation in in vitro rumen fermentation systems. Standard herbage samples must be included in each in vitro run to measure variability among runs and to determine when an entire run should be discarded. Digestive capacity of rumen inoculum may be influenced by animal species, breeds within species, individuals, and within animal variation from time to time. Form or type of donor diet has frequently been found to influence inoculum efficiency. While some investigators have contended that variability in inoculum digestive capacity may be controlled by feeding the donor animal a ration similar to the substrate being tested, Nelson et al. (1972) reported that their study (including donor diets of legume or grass hays or maize silage) did not support this thesis. Also, Grant et al. (1974) found no differences among rumen fluid sources (Philippine water buffalo, Holstein-Red Sindhi cow in the Philippines, or Holstein cow in New York) in capacity to digest a great diversity of forages. On the other hand, the Philippine ruminants provided a more effective rumen fluid for digesting tropical grasses, rice straw, and pineapple pulp to which they were adapted and to which the New York cow was not adapted.

Slyter and Weaver (1972) reported that cellulolytic bacteria possessed less cellulase activity, but no reduction in numbers, when grain was added to the forage diet of the donor animal. Researchers generally agree that donor animals should not receive grain in their diets to achieve best in vitro digestion with minimum variability.

Rumen (cow) microbes were more efficient in digesting grass hays and wheat straw than were cecum (pony) microbes in both in vitro and nylon bag procedures (Koller et al. 1978); however, high-quality alfalfa hay was equally well digested by both inoculum sources.

Attempts have been made to improve the uniformity of rumen inoculum by various processing methods. However, simple straining of collected rumen fluid through cheesecloth is a satisfactory approach, and more complex procedures have not proved greatly advantageous.

The amount of inoculum above a minimum does not affect in vitro values if its ratio with the substrate and buffer is held constant. The rate of fermentation may increase if inoculum amounts are increased without a commensurate increase in buffer-nutrient solution (McLeod and Minson 1969).

Anaerobiosis

Although the freshly collected inoculum can be aerated considerably without loss of activity, exposure to air and unnecessary delays in inoculation should be avoided. Most researchers recommend CO_2 gasing over the inoculated substrate before stoppering of the fermentation vessel with gas release valves or before sealing of screw cap culture tubes. The earlier-used continuous bubbling of CO_2 through the inoculated substrate during incubation is not needed.

pH

The volatile fatty acids produced during in vitro fermentation depress pH; however, pH will normally be maintained within recommended limits of 6.7–6.9 if the donor animal is fed hay. McLeod and Minson (1969) found the highest in vitro digestion at pH 6.7 and lowest at 6.1; a pH of 7.2 gave intermediate digestion. During the acid-pepsin second stage, a pH of 1.2 should be maintained for optimum results.

Temperature

Because the first stage of the in vitro rumen fermentation procedure is temperature-sensitive, incubation should be at 38.5 or 39 °C.

Sample Size

Numerous researchers have found that sample size can at times influence in vitro values. For example, Sayre and Van Soest (1972) found that increasing the substrate sample size from 250 to 500 mg did not change digestibility in centrifuge tubes, but digestibility increased with sample size in Erlenmeyer flasks while it decreased in screwcapped tubes. However, variation can be controlled if the concentrations of buffer-nutrient and rumen inoculum are maintained in constant ratio with the amount of substrate. Although the Tilley and Terry (1963) method calls for a 500-mg sample, many laboratories have successfully utilized 250-mg samples, and our recommended procedures include the smaller sample size.

Sample Preparation

Drying and grinding procedures influence in vitro rumen fermentation much as they do numerous other laboratory assays. Oven drying at excessively high temperatures produces indigestible artifacts. Usually, drying at 65 °C or less is recommended. Fine grinding (about 0.5 mm) is highly desirable, but 1-mm particle size is also satisfactory. McLeod and Minson (1969) found that digestibility of five grass species increased when particle size was reduced from 2 mm to 0.4 mm, except for *Setaria* spp. for which the largest particle size gave the highest digestibility.

Length of Incubation

Usually the curve for rate of digestion in stage one of in vitro rumen fermentation systems is sigmoid, with an initial lag phase of up to 12 h. The curve plateaus at 18–24 h and often becomes asymptotic at about 48 h. Therefore, a 48-h first stage is usually recommended. However, because of greater concentrations of soluble cell contents, the initial rates of digestion of legumes are faster than those of grasses, and the digestion ceiling is reached sooner for legumes. Also, Grant et al. (1974) reported that true DM digestion in vitro increased when incubation time was increased from 48 to 96 h when substrates were tropical grasses, rice straw, and pineapple pulp.

Reduction of incubation time during the second stage from 48 to 24 h has been proposed to facilitate scheduling for routine analyses. Slightly reduced IVDMD values are obtained from a 24-h second stage incubation; however, satisfactory in vitro – in vivo relationships have been reported (Barnes 1966; Larsen and Jones 1973).

[3]These procedures were developed jointly with other members of a subcommittee of NC-64; we have made further additions and modifications.

Recommended Procedures for Determination of In Vitro Dry Matter Digestibility[3]

One of the primary accomplishments of the NC-64 North Central Regional Research Committee in the United States was the documentation of an in vitro rumen fermentation system that could be recommended for estimating in vivo digestibility. The proposed methods outlined below are suggested as having potential for laboratories just initiating an in vitro system of forage evaluation and for use by on-going laboratories to compare with their current procedure. The methods are not claimed to be superior to others, because there is no perfect method adaptable to all circumstances. Indeed, we have already modified the original direct acidification method (method B) to best adapt it to the forage quality laboratory facility in the Agronomy and Plant Genetics Department at the University of Minnesota. However, the procedures described give reproducible in vitro dry matter and organic matter results, as verified by collaborative trials.

Any laboratory engaged in forage quality evaluation must establish its own "standard" procedure, which has been tested and proven reliable through the use of forage samples with known in vivo and in vitro results. Thus, methods other than those outlined below may be more appropriate for use in a specific laboratory.

Modification of the Two-Stage Tilley-Terry Method

Apparatus

(1) Polyethylene or glass 50-ml centrifuge tubes and appropriate racks to hold tubes upright.

(2) Rubber stoppers for item 1 fitted with a gas release valve (Tilley and Terry 1963; Harris 1970).

(3) Fritted glass filtering crucibles (coarse porosity, 40–60 microns and crucible holders for use during filtration.

(4) Permanent laboratory equipment, including pH meter preferably with combination electrodes (usable in centrifuge tubes), analytical balance, drying oven, muffle furnace, centrifuge, incubator, incubation bath, and oxygen-free CO_2 from a regulated source.

(5) Other expendable laboratory supplies such as beakers, Buchner funnel, cheesecloth, Erlenmeyer flasks, glass tubing, graduated cylinders, insulated flask, side arm suction flask, thermometer, and tongs.

Reagents

(1) Buffer-nutrient solution (McDougall 1948). The following quantities are used for 1 litre of

buffer: 9.8 g $NaHCO_3$; 7.0 g $Na_2HPO_4 \cdot 7H_20$ (3.71 g anhydrous); 0.6 g KCl; 0.5 g NaCl; 0.1 g $MgSO_4 \cdot 7H_20$; and 0.5 g urea (optional). Mix in ± 500 ml of distilled water in a 1 litre volumetric flask and stir until dissolved. Use distilled water to bring to volume, and then store. *Just prior* to use, add 0.04 g $CaCl_2$, keep at 39 °C and bubble CO_2 into the solution until pH is 6.8–7.0.

(2) 5% weight/volume mercuric chloride: add 5 g $HgCl_2$ to 100 ml volumetric flask and bring to volume with distilled water.

(3) 1 *N* Na_2CO_3: add 143 g $Na_2CO_3 \cdot 10\ H_2O$ to 1 litre volumetric flask and bring to volume with distilled water.

(4) 1 *N* HCl: add 86 ml concentrated HCl to 1 litre volumetric flask and bring to volume with distilled water.

(5) Acid-pepsin solution (must be freshly prepared for each run): add 2 g of 1:10 000 pepsin or equivalent and 100 ml of 1 *N* HCl to 1 litre flask and bring to volume with distilled water.

(6) Strained whole rumen fluid inoculum: a cow or steer fitted with a rumen fistula should be fed alfalfa hay, or a forage similar to the sample substrates, twice daily. Attention should be given to providing minerals and nitrogen if needed. Feed intake of the donor animal should be limited to approximately 1 kg hay/100 kg of liveweight per feeding. Time of daily feeding and the sampling of rumen contents should remain constant relative to time of feeding. Rumen contents should be obtained in a manner that is routine and standardized for each laboratory. The rumen contents should be processed by squeezing through four layers of cheesecloth and collecting the rumen fluid in a prewarmed insulated container. Rumen fluid should not drop below 39 °C and it should be exposed to an atmosphere of CO_2 whenever possible, preferably by bubbling the CO_2 throughout the fluid.

Inoculum blanks

The strained whole rumen fluid inoculum contains indigestible material that must be taken into account when calculating results. Therefore, inoculum blanks containing buffer-nutrient solution and rumen fluid inoculum are processed through both the fermentation and pepsin incubation stages. Six inoculum blanks interspersed throughout the forage samples are suggested for each in vitro run. The average dry matter residue of the inoculum blanks is used in calculating in vitro dry matter digestibility values.

Procedure

Weigh about 250 mg of sample on weighing paper or similar material and quantitatively transfer it to a 50-ml centrifuge tube. Weigh duplicate samples into dry tared containers for dry matter determination. The dry matter samples are dried for 24 h at 105 °C and hot weighed or weighed after cooling 30 min in a desiccator. Whenever possible, all samples for all runs should be weighed out within a short period of time. Add two 10-ml portions of buffer-nutrient solution to the centrifuge tube containing the 250 mg sample. Gently mix the contents between additions of buffer and wash down the sides of the tube with the second portion of buffer. Allow the tubes to stand at 39 °C for a short period to permit saturation of the substrate. Collect and prepare the rumen fluid inoculum during this time, which should not exceed 30 min. Care must be taken to maintain the pH of the buffer-nutrient solution between 6.8 and 7.0.

Add 5 ml rumen fluid inoculum per tube. Flush the surface of tube contents with CO_2 for approximately 10 sec before stoppering with the gas release valve. Incubate the tubes at 39 °C for 48 h.

Gently rotate the tubes at approximately 2, 4, 20, and 28 h after initiation of incubation to disperse the forage particles.

After 48-h incubation, add 1 ml $HgCl_2$ solution, 2 ml of Na_2CO_3 solution, and centrifuge for 15 min at 2000 × gravity to sediment the suspended dry matter. Decant supernatant carefully to avoid loss of dry matter.

Add 25 ml of acid-pepsin solution and mix gently. Incubate tubes without stoppers for 48 h at 39 °C. Gently rotate tubes to resuspend the residue at approximately 2, 4, 20 and 28 h after initiation of incubation.

After 48 h, filter the tube contents through a tared fritted glass crucible. Dry to constant weight at 105 °C. The residue retained on the filter is undigested dry matter. Crucibles should be weighed after cooling in a desiccator or weighed hot directly from the 105 °C oven on a single pan analytical balance.

Calculations

In vitro dry matter digestibility:

$$\text{IVDMD (\%)} = 100 \times \frac{\text{Samp. DM} - \left(\text{Resid. DM sample} - \text{Mean resid. DM inoc. blank}\right)}{\text{Sample DM}}$$

where DM = dry matter.

Verification

Nineteen laboratories participated in a study using this procedure to estimate in vivo digestibility of 12 hays of temperate species (*Medicago sativa*, *Phalaris arundinacea*, *Bromus inermis*, and *Festuca arundinacea*) having in vivo digesti-

bility ranging from 50 to 67% DMD. Average in vitro DMD ranged from 50 to 70%. The correlation coefficients between in vivo and in vitro DMD ranged from 0.79 to 0.97 for individual laboratories, with an average r value of 0.93 and a standard error of estimate of 2.5 (Barnes 1970).

Direct Acidification Method

The direct acidification method is a modification of the method outlined above in that the centrifugation step following the initial 48-h fermentation is eliminated. The use of a phosphate buffer as outlined below greatly facilitates the procedure through avoidance of the excessive frothing that occurs with a bicarbonate buffer.

Reagents

(1) Buffer-nutrient solution (Kansas State buffer). Solutions A and B outlined below can be made in the volumes desired and stored separately for several weeks. *Just prior* to use, add 20 ml of solution B to each litre of solution A. The exact amount of solution B added to solution A should be adjusted so as to obtain a final pH as close to 6.8 as possible. No further adjustment of pH is necessary. Prewarming of solution A to 39 °C is recommended. Solution A (quantities in g/litre distilled water): KH_2PO_4 10.0; $Mg_2SO_4 \cdot 7H_2O$ 0.5; NaCl 0.5; $CaCl_2 \cdot 2H_2O$ 0.1; and Urea (reagent grade) 0.5 (optional). Solution B (quantities in g/100 ml distilled water): Na_2CO_3 15.0; and $Na_2S \cdot 9H_2O$ 1.0.

(2) 6 *N* HCl: add 516 ml concentrated HCl to 1 litre volumetric flask and bring to volume with distilled water.

(3) Pepsin powder: 1:10000 pepsin or equivalent.

Procedure

Using the phosphate buffer, follow the two-stage method outlined above until the end of the initial 48-h fermentation.

After 48-h fermentation, directly acidify by adding 1 ml 6 *N* HCl to each tube and mixing gently. Then add 0.1 g pepsin powder to each tube and mix. (A 1 ml HCl solution containing 0.1% pepsin may be used instead of adding acid and pepsin separately.) Incubate at 39 °C for 48 h without stoppers. Gently rotate tubes to resuspend the residue at about 2, 4, 20, and 28 h after starting incubation.

After 48-h incubation, filter the tube contents through tared fritted glass crucibles. The residue retained on the filter is undigested dry matter. Dry to a constant weight at 105 °C. Crucibles should be weighed after cooling in a desiccator under vacuum or weighed hot directly from the 105 °C oven on a single-pan analytical balance. Calculations remain the same as the two-stage method outlined above.

Verification

We compared methods A and B in Minnesota (using the urea reagent option in both cases) for predicting in vivo DMD of eight grass and alfalfa hays provided by the NC-64 committee and of 25 maize and 26 sorghum silages (Schmid et al. 1975). Mean DMD of the hays measured by in vivo, method A, and method B was 56, 54, and 58%, respectively. Correlations of $r = 0.85$ and 0.87 occurred between A or B and in vivo methods, respectively.

In the silage study, DMD means, standard deviations, and correlations (r) with in vivo DMD were as follows:

	Maize silages			Sorghum silages		
	Mean DMD (%)	S.D.	r	Mean DMD (%)	S.D.	r
In vivo	64	3	–	56	5	–
Method A	72	3	0.83	61	7	0.91
Method B	70	2	0.50	58	6	0.91

The correlations of A versus B values were $r = 0.67$ and 0.95 for maize and sorghum silages, respectively. While all correlations were statistically significant ($p \leq 0.05$), the relationship between method B and in vivo DMD for maize silages was too low for predictive purposes. Thus, we concluded that direct acidification in vitro rumen fermentation cannot be recommended for use with maize silages. We presented simple regression equations (Schmid et al. 1975) to predict in vivo DMD from in vitro DMD in the other three cases; standard errors of prediction ranged from 1.78 to 1.95.

Other Alternatives

Modification of the Filtering System

The use of filter paper and a filter funnel is suggested as an alternative to the use of fritted glass crucibles. Hardened filter paper such as Whatman No. 54[4] is recommended. A filter cone, such as the nickel filter cone (Sargent S-32615)[4] placed

[4]Mention of a trade name, proprietary product, or vendor does not constitute a guarantee or warranty, and does not imply their approval to the exclusion of other products or vendors that may also be suitable.

Table 1. Proposed schedule for routine two-stage in vitro rumen fermentation analyses (direct acidification and 24-h second-stage period).[a]

Day	Morning	Afternoon
Monday	Inoculation (1)	Weigh samples and place in oven (3)
	Weigh residue (0)	
	Calculate results (0)	
Tuesday	Inoculation (2)	Weigh samples and place in oven (3)
	Weigh sample (3)	
	Weigh oven-dry samples (3)	
Wednesday	Add HCl-pepsin (1)	Weigh samples and place in oven (3)
	Weigh oven-dry samples (3)	
Thursday	Add HCl-pepsin (2)	Weigh oven-dry samples (3)
	Filtration (1)	
Friday	Filtration (2)	Calculate results (1)
	Weigh residue (1)	

[a]Based on two runs per week of approximately 150 samples per run, where: 0 = previous week; 1 = run 1 of current week; 2 = run 2 of current week; and 3 = future week.

in a fluted filter funnel, may be used with 9-cm filter papers. Filter paper may be tared as follows: (1) break seal on box of 100 filter papers and allow them to equilibrate to lab conditions; (2) weigh (air dry) individual filter papers indicating weight on each paper; (3) select 10 filter papers at random, dry 24 h at 105 °C, and hot weigh to determine oven-dry weight; and (4) use the dry matter percentage of these 10 filter papers to calculate the oven-dry tare weight of the entire box of filter papers.

Hot weighing individual filter papers after drying reduces the variation in tare weights that may occur from the absorption of moisture by papers during the process of weighing. This is more of a problem when large numbers must be weighed under conditions of high humidity.

Modification of Length of Second-Stage Incubation

Reducing the length of incubation in the acid-pepsin stage from 48 to 24 h is suggested as an alternative for individual laboratories to consider. Such a modification greatly facilitates scheduling for routine analysis of large numbers of samples by allowing in vitro runs to be completed within one normal work week.

Laboratories attempting to adapt such a modification should verify the reliability of their in vitro results, particularly if interlaboratory comparisons are contemplated.

Proposed Schedule

The proposed schedule for routine two-stage in vitro rumen fermentation analyses (direct acidification and 24-h second-stage incubation period) is given in Table 1.

Use of Standard or Index Samples

Standard or index forage substrates in duplicate or triplicate are recommended for inclusion in each in vitro run. Standard forage samples of known high and low in vivo digestibility relative to the forages being tested should be used.

The digestive efficiency of the rumen fluid inoculum and pepsin solution used in a given in vitro run may be assessed with such standards. Procedures for adjustment of in vitro values to correct for run-to-run variation have been suggested

(Baumgardt et al. 1962; Minson and McLeod 1972; Tilley and Terry 1963).

Nitrogen Supplementation of In Vitro Medium

Nitrogen supplementation in the form of urea is included as an optional part of the buffer-nutrient solution in the above procedures. Nitrogen supplementation is particularly recommended when studying feedstuffs containing large quantities of readily available carbohydrates (Schmid et al. 1969). It may also reduce the analytical error associated with variations in rumen inoculum source and amount (Alexander and McGowan 1966). However, N supplementation should be used with caution when evaluating forages and feedstuffs that are not supplemented under practical feeding conditions.

Application of In Vitro Rumen Fermentation Methods

We agree with Pigden (1969) that estimation of absolute digestibility is not the primary application of in vitro rumen fermentation procedures. Rather, these procedures are unparalleled for predicting relative digestibility differences among a wide range of forage species and types. Plant breeders and forage production and management researchers find in vitro rumen fermentation to be a valuable technique, as do ruminant nutritionists who need to predict forage quality of vast numbers or greatly varying forage samples grown in limited quantity. The wide acceptance of in vitro rumen fermentation methods by researchers is evidenced by our recent title search of journal publications. Over 300 publications since 1970 include "in vitro digestibility" or "in vitro dry matter disappearance" in their titles.

Burton and Monson (1978) provided a recent example of success in using in vitro rumen fermentation in a forage breeding program (numerous other examples could be cited). They released 'Tifton 44' Bermuda grass partly on the basis of its 5–6% greater IVDMD than the cultivar 'Costal'. Tifton 44 gave 19% better average daily gain of steers than did Coastal when both were grazed or fed as pellets.

Richardson et al. (1976) reported that the in vitro effect of monensin was reproducible in vivo when they fed both concentrate and forage rations to cattle. When maize cobs and alfalfa were used as substrates in either case, propionic acid production increased while acetic and butyric acid production decreased.

In the Plant Science Research Unit (USDA-SEA-AR) at the University of Minnesota we have published more than 20 journal articles based on use of in vitro rumen fermentation to predict forage digestibility in grazing, plant breeding, and forage management trials.

We agree with McQueen (1978a) that in vitro rumen fermentation has only limited application to routine quality testing of producer samples, because most feed analysis laboratories consider the method to be too time-consuming and too difficult and expensive to use for farm feeds. The difficulty in getting commercial laboratory analysis via the in vitro technique has led the American Forage and Grassland Council's Hay Marketing Task Force to recommend acid-detergent fibre for routine prediction of forage digestibility of farmers' samples.

Status of Nylon Bag Methods for Forage Quality Evaluation

The "nylon bag" methods involve placement of forage substrate, in bags made of indigestible fabrics such as nylon, Dacron, or silk, directly in the rumen (in vivo). Both rate and extent of substrate digestion may be measured by loss of dry matter or specific nutrients after specific incubation periods. Barnes (1973) and Pigden (1969) reviewed the literature concerning development and application of nylon bag methods. Both pointed out that a major shortcoming of these methods is that they are difficult to standardize and that they are subject to considerable variability. Another disadvantage is that they require a large sample size (about 10 g dry matter).

However, several researchers reported high correlations between nylon bag in vivo digestibility and conventional in vivo digestibility and/or in vitro rumen fermentation values of a variety of forages (Barnes 1973; Monson et al. 1969). Also, Burton et al. (1967) released 'Coastcross-1' Bermuda grass largely because of its 12% greater digestibility than 'Coastal,' and the digestibility improvement was accomplished via a selection program using a nylon bag method.

Status of Fungal Enzyme Methods for Forage Digestibility Evaluation

Establishment of Cellulase and Related Techniques

Donefer et al. (1963) compared the efficacy of using the purified enzyme "Cellulase 36" (Rohm and Haas, Philadelphia), aqueous solutions, or both to replace the relatively inconvenient and difficult-to-standardize in vitro rumen fermentation systems for predicting energy digestibility of forages. They used either cellulase or cellulase + pepsin in KH phthalate solution (pH control) as

Table 2. Results obtained by Donefer et al. (1963) using eight temperate legumes and six grasses as substrates.

Laboratory treatment	Correlation (r) with in vivo energy digestibility (%)	Range of absolute digestibility (%)
Cellulase	0.68	22–45
Cellulase + pepsin	0.70	23–49
Acid-pepsin	0.73	24–46
Distilled water	0.61	20–33
12-h in vitro cellulose digestion (rumen fermentation)	0.73	27–55

cellulolytic media, and obtained the results given in Table 2 with eight temperate legumes and six grasses as substrates.

The in vivo energy digestibility of these forages ranged from 53 to 67%. Obviously, the cellulase treatments were not accomplishing anything beyond that of acid-pepsin alone.

In contrast, Jarrige et al. (1970) in France and Guggolz et al. (1971) in Nebraska reported correlations between solubility in cellulase and in vivo DOM or DDM of up to 0.92 and 0.90, respectively, for mixed temperate forages. Jarrige et al. (1970) used a one-stage (24-h) procedure and cellulase (*Basidiomycete* source) supplied by a French company; they found a better prediction of in vivo digestibility via their cellulase procedure than via the Tilley and Terry (1963) in vitro procedure. Guggolz et al. (1971) used a two-stage procedure that employed Onozuka SS cellulase in stage one (72-h) and "Pronase" (protease) in stage two (overnight); they found a poorer prediction of in vivo digestibility by this method than by a modified Tilley and Terry in vitro method. In Minnesota, Schmid et al. (1975) obtained variable correlations (r= –0.42 and +0.72) between solubility of maize silages and sorghum silages, respectively, in cellulase (48-h stage one) and acid-pepsin (24-h stage two) compared to in vivo digestibility. We used Onozuka SS cellulase; our modified Tilley and Terry in vitro method predicted in vivo digestibility of both types of silages far better than did cellulase-acid pepsin. However, Autrey et al. (1975) found that cellulose content of maize silage that had been ensiled with *Trichoderma viride* cellulase was lower (31%) than that of untreated silage (34%).

Other recent reports have substantiated the merits of cellulase techniques for estimating digestibility of many forages. Jones and Hayward (1973) described a one-stage procedure based on a *T. viride* preparation (BDH Ltd., Poole, Dorset, England) that had cellulase, hemicellulase, and proteolytic activity. They reported very satisfactory prediction of in vivo DMD with this assay for five temperate grass species (Table 3), although absolute digestion values were more than 20 percentage units lower than those for in vitro or in vivo DMD. Pulli (1976) confirmed the merits of this approach for grass and a grass-clover mixture in Finland (Table 3). Dowman and Collins (1977) modified the one-stage procedure of Jones and Hayward (1973) by using a finer sample grind (0.75 mm), by increasing the concentration of cellulase, and by reducing the digestion time from 48 to 24 h. This modified method was as good a predictor of DOMD of grass silages as was Tilley and Terry (1963) in vitro, and it was highly correlated with in vivo DOMD (Table 3).

McQueen and Van Soest (1975) found a significant correlation between enzyme digestion and in vivo digestion of 18 grass and legume hays (Table 3), but their endorsement of enzymatic procedures was restricted by the need for separate estimates for individual species or groups of species. They also reported that enzyme sources varied in digestive capacity.

Jones and Hayward (1975) modified their 1973 method to include pretreatment of herbage with acid-pepsin before digestion in cellulase; this method allowed similar prediction equations for both grasses and legumes (Table 3). They tested four fungi sources, and concluded that *T. viride* (BDH Ltd.) was the most active on both herbage and cellulose paper. Adegbola and Paladines (1977) confirmed the observation of Jones and Hayward (1975) that predigestion with acid pepsin improves the solubility of herbage DM in cellulase solutions (Table 3); they used a *T. viride* cellulase from a New Jersey source to digest 11 tropical grasses and legumes. Goto and Minson (1977), using Onozuka SS cellulase and 48-h pepsin pretreatment (rather than the 24 h used by Jones and Hayward 1975), also concluded that in vivo DMD of both tropical and temperate grasses could be accurately predicted by the pepsin-cellulase assay (Table 3).

Terry et al. (1978) further tested the pepsin-cellulase (BDH Ltd.) procedure of Jones and Hayward (1975). They confirmed the reliability, accuracy, and precision of the pepsin-cellulase method for predicting in vivo DMD of grasses (Table 3). However, Terry et al. (1978) agreed

Table 3. Procedures and digestibility relationships for selected fungal enzyme assays of forages reported between 1973 and 1979.

Reference	Forage type	Primary type of digestion	Correlation with in vivo or in vitro digestibility (r)	Prediction equation (y=% in vivo DMD; x=% enzyme DMD)	Error expression
Jones & Hayward (1973)	Temperate grasses	Cellulase	0.92 (vivo)	$y = 0.72x + 33.0$	RSD 2.5
Pulli (1976)	Temperate grass	Cellulase (Jones & Hayward 1973)	0.99 (vitro)	—	RSD 1.3
	Grass & red clover		0.99 (vitro)	—	RSD 0.9
Dowman & Collins (1977)	Grass silage	Cellulase (modified Jones & Hayward 1973)	0.89 (vivo)	$y = 0.58x + 31.6$ (organic matter)	RSD 2.3
McQueen & Van Soest (1975)	Temperate grasses & legumes	Cellulase + hemicellulase	0.80 (vivo)	—	S.E. 6.0
Jones & Hayward (1975)	Temperate grasses	Pepsin + cellulase	0.96 (vitro)	$y = 0.61x + 30.4$	RSD 2.4
	Temperate legumes		0.94 (vitro)	$y = 0.60x + 31.6$	RSD 2.7
Adegbola & Paladines (1977)	Tropical grasses & legumes	Pepsin + cellulase (Jones & Hayward 1975)	0.98 (vivo)	—	$s_{y \cdot x}$ 2.3
Goto & Minson (1977)	Tropical & temperate grasses	Pepsin + cellulase (modified Jones & Hayward 1975)	0.94 (vivo)	$y = 0.69x + 20.3$	RSD 2.7
Terry et al. (1978)	Temperate grasses	Pepsin + cellulase (Jones & Hayward 1975)	0.92 (vivo)	$y = 0.56x + 34.7$	RSD 1.8
McLeod & Minson (1979)	Tropical & temperate grasses	Pepsin + cellulase (Goto & Minson 1977)	0.94 (vivo)	$y = 0.70x + 18.2$	RSD 2.6
	Tropical & temperate legumes		0.91 (vivo)	$y = 0.60x + 22.2$	RSD 3.1
Roughan & Holland (1977)	Temperate grasses & legumes	Neutral-detergent fibre + cellulase	0.98 (vivo)	$y = 0.98x - 10.12$	RSD 2.8

with McQueen and Van Soest (1975) when they reported that the pepsin-cellulase method was decidedly less accurate than the Tilley and Terry (1963) in vitro method for predicting digestibility of temperate legumes or grass-legume mixtures (separate regression equations were needed for each legume species). On the other hand, McLeod and Minson (1979) found that their modified Jones and Hayward (1975) pepsin-cellulase method could be used to estimate the in vivo DMD of legumes with an error only slightly higher than that for grasses, and that the regressions for legumes and grasses were similar (Table 3). They concluded that their results may have differed from those of Terry et al. (1978) in that they used much higher concentrations of cellulase and a superior "broad spectrum Onozuka cellulase." McLeod and Minson (1979) also concluded that in vivo DMD of legumes and grasses can be predicted by the pepsin-cellulase method while using the same equation; however, to eliminate bias they suggested that samples of known digestibility similar to those being tested should be included as standards in each run (some species may require completely different regressions).

Further tests by McLeod and Minson (1979) indicated that fineness of sample grind (1 mm or 0.4 mm), incubation temperature (39 or 50 °C), cellulase concentration (2.5% or 0.625% w/v cellulase solution), and incubation time (24 h or 48 h for each stage) had very little effect on pepsin-cellulase prediction of digestibility. Use of a 0.5 g, rather than 0.2 g, sample size provided lower standard deviations.

Roughan and Holland (1977) in New Zealand claimed that none of the cellulase methods proposed in the literature (including that of Jones and Hayward 1975) solubilized nearly as much organic matter as is digested in vivo, so they decided to try a different approach; this approach was to take the cellulase digestion to completion by using a highly active enzyme preparation. They selected a "potent cellulase solution" prepared from culture filtrates of an artificially-produced mutant of *Trichoderma* identified as the new species, *T. reesei* Simmons (obtained from NLABS Culture Collection of Fungi, Department of Botany, University of Massachusetts). While cell walls of untreated whole, dried forage were either not attacked by this cellulase or only very slowly, cell walls isolated by neutral detergent extraction were readily hydrolyzed. Thus, they substituted neutral detergent for the acid-pepsin of earlier methods (much as Van Soest et al. 1966, substituted neutral detergent solution for acid-pepsin in the Tilley and Terry in vitro rumen fermentation procedure).

This two-stage neutral detergent extraction followed by "exhaustive hydrolysis" with standardized cellulase (Roughan and Holland 1977) was highly correlated with in vivo DMD of grasses and legumes (Table 3). This procedure gave absolute values higher than in vivo DMD! Because they decided that the best way to ensure a continuing supply of active enzyme was to produce it from fungal cultures grown in their own laboratory, they described a detailed procedure for producing the cellulase.

Conclusions Regarding Fungal Enzyme Techniques

We have made the following conclusions regarding the use of fungal enzymes for predicting energy digestibility of forages:

1. Recent evidence indicates that fungal cellulases are often able to predict the digestibility of forages (after pretreatment with either acid-pepsin or neutral detergent) nearly as well as in vitro rumen fermentation.

2. The application of the pepsin-cellulase method described by Jones and Hayward (1975) and the modifications thereof by Goto and Minson (1977) and McLeod and Minson (1979) to legumes as well as grasses needs confirmation in other laboratories. Fungal cellulases appear to be more sensitive to forage species variations than are rumen inocula.

3. The added benefits of use of neutral detergent pretreatment of substrates followed by a standardized "potent cellulase solution" such as described by Roughan and Holland (1977), in order to take the digestion by cellulase to completion, need confirmation.

4. Because some forage species respond differently than others to cellulase enzymes, standard samples of each species under test with known digestibility should be included in each cellulase assay.

5. The activity of the selected cellulase should be measured before routine use via incubation of standard forage samples and/or cellulose paper at several enzyme concentrations.

6. Because cellulases may vary greatly in their capacity to digest forage fibre, further research is needed to standardize the activity of marketed cellulase preparations. Production of standardized cellulases within each analytical laboratory may also resolve this problem.

Relationships of Conventional and Preferred Fractions to Determined Energy Values

D. J. Minson[1]

The digestibility of forages may be estimated by conventional chemical analysis with an error varying from ± 4.1–7.6%. This error may be reduced if the regressions for estimating energy value are restricted to a narrow range of forages. The preferred methods of laboratory analysis are based on in vitro methods using rumen fluid or cellulase and these give estimates with errors of ± 2.3–2.7%. The most accurate predictions of energy value can be obtained by using the in vitro method as a true bioassay, comparing the test sample with a standard of known in vivo energy value.

New feeding systems are now based on metabolizable energy (ME) and there is a need for more forage samples of known ME value for use in standardizing laboratory methods for predicting ME. In the absence of ME standards, ME may be estimated from the digestible organic matter percentage (D) of the feed. Conversion factors relating ME to D are similar for both temperate and tropical forages.

There is need for more standard forages of known ME value, particularly tropical forages. However, the most urgent need is to determine the factors associated with differences in ME utilization by animals.

It is now recognized that the metabolizable energy (ME) in different feeds is utilized with different efficiencies according to the form of the animal production, the ME concentration of the diet, and the feed type (Blaxter 1977). This discovery has led to the development of new feeding systems that have three basic components: (1) lists of ME values of feeds; (2) conversion equations relating net energy (NE) to ME; and (3) tables of animal requirements in terms of NE.

In the new NRC system the feed tables contain not only the ME value but also the best estimate of the NE values of each feed for different forms of production. These NE values are estimated from the ME value and the most appropriate conversion equations. Using this approach the three component system is effectively reduced to a two component system, thus facilitating the calculation of least cost rations. In the United Kingdom no attempt has been made to list estimated net energy values alongside the ME values of individual feeds. This has led to a rationing system (MAFF 1975) that is more complicated and lacks the desired flexibility inherent in the NRC system (Minson 1978).

Tables of ME values can readily be produced for grains and roots, which have relatively constant ME values. There are difficulties with roughages, which vary widely in energy value according to stage of growth and method of conservation. Feed tables will list the ME values of roughages, but for many advisory and research purposes more accurate estimates of ME will be required.

In this paper I will consider laboratory methods for predicting ME, the error associated with the use of these equations, and the ways this may be reduced. Finally, I will be outlining future work that I believe is required in the area of the laboratory prediction of forage energy value.

Regression Equations for Predicting ME

The United Kingdom Ministry of Agriculture, Fisheries and Food has published (MAFF 1975) fourteen different equations for predicting the ME value of roughages (Table 1). The need for this large number of equations appears to be based on the assumption that the relation between ME and feed composition varies between feeds and that ME can be predicted satisfactorily by many different laboratory methods. Morgan (1974) describes the way many of these equations

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were derived and provides an estimate of the error associated with any predicted ME value (Table 1). The first four equations are based on 47 hays fed at maintenance level to sheep in digestion trials. Digestible energy (DE) was measured and ME values calculated as proposed by Armstrong (1964) (ME = 0.81 × DE). Other equations appear to be based on ME values calculated from in vivo determinations of the quantity of digestible organic matter in the feed (DO). DO is converted to

Table 1. Regressions for predicting the metabolizable energy value (kcal/g/feed) (y) from the composition of the dry matter (%).

Feed	Regression[a]	RSD	RSD as % of mean
47 Grass hays[b,c]	y = 4.08 – 0.053 MADF	± 0.18	9.1
	y = 3.23 – 0.37 ADF + 0.033 CP	± 0.17	8.4
	y = 0.20 + 0.033 IVD	± 0.15	7.1
	y = 0.11 + 0.03 IVD + 0.03 CP	± 0.13	6.5
Grass hays[c]	y = 3.18 – 0.45 CF + 0.041 CP	—	—
Fresh Grass[c]			
All samples	y = 3.80 – 0.045 MADF	—	—
Regrowths only	y = 3.97 – 0.053 MADF	—	—
Fresh legume	y = 2.94 – 0.029 MADF	—	—
All feeds	y = 0.05 + 0.033 IVD + 0.024 CP	—	—
Dried grass	y = 3.35 – 0.033 MADF	—	—
Dried grass	y = 3.32 – 0.041 CF	—	—
Silage[c]	y = 2.61 + 0.05 CP – 0.011 MADF – 0.014 DM	—	—
Silage[c]	y = 1.29 + 0.053 CP + 0.014 IVD – 0.014 DM	—	—
Primary growth only[c]	y = 1.20 + 0.045 CP + 0.017 IVD – 0.012 DM	—	—
Maize silage[c]	y = 3.35 – 0.031 MADF – 0.007 DM	—	—

[a]MADF = modified acid-detergent fibre %; ADF = acid-detergent fibre %; CP = crude protein %; IVD = in vitro digestible organic matter in the dry matter %; CF = crude fibre %; and DM = dry matter %.

[b]Morgan 1974.

[c]MAFF 1975.

Table 2. Regressions for predicting metabolizable energy value (kcal/g) (y) from the composition of the feed.

Feed	Regression[a]	RSD	RSD as % of mean
	DRY MATTER BASIS		
16 grasses[b]	y = 1.81 + 0.064 CP	± 0.158	5.8
	y = 4.64 – 0.083 cellulose	± 0.137	5.0
	y = 3.52 – 0.147 lignin	± 0.127	4.7
	y = 4.20 – 0.057 CF	± 0.109	4.0
	y = 4.06 – 0.063 hemicellulose	± 0.103	3.8
	ORGANIC MATTER BASIS		
16 grasses[c]	y = 1.89 + 0.067 CP	—	6.0
Excluding orchard grass[c]	y = 1.76 + 0.082 CP	—	—
16 grasses[c]	y = 4.65 – 0.062 CF	—	4.7
Excluding meadow fescue[c]	y = 3.92 – 0.175 CF	—	4.7
16 grasses[c]	y = 0.042 IVD – 0.05	—	3.1

[a]CP = crude protein %; CF = crude fibre %; L = lignin (Ellis) %; IVD = organic matter digestibility in vitro %.

[b]Armstrong 1964.

[c]Armstrong 1966.

ME by assuming a constant calorific value of 4.4 kcal/g for the DO to give DE, from which the ME can be calculated (ME = DO × 4.4 × 0.81). This equation simplifies to ME = DO × 3.56 kcal/g, which is similar to the value of 3.6 published by van Es (1978) for green fodders and conserved green fodders. For corn silage a conversion factor of 3.7 is recommended (van Es 1978).

Armstrong (1964) *measured* the ME value of the dry matter of sixteen grasses and published equations relating ME at maintenance to the concentration in the dry matter of five chemical fractions (Table 2). Using these equations ME values of low temperature (inlet temperature 120 °C) dried spring growths could be estimated with an error of ±3.8% from hemicellulose analysis and ±5.8% for protein analysis. Armstrong (1966) used the same measured ME values to derive regressions relating ME of the feed organic matter to the chemical composition of the feed organic matter and the quality of organic matter that disappeared in vitro (Table 2). The lowest error (±2.5%) was found when ME was estimated from the organic matter disappearance in vitro, compared with 6.0% for a regression based on crude protein. No other equations appear to have been published relating measured ME values and chemical composition of the feed.

Table 3. Laboratory analyses used to estimate digestibility of forages.

	Energy component[a]	Correlation coefficient (*r*)	RSD
Conventional fractions			
Crude protein[b]	OMD	—	± 6.2
Crude protein[c]	ED	+ 0.79	± 5.2
Lignin (ADF)[d]	OMD	- 0.83	± 4.8
Methoxyl[e]	OMD	- 0.42	± 3.8
Neutral-detergent fibre[d]	OMD	- 0.49	± 7.6
Acid-detergent fibre[f]	OMD	- 0.76	± 5.3
Modified acid-detergent fibre[g]	DMD	- 0.75	± 4.8
Normal acid fibre[h]	OMD	- 0.84	—
Crude fibre[i]	OMD	- 0.94	± 5.2
Crude fibre[c]	ED	- 0.87	± 4.1
Preferred fractions			
Dry matter disappearance			
(a) Rumen fluid[j]	DMD	—	± 2.3
(b) Cellulase[k]	DMD	+ 0.94	± 2.7

[a] OMD = organic matter digestibility %; DMD = dry matter digestibility %; and ED = energy digestibility %.

[b] Minson and Kemp 1961.

[c] Phillips and Loughlin 1949.

[d] Bosman 1970.

[e] Kivimae 1960.

[f] Sullivan 1964.

[g] Clancy and Wilson 1966.

[h] Griffith and Thomas 1955.

[i] McMeekan 1943.

[j] Tilley and Terry 1963.

[k] Goto and Minson 1977.

Regression Equations for Predicting Energy Digestibility

Only a few regressions have been published relating measured ME values of roughages and their chemical composition. Eventually more forage samples of measured ME value will become available for calculating regressions, but until then reliance will be placed on regressions used to predict energy digestibility or the closely correlated parameters of organic matter and dry matter digestibility. These estimated digestible energy values will then be converted into ME values using the constants mentioned in the previous section.

Many plant fractions have been used to predict digestibility and a representative sample of these regressions is presented in Table 3 together with the residual standard deviation (RSD), which is the most useful criteria for comparing analytical methods. The RSD, also called the standard error of the regression, is a measure of the scatter about the line and is the minimum error that must always be applied to any energy value predicted from the regression. The value of the RSD is relatively independent of the range of energy values in the forages chosen for the regression. The RSD is calculated as follows:

$$RSD = \sqrt{\frac{SSy - \frac{(SPxy)^2}{SSx}}{n-2}}$$

where: x = independent variable, i.e. analytical result; y = dependent variable, i.e. energy value; n = number of pairs of observations; SS = sum of squares; and SP = sum of products.

Residual Standard Deviation

The residual standard deviations of the regressions listed in Table 3 vary from a high of ± 7.6 percentage units for a regression based on neutral-detergent fibre to a low value of 2.3 percentage units for the in vitro method using rumen fluid. Analysis for crude protein is quicker, simpler, and cheaper than the in vitro rumen fluid technique and it is very tempting to dismiss the importance of the higher RSD. The argument put forward in the past to justify this action was that the RSD is caused solely by the errors in estimating the in vivo digestibility value and composition of the feed. It is now realized that these two sources of error are very small compared with that caused by differences between forages. In 1959 we showed that the regression relating OMD to crude protein level in the forage was very different for grasses and alfalfa (Minson and Brown 1959). We also found different regressions applied to different months of the year (Minson and Brown 1959; Minson and Kemp 1961). Subsequent work has shown that similar differences among forages occur with all laboratory methods of estimating the digestibility of forage. Even with the in vitro method, which has the lowest RSD, pasture species differences have been found (McLeod and Minson 1969). Once regressions are based on a restricted range of forages there is usually a much lower RSD (Table 4).

Table 4. Residual standard deviation of regressions relating dry matter digestibility to different fractions of five legumes (adapted from McLeod and Minson 1976).

	All legumes	*Desmodium* spp.	*Lablab purpureus*	*Macroptilium atropurpureum*	*Vigna sinensis*	*Medicago sativa*
Number of samples	32	5	7	8	4	8
DMD in vitro	± 2.7	± 2.9	± 2.7	± 1.1	± 1.6	± 1.4
ADF	± 2.2	± 1.3	± 1.7	± 2.0	± 1.2	± 1.9
Modified ADF	± 2.1	± 1.9	± 1.8	± 1.4	± 1.4	± 1.7
Lignin (Van Soest)	± 3.7	± 1.0	± 2.9	± 2.0	± 3.0	± 3.5
Lignin (Christian)	± 2.8	± 3.0	± 2.2	± 1.5	± 2.4	± 2.5
Crude fibre	± 3.7	± 2.2	± 2.2	± 2.3	± 1.6	± 2.5
Nitrogen	± 4.0	± 2.3	± 2.4	± 3.2	± 3.2	± 3.5

Preferred Fraction

There is no simple answer to the question: "Which is the best method of estimating energy value?" The quickest and simplest methods have the highest RSD and for some advisory purposes this possible bias may be quite acceptable. However, where estimates are required with the lowest RSD then there is little doubt that the in vitro method based on rumen fluid or cellulase is obviously the most desirable, being applicable to a wide range of feeds. The data for ME regression are limited (Tables 1 and 2) but this also shows that the in vitro digestion technique provides more reliable estimates of ME value than can be achieved with any chemical fraction.

As indicated by Marten and Barnes (1979) the in vitro method based on rumen fluid has many problems arising from the source of the rumen fluid. These problems may be overcome by dispensing with published regressions relating in vivo to in vitro results and considering the in vitro method as a true bioassay (McLeod and Minson 1969). When used as a bioassay technique the sample to be tested is compared with a standard sample for which the in vivo energy value has previously been measured. For the most accurate results the standard used should be as similar as possible to the forage being tested and the energy value measured with the same class of stock as will be finally used when the forage is eventually fed. Where the forage has a digestibility greater than 60% there is little difference in dry matter digestibility between sheep and cattle but with lower digestibility feeds large differences are common and results cannot be safely transposed.

The problem with the in vitro method based on rumen fluid is not only the unavoidable week to week variation in the activity of the rumen fluid but the need to have fistulated animals close to the laboratory. In some countries special licences are required for keeping fistulated animals and their maintenance is often expensive. Jones and Hayward (1975) developed a method in which rumen fluid-pepsin was replaced by pepsin-cellulase. The cellulase used was expensive and the method gave different results for grasses and legumes (Terry et al. 1978). Recently McLeod and Minson (1978) published a method, using much larger quantities of a cheap Onozuka cellulase, that gave similar regressions for grasses and legumes. With this method the agreement between duplicate samples was much better than with the rumen fluid method and abnormal results were absent. The most important feature was the elimination of the week to week variation experienced when using rumen fluid. Another advantage of the pepsin-cellulase method is that it can be speeded up by shortening hydrolysis times to 24 h with only a small increase in the error.

Conversion Factors

The starting point of most new feeding systems is ME so there can be little argument that all regressions should estimate ME. This ideal is impossible to achieve because very few forage samples are available for which in vivo ME values are known. Therefore, there is little chance of deriving regressions, let alone using them as standards in the in vitro methods.

This problem has been overcome in the United Kingdom (MAFF 1975) by assuming that digestible organic matter has a constant energy content of 4.54 kcal/g and that ME = 0.81 DE. These two factors were derived with temperate forages. But, are they applicable to tropical forages?

The energy value of the digestible organic matter in tropical feeds was studied by Minson and Milford (1966). With a wide range of samples of *Sorghum almum*, *Digitaria decumbens*, and *Macroptilium atropurpureum*, they reported mean energy contents of 4.37, 4.19, and 4.53 kcal/g disgestible organic matter, respectively, with an overall mean of 4.36 kcal/g.

In recent studies I have measured the energy value of digestible organic matter in a range of panicum varieties and legumes and in the separated leaf and stem fractions. The results of these studies are summarized in Table 5 and indicate that the *mean* energy value of the digestible organic matter in tropical forages is virtually the same as that found in temperate forages.

The energy value of the digestible organic matter is not constant but varies from 3.86 to 4.89 kcal/g (Table 5). If a mean value of 4.40 kcal were used in all conversions, the DE and hence ME value could have an error of 12%. This variation in energy content appears to be caused by the quantity of protein in the digestible organic matter. Minson and Milford (1966) reported the following relation between energy value of the digestible organic matter and crude protein %(X) of 60 tropical forages.

$$\text{Energy in DOM} = 3.382 + 0.759 \log (X-10)$$
$$r = 0.83^{**}$$

Very little is known about the ME value of tropical forages. Graham (1967) measured with sheep the ME value of three tropical forages and at the maintenance level of feeding ME = 0.807 DE. Using buffalo fed different roughage/concentrate diets Gill et al. (1976) found DE to ME conversions of 0.797–0.852 with a mean of 0.822. Both these results are not sufficiently different

Table 5. The energy content of the digestible organic matter in forages grown in the tropics.

	Number of samples	Energy mean	kcal/g DOM range
Sorghum almum[a]	31	4.37	4.22–4.57
Macroptilium atropurpureum[a]	13	4.53	4.27–4.80
Digitaria decumbens[a]	16	4.19	3.86–4.43
Digitaria decumbens[b]	8	4.24	4.02–4.43
Panicum sp.[b]	57	4.49	4.14–4.77
Vigna sinensis[b]	6	4.26	4.08–4.44
Trifolium repens[b]	1	4.71	
Medicago sativa[b]	3	4.79	4.60–4.89
Digitaria decumbens[b]			
Leaf	2	4.47	4.45–4.48
Stem	2	4.33	4.20–4.47
Chloris gayana[b]			
Leaf	2	4.44	4.42–4.45
Stem	2	4.49	4.35–4.62
Mean			
Grasses	120	4.40	3.86–4.77
Legumes	23	4.50	4.08–4.89
All samples	143	4.42	3.86–4.89

[a] Minson and Milford 1966.

[b] Minson unpublished.

Table 6. The efficiency of utilization of metabolizable energy for fattening or gain.

	ME (kcal/g)	Net availability of ME for fattening	NE kcal/g
80% barley[a]	3.07	62.7	1.93
All dried grass[a]	2.94	38.1	1.01
Early cut grass[b]	2.34	43.5	1.02
Late cut grass[b]	2.31	32.5	0.75
1st cut grass[c]	2.84	45.2	1.28
3rd cut grass[c]	2.58	32.8	0.84
White clover[d]	2.76	51.0	1.41
Ryegrass[d]	2.91	33.0	0.96

[a] Wainman et al. 1970.

[b] Corbett et al. 1966.

[c] Blaxter et al. 1971.

[d] Rattray and Joyce 1974.

from the 0.81 used in the United Kingdom to justify recommending any conversion factor other than 0.81.

Future Research

The accuracy of an ME estimate will always depend on the availability of reliable in vivo standards of known ME content. The most urgent need, especially in the tropics, is the in vivo measurement of the ME of a wide range of forages and the distribution of these standard samples to laboratories wishing to estimate ME values of forages. I believe these standards must be *measured* ME values and not DE values doctored for energy losses in the urine and as methane. If we cannot afford to produce these standards properly then we should use a feed system based on DE and not ME.

Many analytical fractions have been examined in the past 40 years, and it is nearly 20 years since the breakthrough was achieved with the in vitro method based on rumen fluid. The in vitro method has been simplified by the use of Onozuka cellulase and the low RSD is unlikely to be further reduced. It only remains to test the method under a wider range of conditions to ensure that it is completely free of any legume/grass differences. It is most unlikely that any chemical method will give lower RSD values than the existing methods and attempts to find a new chemical method should be given a low research priority.

The estimation of ME is only the first requirement of the new feeding systems. To be of any value the ME values must be accurately converted into Net Energy (NE) values. The conversion factors vary widely according to feed type, even when the ME concentration in the feeds are similar (Table 6). There is an urgent need to determine the cause of these differences. Until we know what factors affect this conversion and how we can accurately predict NE from ME there is perhaps no need to further improve the prediction of ME from laboratory analyses.

Description of Sugarcane Feeds: Nomenclature and Nutritional Information

E. Donefer and L. Latrille[1]

Different types of sugarcane (*Saccharum officinarum*) feeds are described and suggestions made to standardize their nomenclature. Feeds described include sugar factory by-products (molasses, bagasse, bagasse pith) as well as feeds derived from unprocessed plants (cane tops, whole plant, derinded stalks). Some inconsistencies in published (NRC) values for chemical composition and energy values are identified and recommendations made to improve the evaluation of the original data prior to dissemination. Standard analytical methods used by the sugar industry (total sugars expressed as invert and degrees brix), and the neutral-detergent soluble fraction, are used to describe the feeding value of sugar-containing feeds such as molasses and whole cane. Forage-type sugarcane feeds, such as cane tops and bagasse, can be nutritionally described by chemical and in vitro procedures used for forage evaluation purposes. It is recommended that prior to publication original technical information be evaluated by individuals experienced with the different feed types and that generalized regression equations to calculate nutrient availability from chemical data be used with caution. As direct determinations of energy values for sugarcane feeds are very limited, efforts to produce this specific information should be encouraged.

By way of definition, the term sugarcane feeds can be used to describe feedstuffs obtained as a by-product of the manufacture of sugar, or whole plant or plant parts used directly as feeds (i.e. no sugar extraction is involved).

To be specifically recognized is the considerable information on the use of sugarcane feeds (molasses, bagasse, whole plant) contributed since the mid 1960s by T.R. Preston and his associates in Cuba, Mexico, the Dominican Republic, and other countries.

Increasing interest in the last decade in the direct feeding of the whole sugarcane plant has also resulted in part from studies in the Caribbean, supported by the Canadian International Development Agency (CIDA), that culminated in late 1976 in the establishment in Trinidad of a Sugarcane Feeds Centre (SFC)[2] whose terms of reference include the determination of the technical and economic feasibility of cattle production systems based on sugarcane-derived feeds.

Of particular relevance is the system of documenting the worldwide use of sugarcane feeds, which our group has been developing, and which presently contains references to over 500 sugarcane feed-related documents contained in a computer-aided retrieval system that forms part of a Sugarcane Feeds Information Service (SFIS). References to this data source will be brief as detailed considerations are not within the scope of this paper.

National Research Council Feed Nomenclature and Nutritional Information Service

Over a period of many years, the Sub-Committee on Feed Composition of the U.S. National Research Council has been developing a standardized system of feed nomenclature and has collected nutritional information from a wide variety of sources for a large number of feeds. We will be referring in this paper to information on sugarcane feeds derived from this NRC system as it has appeared in several sources, but with an assumed common origin from the documentation collection maintained at the International Feedstuffs Institute at Utah State University.

It is also assumed that the NRC system and data form an important part of the International Network of Feed Information Centres (INFIC) program as described by Haendler (see p. 114).

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[2]McGill University has been contracted by CIDA to be its agent in the execution of this project.

Although our remarks about the publication of data from the NRC system in relation to sugarcane feeds are critical, they are offered in the most constructive spirit, and in the belief that such feeds documentation systems should play a crucial role in the development of animal production systems in all countries. Indeed, we hope that our comments will lead to discussions as to what positive steps might be taken to ensure the technical validity of the information offered as services to others who do not have the resources available to critically evaluate the accuracy of such computations.

Types of Sugarcane Feeds

Upon our request, the International Feedstuffs Institute (Utah) recently supplied a list of 45 different international names for feeds derived from sugarcane (*Saccharum officinarum*). We have taken the liberty to condense this list to the format presented in Fig. 1, and would indicate that many more permutations can be obtained by considering different forms (i.e. fresh, dry, ensiled), plant maturities, treatments, and other factors.

In comparing the various sources of sugarcane feeds data published over the past seven years (1971–78), there are apparent changes in nomenclature and many apparent contradictions and errors in the data presented. It can be concluded that with any new system there is a natural evolution, so that newer versions are more accurate. As will be seen later in the presentation of specific nutritional data, the magnitude of these changes and errors cannot be easily explained (in fact no explanation is generally given with tabular presentations), and thus they constitute a serious impediment to the credibility of "standardized" documentation systems.

The left-hand portion of Fig. 1 indicates the International Feed Name (or names, when more than one has been used), and only one reference number, although several are available if different aspects (processes, maturity etc.) are used to describe the feed. The International Feed Number cited in Fig. 1 for the first six sugarcane feeds represents the fresh or "wet" form.

In this section only aspects of nomenclature will be dealt with; nutritional considerations will be presented later. The most common initial practice in harvesting mature sugarcane for sugar factory use is to cut off the top of the plant ("*top of aerial part*") which can be left in the field or gathered for use as livestock feed. The *cane stalk* thus represents the plant part that enters the sugar factory (A), where it is processed to extract as much of the sucrose as possible. The two major factory by-products are the plant's fibrous residues (*bagasse*), and the residual syrup (*molasses*) remaining after the removal of as much of the sugar as possible through crystallization and centrifugation processes. (The older "evaporation process" for sugar preparation, still in use in many parts of the world, would result in a much less complete separation of the sugar from other components of the syrup).

It is fortunate that NRC appears to have dropped their earlier use of the term "pulp" and has accepted the conventional term "bagasse" in later publications as the description for this plant part. It is less fortunate that a satisfactory NRC term has not been presented for a product prepared from bagasse of which there is some interest as a

Int. Feed Name	Int. Feed No.	Common Names
aerial part	(2-04-689)	whole plant
├→top of aerial part	(2-13-568)	cane tops
└→stems or stalks	(2-13-248)	cane stalks
(B) ├→ pith	(2-13-564)	derinded stalks, sugarfith, Comfith
(stalks wo rind)		
└→rind	(2-20-761)	rind
(A) ├→bagasse or pulp	(2-09-909)	bagasse
└→pulp, sifted	(1-04-700)	bagasse pith, bagacillo
├→molasses	(4-04-696)	cane, blackstrap or final molasses
└→molasses, dehy	(4-04-695)	dry (dehydrated) molasses
└→sugar, raw	(4-13-569)	raw sugar
├→sugar, brown	(4-13-578)	brown sugar
└→sugar	(4-04-701)	white (refined) sugar

Fig. 1. International and common names for sugarcane-derived feeds.

potential livestock feed (bagasse pith or bagacillo). The term "pulp, sifted" (McDowell et al. 1974) is neither satisfactory nor accurate because it does not clearly indicate which portion constitutes the material after the "sifting" or depithing process (and actually infers the wrong fraction). Actually, depithing is used to obtain a product consisting primarily of external (rind) fibres, which is a superior product for board or paper manufacture, leaving the fine siftings (internal fibres) as a by-product. It is thus recommended that the conventional term "*bagasse pith*" be used to designate this latter fraction.

It should also be indicated that the cane molasses referred to by NRC is not the only type produced, as in its widest definition molasses can be used to refer to any of the sugar-containing syrups obtained during processing. Cane (blackstrap) molasses is the final product in that further sucrose cannot be effectively crystallized from this syrup. Other molasses types are characterized by higher sugar and lower ash contents, and although primarily for human consumption, their use has been investigated in swine and poultry rations.

Also produced primarily for human consumption are the crystallized sucrose forms such as raw and refined sugar. These sugars have also been successfully incorporated into animal rations, but such use is highly influenced by cost factors. The NRC use of the term brown sugar is not clear, because in many countries this human food product is produced by adding pigmentation (from sugar processing sources) to refined sugar.

An alternative pathway (B) in sugarcane processing (Fig. 1) is related to a "new" Canadian-developed "separation" system. This process, developed for potential sugar factory use, would replace the energy-intensive cane crushing with a procedure that derinds the cane stalks, thus making the plant interior available to subsequent sugar extraction procedures. Relevant to this paper is the possible direct use of the derinded sugar-containing cane stalks as a livestock feed. The NRC term "*pith, chopped*" (McDowell et al. 1974) for this feed, is *not satisfactory or accurate*, as it confuses this product with the sugar-free pith fraction from bagasse, and does not imply the presence of the sugar, which, of course, has the most significant effect on its potential feeding value. It is recommended that the International Feed Name for this plant part be "*stalks wo rind.*" Some authors commonly refer to this feed as derinded sugarcane (not completely accurate as only the stalk is derinded and cane tops need not be present), "Comfith" (a trade name whose use should be discouraged in technical literature), and sugarfith, a common name that designates the major components of the feed. Sugar represents over 50% of its dry matter content and the term "fith" has been introduced as a description of the combination of two physically different ligno-cellulosic materials that constitute the sugarcane stalks' internal fibre component, i.e. the *fibre* vascular bundles and *pith* (hence the derivation of the term fith).

Nutritional Characteristics of Sugarcane Feeds

Sugar Factory By-Products

Molasses

Cane molasses is extensively used as an ingredient in the formulation of dry concentrate mixtures or liquid supplements for cattle, particularly in the technically developed countries. It is ironic that, whereas this feedstuff as an inexpensive source of energy is transported long distances, it is much less commonly used as a livestock feed in its tropical countries of origin, with reports that in some areas the molasses may actually be discarded at the sugar factory. It is also ironic that the nutritional information available for this most commonly used sugarcane feed is as variable and contradictory as it appears.

Table 1 presents summaries for chemical composition (proximate analyses) and energy content, as listed in references that draw their information from the NRC system, with the same NRC feed reference number being used in all cases (4-04-696). Only energy values for ruminant species are presented.

As the composition of molasses is controlled in the sugar factory, its analyses usually appear to be quite standardized, as indicated by the quality description in the NRC name of "mn 46% invert sugars mn 79.5 degrees brix." One exception is the erroneous crude fibre value of 9.9% presented by McDowell et al. (1974) (CF should be 0), with the stated nitrogen-free extract also being markedly affected because it is calculated by difference.

From the energy contents presented, it would appear that between the 1974 and 1976 publications, a major shift was made, with the latter reported values being about 25% lower than the former. The existence in the literature of such widely varying values, supposedly originating from a common NRC source, can only play havoc with any attempt to accurately formulate molasses-containing rations.

In the bottom part of Table 1, information is presented dealing with experimental determinations of the energy value of molasses for ruminants. The data are listed in order of increasing

Table 1. Feed analysis and energy content: sugarcane, molasses (cane molasses) (4-04-696).

	Chemical composition (% DM)							Energy content (Mcal/kg DM)					
Reference	DM	Ash	CF	EE	NFE	CP	Species	DE	ME	NE_m	NE_g	NE_l	TDN
NRC 1971a	77.0	10.1	0	0	84.0	5.9	Cattle	4.23	3.47	2.27	1.48	2.60	96.0
NRC 1971b	—	—	—	—	—	4.3	Dairy	4.01	3.43	2.27	1.48	2.42	91.0
McDowell et al. 1974	77.2	10.3	9.9	0.3	74.0	5.4	Cattle	4.23	3.47	2.49	1.57	(2.59)	96.0
NRC 1976	75	—	—	—	—	4.3	Beef	—	2.75	1.91	1.20	—	72.0
NRC 1978	75	—	—	—	—	4.3	Dairy	3.17	2.76	1.60	1.03	1.64	72
Ensminger & Olentine 1978	75	10.3	—	0.1	85.7	5.2	Ruminant	3.22	2.64	1.64	1.06	1.67	73
SFIS No.[a]	*Literature values — levels in ration (%)*												
568	—	—	5–15	—	—	—	Beef heifers	—	—	1.37	0.78	—	—
31	—	—	10	—	—	—	Beef steers	2.86	—	—	1.52	—	62.5
32	—	—	10	—	—	—	Dairy cows	—	—	—	—	1.50	—
568	—	—	20	—	—	—	Beef heifers	—	—	1.23	0.70	—	—
31	—	—	25	—	—	—	Beef steers	2.77	—	—	0.83	—	62.0
32	—	—	30	—	—	—	Dairy cows	—	—	—	—	0.51	—
31	—	—	40	—	—	—	Beef steers	2.60	—	—	0.77	—	59.3

[a]31 = Lofgreen and Otagaki 1960a; 32 = Lofgreen and Otagaki 1960b; and 568 = Lofgreen 1965.

molasses level, the effect of which is to decrease energy utilization. As the experimentally produced values are lower than those compiled from NRC sources, even after their downward correction, a serious question must be raised as to the origin and validity of the NRC energy data.

Perhaps the most extensive determination on the use of high molasses ration levels for growing cattle have been conducted at the Cuban Institute of Animal Science (ICA), with a series of over 10 papers on intensive beef production for urea-molasses based rations published in the Cuban Journal of Agricultural Science (Revista Cubana de Ciencia Agricola). In all the Cuban publications the ME ration contribution of molasses is calculated, based on the ME content listed in NRC tables, with no apparent attempt made to experimentally define molasses energy content. Animal productivity, in terms of liveweight gain, was determined as the major criteria of their studies, and the molasses-urea feeding system was widely adapted in Cuba in beef production schemes. The above can be interpreted to suggest that determining the productive potential of animal feeds, particularly in developing countries, is far more important than being concerned with refining coefficients of nutrient (energy) utilization.

Dry (Dehydrated) Molasses

NRC tables of feed composition also include analyses for a dry molasses product (molasses, dehy, 4-04-695). Although the name implies that drying is the only difference between the two types of molasses, examination of the chemical content indicates compositional changes (Table 2). The crude fibre content of the dry molasses is about 3%, indicating that a fibre source has been

Table 2. Feed analysis and energy content: sugarcane, molasses, dehydrated (4-04-695).

	Chemical composition (% DM)							Energy content (Mcal/kg DM)					
Reference	DM	Ash	CF	EE	NFE	CP	Species	DE	ME	NE_m	NE_g	NE_l	TDN
NRC 1971a	93.5	14.4	2.9	0.6	71.5	10.7	Cattle	3.76	3.08	—	—	—	85.3
NRC 1971b	96.0	—	5.2	—	—	10.7	Dairy	—	—	1.78	1.18	—	68
McDowell et al. 1974	94.7	(23.5)	(3.6)	(2.7)	(52.2)	(18.0)	Cattle	(0.92)	(0.76)	(0.63)	(–1.33)	(0.00)	(20.9)
NRC 1976	96.0	—	5.2	—	—	10.7	Beef	—	2.81	1.78	1.18	—	68
Ensminger & Olentine 1978	90	12.3	5.0	1.0	72.3	9.3	Ruminant	3.00	2.46	1.49	0.92	1.55	68
NRC 1978	96	—	5	—	—	10.7	Dairy	2.99	2.58	1.49	0.92	1.54	68

added during the drying process. This additive is most likely to be bagasse pith, and it is recommended that its level of inclusion should be part of the NRC name. Although dry molasses should be a product of relatively uniform composition, this is not indicated in Table 2, with some values (particularly from McDowell et al. 1974) definitely erroneous.

Bagasse

This sugarcane by-product is primarily used as a low-grade material to fuel the sugar factories' steam generators, with the surplus produced over fuel requirements generally resulting in disposal problems. The potential for board and paper manufacture from bagasse is dependent on the local availability of manufacturing technology, and is becoming an increasing area of interest in many developing countries.

As can be seen in Table 3, in its "raw" form, bagasse is a low-quality roughage, with its feeding value fitting in between wood and straw. Its use as an animal feed has thus been limited, because as a major component of a ration it cannot meet the animals' maintenance requirement. Bagasse has been more effectively utilized at low levels in ruminant rations (< 10%) where it would primarily serve as a nonnutritive roughage factor, with such rations generally predominating in high-digestibility energy feeds.

Potential for the use of bagasse as a ruminant feed may be realized through the development of chemical or physical processing methods that disrupt the ligno-cellulose complex, and thus markedly increase the availability of energy. Such treatments (i.e. alkali, high-pressure steam), although tested at laboratory levels, have not seen large scale commercial application, possibly due to the cost involved in establishing and operating treatment plants.

A summary of NRC-derived information on bagasse (Table 3) indicates a wide divergence of values, supposedly being used to describe the same feed. It is certainly not conceivable how bagasse can be described in some of these references as having energy values approaching normal forage sources.

A by-product from bagasse that has a higher feeding value is the pith (inner fibre) component, which is separated from rind particles by depithing procedures. The removal of the more highly lignified rind results in a ligno-cellulosic feedstuff of a much higher feeding value, although literature references are limited. Bagasse pith, due to its higher initial energy digestibility (compared to bagasse), has been used as a material for delignifying treatments.

There is production in many areas of "dry" molasses-bagasse pith mixtures, but the bagasse component can only be regarded in these cases as serving as a nonnutritive absorptive carrier for the molasses. It is recommended that when data are presented for a feed composed of several ingredients, the percentage composition of the mixture should be cited as part of the international feed name.

Sugarcane Feeds Derived from Unprocessed Plants

Cane Tops

This has been the part of the sugarcane plant traditionally used as a livestock feed, as the tops can be gathered in the cane fields after the stalks are transported to the sugar factory. The data from NRC sources listed in Table 4 indicate that cane tops approximate an average forage in feeding value. The highest concentration of protein in

Table 3. Feed analysis and energy content: sugarcane, bagasse (pulp), dehydrated (1-04-686).

	Chemical composition (% DM)							Energy content (Mcal/kg DM)					
Reference	DM	Ash	CF	EE	NFE	CP	Species	DE	ME	NE_m	NE_g	NE_l	TDN
NRC 1971[a]	91.5	3.1	48.6	0.7	45.9	1.7	Cattle	1.24	1.02	—	—	—	28.1
McDowell et al. 1974	93.6	4.5	41.6	1.2	50.8	1.9	Sheep	0.98	0.80	—	—	—	—
McDowell et al. 1974[a]	46.4	4.2	49.2	3.7	37.0	5.9	Cattle	2.18	1.79	1.06	0.26	0.99	49.5
NRC 1978	92	—	48	—	—	1.8	Dairy cows	1.24	0.80	0.71	0	0.57	28
Ensminger & Olentine 1978	92	3.3	47.9	0.8	46.3	1.8	Ruminant	2.09	1.72	1.02	0.17	1.04	47
NRC 1971[ab]	90.3	7.9	45.0	1.0	44.2	1.9	Cattle	2.00	1.64	—	—	—	45.4

[a] Dehydrated, ground (1-09-757).

[b] Bagasse pulp, sifted (bagasse pith) (1-04-700); mistakenly cited as (4-04-700).

Table 4. Feed analysis and energy content: sugarcane, top of aerial part (cane tops).

	Chemical composition (% DM)							Energy content (Mcal/kg DM)					
Reference	DM	Ash	CF	EE	NFE	CP	Species	DE	ME	NE_m	NE_g	NE_l	TDN
NRC 1971a[a]	29.6	9.5	35.8	2.0	47.6	5.1	Cattle	2.24	1.84	—	—	—	50.8
McDowell et al. 1974[b]	32.6	8.7	32.1	1.4	52.2	5.5	Cattle	2.40	1.97	1.16	0.45	1.16	54.4
McDowell et al. 1974[c]	30.0	5.8	28.5	4.0	56.0	5.7	Cattle	3.45	2.83	1.80	1.19	1.98	78.2
McDowell et al. 1974[d]	55.5	11.2	31.8	2.5	48.7	5.7	Cattle	2.45	2.01	1.18	0.50	1.20	55.6
McDowell et al. 1974[e]	85.5	5.4	39.3	2.0	51.0	2.3	Cattle	2.34	1.92	1.13	0.40	1.11	53.0
Ensminger & Olentine 1978[f]	26	11.8	33.1	1.7	48.1	5.2	Ruminant	2.14	1.76	1.05	0.22	1.07	49
McDowell et al. 1974[f]	25.9	5.9	30.4	3.4	56.3	3.5	Cattle	2.91	2.38	1.43	0.85	1.55	65.9
			Literature values										
SFIS summary	—	7.0	32.6	1.8	50.3	—	Cattle	—	—	—	—	—	52.6

[a] Ensiled (3-08-528).
[b] Fresh (2-13-568).
[c] Chopped (2-13-563).
[d] Ensiled (3-08-528).
[e] Dehydrated (1-13-565).
[f] Leaves with top of aerial part (2-04-692).

Table 5. Feed analysis and energy content: sugarcane, aerial part (whole plant), fresh (2-04-689).

	Chemical composition (% DM)							Energy content (Mcal/kg DM)					
References	DM	Ash	CF	EE	NFE	CP	Species	DE	ME	NE_m	NE_g	NE_l	TDN
NRC 1971a	25.1	6.8	31.3	2.2	53.5	6.2	Cattle	2.52	2.07	—	—	—	57.2
NRC 1971a[a]	27.2	6.0	32.0	2.1	52.7	7.3	Cattle	2.56	2.10	—	—	—	58.0
McDowell et al. 1974	20.3	3.2	30.5	2.5	60.1	3.5	Cattle	2.67	2.19	1.30	0.67	1.37	60.5
McDowell et al. 1974[b]	25.5	4.5	24.2	1.6	67.0	2.5	Cattle	3.07	2.51	1.51	0.96	1.68	69.6
McDowell et al. 1974[c]	20.9	13.9	34.0	1.5	38.2	12.4	Cattle	2.57	2.11	1.25	0.60	1.30	58.4
Ensminger & Olentine 1978	26.0	6.1	30.9	2.1	54.9	5.9	Ruminant	2.59	2.12	1.27	0.60	1.32	59
			Literature values										
SFIS summary	—	4.9	28.2	2.1	61.1	3.3	—	—	—	—	—	—	55
U. of Florida[d]	—	4.3	28.1	1.2	64.0	2.3	In vitro	—	—	—	—	—	56.6

[a] Mature (2-04-687).
[b] Chopped (2-09-700).
[c] Late vegetative (2-10-258).
[d] Pate and Coleman 1975.

the sugarcane plant is found in the growing green leaves, which constitute part of the tops of the mature plant. A factor influencing the available energy content of this feed is the amount of sugar present, because the cane tops include the upper part of the sugar-containing stalk. As in previous cases, the published data present a contradictory picture, particularly from McDowell et al. (1974). Two of the energy values are definitely erroneous (two other listings from this source, not reproduced, give TDN values of 148 and 156%). Also included in Table 4 are average values calculated from about 30 SFIS documents that give directly determined data.

Whole Plant

Over the past 65 years, there have been scattered reports in the literature describing the use of the whole sugarcane plant as a livestock feed. Particularly in times of feed shortage (dry season) there are indications of the use of the whole sugarcane plant as an animal "survival" feed, although apparently not supplying much more than maintenance requirements. The poor animal performance would appear related to the deficiencies of protein and other nutrients when this energy-rich plant is fed unsupplemented.

The summary in Table 5, indicates fibre and protein levels similar to grass forage, but with a higher available energy content than that normally found in cellulosic feeds. The latter is due to the high level of sucrose found in the mature plant.

Also included in Table 5 are averages calculated from about 30 SFIS documents, listing original data for the whole plant. In addition, average values are presented from a University of Florida study in which 66 sugarcane clones representing many standard varieties grown throughout the world for sugar production were chemically analyzed. These samples were collected from 10-month-old cane, which would be slightly younger than the 12-month or older mature cane used for sugar production.

Literature data indicate that the digestibility of the sugarcane plant varies little with plant age, probably due to the increase in highly digestible sugars with maturity, which compensates for the decreasing digestibility with maturity of the lignified leaf and stem portions of the plants. Sugarcane is one of the few "forage" plants (maize is another) that is at its highest content of available energy at maturity, and in the case of sugarcane this energy content is maintained throughout the dry season, when the plant is normally harvested for sugar extraction.

Regarding terminology, although referred to as the whole plant, indicating that all the aerial parts are used, in actual practice it is necessary to chop the plant prior to feeding.

Derinded Stalks

As the rind represents the most lignified portion of the sugarcane plant, its removal would be expected to increase the digestibility of the resultant feed (sugarfith) (stalks wo rind). Very limited data are available on the effect of derinding or chemical analysis and digestibility as most observations have been made in growth studies. A factor minimizing the effect of derinding is the relatively small percentage of the total plant that constitutes the rind. Derinded stalks are also usually fed in combination with cane tops, normally in a 70:30 ratio approximating the ratio in whole cane.

A survey of the literature indicates an average dry matter digestibility of 65% for sugarcane stalks (w rind), 73% for derinded stalks, and 68% when derinded stalks are fed in combination with cane tops (thus constituting the entire plant minus the rind).

In the limited number of sheep and cattle feeding trials in which whole and derinded cane have been compared, liveweight gains have been similar. These results, together with the relatively high costs associated with derinding, would suggest that the use of this process would be most justified when the separated rind is utilized for board manufacture.

Analytical Methodology for Sugarcane Feeds

The same chemical procedures found effective in describing the nutritive value of most animal feeds, may also suffice for sugarcane feeds.

In the case of molasses, the International Feed Name already includes a definition of the minimum contents of invert sugars[3] and degrees brix (approximating total solids). The sugar industry thus standardizes and defines different molasses products with these measurements, which thus serve as a definition of quality. Brix measurements are also made on whole mature cane to obtain an approximation of its sugar content.

Perhaps the most effective analysis for sugarcane feeds derived from the whole plant (sugar containing) are the "detergent fractions" as devised by Van Soest. In particular, the cell contents

[3]The IFN name does not clearly indicate that the total sugars present are *expressed as invert*, and sucrose, which is the predominate sugar present in molasses, is not an invert sugar.

as measured by the neutral detergent solubles (NDS) fraction would appear to be a good measure of total sugars present, and thus would characterize this special sugar-containing property of sugarcane derived feeds. The University of Florida study of 66 cane varieties (Table 5) (10-months-old) indicates an average NDS content of 47.3%± 4.4 (S.D.), with a surprising range of 32–57%, indicating a wide variation in sugar content among varieties of similar age.

Regarding the forage-type sugarcane feeds (i.e. bagasse and cane tops), it would appear that chemical and in vitro procedures used generally for forage evaluation purposes would also be satisfactory to supply information on their nutritive value. Studies on the effect of different treatments on increasing the cell-wall availability of low-quality roughages, such as bagasse and bagasse pith, have made extensive use of in vitro rumen fermentation techniques.

Recommendations

This paper has in fact dealt with two subjects, one related to the specific question of sugarcane derived feeds, and the other to the publication in various sources of feed data information as related to the NRC (or INFIC) system. It might be felt by some that undue emphasis has been placed on this latter aspect, but we are of the opinion that this critique is much more important to the theme of the workshop. Although we have pointed out some of the errors and inconsistencies of the published data in relation to sugarcane-derived feeds, we would have no reason to believe that they do not also apply to many other feeds.

Also relating to a theme of this workshop, the problem of lack of consistency in published nutritional information can be particularly severe in developing countries, as a limiting factor in such areas is lack of up-to-date reference material. So whereas corrected values may be available in more recent publications, the older references may constitute those most available in countries lacking facilities and funds for obtaining the most recent publications. There also appears to be the tendency (mistaken, we believe) to regard data from the technically developed countries as infallible, which further complicates the production and distribution of possible erroneous information.

We believe that the development of international systems of feed nomenclature and nutritional descriptions may play a useful role in improving worldwide agricultural production. We would hope that the developers of such systems would pay as much attention to the evaluation of the accuracy of the original technical information as to the sophistication of the hardware necessary to handle and disseminate the data.

An extremely vulnerable point would seem to be the dependability of the source data. It must be accepted that data originating from a wide variety of types and distribution of sources is also widely variable in the accuracy and precision with which it is produced. The adoption and adherence to standardized procedures might do much to correct that situation but it must be recognized that the generalized adoption of these methods will take time, particularly in developing countries. At this time we would recommend that continued emphasis be given to the compilation of nutritional data. However, we strongly recommend that, prior to publication, careful scrutiny of the original data should be made by individuals with experience with the particular group of feeds (e.g. energy feeds, protein supplements, sugarcane feeds, etc.). Publication of results from a small number of citations should indicate their limitation.

We would also recommend caution in the use of generalized regression formulas to calculate "missing values" for nutrient utilization when only chemical analysis results are available. As direct determination of energy values for sugarcane feeds are very limited, efforts to produce this specific information should be encouraged.

Appreciation of the Nitrogen Value of Feeds for Ruminants

R. Vérité[1,2]

Crude protein and digestible crude protein have long been used to express the protein value of ruminant feeds. But although generally suitable, these expressions are inadequate in some situations. Over the last 10–15 years a fairly good understanding of the main phenomena of nitrogen digestion and metabolism in ruminants has been obtained, and in the last 5 years attempts have been made to systematize prediction of the amount of amino acids absorbed. The models that have been developed are of two kinds: (1) those that are very sophisticated and incorporate, in a dynamic way, all digestive phenomena and require the use of a computer; and (2) those that are simpler and are more practical for formulating diets and comparing feeds. These new systems are all based on the same principles although the ways of expressing the protein value of the feeds are apparently different: metabolizable protein; absorbable protein; protein digested in the small intestine; undegraded protein; and rumen degradable protein. The fundamentals and limits of these systems are reviewed to try to specify which parameters should be assessed for improving the prediction of the protein value of the feeds, and which methods seem most appropriate.

In most countries, the protein value of ruminant feeds has for a long time been expressed as crude protein (CP) or as digestible crude protein (DCP). Though generally suitable, this expression is inadequate in some situations: for example with diets of grass silage, or ones using nonprotein nitrogen (NPN) or protected proteins (heat, tannins, formaldehyde). This arises partly because the actual protein value of a feed depends in fact on the amount and relative proportion of amino acids absorbed in the small intestine.

The tremendous amount of research that has been carried out over the past 10–15 years in the field of nitrogen digestion and metabolism in ruminants has led to a fairly good understanding of the main phenomena (Cole et al. 1976; Haresign and Lewis 1977; Jarrige et al. 1978; Kaufmann 1977a,b; Smith 1975; Sutherland et al. 1975; Tamminga 1977a,b). Attempts have been made during the last 5 years to systematize prediction of the amount of amino acids absorbed. This has involved either very sophisticated models incorporating, in a dynamic way, all digestive phenomena but necessitating the use of a computer (Baldwin et al. 1977; Black et al. 1975) or simpler but more practical systems for formulating diets and comparing feeds (Burroughs et al. 1975; Chalupa 1974; Jarrige et al. 1978; Kaufmann 1977a; Roy et al. 1977; Satter and Roffler 1975).

By reviewing the fundamentals and limits of these systems, I will try to specify which parameters should be assessed for improving the prediction of the protein value of feeds, and which methods seem most appropriate.

System Fundamentals

The proposed new systems are all based on the same principles, although the ways of expressing the protein value of feeds are apparently different: metabolizable protein for Burroughs et al. (1975); absorbable protein for Chalupa (1974); protein digested in the small intestine (PDI) for Jarrige et al. (1978); and undegraded protein (UDP) along with rumen degradable protein (RDP) for ARC (Roy et al. 1977). They have a common factorial structure to which even the system of Satter and Roffler (1975) can be linked, although its presentation and method of obtaining some of the parameters are different from the others (see Fig. 1).

Starting with feed characteristics, these methods attempt to assess the amount of amino acids absorbed in the intestine by considering their threefold origins: endogenous proteins; dietary proteins nondegraded in the rumen; and microbial proteins synthesized in the rumen. The importance of endogenous nitrogen is not well known and furthermore is less directly dependent

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[2]I am most grateful to M.P. Journet for reading the paper and giving useful advice.

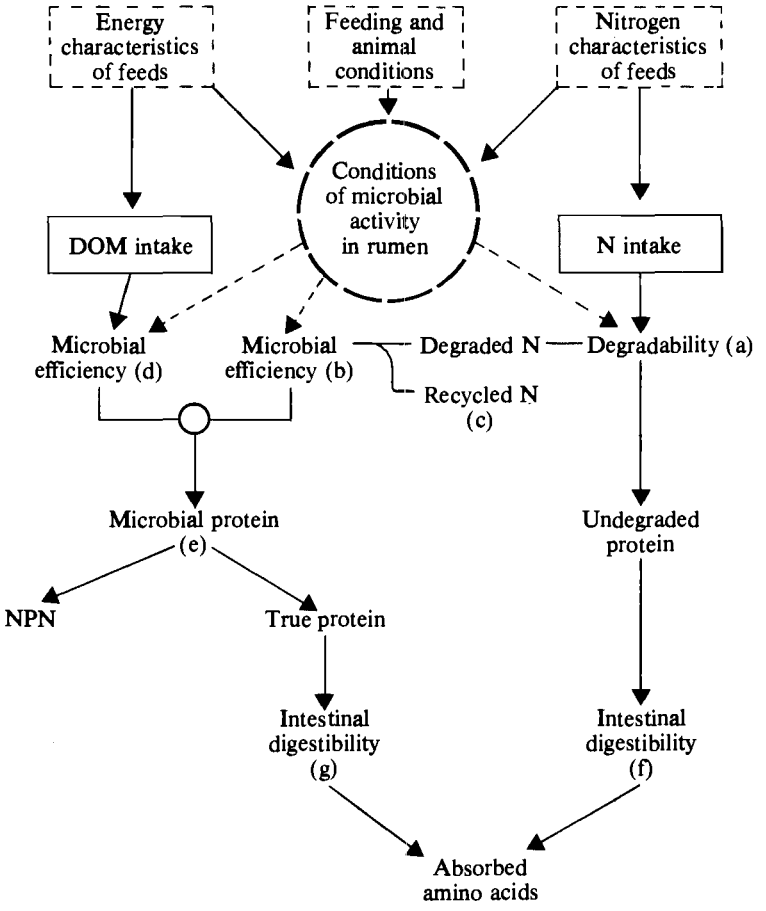

Fig. 1. Principles for calculating the nitrogen value of feeds (letters in parentheses refer to Table 1).

on the nature of the feed than the other two fractions. It is indirectly taken into account either in the value of feeds, by way of apparent digestibility, or in animal requirements.

The proteins in the feed are partly degraded by microbial enzymes in the rumen and yield mainly ammonia as an end product. The importance of degradation varies from one feed to another and largely depends on the intrinsic characteristics of the feed, summed up in the term "degradability." Undegraded feed proteins are then exposed to animal enzyme action in the small intestine. The intensity of this digestion, and the amino acids that are made available, probably also vary according to the type of feed.

The importance of microbial synthesis in the rumen depends directly on the feeds, through the amount of nutrients that they supply the microbes. With normal diets, available energy and ammonia (or rather, nitrogen precursors of microbial proteins) are most probably the limiting factors in the rumen. Each system considers this alternative and estimates microbial proteins from: (1) the digestible organic matter content or a similar criterion; and (2) the degradable nitrogen supplied by the feed (see Fig. 1). Differences in the systems do, however, exist (Table 1).

Degradability of Proteins

The supposed ranges of variations between feeds differ widely. In particular, Satter and Roffler (1975) supposed no variations between diets and only the British (Dacron bags) and French (solubility and in vitro fermentability) systems refer to a method for determining degradability.

Origin, and Efficiency, of Ammonia Utilization

The possibility of N recycling by saliva and

Table 1. Comparison of systems recently proposed for evaluating the nitrogen value of feeds for ruminants.

	Jarrige et al. 1978	Roy et al. 1977	Satter and Roffler 1975	Burroughs et al. 1975	Kaufmann 1977	Chalupa 1974
Nitrogen degradability (a)		4 classes :				
Range for natural feeds[1]	0.35–0.90	0.4–0.8	0.66	0.52–0.95	0.60–0.80	0.30–0.60
Methods of estimation	Solubility+ in vitro degradability	Nylon bag	—	—	—	—
Intestinal digestibility of by-passed protein	variable	constant	constant	constant	constant	variable
Microbial utilization of degradable N						
Efficiency of conversion of degradable N (b)	1.0	1.0[2]	0.9	1.0	—	0.8
Takes account of N recycling (c)	In the formulation of diet eventually	No	0.12 N intake	No	No	No
Takes account of degraded N required by rumen microorganisms	Yes	Yes	No	No	No	No
Microbial utilization of energy						
Theoretical basis[8]	DOM	ME	TDN	TDN	SE	
Yield: g CP/kg DOM[3] (d)	135	122	120–125[4]	130	135	70
Proportion as true protein (e)	0.8	0.8	0.8	0.8	0.9[5]	0.85
Digestibility in the intestine						
Site[6]	SI	SI	TI ?	?	SI	SI
Apparent or true digestibility	True	Apparent	True (?)	True	True	Apparent
Value: dietary protein (f)	0.60 to 0.95	0.70	0.87	0.90	0.85	variable[7]
microbial true protein (g)	0.70	0.70	0.80	0.80	0.85	0.78

[1]1.0 for industrial NPN in all systems.

[2]0.8 for industrial NPN.

[3]In all the systems, except that of Satter and Roffler, the yield of microbial protein is directly related to DOM, even when expressed differently (ME, SE, TDN).

[4]Value calculated from the upper limit for NPN utilization.

[5]Utilizable N = amino acid N + 0.5 non amino acid N.

[6]SI, small intestine; TI, total intestine.

[7]The apparent digestibility of dietary protein is the same as the digestibility of CP in the feed tables.

[8]DOM, digestible organic matter; ME, metabolizable energy; SE, starch equivalent; TDN, total digestible nutrients.

NOTE: Letters a-g refer to Fig. 1.

through the rumen wall is considered only by Satter and Roffler (1975) and Jarrige et al. (1978). Microbial needs are, moreover, occasionally taken into consideration in diet formulation; indeed, a minimum (French system) or an optimum supply (British system) must be given to the microbes even if the animal requirements are already satisfied.

Parameter Representing Energy

Depending on the system, digestible organic matter, total digestible nutrients, metabolizable energy, or starch equivalent (Table 1) is used, but the authors assume a set connection between these parameters and the DOM content. Therefore, this difference is only an apparent one.

Digestibility of Proteins within the Intestine

The site considered (small or whole intestine) and the calculation method (apparent or real digestibility) vary. Sometimes it is supposed that protein digestibilities differ between microbial

and feed proteins and, even occasionally, between feeds (Chalupa 1974; Jarrige et al. 1978).

Assumed Values of Different Coefficients

Except for Chalupa (1974), assumed efficiencies of energy utilization by microbes are similar: 120–135 g microbial crude protein per kilogram DOM. All authors, however, point out the importance of the interference of rumen conditions on this yield. Indeed, the flow of proteins into the intestine depends not only on the characteristics of the feed as considered by the systems, but also on many factors that affect microbial activity (Fig. 1). Thus, the time spent by the feeds in the rumen influences the degradation of their proteins. This is more evident when the degradation is low and slow, as suggested by the results of Miller (1973) and Orskov and Fraser (1973) with

Table 2. Ruminal bypass of protein and microbial protein synthesis (from Jarrige et al. 1978).

	Animal[a]	Microbial N estimation[b]	Feed N nondegraded in the rumen: % N ingested	Feed N nondegraded in the rumen: % N leaving rumen	Synthesized microbial N[c]: ‰ RDOM	Synthesized microbial N[c]: ‰ DOM	
Green Forages							
Ray grass	S	DPA	30	35 & 46	25.9 & 49.0	16.5 & 26.8	Ulyatt et al. 1975
Ray grass	C	DPA	56 & 57	45 & 54	22.4 & 26.0	15.5 & 18.4	Hagemeister et al. 1976
Ray grass	S	^{35}S	48	58	—	—	Harrison et al. 1974
White clover	S	DPA	27	37	31.7	20.7	Ulyatt et al. 1975
Red clover	S	DPA	35	34	—	—	Harrison et al. 1973
Clover + grass	C	DPA	46	46	25.5	15.9	Hagemeister et al. 1976
Hays							
Lucerne	S	DPA	35	42	37.0	23.2	Lindsay and Hogan 1972
Lucerne	S	^{35}S	44	51	31.0	23.3	Walker et al. 1975
Lucerne	S	^{15}N	21	27	—	—	Pilgrim et al. 1970
Lucerne	S	^{15}N	32	35	—	—	Nolan 1975
Subterranean clover	S	^{35}S	47–57	49–55	32.2	19.7	Hume and Purser 1975
Ray grass	S	DPA	41–57	15–20	—	—	Harrison et al. 1973
Wheaten hay	S	^{35}S	40–54	35–40	20.6 & 24.1	14.4 & 17.2	Walker et al. 1975
Lucerne — Bromus	S	^{15}N	21	22	25.5	21.0	Mathison and Milligan 1971
Dehydrated Forages							
Lucerne — long	S	DPA	19	21	—	—	Harrison et al. 1973
Lucerne — chopped	S	^{35}S	28	32	—	—	Mathers and Miller 1977b
Subterranean clover	S	^{35}S	27	38	30.9	25.4	Hume and Purser 1975
Subterranean clover	S	DPA	32	37	39.5	25.6	Hogan 1973
Subterranean clover	S	^{15}N	43	58	20.1	12.4	Walker et al. 1975
Red clover	S	DPA	72	60	—	—	Harrison et al. 1973
Sainfoin, chopped	S	DPA	80	63	—	—	Harrison et al. 1973
Bermuda grass	S	DPA	44–62	46–48	—	—	Amos et al. 1976
Ray grass	S	^{35}S	71–73	57–66	—	—	Harrison et al. 1974
Grass	S	^{35}S	48–50	46–50	—	—	Mathers and Miller 1977
Silages							
Ray grass — direct cut	S	^{35}S	15	18	26.7	19.4	Beever et al. 1977
Ray grass — formol	S	^{35}S	78	71	10.6	6.8	Beever et al. 1977
Clover + grass — direct cut	C	DPA	23–42	18–33	32	19	Hvelplund and Moller 1976
Clover + grass — formic acid	C	DPA	44–47	41	21	13.5	Hvelplund and Moller 1976

(continued)

Concentrated Feeds							
Barley	S	^{35}S	11	11	—	—	Mathers and Miller 1977b
Barley	S	DPA	57	43	32.0	17.2	Orskov et al. 1972
Peanut meal	S	^{35}S	37	38	—	—	Hume 1974
Peanut meal	S	DPA^{35}S	22	12	—	—	Miller 1973
Soybean meal	S	^{35}S	61	48	—	—	Hume 1974
Sunflower meal	S	DPA^{35}S	19–28	—	—	—	Miller 1973
Fish meal	S	^{35}S	71	59	—	—	Hume 1974
Fish meal	S	DPA^{35}S	69	29	—	—	Miller 1973
Lucerne meal	S	^{35}S	38	57	11.6	9.4	Leibholz 1972
Mixed Diets							
Forages:							
+Copra meal	C	DPA	57	61	19.8	14.0	Hagemeister et al. 1976
+Soybean meal	C	DPA	45	44	33.0	22.2	Hagemeister et al. 1976
+Fish flour	C	DPA	33	33	35.6	25.0	Hagemeister et al. 1976
+Yeast	C	DPA	42	35	35.2	24.5	Hagemeister et al. 1976
+Rapeseed meal	C	DPA	40	40	33.0	21.7	Hagemeister et al. 1976
+Peanut meal	C	DPA	45	44	31.2	21.0	Hagemeister et al. 1976
+Horse-beans	C	DPA	46	45	30.4	19.3	Hagemeister et al. 1976
+Urea	C	DPA	23	27	33.2	23.8	Hagemeister et al. 1976
Hay (4.5 kg) + Maize (2 kg)	H	f	27	32	—	—	Neudorffer et al. 1971
Hay (2 kg) + Maize (4 kg)	H	f	41	45	—	—	Neudorffer et al. 1971
Barley + soybean meal	CV	^{35}S	26	26	25.8	15.8	Leibholz 1975
Beet pulp + barley	S	DPA	21	20	22.9	17.8	Chamberlain et al. 1976

[a]S = sheep: C = cows: H = heifers: CV = calves.
[b]Estimation of microbial nitrogen by diamino-pimelic acid (DPA), radioactive markers (^{35}S or ^{15}N), or fractionation by centrifugation (f).
[c]Microbial nitrogen per kilogram organic matter apparently disappearing in the rumen (RDOM) or in the total digestive tract (DOM).

different levels of intake. Microbial synthesis depends even more on microbial population and conditions in the rumen.

The conditions established in the rumen, and which regulate microbial activity, result both from the nature of the feeds and, above all, from the general pattern of feeding: e.g. feeding levels, physical form of the diet, feeding rhythm, feed interferences, general balance of the diet, and physiological state of the animal.

Thus, at present, the determination of protein degradability is an important point: DOM content must also be known but raises no new problems. In the future, two other aspects will have to be considered: estimation of digestibility in the intestine due to varied effects of conservation, technology, etc.; and improvement in predicting microbial synthesis according to the nature of the feeds.

Estimation of Protein Degradability

Determination of the amount of feed proteins escaping degradation in the rumen is now possible with postruminal fistulas. This is calculated indirectly by differentiating between the total flow of nitrogen into the intestine and: (1) making a simultaneous assessment of the microbial fraction with markers; or (2) measuring the total nitrogen flow measured with a similar protein-free diet (Miller 1973). Although the accuracy of this method is limited, the large variations observed obviously reflect actual differences between feeds or diets (Table 2).

Degradability depends mainly on the physical accessibility of the proteins and their sensitivity to enzyme attack. As to the physical accessibility of the proteins, an important but probably not necessary stage is solubilization in the rumen fluid because bacterial enzymes act within the cell or on immediate contact with it. Solubilization depends on the characteristics of the feeds, which can be summed up by the term "solubility," and on the environment in the rumen (pH, degrees of wetting and chewing, ionic strength, etc.). Solubility varies with the nature of the nitrogen constituents, which varies widely (see Jarrige et al. 1978) — this is particularly true for NPN constituents, which are solubilized very rapidly and are abun-

Table 3. Biological methods for estimation of protein degradability.

	Duration (h)	Assay sample (g/100 ml rumen inoculum)	Calculation method	
			Final test	Taking account of microbial effect[a]
Dacron bags				
Mehrez and Orskov 1977	Variable[b]	—	Residual N	No
Mathers and Miller 1977	4-6	—	Residual N	No
Crawford et al. 1978	up to 6	—	Residual N	No
Stern et al. 1978	up to 24	—	Residual N	No
In vitro fermentation				
Little et al. 1963	up to 24	2[c]	NH_3 conc	A
Melosch 1974	8	—	NH_3 conc	B
Dinius et al. 1974	21	0.5	NH_3 conc	A
Driedger and Hatfield 1972	up to 24	1.6	NH_3 conc	A
Amos et al. 1974	24	2.5	NH_3 conc	No
Schmidt et al. 1973	up to 4.5	4	NH_3 conc	A
Crooker et al. 1978	up to 6	1.5–6[c]	NH_3 conc	No
Vérité and Demarquilly 1978	6	5[d]	NH_3 conc	C

[a]A = control without feed protein; B = control with urea replacing feed protein; C = control without feed protein but with additional precautions (equalized energy supply and NPN pulse) to ensure that microbial activity is similar to that of tested feed.
[b]Corresponding to 85% DM disappearance.
[c]Constant nitrogen weight.
[d]Constant nitrogen weight but equalized DM supply with extra wheat starch.

dant in roots and conductive tissues, and with protein localization in the cells and tissues, and with the degree of lignification of the plants. Solubility can be modified by the method of conservation (hay, silage) (Goering and Waldo 1974), and by grinding or by heat treatments that can alter proteins.

Sensitivity to enzyme attack also varies. Some proteins, although very soluble, are not very sensitive to bacterial proteases due to a special structure (ovalbumin, Mangan 1972). Treatments by heat or tannins (Leroy et al. 1964) or aldehydes (Ferguson et al. 1967; Zelter et al. 1970) reduce this sensitivity.

The different methods that are used to estimate degradability vary in the account they take of this phenomenon depending on whether they involve microbial, enzymatic, or solubility measurements. Their common objective must be to correctly classify feeds whatever the causes of their differences: nature of feed either between (grains, oilmeals, hays, silages) or within categories; batches of the same feed; or technological treatments. Furthermore, a common feature of these different methods is that they all give only a relative value for degradability and must be calibrated to give the value of ruminal degradation in vivo.

Microbial Methods

Measurements of the rumen ammonia level of fistulated animals after feeding have often been used to compare different forages, different methods of conservation, or different technological processes. It is a tedious method, difficult to interpret, and suitable only for comparisons.

The Dacron bag method (Table 2) although rather laborious has the advantage of measuring protein degradation in the rumen, but under conditions differing from those of normal digestion (food sequestration). In vitro fermentation is easier and has been used for a longer time, mainly for comparing the effects of different technological treatments (Table 3). With these techniques, the values obtained have different meanings according to the duration of contact with the rumen fluid as degradation is a function of time (Pichard and Van Soest 1977; Waldo 1978). The readily degradable fraction (2–4 h) is probably not efficiently utilized by microbes because ammonia is generally in excess in the rumen after feeding. Long-term incubations (15–24 h) are basically designed to estimate the undegraded fraction; whereas, short-term incubations (2–6 h) estimate it along with the slowly degraded fraction, which is probably efficiently used by microbes. The latter may be a good criterion of the overall effi-

ciency of N utilization in the rumen. Monitoring (Mehrez and Orskov 1977) is an interesting technique but requires numerous measurements.

With in vitro techniques, the amount of ammonia apparently produced is the final measurement. It represents the balance between the actual amount produced and the consumption by microbes. The latter must, therefore, be inhibited (Broderick 1978) or taken into account in the calculation, usually by comparison with a control (Table 3). The intensity of microbial activity must also be as constant as possible. Because of this, energy supply must be the same for all the feeds tested as well as for the control, and enough ammonia must be present even at the end of the period. Unfortunately these precautions are not always taken and some results are biased. Differences noted with oats between in vitro fermentation (Crooker et al. 1978) and Dacron bags (Crawford et al. 1978) may arise from this.

Comparisons of intestinal flow with these methods have only been made with a small number of feeds. There was good agreement with degradability in Dacron bags determined over a short period (Mathers et al. 1977). Agreement with in vitro fermentations was quite good for a wide range of mixed diets given to cattle, but was somewhat inaccurate when variations in degradability were low (Hagemeister et al. 1976).

Enzymatic Methods

Proteolytic enzymes (pepsin, trypsin, etc.) are frequently used to estimate nitrogen digestibility. They allow an estimation of the damage caused to proteins by techniques of conservation (Goering and Waldo 1974). This can perhaps be extended to an estimation of intestinal digestibility of non-degraded dietary proteins.

Another interesting possibility is the use of low levels of enzymes to estimate degradability. Degradability in pepsin (0.01%; 16 h) was in close negative correlation with intestinal flow of four forages (Beever et al. 1976). Further data obtained from the same group with eleven forages indicate that, among six methods, pepsin and in vitro fermentability were the most accurate in predicting protein bypass (Siddons and Beever 1978). Similar enzymatic techniques are currently being studied in France. Pichard and Van Soest (1977) monitored enzymatic degradation (protease of *Streptomyces griseus*) to distinguish protein fractions according to their degradation rate, and tried to relate them to the usual detergent fractions.

Solubility

Many aqueous solutions are used, the most common being: autoclaved rumen fluid (Crawford et al. 1978; Crooker et al. 1978; Issacs and

Table 4. Protein solubility and fermentability of some feeds.

	Solubility[a]						In vitro ferment.[e]	Dacron bags[d]
	WB[b]	WB[c]	WB[d]	NaCl[d]	ARF[d]	PB[e]		
Energy feed								
Barley	—	17	—	—	—	25	46	—
Oats	—	26	27	9	13	35	40	69
Corn	16	12	5	11	2	15	25	9
Wheat	35	30	22	26	21	30	58	63
Buckwheat	44	39	34	36	30	—	—	37
Beet pulp	35	4	—	—	—	11	20	—
Protein supplements								
Peanut meal	57	40	37	20	11	61	57	41
Soybean meal	15	13	20	13	6	15	32	23
Linseed meal	45	51	—	—	—	36	58	—
Rapeseed	45	39	—	—	—	40	31	—
Sunflower meal	—	30	34	31	24	34	48	37
Alfalfa	—	23	—	—	—	25	22	—
Brewers dried grains	5	3	3	4	1	3	10	13

[a] WB = W. Burroughs buffer; ARF = autoclaved rumen fluid; PB = phosphate-bicarbonate buffer.
[b] Veen and Bakker 1977.
[c] Wohlt et al. 1973.
[d] Crawford et al. 1978.
[e] Vérité and Demarquilly 1978.

Table 5. Calculation of the flow of nonammonia nitrogen entering the duodenum (ND) from the daily intakes of digestible organic matter (DOM) and of nitrogen (NI) or insoluble nitrogen (INI) (all values in g/day) (from Jarrige et al. 1978).

	Sheep (n = 158)	Cattle (n = 72)
With NI		
a	0.0199	0.0138
b	0.41	0.65
r	0.83	0.68
CV[a]	15.2	13.3
With INI		
a	0.0224	0.0207
b	0.57	0.72
r	0.87	0.77
CV	13.4	11.0

[a]CV = residual coefficient of variation.

NOTE: Model also takes account of the differences between laboratories (l_i):

$$\frac{ND}{DOM} = a + l_i + b\,\frac{NI \text{ or } INI}{DOM}$$

Owens 1972; Little et al. 1963; Waldo 1978; Wohlt et al. 1973); water (Little et al. 1963; Waldo 1978); sodium chloride (Crawford et al. 1978; Crooker et al. 1978; Whitelaw and Preston 1963); dilute alkali (Dinius et al. 1974; Little et al. 1963); Burroughs solution (Crawford et al. 1978; Crooker et al. 1978; Veen and Bakker 1977; Wohlt et al. 1973); phosphate buffer (Mc Rae 1970); and phosphate bicarbonate buffer (Crooker et al. 1978; Vérité and Demarquilly 1978).

Many factors affect solubility (temperature, duration of contact, mixing, grinding, soluble protein concentration, pH, and ionic strength); therefore, complete standardization is necessary. Their effect is not always well known. For example, pH affects solubility differently according to the feed (Isaacs and Owens 1972; Whitelaw and Preston 1963). Purified protein solubility decreases with pH (Wholt et al. 1973), but solubility of natural foods does not vary between pH 8.5 and pH 3.8 according to Blanca and Sutherland (1969). With most common methods, pH and ionic strength are similar to those in the rumen.

Only a few series of measurements on a large number of foods have been published (Crawford et al. 1978; Crooker et al. 1978; Veen and Bakker 1978; Vérité and Demarquilly 1978; Wohlt et al. 1973). Although food origins and methods were different, these classifications were roughly the same (Table 4). Variations between batches of one feed were sometimes large, especially with oil meals (Vérité and Demarquilly 1978).

Different solubility methods were compared using the same set of foods (Blanca and Sutherland 1969; Crooker et al. 1978; Little et al. 1963; Wohlt et al. 1973). Agreement between the methods was not always good, but the number of feeds and the range of their values were often not great enough. Furthermore, the choice of autoclaved rumen fluid as a reference for the other solubility methods may be questioned because the agreement between the former and microbial methods is far from being perfect (Crawford et al. 1978).

Reference to a microbial method is probably better. Two comparisons of solubility with microbial methods have been done: (1) with the Dacron bag method (Crawford et al. 1978); and (2) with an in vitro fermentation (Vérité and Demarquilly 1978) modified from Vérité and Journet (1973). They give similar conclusions: classification of feeds by both biological methods is quite similar and is fairly accurately reflected by solubility. In these two studies, however, the solubility of some cereals (wheat, barley, oats) greatly underestimates their fermentability. Indeed, intestinal flow measurements (Orskov and Fraser 1973; Miller 1973) show that the proteins in these cereals are highly degradable.

Solubility in a buffer was indirectly compared with the intestinal flow measurement in sheep and cattle (Jarrige et al. 1978). For this work, many results from the literature were used (Table 5). Total protein flow into the intestine was related to DOM and insoluble protein intakes. Insoluble protein intakes were usually unknown and had to be predicted using the solubility value in our own feed catalogue (Vérité and Demarquilly 1978). Nevertheless, correlations indicate that solubility is of value for increasing the accuracy of prediction of intestinal nitrogen flow. Furthermore, these regression techniques represent a calibration of this solubility method: 0.65 insoluble nitrogen would escape ruminal degradation, as indicated by the regression coefficient.

For research purposes, microbial methods (Dacron bags or in vitro fermentation) appear to more accurately estimate degradation of proteins in the rumen. Nevertheless, as indicated previously, a number of precautions relative to microbial activity should be taken. These methods are however tedious and expensive, and have only moderate repeatability.

Solubility methods are apparently more adapted to routine, and among them, buffer solutions are often used most. They provide a significant improvement in predicting the protein value

of most feeds. For the exceptions, correction factors can be calculated by comparing the solubility values with in vitro values (or other biological values) as has been done in France for the PDI system. Enzymatic methods could perhaps provide a solution to this problem. More rapid progress could be made if degradability criteria of the feed's nitrogen were also given when intestinal flow measurements were obtained.

Microbial Synthesis and Energy Utilization

Microbial protein synthesis depends on the amount of energy available in the rumen. When estimated in vivo, it has been related to the organic matter that apparently disappeared in the rumen (RDOM). Average values for the efficiency of energy utilization by microbes are then close to 27–32 g microbial nitrogen per kilogram of RDOM (Kaufmann 1977; Thomas 1973; Van Nevel and Demeyer 1977). In fact, variations are great (Table 2) and arise from: (1) the diversity and sophistication of the methods of measurement (sampling, measurement of intestinal flow, microbial fraction determination — inaccuracy exists at each step and errors are cumulative); (2) the inadequacy of the criterion representing the energy (ATP) available to the microbes — more elaborate criteria have been suggested for estimating the amount of substrate really fermented (Czerkawski 1978), but improvement is not conclusive, and on the contrary, a less accurate criterion, total digestible organic matter, has been retained in different systems because its prediction from food analysis is easy; and (3) actual variations among the feeds in the diets. Thus, with forages, microbial efficiency would be better than with diets having a high proportion of concentrate (McMeniman et al. 1976; Thomas and Rook 1977); however, one should notice that with these diets, the ammonia level in the rumen is often too low.

In addition to the balance between the nutrients supplied to the microbes, some other primary causes of these variations in microbial synthesis have now been identified. Increased efficiency with the growth rate of bacteria is now well established (Bergen and Yokohama 1977), and a reduction in the turnover time of microbial proteins in the rumen (lysis, protozoa engulfment) could have the same effect. This could result from the decrease in the relative incidence of maintenance needs of the microbes (Isaacson et al. 1975), which represents 55% for a dilution rate of 0.02 and only 15% for a dilution rate of 0.12. In vivo, the positive relation generally observed between the turnover rate of ruminal fluid and the efficiency of microbial synthesis (Cole et al. 1976; Harrison et al. 1975) results from the same mechanism. This relation can, however, be modified by concomitant changes in microbial facies (Thomas 1977).

Diet compositions or methods of feeding that give a dense microbial population, which has a high growth rate and flows rapidly out of the rumen, are therefore likely to promote greater efficiency of energy utilization for microbial protein synthesis. Thus, with green forage of good quality (ray grass or white clover) the efficiency is much greater than with low quality hay or straw (Walker et al. 1975).

Efficiency of Microbial Synthesis and Carbohydrate Constituents

Although ATP yield is dependent on biochemical processes in the rumen, a single and clear relation between microbial efficiency and the rumen VFA pattern does not exist (Thomas 1977). In the same way, microbial efficiency is the same for corn starch and cellulose paper (Offer et al. 1978).

Therefore, type of carbohydrate has no definite effect, but degradation rate could be very important, as suggested by the theoretical scheme of Johnson (1976). In vitro, variations have been observed in microbial yield with the proportion of nonstructural carbohydrates (Stern et al. 1978) and with treatment of starch by heat, pressure, and humidity (Bartley and Deyoe 1977; Durand et al. 1972). In vivo, associative effects have been noticed between starch and cellulose (Offer et al. 1978) and between lucerne hay (alfalfa) and barley (Mathers and Miller 1977). For a long time, improvement in NPN utilization has been obtained when the rate of ammonia release matches the rate of carbohydrate degradation (Helmer and Bartley 1971; Journet 1975). Another related observation is that Grenet and Demarquilly (unpublished) obtained a correlation between nitrogen balance in growing sheep and soluble carbohydrate content of grass silages.

Consequently, the prediction of the nitrogen value of a diet can be improved if the degradation rate of the feeds is considered. The analytical fractions usually determined to predict energy value of the feeds may be convenient. A microbial criterion for organic matter degradation rate (Dacron bags or short-term in vitro digestibility) will probably be better, and could therefore, be associated with determinations of protein degradability.

Variations in Microbial Yield and Nitrogen Supply

The efficiency of energy utilization for microbial synthesis decreases when nitrogenous pre-

cursors of microbial proteins are limiting (Hume et al. 1970). Ammonia is the most important of these precursors (Kennedy and Milligan 1978a,b). Amino acids and peptides are required by some bacteria species, but in most diets, they are probably not limiting. Reported optimum ammonia levels in rumen fluid vary from 4 mmoles/litre (in vitro trials by Okorie et al. 1977 and Satter and Slyter 1974) to 10–17 mmoles/litre (in vivo trials of Allen and Miller 1976; Hume et al. 1970; Mehrez et al. 1977). In fact, these differences are more slight than they seem. With the system of Satter and Roffler (1975) it can be calculated that the diet should provide about 115 g degradable proteins per kilogram DOM in order to maximize microbial synthesis; whereas, Allen and Miller (1976) indicate 160 g. The optimal level may also be different if effects on microbial synthesis, on organic matter digestibility, or even on voluntary intake are considered.

The contribution made by endogenous recycling of nitrogen in the rumen may be important as is indicated by the frequently observed increase in nitrogen flow into the duodenum compared to nitrogen intake with diets having a low nitrogen content. The site (saliva or rumen) and the importance of N constituents (urea or other secretions) are subject to controversy (Kennedy and Milligan 1973; Mc Rae et al. 1977; Nolan et al. 1976). Nevertheless, some of the systems take direct account of recycling; for example, Satter and Roffler (1975) consider N recycling to be proportional to the amount of nitrogen ingested. Other systems, on the contrary, do not take account of recycling perhaps because they assume that, under favourable conditions, microbes can take up all the ammonia present in the rumen. Jarrige et al. (1978), however, distinguish between an optimal supply (135 g of degradable protein per kilogram DOM) allowing maximal microbial synthesis, and a minimal supply (110 g) below which microbial activity is impaired (decrease in digestibility and voluntary intake).

Conclusions

The new systems for estimating the nitrogen value of feeds take account of their energy characteristics. Other important interactions between energy and nitrogen, not treated here, operate at the metabolic level. These systems could be improved but most of them do allow improvements in the formulation of diets (Vérité et al. 1979). Feeding of high producing animals such as dairy cows, which need a large supply of undegraded dietary proteins, would be one of their main area of use. The new systems would also allow more attention to be paid to the effects of the level and quality of nitrogen supply on the energy value and voluntary intake of the diets.

Improvements in these systems would be related to improvement in: (1) prediction of nitrogen degradability of feeds and digestibility of proteins in the intestine; and (2) efficiency of energy utilization for microbial protein synthesis. More rapid progress could be made if simple analytic fractions (energy and nitrogen characteristics) were determined each time a digestion study was done with fistulated animals.

Trade and Legal Aspects of Analytical Techniques for Feeds

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The criteria applied to analytical techniques for feeds are discussed from the point of view of industry, trade, and the law. Industry uses its own methods of analyses. These are suited to the circumstances of the plant and the capacity of its laboratory, and are usually relatively quick and simple. Their main goal is to obtain reliable nutrient tables for the raw materials and to have easily determinable parameters for the regression equations. For trade it is very important that standard methods of analysis be available for the criteria that are to be used to determine the real value of the raw materials. These methods must be available to the buyer and the seller and be stipulated in the contract. Analyses for legal aspects are more exacting and thus have their own standardized methods, which must stress reproducibility. Circular tests are shown to be the best for the establishment of the magnitude of these legal tolerances.

It is possible to divide the method used by the industry to analyze feeds into two parts: those analyses necessary to optimize the quality of the feed that is sold to the farmer; and those that are required by the government to safeguard the wishes of the consumers, regarding on the one hand good quality of the end products and on the other hand the health of the consumer.

I will use the situation in Holland to discuss the first point; whereas, for the legal aspects I shall try to extend the matters to the European Economic Community, where the harmonization of the legislation is going on in an interesting way. Although forages (except for beet-pulp and alfalfa meal) are normally not of much direct interest to the feed industry, indirectly their analyses are used as a service object by a lot of the producers of concentrates for dairy and beef cattle. I will also discuss the tolerances acceptable for the law because they are a matter of great concern at this moment in the E.E.C.

Industry

The feed industry buys raw materials partly on the world market and partly on the local market. With these raw materials we try to make as economic as possible our total arsenal of feeds. This arsenal consists on the one hand of special feeds such as milk replacers and pet foods that have a very rigid composition as far as both the quality and quantity of their raw materials are concerned. On the other hand we have the feeds for layers and for finishing pigs where a great variety of raw materials can be used and where we can easily compensate for poor quality when we know it exists. The rest of the feeds are somewhere between these two groups; for example, in feeds for cattle where palatability requires restrictions in the type of possible raw materials as well as in their quality.

Other restrictions that we have are very often dictated by the physical quality needed for the end product. Bulk transport, which is about 80% of our total production, requires very good hardness of the pelleted feed, which urges the use of materials with good binding capacity like molasses and certain pulps and which forbids the use of high amounts of fats. On the other hand, we have the feeds that are delivered in meal form. These are also limited in the use of fats and molasses because of the bridging effect of these ingredients in the meal. Depending on the size of the mill and the intentions of the producers, combined with the laboratory facilities available and the skillfulness of the employers, there are many ways to obtain a reasonable product. All of them are guided by a computer. The small millers are using the computers of their vitamin suppliers or those of their advisory organization. These computers are using simple linear programing, based on the national feed ingredient tables for

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the nutrients and on local market prices, to optimize the economics.

The large, more sophisticated, firms are using their own tables based on data of their total feed package using a computer technique called "blend of blends." With these techniques one can build in the optimal use of the stocks already bought and get an indication of which additional raw materials to buy. The big advantage of this system is that those materials that are scarce can be used in the feeds where they are the most economical. These computer systems, the complicated ones as well as the simple ones, are completely dependent on their inputs. This means that one must always try to get the most adequate information in the input. Once this input is correct, the system gives the optimal information one needs. The larger firms, who normally run their own computer program, normally have the ability to incorporate figures deviating from the standard list very quickly. This is not true of the smaller companies that are using a more general system that is much less flexible.

Keeping these basic principles in mind, one can easily understand the most important consideration for a large feed compounder as compared to a small one. The smaller one must try to buy from his dealer those raw materials that are, as close as possible, of the same quality as those in the general list. He very soon runs into problems when he has to recalculate his rations because of a raw material that has an aberrant quality. This is even more important when he has bought a product with different physical characteristics than he expected. Another reason that the normal miller must rely on his dealer is that his own laboratory is normally very small. He must send his samples to a control lab and normally gets the results too late to introduce them in an effective way into his feeds. The normal methods of analyses used at the smaller mills are physical ones, to see if their installations work well, combined with a moisture determination with an infrared heater. The rest they get afterwards from their control laboratories. The big firms, who normally have their own more specialized laboratories, use all the analyses that are important for the inputs into their own computer program. Table 1 gives an example of the data used in a computer input for a normal product.

Crude protein is analyzed because in most raw materials it is used in the regression equations for calculating energy values and for those predicting digestible protein and amino acid values.

The crude fat content is very often correlated with the essential fatty acids and of course with the energy values of a feedstuff (in addition we need to know fat content in relation to the pelletability of the feeds). Crude fibre is used in most of the digestibility equations, and very often its amount is restricted, for instance for young animals, but on the other hand a minimum amount is required in the case for sow, cattle, and rabbit feeds. The moisture in a feed has no feeding value at all. It however has a well-known effect on the keeping qualities of the feed.

Table 1. Data used in a computer input for a normal product.

Crude protein	ME poultry
Crude fat	NE pigs
Crude fibre	VEM dairy
Moisture	VEVI beef
Ash	Palatability
Calcium	Minimum required
Phosphorus	Maximum allowed
Available phosphorus	
Sodium chloride	
Essential fatty acids	
Lysine (av.)	
S-amino acids (av.)	Amount in stock
Tryptophane	Amount available in market
Threonine	Price in stock
Xanthophyll	Price in market

It is sometimes essential to know the ash content for predicting the energy content and very often it is correlated with the calcium and/or phosphorus content. Of the other items in Table 1, the essential fatty acids are only determined in cases where there are no figures available, related to the fat content, or where we do not trust the source of the figures or the supplier of the fat. The same is true for the minerals, calcium, phosphorus, and chloride. The energy values are always calculated. We use our own analytical results and substitute them for the x_1, x_2... etc. in the different regression equations.

In these equations one always works with digestibility figures, as shown by the work of Härtel (1977) from Hohenheim and of Janssen and Terpstra (1979). We need the content of starch

and sugars to predict the digestibility of the nitrogen-free extract. To do this, we assume that the starch and sugar for poultry have a digestibility of 100%. The rest of the NFE has a fairly low digestibility, somewhat different, but constant for the various groups of raw materials. This is why we routinely analyze the starch and sugar content of some of our raw materials, although they are not directly used in the computer program.

I would strongly emphasize once more that the nutritional data must be additive, otherwise the computer cannot work with them. To get these data for the computer input we only do a few analyses directly in our laboratory because we start with available nutrient tables produced by authors who we think are reliable. Having these tables we look for parameters that we can use to find the right figures for our own products.

Depending on the type of raw material, and the feed we use it in, we need a special routine, and I will now look somewhat closer at the different groups.

Cereals

When we have for example a relative simple cereal like yellow corn 3, U.S.A. harvest 1978, the best parameter is moisture. In our table we have calculated every nutrient of corn on the basis of 14.5% moisture. If we buy a shipload with a moisture content deviating more than 1% from this value, we correct our nutrient values for as long as we are using corn from that ship. Experience has shown that for this product it is not worth doing more analyses.

For some of the other cereals we need additional parameters for prediction. Barley requires, in addition to moisture, figures for starch and crude fibre. Milo (sorghum) can contain a high amount of tannins so that its use has to be restricted, and in addition we also have to control fibre. The decision as to what to analyze in addition to moisture is taken normally by the microscopist when samples enter the laboratory. He judges the sample on purity and on normality. His task is to minimize the extra work that must be done by the rest of the laboratory staff. In the second half of every year, new harvests for the cereals from different origins are analyzed more frequently to determine if it is necessary to change the variables in the regression formulas for the new season. These analyses include for the cereals: crude protein; crude fat; crude fibre; ash and moisture; and also starch and sugar, which are enough to calculate the other nutrients in our tables.

By-products

These products are very important within the common market. Due to the levy system, cereals are much more expensive than on the world market. However, the by-products, and at the same time products like tapioca, and beet and citrus pulp, are relatively much cheaper. Often the value of the by-products in the common market is higher than the value on the world market of the cereals they are derived from. For this reason, the flow of these products into the E.E.C. is thus very strong and it is very important for us as users to have a good knowledge of these feedstuffs because their variations in quality are very large. We *normally* analyze these products for protein, fibre, starch, and moisture. Sometimes, as for example with rice polishings, we also examine the fat content.

For a product like beet pulp we determine the ash and the sugar content to determine if the product is mixed with molasses and/or vinasses, which is often done. The microscopist is also an important man for this group of products. Here, however, his first task is to find out if a product is adulterated with hulls or other unwanted materials. If he finds this to be the case, he can ask for additional analyses.

Proteins

Vegetable

These are of course always investigated for their protein content because that is what they are bought for, and variation is often large. Depending on the product, and again microscopic examination, further analyses for fibre, fat, moisture, or ash may be done. Routinely, on the other hand, some products are always analyzed for their fat content — oil cakes, for instance, and some are always analyzed for their ash content — for example, sesame products. Again we must do this because variation is normally very large for these items. Soybean oil meal, being the most important protein source for our nonruminant feeds, requires special attention because of the toasting. Here we analyze the urease activity. Some firms are analyzing trypsin inhibiting activity because it is more scientifically correct; however, I feel it is too difficult for routine use.

Animal

Here we routinely analyze protein, fat, and ash for fish as well as for tankage-type products. When the quality is different from what we expected or when we have an unknown quality,

we further analyze for calcium, phosphorus, and salt, and very often we look for available lysine (Carpenter) and pepsin-HCl digestible protein to see if the products were overheated. From time to time we have to analyze for urea as this is a very lucrative adulterant because, by contract, we pay by percentage protein.

Other animal proteins that are used in our feeds are milk products. Normally their quality is controlled by the government as far as they are fit for human consumption. The remainder are the whey powders, and their quality is determined by their protein, ash, and moisture content. When these products are bought as ingredients for milk replacers then of course their solubility is an important quality criterion.

Besides these three important groups of basic materials used in our feeds, there are some minor products that need special treatment. First of all there are the colouring agents incorporated into layer mashes to influence yolk colour. These are analyzed for their xanthophyll content. We must also analyze minerals, when necesary, for their content of calcium, magnesium, and/or phosphorus. Last but not least, we have the premixes. People who make their own premixes control the separate ingredients, because these analyses are much simpler, prepare the premixes using normally stabilized products, and take care that the ingredients are not too old.

At present, the industry uses those analytical methods that suit it best, which means that very often short methods are used instead of official ones. For example: when we analyze carbonate in the feed, we calculate it as limestone; when we analyze Cl^- ions, we call it salt; and for fat and moisture shorter extraction and evaporation times are normally used than are prescribed in the official tests. However, this requires a lot of experience.

Trade

It is a different problem when analyses are done to control guarantees between the buyer and seller, especially when payments are made per percentage of protein or per percentage of sugar as is often the case with animal proteins or molasses.

Table 2 gives the results of a circular test on a sample of molasses. The first eight columns are from Dutch industries that knew a test was being conducted, the next four are from official labs in Belgium, Holland, and the United Kingdom ($\overline{X}$ is the average figure, and s is the standard deviation.

This test was done because a shipment of molasses was sold with a guarantee of a maximum of 28% moisture and a minimum of 48% total sugars. The buyer had as his control lab B, and the seller had lab G. The agreement was an allowance of 1:1 for both moisture and sugar. This meant 6% for B and only 1% for laboratory G. For this shipment of 1000 tonnes, it meant a difference of 7500 gilders. The method called "moisture 103" is a quick method in which the sample is dried at 103 °C for 4h, after mixing with sand, as is normally done in the United Kingdom. The second moisture method was the one declared as officially accepted in the E.E.C. (E.E.C. 1971a). The analytical procedure we requested when analyzing for glucose and saccharose was the official E.E.C. method as described in E.E.C. (1971b).

Table 2 shows that it is absolutely necessary to put in the contract the standardized analytical procedure that must be used. Even then, one cannot be sure of getting the right answer, so it is wise to have a spare sample available to send to another laboratory in case of disagreement. As an illustration of the type of contracts that are used

Table 2. Results of a circular test taken by the VNMF in 1977 on a sample of molasses.

	D	S	P	K	H	M	T	W	G	V	A	B	$\overline{X}$	s^a	n
Moisture 103	29.3	—	27.2	28.5	28.6	29.8	29.0	28.9	—	—	30.2	31.0	29.2	1.09	9
Moisture E.E.C.	26.6	31.5	23.5	27.4	27.7	—	—	26.8	26.9	27.6	29.5	—	27.5	2.17	9
Invert sugar	13.4	13.9	13.9	14.1	14.2	13.5	13.9	15.1	14.8	14.2	14.6	—	14.2	0.52	11
Saccharose	30.5	30.6	31.2	30.0	30.4	30.6	30.5	32.7	30.4	29.8	32.8	—	30.9	1.00	11
Total sugars as invert sugar	45.5	46.1	46.7	45.7	46.2	45.7	46.0	49.5	46.8	45.6	49.1	45.0	46.5	1.41	12

$$a_s = \sqrt{\frac{(X - \overline{X})^2}{n - 1}}$$

Table 3. Part of the new soybean oil meal contract used by the Grain and Feed Trade Association.

ANALYSIS AND ALLOWANCES OF U.S. SOYBEAN OIL MEAL AND/OR PELLETS

A. Analysis of the sample at arrival

Guaranteed content:

B. Crude protein:

not less than 44.0 %, allowances in a 1:1 ratio in case of deficits.

C. Crude fibre:

not over 7.0 %, no allowances in case of exceeding, till 7.5 %;
over 7.5 % allowances start at 7.0 % as follows:
7.0–7.5 %, in a 1:1 ratio
over 7.5–8.0 %, in a 2:1 ratio
over 8.0–9.0 %, in a 3:1 ratio
over 9.0 in a 4:1 ratio

D. Moisture:

not over 12.5 %, allowances in a 1:1 ratio in case of exceeding.

Valid from 1 February 1979.

in the trade, Table 3 gives part of a new soybean oil meal contract used by the Grain and Feed Trade Association (GAFTA). Here we see that there are independent allowances for protein, moisture, and crude fibre.

As one can see, a redress of 1 to 1 for a too high moisture content is quite realistic. When we have standard material, however, then the protein content will be 0.44% too low at the same time. So in fact you get a bonus allowance of about half a percent in cash for each percent of moisture. When we go to the crude fibre, we see that the punishment for adding too many hulls to a shipment is very severe. Adding an extra 5% hulls to a standardized batch makes the fibre content go up by 1.7%, and at the same time the protein content goes down 1.6% to 42.5%. This means an allowance by this contract of 5.2%. When the "error" goes up to 10%, the allowance becomes 3.2% for the protein and 9.7% for the fibre, thus a total 12.9%. One can clearly see that with the new contract, it is no longer advantageous to add hulls to the soybean oil meal.

Seeing the large allowances that are to be paid for crude fibre it is evident that we need a standardized method so that we can get reproducible values. In this contract we have a tolerance of 7.0–7.5%, but one still needs very good laboratories.

We have a clause for toasting, but it has not yet been included in the contracts because it is very difficult to interpret the analytical results and the trading value. This accounts not only for undertoasting but for overtoasting as well. This is the reason "urease activity" and protein solubility have not yet found their way into these types of contracts.

It is, therefore, absolutely necessary to have the methods that must be used in the analyses of the samples, and the method of sampling, stated in the contract.

Law

So far we have seen that it is important to have reliable methods of analysis. Depending on the laboratory, as far as the feed-industry is directly concerned, or depending on the agreement between buyer and seller, as far as the trade is concerned, either one method or another is chosen without many problems.

This becomes totally different when the *law* is considered. The law requires definite figures and the law permits definite tolerances. Therefore, the law needs strictly standardized analytical procedures. The government, which normally has its own laboratories that have to look after the legal aspects of the regulations, can be considered as an involuntary partner. This partnership has to be

Table 4. E.E.C. methods of analysis and their application dates.

Methods for ingredients		Methods for additives	
Calcium	1-7-72	Detection and identification of tetracyclines	1-7-73
Carbonates	1-7-72	Chlortetracycline	1-7-73
Crude ash	1-7-72	Oxytetracycline	1-7-73
Ash insoluble in hydrochloric acid	1-7-72	Tetracycline	1-7-73
Chlor of Chlorides	1-7-72	Oleandomycin	1-7-73
Lactose	1-7-72	Tyrosine	1-7-73
Potassium	1-7-72	Virginiamycin	1-7-73
Sodium	1-7-72	Retinol (Vitamin A)	1-1-74
Sugar	1-7-72	Thiamine (B_1)	1-1-74
Urea	1-7-72	Ascorbic and dehydroascorbic acid	1-1-74
Urease activity in soybean products	1-7-72	17bis — Starch — Pancreatine method	1-11-74
Moisture	1-1-73	Amprolium	1-11-74
Determination of moisture in animal and vegetable fats and oils	1-1-74	Ethopabate	1-11-74
Volatile nitrogenous bases	1-1-73	DOT	1-11-74
Crude fats	1-1-73	Menadione (Vitamin K)	1-11-74
Phosphorus	1-1-73	Buquinolate	1-11-75
Starch	1-7-73	Furazolidone	1-11-75
Crude protein	1-7-73	Sulfaquinoxaline	1-11-75
Crude protein soluble in pepsin and hydrochloric acid	1-7-73	Fixing of sampling methods	1-3-76
Determination of pepsin activity	1-7-73		
Determination of magnesium	1-1-74		
Crude fibre	1-1-74		
Methods for undesirable substances and products			
Hydrocyanic acid	1-7-72		
Mustard	1-7-72		
Theobromine	1-7-72		
Alkaloids in lupines	1-7-72		
Free and total gossypol	1-7-73		
Aflatoxin B_1	1-3-76		

one-sided as far as it concerns the choice of analytical methods. Here, the criteria cannot be first simplicity, rapidity, and repeatability; here, reproducibility among the different laboratories is much more important.

In the common market a special committee looks after analytical procedures. They have, I think, adopted the very sound principle that they need an official method of analyses for every item covered by law. When the E.E.C. started, it was not possible to do this for all additives and for all unwanted or undesirable substances because they could not afford to wait until all methods were harmoniously accepted. However, a great deal has been covered, as can be seen from Table 4, which gives a list of the E.E.C. methods of analysis and their application dates. This table gives the situation in 1977, but during the last 2 years several new methods for trace minerals and for additives and undesirables have been investigated, but they have not yet been finalized (they are due for completion in 1979).

The system that is used before a method is officially adopted is the one in common use. Proposals for "official" methods are made by one of the members of the "standing committee." After initial acceptance of the draft, it is sent to interested groups for comment. With these comments the final draft is prepared and the application date is set.

Normally the first draft is derived from a method already in practical use in some of the member states; therefore, the whole procedure can proceed very quickly. This is in contrast to the International Standardization Organization (I.S.O.), which wants to get uniformity and in addition wants not only a good reproducible method of analysis but also the best one from a technical standpoint, which takes a tremendous amount of work and time. One of the other problems with I.S.O. is that they want their methods accepted as the official ones by everyone. I do not think that this is right. The authorities are free to select those procedures they think are the most suitable for legal use. The I.S.O. should produce standard methods and offer them to the legislator under the motto: take it or leave it. The main purpose of the I.S.O. should be standardization from a technical point of view, and solving analytical problems for their own organization.

One of the big problems we have at this moment with the analytical standard procedures of the E.E.C. is that they do not give, in all cases, repeatability and reproducibility. This is partly because the range is not known, as is the case with aflatoxin, and partly because it was not always usual up till now, as is the case with the pancreatin-starch method.

In the new feed laws, especially those dealing with straight feeds and compound feeds, we are confronted with obligatory declarations surrounded by tolerances. Those tolerances have to cover analytical tolerance, sampling tolerance, and manufacturing tolerance. In laws concerning additives and those for undesirable substances we must give guarantees on the one hand, but are not allowed to exceed maximum limits on the other hand. The magnitude of the problem is demonstrated in Tables 5 and 6. Table 5 gives data from a circular test held in eight of the nine member countries of the common market regarding aflatoxin B_1 in groundnut oil meal. Table 6 gives data from a circular test in the same countries to control normally required analytical figures in an all-mash layer feed.

To get these results, we did not tell the laboratories that it was a circular test; we just sent the sample with a request for the content of aflatoxin B_1 as determined by the official method of the E.E.C. The laboratories were almost all officially recognized state laboratories. The results show very clearly that we need a tolerance of about 50% at this level, or about 0.2 ppm. At the extremely low level of 10 ppb, which we have to respect as a maximum in our feeds for young animals (E.E.C. 1974), this tolerance probably has to be over 100%. In the past, it has been left to the member states to decide their own tolerances, but eventually the E.E.C. should give these tolerances.

Table 5. Results of a circular test of groundnut oil meal for aflatoxin B_1 content (conducted by FEFAC in 1978).

	Aflatoxin B_1 (ppm)	Cost in gilders
Belgium	0.2	140
Denmark	0.5	160
France	0.17	—
Germany	0.26	110
Holland I	0.38	300
Holland II	0.37	130
Italy	0.35	—
Luxemburg	0.3	85
U.K.	0.27	85

Table 6. Results of a circular test of all-mash layer feed (conducted by FEFAC in 1978).

	Country												
	A	B	C	D	E	F	G	H	I	Ave.	Stand. dev.	Calculated value	Tolerance
Moisture (%)	10.4	10.4	10.5	10.5	10.6	10.2	9.9	*7.5*	10.0	10.00	(0.97)	11.3	1.1
In dry matter													
% Protein	20.3	20.1	19.4	19.4	**21.2**	20.6	21.0	20.6	20.4	20.33	(0.63)	19.8	1.2
% Crude fibre	6.6	**4.7**	8.1	—	6.9	6.8	**9.5**	6.7	6.7	7.00	(1.37)	7.4	1.1
% Crude fat	8.0	8.4	8.0	8.3	8.1	7.9	7.8	8.1	8.1	8.08	(0.19)	8.1	0.8
% Ash	12.8	12.2	13.3	—	12.9	13.6	12.4	12.6	12.2	12.75	(0.51)	14.1	1.0
% Sand/sil.	0.9	1.1	—	—	1.4	1.0	1.1	0.9	0.9	1.04	(0.18)	1.3	0.3
% Ca	3.5	3.5	3.6	**3.0**	3.8	3.5	3.6	**2.8**	3.5	3.42	(0.32)	3.6	0.4
% P	0.8	0.7	0.8	0.7	0.9	0.8	0.8	0.8	0.8	0.79	(0.06)	0.8	0.1
% Na	0.22	0.23	0.20	—	—	0.21	0.21	0.17	0.20	0.21	(0.02)	0.25	0.1

The problem becomes even larger when we consider the pesticide residues that are to be tolerated in the feed (E.E.C. 1977a). The maximum levels allowed here vary from 10 to 1000 ppb, and the analytical techniques are much more difficult. It is not only the gas chromatograph that gives problems. The steps used during the purification process that gets rid of the interfering impurities are very often the cause of very large variations between different laboratories. Here we also need tolerances of at least 100%.

Table 6 presents the results from a circular test with a layer feed. Again we did not indicate that it was a circular test; we just asked for an analysis of the feed following the official E.E.C. methods. We did not check to see if they actually did use E.E.C. methods; we just collected and tabulated the results. In the E.E.C. directive for straight feeds (E.E.C. 1977b), and in the draft of the one for the compound feeds (E.E.C. 1978), tolerances are proposed for the declared feed values. The first nine columns of Table 6 give the figures produced by the labs in the different countries. Column 10 gives the average; 11 the standard deviation; and 12 the figures taken from the computer and based on the input. The last column gives the tolerances proposed by the E.E.C.

When we get a one-sided tolerance of the magnitude of the E.E.C. proposal we have no problems. We can always adapt the figures on the label of our feed bags in such a way that we are legally always correct. When we have a two-sided tolerance, however, then we run into problems, as can be seen from the table. The main cause of discrepancies between the E.E.C. and the industry concerning tolerances is based on the extra room needed for manufacturing deviation. On the other hand, we understand that the E.E.C. wants the double-sided tolerance because without it lots of people would declare every maximum 100% and every minimum 0%, which would mean that most of the obligatory items would not say anything realistic about the quality of the feed.

The only thing that really counts about the quality of the feed is its balanced energy level. Until now it has not been realistic to require a declaration of energy level because it is too difficult to analyze. Perhaps in the future, if the system of Sibbald (1979) is accepted, the situation will be better, but for this everyone needs a bomb calorimeter and that will be difficult for the smaller manufacturers.

Last but not least: what are we doing with the forages? As I said it is a matter of service for our farmers. In Holland we have special laboratories, where all roughages are analyzed. The farmer sends a sample from every batch of roughage he has made. Then the laboratory analyzes the crude protein, the crude fibre, the ammonia, the dry matter, and the organic matter. Depending on the product the digestible organic matter is calculated and with the DOM, the VEM (feed unit for

dairy cattle with average production of 15 kg milk/day) or the VEVI (feed unit for beef cattle with intake of 1.5 times maintenance). We work quite well with this method and we do not feel the necessity to switch to a more sophisticated system. When we must switch, we will. But, the institutes must prove it is worthwhile, and we must have *better* predicting regression equations than we now have.

Conclusions and Recommendations

The INDUSTRY uses its own methods of analysis, suited to the circumstances of the plant and to the capacities of its laboratory. Its main purpose is to have reliable nutrient tables of the raw materials, and in addition we have easily determinable parameters to fill in the figures in the regression equations.

For the developing countries it is the task of the advisers to supply the right tables and to give the system for correcting those tables if necessary and possible. At the same time these advisers have the task to make sure that the microingredients, the vitamins, trace-minerals, coccidiostats, etc. are fully guaranteed.

Roughage control is done by specialized laboratories. They are using those methods that are used in the regression equations given by the national or international research institutes. This is a task for the government or the universities of the developing countries, because industry itself is not yet strong enough at this moment.

The TRADE has its own rules, but it is very important that there are standard methods available for those criteria that are necessary to determine the real value of the raw materials. These standard methods must be available to the buyer as well as the seller. The contracts must contain the phrase: guaranteed at arrival.

The LAW has its own standardized methods. These must be used to control the legal aspects of the industry. It is necessary to determine the magnitude of the tolerance by means of circular tests without foreknowledge, because this is the practice. Of course one has to include afterwards the manufacturing tolerance.

Standardization of Procedures

Elwyn D. Schall[1]

The shortcomings of crude fibre as a meaningful nutrition labeling guarantee are generally acknowledged. The same is true, to a lesser extent, of crude protein and crude fat. However, the literature contains a century of accumulated data for these components in feed ingredients and they serve a useful purpose in the comparative analysis of feeds even though their use in absolute nutritional evaluations has many limitations. For these reasons it may be more expedient to supplement present labeling rather than attempting its displacement.

The general acceptance of additional labeling, particularly by regulatory agencies, requires tested and approved methods of analysis applicable under widely varying conditions. These agencies generally do not permit unenforceable guarantees.

Method development, testing, and approval is a function of organizations such as the Association of Official Analytical Chemists. The AOAC is a volunteer scientific organization and welcomes participation of all qualified and interested scientists in providing leadership for method development and evaluation.

The application of approved methods is a function of regulatory agencies, nutritionists, trade associations, feed manufacturers, and others interested in evaluating the quality of feeds and feed ingredients.

The mechanisms for developing and adopting new methods of analysis for feedstuffs are well established through organizations such as the Association of Official Analytical Chemists (AOAC). However, bringing these better methods into general use is frequently a more difficult problem, particularly where they are to substitute for long established methods. It is a little like discarding an old pair of shoes of proven comfort for a new pair whose performance is still in question. We tend to feel more secure with the old, known quantity than with the new. We have many examples of this in the AOAC Official Methods of Analysis (AOAC 1975), commonly referred to as "The Book of Methods." Developing and testing methods are one thing; getting them into general use is quite another.

The role played by associations such as the AOAC and the Association of American Feed Control Officials, as well as other testing bodies and regulatory agencies, in developing methods on the one hand and using them on the other, is frequently confused and I should like to clarify this point.

The AOAC is a scientific and educational organization whose function is to make available tested methods of analysis within its area of interest. The Association does not promote methods once they are developed nor does it recommend one method over another where two or more methods for the same component may be approved. This choice is left to the user who selects a method most nearly meeting his particular needs and conditions.

The AOAC methods are occasionally referred to as standards, although this is a misnomer in the strict interpretation of the word. The term "standard" implies a minimum degree of quality in a product; whereas, a chemical method is a means of measuring compliance with such a standard. I think it particularly important to make the distinction in feedstuffs because certain areas of the United States have had established standards for these products for many years. These standards were regulatory decisions and not chemical methods.

The function of the AOAC is to provide a means of measuring the level of a chemical entity in a sample. Whether that entity is a useful factor to the nutritionist, manufacturer, consumer, regulatory agency, or other user of the method is for them to decide. We can illustrate this by an example. The acid-detergent fibre method developed by Van Soest was adopted as an official method by the AOAC 6 years ago and is included as an official procedure in the Official Methods of

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Analysis (AOAC 1975). Included in the same chapter is the time honoured crude fibre method, but the AOAC does not pass judgement on the relative nutritional merits of crude fibre and acid-detergent fibre. If the user is interested in measuring one or the other, a proven method is provided for his use. The judgement as to what he wishes to measure is his.

There is a growing dissatisfaction with the older crude fibre method, particularly as it is applied to forages, as a measure of nutritional quality of these products. The goal of this workshop, as I see it, is to explore avenues of: (1) developing methods to measure the nutritional qualities of feedstuffs more accurately, and (2) equally important, bringing them into general use and applicability once they are developed. The development of the method is a scientific matter. Bringing it into general use is a matter of judgement and choice on the part of the user.

Who, then, determines whether and how frequently a method is used, particularly methods relating to the analysis of feedstuffs. Obviously, nutritional researchers are an important segment of this group. You are seeking very specific responses and you are quite selective and critical in your search for and use of methods that will measure the nutritional attributes in which you are interested. Methods can serve research purposes very adequately and yet not become widely used in the feed industry. I suspect that the acid-detergent fibre is an example of this.

Other users include laboratories concerned with quality control in feed manufacturing operations, commercial laboratories involved in developing and implementing trading rules between sellers and purchasers, and regulatory agencies, among others. Of all of these, I suspect the influence of the regulatory agency has the greatest impact on whether a method becomes widely used. This suggests that the pathway to better nutritional labeling of feedstuffs on a routine basis consists of two steps: (1) scientifically sound methods must be developed, which are generally applicable to the products found in the commercial trade; and (2) regulatory officials must be convinced of the value of these newer methods and designations so that they become a part of the required guarantees and labeling of the products.

I should like now to describe the operation of the AOAC for you and suggest how it can respond to your requests for better methods of analysis for feedstuffs. The AOAC is the primary source of methods for North American regulatory agencies. It also serves other areas of the world to a lesser extent, although regulatory posture in the matter of method selection varies greatly throughout the world. I shall then discuss the regulatory aspects and the procedures involved in moving toward acceptance by the regulatory arm for a requirement to include specific guarantees in the labeling.

Collaborative Study Procedures of the AOAC

The AOAC ia a unique, nonprofit scientific organization whose primary purpose is to serve the needs of government regulatory and research agencies for analytical methods (AOAC 1978). The goal of the Association is to provide methods that will perform with the necessary accuracy and precision under usual laboratory conditions (AOAC 1977). Since its formation in 1884 the AOAC has provided a mechanism to select methods of analysis from published literature or develop new methods, collaboratively test them through interlaboratory studies, approve them, and publish the approved methods for a wide variety of materials relating to foods, drugs, cosmetics, agriculture, forensic science, and products affecting the public health and welfare. Its membership is composed of scientists from Federal, State, Provincial, and other regulatory bodies who work within the AOAC's established procedures as researchers, methods collaborators, and committee members. Although most of the members are from North America, many nations throughout the world are represented.

The AOAC has almost a century of experience in utilizing collaborative study as a means of determining the reliability of analytical methods for general purposes and, especially, for regulatory purposes. In fact, the AOAC's major contribution to analytical science has been to bring the collaborative study technique for the validation of analytical methods to a high degree of perfection. In such a study, laboratories analyze identical sample sets that cover the range of applicability of a method previously selected as being useful and practical. The purpose of the study is to establish the characteristics of the methods with respect to accuracy, precision, sensitivity, range, specificity, limit of detection, limit of reliable measurement, selectivity, practicality, and similar attributes, as required.

Organization and Procedures for AOAC Collaborative Studies

The collaborative study is organized and directed by an analyst designated as the associate referee for the specific subject under investigation. Currently, some 600 associate referees appointed by the association are responsible for as

many topics. An associate referee is selected for his knowledge, interest, and experience in the subject matter field. He operates under the scientific guidance, support, and administrative supervision of a general referee, who is in turn responsible for a product area. The associate referee reviews the literature and selects one or two of the better analytical methods available, modifying them as needed. Alternatively, he may develop or adapt a method used in his laboratory for the analyte and matrix under study, testing it thoroughly in his laboratory before designing a collaborative study. The general referee is kept informed of such preliminary studies.

The samples analyzed in a collaborative study are normally prepared and distributed to the participants by the associate referee. The association follows the recommendations of Youden (1962) that not fewer than five laboratories participate and that a minimum of six sample materials be sent to each. These are minimal and, in practice, both are usually exceeded. In addition, a reference or practice sample is included, where possible.

Laboratories with at least some experience in the general subject matter are selected as collaborators. Because the objective of the study is to standardize the method, as contrasted to standardizing the analyst (Egan 1977), all analysts are instructed to follow the method exactly as written even though they may not concur with the associate referee's selection among possible alternatives. The level of the analyte in the samples is usually unknown to the participants.

All individual results obtained by the collaborators are reported to the associate referee, who compiles and evaluates them. Because statistical treatment of the data is considered essential in a rigorous evaluation of the method for accuracy, precision, sensitivity, and specificity, it is now required for all studies. The association considers this of such importance that it provides statistical assistance in all cases where it is otherwise unavailable to the associate referee. A statistical manual (Youden and Steiner 1975) is also provided.

The associate referee makes the initial judgment on the performance of the method. If he recommends approval, it passes to the general referee and then to a committee of experts. If both recommend approval, the method is presented at the association's annual business meeting for vote by the membership. Approved methods and supporting data are published in the *Journal of the Association of Official Analytical Chemists*. They are subject to scrutiny and general testing by other analysts for at least a year before final adoption. They may be modified and restudied collaboratively as needed, should feedback from general use reveal flaws in the method or in its written set of directions. Approved methods are included in the Association's "Official Methods of Analysis," a book of some 1000 pages, which is updated every 4-5 years.

The preceding summary of AOAC's modus operandi recognizes the need for healthy skepticism toward results obtained by analytical methods that have not undergone such rigorous scrutiny and interlaboratory testing of their accuracy, precision, dependability, specificity, and practicality.

Selection of Methods for Study

A certain degree of variability is associated with all measurements. Much of the research on analytical chemistry is an attempt to minimize that variability. But there are many different types of variability in analytical work. We often find that when we attempt to minimize one kind, we must necessarily permit expansion in another kind. In practical analytical chemistry, the problem often comes down to which variability is to be minimized.

Some examples of this point may be helpful. In atomic weight determination, everything — especially *practicality* — is sacrificed for *accuracy*. A high degree of accuracy and practicality is required in the assay of precious metals, but the fire assay used is generally *applicable* to little else besides metals and minerals. In clinical chemistry, within-laboratory *precision* (repeatability) is critical, and often is of greater interest to clinical laboratories than absolute accuracy or agreement with the values of other laboratories (reproducibility). In drug analysis, a high degree of accuracy is required in the therapeutic *range* because the analytical values determining the identity, strength, quality, and purity of pharmaceutical preparations, as laid down in pharmacopoeial specifications, are directly related to clinical value. With polynuclear hydrocarbons, *specificity* is important, because some of these compounds are carcinogenic while others are not. In applying the famous Delaney clause of the United States Federal Food, Drug, and Cosmetic Act, all attributes of the analytical methods are secondary to the detection of extremely small concentrations (*detectability*), or to exhibiting a high degree of response for small changes in concentration (*sensitivity*).

There is a very special case involving accuracy, where the "true value" is determined by the method of analysis. Many legal specifications and standards for food and agricultural products de-

fine ill-defined components such as moisture, fat, protein, and crude fibre in terms of reference methods. Therefore, the precision of these methods becomes the limiting factor for their performance. In fact, most analyses involved in commercial transactions require primarily that the buyer and seller agree on the same value (analytically and economically), regardless of where it stands on an absolute scale.

The point of these examples is that although methods of analysis are characterized by a number of attributes — accuracy, precision, specificity, sensitivity, detectability, dependability, and practicality — no method is so flawless that all these qualities can be maximized simultaneously. For any particular analysis, the analyst must determine, on the basis of the purpose of the analysis, which attributes are essential and which may be compromised.

Unfortunately, the literature is replete with examples indicating that an individual analyst, and especially the orginator of a method of analysis, is not an unbiased judge of the relative merits of the methods of analysis he develops and uses. In our experience, the collaborative study provides impartial data on the suitability of the method. The data, in many cases, speak for themselves.

The collaborative study, or ring test or round robin test, as it is called in other organizations, provides the basic information on the performance of analytical methods. The extent of the information will depend on the number of samples provided, the number of analyses performed, and the number of laboratories participating. The data should be unbiased because the composition of the samples is known only to the administrator of the study.

Some of the requirements of the study and their relationship to the characteristics and attributes in the method are as follows: (1) *accuracy* — samples must be of defined composition (by spiking, by formulation, or by analytical consensus); (2) *specificity* — samples should contain related analytes; (3) *sensitivity* — samples should differ from each other or from negative samples by a known amount; (4) *applicability* — samples should include the concentration range and matrix components of interest; (5) *blanks* — samples should include different matrices with "none" of the component of interest; (6) *precision* — instructions should request replicate analyses by the same or different analysts in the same laboratory, preferably on different days. By far a better procedure is to include "blind" (unknown to the analyst) replicate samples in the series; and (7) *practicality* — instructions should request information as to the actual and elapsed time required for the analyses, the availability of reagents, equipment, and standards, and any necessary substitutions. When practice samples are included, the number of analyses required to achieve the stated recovery and repeatability should be reported.

Procedural Details of Collaborative Study

As numerous beginners in this field have discovered, much preliminary work must be done before sending out samples.

(1) The method must be chosen and demonstrated to apply to the matrices and concentrations of interest.

(2) The critical variables in the method should have been determined and the need for their control emphasized (a ruggedness test (Youden 1963) is useful for this purpose).

(3) The method should be written in detail by the associate referee and tested by an analyst not previously connected with its development.

(4) Unusual standards, reagents, and equipment must be available from usual commercial sources of supply, or sufficient quantities must be prepared or obtained to furnish to the participants.

(5) The samples must be identical and homogeneous so that the analytical sample error is only a negligible fraction of the expected analytical error.

(6) A sufficient number of samples must be prepared to cover typical matrices and the concentration range of interest (tolerance, maximum or minimum specifications, likely levels of occurrence, etc.).

(7) Samples must be stable and capable of surviving the rigors of commercial transportation.

(8) Reserve samples should be prepared and preserved to replace lost samples and to permit reanalysis of samples considered as outliers to attempt to discover the cause of abnormal results.

(9) The instructions must be clear. They should be reviewed by someone not connected with the study to uncover potential misunderstandings and ambiguities.

(10) If the analyte is subject to change (e.g. bacterial levels, nitroglycerin tablets), provision must be made for all participants to begin the analysis at the same time.

(11) Practice samples of a known and declared composition should be furnished with instructions not to analyze the unknowns until a specified degree of recovery and repeatability (or other attribute) has been achieved.

(12) Provision should be made when necessary for submission of standard curves, tracings of recorder charts, or photographs of thin-layer plates to assist in determining possible causes of error.

Other Types of Interlaboratory Studies

This type of collaborative study, which is designed to determine the characteristics of a method, must be carefully distinguished from other types of interlaboratory studies that by design or through ignorance provide other kinds of information. The most important types of other studies are listed below.

(1) Those studies that require the collaborators to investigate the variability of parts of methods or applicability to different types of samples. (An interlaboratory study is usually an inefficient way of obtaining this type of information.)

(2) Those studies that permit an analyst to use any method he desires. Such studies invariably produce such a wide scatter of results that the data are of little value for evaluation of methods. They may be useful in selecting a method from a number of apparently equivalent methods, provided the purpose is emphasized beforehand and the participants provide a description of the method used to permit a correlation of the details of the methods with apparent biases and variabilities.

(3) Those studies that are used for quality control purposes, whose participants are not permitted sufficient time to gain familiarity with the method, or who permit deviations to enter into the performance of the analyses on the grounds that the deviation is obviously an improvement that could not possibly affect the results of the analysis, or who claim to have a superior method.

With this background information, it is now appropriate to introduce the following definitions, which were agreed upon as part of the guidelines for collaboration between the AOAC and the Collaborative International Pesticide Analytical Council Ltd. (CIPAC) (AOAC 1974).

Collaborative study. An analytical study involving a number of laboratories analyzing the same sample(s) by the same method(s) for the purpose of validating the performance of the method(s).

Preliminary interlaboratory study. An analytical study in which two or more laboratories evaluate a method to determine if it is ready for a collaborative study.

Laboratory performance check. The analysis of very carefully prepared and homogeneous samples, normally of known active ingredient content, to establish or verify the performance of a laboratory or analyst.

Regulatory Labeling Requirements

Following the development and testing of new methods by the AOAC or similar organizations, the next step should be the consideration by the regulatory branch of the government of incorporating these new parameters into required labeling. The initial contact in North America is the Association of American Feed Control Officials, commonly known as AAFCO. The purposes of this 70-year-old association, as quoted from its bylaws (AAFCO 1978), are as follows:

"The purposes of the corporation shall be to establish and maintain an Association through which officials of any state, dominion, federal or other governmental agency on the North American Continent, and employees thereof charged with a responsibility in enforcing the laws regulating the production, labeling, distribution, or sale of animal feeds or livestock remedies may unite to explore the problems encountered in administering such laws, to develop just and equitable standards, definitions and policies to be followed in enforcing such laws, to promote uniformity in such laws, regulations and enforcement policies, and to cooperate with members of the industry producing such products in order to promote the effectiveness and usefulness of such products."

As a part of their function in promoting uniformity between the various governmental units regulating the sale of commercial feeds, the association has developed a Uniform State Feed Bill, which it encourages the states and other governmental units to follow in adopting laws governing the sale of commercial feeds. The model bill was completely redrafted about 10 years ago and to date some 24 states have passed legislation identical to, or essentially the same as, the model bill. Section 5, dealing with the subject of required labeling on commercial feeds, reads in part:

"Section 5. Labeling

A commercial feed shall be labeled as follows:

(a) In case of a commercial feed, except a customer-formula feed, it shall be accompanied by a label bearing the following information:
  (1) The net weight.
  (2) The product name and the brand name, if any, under which the commercial feed is distributed.
  (3) The guaranteed analysis stated in such terms as the (Administrator) by regulation determines is required to advise the user of the composition of the feed or to support claims made in the labeling. In all cases the substances or elements must be determinable by laboratory methods such as the methods published by the Association of Official Analytical Chemists."

Two points are of interest. First, the requirement for the product to be labeled is a part of the law, but the specifics as to how the guaranteed analysis must be stated is delegated to the administrator of the act who is authorized to adopt regulations detailing this requirement. This provides the administrator maximum flexibility to meet changing needs and conditions because he can amend regulations easily and promptly. Amending a law requires an action by the legislative body and is a much more time consuming process.

A second point of interest in the labeling provisions (Section 5(a) (3)) is the requirement that "in all cases the substances or elements must be determinable by laboratory methods such as the methods published by the Association of Official Analytical Chemists." Some latitude is provided in the model regulation in that methods "such as" those of the AOAC are specified. Some states tend to be more restrictive and require only AOAC methods to be used, if they are available for the component in question. In view of these requirements, any method proposed for general adoption in North America will have a much greater chance of acceptance in the regulatory channels if it has been approved as an official method by the AOAC.

The AAFCO model regulation covering the specifics of stating the guaranteed analysis is included under Regulation 2, which reads in part as follows:

"Regulation 2. Label Format

(a) Commercial feed, other than customer-formula feed, shall be labeled with the information prescribed in this regulation on the principal display panel of the product and in the following general format.

(1) Net weight.

(2) Product name and brand name if any.

(3) If a drug is used:

I. The word "medicated" shall appear directly following and below the product name in type size, no smaller than one-half the type size of the product name.

II. The purpose of medication (claim statement).

III. An active drug ingredient statement listing the active drug ingredients by their established name and the amounts in accordance with Regulation 4(d).

IV. The required directions for use and precautionary statements or reference to their location if the detailed feeding directions and precautionary statements required by Regulations 6 and 7 appear elsewhere on the label.

(4) The guaranteed analysis of the feed as required under the provisions of Section 5(a)(3) of the Act include the following items, unless exempted in (VIII) of this subsection, and in the order listed:

I. Minimum percentage of crude protein.

II. Maximum or minimum percentage of equivalent protein from non-protein nitrogen as required in Regulation 4(e).

III. Minimum percentage of crude fat.

IV. Maximum percentage of crude fiber.

V. Minerals, to include, in the following order: (a) minimum and maximum percentages of calcium (Ca), (b) minimum percentage of phosphorus (P), (c) minimum and maximum percentages of salt (NaCl), and (d) other minerals.

VI. Vitamins in such terms as specified in Regulation 4(c).

VII. Total sugars as invert on dried molasses products or products being sold primarily for their sugar content."

Section (a)(4) is of particular interest because it contains the guidelines for the crude protein, fat, and fibre guarantees.

The question has been raised of whether AAFCO could substitute neutral-detergent fibre for crude fibre in the labeling requirement. The answer in one word is "yes," but there are a number of requirements that must be met before this can be done. First of all, we must have a method that is applicable to the general run of commercial feeds moving in commerce. Because the regulatory official encounters samples of all conceivable composition and ingredients, the method must be able to handle these complex mixtures with minimum interference from the variable quantity and quality of these ingredients. Methods with limited applicability can serve research needs quite adequately but they are not very useful for regulatory purposes. One of the factors

contributing to the slow adoption of the detergent fibre procedures has been their limited applicability to hays and forages, which are relatively simple mixtures of ingredients. In addition, these commodities are largely exempt from feed control laws. These methods serve the researcher very well for their intended purpose, but they have not been developed to the point where they are generally applicable to mixed feeds. As indicated earlier, reliable laboratory methods are needed before additional guarantees are required.

Secondly, the matter of displacing crude fibre guarantees has other ramifications. Although only an empirical method with many shortcomings generally acknowledged, it has over a century of accumulated data in the literature and is a useful reference point in the comparative analysis of various ingredients. You may not know its precise nutritional meaning but it does provide a basis of relating quality, in a general way, of one ingredient with another. Crude fibre data are so widely spread through the literature, and are so readily available and firmly entrenched, that displacing crude fibre as a required guarantee is going to be a very difficult and time consuming process.

I suggest that a more fruitful procedure is to approach regulatory officials with the concept of supplementing crude fibre guarantees with the newer, more meaningful values. I think they would be amenable to including guarantees, for example, for acid-detergent fibre or neutral-detergent fibre if the nutritionists can demonstrate the need for and the benefit of these additional guarantees. Concurrently, of course, approved methods of determining these components would be needed for enforcement purposes. Regulatory officials generally do not permit unenforceable guarantees.

The procedures for method approval through the AOAC was presented above. The initial requirement is the appointment of an interested investigator as the associate referee for the subject in question. The AOAC stands ready to make these appointments upon request. A list of interested investigators should accompany the request together with a brief summary of their qualifications so that qualified appointments can be made to better the chances of success of the work. Associate referees are not limited to North American residents. In fact, we have an increasing number from other countries, with representatives currently from Germany, The Netherlands, Mexico, Kuwait, and Venezuela.

Speaking from the regulatory standpoint there is occasional pressure to expand the number of guarantees permitted on commercial feeds in the U.S. This comes primarily from a limited number of feed manufacturers at the present time. To some extent this is a sales promotion gimmick but some of it also has nutritional support. As examples of the type of requests that we receive I would cite the demand for amino acid guarantees. These range from the limiting amino acids, particularly lysine, tryptophan and methionine, to requests to guarantee all known amino acids. Control officials are becoming much more sympathetic toward permitting guarantees for lysine, tryptophan, and methionine but methodology is still presenting problems to their general acceptance.

The second most prevalent request is for energy guarantees. The general reaction among regulatory officials at the present time leans toward the view that energy guarantees are misleading and are unenforceable. Further work is needed in defining and measuring energy values before they will be permitted on labels as guarantees.

A third area of increasing interest is that of guarantees for "available" nutrients. This includes such items as digestible protein and guarantees for available mineral elements. Nutritionally, of course, these are very important parameters because the animal response depends upon the availability of the nutrients. Practical procedures to monitor these properties in commercial feeds are not available and the general regulatory attitude is that including these guarantees in the labeling is premature at the present time. This represents an area where new methodology is needed before much progress can be expected toward including these guarantees in the required labeling.

Finally, I would call your attention to a developing trend in the United States that may have a bearing on the acceptance of additional guarantees. This may well be limited to the U.S. but it is a factor of which you should be aware. Even though there is some interest in additional guarantees, as discussed above, there is also a growing concern by the feed manufacturer regarding the liabilities assumed or implied by a guaranteed analysis. The view is held that the greater the number of guarantees the greater the chances of a deficiency being found and thus the greater the chances of legal suits being instituted against the company. There is some justification for this concern. It is supported by the growing consumer protectionist climate that is developing and that encourages an increasing number of liability suits, whether justified or not. The greater the number of guarantees the greater the exposure to these liability suits. Reputable manufacturers

willingly back the quality of their products and handle cases promptly and voluntarily based upon merit. They are understandably reluctant to invite an outpouring of nuisance suits that are very expensive both in time and money. They are quite willing to guarantee their products with meaningful labeling but any additional guarantees proposed should serve a meaningful purpose.

Relationship to INFIC: Feed Data Documentation and Standardized Methods

H. Haendler[1]

The worldwide need for feed data has led to the development of centres for feed data documentation and to international cooperation within INFIC. The principles of feed data documentation include generalization of statements that are collected in the form of data. Generalization requires identification of all constituents of the statements.

For the identification of the "object" of the statement, i.e. the feed, a system for conceptual analysis and description has been developed. A multilingual feed vocabulary, consisting of six facets with descriptors for denoting the different types of characteristics, allows the systematic analysis and description of a feed. Within the recording and coding system codes are provided for an additional ("individual") description of the feed samples.

For generalizing the second constituent of the statements, i.e. the property of the feed as found by chemical analyses, standardized analytical methods and standardized designations for the results are demanded. Special attention should be given to an international standardization of the systems for energetic evaluation of the feed value. Efforts to adopt accurate and simple methods — at least for estimating digestibility in vitro — should be endorsed.

Within agriculture there exists a special need for information dealing with results of feed analyses. The units of information are called informemes, and can be regarded as statements that inform users. Informemes are represented by data. Data on feed composition are a necessary ingredient for all decisions concerning the feeding and nutrition of farm animals, the preparation of diets, the manufacture of mixed feeds, and the planning of forage production.

In an exceptional case it may be possible to "produce" such information on feeds by carrying out experiments with animals and chemical analyses in a laboratory. This requires time and money, mostly much more than available, and the more complete and exact the knowledge that is demanded, the more time and money that are required. The costs for the production of such information increase considerably from one level of analyses to the next: the lowest in cost are simple analyses of nutrients; the next, detailed analyses (minerals, amino acids, vitamins etc.); considerably increased are the costs for trials with animals for determining digestibility; and finally, obtaining energy values with respiratory equipment is extremely expensive.

This way of "self-producing information" therefore is not practicable for answering regular demands of information. But, the results of different experiments and analyses, being the single pieces of knowledge, can be used again and again for the information requirements of many users. Information — contrary to other commodities — has the advantage of not becoming exhausted by being used; it can be stored for unlimited periods and can be used wherever and whenever it is demanded.

For more than 100 years, feeds have been analyzed in laboratories all over the world. Experiments have been carried out with animals to determine nutritive value, digestibility, or particular effects of feeds in diets. This work was done initially to answer a particular question, and the results of this experimental and analytical work were used for actual research on, or control of, a special feed and were not stored for general information and retrospective retrieval. Later it was recognized that these data, scattered in many institutions around the world, constituted an enormous source of information. But, to make use of this information it must be collected systematically and processed using specific

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methods. This kind of work comprises feed data documentation.

There is no question that efficient work in data documentation, especially the utilization of data sources distributed worldwide, demands international cooperation. This was started after valuable preliminary efforts made by FAO, with the foundation of the International Network of Feed Information Centers (INFIC) in 1971 (INFIC 1979). The preliminary work has been carried out to develop a new international system and to prepare methods for worldwide data exchange and for data distribution in different languages. This coordination required hard work to overcome the incompatibility of single systems and to prepare new documentation tools and methods acceptable to and usable by all INFIC members.

The Principles of Feed Data Documentation

Analytical work in laboratories and feed data documentation have the common goal of providing information on the composition and/or nutritive value of feeds. The difference is that analytical work in the laboratories leads to a specific statement about a special sample of a feed concerning a specific property or value; whereas, feed data documentation communicates general information to different types of users. Hence, the documentalist must generalize the results of laboratory work: i.e. the special statement (given as the result of a single observation) must be modified to a general statement (useful beyond the special situation). To do this work of induction (in the philosophical meaning of the term) without affecting the reliability of the information, some presuppositions must be made.

The first problem is statistical and well known: How many single observations are necessary to allow an inductive conclusion? This problem is very closely combined with the question: How representative are the single observations? To give an example, one does not need many observations to come to statements like "swans are white" or "ravens are black" because black swans and white ravens are very rare birds. Thus, relatively small populations of swans and ravens are representative enough to permit the conclusion that these birds are white and black, respectively. But, to make a real statement like that given in the example, one must be able to distinguish between swans and ravens. This is not as simple as it looks at a first glance, and stresses a very important point concerning the relationship between laboratory and documentary work. To keep the example: If one "analyst" reports an observation on a white bird and another observation on a black bird — and both may be exactly right — the "documentalist" cannot conclude "birds are gray."

Returning to feeds, let us assume that an analyst has received a sample of an unknown feedstuff, analyzed it, and told the customer its protein content and that he did not find toxic substances in it. This may be good and valuable information for the customer (he may have 100 tonnes of this material and want to feed it to his pigs), but it is not the information required by the user of the feed information centre. Therefore, the centre will not record and process such data.

The analyst may use the best and most reliable method to determine a component or the nutritive value of a feedstuff, and his work may be very successful for his customer, but to make further use of the results via data documentation a very clear and exact identification of the "object of observation," i.e. the analyzed feed, must be given. Generalizing specific statements is feasible only if the object of the single case is well identified. Identification here is meant in its strictest meaning: Is one object identical or equal to the object one wants information about? Someone who wants information about ravens cannot use any information about swans; he can only use information on the identical bird!

However, identification in the case of feeds is much more difficult that in the case of swans and ravens or of any other birds or animals or things. This is because swans, ravens, and things like these are objects in the narrower sense of the word, objects having a form, a body with a shape. On the contrary, feeds are amorphous substances. One cannot really distinguish between single individuals, and therefore one cannot really group individuals into classes. Nevertheless, if we want to process data from different observations, made at different places of the world, we have to decide exactly which data can be brought together for generalizing statements and for calculating averages and which data have to be handled separately.

An observation on a feed is the result of the chemical analysis on a specific sample. There is no other choice but to regard such a sample as a quasi-individual even if it is not an object in the strong sense of the word.

If we want to identify or classify something, we have to look for its characteristics. The sum of its essential characteristics determines the concept of the "thing," its relationship to other concepts, and, therefore, its place in a conceptual system (classification system). The determination of the characteristics of a feed may be regarded as

its conceptual analysis. The history of philosophy shows that this type of analysis is much older than chemical analysis — say 2000 years. Aristotle, the father of logic, introduced the term "analytics" for what was later called "formal logic."

Although chemical analysis of a feed sample leads to results like protein content, fibre content, etc. and definitely to its nutritive value, conceptual analysis leads to characteristics (conceptual factors) and definitely to the concept.

Just as the result of a chemical analysis can be made into a statement, so can the result of a conceptual analysis. The generalization of statements needs standardization. This is true for statements on both chemical composition and conceptual composition. In both cases standardization concerns the methods of the analysis as well as the description of the results. Only materials designated in the same manner can be compared or summarized.

The first step toward generalizing statements must be the standardization of the constituents of these statements. These are, as has been shown, the results of the conceptual analysis (including their description) on the one hand and the results of the chemical analysis (including their designation) on the other. Both sides are equally important for feed data documentation and information.

Systematic Feed Description

The methods and tools for conceptual feed analysis and the description of its results have been developed for practical purposes by INFIC (INFIC 1979). Although this work has been carried out by members of the INFIC group, using the experiences of Hohenheim University in Stuttgart and Utah State University, the principles of the methodology date back to Aristotle's theory of categories. These principles have led to the elaboration of faceted indexing languages and classification systems. All these systems not only assign the concepts (represented by its denominations or terms) to different categories or facets but also distinguish sharply between the "basic category" of substance (according to Aristotle; named entities, objects, concretes, etc. by others) on the one hand and attributes, properties, processes, etc. on the other.

The necessity to differentiate in faceted indexing languages between "concretes" and "processes" was pointed out by Kaiser (1911), and has been recently discussed by Svenonius (1978). It should also be remembered that the addition of concepts (like properties, processes) as new characteristics to "concretes" means specification. Both these principles are included in the faceted system for systematically describing feeds. This is the "International Feed Vocabulary," which has been extended for practical use to the "INFIC Feed Thesaurus."

For the special purposes of feed description, the facets had to be adapted to those types of characteristics essential for determining a specific feed. The "concretes" in our case could be well described by determining the raw or original material of the feed (plants, animals, or other). In most cases not the whole plant or animal is prepared as feed. Thus, for feed description the category "concretes" must be divided into two facets, one for the "original material" and another for determining the part used as feed. This second facet consists of the terms that are necessary for the more specific characterization of the first facet. The next constituent for analyzing and describing the characteristics of a feed is, according to Kaiser, the process the "concrete" has undergone. Thus, the processes or treatments must be considered as a third facet.

Although the essential characteristics of a feed are covered by these three facets, it has been found that in some cases additional characteristics must be analyzed to describe the feed completely. This is especially true in the case of plants, and under certain circumstances in the case of animals too. For an exact characterization of a feed, therefore, the stage of maturity, the time of cutting or the crop, and eventually the degree of quality, if not already characterized by the preceding description, must be considered.

The system for analyzing (conceptually) and describing feeds therefore consists of six facets: (1) original material (plant, animal, or other basic material); (2) parts of this material used as feed; (3) processes or treatments the material has been subjected to; (4) stage of maturity in which plants or animals are used; (5) the cutting or crop (for plants only); and (6) grade (quality). Each of these facets is a list of controlled and fixed terms called "descriptors," which represent a characteristic (as a concept element) for analyzing (conceptually) and describing a special feed. Thus, the feed description is the synthesis of the adequate descriptors taken from the facets, at least of facets one, two, and three. Faceted indexing systems are therefore regarded as analytical-synthetical systems.

For the correct use of a vocabulary, grammar is necessary, and it is this that makes the vocabulary into a language, in our case a documentary language. Grammar consists of syntactical rules (syntax) for combining the terms/descriptors and

making combinations (syntagmas) understandable. The syntax of the feed description systems lies in the facet structure, which gives each type of characteristic the right place in the syntagma and fixes its syntagmatical relationship to the other constituents. Therefore, a faceted description system ensures proper concept analysis and description as well as high retrieval performance (recall and precision). To exclude polysemy of terms the thesaurus allows the proper use of descriptors by offering scope notes and concept relationships.

Because the system for feed description was to be used for the purposes of an international network, it was necessary to use a multilingual thesaurus. Therefore the vocabulary, i.e. the totality of descriptors, was established in three versions: English, German, and French (a Spanish version is in preparation). This means that each descriptor has three different lingual equivalences, all of which represent exactly the same concept. Great care was taken to obtain this semantic equivalence even in cases where homonymy or polysemy existed in a term in one of the languages. The whole vocabulary provides multilingual descriptors for about 5700 concepts.

Besides the vocabulary, the thesaurus contains other terms (nondescriptors) from which reference is made to the adequate descriptor. In this part are included — beside synonyms within the "system languages" — referring terms from other languages, especially those from tropical and subtropical countries, that concern plants used as feeds in those regions. A provisional version of the "INFIC Feed Thesaurus" includes about 25000 entries. By using the methods and tools that have been developed for identifying feeds one can produce systematic concept analyses using unambiguous descriptions that are independent of national languages and local idioms.

Another point should be kept in mind. This method of describing feeds systematically makes it possible to distinguish (artificially) between "classes" or "species" and "individuals." The facets of the vocabulary provide descriptors that represent essential characteristics of a feed. An individual may have individual characteristics beside the essential characteristics that are typical for the species. If a swan has a broken wing, then this is an individual characteristic restricted to this particular bird, and it cannot be considered as typical for the species.

Beside the essential characteristics of feeds, there may also be "individual" characteristics of different samples. These may be influenced by environmental conditions. To consider these individual characteristics a very sophisticated system for sample description has been developed. Thus, where necessary, individual characteristics of a single feed sample can be used as selection factors for data selection.

Standardization of Analytical Techniques

Having treated the first constituent of a statement, we must now turn to the other constituent, the property of the "object" determined by chemical analysis or by trials with animals. What has been said before about generalization of statements is also appropriate here. Only the same type of properties can be compared or can be used for generalizing, for calculating averages, or for deducing values. The problem of standardizing analytical methods has been discussed in previous papers; therefore, this section will be restricted to the information aspects of this standardization.

Feed data documentation in Germany started 30 years ago with the recording of results from the "classical" analyses according to the "Weende" method. Soon the spectrum of data that had to be recorded began to increase constantly. In addition to information on the 470 substances that occur in the feeds, other factors like digestibility by different animals, availability, and biological value had to be considered. In all there are about 800 items. With this increase in volume, more and more problems were encountered. The first of these involved general problems of recording and coding the different kinds of values. Thus, a new system for data recording within INFIC was developed. A second problem soon developed — the consideration of nutrients or values for which different analytical methods were used. The more different two methods are in their results, the less comparable are their results. This fact made it necessary to include in the recording and coding system separate codes for different analytical methods, which had to be added to the codes for the nutrient and its content figures. These codes can be used to separate data originating from different analytical methods. If for instance an unsatisfactory method has been replaced by a new and better one, the data derived from the ancient method can be excluded for cases of information output, provided enough single results based on the new method are available.

There is no question that the use of different methods for analyzing the same nutrient of a feed makes the recording and coding system complicated. However, the user would be confused if he were provided with different figures for

the same property, and if the information centre mixed data resulting from different methods the information would be unreliable. Therefore, from the point of view of the information system and its users it can be concluded that analysts should standardize the methods for feed analyses on a worldwide basis. The most exact methods should be used, but of course the possibility of applying these methods in all laboratories of all countries, including developing countries, should be considered as well.

When enough data from these standardized methods are stored in the data bank, former data gained from unreliable methods can be neglected. But, until that time, data documentation must use exact coding of different methods and take this into consideration for all information purposes.

If the efforts of the analysts to promote and standardize the methodology of analytical techniques lead to new and better methods, it is no problem to denote the results of these new methods with new codes; thus, they can be correctly processed. In this case, it is desirable that enough data from the more exact method be collected and stored as soon as possible, so as to have a broad basis for reliable averages and for generalizing statements.

Not only does the analysis have to be standardized, but the way of designating the result has to be standardized because many different methods are practiced. The INFIC system for data recording, therefore, provides codes for the different bases on which data are recorded, for example, dry matter basis, "as fed" basis, or, for amino acids, a 100-g protein basis. These codes are used to standardize recorded information a posteriori by computer. Nevertheless, a priori standardization of the designation of analysis results in the laboratories could reduce the work in the information centres and probably reduce mistakes.

Standardization of Energy Value Determination

The heterogeneity of methods for the determination and designation of energy values is extremely high. The first level of this heterogeneity exists in the different kinds of energy that are calculated: gross energy, digestible energy, metabolizable energy, and net energy. Another level of differentiation is caused by the method for determining or calculating the energy content of a feed. A distinction must be made between direct trials with animals in respiratory equipment or similar methods, and different methods for calculating energy content by using regression equations on the basis of raw nutrients or of digestible nutrients. A third level of differentiation concerns the designation of energy values. The first measurements of energy value were compared with a standard feed (hay value, barley unit, oat unit, nordisk feed unit etc.) or with a standard nutrient (Kellner's starch equivalents), but later, caloric values were used, and more recently the Joule has been introduced.

The preferred method differs from country to country because the systems developed in a region are most used and well-known there. The degree of confusion concerning energetic feed evaluation is reflected in the literature on this special subject. In a retrospective search of the literature in the Hohenheim Documentation Center, which covers only the last 20 years, about 500 documents dealing with energy evaluation of feeds were retrieved.

What was said regarding the different methods of analytical techniques is much more pertinent with regard to the determination of energy. In data documentation different formulae and different computer programs can be used to calculate different types of energy. Indeed we did so, and by using different calculation methods in different tables and for special purposes we produced tables in which different energy values could be compared.

Although this makes the system complicated, this is not the most important problem. The user can be confused by so many figures on the energy value of a feed; therefore, worldwide standardization of the systems for energetic feed evaluation would be very desirable. We are observing with great interest the efforts of competent organizations in this field like the EAAP working group "Feed Evaluation for Practical Application." Based on the report by van Es (1976) about the activities of this group one cannot be optimistic regarding the possibility of finding a satisfactory solution in the near future. It is hoped that international cooperation on a large scale — as promoted by this workshop — will soon lead to more success.

From the point of view of feed data documentation some points should be remembered. First, the kind of energy must be the same. It should not be forgotten that for a general statement concerning energetic value, different kinds of production can hardly be considered. A measure must be used that can designate a general nutritive value. For practical purposes, the requirement of energy for different kinds of production must be designated in an adequate way.

Second, results of direct determinations of energy content — using animals in respiratory

equipment — are restricted to the special feed sample used in the trial. These results can be used only for exactly the same feed (not only as described according to the conceptual analysis but also found to have exactly the same content of nutrients). Because this cannot be realized, the average energy value of a feed must be calculated on the basis of the average content of nutrients of that feed. This is the reason the Hohenheim Documentation Center did not record single directly determined energy values, but rather calculated values in each case by using the average values of digestible nutrients. However, we have recently started to record directly determined values so as to complete our data bank and to have data for comparison.

This does not mean that direct trials for the determination of energy values are not necessary. On the contrary, they should be applied to provide better knowledge about the energy values of different kinds of feeds with the view toward correlating the content of digestible nutrients with the energy value.

This leads to the question of the determination of the digestibility of different nutrients. From the beginning of our feed documentation work in Germany we were very keen to collect and record all existing data about the digestibility of the nutrients of different feeds by different animals. Such data are very valuable and become a broad basis for averages. We preferred to have such data from animal trials. Recently, however, only a few institutions are carrying out such expensive and complicated trials. Instead, in vitro methods for determining the digestibility of the nutrients are being increasingly used. For a long time we hesitated to record them, being afraid of unreliable data.

We think that the new methods promise to be reliable. Besides accuracy, the simplicity of a method must be considered if it is to be used in the various countries of the world. We are hopeful that scientists will agree to use the method developed by Menke et al. (1979), which uses gas production in vitro with rumen liquor for determining digestibility, and allows the calculation of the metabolizable energy of a feed.

It would be a great benefit to feed data documentation and information, if the standardization of such methods and their worldwide use produced new data that would broaden the data base and allow more generalized statements concerning the feeding value of all feeds. This would be valuable to INFIC in its task of supplying information to users in all parts of the world. But such standardization would also help the different feed information centres within INFIC to work closely together, to exchange data, to develop these to reliable information by using adequate processing methods, and to make them available to users in all countries for promoting animal production, agriculture, and the welfare of man.

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