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SUMMARY - To begin with, the importance of the prediction of the nutritive value and intake of feeds for the ruminant animals is stressed. The prediction is mainly correlated to digestibility, NDF content and lignin content. Then the most important chemical methods, enzymatic methods and biological methods (*in vitro, in vivo, in sacco*) which can be used as predictors, are reviewed and commented. It is concluded that each method has its own validity and limitations in predicting the nutritive value of feeds. Among all the described methods, it is the authors' opinion that the gas production technique was the most adequate in the present project.

Key words: Nutritive value, prediction methods.

RESUME - *"Commentaires sur les méthodes d'évaluation des aliments utilisées dans le projet". Au début, les auteurs soulignent l'importance de la prévision de la valeur nutritive et de la capacité d'ingestion des aliments pour les ruminants. La prévision est corrélée principalement avec la digestibilité, le niveau de NDF et le niveau de lignine. Ensuite, les plus importantes méthodes chimiques, enzymatiques et biologiques* (in vitro, in vivo, in sacco)*, qui peuvent être utilisées pour la prévision, sont passées en revue et commentées. En conclusion, chaque méthode a sa validité et ses limitations en ce qui concerne la prévision de la valeur nutritive. Entre les méthodes décrites, selon les auteurs, la technique qui mesure la production de gaz a été la méthode la plus convenable dans ce projet.*

Mots-clés : *Valeur nutritive, méthode de prévision.*

Introduction

Feed evaluation is needed either to assess the value between feeds or to assess prospective production levels and food import and export strategies.

The methods to express the feed value tend to simulate or measure the effect of the digestion on that foodstuff.

The most common definition of a feed states that it is a product that contains nutrients and possibly other components that are not nutrients. The digestion aims to separate the ones from the others being therefore the potential of a feed associated with the amount of nutrients that can be provided to the animal.

Having this in mind most of the feed evaluation methods tend to reproduce what is happening at the gastro-intestinal tract when the feed is eaten by the animal.

Roughages are poor in digestive nutrients either because nutrients such as nitrogen and non structural carbohydrates are present at low concentrations or because they are poorly digestible, due to the presence of various physical or chemical factors such as lignin and polyphenols.

In warm climates, the diet of many ruminants is largely composed (between 90 and 100%) of forages such as the aerial parts of herbaceous mature plants, crop residues after harvest, trees and shrubs.

In temperate zones, grasses form the basis of grazing land but grass straws and cereal straws (wheat, maize, sorghum, rice) are also consumed by ruminants usually as a complement.

Because of their botanical diversity and the pronounced differentiation of their organs and tissues, roughages show considerable morphological, anatomic and physicochemical heterogeneity. This

heterogeneity occurs seasonally among plant species and among organ tissues. Hence the characteristics of these forages are neither permanent nor uniform for any particular plant species.

Most of the methods used to predict the nutritive value of forages have been developed in temperate zones, usually for grasses and legumes in which the range of variation of this value is less wide than in roughages. The composition of these forages usually enables microbial or enzymatic digestion to take place under optimal physiological conditions.

There are mainly three types of methods for the evaluation of feeding value, which are the chemical, the enzymatic and the biological ones. All of them tend to simulate what is happening in the animal during the digestive process. This simulation is by definition an approach and not the real value, which always carries a certain degree of error. It is not our purpose to describe fully all the methods that have been used in the present project but describe each group of methods on their main characteristics limitations, ideal conditions to be used and when they should or should not be recommended as the best choice.

Chemical methods

Chemical methods give information on the main chemical composition present in the feeds, which as such is very little. However this information has been used to predict the nutritive value and it is for this purpose that most of the chemical methods are still used in animal nutrition. Just some of the parameters have the impact or the reflex on digestion, which justifies their utilization to predict the nutritive value. Among those it should be mentioned the nitrogen (crude protein), the crude fibre and the cell wall constituents.

Nitrogen

The crude protein (CP) content is often a good predictor of organic matter digestibility (OMD) in temperate grasses since it diminishes as the plant grows and also varies inversely with the indigestible cell wall fraction. However, OMD predictions are only accurate if they are carried out on individual plant species, production cycles or seasons, and soil types (Aufrère and Guérin, 1996).

The nitrogen supply from the soil where they are growing on, can be a limiting factor in tropical zones. Guérin in 1987 (Aufrère and Guérin, 1996) observed a wide variation in CP for the same digestibility within the same species and in these cases this parameter could give less reliable predicted values.

Crude fibre

Cell wall indigestibility is mainly due to the lignin content. However the Weende crude fibre (CF) values in temperate forages increase according to the cell wall content, at least in the course of any given growth cycle. Therefore CF is generally a good criterion of cell wall indigestibility for a particular plant, but for the same CF content plants can exhibit different digestibilities according to the plant species and even to the re-growth cycle number. Prediction is improved if nitrogen content is included in the prediction equation (Andrieu and Weiss, 1981).

For hays harvested at the end of the first cycle, straws and old tropical forages in which CF varies relatively little, it is preferable to use lignin content to estimate the OMD.

Today CF is of very little nutritional meaning and just a few laboratories maintain the method at work, therefore it is becoming more an historical approach rather than a useful or routine method of feed evaluation even when it is providing good statistical predictions.

Cell wall constituents

Van Soest fractionation (van Soest and Wine, 1967) makes it possible to measure the total cell wall content (neutral detergent fibre, NDF), ligno-cellulose content (acid detergent lignin, ADL) of forages.

In particular acid detergent fibre (ADF) determination allows the evaluation of practically all the cellulose and lignin.

To predict the digestibility of forages, total cell wall content or NDF is a less accurate predictor of OMD than ADF or CF. Lignin content is the variable most closely linked to *in vivo* digestibility. It is necessary to establish separate relations, at least for grasses and legumes.

For the same lignin content, legumes have less indigestible cell wall material and higher digestibility than grasses (Demarquilly and Andrieu, 1987).

However this analytical procedure is still imperfect because in legumes the neutral detergent solution used to determine NDF solubilises pectic substances present in stems at levels between 11 and 22% (Hatfield, 1992). Besides cell wall content (NDF and ADF) can be overestimated if a larger proportion of nitrogen remains bound to the cell walls, which is the case in temperate zone for legumes and young grass leaves (van Soest and Robertson, 1980). The presence of varying quantities of tannins can also lead to an overestimation of the ligno-cellulose content (Makkar *et al.*, 1995).

Finally the results of ADL determination can be adversely modified by errors caused by products of Maillard reactions (van Soest and Mason, 1991) or by tannins (van Soest, 1994). These errors are important, and ADL is certainly overestimated in shrub forages for example (ADL 40% of dry matter, DM).

Acid detergent insoluble nitrogen (ADIN) indicates the general level of indigestibility factors that are important in roughages and this explains why ADIN is a more efficient predictor of OMD than total nitrogen or ADF.

Preparations of crude fibre and ADF are overdigested, dissolving indigestible components, lignin and some hemicelluloses, respectively. Neutral detergent, on the other hand, dissolves mainly nutritionally available substances, and isolates the incompletely available fraction. NDF gives good estimates of digestibility in animal species with minimal gut fermentation, but becomes inefficient where extensive fermentative digestion of fibre occurs. Fibre digestibility may not be related to fibre content. This is due to the differing environmental factors promoting lignification as opposed to cell wall content (van Soest, 1996). Therefore and as a conclusion it must be said that the use of fibre (particularly of ADF) to predict digestibility is a gross misuse and a prevision of scientific understanding. The problem could be partly alleviated if lignin was determined along with the fibre measurement. NDF is the pertinent measure of fibre relatively to rumen requirement. ADF was promoted largely from its higher association with digestibility in spring-harvested forages, although ADF is not a very useful indication of ration in regard to fibre.

From an ideal point of view required analysis for rations balancing would include NDF and lignin. ADF could be dropped, since digestibility can be more accurately estimated from NDF and its lignin content (van Soest, 1994).

Generally speaking the chemical methods are less reliable than the enzymatic or biological ones as they are far from what happens within the animal, however sometimes, they are the only alternative and so they should be used but care must be taken on the conditions and feeds to which they are applied.

Enzymatic methods

Ruminal digestion is mainly linked to the cellulolytic activity of the microbial flora, which represents its specificity and advantage. In the 1960's Donefer *et al.* (1963) used commercially available cellulolytic enzyme preparations, often extracted from fungi, to reproduce this activity. Since then many enzymatic methods have been proposed to predict feed digestibility. They differ in the nature of the enzymatic preparations and whether a pre-treatment (chemical or enzymatic) is necessary or not (Aufrère and Michalet-Doreau, 1990). These methods are widely used for forages, and have been applied to by-products, concentrates and mixed feeds produced by agro-food industry. For various types of forages prediction is higher than with chemical methods and comparable to that obtained *in vitro* (Aufrère and Guérin, 1996).

In addition, cellulase methods can be used for mixtures and permanent pastures. For forages with high cell wall contents (i.e. straws) a simplified method can be applied with no pre-treatment (Rexen, 1977). With forages containing tannins, OMD prediction is poor when cellulolytic enzymes are used.

This can be due to the fact that enzymes are used at pH values different from that prevailing in the rumen, enabling possible the release of tannins and their subsequent linkage to proteins. Another possible explanation is because some kind of tannins might have inhibiting effects on the enzyme activity especially on cellulase (Mandels and Reese, 1963).

Enzymatic methods shows a favourable repeatability and reproducibility allowing an accurate prediction of *in vivo* digestibility, except for forages containing tannins (Aufrère and Guérin, 1996) for which an adaptation period is necessary. Enzymatic methods can find important applications in laboratory analyses to predict the nutritive value of roughages. They are inexpensive relative to the reference *in vivo* methods and are also faster and easier to implement routinely. As a conclusion it can be said that enzymatic methods do perform better than chemical methods to predict the feed value and they are a better approach to the animal digestion process.

Biological methods

The biological methods were created to represent and simulate a part or a series of parts of the digestive tract and digestion process in animals. Among the biological methods we have considered: (i) the digestibility with *in vivo* trials; (ii) the *in vitro* two stage technique (Tilley and Terry, 1963); (iii) the *in situ* technique (Meherez and Ørskov, 1977; Ørskov and McDonald, 1979); (iv) the *in vitro* semi-continuous culture (Czerkawski and Breckenridge, 1977); and (v) the *in vitro* gas production (Menke and Steingass, 1988).

There is a great similitude among all biological methods and therefore we decide to discuss them together and not on an individual basis as we have done before for the chemical methods. The "basic model" which gives the value utilized for defining the nutritive value of a feed is the *in vivo* digestibility, which represents the entire process occurring in the gastro-intestinal tract. Alternatively the other methods simulate the rumen tract in the case of cannulated animals being with the two stage technique, the gastric digestion integrated with the rumen digestion.

The methods consider a series of biological and physical processes, which differ considerably between themselves.

The basic value obtained with *in vivo* trials is the result of the integration of two main processes: digestion and absorption; the other systems consider degradation *(in situ)*, fermentation (gas test) and digestion (two stage technique). The consequences of these different approaches are that the information provided is different in type, unit of measurement and its distribution over time.

The *in vivo* digestibility of roughages depends on their specific characteristics, and also on the conditions in which they are used. Forages poor in fermentable nitrogen and in certain minerals such as P, Mg, S and Cu have to be supplemented so that the rumen can function correctly and these forages reach their full potential digestibility. Roughages are usually associated with other feeds in animal diets and numerous interactions can therefore take place.

Forages containing tannins have *in vivo* digestibilities that depend on their proportion in the diet. Moreover samples taken for analysis usually correspond to the forage offered; whereas *in vivo* digestibility corresponds to the forage ingested whose chemical composition is different due to the choice made by the animal. This difference is more marked for roughages and depends on the amount of feed on offer (refusal rate) by the experiments. This partly accounts for the lower accuracy of prediction of the digestibility of these forages compared to those of temperate zones using laboratory methods.

The cytoplasmic constituents of forage grasses and legumes in temperate zones have a true digestibility of practically 100%. The non cell wall constituents such as nitrogen and lipids recovered in faeces are essentially of endogenous or microbial origin, and their quantities vary slightly as a function

of the dry matter intake (DMI): 120 g to 140 g faecal organic matter (OM) per kg DMI (Demarquilly and Jarrige, 1981). A relationship was established by van Soest (1967) from 18 hays:

SD = 0.98 x S - 129; R = 0.99

where SD: soluble digestible (g kg^{-1} DM), and S: soluble = 1000 - NDF (g kg^{-1} DM).

The amount of apparently indigestible OM found in faeces thus mainly depends on the amount of indigestible cell wall ingested. For these temperate forages, predicting OMD requires the prediction of the cell wall indigestible content, which highly depends on the amount and nature of the lignin contained in these cell walls.

In contrast, for some forages, especially those rich in tannins and other often badly known as secondary compounds, the true digestibility of the cytoplasmic constituents is not total, but highly variable according to the amounts and nature of those secondary compounds. In addition, the digestibility of the cell walls of these forages is also affected by tannins.

Therefore the OMD of these forages depends not only on their indigestible cell wall content, which is highly variable, but also on their concentrations in truly indigestible cytoplasmic constituents.

The *in situ* technique is used to obtain an estimate of the parameters of the kinetics of degradation with an application of an exponential equation (Ørskov and McDonald, 1979). The same technique can be used for the continuous culture method in which samples can be incubated for different times to obtain a kinetic or an absolute value.

A slight variation of the *in vivo* method is the use of markers to measure digestibility parameters and, of particular importance, has been the use of markers monitored in the faeces, an approach used for more than 120 years. The use of urinary markers is a more recent development reflecting advances in knowledge of the metabolism of various dietary materials that can result in metabolites being excreted via the urine.

Progress in the marker technique, both faecal and urinary, has been greatly facilitated in recent years by the introduction of new or improved analytical procedures. Among the faecal markers it should be mentioned the recent developments in the use of plant cuticular wax compounds for estimating diet composition and intake of plant material and of supplementary feeds. In the case of urinary markers attention must be on the total urinary purine derivatives excretion as a method for estimating rumen microbial protein production. Besides recent results (Mayes *et al.*, 1995) do indicate the potential to use the urinary excretion of aromatic and phenolic derivatives as markers of total feed intake and in certain instances, for the identification of ingested plant species.

Kotb and Luckey (1972) listed a number of criteria for a substance to be an effective faecal marker, with inertness and lack of absorption or metabolism occurring in the digestive tract, and considered as the most important, since it effectively meets the main criteria of quantitative faecal recovery, inertness and accuracy of analysis, chromium sexquioxide (Cr_2O_3), the most widely used. However, titanium oxide (TiO_2) has also been used as a faecal marker for estimating soil ingestion (Healy, 1968) and barium sulphate (Kotb and Luckey, 1972) may also be a potential replacement for Cr_2O_3.

Because of their high faecal recovery rates rare earth salts especially Ytterbium (Yb) have been used in recent years as markers for faecal output estimation (Hatfield *et al.*, 1990).

Alternatively one can use the so-called internal markers. However until recently such internal markers have been generally unsatisfactory, because most of the indigestible examined as potential markers have not been discrete chemical entities; what is measured in the faeces may not be the same substance as that in the diet. As a consequence, certain markers, such as lignin or indigestible acid detergent fibre, may give good results in some situations but poor results in others (Dove and Comb, 1992). When the conditions are appropriate silica can also be a good internal marker providing the animals have not access to the feed contaminated with soil. Our colleagues at Florence University did use such an approach with success because the animal beds were made of an unpalatable commercial compound of known composition, based on peat (Antongiovanni *et al.*, 1996).

The analytical procedure for silica determination is very satisfactory being the digestibility results obtained good.

Recently there is a growing interest on the plant-wax compounds, namely hydrocarbons as a potential marker. The predominant hydrocarbons of most plants are n-alkanes, which usually occur as mixtures ranging in chain length from 21-37 carbon atoms. The relative simplicity of analysis of hydrocarbons by gas-chromatographic methods and their inertness were the primary reasons for considering n-alkanes as interesting markers of the digestibility of the pasture intake by grazing ruminants (Mayes *et al.*, 1986).

The gas test represents a system adapted to yield continuous information (Menke and Steingass, 1988) and the high number of observations enables the application of models with the presence of several feed components and a sigmoidal approach.

The biological methods can also be divided in "open" and "closed" systems. The level of openness represents the possibility of entering and modifying the process over time; generally in open systems, the input can be introduced at any time. It can also be modified and the final metabolites can leave the system without interfering with the biological phenomena.

The methods have different levels of complexity because of the number of variable involved; it is possible to consider four variables and their relative effects: (i) level of intake; (ii) associative effects; (iii) recycling of nitrogen; and (iv) transit time (Susmel and Filacorda, 1996).

The level of intake for *in vivo* methods is a constitutional part of the system while *in vitro* methods are unable to consider these aspects. The transit time, a variable closely associated with the level of intake, is an intrinsic variable in the *in vivo* methods and in semi-continuous culture techniques it can be simulated and integrated (Czerkawski and Breckenridge, 1977). In the *in situ* the passage rate is integrated in the model used to estimate the effective degradability (Ørskov and McDonald, 1979). The value of the rate used in the *in situ* method is constant and has been generally measured for temperate feeds. *In vitro* closed systems lack the rate variable and this represents a limit to the dynamic interpretation of the phenomena.

Associative effects are considered in the *in vivo* digestibility as an intrinsic aspect. Fistulated animals are used to detect the associative effects in the reticulo-rumen, and in the *in situ* technique these effects can be studied but the results do not consider the rumination process, passage time of feeds and the phenomenon of compensation of digestion. The *in vitro* closed techniques are considered adequate to examine this effect (Meherez *et al.*, 1983) although there are possibilities of using the continuos culture method for such investigations.

The recycling of nitrogen represents another important factor for understanding the nutritional ecology and the adaptability of species that eat roughages. The open systems take this into account as an intrinsic factor; otherwise, the closed *in vitro* methods cannot replicate this aspect in dynamic terms and with continuos culture the NH_3-N represents an unstable fermentation characteristic (Mietinen and Setala, 1989).

In a recent study (Adesogan *et al.*, 1995) aimed to measure the suitability of three less animal dependent techniques: (i) *in vitro* digestibility (Tilley and Terry, 1963); (ii) *in situ* degradability (Ørskov and McDonald, 1979); and (iii) gas production (Theodoron *et al.*, 1994) for predicting the *in vivo* digestibility of whole crop wheat and the results are present on Table 1.

From those results it seems that the rate of degradation from *in situ* or gas production appears better than either potential degradability or gas pool size for predicting the *in vivo* dry matter digestibility of the whole crop wheat.

Besides it seems that the *in vitro* digestibility technique appears superior to both *in situ* degradation and gas production techniques for estimating digestibility.

Factors affecting the biological methods

In the *in vivo* methods, the initial trial conditions generally have a low weight because the final result is a combination of the adaptation period and the observation period. The other methods can be influenced to variable degrees by the initial conditions. For the *in vitro* methods the initial conditions

and variability of the results depends on the composition and activity of the rumen liquid (Aerts *et al.*, 1977) and in turn these are dependent upon time of collection and type of diet (Judkins *et al.*, 1990).

Table 1. Relationship between the *in vivo* apparent digestibility (g kg^{-1}) of dry matter (y variate) of nine whole crop wheat forages and their *in vitro* dry matter digestibility values (g kg^{-1}), kinetic parameters of gas production (ml kg^{-1} DM) and *in situ* degradation (g kg^{-1} DM) (Adesogan *et al.*, 1995)

Technique (x variate)	Equation and factors used†	R^2	Significance
In situ degradability	0.772 - 0.000362 a	0.37	0.084
	0.456 + 0.000464 b	0.49	0.035
	0.552 + 0.232000 c	0.60	0.015
	0.402 + 0.000230 A	0.09	0.409
	0.668 - 0.000083 U	0.06	0.526
	0.416 + 0.000393 p	0.00	0.576
Gas production	0.449 + 0.000729 A ∞	0.22	0.205
	0.546 + 0.044200 Cr	0.75	0.000
	0.549 + 1.300000 Cs	0.45	0.033
	0.470 + 0.492000 Dr	0.58	0.017
	0.420 + 3.740000 Ds	0.64	0.010
	0.717 - 0.003970 Ti	0.62	0.011
	0.541 + 0.000115 Egas	0.00	0.858
In vitro digestibility	0.106 + 0.831 DMDi	0.00	0.830

†a = immediately soluble fraction; b = potentially degradable but not immediately soluble fraction; c = rate of degradation of b per hour; A = a + b = potential degradability; U = 1000 - (a + b) = undegradable fraction; p = effective degradability; A ∞ = upper asymptote representing gas pool size; Cr and Cs are specific initial and final rates per hour of gas production respectively; Dr and Ds are fractional constants governing the decay per hour; Ti = point of inflection (h); Egas = effective gas production calculated using parameters from the Ørskov and McDonald (1979) model; DMDi = dry matter digestibility *in vitro*

The basal diet represents a strong source of variability in the *in situ* and *in vitro* methods that need a source of rumen inoculum. For the *in vitro* closed systems, the type of diet represents a strong source of modulation of the activity of rumen inoculum (Judkins *et al.*, 1990). The end products represent another source of variability; with *in vitro* closed and open systems, they can be a disturbance factor for the estimate, while in open systems this influence is limited, mainly due to the impossibility of the *in vitro* methods to absorb the end products, causing a shift in the microbial population (Mansifield *et al.*, 1995).

Level of prediction

Each system has different prediction objectives. The *in vivo* digestibility trials represent the basic value for digestibility and dry matter intake for voluntary intake. The digestibility trials are often conducted with mixed diets because of the impossibility of having a maintenance level; in this case the estimation of the digestibility of fibre rich feeds given in mixed diets, should not be considered as an absolute value relative to the ration in which it is included (Pigden *et al.*, 1980).

The degradability of dry matter and NDF seems to be the most important variables describing the nutritive value of some tropical feeds (Vadiveloo and Fadel, 1992).

The cannulated animal methods represent a good technique for estimating the digestibility, especially for feeds rich in structural carbohydrates (Archimed *et al.*, 1995) but this technique is less accurate than those involving the whole tract (classic digestibility trial).

For dry matter intake the levels of predictability are good with the parameters obtained *in situ* (Ørskov, 1988) and the gas test (Khazaal *et al.*, 1993) while the one and two stage techniques have a low correlation with the observed intake for hay (Khazaal *et al.*, 1993) shrubs and Mediterranean feeds (Susmel *et al.*, 1993). The semi-continuous culture and cannulated animal techniques are generally not used for estimating intake.

The results obtained with the semi-continuous culture (Rumen Simulation Technique, RUSITEC) have been shown to reproduce the rumen or *in vivo* degradation patterns of carbohydrate and protein.

The prediction of the energy value, expressed as the net energy (NE) or metabolizable energy (ME) usually consists of the estimation of OMD which is the main factor of variation in the energy value.

The methods used to predict OMD basically consists of defining mathematical relationships to link *in vivo* digestibility with the results of chemical or enzymatic laboratory tests.

Trends in feed evaluation

The feeding value is the main step for the validity of feed evaluation systems. A long debate is still going about the choice of an adequate feeding system and again it looks almost impossible to obtain a consensual option mainly because it has been a political decision to produce country or regional systems which sometimes do not express substantial differences between them, but also because there is a permanent conflict between the need to produce a simplified proposal and to accommodate most of the dynamics of the biological process.

For the objective of the present publication the basic problem would be the biological processes which are not static. The feed value systems provide also little or no information on how much the animal consume of that feed (Ørskov, 1996). Thus if a farmer finds that the cheapest metabolizable energy can be with straw he can only feed a limited amount as the animals will only consume limited amounts. The feed values therefore do not give a proper exchange rate and they are on the whole best used only for concentrates that are rationed. For roughage where the feed intake is crucial, they are of limited value.

Besides there is the problem of intake. To predict intake from digestibility measurements and chemical analysis seems that they do not give good relationships.

Various techniques and applications have been proposed to predict the nutritive quality of feedstuffs as we have seen before. Most of these attempts to predict nutritive value of feeds apply statistic regressions between measured nutritive value (usually digestibility) and some measured parameters. This may be a fibre value, a proximate composition, a nylon bag digestion, an enzymatic digestion or a longer array of chemical analyses. This basis of comparison assumes that measurement of the predicted characteristic will be a reliable indicator of nutritional quality. Within this aspect there are several problems. One is that the measured characteristic had a true and consistent biological effect upon quality. A second problem is that many procedures depend on a solubility measurement with the assumption that solubility indicates availability. Third, that the literature on feed composition have a varying data base. A fourth problem is that feed composition is environmentally and regionally influenced so that calibration equations will vary.

Consistency of biological effect is complicated and involves various aspects that include classification of nutrients available to animal enzymes and the completeness of laboratory digestive systems. Uniformity of digestibility of chemical components was the basis of the Lucas analysis and the evolution of the detergent system (van Soest, 1996).

Regarding the solubility problem one must remember that all systems that depend on the assay of an insoluble residue involve the assumption that soluble matter is nutritionally available and digestible. This assumption particularly applies to *in vitro* rumen, nylon bags placed in the digestion tract and enzymatic assays. Soluble substances that are not digestible include some Maillard products, soluble tannins and other phenolics and probably other secondary substances. Even available soluble substances when placed in a rumen medium dissolve but are not yet utilized by rumen bacteria which may take some time. The disappearance models assume that digestion is

instantaneous. Lignin rendered soluble by alkaline treatments appears to remain indigestible and can be assayed by ultraviolet absorbance at 280 nm (Lau and van Soest, 1981).

A particular problem with nylon bags is in the integrity of the membrane. Most of the interactions had been concerned with microbial contamination. However fine plant cell walls can also enter and these can be highly lignified (van Soest, 1994).

Similarly fine feed particulate matter can be lost from the bag. In the case of time-sequence digestions, systematic overestimation of rates can occur through this mechanism.

The problem of an adequate data base is an old debate around the importance of a proper feed identification. This may include plant species, plant parts, stage of growth and other descriptions of the product.

Plant composition varies with climate, weather and soil factors (van Soest, 1994). This leads to variation in regrowth as well as from season and year.

Generally any environmental factor that limits plant development will increase digestibility, decrease fibre and lignin. An exception is light and day length that promotes photosynthesis, sugar production and plant growth (van Soest, 1996).

Day length in spring and summer vary with latitude along with temperature differences from north to south, so that calibration of forage quality in northern Europe will not apply to Portugal, Spain and Italy. Examples of climate and weather effects are shown in Table 2.

Table 2. Effect of season and year on alfalfa quality in New York State (data of G. Flick, cited in van Soest, 1994)

Harvest date						
	Cold spring				Hot summer	
	5/30	6/10	6/20	6/30	7/12†	7/19††
1983						
Degree days	444	616	913	1167	–	–
CP %	22	21	20	19	19	19
NDF %	31	35	41	45	43	46
1986						
Degree days	828	1061	1274††	1505††	–	–
CP %	19	19	18	19	21	20
NDF %	39	43	46	48	35	41

†Second cuttings, previously harvested June 10
††Tillering

Current practice is to use ADF to predict digestibility and forage quality. This practice is not founded on sound Science, because the predictor of forage digestibility and quality is not related to fibre contents in post June 21 cuttings (Table 2). Lignification is the dominant factor affecting NDF digestibility and is sensitive to environmental temperature and soil moisture. As a result, two forages of the same ADF contents can differ considerably in digestibility. It must be understood that fibre (NDF) is a nutrient requirement and has positive associations with feeding and rumen function, so the use of fibre to indicate poor quality is a further confusion in the current practice (Table 2) (van Soest, 1994). Although ADF is highly correlated with the overall digestibility, individual year calibrations (regression equations) can deviate significantly due to annual weather variations. This reliability of regression equations using ADF is questionable (van Soest, 1996).

Final comments

Feed evaluation can be made through different techniques usually to predict feeding value of the different feeds.

From what we have already seem the main techniques are based on chemical, enzymatic and biological approaches, having each one its advantages and its limitations. Besides some of them were proposed and tested for specific situations and therefore they should not be used outside of their recommended conditions.

Another comment we want to make is the choice of the method according to the objective as the same method can be used for predicting *in vivo* digestibility or fermentation kinetics and, for instance, the high loss of fine particles observed in the *in situ* degradation technique which limits its validity for describing fermentation kinetics of some feeds, even roughages. The gas production technique may be a valid alternative in those cases.

Another main problem is the prediction equations chosen to calculate the *in vivo* digestibility or even the feeding value. As we have seen before there are a lot of factors affecting those predictions and interactions. Among those we shall mention the environmental ones as the most important. Therefore it is also critical to choose the adequate prediction equation to reach a representative value for the feed that is being analysed.

Finally it should be said that all major feed evaluation systems are static. They provide little or no information on how much the animals will consume of the feeds. To overcome this limitation several attempts have been made like the recent potential feed concept proposed by Ørskov (Ørskov, 1996). The idea is to describe feeds in a way that potential consumption can be predicted as well. This would also have the distinct advantage that feed potential could be related to animal potential, so that animal and their type of product (e.g. maintenance, fattening, reproduction and lactation), can be matched to the available feed potential.

One of the problems with this type of proposal is the fact that it is not yet known if these feed potential will or will not be an adding entity.

The different techniques available are important methodological tools to measure feeding value but one must choose the adequate one for the objective under study and one must remember that all of them have limitations, being sometimes necessary to introduce some adjustments according to the animal, the feeds or the conditions.

The present project provided an excellent opportunity to test different methodologies in different conditions and measure their adequacy and their main advantages compared to alternative methods of prediction.

Methodological approach (laboratory methods) to the assessment of the nutritive potential value of fibrous feeds

To perform most of the experiments during the present project several methods and techniques were used to measure the nutritive potential value of fibrous feeds.

Some of them were just simple indications of such potential quality like the chemical parameters, but others like the enzymatic or the biological methods were approaches of what really happens in the digestive tract of the animal. Among those we must separate the ones where the animal is the model like the *in vivo* digestibility trials or even the *in sacco* degradability from the others where the animal is a donor of rumen fluid either for the *in vitro* digestibility either for the gas production technique or for the RUSITEC.

Summarizing what has been said one can present the used methodologies as follows:

(i) Enzymatic methods

(ii) Biological methods
- *In vitro* digestibility
- *In sacco* degradability
- *In vitro* digestibility
- Gas production
- Semi-continuous system (RUSITEC)

The values so obtained will not give a direct indication of the potential nutritive values of the feeds but they are then used to predict the real value through the prediction equations made to achieve a reference value, which in most cases is the *in vivo* digestibility.

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