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Energy and protein evaluation of conventional and non-conventional feedstuffs. Possibilities and pitfalls

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SUMMARY – Feed evaluation is essential as it allows for optimisation of ration composition and, by this, prevention of malnutrition. For non-conventional feeds, where experience is scarce or lacking, it is even more important. Feed evaluation includes assessment of chemical composition, energy and protein value, feed intake potential and content of eventually antinutritional or even toxic substances. Uncritical use of laboratory methods for analysing non-conventional feeds can result in severe errors in feed values. Especially for low digestible feeds and feeds containing antinutritional substances, different methods may differ considerably in their prediction of digestibility. Conventional concentrates and temperate forages have generally high total true protein availabilities; around 93%. However, many non-conventional feeds like some tropical forages and browses can be very low in total true protein availability, and even protein rich feeds can result in a negative protein supply for the animal.

Keywords: Energy evaluation, protein evaluation, *in vitro, in situ*, ruminants.

RESUME – *"Evaluation d'énergie et de protéine des aliments conventionnels et non conventionnels. Possibilités et difficultés". La détermination de la valeur nutritive est indispensable pour optimiser la composition du régime alimentaire, ce qui permet entre autres d'éviter la mauvaise nutrition. Cette opération est importante surtout pour les aliments non conventionnels qui n'ont pas été largement étudiés. La caractérisation nutritionnelle concerne la détermination de la composition chimique, de la valeur énergétique et azotée, de l'ingestion potentielle et éventuellment l'identification des substances anti-nutritionnelles voire toxiques. L'utilisation non critique des méthodes de laboratoire pour l'analyse des aliments non conventionnels peut engendrer des erreurs importantes concernant leur valeur nutritive. La situation devient complexe dans le cas de la prédiction de la digestibilité d'aliments peu digestibles et/ou contenant des composés secondaires. Les concentrés et les fourrages conventionnels ont, généralement, une disponibilité de l'azote très élevée, qui est d'environ 93%. Par contre, beaucoup d'aliments non conventionnels, comme certains fourrages tropicaux et les arbustes, peuvent être très pauvres en azote disponible à cause de leur faible teneur en azote et/ou de la présence de composés secondaires qui empêchent l'utilisation des protéines par l'animal et sa microflore.*

Mots-clés : *Évaluation énergétique, évaluation azotée,* in vitro*,* in situ*, ruminants.*

Introduction

In ruminant production it is important to optimize production, which in economic terms means to minimize costs per produced unit of e.g. milk or meat. However, in many countries other criteria like animal welfare, ethical considerations and environmental effects have gained increasing importance for animal production in the past years. However, the ration fed to the animals is important for reasons related to economy, animal welfare, ethics and environment. Feed costs are the major production costs and the main factor affecting production. Many welfare problems arose for production diseases due to problematic feed rations, and major environmental problems arising from ruminant production are due to excretion of nitrogen and emission of methane. Therefore, feed evaluation is essential to optimize rations with regard to all the important criteria.

Different feeds having different feeding value have been known for a long time. Thaer (1752-1828) started a systematic evaluation of feeds used for ruminants and produced feed tables based on the hay value. Thaer's feed evaluation was based on solubility of feeds in different solvents. This was the first attempt to make a chemical fractionation, which developed fast in the 19th century followed by an evaluation of the energetic value of different feeds obtained in calorimetric measurements in respiration chambers. The use of digestibility as a basis for the availability of the nutrients started in the middle of the 19th century, when Henneberg and Stoman (1860) combined digestibility trials with chemical fractionation (Weende analysis). The Weende analysis, where the feed is fractionated in

crude ash, crude protein, crude fat, crude fibre and nitrogen free extract, has since then been the basis for all classical feed evaluation systems based on digestible nutrients. The history of feed evaluation has been described in detail by Flatt (1988).

The classical energy evaluation systems based on amounts of digestible fractions estimated in the Weende analysis system has been further developed with generation of new knowledge and several so called modern systems have been introduced in many countries. Realizing that the microbial fermentation of feeds in the rumen had a major impact on the protein value of feeds led to introduction of the 'new' protein evaluation systems (Vérité and Peyraud, 1989; AFRC, 1992; Tamminga *et al.,* 1994; Madsen *et al.,* 1995; NRC, 2001). Introduction of the protein evaluation systems increased the need for new feed analysis, as values for rumen protein degradability, intestinal digestibility of rumen undegraded feed protein, and potential for microbial protein synthesis are necessary information. A new generation of feed evaluation systems have been introduced, and they integrate energy and protein evaluation by modelling the nutrient metabolism in the digestive tract (e.g. Danfær, 1990). These systems increased the needs for additional feed analysis, especially for determination of rate of degradation in the rumen of carbohydrate and protein fractions. Information on concentration of amino acids, vitamins and minerals is also important. In many tropical feeds the content of antinutritional factors can be problematic. This paper, however, will only deal with energy and protein evaluation, and will discuss methods together with some of their possibilities and pitfalls for use in feed evaluation.

Energy evaluation

The classical energy evaluation systems are all based on amounts of digestible nutrients as the first step in the evaluation of the energy value. As most organic components except fat in different feeds are rather similar in energy content, the main factor determining the energy value is the digestibility of organic matter (OM). Therefore, methods for determination of OM digestibility are essential for energy evaluation. The digestibility obtained for OM will depend on the situation in which it is estimated (type of animal, ration composition, feeding level). With increased feeding level, especially the digestibility of cell wall carbohydrates (NDF) is decreased. This decrease is due to a combination of reduced retention time in the rumen and less favourable rumen environment for fibre digestion. The less favourable rumen environment for fibre digestion, when feed level is increased, is caused by both a decreased rumen pH, and by microbial substrate preference (Weisbjerg *et al.*, 1999), as increased feeding level normally is followed by an increased concentrate/forage ratio. Therefore, the effect of increased feeding level will also depend on the ration composition, and the magnitude of the decrease in digestibility will increase with increased concentrate/forage ratio (Kristensen and Aaes, 1989; Colucci *et al.*, 1982). Cattle compared to sheep seems to be similar in OM digestibility, however, cattle seems to have a higher NDF digestibility and lower protein digestibility than sheep, both at maintenance level (Woods *et al.*, 1999) and when fed ad libitum (Südekum *et al.*, 1995). The decrease in digestibility with increased feeding level seems to be more pronounced for cattle than for sheep (Südekum *et al.*, 1995).

The new feed evaluation systems, based on the modelling of the metabolism in the digestive tract, aim at predicting digestibility in the actual production situation. In contrast, classical systems are based on standard digestibility, and most systems rely on digestibility obtained using sheep fed at maintenance level.

Methods for estimation of digestibility

The sheep have been the preferred animal to use for obtaining standard digestibility for ruminants. Sheep are normally fed at maintenance level during digestibility trials, and the obtained digestibility can therefore be regarded as a 'potential' *in vivo* digestibility. However, many feeds cannot be fed alone or will give rise to reduced digestibility if fed alone, due to e.g. lack of structure or protein in the test feed, due to content of antinutritional factors or due to bad palatability. Digestibility trials where such feeds are fed alone will therefore either give no results due to lack of feed intake or give low digestibility due to impaired rumen fermentation. The obtained digestibility will of course tell what would happen in practice if the respective feeds make up the whole ration, but it will not inform about the potential digestibility of the feed if fed as a part of a ration, which would be the normal situation.

Feeds low in rumen degradable protein are known to impair rumen fermentation severely, resulting especially in reduced feed intake and reduced fibre digestion (Weisbjerg *et al.*, 1998). Recommended minimum level of CP in digestibility trials is 12% in DM (Rymer, 2000). Therefore, feeds low in protein are normally supplemented with a protein source like urea which can be assumed as 100 % digestible, or a protein source where the digestibility has been determined on beforehand (e.g. soybean meal). The digestibility of the test feed is then calculated by difference assuming no other associative effects than the protein effect. Feeds low in structure, e.g. concentrates, need supplementation with a proper forage like hay, and as for protein supplementation digestibility of the hay must be determined on beforehand, and test feed digestibility is then determined by difference.

For feeds, which can only be fed as a small part of the total ration due to bad palatability, anti-nutritional factors or even toxic components, the difference method may be problematic if the feed can only make up a small part of the ration. Then the regression method can be used, where digestibility of rations with different ratios between test feed and supplement are determined, and then the digestibility of the test feed is found by regression analysis and extrapolation. This method has the advantage that if enough different ratios between test feed and supplement have been examined, then the threshold, where the test feed affects the ration digestibility negatively, can be seen as a deviation to the right line for digestibility plotted against ratio. However, if the test feed can only make up a small part of the total ration, the precision of the estimate obtained by extrapolation may be poor. An example of a nutrient, which can be detrimental to rumen fermentation if fed in to high amounts, is fatty acids and especially polyunsaturated fatty acids and medium chain length fatty acids (Weisbjerg and Børsting, 1989). For more detailed descriptions of *in vivo* digestibility trials and guidelines see e.g. Rymer (2000) and Cochran and Galyean (1994).

In vivo trials are resource demanding, regarding animals, labour, time, and amount of test feed needed. *In vivo* trials are therefore expensive to use in research and impossible to use in practical feed evaluation. Therefore a number of laboratory methods have been developed for prediction of OM digestibility. The most used methods are *in vitro* solubility based on either rumen fluid or on commercial enzymes. Generally, the laboratory methods need to be calibrated to *in vivo* digestibility to make prediction equations for *in vivo* digestibility. The early, and still very alive method is the *in vitro* rumen fluid method introduced by Tilley and Terry (1963). Tilley and Terry (1963) found an *in vitro* solubility:*in vivo* digestibility ratio for DM very close to 1:1 [*in vivo* digestibility (%) = -1.01 + 0.99 (*in vitro* solubility, %)].

True vs apparent digestibility

A 1:1 ratio between *in vitro* solubility and *in vivo* digestibility is not obvious for several reasons. Published prediction equations, using similar *in vitro* methods, have therefore shown to be very variable according to both laboratory and feed type (Weiss, 1994). The reason why the 1:1 ratio is not obvious is partly due to the fact that the *in vivo* digestibility obtained as feed-faeces difference is apparent digestibility, whereas *in vitro* solubility can be regarded as 'true' digestibility. Apparent digestibility is lower than true digestibility, as some endogenous material not originating from the feed is excreted in the faeces. A large part will be microbial matter from hind gut fermentation. Fermentable matter reaching the hind gut is feed, microbial matter and endogenous secretions (cell slough, enzymes) not digested before the hind gut. Endogenous material does not contain fibre, and can therefore be regarded as cell content or neutral detergent solubles (NDS). NDS obey the Lucas principle (Van Soest, 1994). The Lucas principle means that a nutrient (e.g. NDS) of all feeds has the same true digestibility, and an endogenous loss which is a constant proportion of feed DM intake. When digestible NDS in % of feed DM is plotted against NDS in % of DM, true digestibility can be estimated as the slope, and endogenous loss as the negative intercept. Using this principle on 2389 sheep digestibility observations for feeds ranging from straw to soybean meal gave a true digestibility of 105% and an endogenous loss of 106 g NDS per kg feed DM (Weisbjerg *et al.*, 2002a). The reason for the true digestibility above 100% is probably that endogenous loss increases with increased NDF content in the feed, and therefore there will be some deviation from the simple linear relationship. For protein a true digestibility of 93.6% and an endogenous loss of 33.6 g per kg feed DM was found (Weisbjerg *et al.*, 2002a). Earlier studies have shown that crude fat has a true digestibility of 96% and that the endogenous loss of fat in faeces amounts 10 g per kg feed dry matter (Weisbjerg *et al.*, 1991). According to conventional feed chemistry, organic NDS – (crude protein + crude fat) can be regarded as carbohydrates. This shows us that the endogenous loss of carbohydrates is

approximately 62 g per kg ingested dry matter (106 – (34+10)). A loss of approximately 100 g of endogenous material in the faeces per kg feed DM means that apparent digestibility underestimates true feed digestibility of OM with 11 – 13 %units, as shown in Table 1.

Table 1. Example of difference between true and apparent OM digestibility with different combinations of feed digestibility and ash concentration, assuming an endogenous loss (E-NDS) of 100 g NDS per kg feed DM

Amounts (g/day)					Digestibility of OM (%)		
Feed			Faecal		Apparent	True	Difference true-app.
DM	Ash	OM	OM	E-NDS			
1000	100	900	200	100	77.8	88.9	11.1
1000	100	900	400	100	55.6	66.7	11.1
1000	200	800	200	100	75.0	87.5	12.5
1000	200	800	400	100	50.0	62.5	12.5

This further means that when Tilley and Terry (1963) using their rumen fluid *in vitro* system found a 1:1 ratio to *in vivo*, then the *in vitro* system gave solubilities which were more than 10% units lower than the true *in vivo* digestibility of the feeds. Therefore, for an *in vitro* solubility method to be as effective as the *in vivo* systems, it should give solubilities which are 11–13 % units higher than the corresponding *in vivo* apparent digestibilities.

Laboratory methods for OM digestibility

The *in vitro* rumen fluid method (Tilley and Terry, 1963) was designed to simulate the rumen and intestinal digestion, with first 48 h incubation in a buffered rumen liquor solution, followed by a pepsin-HCl incubation for another 48 h, thereafter the DM or OM residue not solubilised is determined. The consequence of using DM instead of OM is discussed in the next section. Enzymatic *in vitro* methods using cellulase or a mixture of enzymes after a pepsin-HCl incubation has gained increasing popularity in recent years (De Boever *et al.*, 1986; Weisbjerg and Hvelplund, 1993). Also combination of NDF boiling and cellulase treatment is used (Givens *et al.*, 1990). The *in situ* technique, where the feed is incubated for a certain time in the rumen in a nylon bag with small pores which allows degraded feed to leave the bag, can also be used (Ørskov, 2000) and should in theory be close to the *in vitro* rumen fluid method. Other methods are gas production, where the gas produced after a certain incubation time is correlated to *in vivo* digestibility (Menke *et al.*, 1979). During the last decade this technique has been developed further to measure gas production profiles, which allow for interpretation of fermentation of different feed fractions (Cone, 1998). Gas production methods are especially valid for estimating digestibility of liquid feeds, which cannot be judged by a solubility method. For practical feed evaluation, NIRs is today the choice for laboratories that analyse a large number of samples within the same feed type (Givens *et al.*, 1997). However, to run NIRs in a proper way, it is necessary to have a biological reference method running for continuously control and calibration of the NIRs. For a more extensive description of the different methods for estimation of digestibility, see Givens *et al.* (2000).

Pitfalls

The present section will not try to cover all pitfalls with respect to evaluation of digestibility, but will highlight some examples. DM digestibility is often measured instead of OM, as it does not involve estimation of crude ash and is therefore easier. However, it can also result in severe errors when feeds are heavily polluted with e.g. soil. If a silage sample is polluted with 5% sand (acid insoluble ash) in DM, use of DM solubility instead of OM will results in an underestimation of energy value with 5-7% depending on the "normal" ash concentration in the feed, if the DM digestibility is used as estimate for energy or OM digestibility.

As discussed previously, laboratory methods necessarily need to be calibrated against *in vivo* data (eventually indirectly), and the equation obtained will normally be valid only for the population of samples used for calibration. Therefore, available calibrations will normally not cover non-conventional feeds, and extrapolating the method to cover these feeds might be problematic.

An example of this is the use of the *in vitro* rumen fluid method on concentrates. Many concentrates have a high fat level, and fatty acids may impair *in vitro* fermentation like they impair rumen fermentation. Secondly, long chain fatty acids are not degraded during fermentation and the original (Tilley and Terry, 1963) washing procedure with water only extract modest amounts of the fat. Therefore, the method has to be modified before used on fat rich samples. Modifications could be extraction of the residue with e.g. acetone, or it could be pre-extraction of the sample with e.g. acetone before incubation, which would also remove the risk of fatty acids impairing *in vitro* fermentation. Results from a study with 40 concentrates (18 straights and 22 compounds) showed a large effect of extraction with acetone on the amount of residue, or pre-extraction with diethyl ether on the OM solubility obtained (Weisbjerg *et al.*, 1992), as shown in Fig. 1. From the figure it is clear that washing with water give much lower solubility values than extraction with acetone or pre-extraction with diethyl ether, when total solubility was calculated in % of original feed OM. Pre-extraction did not give higher values than extraction after incubation, indicating that the fatty acids from the present feeds did not inhibit *in vitro* fermentation noteworthy. Further, Fig. 1 indicates that washing with water only extracts 20-30 % of the fat, and as fat normally will be highly digestible, this will impose a considerable underestimation of digestibility of fat rich feedstuffs. Therefore, the two modified methods increased the correlation to *in vivo* digestibility, from R^2=0.80 for standard Tilley and Terry (1963) to 0.84 for acetone and 0.83 for pre-extraction with diethyl ether. The reason why R^2 did not increase more was that in the present sample population *in vivo* digestibility and crude fat concentration was correlated.

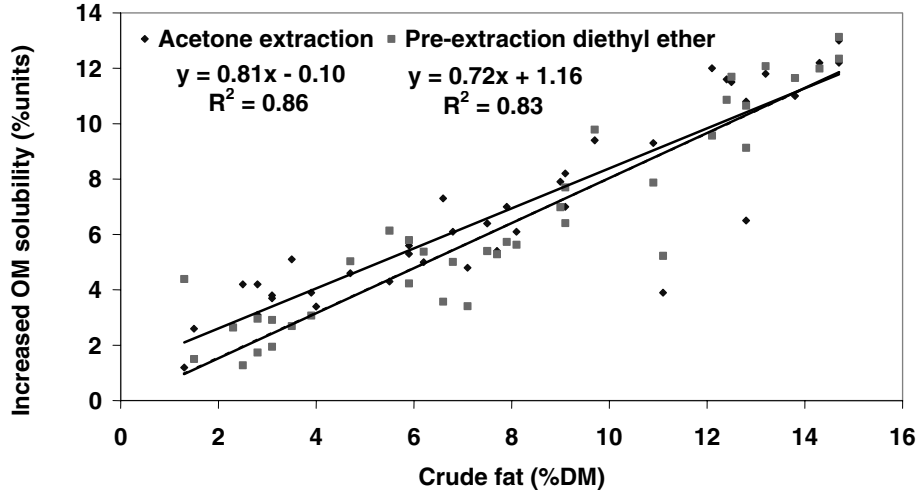

Fig. 1. Increase in *vitro* solubility of OM plotted against crude fat concentration of different concentrates. Increase was the effect of acetone extraction of residue after incubation or pre-extraction using soxhlett with diethyl ether compared to standard wash with tap water (mod. a. Weisbjerg *et al.*, 1992).

Another example of pitfalls in estimating OM digestibility *in vitro* is the possible content of antinutritional factors, which might affect the microbes in the *in vitro* rumen fluid method, but not influence the *in vitro* enzymatic method, as shown in Table 2. The table shows, that the two *in vitro* methods rank OM solubility very differently when grasses are compared with browses/legume trees. For grasses the rumen fluid method gives slightly higher values, whereas for browses/legume trees the enzymatic method gives much higher (as mean 28% units higher) solubilities than the rumen fluid method. The reason for this is probably that browses/legume trees contain antinutritional factors, which inhibit the microbes during *in vitro* fermentation. The question is then, what are the true values? From the results in Table 2 we cannot decide the true value, as the true value probably also will depend on in which proportion the test feed is included in the total ration. However, the true value will

probably be somewhere in the range between the solubility obtained with the two methods, and therefore the use of two methods has given an idea about the value as well as problems involved in the prediction.

Table 2. Comparison of OM solubility estimated from *in vitro* methods based on rumen fluid (IVOM) and enzymes (EZOM) of some Tanzanian and Indonesian forages (mod. a. Mlay *et al.*, 2002)

Latin name	Common name	IVOM[†]	EZOM[††]
Grasses			
Andropogon timorensis		68.3	66.5
Brachiaria brizantha	Signal grass	56.5	50.6
Bothriochloa radicans	Veld grass	35.7	35.0
Cynodon dactylon	Star grass	38.7	29.2
Panicum maximum	Tanganyika grass	53.9	56.1
Mean		*51*	*47*
Browses/legume trees			
Acacia catechu	Catechu	50.9	86.6
Gliricidia sepium	Gliciridia	56.0	77.0
Leucaena leucocephala	Leucaena	46.8	69.2
Sesbania grandiflora	Sesbania	58.4	84.9
Zizyphus mauritania	Bidara	27.1	61.3
Mean		*48*	*76*

[†]Tilley and Terry (1963).
[††]Weisbjerg and Hvelplund (1993).

Similar problems with low *in vitro* values using the rumen fluid method were found by Mlay *et al.* (2003) with cassava meal. *In vitro* rumen fluid OM digestibility was in this study found to be 33 and 39 when analysed in two different laboratories, although *in vivo* digestibilities indicated a high digestibility of this starch rich meal indicating antinutritional factors in the feed which inhibit fermentation when the feed is the only substrate (*in vitro*) but not when used in a diet in a smaller proportion (*in vivo*).

A third example of pitfalls in estimation of OM digestibility is incubation time applied. The Tilley and Terry (1963) method was developed with grass, clover and lucerne products, which all are reasonable readily digested. Therefore, a plateau for digestion was reached within 48 h. If samples are digested more slowly, or contains factors, which inhibit fermentation, the plateau might not be reached within 48 h. This means both lower repeatability and reproducibility of the analysis, and maybe also underestimation of digestibility. In Denmark, we had severe problems in the evaluation of samples of fresh (un-ensiled) maize whole crop samples from field trials, as repeatability and reproducibility were very poor. Estimated digestibility was often much lower than expected when compared to values obtained for ensiled maize of similar quality within year. The reason for this was probably that the plateau was not reached within 48 h, and also that maize starch from fresh samples delayed the fermentation compared to the samples of ensiled maize whole crop, where the starch is degraded and solubilised during ensiling. The method used for collection of rumen fluid may also have an impact. Using hand squeezed rumen fluid collected from the top layer in the rumen instead of rumen fluid harvested by suction increased the *in vitro* solubility at short incubation times for fresh maize whole crop, whereas the difference between type of rumen fluid was negligible for ensiled maize whole crop, as shown in Fig. 2. An extensive study on fresh and ensiled whole crop of barley and maize where also sheep digestibility was measured on 35 fresh or ensiled samples resulted in a change from a rumen fluid based to an enzymatic based *in vitro* method (Søegaard *et al.*, 2001).

Protein evaluation

In modern protein evaluation the feed is evaluated both with respect to its ability to supply the

ruminant with amino acids originating from rumen undegraded feed protein, and its ability to supply the rumen microbes with N originating from rumen degradable protein, and with fermentable organic matter (carbohydrates) as an energy source for microbial synthesis and thereby supply to the ruminant with amino acids from microbial protein. To predict the protein value of a feed the following information on the feed are therefore needed: Crude protein concentration, amino acid concentration, rumen protein degradability, intestinal digestibility of rumen undegraded feed protein and content and fermentability of organic matter.

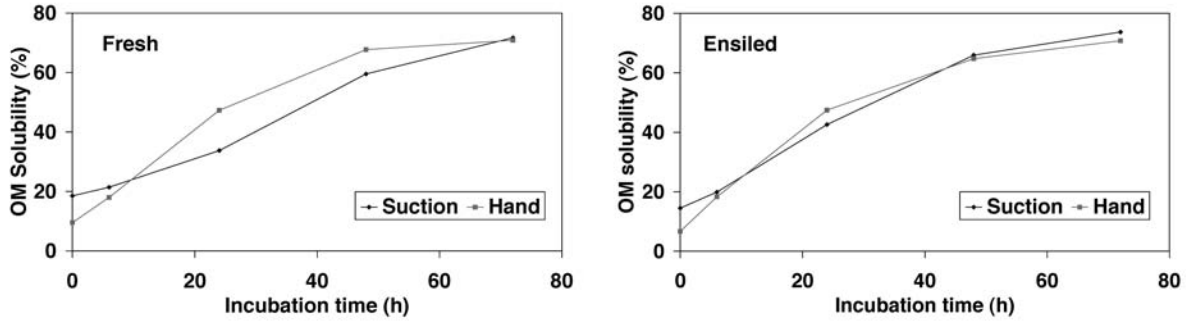

Fig. 2. Degradation profile of OM *in vitro* for fresh (un-ensiled) and ensiled maize whole crop using rumen fluid harvested by suction or hand squeezed (mod. a. Søegaard *et al.*, 2001).

Crude protein fractions

The protein content of a feed is often only based on an analysis of crude protein (N x 6.25). However, the proportion of crude protein, which is amino acid protein, and therefore of biological value for the ruminant if not degraded in the rumen but digested in the small intestine, can vary substantially. For non-conventional feeds where information on amino acid concentration cannot be found in feed tables or other literature sources, an amino acid analysis is very valuable not only to inform about the total amino acid concentration, but also about the concentration of individual essential amino acids, which especially is of importance if it is a protein rich feed with low rumen degradability. The amino acid proportion in crude protein is normally lower in forages than in concentrates, and ensiling normally further decrease amino acid proportion.

Protein degradability in the rumen

In vivo assessment of protein degradability in the rumen is difficult, as duodenal flow is made up by three sources, which are undegraded feed protein, microbial protein and endogenous protein. Further, the undegraded feed fraction normally originates from several feeds making up the ration. *In vivo* assessment relies on a partitioning of the duodenal flow in these three origins, and technically this is difficult (Larsen *et al.*, 2000). Therefore, the *in situ* (nylon bag) method (Hvelplund and Weisbjerg, 2000) has become the '*in vivo*' reference method. The *in situ* method traditionally includes an estimation of the protein degradation profile (soluble fraction, non-soluble but potentially degradable fraction, and rate of degradation), and afterwards a calculation of the effective degradability assuming that soluble fraction is instantaneously degraded, and that the degradable fraction leave the rumen with a certain constant fractional passage rate.

The *in situ* method has several shortcomings. The reproducibility between laboratories is shown to be low (Madsen and Hvelplund, 1994), and loss of small particles through the bag pores can be substantial as well as microbial contamination of the residues of especially fibre rich feeds. The profile obtained is also dependent on bag pore size, sample size etc. (Hvelplund and Weisbjerg, 2000). Also the assumptions used for calculation of effective degradability can be problematic. Recent research has shown that a significant part of soluble protein can pass to the small intestine (Choi *et al.*, 2002). Also the use of one constant for fractional rate of passage can result in poor estimation of supply of rumen undegraded feed protein, as passage rate may differ between feeds and increases with

increasing feeding level (Weisbjerg *et al*., 2002b). Some protein evaluation has partly acknowledged this by introducing variable passage rate depending on feeding level (AFRC, 1992) or different passage rates for concentrates and forages as proposed by Tuori *et al.* (1998).

Digestibility of rumen undegraded protein

Only few data are available on *in vivo* small intestinal digestibility of rumen undegraded protein (Hvelplund and Weisbjerg, 2000), as assessment of this parameter is as difficult as assessment of rumen degradability due to the same problems of tracking origin. Further, the small intestinal digestibility of the undegraded protein will depend on the rumen degradability, as shown by Hvelplund *et al.* (1992). The mobile nylon bag method has shown to give disappearances, which are good estimates of *in vivo* true digestibility (Hvelplund *et al*., 2001). In the mobile bag method a feed sample is placed in a heat sealed nylon bag with small pore size, incubated in the rumen for some time and afterwards incubated in pepsin-HCl to mimic abomasal digestion, before introduced to the small intestine through a duodenal cannula and then left to follow the digesta flow to the faeces. The mobile bag method is a potential reference method for other laboratory methods. The mobile bag used as described above gives an estimate of total tract digestibility, and then small intestinal digestibility of rumen undegraded protein can then be calculated after estimation of the rumen degradability as shown by Hvelplund *et al.* (1992).

Pitfalls

For many non-conventional feeds information on rumen degradability and total digestibility is non-existing, and the crude protein concentration is the only value available. However, total tract digestibility can be low even for protein rich feeds. In a recent study by Weisbjerg *et al.* (2003), it was shown by using the mobile bag technique that protein digestibility of mango leaves (6.7 and 9.5 % crude protein in DM) and acacia leaves (15.7 % crude protein in DM) were so low (Table 3), that the true digestible crude protein was less than the inevitable endogenous faecal loss of 3.4 % of feed DM described above. This shows the importance of estimating total protein digestibility of feeds.

Table 3. Crude protein (CP) concentration and true CP digestibility measured as total tract mobile bag digestibility of some Ugandan forage tree leaves

	CP (% DM)	True CP digestibility (% CP)	True CP digestibility (% DM)
Grewia similis (Bukomakoma)	28.5	89.4	25.5
Bujubwa	9.0	80.5	7.2
Rhus natalensis (Musese)	20.4	66.4	13.5
Grewia mollis (Bukomakoma)	22.3	56.3	12.6
Vernonia amygdalina	28.3	55.0	15.6
Sepium ellypticum	20.1	50.6	10.2
Jackfruit	14.0	29.8	4.2
Mango	9.5	26.8	2.5
Acacia hockii	15.7	14.6	2.3
Mango	6.7	5.3	0.4

This is not only important for untreated feeds but also if heat treatment or other types of treatment (chemical) is used to protect the protein against rumen degradation. A measure of rumen degradability is not information enough, as the decrease in rumen degradability might have caused a decrease in total digestibility, and in such case the protection has only resulted in lower supply with degradable protein in the rumen, and increased the excretion of protein in the faeces. In our laboratory, we have examined several commercial products with "by pass" protein, where total protein digestibility had been severely impaired by treatment. Our standard method for mobile bag digestibility

described above was developed for concentrates and temperate forages. For tropical forages, the 16 h incubation in the rumen is probably too short to obtain total potential digestibility, as shown by Mgheni *et al.* (1994). As also shown by Mgheni *et al.* (1994) many tropical forages will only supply the rumen with degradable protein, as the digestibility of the rumen undegraded protein is very low or zero. Acid detergent insoluble nitrogen (ADIN) has been used as a measure of protein availability, and e.g. the British protein evaluation system (AFRC, 1992) use a digestibility of 90% of the rumen undegraded protein, which is not ADIN. However, use of ADIN is problematic for some feeds, e.g. distillers grains (Kusumanti *et al.*, 1996; Waters *et al.*, 1992).

Discussion and conclusion

Today we have many tools to use in feed evaluation, to help in a proper estimation of the feed value of a feedstuff. For many conventional feeds these methods can give rather exact estimates of the energy and protein value. The trend in feed evaluation is towards ration evaluation systems (models) which can predict the feed value as amounts of digested nutrient supplied from a diet taking ration composition and feeding level into consideration in the prediction.

However, in a major part of the world feed evaluation laboratories are not well equipped, feed table information values are scarce, and many non-conventional feeds are used where little or nothing is known about the feeding value, sometimes not even the name of the feed is known. Often these feeds further contain anti-nutritional factors. It is therefore a big challenge for scientists working with feed evaluation to come up with methods and systems, which can be used in these situations, and which are not too resources demanding.

This paper has discussed some of the most common methods in energy and protein evaluation, and highlighted some problems with the methods, especially problems that might arise when the methods are used on feed types for which the methods have not been developed.

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