DEVELOPMENT OF IMPROVED METHODS FOR ESTIMATING THE NUTRITIVE VALUE OF TROPICAL FORAGES

PROJECT A0316 (R5180;Z0021)

1 APRIL 1992 TO 31 MARCH 1997

Report prepared by Dr C D Wood, Natural Resources Institute, University of Greenwich, April 1997

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OUTPUTS **41**

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EXECUTIVE SUMMARY

Some of the major problems of ruminant nutrition in LDCs are nutrient imbalances, particularly shortages of dietary protein, commonly found during the dry season when diets are often based on crop residues and poor quality pasture. Existing nutritive value assessment methods do not take into account the effects of anti-nutritive factors or interactions between feeds, factors which can be particularly important in ruminant diets in LDCs. This project sought to develop improved methods for estimating nutritive value applicable to LDC diets. Two simple laboratory assays have been shown to indicate the inhibitory effect of tannins on rumen microbes. The project has used *in vitro* gas production as a core technique to evaluate feeds and feed mixtures. It has demonstrated that: the technique is inhibited by tannins; is very sensitive to differences between feeds; is sensitive to interactions between feeds in mixtures and responds to nitrogen supplementation. Strong relationships were found between the extent of degradability of pasture samples during *in vitro* gas production and in the rumen (as measured by the nylon bag technique). Relationships between gas production, other *in vitro* methods and *in vivo* digestibility were, however, less strong for fodder tree leaves.

During a study on Nepalese fodder tree leaves, no strong positive correlations were found between farmers' rankings of fodder tree leaf nutritive value and *in vitro* digestibility. Farmers' rankings appeared to be related to protein supply and dung quality (which was inversely related to *in vitro* digestibility). This was a surprise as it had been expected that digestibility would be the primary positive indicator of fodder tree quality. The *in vitro* gas production method was modified to provide information on the nitrogen status of feed mixtures. The ability of the method to ferment feed mixtures under controlled nitrogen limiting conditions, while being able to monitor fermentation kinetics where nitrogen scarcity has major effects, gives *in vitro* gas production advantages over existing nutritive value assessment methods for studying the supplementation of feeds. The approach has been applied to feeds used in three *in vivo* supplementation experiments and one single feed experiment to help validate the technique, and the initial screening of fodder mixtures from N W India and Costa Rica to illustrate its use.

Variable responses were found in both intake and digestibility during *in vivo* trials. The interactions observed *in vitro* appeared to correspond to interactions observed *in vivo* in some trials, but were at times difficult to interpret. Strong correlations were found between intake and the gas production rate constant (b), and between digestibility and the gas production lag time (T+) for roughages and supplemented roughages in the final, most comprehensive trial. A more mechanistic approach to integrating and interpreting *in vivo* and *in vitro* data is required, together with a more

detailed integration and consideration of data obtained by this and collaborating projects.

The gas production method did not appear, in practice, to give reliable indications of the effects of all the major relevant anti-nutritive factors in fodder trees. Two simple bioassays were found to be potentially useful for screening fodder trees for such factors. These methods need to be used more widely to assess their usefulness in evaluating tree fodders. Laboratory methods aimed at indicating the supply of by-pass protein in tanniniferous feeds appeared unconvincing and require further development.

The project covered technically complex fields of work using new approaches; there is considerable scope for further strategic research in this field. The gas production method is being actively researched for application to intensive feeding systems by several other groups in the UK and elsewhere; doubtless there will be further developments in the technique. The most recent findings of this project indicate that useful indicators of performance are obtainable for supplemented roughages. The gas production method appears to be particularly suitable for investigating the key areas of interest in improving LDC diets, where traditional *in vitro* techniques are inappropriate for providing the required information, and is sufficiently developed to start applying it to practical problem solving.

BACKGROUND

There are widespread seasonal scarcities of feed resources for ruminants in the tropics, exacerbated by the poor quality of the feeds which are available. It was recognised in the ODA Renewable Natural Resources Research Strategy (RNRRS) 1995 - 2005 (and elsewhere) that inadequate feed supplies are a major constraint to increased livestock production in many less developed countries (LDCs). Various strategies may be used to alleviate feed shortages, but for any of these strategies to be implemented methods of assessing nutritive value of tropical feeds need to be re-addressed, to be capable of assessing feeds such as high protein leguminous forages (which often contain anti-nutritive factors) and assessing the value of feeds in mixed diets (which may not be balanced).

At present, largely due to the limited availability of appropriate techniques and the traditional single disciplinary approach to research, estimates of nutritive value tend to be restricted to those relating to chemical composition and rumen digestion of single feeds as part of balanced diets. This approach may be appropriate for feeds grown in temperate climates in developed country feeding systems. However, feeds grown in the tropics have additional components which may have deleterious effects on animal production, but which are not detected by the standard chemical methods currently used to assess nutrient content. Tropical feeding systems may include the use of unbalanced diets at times of feed shortage, hence conventional evaluation techniques may be misleading.

An *in vitro* fermentation (gas production) method for estimating the fermentation kinetics of tropical feeds was developed under a previous project (EMC X0162) by Dr M Theodorou and co-workers. This method has been adopted by NRI as the core

technique for evaluating feeds and feed mixtures, and forms the major focus of this project.

Laboratory analyses, sometimes supported by *in vitro* digestibility assays, are used in many LDCs to evaluate feeds. This indicates a high level of demand for laboratory feed assessment. The techniques used are those developed for temperate feeds and feeding systems. The weaknesses of these techniques are indicated above. If improved techniques can be shown to give more useful indicators of feed quality and are generally suitable they would be of use to LDC laboratories. Furthermore, the output of such work, advice on improved feeds and diets, is of interest to many livestock keepers as feed constraints are widely acknowledged as being a major limitation to livestock production.

PROJECT PURPOSE

The project pre-dated the RNRRS 1995 - 2005, however its goal can be stated as: performance of livestock (including draught animals) in forest-agriculture interface and hillside (crop/livestock or livestock) production systems improved. The project is also relevant to the other livestock production systems although it was formally included in the forest-agriculture interface system. The purpose (indicative output 1.4) was to develop techniques to improve the contribution to animal production of crop residues, tree fodders and fodder crops, although again it was highly relevant to several indicative outputs across the range of farming systems.

To improve animal production farmers need advice on how to use the available feeds in improved diets, or to incorporate novel or unfamiliar feeds and, if there are particular times of feed shortage, advice on how this may be alleviated. The specific purpose of this project was to provide laboratory tools which can be used to generate information on nutritive value as part of the process of developing appropriate advice to farmers.

RESEARCH ACTIVITIES

This was a large, complex project with several strands of activities. Many of the activities (and outputs) have been written up as scientific papers (draft and published) or reports. The papers and selected reports have been attached as a Technical Annex in six parts. The main text of the Final Technical Report gives an overview of activities and outputs. The text has been organised on a loosely chronological basis and also by technical theme. The chronology was in practice by no means as clearly partitioned as the text may indicate as many activities were ongoing simultaneously. However, the report is organised in this way to describe the reasoning behind the activities and how this developed as the project progressed.

A detailed review on the background and potential use of the *in vitro* gas production method was prepared during the course of this project:
Wood C D, Thorne P J, Romney D L and Rosales M (1997) Laboratory techniques appropriate for evaluating ruminant feeds in less developed countries, with particular reference to the potential use of in vitro gas production methods (Technical Annex, part 6).

Nutritive value assessment, including gas production techniques, has been a area of active research by many groups worldwide during the period of this project. Some of these more recent findings have been included in the review. The conclusions of the literature review include the following:
a) Existing *in vitro* techniques can be used to predict *in vivo* digestibility, intake and animal performance with varying degrees of success. The gas production technique shows promise as a simpler and more versatile method for predicting these parameters, and as a more general research tool, but requires further development and calibration against feeding trial data.

b) Feed evaluation has a history of different research groups independently developing feed evaluation systems, leading to different protocols for laboratory methods and different equations for interpreting data. A more collaborative approach to developing the gas production method would avoid unnecessary duplication and confusion.

c) Some of the major problems of ruminant nutrition in LDCs are nutrient imbalances, particularly shortages of dietary protein. Existing feed evaluation and *in vitro* techniques are not designed to assess feeds as part of protein-deficient diets. New approaches are required for the investigation of interactions between feeds to formulate more balanced diets. The Theodorou gas production technique appears to be particularly suitable for this use as feed mixtures can be fermented under controlled conditions.

d) There is no framework for accounting for the effects of anti-nutritive factors in ruminant feeds and more work is required to evaluate such factors.

These conclusions broadly set the technical agenda for this project.

1 Initial findings (1 April 1992 - mid 1993)

1.1 Tannins

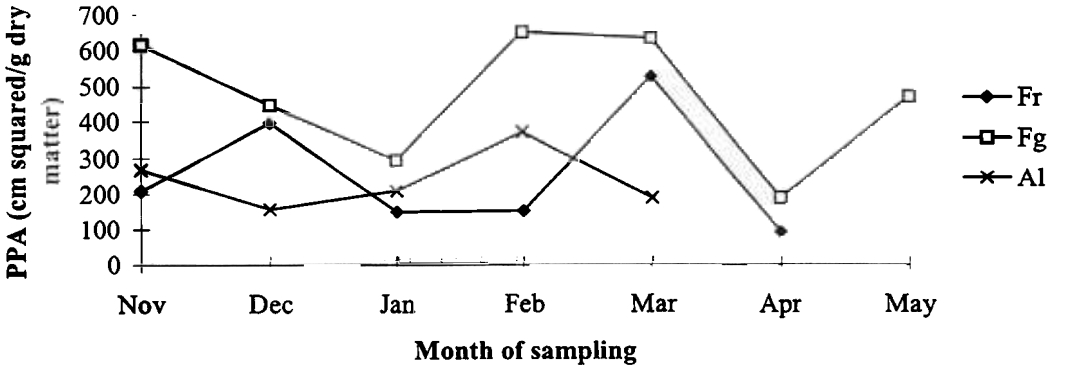
The initial focus of the project was on anti-nutritive factors, particularly tannins in tree fodders as those are very commonly used in LDC feeding systems. For technical background to this work see the literature review prepared during the project (*Effects of tannins in ruminant nutrition, by C. D. Wood, in the Technical Annex part 1*). This was written late in the project and includes some of the outputs of the project as well as background material. In summary, tannins are chemically diverse and there are many published methods which can be used to assay them. At the start of this project there were few indications as to which methods most reliably indicated the effects of tannins on animals, although protein precipitation assays were recommended by some groups. Tannins are widely found in trees, shrubs and some agricultural by-products. Many ruminants in LDCs consume tanniferous feeds. Tannins decrease the availability of nutrients in feeds and can have direct toxic effects on animals, but the balance of importance between these two effects is unclear.

There is very limited information in the scientific literature on the composition of tree fodders in general and tannins in particular. Data tend to be of single samples with no indication of the variability of composition. Therefore, as a first stage, the variability

of tannins and other major nutrients (ash and crude protein) of tree leaves from different positions within the tree and of different ages was investigated using two Nepalese fodder tree species. The major conclusions were that extractable tannins can vary significantly between trees of the same species, between leaves from single trees and with time. Some apparently random variations were also apparent in the ash and crude protein contents. Multiple samples taken from different trees at different times and from different positions are required for the assessment of nutritive value; representative samples must include leaves of different ages and from different positions. The work is described in detail in:
Wood C D, Tiwari B N, Plumb V E, Powell C J, Roberts B T and Gill M (1995) Differences in protein precipitation activity of extractable tannins, crude protein and ash contents of leaf samples from Nepalese fodder trees. Tropical Science 35: 376-385 (Technical Annex, part 1).

The study was extended to include 13 Nepalese fodder species over the November to May period when tree fodders are particularly important. As in the first study, the major nutrients (dry matter, ash and crude protein) and extractable tannins were assayed. Nutrient levels were relatively stable with some variability with time related to leaf senescence. Tannin levels fluctuated with time, with trees from similar altitudes fluctuating in similar ways. Tannin levels also tended to fall prior to leaf shedding in 6 species. The fluctuations in three species, *Ficus roxburghii* (Fr), *F. glaberrima* (Fg) and *Artocarpus lakoocha* (Al) are illustrated in Figure 1, where PPA = protein precipitation activity of tannins extracted into aqueous acetone.

Figure 1 Fluctuation of tannins with time



Fluctuations in tannins were apparently due to changes in the extractability and quantity of condensed tannins. It was suggested that these fluctuations could be weather related, and could be of practical importance to farmers. The study was reported in part in:
Wood C D, Tiwari B N, Plumb V E, Powell C J, Roberts B T and Gill M (1993) Interspecies differences in tannin activity of leaves from 13 species of Nepalese browse trees p212-213 In Animal production in developing countries. An occasional publication of the British Society of Animal Production, No 16 Editors Gill M, Owen E, Pollott G E and Lawrence T L J (Technical Annex, part 1)
and in full in:
Wood C D, Tiwari B N, Plumb V E, Powell C J, Roberts B T, Sirimane V D, Rossiter J T and Gill M (1994) Interspecies differences and variability with time of protein precipitation activity of extractable tannins, crude protein, ash and dry matter

contents of leaves from 13 species of Nepalese fodder trees J Chemical Ecology 20: (12) 3149 - 3162 (Technical Annex, part 1).

1.2 Gas production

This method was developed by Dr Theodorou, Institute of Grassland and Environmental Research (IGER), under an ODA contract funded through NRI (project X0162). The method was found to be simple to do yet sensitive, and suitable for providing information on the fermentability of tropical crops and residues intended for use as ruminant feeds. The gas production method was seen as potentially superior to existing *in vitro* digestibility methods which all have weaknesses. Most importantly, it provided data on rates of degradation which were seen as being particularly important for evaluating the nutritive value of poor quality tropical forages. Existing *in vitro* techniques aimed at providing estimates of the end point of digestion and have been applied successfully to estimating the *in vivo* digestibility of relatively good quality temperate forages. The background to the uses of the gas production method, together with some more recent work, have been reviewed during the course of this project (*Wood C D, Thorne P J, Romney D L and Rosales M (1997) Techniques for evaluating ruminant feeds in less developed countries, with particular reference to the potential use of in vitro gas production methods. Technical Annex, part 6*). The method was set up at NRI at the start of this project.

The gas production method was used initially (to about mid 1993) in 3 areas of work:
- investigating the effects of tannins on *in vitro* digestibility (as indicated by gas production)
- as a ranking tool for tree fodders
- to investigate interactive effects.

A strategy of simultaneously increasing our understanding of the processes occurring during *in vitro* fermentation and making comparisons with *in vivo* data was adopted throughout the course of the project.

1.3 Effects of tannins on digestibility

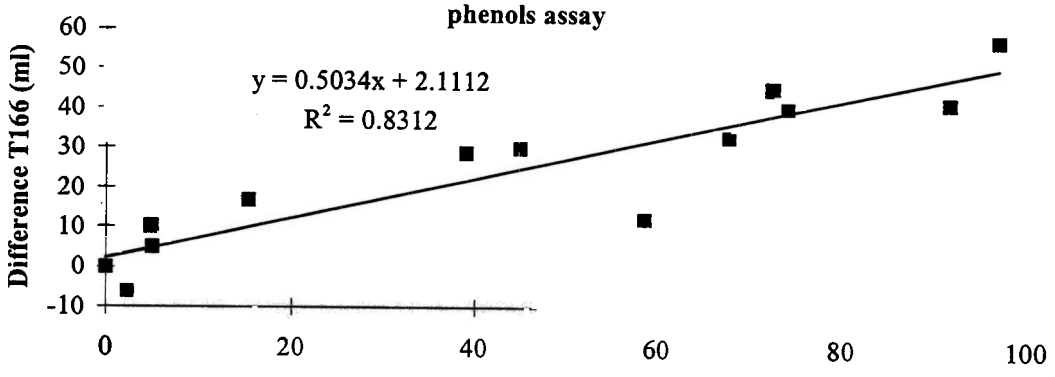
Tannin-binding agents, polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP), were used to bind and neutralise the effects of tannins in tree leaves. By comparing gas production with and without tannin-binding agents, a measure of the effects of tannins on gas production, and hence digestibility, was obtained.

PEG was found to overcome most, but not all, of the inhibitory effects of tannic acid on gas production. Treatment of Colombian tree leaves produced variable responses with no strong correlation between the response and tannin levels assayed (reported in detail in: Rosales M (1996) Ph D Thesis, Univ of Oxford. In vitro assessment of the nutritive value of mixtures of leaves from tropical fodder trees).
This finding was open to interpretation:
- were the tannin assays poor indicators of the effect of tannins on gas production?
- was PEG unable to bind all the tannins, especially those already bound to leaf components?

Both interpretations could be supported from the scientific literature.

To distinguish between these two interpretations, further work was conducted using tannin extracts where none of the tannins would be bound prior to treatment with tannin-binders. A range of tree leaf samples from Bolivia were analysed for extractable tannins by three methods (total phenols, condensed tannins and protein binding activity). From these, 12 samples were selected so as to give a range of phenolic compositions. Extracts were then fermented with and without PVP. It was found that the inhibitory effects of tannins on gas production were significantly correlated with the total phenols and protein binding activity assays, but not with the condensed tannin assay. Figure 2 illustrates the relationship between one measure of the inhibition of gas production (Difference T166) and the total phenols assay.

Figure 2 Correlation between inhibition of gas production and the total phenols assay



It was concluded that the total phenols assay and, less accurately, protein precipitation activity were useful indicators of the degree of inhibition of rumen micro-organisms by phenolic compounds in tree leaves. The acid butanol assay for condensed tannins was not an indicator of rumen micro-organism inhibition when used for a range of tree species.
For full details see:
Wood C D and Plumb V E (1995) Evaluation of assays for phenolic compounds on the basis on in vitro gas production by rumen micro-organisms. Animal Feed Science and Technology 56: 195-206. (Technical Annex, part 1).

Another study (project I0046) conducted by NRI at the same time demonstrated that *in vitro* gas production was also inhibited by cyanide compounds in the leaves of cassava.

The technique was, therefore, clearly sensitive to at least some of the more important anti-nutritive factors. This property appeared to make it particularly suitable for ranking, screening or evaluating tree fodders which often contain tannins and other anti-nutritive factors. These conclusions were reported to the Livestock Strategy Area Advisory Committee in February 1995 (*Wood C D, 1995; Feed evaluation: Recent developments. Summary of presentation given to LSAAC, February 1995. Technical Annex, part 1*).

This positive conclusion was qualified by the observation that the *in vitro* system was not susceptible to any toxic effects that tannins may have directly on animals and effects which may be exerted by their binding to feeds. Also, when the relationships

between gas production characteristics and composition for 70 tree leaf samples from Bolivia, West Africa and Colombia were investigated it was found that more than half of the variability in gas production could not be explained by the variability in components analysed. As expected, fibre and total phenol contents were significant factors in determining gas production. Nevertheless, we were clearly some way away from understanding all of the factors which are important in contributing to the *in vitro* gas production characteristics. This study is reported in more detail in:
Wood C D, Grillet C, Rosales M and Green S (1995) Relationships between in vitro gas production characteristics and composition of tree leaf fodders from Bolivia, West Africa and Colombia. Abstract Animal Science 60: 541 (and summary) (Technical Annex, part 1).

1.4 Ranking of tree fodders

The gas production method was applied to Bolivian leaf samples to see if it was able to distinguish between tree species and within tree species. Samples from 19 species were categorised by local farmers as being of high or medium palatability, or as being unpalatable, and evaluated by the gas production method. Large differences were found in gas production characteristics between species, but there was no relationship between palatability and gas production characteristics. In fact some species of low fermentability were said to be highly palatable whilst some unpalatable species were highly fermentable.

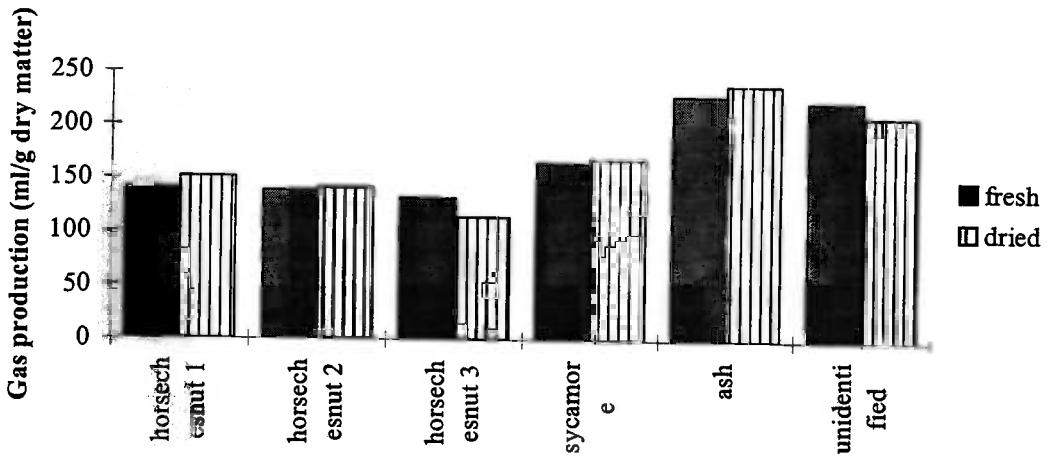
The gas production method was sufficiently sensitive to detect significant differences between genetically identical trees grown at different sites. There was an apparent relationship between soil fertility and gas production, the more fertile the site the higher the gas production tended to be. The study demonstrated that the gas production method was sufficiently sensitive to rank tree fodders and even to undertake more detailed studies of their characteristics. It also indicated that there was no apparent relationship between palatability and fermentability over a wide range of tree leaves (including some which were not regarded as fodders). The study is reported in more detail in:
Wood C D, Johnson J and Powell C (1993) Evaluation of Bolivian tree leaves as fodders by an in vitro fermentation technique Agroforestry Forum 4: 28-34 (Technical Annex, part 1).

1.5 Sample preparation

There are several references in scientific literature to the modification of tannins during sample preparation, although different tannins appear to behave in different ways. Tannins are known to be susceptible to oxidation, can polymerise and are generally fairly reactive. Such reactions could affect the values obtained in tannin assays and affect the fermentation characteristics of tanniniferous samples. Therefore some trials were undertaken to investigate the extent of modification during sample drying.

Differences in gas production characteristics between fresh tree leaves and leaves dried at 50°C were investigated by taking samples of leaves from four UK tree species. Drying made little difference to the gas production characteristics and did not affect their ranking (selected data presented in Figure 3 below).

Figure 3 Gas production of fresh and dried leaves after 52 hours incubation



Leaf samples from twelve tree species from the Santa Cruz province of Bolivia were obtained during NRI project Q0010. Samples were subdivided and tannins extracted from fresh material and after oven drying at 50°C for 16 to 24 hours. Losses of extractable tannin protein precipitation activity caused by drying ranged from 18.7% to 70.1% (relative to the extractable tannin protein precipitation activity extracted from fresh leaves). Leaves of *Erythrina poeppigiana* were dried at 50°C for 24 or 48 hours after being kept at ambient temperatures of 2, 24 or 96 hours. No significant differences in gas production characteristics were observed between these different treatments.

Therefore, as long as the drying temperature was sufficiently low, 50°C appearing to be appropriate, then tanniniferous material could be dried without major modification to the fermentation characteristics. In contrast, extractable tannin levels clearly could be modified during this process. This may have been due to changes in the tannins, or reactions between tannins and other feed components, which rendered them less extractable, but which made little difference to the fermentation properties of the feeds. The apparent relative vulnerability of tannin analysis to artifacts was one factor in prompting a change of approach towards looking at nutrient availability directly (using *in vitro* techniques) rather than to attempt to predict it from tannin and other assays.

1.6 *In vitro* interactive effects

Rumen microbes require a complex balance of nutrients and optimal conditions within the rumen in order that they can grow quickly and degrade the fibrous fraction of the feed. Deficiency of any essential nutrient will inhibit microbial growth and function even if other nutrients are amply supplied. Therefore, feeds which are deficient in a particular nutrient (or nutrients) can be supplemented by other feeds which provide these nutrients and balance the diet. Feeds can, therefore, interact with each other; that is the fermentation properties (or digestibility) of feed mixtures is not necessarily the sum of the of the fermentation properties of the individual components. Under certain conditions these interactions can be negative or positive (the background to this is considered in more detail in: *Wood C D, Thorne P J, Romney D L and Rosales M (1997). Techniques for evaluating ruminant feeds in less developed countries, with*

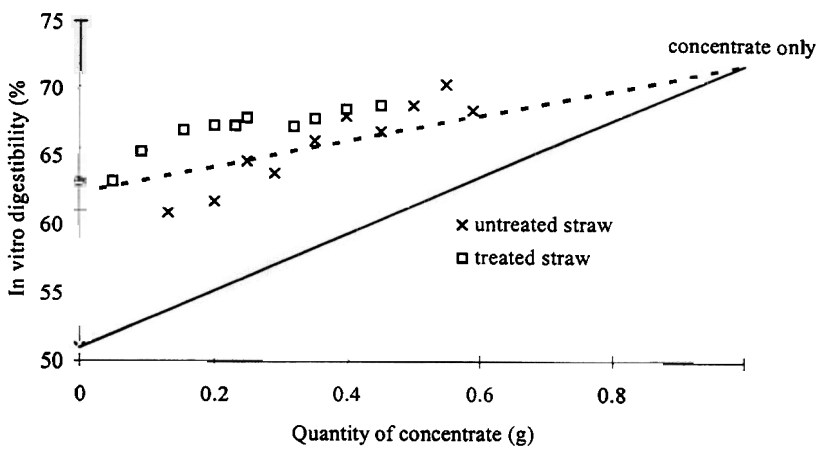
particular reference to the potential use of in vitro gas production methods. Technical Annex, part 6). The gas production method allows individual feeds and feed mixtures to be fermented so that the actual fermentation characteristics of the mixtures can be measured experimentally and compared with the properties of the mixtures predicted (assuming no interactions) from the properties of the individual components, weighted according to their proportions in the mixture. This comparison can be calculated as a percentage difference:

$$\text{Difference (interaction) \%} = \frac{100 \times (\text{Measured gas production} - \text{Predicted gas production})}{\text{Predicted gas production}}$$

Interactions also reveal themselves in graphs of any selected fermentation parameter plotted against the proportion of supplement. If there are no interactions, a straight line linking the gas production parameter values of the individual feeds will predict the value of the feed mixtures if there are no interactions. Experimental data for feed mixtures lying away from this straight line will indicate the presence of interactions. When interactions occur the relationship between gas production parameter and proportion of supplement will be better represented by a curve than by a straight line. These approaches, sometimes in combination, have been used in analysing data on interactions between feeds.

The project benefited by the arrival in Spring 1992 of Drs Sampath and Prasad from the National Dairy Research Institute, Bangalore, India. They were conducting a programme of feeding trials investigating the urea treatment and supplementation of finger millet straw in India. During their stay in the UK, the *in vitro* gas production method was used to investigate interactions between straw and supplements.

Figure 4 Measured in vitro digestibility compared with that predicted assuming no interactions between treated or untreated finger millet straw and concentrate



Firstly, untreated and urea treated straw was supplemented with different levels of a concentrate rich in protein and digestible energy. Similar trends were observed in *in vitro* gas production and in *in vivo* digestibility. *In vitro* and *in vivo* digestibilities were best matched at 45.8 and 47.9 h incubation for untreated straw and 43.5 and 61.0 h for urea treated straw for *in vitro* trials 1 and 2 respectively. Significant interactive effects were observed between supplement and both straws. The fermentability of

untreated straw was particularly stimulated by small quantities of supplement. The fermentability of treated straw was higher than that of untreated straw and less stimulated by supplementation. A comparison of measured *in vitro* digestibility to that predicted from characteristics of the single feeds is given in Figure 4. In the figure the scatter points indicate the measured *in vitro* digestibility and the straight lines (doted for treated straw, continuous for untreated straw) indicate the *in vitro* digestiblities of feed mixtures assuming no interactions.

These findings were consistent with the hypothesis that fibre digestibility can be increased by providing a supplement which provides sufficient nutrients to stimulate the activity of rumen microbes. This work is reported in detail in:
Prasad C S, Wood C D and Sampath K T (1994) Use of in vitro gas production to evaluate rumen fermentation of untreated and urea treated finger millet straw (Eleusine coracana) supplemented with different levels of concentrate. Journal of the Science of Food and Agriculture 65 457-464 (Technical Annex, part 2).

The second piece of work looked at the supplementation of the same straw samples with urea, rice bran, cottonseed and groundnut cakes. Significant positive interactions were observed between groundnut cake and untreated straw at the three levels of supplementation studied, that is gas production was increased compared to that predicted by the no interaction model. Some positive interactions were also observed between groundnut cake and treated straw, and between cottonseed cake and untreated straw, although statistical significance was not achieved at all levels of supplementation at all incubation times studied. No consistent interactions were observed between cottonseed cake and treated straw; rice bran had little effect on either type of straw. Urea inhibited gas production from both straws. Interactive effects on gas production were most pronounced in the early stages of fermentation and appeared to be related to the high content of highly fermentable material particularly in groundnut cake but also in cottonseed cake. This work is reported in detail in:
Sampath K T, Wood C D and Prasad C S (1995) Effect of urea and by-products on the in vitro fermentation of untreated and 5% urea treated finger millet (Eleusine coracana) straw. Journal of the Science of Food and Agriculture 67 323-328 (Technical Annex, part 2).

After the *in vitro* work had been completed, *in vivo* trials were conducted in India by Drs. Sampath and Prasad (as part of a separate project) using cottonseed cake and groundnut cake as supplements to finger millet straw. Again the best match between *in vivo* and *in vitro* data was found after about 39 to 45 hours incubation and similar trends were observed *in vivo* and *in vitro*.

1.7 Initial conclusions

Given the demonstrated sensitivity of *in vitro* gas production to anti-nutritive factors discussed above, coupled to its ability to respond to interactions between feeds, it was concluded that the technique appeared to be a suitable core method for investigating the types of interventions which may be appropriate in LDC farming systems. The technique was very rapid compared with *in vivo* trials, and was capable of investigating feed properties which could not be readily investigated using conventional *in vitro* techniques.

Nevertheless there were a number of uncertainties, several of which by mid 1993 were already being investigated.
- It was unclear what components of the feeds were interacting with each other. An understanding of this would clearly help in the interpretation of the data. Therefore a more detailed investigation of these, using model substrates and feeds, was undertaken.
- There was a growing unease that the nitrogen rich medium used in the original Theodorou protocol (following the conventional approach used for *in vitro* digestibility assays) may not always be appropriate. In particular, the studies with Drs Sampath and Prasad reported above were conducted using a nitrogen rich medium. For energy supplements this may be appropriate, but for nitrogen or protein supplementation a nitrogen limited environment may be required (particularly in LDC diets where protein levels are usually low).
- Comparisons between *in vivo* and *in vitro* gas production data was required to see that interactions and rankings obtained *in vitro* did have some meaning in terms of animal performance. Therefore it was decided to seek collaborators who were conducting *in vivo* trials who would be willing to provide samples and data to enable such comparisons to be made.

2 Intermediate phase (mid 1993 - early 1996)

2.1 Interactions between feeds and feed components

High fibre, low protein barley straw was supplemented *in vitro* (in nitrogen-rich medium) with lucerne, meadow, rye and timothy hays which are all considered to be of superior quality. All the mixtures showed some evidence of positive interactions (i.e. a bringing forward of the fermentation). Interactions were highest in the early stages of fermentation. Interactions were highest with lucerne and timothy hays, which had the highest initial rates of gas production. Extent of interaction appeared to be related to initial fermentation rate, which in turn may have been due to the presence of soluble, highly fermentable sugars. Compared to the hays, barley straw had a high fibre content, but mid-range lignin content. The fibre fraction was degraded slowly, but relatively extensively. These studies are described in more detail in two unpublished reports (*To determine the effect of supplementation of barley straw with lucerne hay, meadow hay, rye hay and timothy hay on gas production during in vitro fermentation; To determine the effect on the in vitro fermentation technique on the fibre fraction of five samples of temperate hay and straw, both by C Powell, Technical Annex, part 2*).

The above work was intended as an initial study which would lead to a PhD programme, but due to the departure of the member of staff involved this general area of work was incorporated into the programme of a second PhD research student/visiting scientist as described below.

The chemical components of fodder tree leaves that affect fermentation, and the time at which the effect occurs were identified. In a nitrogen-rich medium, the soluble components made a more significant contribution to gas production from 3 to 16 h incubation, the less soluble material was more significant from 20 to 33-39 h. From 45 h onwards significant correlations were not found between chemical composition

and gas production. The main components which affect fermentation were found to be: soluble protein, soluble carbohydrates, starch, acid detergent fibre and phenolic compounds. Insoluble protein was a factor when feeds were fermented in a nitrogen-free medium, but not in a nitrogen-rich medium. In a nitrogen-free medium, soluble components were less important during the early fermentation stage but the main components which affected fermentation were the same.

The fermentation of mixtures of model substrates, and mixtures of different fodder trees with glucose, starch or cellulose, indicated that the greatest interactions occurred when mixtures had components of similar fermentability, that is the periods of maximum fermentation of carbohydrate and protein were synchronous. Interactions found between tree leaf mixtures were also consistent with this view.

Using quebracho tannin as a model, it was shown that tannins reduce the extent of fermentation even in small quantities. Substrates of intrinsically low fermentability (starch and cellulose) were affected more by quebracho tannin than highly fermentable glucose. Quebracho bound both soluble and insoluble protein.

This work is described in considerable detail in: *Rosales M (1996) Ph D Thesis, Univ of Oxford. In vitro assessment of the nutritive value of mixtures of leaves from tropical fodder trees.* Due to its length, the abstract and general discussion only have been included in the Technical Annex (part 2). Some of the key aspects of the study are described in two draft summary papers and a draft full length paper which are included in the Technical Annex part 2:

Rosales M, Gill M, Wood C D, Romney D, Speedy A W and Stewart J (under review) The contribution of chemical constituents of fodder tree and shrub leaves to gas produced during in vitro fermentation in nitrogen free and nitrogen rich media. Submitted for presentation to In vitro techniques for measuring nutrient supply to ruminants, Occasional Meeting of the British Society of Animal Science, Reading, July 1997.

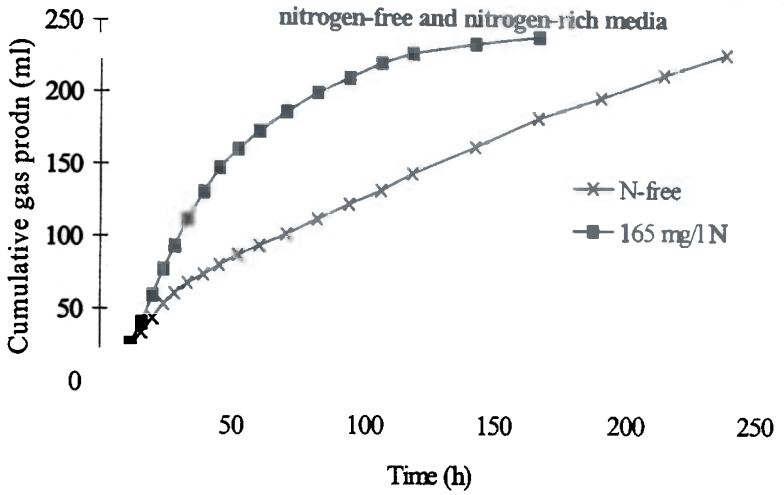
Rosales M, Gill M, Wood C D, and Speedy A W (under review) Associative effects of in vitro mixtures of tropical fodder trees. Submitted for presentation to In vitro techniques for measuring nutrient supply to ruminants, Occasional Meeting of the British Society of Animal Science, Reading, July 1997.

Rosales M, Gill M, Wood C D, Romney D, Speedy A W and Stewart J (in preparation) The contribution of chemical constituents of fodder tree and shrub leaves to gas produced during in vitro fermentation in nitrogen free and nitrogen rich media. Draft paper.

2.2 Nitrogen in medium

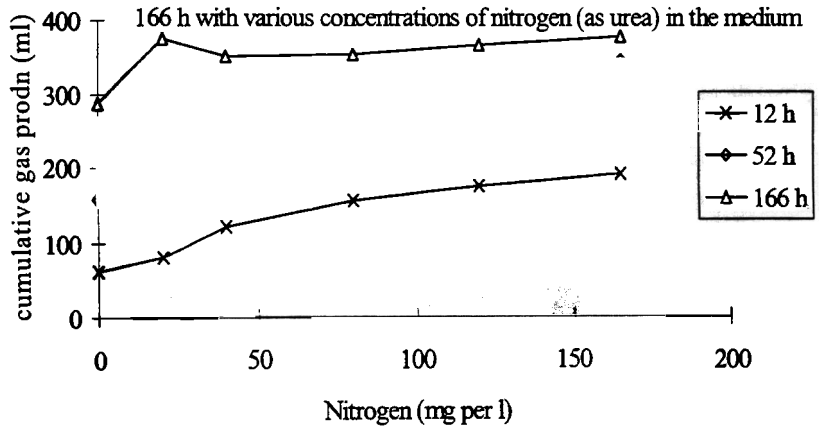
The effects of nitrogen on an *in vitro* gas production method were investigated by fermenting two nitrogen-deficient substrates (glucose or barley straw) in media with different concentrations of nitrogen (from 0 to 165 mg l^{-1}) using two nitrogen sources (ammonium sulphate or urea). Such substrates are fermented more slowly when nitrogen supply is limiting as illustrated in Figure 5.

Figure 5 Fermentation characteristics of barley straw fermented in nitrogen-free and nitrogen-rich media



The effect of nitrogen was complex and defining minimal nitrogen concentrations may be misleadingly simplistic. Figure 6 illustrates how the response to nitrogen changes during the course of the incubation. Thus, at low nitrogen levels longer fermentation periods were required to achieve maximum fermentation. Earlier the work (described in section 1.6) suggested that, when the nitrogen rich medium was used, about 52 h was the period most related to *in vivo* data. At 52 h, the response of the *in vitro* gas production system to nitrogen supplementation was broadly consistent with earlier *in vitro* work, indicating that a minimum of 50 to 100 mg l^{-1} nitrogen is required to achieve the maximum degradation of carbohydrate by rumen microbes.

Figure 6 Cumulative gas production from glucose incubated for 12, 52 and 166 h with various concentrations of nitrogen (as urea) in the medium



The effect of nitrogen appeared to be mainly, if not entirely, on the rate of degradation, and did not greatly affect final cumulative gas production. The *in vitro* gas production method is convenient for monitoring the rate of degradation of carbohydrate and appeared to be generally suitable for investigating interactions between feeds. This work is described in more detail in:

Dryhurst N and Wood C D (under review). The effect of nitrogen source and concentration on in vitro gas production using rumen micro-organisms. (Technical Annex, part 2)

2.3 *In vivo/in vitro* comparisons

2.3.1 Comparisons with nylon bag (*in sacco*) data

The opportunity arose to evaluate various pasture samples from Mongolia which had previously been evaluated using the nylon bag method. Indicators of the extent of degradation obtained from the two techniques were generally highly correlated over what was a limited range of degradabilities, as illustrated in Figure 7. Rate constants were poorly correlated as shown in Figure 8, the gas production rate constants being generally higher than those found using the nylon bag technique.

Figure 7 Correlation between nylon bag degradability (a + b) and gas production after 70 h incubation

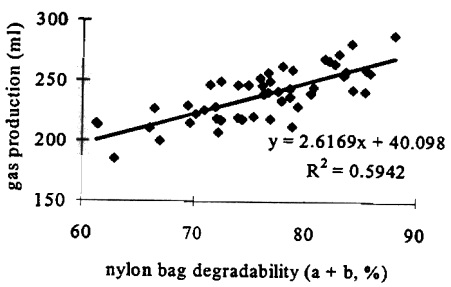
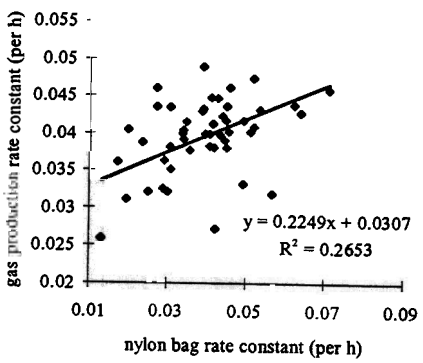


Figure 8 Correlation between rate constants of nylon bag degradation and gas production



Both techniques detected similar trends in pasture degradabilities. This work is describe in outline in:
Murray A H, Daalkhaijav D and Wood C D (1996) Rumen degradability of Mongolian pastures: a comparison of in situ and in vitro gas production techniques. Abstract, summary and poster presented at the British Society of Animal Science Winter Meeting 1996. Animal Science 62: 684. (Technical Annex, part 3)
and in detail in:
Murray A H, Daalkhaijav D and Wood C D (in preparation) The rumen degradability of Mongolian pastures measured by in sacco and in vitro gas production techniques. (Technical Annex, part 3)

In another experiment, 19 shrub and tree leaf samples from Colombia were evaluated by gas production and the nylon bag technique. Values of the extent of degradation (gas pool size and nylon bag degradability) were correlated (gas pool size = -33.35 + 2.78*degradability; $R^2 = 0.71$, $P<0.001$). The rate constants given by the two methods were not correlated. These findings were fully consistent with those of the Mongolian pasture study. This work is described in: *Rosales M (1996) Ph D Thesis, Univ of Oxford. In vitro assessment of the nutritive value of mixtures of leaves from tropical fodder trees.*

2.3.2 Comparison between ranking of *Gliricidia sepium* accessions *in vitro* and *in vivo*

As part of an ODA funded Forestry Research Programme project conducted by the Oxford Forestry Institute (OFI), *Gliricidia* accessions from five different seed sources were grown at five sites (Colombia, Costa Rica, Indonesia, Nigeria and Sri Lanka), feeding trials conducted and replicate leaf samples taken for chemical analysis and evaluation by *in vitro* gas production (by project A0316 in collaboration with the OFI project). Leaves from accession 32/92 were the most fermentable (as judged by cumulative gas productions after 12, 52 and 70 h incubation), followed by accession 4/92 then accessions 126/91, 125/91 and 124/91 which were similar. Differences in fermentability between accessions generally achieved statistical significance ($P<0.05$) at all five sites, but were not consistent at all sites. Significant ($P<0.05$) genetic variation was found in CP and ADF, but not in NDF and coumarin levels. With the possible exception of gliricidia grown at Costa Rica, accession related differences in nutritive value were considered unlikely to be of importance to livestock keepers. Significant differences ($P<0.05$) were found between samples of young and old leaves, leaves from sunny and shady plots and between air dried and freeze dried samples. Differences between samples from different sites were generally greater than differences between accessions for trees grown at a single site and sampled in the same way. Site differences may have been related to differences in environment, but could have been caused by other factors.

During the early stages of gas production, differences were observed between young and old leaf samples from Colombia, the young leaf samples producing less gas. This was perhaps a surprising finding as young plant material, such a grasses, are more fermentable than old material. This was interpreted as a possible indication of the presence of unidentified anti-nutritive factors in the young plant material. The young gliricidia leaf samples with the highest and lowest gas productions were used to supplement (at 26% of substrate) sugarcane tops to mimic feeding trials conducted in Colombia. No significant differences were observed in the gas productions of the feed mixtures, consistent with the feeding trials. The lowest gas producing young leaf sample had a very low gas production compared to its dry matter disappearance when fermented alone. The reasons for this are unclear, but have been found with other samples during this project (such as the two tree fodders from West Africa described in section 2.3.4). The low gas producing sample was relatively low in protein, a factor which would be expected to increase the gas production. VFA analysis indicated that VFAs were being produced in increased quantities in the low gas producing samples, with increased acetate production compared with the higher gas producer (390 mg l^{-1} compared with 294 mg l^{-1}), which would be expected to increase gas production. Hence it was unclear why this particular sample produced relatively little gas as it was fermented.

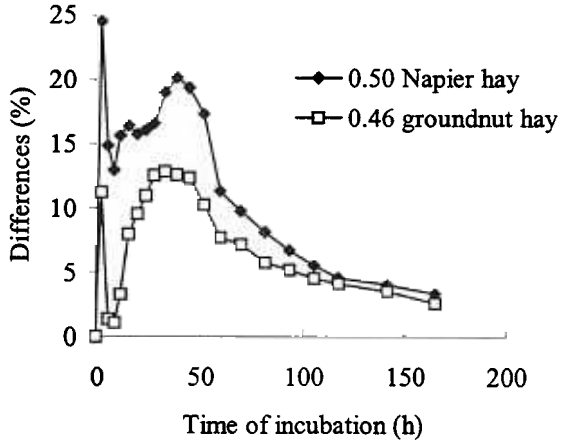
A detailed account is contained in:

Wood C D, Stewart J L and Vargas J E (in preparation). Genetic variation in the nutritive value of Gliricidia sepium. 2. Leaf chemical composition and fermentability by an in vitro gas production technique. (Technical Annex, part 3)

2.3.3 Comparisons between *in vitro* interactions and *in vivo* data (Zimbabwe hay mixtures)

Measurement of gas produced during *in vitro* fermentation was used to investigate the fermentability of poor quality natural pasture (veld) hay from Zimbabwe mixed with different amounts of Napier hay or groundnut hay. *In vitro* fermentations were conducted in nitrogen-rich and nitrogen-free media to parallel *in vivo* trials which had been conducted by another LPP project. Groundnut hay was more rapidly fermented than Napier hay, the nitrogen content of the medium making little difference to the fermentation characteristics of either hay. Veld hay was the least fermentable substrate, particularly when nitrogen-free medium was used. Statistically significant positive interactive effects were observed between both supplements and veld hay fermented in both media as gauged by gas production and dry matter disappearance. This implied that the feed mixture was fermented more quickly than predicted from the fermentation characteristics of the individual feeds. Interactions were time dependent as shown in Figure 9 (feeds fermented in nitrogen-rich medium in this case). The figure shows differences between observed gas production and predicted production if there were no interactions (expressed as a % of the predicted gas production). Two feed mixtures have been taken in this illustration, 0.50 g Napier hay + 0.50 g veld hay and 0.46 g groundnut hay + 0.54 g veld hay. The initial (3 h) interaction may have been artefactual; 3 h gas production is low and relatively variable so small differences can appear as large % differences. Interactions were generally at their highest between 33 and 52 h incubation.

Figure 9 Differences (interactions, %) at different times of incubation

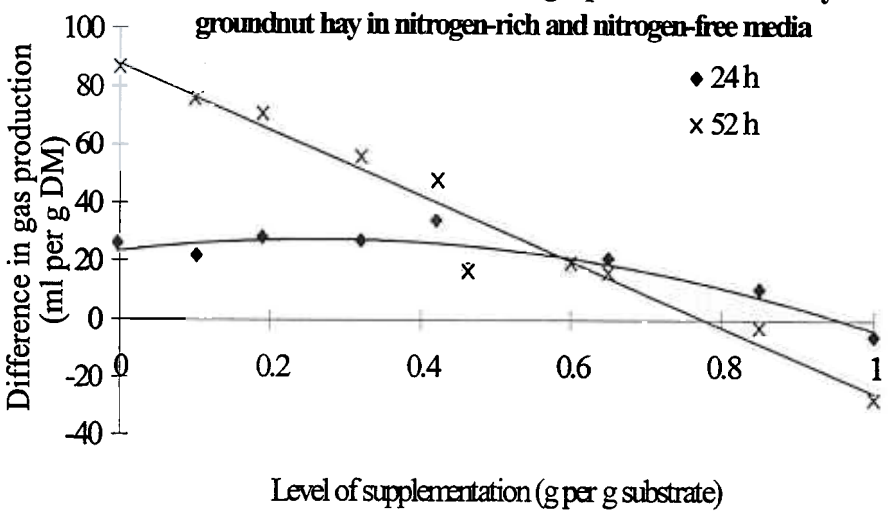


Evidence of significant interactions had not been obtained from earlier measurements of *in vivo* digestibility using the same feeds, but they may have been obscured by increased rates of passage with increased supplementation.

Differences between gas productions in nitrogen-rich and nitrogen-free media were explored as possible indicators of nitrogen deficiency in feed mixtures. Figure 10 illustrates the differences in cumulative gas production after 24 h and 52 h incubation of veld hay supplemented with groundnut hay. After 24 h only high levels of supplementation, greater than 0.6, were having any appreciable effect on reducing the difference in gas production due to the nitrogen in one of the media. In other words, low levels of supplementation were doing little to relieve nitrogen deficiency at this

time, and the rate of degradation of supplement protein appeared to be limiting the rate of degradation of the rapidly fermentable carbohydrate. At 52 h the linear relationship between supplement and difference in gas production indicated that degradation of supplement protein had ceased to be rate limiting. It also indicated that groundnut hay was approximately balanced in terms of fermentable carbohydrate and protein. Comparisons of gas production in nitrogen rich and nitrogen free media appeared to be a useful, relatively direct way of investigating the balance between fermentable carbohydrate and protein in feeds and feed mixtures.

Figure 10 Difference in cumulative gas production of veld hay + groundnut hay in nitrogen-rich and nitrogen-free media



The work was reported in part as a poster presentation at a conference in Zimbabwe and a detailed account is contained in: *Wood C D and Manyuchi B (in press) Use of an in vitro gas production method to investigate interactions between veld hay and Napier hay or groundnut hay supplements. Animal Feed Science and Technology. (Technical Annex, part 3)*

2.3.4 Comparisons between the Theodorou gas production method, other *in vitro* methods, and *in vivo* digestibility

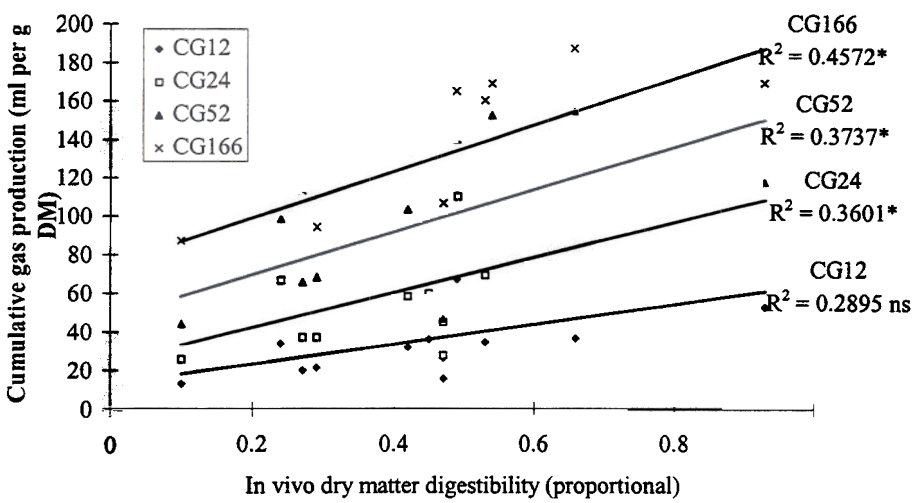
20 leaf samples from West Africa were evaluated by the Menke and Theodorou gas production methods in collaboration with CIRAD-EMVT. Theodorou gas production and some tannin assays were done as part of project A0316, other data and samples were obtained as part of an European Union funded project. End point gas productions by the two methods were in general highly correlated except for two samples (*Faidherbia albida*, green fruit; *Acacia seyal*, young leaves and green stalks), which produced considerably less gas in the Theodorou method than in the Menke method. Excluding these two samples the correlation obtained was:
Menke gas production (ml) = 0.1562*Theodorou gas production (166 h, ml per g DM) - 3.937 (R^2 = 0.74). Interestingly, these two samples also produced less gas than expected from their dry matter disappearances using the Theodorou method. There were no obvious outliers on the Menke gas production - Theodorou dry matter disappearance correlation (R^2 = 0.71 using all data). It was unclear why these samples should behave in this unexpected manner in the Theodorou method, or whether this

was some artifact of the method or an indicator of an important property of these feeds.

Pepsin cellulase digestibility for 18 of the leaf samples from West Africa (including the two outlying samples identified above) was highly correlated with the dry matter disappearance during the Theodorou method ($R^2 = 0.88$), and, after excluding the two outlying samples identified above, was highly correlated with gas production by the Theodorou method ($R^2 = 0.74$).

Correlations between cumulative gas production (CG) at selected times (in hours) and *in vivo* dry matter digestibility are given in Figure 11. Linear relationships achieved statistical significance ($P<0.05$) after 24 h, and longer, incubation. Significant ($P<0.05$) relationships were also found with DMD96 ($R^2 = 0.50$), the France* parameters gas pool (A; $R^2 = 0.53$) and lag time (T+; $R^2 = 0.56$). A report on the work with CIRAD is included in the Technical Annex part 3. (An analysis of the CIRAD data was not available at the time of writing, but from the data supplied the R^2 values for the correlations between *in vivo* dry matter digestibility and the Menke gas production data and pepsin cellulase digestibility were found to be 0.09 and 0.15 respectively, considerably lower than those found for the Theodorou gas production data.)

Figure 11 Relationships between in vivo digestibility and gas production



Thus, while the Theodorou *in vitro* gas production method was generally giving some indications of *in vivo* dry matter digestibility of tree leaves, these indicators did not appear to be particularly accurate or reliable. Use of the France model appeared to increase the accuracy of the predictions using the gas production method, the lag time (whose physiological importance is unclear) surprisingly giving a marginally better correlation than the gas pool (a measure of the end point of fermentation). The improving correlation with increasing incubation time is consistent with the improved correlation with the gas pool. While there is scope for a more sophisticated statistical analysis of these data in collaboration with CIRAD, the findings of the Nepalese tree

* Obtained by fitting the France *et al.* (1993) Journal of Theoretical Biology 163: 99 - 111 mathematical model to cumulative gas production data.

leaf fodder work (described in section 2.3.6 below) called into question whether the approach used in this study was appropriate to the role of tree leaf fodders in all farming systems.

2.3.5 Comparisons between gas production data and neutral cellulase digestibility (NCD): Nepalese tree leaf fodders

As part of a wider study on Nepalese tree fodders (reported in section 2.3.6), data were obtained on tree fodder composition and *in vitro* digestibility. NCD was significantly ($P<0.01$) linearly correlated with gas production at 24, 52 and 70 h of incubation. Even so, the correlation was not particularly strong as illustrated in Figure 12 for NCD against the cumulative gas production after 70h incubation (CG70). A stronger correlation was obtained between the two gravimetric assays, NCD and the dry matter disappearance during the gas production method (70 h incubation, DMD70), as illustrated in Figure 13. These correlations were less strong than those found with the West African samples (section 2.3.4 above), but again a stronger correlation was found between the enzymic method and dry matter disappearance than with gas production. The data show that there are broad similarities but detailed differences between the different *in vitro* indicators of digestibility.

Figure 12 Nepal leaves: NCD vs CG70

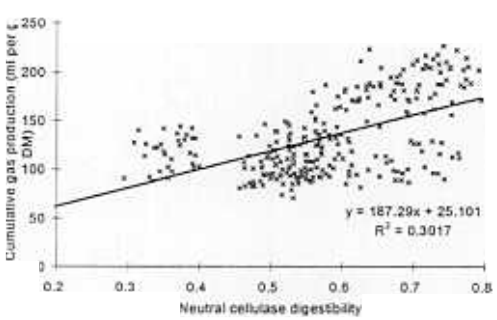
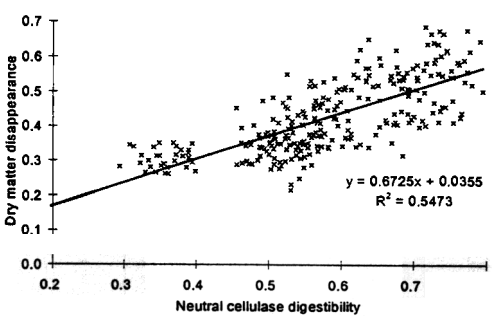


Figure 13 Nepal leaves: NCD vs DMD70



2.3.6 Nepal PAC studies

Contacts were made with the School of Agricultural and Forest Sciences, University of Wales, who have been investigating farmers' knowledge on the nutritive value of tree fodders (and other aspects of the farming system) found in the middle hills of Eastern Nepal for the ODA Forestry Research Programme. It had been found that Nepali farmers used two scales to describe nutritive value. Posilo - kam posilo could be translated as highly nutritious and low nutritive value respectively. Obano - chiso was translated as "dry and warm" and "cold and wet" respectively, apparently referring to the qualities of the dung produced. A range of tree fodder samples were collected and evaluated by chemical assays and *in vitro* techniques in order to:
- examine the consistency of laboratory nutritive value assessments and farmer classifications
- compare farmers' classifications with laboratory indicators
- identify nutritive value assessment methods that individually, or in combination, might be used to supplement farmers' assessments of tree fodder quality.

Large differences in nutritive value of the different fodder types studied were apparent, both from farmers' perceptions and laboratory results. The principle characteristics of the different species is given in Table 1.

Table 1: The principal nutritional characteristics of the five fodder tree species studied.

Species	Principal Characteristics
Ficus nemoralis	Most digestible and low in tannins, but relatively low protein content.
Ficus roxburghii	Intermediate in most respects.
Albizia julibrissin	Very high crude protein and low in tannins but high fibre content and very low digestibility.
Ficus semicordata	Lowest in protein, low tannins and intermediate digestibility.
Prunus cerasoides	Digestible but low in protein and very high tannin content.

The extent of farmers' abilities to discriminate the different fodder types on the basis of nutritive value appeared to be broadly similar to the discriminatory powers of combinations of laboratory nutritive value assessment methods. Seasonal changes in fodder quality were also evident from the assessment made by farmers' and in the laboratory assessments. However, individual instances of farmers predicting differences that were not apparent from the suite of techniques applied in the laboratory, and *vice versa*, were recorded. It is concluded that farmers have an extensive knowledge of tree fodder quality that programmes aimed at the improvement of fodder quality and feeding strategies should seek to build upon rather than replace. On the other hand, laboratory assessments of nutritive value clearly have the potential to describe fodder quality in terms that smallholder farmers can comprehend providing that they are applied in line with farmers' objectives. This work is described in detail in:
Thorne P J, Subba D B, Walker D H, Thapa B, Wood C D and Sinclair F L (under review). Indigenous and laboratory assessment of the nutritive value of tree fodder. Part 1: Discrimination amongst and within species. (Technical Annex, part 4).

A first step towards determining the consistency and complimentary of nutritive value assessments made in laboratories and by farmers was explored in a companion paper:
Walker D H, Thapa B, Thorne P, Sinclair F L, Wood C D and Subba D B (under review) Indigenous and laboratory assessment of the nutritive value of tree fodder. Part 2: Comparison of farmer and laboratory assessment. (Technical Annex, part 4).
The work has also been briefly reviewed:
Thorne P J, Walker D H, Subba D B, Wood C D, Sinclair F L and Thapa B L (in press) Predicting the nutritive value of tree fodder: consistency and complementarity between assessments made by Nepalese, smallholder farmers and by laboratory

techniques. Summary for Animal Science (for presentation at BSAS Winter Meeting 1997). (Technical Annex, part 4).

It was found that the posilo - kam posilo scale appeared to be related to protein supply while the chiso - obano scale was related to digestibility, as illustrated in Table 2.

Table 2 Correlations (r) of farmers' rankings with laboratory indicators of nutritive value

Laboratory parameter	obano ranking	posilo ranking
Neutral cellulase digestibility	-0.84	0.34
Duodenal protein supply index	-0.34	0.80

It came as a major surprise that there did not appear to be a strong positive correlation between perceived nutritive value and *in vitro* digestibility measurements. High *in vitro* digestibility was a property of chiso feeds. Farmers did not, however, regard chiso feeds as necessarily good quality and tended to use them mixed with obano feeds. The reasons for this were unclear but could relate to the importance farmers give to dung properties. Clearly, the ability of the tree fodders to supply protein was of major importance.

While the above inferences need to be confirmed in *in vivo* trials, these findings have considerable implications as they indicate care must be taken in defining the role of feeds in the farming system in order to evaluate them realistically. Simplistic rankings on the basis of dry matter digestibility (*in vitro* or *in vivo*) could be very misleading.

2.4 Intermediate phase conclusions

Interactions (mainly changes in kinetics of fermentation of mixtures compared to the rates predicted from the fermentation of individual feeds) were found between feeds in nitrogen-rich medium which appeared to be due to highly fermentable soluble sugars accelerating the fermentation of more complex carbohydrates. Interactions were found between fermentable protein and fermentable carbohydrate, interactions being maximised when the fermentation of protein and carbohydrate was synchronised. Protein (or nitrogen) appeared to bring forward the degradation of carbohydrate under nitrogen limiting conditions. "Minimum" nitrogen levels of 50 - 100 mg l^{-1} in order to achieve the maximum rate of fermentation were broadly consistent with earlier estimates i.e. the *in vitro* gas production method responded as expected.

Comparisons with other *in vitro* methods indicated that there was generally highly significant correlation between the extents of fermentation/degradation measured by different methods. Even so, the linear relationship between gas production and neutral cellulase digestibility was not always particularly strong for tree leaf samples. This may have been due to anti-nutritive factors affecting enzymes and rumen microbes differently. Comparison with nylon bag data indicated that the Theodorou method generally gives a good *in vitro* model of the rumen and was encouraging. Certain samples behaved anomolously, giving reduced gas productions, for unknown

reasons. Such samples were rare and readily identified, so the implications of this were unclear but possibly not very important. The rate constants obtained by gas production and nylon bag methods were poorly correlated, perhaps not surprisingly as the nylon bag measures the rate of degradation of the insoluble fraction only and gas production measures fermentation of the whole feed.

Quebracho tannin reduced the fermentability of protein and complex carbohydrates, even in small quantities. Glucose fermentation was less inhibited by quebracho tannin. This may indicate that inhibition of fermentation by binding to substrate may be more important than direct inhibition of microbes.

The animal feeding trials using *Gliricidia* did not in general reveal significant differences. This was broadly consistent with the *in vitro* gas production data. The Zimbabwe trial revealed interactions *in vitro*, but not in *in vivo* digestibility. There were indications that interactions could have been expressed as changes in rates of passage rather than digestibility, but rates of passage were not measured in that trial.

The Nepal tree fodder studies indicated that laboratory methods could complement farmers' knowledge and gave some insights as to what farmers' classifications may signify in nutritional terms. There were indications that *in vitro* gas production may be inadequate in indicating the effects of anti-nutritive factors. There was strong (and very unexpected) evidence that dry matter digestibility by itself was not a good indicator of the nutritive value of the leaves within the Nepalese feeding system, but protein supply was very important. This probably related to the role of tree fodders in Nepalese feeding systems and the role of livestock as providers of manure within the farming system.

Taking digestibility as a key indicator of nutritive value was looking increasingly inappropriate. Assessing tree fodders, or other protein supplements, for their ability to interact with protein deficient roughages appeared to be much more appropriate to LDC feeding systems during the problematic periods of feed shortages.

Thus, the findings of the project continued to encourage the belief that the *in vitro* gas production technique was a suitable core method which was representative of the digestive processes in the rumen and responded to interactions between feeds and anti-nutritive factors in ways which appeared to make sense biologically. However, there was a general shortage of data which demonstrated how accurately the gas production method mimicked these processes. Interactions between protein-deficient roughages and feeds with relatively high protein contents would be particularly important. It was decided that priority should be given to comparing gas production data with *in vivo* data to validate the technique for this application. To avoid the complications of anti-nutritive factors and ensure close control over the conditions of the *in vivo* trials, these were conducted in the UK using temperate feeds. Further, simple bioassays were investigated to see if they could be used as potential screening tools for anti-nutritive factors as there was growing evidence that the gas production technique by itself was inadequate. Also further work was conducted on *in vitro* techniques to estimate the extent of protein degradation in tree leaf fodders.

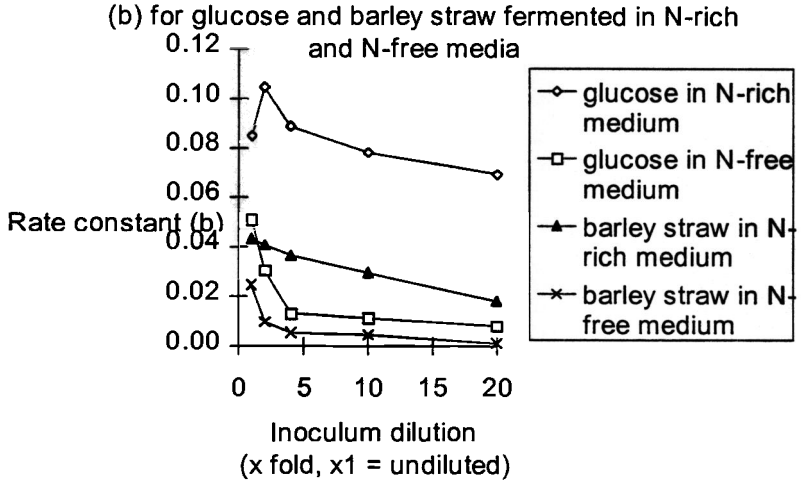
3. Final phase (early 1996 - 31 March 1997)

3.1 ADAS standardised methodology, response to nitrogen

As part of a collaborative ring test involving various UK and overseas organisations which use the Theodorou gas production method, NRI modified the methodology which had been used in the earlier studies and adopted a standardised methodology devised by ADAS. This involved using an inoculum about four times more concentrated than had been used previously. A major effect of this was that, even when a nitrogen-free medium was used, the mixture fermented now proved to be almost nitrogen-sufficient. This stimulated renewed interest in the response of the *in vitro* method to nitrogen and inoculum concentration.

Inoculum was used undiluted and diluted x2, 4, 10 and 20 with nitrogen-free medium and used to ferment glucose and barley straw in both nitrogen-rich and nitrogen-free media. Dilution of the inoculum resulted in a modest reduction of gas production when nitrogen-rich medium was used, although the fermentation curves were broadly similar up to and including the x10 dilution. However, in the nitrogen-free medium gas production was greatly slowed when the x4 dilution, or more dilute, inocula were used. This is illustrated in Figure 14 by the relationships between the rate constant (b) and inoculum dilution. For both barley straw and glucose, dilution results in a gradual, approximately linear, decline in rate constant when the N-rich medium is used, but a rapid drop when the inoculum was diluted x4 in the N-free medium.

Figure 14 Effect of inoculum dilution on rate constant (b) for glucose and barley straw fermented in N-rich and N-free media



A x4 diluted inoculum was considered to be a suitable compromise as at this dilution the fermentation curve was similar to that obtained with the undiluted inoculum, but the incubation mixture was very nitrogen deficient and therefore highly responsive to substrate nitrogen. Such an inoculum provided about 30 mg l^{-1} nitrogen to the incubation mixture. The inoculum was largely equivalent to that used in the earlier experiments using the original Theodorou protocol inspite of some modifications to the protocol for its preparation. The work has been reported in brief by: *Wood C D, Prathalingam N S, Murray A M and Matthewman R W (under review). Use of the gas production technique to investigate the supplementation of nitrogen deficient feeds. British Society of Animal Science Occasional Paper (In vitro techniques for*

measuring nutrient supply to ruminants, University of Reading, 8 - 10 July 1997) - Technical Annex part 3.

3.2 Bioassays for anti-nutritive factors

One possible interpretation of the Nepalese fodder tree work in section 2.3.6 was that anti-nutritive factors were confounding the relationship between digestibility and nutritive value, and that the *in vitro* gas production technique was not adequately accounting for these. As noted by Wood and Plumb (1995; technical annex paper 7) "...the *in vitro* system used here was not susceptible to any toxic effects that tannins may have directly on animals...". It was therefore decided to look at some simple bioassay techniques to see if they could be used as indicators of toxicity, perhaps being susceptible to factors which did not inhibit rumen microbes but which could affect animals directly. This study used samples obtained during the Nepalese fodder tree study described above.

Two techniques were used: TLC/fungal inhibition and brine shrimp mortality. Both techniques indicated the presence of a toxic factor in *Prunus cerasoides*, and in 1 out of 4 samples of *Ficus nemoralis*. The consistency between the techniques was encouraging, particularly as *Prunus cerasoides* was also considered to be of low nutritive value by farmers. This species was, however, quite highly fermentable, so rumen microbes did not appear to be inhibited by this factor. Reports on the two assays have been combined to produce a draft paper:
Wood C D, Panigrahi S, Goodenough L, Rossiter J T and others (in preparation). Use of bioassays to detect anti-nutritive factors in Nepalese fodder trees. (Technical Annex, part 4).

The abstract is given below:
8 tree leaf fodders commonly used in Nepal, which had been previously ranked for nutritive value by farmers, were evaluated for the presence of anti-nutritive factors by *in vitro* gas production, brine shrimp and thin layer chromatography (TLC)/mould inhibition bioassays. The brine shrimp and TLC/mould inhibition techniques were found to be sensitive to a toxic factor in samples of *Prunus cerasoides*. This factor appeared to affect the nutritive value of the leaves as assessed by farmers in Nepal. The *in vitro* gas production technique did not appear to be sensitive to this factor. It was concluded that the brine shrimp and TLC/mould inhibition techniques are potentially useful for screening fodder trees for anti-nutritive or toxic factors, to be used in conjunction with other nutritive value assessment methods.

3.3 Protein degradation

Different types of protein are degraded at different rates and to different extents by rumen microbes. Tree fodders are important as sources of protein, yet there is little information published on the degradation of tree leaf protein. While tannins have been implicated in reducing protein degradation, it was unclear to what extent the intrinsic properties of leaf proteins are important in determining degradation characteristics. Studies were undertaken on the degradation of protein by rumen microbes (using the gas production method as an *in vitro* model) and then using a two stage enzymic treatment to mimic digestion in the lower gut.

Work on the degradation of protein by rumen microbes is described in: *Whetton M, Rossiter J T and Wood C D (under review) Nutritive evaluation of nitrogenous fractions in leaves of Gliricidia sepium and Caliandra calothyrsus, in relation to tannin content and protein degradation by rumen microbes in vitro. (Technical Annex part 5).* This paper describes a study of two fodder tree species, *Gliricidia sepium* and *Caliandra calothyrsus*, which was undertaken in order to investigate the degradation of leaf protein (crude and soluble protein), by rumen microbes during *in vitro* fermentation and the effect of total tannin content on protein degradation. Tree leaf samples were freeze dried, ground and fermented as sixteen replicates in a nitrogen free medium using the Theodorou gas production procedure. At each sampling time (0, 1, 3, 6, 9, 24, 48 and 70 h) the residues from duplicate incubations were recovered by filtration, washed and dried. Soluble protein was extracted and analysed for protein and by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE gels were stained to detect glycoproteins. Differences in dry matter disappearance and protein degradation were observed between the two leaf species. *C. calothyrsus*, after extraction with 70% acetone to remove tanniniferous material, showed greater dry matter disappearance and crude protein losses during fermentation (40% and 20% greater, respectively). The cumulative gas production of *C. calothyrsus* was increased after extraction with 70% acetone. It was concluded that the differences in protein degradation characteristics were mainly due to the inhibitory effects of tannins. The apparent increased resistance of glycoproteins to degradation appeared to be of relatively minor importance to nutritive value of the fodder.

Hence, the intrinsic properties of the protein, which are know to be capable of having a major influence on their degradation, appear to be of secondary importance in tree leaf fodders, if the two species studied are at all typical. The importance of tannins in assessing the nutritive value of tree fodders is again emphasised, whether this is done by analysing for tannins directly or by measuring the degradation of protein by rumen microbes.

In a second piece of work, residues of feeds fermented in nitrogen-free medium were collected, analysed for nitrogen, and subjected to acid-pepsin or acid-pepsin + pancrease treatment using a recently published *in vitro* technique developed for estimating the digestibility of protein from concentrates in the lower gut. This was in order to see if the method may be suitable for providing *in vitro* estimates of the supply of by-pass protein (i.e. protein which resisted degradation in the rumen but was degraded in the lower gut) from tanniniferous feeds. The method was applied to the *in vitro* protein degradation of the Nepalese fodder samples, also used for the anti-nutritive factor bioassay study. This is described in detail in a draft paper: *Wood C D and others (in preparation). In vitro protein degradation in eight fodder tree species from Nepal. (Technical Annex part 5).*

Samples of eight types of Nepalese fodder tree were obtained together with information on their nutritive value as perceived by local farmers. *In vitro* protein degradation by rumen microbes during a gas production procedure and a two-stage enzymic treatment procedure were compared to that predicted by the composition, crude protein (CP) and acid detergent insoluble nitrogen (ADIN), and to the farmers' rankings. Other parameters derived from the gas production procedure which were considered to be possible indicators of protein degradation were also investigated. Estimates of the total degradable protein fraction by enzymic and compositional

methods were significantly (P<0.001) linearly related, but enzymic methods gave consistently lower estimates. Significant linear relationships were also found between both measures of total protein digestibility and farmers' posilo ranking, believed to relate to protein supply to ruminants. The two stage enzymic treatment may not, however, be suitable for tanniniferous feeds as tannins appeared to interfere with the second (pancreatin) digestion stage. Fodder trees are potentially an important source of dietary by-pass protein and a reliable *in vitro* indicator would be a useful addition to existing feed evaluation methods, but as yet there is no such method established for evaluating tanniniferous feeds.

3.4 Use of *in vitro* gas production technique for predicting intake and digestibility of single feeds.

These trials were conducted in collaboration with project A0384 (ZC0005), and the results have been described in a summary paper and a draft full length paper, both included in Technical Annex part 5:
Romney D L, Cadario F C, Owen E and Murray A H (under review). Comparison of parameters from the Theodorou gas production technique using nitrogen-free and nitrogen-rich media as predictors of DM intake and digestibility. British Society of Animal Science Occasional Paper (In vitro techniques for measuring nutrient supply to ruminants, University of Reading, 8 - 10 July 1997).

Cadario F (in preparation). Use of in vitro gas production technique for predicting in vivo apparent digestibility and voluntary intake of feedstuffs for sheep. (Note: some of the statistical analysis in this draft is incorrect, values indicated in Table 3 below were derived from a re-analysis of the data).

Sheep were offered one of 12 feeds, six hays or straws and six other feeds: soaked sugarbeet pulp, chopped alfalfa, alfalfa pellets, maize gluten feed, wheat feed and a chopped alfalfa/oat straw mixture. Dry matter intake (DMI) and digestibility were

Table 3: Values of R^2 for the relationships between *in vitro* parameters and DM intake and digestibility

		Dry Matter Intake		Digestibility	
		Hays and Straws	Other feeds	Hays and Straws	Other feeds
N-rich	CG12*	0.411	0.410	0.806	0.655
	CG24	0.340	0.487	0.852	0.848
	CG52	0.134	0.524	0.766	0.750
	CG70	0.091	0.495	0.721	0.640
N-free	CG12	0.466	0.116	0.869	0.202
	CG24	0.604	0.016	0.895	0.099
	CG52	0.716	0.008	0.855	0.067
	CG70	0.712	0.063	0.853	0.478
	CP	0.740	0.443	0.387	0.535
	ADF	0.755	0.236	0.698	0.065

* CG12 = cumulative gas production after 12 h incubation, CG24 = cumulative gas production after 24 h incubation etc.

determined *in vivo*, and feed samples were fermented in both nitrogen-free and nitrogen-rich media using the Theodorou gas production method. Coefficients of determination (R^2) were calculated for cumulative gas production, CP and ADF contents on *in vivo* parameters. The main correlations investigated are given in Table 3.

The DMI of hays and straws was related to both CP and ADF, CG52 and CG70 in nitrogen-free medium being similarly (but slightly less strongly) related. For hays and straws, relationships between digestibility and gas production from the nitrogen-free medium were particularly strong, and they were also strong with the nitrogen-rich medium gas production data. The relationships were most strong at 24 h incubation in both cases. The potential for predicting DMIs of "other" feeds when fed alone appeared to be relatively poor with both media. Relationships between digestibility and gas production in nitrogen-rich medium, particularly CG24, were strong for the "other" feeds.

Gas productions were greatest with the nitrogen-rich medium with differences between the media decreasing as CP content of the feed increased. The relationship between CP content and the difference between the nitrogen-free and nitrogen-rich media in gas production strengthened with incubation time (R^2 = 0.06, 0.41, 0.75 and 0.82 at CG12, CG24, CG52 and CG70 respectively).

For fibrous feeds such as hays and straws, digestibility might be predicted from gas production parameters with similar accuracy regardless of the medium used. However, where CP explained a relatively high proportion of the variation in *in vivo* parameters, such as intake of hays and straws, a N-free medium may be more suitable. Amongst the 'other' feeds, some were chopped or ground. In this case, small particle sizes, resulting in rapid rates of passage, are likely to have affected intake. Parameters derived from gas production techniques may not predict the DMI of such feeds effectively. Gas production parameters from fermentations conducted with N-rich medium appeared to offer the greatest potential for predicting the digestibility of a wide range of feeds.

3.5 ADAS trial: Use of the gas production technique to investigate responses of supplementing low quality forages (first trial)

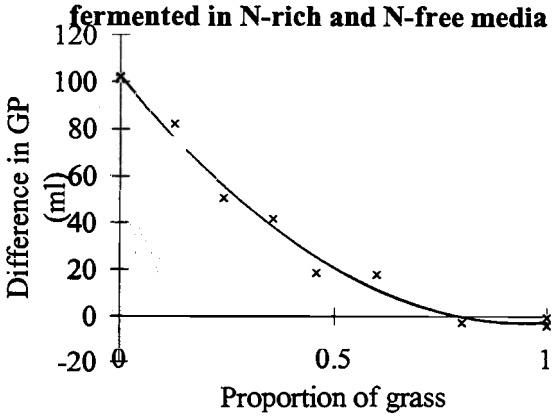
Winter wheat (3.4% crude protein) was supplemented with either high temperature dried grass (grass) or high temperature dried lucerne (lucerne) in *in vivo* feeding trials conducted by ADAS and in parallel *in vitro* gas production trials conducted at NRI. The objective was to assess the ability of the gas production technique to predict *in vivo* responses.

Initial findings of the *in vivo/in vitro* study are given in two summary papers (both in Technical Annex part 5):
Wood C D, Murray A H, Moss A R and Givens D I (under review). Use of the gas production technique to investigate responses of supplementing low quality forages: 1. In vitro interactions. British Society of Animal Science Occasional Paper (In vitro techniques for measuring nutrient supply to ruminants, University of Reading, 8 - 10 July 1997).

Murray A H, Moss A R, Wood C D, Givens D I and Gill M (under review). Use of the gas production technique to investigate responses of supplementing low quality forages: 2. In vivo interactions and comparison with in vitro parameters. British Society of Animal Science Occasional Paper (In vitro techniques for measuring nutrient supply to ruminants, University of Reading, 8 - 10 July 1997).

In vitro data indicated that, using the nitrogen-rich medium, there was a linear relationship between supplementation and gas production. This indicated that there were no interactions, although curvilinear relationships were observed between the proportion of supplement and the rate constant (c) (a measure of the "bend" of the gas production curve) and lag time (a measure of the delay after inoculation until the maximum rate of gas production is reached) of the France *et al.* (1993) model. Using the nitrogen-free medium the relationships were non-linear indicating positive interactions between straw and both supplements. This interaction presumably related to the ability of the supplement to provide nitrogen (protein) to the rumen microbes, facilitating the degradation of the protein deficient straw. By subtracting the gas produced from the feed mixtures in nitrogen-rich and nitrogen-free media, the nitrogen status of the mixtures was investigated: the larger the differences the greater the nitrogen deficiency. The data obtained after 48 h incubation for wheat straw + grass supplement is given in Figure 15.

Figure 15 Difference in gas production (GP) at 48 h incubation for wheat straw + grass mixtures fermented in N-rich and N-free media



Difference in gas production was greatly reduced by increasing supplement up to about 0.5 (50%), but further increases had a declining effect. The data for wheat straw supplemented with lucerne were very similar.

In the *in vivo* trials, a linear increase in dry matter intake was observed for straw supplemented with lucerne, while an accelerating intake was observed with increasing grass supplementation. For *in vivo* digestibility, there were non-linear responses to both supplements indicating interactions of some type. This underlined the inadequacy of existing approaches to feed evaluation which assume that such interactions are not important. The data from the *in vivo* trial is given in Table 4.

Table 4 The effect of supplementing wheat straw with high temperature dried forages on straw intake, and total intake and digestibility

		Level of supplement					
		0	**0.1**	**0.2**	**0.3**	**0.4**	**SED**
HTDG	Straw DMI (gd^{-1})	798	792	799	808	790	
	Total DMI (gd^{-1})	798	907	1054	1256	1457	51.6***
	DM Digestibility	0.44	0.49	0.51	0.53	0.53	0.017***
HTDL	Straw DMI (gd^{-1})	701	742	721	699	701	
	Total DMI (gd^{-1})	701	846	948	1086	1235	48.5***
	DM Digestibility	0.46	0.49	0.49	0.49	0.49	0.009***

HTDG = high temperature dried grass (grass)
HTDL = high temperature dried lucerne (lucerne)
DMI = dry matter intake (intake)

Very strong correlations were found between total intake and the rate constant (b) (related to the maximum rate of gas production) for both supplements. These are illustrated below in Figures 16 and 17.

Figure 16 Wheat straw + grass:rate constant (b) versus total intake

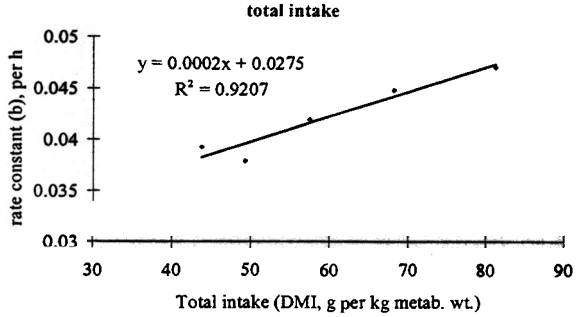
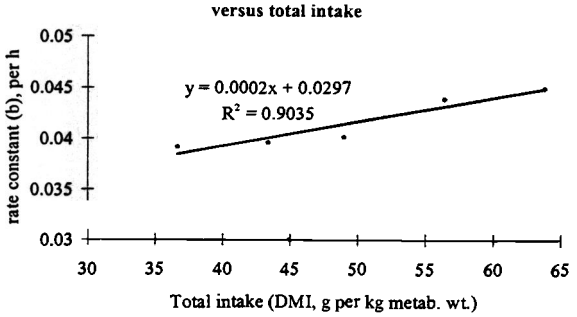


Figure 17 Wheat straw + lucerne: rate constant (b) versus total intake



Perhaps equally noteworthy was the very poor correlation between *in vivo* dry matter digestibility and the gas pool (A; an indicator of the extent of degradation), parameters which might have been expected to be highly correlated.

The *in vivo* responses described here were different to those found for the perhaps comparable feeding trials in Zimbabwe described in section 2.3.3 above, where there were no apparent interactions in *in vivo* digestibility. It was therefore decided to collaborate further with project A0384 to obtain a second, more detailed, data set on interactions between supplements and low quality forages *in vivo* and *in vitro*.

3.6 Wye trials: Use of the gas production technique to investigate responses of supplementing low quality forages (second trial)

The primary objective of this study was to establish if there were interactive effects of some type *in vivo* which corresponded to interactions observed using the *in vitro* gas production technique. Assuming a positive outcome, a second objective was to investigate correlations between *in vitro* and *in vivo* data and provide data for future modeling work if required. This study built on to an *in vivo* trial which was being conducted as part of project A0384 with similar objectives (but excluding the *in vitro* study) in order to extend the scope of the *in vivo* treatments and the data collected, for the mutual benefit of both projects.

The study investigated interactions observed *in vitro* and *in vivo* between two roughages and two high quality forages. Twenty four wether lambs were used to determine intake, digestibility and rate of passage of barley straw (straw) or meadow hay (hay) supplemented with alfa A (AA, an alfalfa/lucerne based commercial feed) or wheat feed (WF) at two levels of supplementation (low and high). Each feed was also fed individually. Samples of feed from the feeding trial were evaluated as individual feeds and as mixtures by *in vitro* gas production in nitrogen-rich and nitrogen-free media.

The proportion of supplement consumed by the lambs is given below:

	AA low	AA high	WF low	WF high
Barley straw	0.32	0.52	0.31	0.47
Meadow hay	0.22	0.37	0.20	0.38

Supplementation of straw with AA or WF increased total daily dry matter intake at both levels. Intake of straw was increased by 10% for AA and 15% for WF by the lower level of supplementation and by 21% for the higher level of WF. However, the higher level of AA led to a decrease of 12% in straw intake. Hay intake was decreased modestly (4 and 6%) by the lower and higher level of AA supplementation, respectively. WF supplementation led to a 2% increase in hay intake at the lower level and an 11% decrease at the higher level.

The digestibility of roughage based diets was increased by supplementation. For all four roughage plus supplement combinations, the relationship between *in vivo* digestibility and level of supplementation was described better using a polynomial than a linear expression. This was consistent with the hypothesis that interactions occurred between the roughage and supplement which affect *in vivo* digestibility; without interactions the relationship would be described best by a linear expression. Table 5 shows the effect of supplementation on dry matter digestibility, together with the effects of interactions between the feeds on digestibility.

Table 5 The effect of supplementation on *in vivo* dry matter digestibility and percentage differences between predicted (assuming no interactions) and observed dry matter digestibility

Straw level of supplement	**AA *in vivo* digestibility**	**% difference (interaction)**	**WF *in vivo* digestibility**	**% difference (interaction)**
no supplement	0.46	0	0.46	0
low level	0.53	4.8	0.56	8.7
high level	0.55	1.0	0.58	5.4
all supplement	0.61	0	0.65	0
Hay				
no supplement	0.58	0	0.58	0
low level	0.58	-0.9	0.58	-1.2
high level	0.59	-1.0	0.61	-1.6
all supplement	0.61	0	0.65	0

$$\%\text{Difference} = \frac{(\text{observed DMD} - \text{predicted DMD}) \times 100}{\text{predicted DMD}}$$

Positive interactions, increasing digestibility by up to 8.7%, were observed for supplemented straw. Interactions between WF and straw were greater than between AA and straw, and were proportionately greater for the lower level of supplementation. Interactions between hay and supplement were relatively low and consistently negative (largest negative interaction = -1.6%).

In vitro gas production in nitrogen rich medium showed that WF was the most rapidly fermentable feed, with AA and hay having similar fermentation rates initially, and straw being slowly fermented. Straw, and to a much lesser extent hay, were more slowly fermented in nitrogen free medium indicating that their fermentation was nitrogen limited. Both supplements had similar gas production characteristics in either medium, indicating that they were at least sufficient in fermentable nitrogen. Comparisons of the fermentation of feed mixtures in nitrogen rich and nitrogen free media indicated that fermentable protein and carbohydrate reached approximate balance at the following proportions of supplement: straw + AA, 0.5; straw + WF, 0.3 to 0.4; hay + AA, 0.1; hay + WF, 0.3.

Positive interactions in gas production were observed between straw and both supplements in both media, but were greatest between straw and WF in nitrogen free medium (up to 51% at 96 h incubation), illustrated in Figure 18. As with *in vivo* digestibilities, interactions between straw and WF were generally greater than those observed between other feed combinations. Although there was some evidence of interactions between hay and AA, particularly in nitrogen free medium, these were very modest (below 5%) as can be seen from the almost linear relationships in Figure 19. Some interactions were observed between hay and WF in both media, these interactions in nitrogen free medium being broadly larger than those found between straw and AA, while in nitrogen free medium interactions were lower than those found between straw and AA.

Figure 18 Barley straw fermented with wheat feed supplement in nitrogen free medium

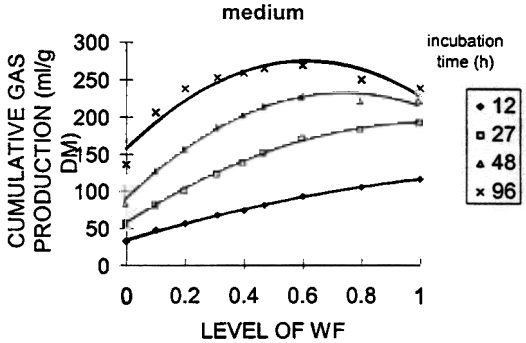
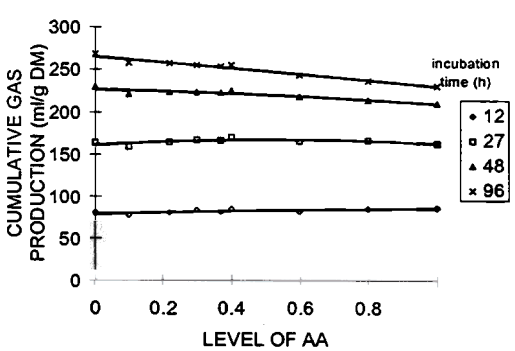


Figure 19 Hay fermented with Alpha A supplement in nitrogen rich medium



The percentage differences (interactions) between barley straw and supplements *in vitro* in the two media at selected incubation times are given in Table 6, and the corresponding data for supplemented hay in Table 7.

Table 6 Percentage difference (interaction) between observed and predicted values for barley straw supplemented with alpha A (AA) or wheat feed (WF) fermented in nitrogen rich and nitrogen free media

	Nitrogen rich medium		Nitrogen free medium	
AA supplement	**low**	**high**	**low**	**high**
Incubation time (h)				
12	4.9	6.0	11.8	10.6
27	4.4	6.3	12.0	12.0
48	2.0	3.8	25.1	22.8
96	-0.1	1.8	37.2	25.4
DMD96	-0.4	-0.3	38.3	26.3
WF supplement	**low**	**high**	**low**	**high**
12	3.8	7.5	15.5	13.4
27	9.1	11.8	24.6	25.6
48	7.7	11.1	47.5	44.5
96	6.4	10.6	50.7	43.7
DMD96	2.9	5.9	46.0	39.1

Table 7 Percentage difference (interaction) between observed and predicted values for meadow hay supplemented with alpha A (AA) or wheat feed (WF) fermented in nitrogen rich and nitrogen free media

	Nitrogen rich medium		Nitrogen free medium	
AA supplement	**low**	**high**	**low**	**high**
Incubation time (h)				
12	-1.4	-1.0	0.0	0.9
27	0.7	1.9	1.9	4.5
48	-0.6	0.3	3.3	4.4
96	-0.9	-0.4	1.6	1.8
DMD96	0.3	2.3	2.5	2.2
WF supplement	**low**	**high**	**low**	**high**
12	8.3	8.9	4.8	9.3
27	6.6	6.6	9.6	16.1
48	5.5	7.0	10.6	16.0
96	5.1	7.6	6.9	11.3
DMD96	3.0	5.1	2.5	4.0

Interactions between straw and AA appeared to be due to the supplement providing fermentable nitrogen, which was deficient in straw. This would account for increases in intake and digestibility at the lower level of supplementation. At the higher level of

supplementation fermentable nitrogen was no longer limiting. There were only modest interactions observed in *in vivo* digestibility, and *in vitro* in nitrogen rich medium. Straw intake may have been inhibited by gut fill due to the consumption of AA. Similar interactions were observed between straw and WF for similar reasons. However, WF was a good source of rapidly fermentable carbohydrate which led to interactions additional to those resulting from the fermentable nitrogen supply. These are reflected in both *in vivo* digestibilities and *in vitro* data. Straw intake was increased by both levels of WF supplementation, presumably a reflection of the accelerated digestibility of the straw and the relatively low gut fill effect of WF which was more rapidly fermented than AA.

Modest interactions were observed between hay and WF *in vitro*, apparently due to the fermentable nitrogen and rapidly fermentable carbohydrate in WF. Interactions were relatively small because hay was almost sufficient in fermentable nitrogen and the carbohydrate was more rapidly fermentable than that in the straw, hence the scope for a supplement to provide deficient nutrients was limited. *In vivo*, WF at the higher level reduced hay intake, possibly due to chemostatic mechanisms as gut fill may have been of secondary importance, and interactions in *in vivo* digestibility were small and negative. Even the lower level of WF supplementation was sufficient to remove the slight fermentable nitrogen deficiency of the hay, and the interactions observed *in vitro* between the rapidly fermentable carbohydrate of WF and the hay appeared to be too small to have a positive effect *in vivo*. No interactions were observed between hay and AA in *in vivo* digestibility and *in vitro* interactions were very small. In general there was a good agreement between the occurrence and relative scale of interactions of feeds measured *in vivo* and *in vitro*, although the *in vitro* gas production method appeared to be more sensitive to interactions between feeds than was found *in vivo*. This may have been a reflection of the reduced microbial density used *in vitro* and the ability of animals to supply some nitrogen to the rumen via urea in the saliva.

As with the first trial reported above, strong linear relationships were found between dry matter intake and the rate constant (b) for all feeds and feed combinations, when fermented in the nitrogen rich medium, with the notable exception of wheat feed fermented by itself. This relationship is shown in Figure 20. When wheat feed was fermented alone the intake was very much lower than indicated by the rate constant b, possibly indicating chemostatic inhibition of intake rather than gut fill which presumably limits the intake of the roughage based diets.

The simple correlations above may have their uses in developing *in vitro* gas production as a predictor of total dry matter intake of supplemented roughages under certain conditions, probably when gut fill is the major factor determining intake. Correlations between *in vivo* digestibility and gas pool size were not significant (R^2 = 0.11, $P>0.05$), and are illustrated in Figure 21. Perhaps surprisingly, a strong correlation was found between lag time (T+) and digestibility (R^2 = 0.73, $P<0.001$) as shown in Figure 22. The strength of the overall correlation was in part due to a remarkably strong correlation (R^2 = 0.99) between *in vitro* lag time and *in vivo* digestibility for the straw and supplemented straw diets. With the hay and supplemented hay diets included (i.e. all the diets except the two supplements fed on their own), the linear correlation remained extremely strong (R^2 = 0.97, $P<0.001$). However, the physiological significance of *in vitro* lag time is unclear. It is suggested that both lag time and digestibility of roughages are determined largely by the same

properties of the fibre, whence the strength of this correlation for these feeds (and conversely why the supplements deviated from the correlation found for roughages). Nevertheless, there is considerable encouragement for the view that both the intake and digestibility of roughages and, perhaps more importantly, supplemented roughage diets may be predictable using the *in vitro* gas production method.

Figure 20 Total dry matter intake versus rate constant (b) in nitrogen rich medium

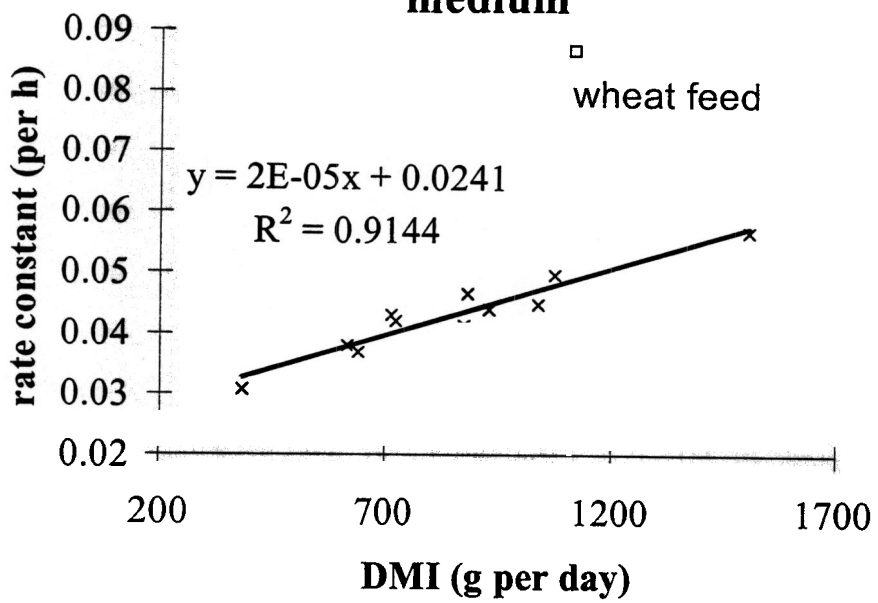


Figure 21 In vivo digestibility versus gas pool size

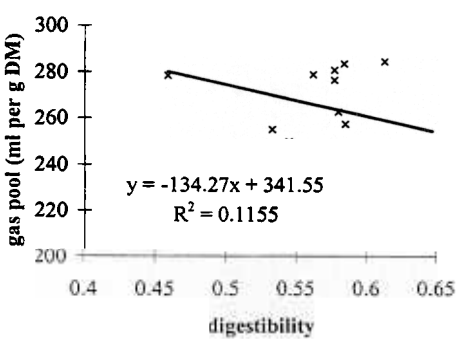
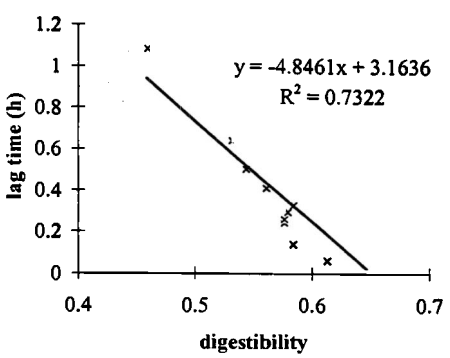


Figure 22 In vivo digestibility versus lag time (T)



These trials are described in greater detail in a draft scientific paper: *The use of the in vitro gas production technique to identify in vivo digestive interactions of sheep fed low quality roughage diets supplemented with high quality forages, by A. H. Murray, D.L. Romney and C.D. Wood (Technical Annex, part 6).*

Data on rates of passage are currently being obtained by project A0384 (which ends in March 1998), which will facilitate the development of a model for interpreting the interactions observed. A more mechanistic approach needs to be developed to integrate data from *in vitro* and *in vivo* trials to develop further the use of *in vitro* gas

production to predict *in vivo* responses. There is also scope for integrating and analysing further some of the earlier *in vivo/in vitro* data to improve any relationships aimed at calibrating *in vitro* data to *in vivo* parameters. Nevertheless, the data obtained to date are generally encouraging and indicate that the larger *in vitro* responses do represent processes which occur *in vivo*.

3.7 India

Samples of a range of fodders used for goat feeding in N W India were obtained, analysed for crude protein and acid detergent fibre, and evaluated using the *in vitro* gas production technique. Samples were evaluated to see if they were nitrogen sufficient and then to see if they could supplement a nitrogen deficient tree fodder. *Acacia* pods (*A. nilotica* and *A. leucophloea*) appeared particularly useful as supplements. There appeared to be much which potentially could be gained by mixing appropriate feeds to produce more balanced diets. This work is described in: *Wood C D and Matthewman R W (1996) Feed samples from NW India: Report on initial evaluation. (Technical Annex, part 5).*

3.8 Costa Rica

Samples of four leguminous shrubs of interest as supplements to roughage (sugar cane waste) were evaluated in collaboration with an ODA animal health project. The following results were obtained and conclusions were reached on the basis of the gas production characteristics of selected feed mixtures fermented in N-rich and N-free media.

Sugar cane tops were rapidly and extensively fermentable, and N deficient. Supplementation with fermentable protein was required to balance the fermentable carbohydrate supplies. On the basis of their ability to provide fermentable protein the supplements were ranked:
Cratylia > Leucaena > Gaucimo > Ramio.
Levels of supplementation to achieve balance between fermentable protein and carbohydrate (i.e. where the response curve starts to level off) were about 0.4 for Cratylia and Leucaena, 0.6 for Guacimo and Ramio, although these may be over-estimates (i.e. lower supplementation levels may be optimal in practice) as no allowance was made for the ability of ruminants to recycle urea to the rumen. As sugar cane was rich in highly fermentable carbohydrate there did not appear to be any major advantage in providing a supplement with this property. In terms of gas production the supplements were ranked:
Ramio>Guacimo>Leucaena>Cratylia.
This was (apparently coincidentally) the reverse of the ranking based on their ability to provide protein. The work is reported in more detail in:
Wood C D and Murray A H (1997) Characterisation of Costa Rican feed samples using the gas production method. Unpublished NRI project report (Technical Annex, part 5).

This study illustrates the versatility of the gas production method in providing information on feed mixtures. By looking at whole diets within the context of the feeding systems, interactions between feeds are taken into account. The contribution of different supplements can then be evaluated in the context in which they are to be

used. In this case different rankings would be obtained depending on the criterion used for that ranking, so it is important to identify the role of the feed being evaluated within the diet (as well as more generally within the farming system).

3.9 Final phase conclusions

The ADAS standardised methodology was inappropriate for studying the supplementation of nitrogen deficient feeds, but could be modified easily for this purpose. This modified procedure, with a diluted inoculum, was adopted for subsequent work. Simple bioassay techniques appear to be potentially useful in evaluating tree fodders which contain anti-nutritive factors. Tanniniferous tree fodders are potentially an important source of by-pass protein. *In vitro* indicators of by-pass protein supply would be a useful addition to feed evaluation techniques, but need further development.

A range of interactions between feeds have been observed in *in vivo* feeding trials and parallel *in vitro* studies. Some strong correlations between the rate constant (b) and intake, and lag time (T+) and digestibility, as well as consistencies in the ranking of the magnitude of interactions between wheat feed or alpha A and barley straw or meadow hay have been found. The *in vitro* protocol using a nitrogen free medium appears to be overly responsive to interactions between feeds and may benefit from the inclusion of low levels of urea to mimic more accurately conditions in the rumen. Nevertheless, these findings encourage the view that estimates of intake and digestibility can be obtained for roughages and supplemented roughages. However, feed interactions remain incompletely understood. A more mechanistic approach to interpreting *in vitro* data is required, which will need further understanding of both the *in vivo* and *in vitro* processes, and the relationships between them. This project, together with related projects, have now obtained a body of data which could be usefully integrated and used in the development of a suitable mathematical model of the digestive processes. Such a model should be able to provide the basis on which predictions of *in vivo* responses can be made from *in vitro* data.

Feeds from N W India were assessed for nitrogen sufficiency, and then their ability to supplement a nitrogen deficient feed. Similarly, four fodder tree leaves from Costa Rica were ranked according to their ability to supplement sugar cane waste. These studies illustrated how the gas production method, in its current state of development, can be applied to problem solving in LDC ruminant feeding systems. The most recent comparisons with *in vivo* trials indicate that predictions of intake and digestibility can probably be included in the future by use of appropriate correlation equations. Such predictions are likely to be limited to supplemented roughage based diets and may not be quantitatively reliable under many circumstances, given the range of animal species, management practices, environments etc. used in LDCs, but nevertheless should provide relative predictions suitable for investigating different feeding options for a very wide range of LDC feeding systems.

OUTPUTS

The Project Memorandum stated that the outputs of the project would be:

a) Methodologies for estimating feed factors which affect the nutritive value of tree fodder, crop residues, supplements and mixtures of these.

b) A suite of combined protocols for estimating the nutritive value of tree fodder, crop residues, supplements and mixtures of these.

c) A new approach to the assessment of nutritive value of tropical feeds tested to demonstrate that it can predict animal performance more closely than conventional techniques.

d) Recommendations on possible new feeding strategies.

The project started as an annually reviewed rolling project with an undefined total duration, which is reflected in its ambitious output targets. At the outset, the project was seen as providing improved (e.g. gas production instead of existing *in vitro* assays) and additional methods (e.g. assays for anti-nutritive factors) within a conventional approach to feed evaluation. This is reflected in phrasing of the output targets. However, as the project progressed there were increasing doubts about the suitability of the conventional *in vitro* approaches in many LDC situations, particularly regarding nitrogen deficient diets. There was, therefore, a shift in approach during the course of the project towards evaluating feeds as parts of diets rather than in isolation, an approach which appeared more suitable for many common LDC situations. With other research groups concentrating on the development of the gas production and other techniques for evaluating temperate feeds such as high quality pasture, silages and concentrates, it was decided to put the emphasis of this project onto the supplementation of nitrogen-deficient roughages which were specifically important in LDCs. Potential supplements included tree fodders, which also received particular attention. It should also be noted that other LPP projects, such the one on intake, are highly relevant to this work and their findings, when available, should be incorporated into the approach recommended below.

The major outputs of the project were:

1. A new approach to assessing nutritive value has been developed, centred on the gas production method, which evaluates diets rather than individual feed components. The gas production method was modified to use a nitrogen limited environment, seeking to mimic conditions which often prevail in LDCs, as well as nitrogen rich environments used conventionally. This new approach provides information on the balance of fermentable carbohydrate and protein in diets directly, without relying on complex models which do not take into account interactions between feeds and may not be appropriate to LDC conditions.

2. A range of interactions between feeds have been observed both *in vivo* and *in vitro* (using the gas production method), although these remain only partially understood. The gas production method appears to be suitable for investigating such interactions.

3. Strong correlations have been obtained between *in vivo* digestibility and intake of supplemented roughages and kinetic parameters derived from the gas production method, indicating that it will be possible to predict of both digestibility and intake for diets of this type. Conventional end point *in vitro* digestibility assays do not look suitable for obtaining such predictions for poor quality roughage based diets.

4. The need to evaluate feeds within the context of the feeding system was illustrated. Laboratory methods have been shown to complement farmers' knowledge on the nutritive value of feeds and are best used in parallel with such knowledge.

5. The role of tannins in reducing the availability of protein and carbohydrate to rumen microbes was illustrated. The Prussian Blue assay for total phenols and protein precipitation gave a good indication of the inhibitory effects of tannin extracts on rumen microbes. Their use is recommended for identifying tannins (e.g in tree fodders), although it is recommended that priority is given to evaluating the positive contribution of tanniniferous feeds to the diet rather than investigating tannin composition.

6. The gas production method was not inhibited by all types of antinutritive factors which appeared to be important in tree fodders. The mould growth/TLC and brine shrimp bioassays appeared to be more generally sensitive to other, non-tannin antinutritive factors. Their use is recommended for screening feeds suspected of containing antinutritive factors (e.g in tree fodders).

7. Using the new approach to investigating fermentable carbohydrate/protein balances, suggestions have been made on improved feeding strategies in N W India and Costa Rica.

These outputs are discussed below in relation to the stated outputs of the project.

Output (a) + (b)

The primary objective of the recommended feed evaluation strategy is to identify diets balanced in rumen fermentable protein and carbohydrate. While this is also an objective of the recently published UK Metabolisable Protein system, this project aimed to do this directly by laboratory methods rather than indirectly using laboratory assays together with a complex feed evaluation system, which may not be fully appropriate to LDC conditions. The new approach is intended for use primarily for assisting dry season LDC feeding situations where interventions will be aimed at alleviating dietary imbalances and deficiencies for animals on relatively low planes of nutrition, but it may be more generally applicable.

Preliminary evaluation of feeds may be made using conventional methods, in order to classify types of feeds. Feeds can be divided into two basic types, roughages (e.g. poor quality pasture, crop residues) and supplements (e.g. tree fodders, good quality fodders, concentrates). Roughages are usually deficient in fermentable protein and have high fibre contents. Supplements will generally be required to provide fermentable protein and possibly readily fermentable carbohydrate. It will usually be possible to classify feeds into these two types from existing knowledge and using conventional proximate and fibre analyses, particularly for feeds which are unfamiliar.

For tree fodders in particular (and feeds suspected of containing antinutritive factors in general), analysis for extractable total phenols and protein precipitation activity, together with screening by TLC/mould and brine shrimp bioassays, will give indications of the presence and possible extent of the effects of anti-nutritive factors.

Additional necessary assessments of individual feeds will include aspects of acceptability to animals. Palatability and/or intake trials of some type may also be desirable for unfamiliar feeds. Work under project A0384 is investigating options for short-term assessments of intakes of feeds. Additionally, for tanniniferous feeds such as tree fodders, some laboratory indicator of its ability to supply by-pass protein would be useful, although this requires further investigation and this project is unable to recommend a method.

Feeds and feed mixtures of particular interest can then be fermented in nitrogen rich and nitrogen free (or nitrogen reduced) media. Levels of supplement required to achieve a balance between fermentable carbohydrate and protein can then be estimated, feeds ranked according to fermentability and/or ability to provide fermentable protein as appropriate. Estimates of the intake and digestibility of the diets may also be obtained when suitable equations have been developed. Various interventions, as considered appropriate, can be investigated relatively rapidly. These may be further investigated by on-station animal feeding trials, farmer participatory on-farm trials, further laboratory trials etc., as appropriate. Judgments based on knowledge of the farming system as a whole will need to be included before recommending particular interventions to help make more balanced diets available.

The project has thus helped to define revised approaches to feed evaluation and to identify useful combinations of analytical and *in vitro* methods for evaluating roughage based diets and the types of supplements used in LDCs.

Output (c)

Conventional feed evaluation evaluates feeds separately and assumes that interactions between them are not of any major importance. However, it is well established that interactions between feeds can be important. For the supplementation of protein deficient roughages the way the roughage and supplement interact is likey to be crucial in improving LDC diets. The conventional approach is, therefore, not valid in this context; a fact which is illustrated by the interactions in feed digestibility observed in some of the *in vivo* trials reported in this project.

With nitrogen-deficient diets, conventional *in vitro* predictors of animal performance, based on end point measurement of roughage digestibility in a nitrogen rich environment, are probably of limited use. Indeed, *in vivo* measurements which evaluate roughages with high levels of supplementation may similarly be of dubious value in assessing the nutritive value of the feed under on-farm conditions. Correlations between the end point of gas production and intake and *in vivo* digestibility have been generally poor in the supplementation studies reported in this project, illustrating the limited value of such measurements. In contrast, for supplemented roughages very strong correlations have been found between both intake and digestibility and certain kinetic parameters (rate constants and lag times) derived from the gas production method. Kinetic parameters cannot be readily

obtained using conventional *in vitro* methods. These correlations could possibly be strengthened further by slight modifications to the gas production protocol, improvements to the mathematical model fitted to the gas production data and the use of a more mechanistic approach to interpret the data.

The results of the project illustrate that the revised approach to evaluating the nutritive value of feeds, within the context of the relevant feeding systems, allow both a better assessment of values and improved understanding of the dynamics of feed utilisation in the feeding system.

Output (d)

Early attempts were made to see if the new approach was generating data which was potentially useful to LDC farmers. This work has started with samples from N W India and Costa Rica, where some recommendations on preferred feed mixtures have been made, but is certainly an area which needs further work in years to come. The whole area of how nutritive value assessment (however done) can be designed and interpreted to reflect farmers' needs and priorities is poorly researched at present.

With hindsight, due to the technical complexity of the subject, it was unrealistic to expect methods to be fully developed, tested and applied to practical situations to a fully satisfactory degree of completeness within five years. Nevertheless, considerable progress has been made towards achieving the projected outputs. The techniques recommended are aimed at generating information highly relevant to LDC feeding systems, they are relatively easy to perform and hence can potentially be done at better equipped laboratories overseas, but the gas production data in particular are not particularly easy to interpret in full at present.

CONTRIBUTION OF OUTPUTS

The RNRRS identified nutrition as an important constraint to livestock development. Livestock production is seen to be increasingly more integrated in mixed farming systems which use crop residues, cut and carry systems, targeted fodder crops and supplements/concentrates rather than open grazing systems. This will mean that farmers, rather than livestock, will increasingly select the feeds offered to the animals. The approach developed above is potentially applicable in all LDC production systems. It will be used to generate information on feeds and feed mixtures to help farmers select feeds, whence to reduce nutritional constraints to production.

The potential areas of impact of the outputs of this project are:
a) In the analysis of feeding systems to identify feed quality constraints and potential ways of overcoming them by supplementation, treatment etc.
b) Evaluation of particular feeds, such as novel supplements, agricultural wastes etc.
c) Selection of fodder or crop varieties with improved nutritive values.
d) Design of rations with balanced fermentable carbohydrate and protein supply.
e) Prediction of animal performance.
f) Assist in the design and interpretation of feeding trials.

The major dissemination pathway for the outputs of this project has been the scientific literature. Details of the seven full-length papers and seven abstract/short papers

published (or in press) are given in the Project Completion Summary Sheet (attached as Appendix 1). Internal reports (eight), student reports/theses (seven) and other dissemination outputs are similarly listed. There have also been a number of visitors who have seen and discussed the methods used; several visiting scientists have had hands-on experience of using the techniques whilst undertaking some of the research described above. The dissemination of this project was recently reviewed by J Morton for the Livestock Production Programme.

The methods need to be applied to practical situations to generate information for farmers, to evaluate their practical usefulness and possibly further develop them. In the author's view, experience should be gained on the practical application of the methods to develop case studies before firmly recommending them for widespread adoption. The general approach should be to use the method in support of projects aimed at developing improved feeds and/or feeding strategies. The project (recently approved) in India with BAIF is one such application, others should include a continuation of the collaboration with Pakhribas Agricultural Centre, Nepal, to build on earlier work; and inclusion of *in vitro* feed evaluation in other projects within the LPP programme. A proposal for further collaboration with Pakhribas Agricultural Centre, Nepal, is at an initial draft stage and the LPP Manager has been contacted to be made aware of these research possibilities.

Some 18 draft papers and summary papers (some are already submitted and under review) have been prepared which need to be finalised, as appropriate, and taken through the process of publication in scientific journals. These have been listed in Appendix 2; copies of recent versions are included in the Technical Annex. There are also data which would benefit from a more detailed consideration and analysis, with the possibility of additional papers being produced. A particularly difficult area is in making comparisons between *in vivo* and *in vitro* data for feed mixtures. Correlations have been investigated in this project (and more widely in feed evaluation), but a more mechanistic approach, probably involving some modeling, is required (and was recommended by the recent external review of the project).

Feed evaluation, and the gas production technique in particular, has been the focus of research by several other groups in the UK and elsewhere. The project has contributed to this process through the scientific literature, and through a ring test and informal symposia organised by ADAS. Recent advances in *in vitro* techniques will be reviewed and discussed at a symposium organised by the British Society of Animal Science to be held in July 1997. Several summary papers on the work of this project have been submitted for presentation at this symposium, and several NRI members of staff will be attending. There will also clearly be lessons to be learned from it. It would be most constructive to revise and update the literature review prepared as part of this project in the light of more recent developments (not least those of this project), to inform scientists in LDCs of these. There is clearly a need for more strategic research on feed evaluation; such a review will also help define better future research requirements. It would also be a useful precursor to the wider dissemination of the gas production technique.

One of the potential advantages of the gas production technique is that it reduces the use of rumen fistulated animals, and potentially could avoid their use completely by using faeces-based inocula. UK research groups are working on the development of

such alternative inocula; if these prove successful early adoption, perhaps after suitable adaptation, is highly recommended.

Laboratory methods aimed at indicating the supply of by-pass protein from tanniniferous feeds appear unconvincing and require further development.

Appendix 1

PROJECT COMPLETION SUMMARY SHEET

DATE sheet completed **20 March 1997**
TITLE OF PROJECT: Assessment of nutritive value of tropical feeds and forages and identification of anti-nutritive factors
R NUMBER: R5180
RNRRS PROGRAMME: Livestock Production Programme
PROGRAMME MANAGER (INSTITUTION): Natural Resources International
SUB-CONTRACTOR: Natural Resources Institute
RNRRS PROGRAMME PURPOSE: Techniques to improve the contribution to animal production of crop residues, tree fodders and fodder crops developed and promoted.
RNRRS PRODUCTION SYSTEM: Forest-Agriculture Interface
COMMODITY BASE: Livestock
BENEFICIARIES: Scientists concerned with ruminant feeds, extension workers, livestock owners.
TARGET INSTITUTIONS: institutions involved in feed evaluation, advising farmers/extension workers on feeds.
GEOGRAPHIC FOCUS: wide ranging.

	Planned	**Actual**
START DATE	1 April 1992	1 April 1992
FINISH DATE	31 March 1997	31 March 1997
TOTAL COST	£1,150,268	£1,150,268

1. Project Purpose:

The specific purpose of this project was to provide laboratory tools which can be used to generate information on nutritive value as part of the process of developing appropriate advice to farmers.

2. Outputs:

The project used *in vitro* gas production as a core technique to evaluate feeds and feed mixtures. It has demonstrated that: the technique is inhibited by tannins; is very sensitive to differences between feeds; is sensitive to interactions between feeds in mixtures and responds to nitrogen supplementation in a manner similar to earlier *in vitro* and *in vivo* studies. Strong relationships were found between the extent of degradability of pasture samples during *in vitro* gas production and in the rumen (as measured by the nylon bag technique). Relationships between gas production and other *in vitro* methods and *in vivo* digestibility were, however, not particularly strong for fodder tree leaves.

No strong positive correlations were found between Nepalese farmers' rankings of fodder tree leaf nutritive value and *in vitro* digestibility. Farmers' rankings appeared to be related to protein supply and dung quality (which was inversely related to *in vitro* digestibility). The *in vitro* gas production method was modified to provide information on the nitrogen status of feed mixtures. The approach has been applied to

feeds used in three *in vivo* supplementation experiments and one single feed experiment to help validate the technique, and the initial screening of fodder mixtures from N W India and Costa Rica to illustrate its use. *In vivo* interactions were found in both in intake and digestibility, however the same responses were not found for different feed combinations. Qualitatively, the interactions observed *in vitro* did generally appear to correspond to interactions observed *in vivo*. However, a more mechanistic approach to interpreting *in vitro* data is required, which will need further understanding of both the *in vivo* and *in vitro* processes. The gas production method did not appear, in practice, to give reliable indications of the effects of the major relevant anti-nutritive factors. Two simple bioassays were found to be useful for screening fodder trees for such factors.

3. Contribution of Outputs to Project Goal:

The gas production technique can be applied to investigating the balance between fermentable protein and carbohydrate in feed mixtures, as well as the extent and rate of carbohydrate degradation by rumen microbes. The supplemention of protein deficient roughages with fodder trees and other supplements can be investigated. Improved supplementation is an important potential intervention in many LDC feeding systems. Simple bioassays and tannin assays for tree fodders will complement the gas production technique and conventional laboratory analyses, providing measures of anti-nutritive factors. These techniques are now available for use to generate information on feeds and feed mixtures to help farmers select feeds, whence reduce nutritional constraints to production.

Interactions between feeds and the actions of anti-nutritive factors are very complex and incompletely understood. There are no laboratory techniques which have been established as reliably predictors of animal responses or performance for tree fodder/roughage diets, or indeed for supplemented poor quality roughage diets more generally. Nevertheless, the laboratory techniques can be applied in conjunction with animal feeding trials (on-station or on-farm), helping design and interpret such trials and extending their scope.

4. Publications (published or accepted)

Wood C D, Tiwari B N, Plumb V E, Powell C J, Roberts B T and Gill M (1993) Interspecies differences in tannin activity of leaves from 13 species of Nepalese browse trees p212-213 In Animal production in developing countries. An occasional publication of the British Society of Animal Production, No 16 Editors Gill M, Owen E, Pollott G E and Lawrence T L J

Wood C D, Johnson J and Powell C (1993) Evaluation of Bolivian tree leaves as fodders by an *in vitro* fermentation technique. Agroforestry Forum 4: 28-34

Prasad C S, Wood C D and Sampath K T (1994) Use of *in vitro* gas production to evaluate rumen fermentation of untreated and urea treated finger millet straw (*Eleusine coracana*) supplemented with different levels of concentrate. Journal of the Science of Food and Agriculture 65: 457-464

Sampath K T, Wood C D and Prasad C S (1995) Effect of urea and by-products on the *in vitro* fermentation of untreated and 5% urea treated finger millet (*Eleusine coracana*) straw. Journal of the Science of Food and Agriculture 67: 323-328

Wood C D, Tiwari B N, Plumb V E, Powell C J, Roberts B T and Gill M (1995) Differences in protein precipitation activity of extractable tannins, crude protein and ash contents of leaf samples from Nepalese fodder trees. Tropical Science 35: 376-385.

Wood C D, Tiwari B N, Plumb V E, Powell C J, Roberts B T, Sirimane V D, Rossiter J T and Gill M (1994) Interspecies differences and variability with time of protein precipitation activity of extractible tannins, crude protein, ash and dry matter contents of leaves from 13 species of Nepalese fodder trees. Journal of Chemical Ecology 20: 3149 - 3162.

Sampath K T, Wood C D and Prasad C S (1993) Effect of sources and levels of nitrogen supplements on *in vitro* fermentation of untreated and 5% urea treated finger millet straw (*Eleusine coracana*). Proc. VI Anim. Nutr. Res Workers' Conf., Bhubaneswar abstract No 309 p151-152

Prasad C S, Sampath K T and C D Wood (1993) Evaluation of untreated and urea treated finger millet straw (*Eleusine coracana*) at different levels of concentrate supplementation using *in vitro* gas production techniques. Proc. VI Anim. Nutr. Res Workers' Conf., Bhubaneswar (1993) abstract No 310 p152

Wood C D and Plumb V E (1994) Total phenols and protein precipitation assay as indicators of the inhibitory effects of phenols on rumen micro-organisms. Proceedings of the 109th meeting of the British Society of Animal Production, Abstract No 75. Animal Production 58: 445

Wood C D, Grillet C, Rosales M and Green S (1995) Relationships between *in vitro* gas production characteristics and composition of tree leaf fodders from Bolivia, West Africa and Colombia. Abstract Animal Science 60: 541 (and summary)

Wood C D and Plumb V E (1995) Evaluation of assays for phenolic compounds on the basis on *in vitro* gas production by rumen micro-organisms. Animal Feed Science and Technology 56: 195-206.

Murray A H, Daalkhaijav D and Wood C D (1996) Rumen degradability of Mongolian pastures: a comparison of *in situ* and *in vitro* gas production techniques. Animal Science 62: 684.

Wood C D and Manyuchi B (in press) Use of an *in vitro* gas production method to investigate interactions between veld hay and Napier hay or groundnut hay supplements. Animal Feed Science and Technology.

Thorne P J, Walker D H, Subba D B, Wood C D, Sinclair F L and Thapa B L (in press) Predicting the nutritive value of tree fodder: consistency and complementarity between assessments made by Nepalese, smallholder farmers and by laboratory

techniques. British Society of Animal Science Winter Meeting 1997 (to appear in Animal Science).

5. Internal Reports

Powell C. (undated) To determine the effect of supplementation of barley straw with lucerne hay, meadow hay, rye hay and timothy hay on gas production during *in vitro* fermentation. Unpublished NRI project report.

Powell C. (undated) To determine the effect of the *in vitro* fermentation technique on the fibre fraction of five samples of temperate hay and straw. Unpublished NRI project report.

Wood C D. (1996) Effects of tannins in ruminant nutrition. Unpublished NRI project report.

Wood C D and Matthewman R W (1996) Feed samples from NW India: Report on initial evaluation.

Note on a workshop held at Pakhribas Agricultural Centre, Nepal, 25 - 29 March 1996.

Wood C D, Thorne P J, Romney D L and Rosales M (1997) Techniques for evaluating ruminant feeds in less developed countries, with particular reference to the potential use of *in vitro* gas production methods. Unpublished NRI project report.

Wood C D and Matthewman R W (1996) Feed samples from NW India: Report on initial evaluation. Unpublished NRI project report.

Wood C D and Murray A H (1997) Characterisation of Costa Rican feed samples using the gas production method. Unpublished NRI project report.

Student reports, theses etc.

Cadario F (1996) MSc Thesis, Univ of Reading. Use of *in vitro* gas production technique for predicting *in vivo* apparent digestibility and voluntary intake of feedstuffs for sheep.

Rosales M (1996) Ph D Thesis, Univ of Oxford. *In vitro* assessment of the nutritive value of mixtures of leaves from tropical fodder trees.

Vargas J E (1995) MSc Thesis, Wye College. Evaluation of the fermentation characteristics of five provenances of *Gliricidia sepium* by *in vitro* gas production technique.

Avornyo F (1995) MSc Thesis, Wye College. An evaluation of the gas production technique in determining interactive effects between ruminant feed mixtures.

Robinson A (1993) Final year student project, Wye College. *In vitro* rumen fermentation studies: standardisation of the Hurley method and comparison with and between earlier procedures.

Clark M (1994) Final year student project, Wye College. An investigation into the ranking of tree leaf species as food supplements for ruminants using modified procedures of the pressure transducer technique.

Goodenough L (1996) Detection of anti-nutritive compounds found in leaves by TLC and fungal inhibition. Student project, Wye College.

6. Other Dissemination of Results

Gill M, Bennison J and Wood C D (1996) The selection of trees for fodder. Advances in Agroforestry. Proceedings of a British Council Short Course, University of Wales, Bangor 29 March - 10 April 1992. Pub. The British Council. p 65 - 73.

Wood C D (1995) Feed evaluation: Recent developments. Summary of presentation given to LSAAC, February 1995.

C. D. Wood and B. Manyuchi (1996) Poster: Use of an *in vitro* gas production method to investigate interactions between veld hay and napier hay or groundnut hay supplements - presented at a conference on Evaluation of Forages for Ruminants in the Tropics held Zimbabwe.

I. R. Armendariz, G. Cadish, K. E. Giller and C. D. Wood (1996) Poster: Nitrogen mineralisation in soils and *in vitro* rumen fermentation parameters as affected by chemical composition of tree fodders presented at a conference "Driven by Nature" held Wye College, UK.

Workshop on fodder tree quality held at Pakhribas Agricultural Centre, Nepal, 25 - 29 March 1996.

7. Follow-up indicated/planned

The methods need to be applied to practical situations to generate information for farmers, to evaluate its practical usefulness and possibly further develop them. In the author's view, experience should be gained on the practical application of the methods to develop case studies before firmly recommending them for widespread adoption. The general approach should be to use the method in support of projects aimed at developing improved feeds and/or feeding strategies. The project (recently approved) in India with BAIF is one such application, others should include a continuation of the collaboration with Pakhribas Agricultural Centre, Nepal, to build on earlier work; add-on activities to other projects within the LPP programme.

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comparisons between *in vivo* and *in vitro* data for feed mixtures. Correlations have been investigated in this project (and more widely in feed evaluation), but a more mechanistic approach, probably involving some modeling, is required.

Feed evaluation, and the gas production technique in particular, has been the focus of research by several other groups in the UK and elsewhere. Recent advances in *in vitro* techniques will be reviewed and discussed at a symposium organised by the British Society of Animal Science to be held in July 1997. Several summary papers on the work of this project have been submitted for presentation at this seminar, and several NRI members of staff will be attending. There will also clearly be lessons to be learned from it. It would be most constructive to revise and update the literature review prepared as part of this project in the light of more recent developments (not least those of this project), to inform scientists in LDCs of these. There is clearly a need for more strategic research on feed evaluation; such a review will also help define better future research requirements. It would also be a useful precursor to the wider dissemination of the gas production technique.

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Laboratory methods aimed at indicating the supply of by-pass protein from tanniniferous feeds appear unconvincing and require further development.

8. Name and signature of author of this report.

C D Wood

Appendix 2

Papers under review and in preparation

Rosales M, Gill M, Wood C D, Romney D, Speedy A W and Stewart J (under review) The contribution of chemical constituents of fodder tree and shrub leaves to gas produced during *in vitro* fermentation in nitrogen free and nitrogen rich media. Submitted for presentation to *In vitro* techniques for measuring nutrient supply to ruminants, Occasional Meeting of the British Society of Animal Science, Reading, July 1997.

Rosales M, Gill M, Wood C D, and Speedy A W (under review) Associative effects of *in vitro* mixtures of tropical fodder trees. Submitted for presentation to *In vitro* techniques for measuring nutrient supply to ruminants, Occasional Meeting of the British Society of Animal Science, Reading, July 1997.

Wood C D, Prathalingam N S, Murray A M and Matthewman R W (under review). Use of the gas production technique to investigate the supplementation of nitrogen deficient feeds. British Society of Animal Science Occasional Paper (*In vitro* techniques for measuring nutrient supply to ruminants, University of Reading, 8 - 10 July 1997).

Wood C D, Murray A H, Moss A R and Givens D I (under review). Use of the gas production technique to investigate responses of supplementing low quality forages: 1. *In vitro* interactions. British Society of Animal Science Occasional Paper (*In vitro* techniques for measuring nutrient supply to ruminants, University of Reading, 8 - 10 July 1997).

Murray A H, Moss A R, Wood C D, Givens D I and Gill M (under review). Use of the gas production technique to investigate responses of supplementing low quality forages: 2. *In vivo* interactions and comparison with *in vitro* parameters. British Society of Animal Science Occasional Paper (*In vitro* techniques for measuring nutrient supply to ruminants, University of Reading, 8 - 10 July 1997).

Romney D L, Cadario F C, Owen E and Murray A H (under review). Comparison of parameters from the Theodorou gas production technique using nitrogen-free and nitrogen-rich media as predictors of DM intake and digestibility. British Society of Animal Science Occasional Paper (*In vitro* techniques for measuring nutrient supply to ruminants, University of Reading, 8 - 10 July 1997).

Thorne P J, Subba D B, Walker D H, Thapa B, Wood C D and Sinclair F L (under review). Indigenous and laboratory assessment of the nutritive value of tree fodder. Part 1: Discrimination amongst and within species.

Walker D H, Thapa B, Thorne P, Sinclair F L, Wood C D and Subba D B (under review) Indigenous and laboratory assessment of the nutritive value of tree fodder. Part 2: Comparison of farmer and laboratory assessment.

Wood C D, Panigrahi S, Goodenough L, Rossiter J T and others (in preparation). Use of bioassays to detect anti-nutritive factors in Nepalese fodder trees.

Whetton M, Rossiter J T and Wood C D (under review) Nutritive evaluation of nitrogenous fractions in leaves of *Gliricidia sepium* and *Caliandra calothyrsus*, in relation to tannin content and protein degradation by rumen microbes *in vitro*.

Wood C D and others (in preparation). *In vitro* protein degradation in eight fodder tree species from Nepal.

Cadario F (in preparation). Use of *in vitro* gas production technique for predicting *in vivo* apparent digestibility and voluntary intake of feedstuffs for sheep.

Rosales M, Gill M, Wood C D, Romney D, Speedy A W and Stewart J (in preparation) The contribution of chemical constituents of fodder tree and shrub leaves to gas produced during *in vitro* fermentation in nitrogen free and nitrogen rich media. Draft paper.

Dryhurst N and Wood C D (under review). The effect of nitrogen source and concentration on *in vitro* gas production using rumen micro-organisms

Murray A H, Daalkhaijav D and Wood C D (in preparation) The rumen degradability of Mongolian pastures measured by *in sacco* and *in vitro* gas production techniques

Wood C D, Stewart J L and Vargas J E (in preparation). Genetic variation in the nutritive value of *Gliricidia sepium*. 2. Leaf chemical composition and fermentability by an *in vitro* gas production technique.

A. H. Murray, D.L. Romney and C.D. Wood (in preparation) The use of the *in vitro* gas production technique to identify *in vivo* digestive interactions of sheep fed low quality roughage diets supplemented with high quality forages.

Appendix 3 Contents of technical annex

TECHNICAL ANNEX PART 1

Wood C D. Effects of tannins in ruminant nutrition (15pp).

Wood C D, Tiwari B N, Plumb V E, Powell C J, Roberts B T and Gill M 1995 Differences in protein precipitation activity of extractable tannins, crude protein and ash contents of leaf samples from Nepalese fodder trees Tropical Science **35** 376-385. (10pp).- reprinted with the kind permission of the journal.

Wood C D, Tiwari B N, Plumb V E, Powell C J, Roberts B T and Gill M (1993) Interspecies differences in tannin activity of leaves from 13 species of Nepalese browse trees p212-213 In Animal production in developing countries. An occasional publication of the British Society of Animal Production, No 16 Editors Gill M, Owen E, Pollott G E and Lawrence T L J (2pp)

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Wood C D and Plumb V E 1995 Evaluation of assays for phenolic compounds on the basis on in vitro gas production by rumen micro-organisms Animal Feed Science and Technology **56** 195-206. (12pp). - reprinted with the kind permission of Elsevier Science, NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands.

Wood C D 1995. Feed evaluation: recent developments. Summary of presentation given to LSAAC, Feb. 1995.

Wood C D, Grillet C, Rosales M and Green S (1995) Relationships between *in vitro* gas production characteristics and composition of tree leaf fodders from Bolivia, West Africa and Colombia. Abstract Animal Science 60: 541 (and summary)

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Powell C (undated) To determine the effect of supplementation of barley straw with lucerne hay, meadow hay, rye hay and timothy hay on gas production during in vitro fermentation. Unpublished NRI report.

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Rosales M In vitro assessment of the nutritive value of mixtures of leaves from tropical fodder trees. PhD thesis, University of Oxford. Abstract and general discussion (p185 - 191) only.

Rosales M, Gill M, Wood C D, Romney D, Speedy A W and Stewart J (under review) The contribution of chemical constituents of fodder tree and shrub leaves to gas produced during in vitro fermentation in nitrogen free and nitrogen rich media. Submitted for presentation to In vitro techniques for measuring nutrient supply to ruminants, Occasional Meeting of the British Society of Animal Science, Reading, July 1997.

Rosales M, Gill M, Wood C D, and Speedy A W (under review) Associative effects of in vitro mixtures of tropical fodder trees. Submitted for presentation to In vitro techniques for measuring nutrient supply to ruminants, Occasional Meeting of the British Society of Animal Science, Reading, July 1997.

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Murray A H, Daalkhaijav D and Wood C D (1996) Rumen degradability of Mongolian pastures: a comparison of *in situ* and *in vitro* gas production techniques. Abstract, summary and poster presented at the British Society of Animal Science Winter Meeting 1996. Animal Science **62** (3) 684.

Murray A H, Daalkhaijav D and Wood C D (in preparation) The rumen degradability of Mongolian pastures measured by *in sacco* and *in vitro* gas production techniques.

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Wood C D, Stewart J L and Vargas J E (in preparation). Genetic variation in the nutritive value of Gliricidia sepium. 2. Leaf chemical composition and fermentability by an in vitro gas production technique.

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TECHNICAL ANNEX PART 4

Thorne P J, Subba D B, Walker D H, Thapa B, Wood C D and Sinclair F L (under review). Indigenous and laboratory assessment of the nutritive value of tree fodder. Part 1: Discrimination amongst and within species.

Walker D H, Thapa B, Thorne P, Sinclair F L, Wood C D and Subba D B (under review) Indigenous and laboratory assessment of the nutritive value of tree fodder. Part 2: Comparison of farmer and laboratory assessment.

Thorne P J, Walker D H, Subba D B, Wood C D, Sinclair F L and Thapa B L (in press) Predicting the nutritive value of tree fodder: consistency and complementarity between assessments made by Nepalese, smallholder farmers and by laboratory techniques. British Society of Animal Science Winter Meeting 1997 (to appear in Animal Science).

Wood C D, Panigrahi S, Goodenough L, Rossiter J T and others (in preparation). Use of bioassays to detect anti-nutritive factors in Nepalese fodder trees.

TECHNICAL ANNEX PART 5

Whetton M, Rossiter J T and Wood C D (under review) Nutritive evaluation of nitrogenous fractions in leaves of Gliricidia sepium and Caliandra calothyrsus, in relation to tannin content and protein degradation by rumen microbes in vitro.

Wood C D and others (in preparation). In vitro protein degradation in eight fodder tree species from Nepal.

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Cadario F (in preparation). Use of in vitro gas production technique for predicting in vivo apparent digestibility and voluntary intake of feedstuffs for sheep.

Wood C D, Thorne P J, Romney D L and Rosales M (1997). Techniques for evaluating ruminant feeds in less developed countries, with particular reference to the potential use of in vitro gas production methods. Unpublished NRI project report.

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TECHNICAL ANNEX PART 6

C D Wood, P J Thorne, D L Romney and M Rosales (1997) Techniques for evaluating ruminant feeds in less developed countries, with particular reference to the potential use of in vitro gas production methods. Unpublished project report.

A. H. Murray, D.L. Romney and C.D. Wood (in preparation) The use of the *in vitro* gas production technique to identify *in vivo* digestive interactions of sheep fed low quality roughage diets supplemented with high quality forages.

DEVELOPMENT OF IMPROVED METHODS FOE ESTIMATING THE NUTRITIVE VALUE OF TROPICAL FORAGES

TECHNICAL ANNEX PART 1

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EFFECTS OF TANNINS IN RUMINANT NUTRITION

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1. Introduction

1.1 Purpose of this review

Tannins are very diverse chemically, are widely found in plant material and can have diverse effects on animals which consume them. There is a large body of scientific literature on various aspects of tannins. This short review seeks to highlight the practical significance of tannins on ruminant nutrition in less developed countries using this literature and the experiences of the staff of the Natural Resources Institute. It will also review approaches to countering the negative effects of tannins, with particular reference to the use of tannin-binding agents. This review does not seek to be comprehensive and where appropriate key references are given containing further details.

1.2 What are tannins?

As the name suggests, the word tannin was originally used to describe plant extracts used to tan animal skins into leather. The important biochemical property of tannins is their ability to bind to protein and form insoluble complexes. General descriptions of the chemical structures can be found in reviews such as Mangan (1988), Jansman (1993) and Kumar and D'Mello (1995). Tannins are generally poorly defined chemically and are found in complex mixtures in many plants. All tannins are polyphenolic compounds, although not all polyphenols have the protein binding properties of tannins. Tannins can be divided into two chemical types, hydrolysable and condensed tannins. Additionally polyphenols of lower molecular weight than the tannins (and not having tannin-like properties) are commonly found. This review will use the term tannin as a general term which may include other polyphenols, and will indicate when comments are specifically about lower molecular weight polyphenols.

2. Measurement of tannins

2.1 Methodologies available

To match the range of tannins, there is a very diverse range of chemical and biochemical assays which has been used to measure the tannin contents of plants. Several methods use the chemical properties of tannins in colorimetric assays. These assays vary considerably in specificity. Their properties and merits have been reviewed by Hagerman and Butler (1989). The most widely used colorimetric assays are given below:

General measurements of tannins (total phenols)
- Folin Denis assay
- Prussian blue assay

Measurements of condensed tannins
- Vanillin assay
- Acid butanol assay

There are several published procedures and modifications to these assays. Based on the recommendations of Hagerman and Butler (1989), the Prussian blue assay using the Price and Butler (1977) procedure and the acid butanol assay by the Porter et al (1986) procedure are used at NRI. These assays are usually used to measure polyphenols which have been extracted in solvent, often aqueous acetone. However, the acid butanol method is also used to measure non-extractable tannins. Again there are various procedures but they involve the treatment of a residue (after extraction) with acid butanol. Terrill *et al.* (1992a) have developed a method which sequentially extracts free, protein-bound and fibre-bound condensed tannins.

Biochemical assays can be used to measure the activity or capacity of tannins to bind and precipitate protein. This is really a measurement of tannin activity and it has been suggested by Hagerman and Butler (1989) that these types of assays give better indicators of the biological activity of tannins than chemical assays. Again there are several procedures available. At NRI, the radial diffusion assay of Hagerman (1987; as modified by Wood *et al.*, 1994) is used as it does not require sophisticated equipment, making it potentially suitable for use in less developed countries.

More sophisticated chemical techniques such as high performance liquid chromatography (HPLC) have been use to identify particular tannins, especially lower molecular weight polyphenols (Mueller-Harvey and Reed, 1992), but also for separating tannins of different molecular weight (Rigaud *et al.*, 1993). The detailed analysis of the higher molecular weight tannins is, however, very challenging.

2.2 Relationships between tannin assays and their anti-nutritive properties

Considering the volume of literature on tannin assays, there is comparatively little on the effectiveness of the different assays as indicators of anti-nutritive effects. This is because such trials are difficult to conduct. The nutritive value of tanniniferous feeds is due to all its components and isolating the effect of tannins is not straightforward. There are no entirely suitable compounds which can be readily obtained and added to feeds as experimental models. With such a diverse group of compounds there is a considerable risk that model compounds may be of limited value. Tannin-binding agents have been used to inactivate tannins thus giving indications of what effects the tannins are having. The use to such agents is reviewed in section 5.2.

Robbins *et al.* (1987) correlated crude protein content to the proportion of digestible protein for feeds such as grasses and agriculturally-produced legumes and grains with very low tannin contents. Using the correlation equation obtained to predict protein digestibility and comparing this to the measured protein digestibility of tanniniferous feeds consumed by mule deer, an estimate of the reduction in digestible protein due to

tannins was obtained. This was found to be highly correlated with the activity of the tannins as assayed by a protein precipitation assay. Hanley *et al.* (1992) used predictive equations developed by Robbins *et al.* (1987) to predict successfully the protein and dry matter digestibilities of tanniniferous forage leaves from seven species and one sample of twigs when fed to black-tailed deer, confirming that a protein precipitation assay gave a useful estimate of the effect of tannins *in vivo*.

McKey *et al.* (1978) have also found that total phenols (by the Folin Denis assay) and extractable condensed tannins (by acid butanol) correlated negatively with the dry matter digested by rumen inoculum during 96 hours incubation. Data were quoted for 30 species of trees from which samples of mature leaves had been taken. For 72 West African fodder trees and shrubs, Rittner and Reed (1992) found that *in vitro* protein degradability was negatively correlated with total phenols (by ytterbium precipitation assay) and extractable condensed tannins (by acid butanol). However, the behaviour of some species deviated greatly from that indicated by the correlation studies. Wood and Plumb (1995) found strong correlations between total phenols (Prussian blue assay) and the inhibition by tannins from Bolivian fodder tree leaves of *in vitro* fermentation (gas production) by rumen microbes. Similar correlations were found using protein precipitation by the radial diffusion assay, but there was no significant correlation with the acid butanol assay.

Other workers have had less success in finding correlations. Makkar *et al.* (1989) were unable to find significant correlations between total phenols (Folin Denis assay), condensed tannins (by the vanillin assay) and protein precipitation with *in sacco* dry matter loss for leaves of 10 species of trees from India. Khazaal and Ørskov (1994) found that the increase in gas production resulting from treating eleven leaf samples with the tannin binding agent PVPP was not related to total phenols (by Folin Denis and by a gravimetric assay), extractable condensed tannins (by acid butanol and vanillin assays) and total condensed tannins (by acid butanol assay). Mole and Waterman (1987) found little correlation between chemical assays for total phenols (by the Folin method), condensed tannins (by the vanillin method), hydrolysable tannins (by various methods) and biochemical activities as assayed by a protein precipitation method and cellulase inhibition.

2.3 Preferred indicators for the anti-nutritive effects of tannins

As may be inferred, there is no clear consensus as to which assays are the most reliable indicators of anti-nutritive effects. Correlations to some extent reflect the selection of samples. There is evidence indicating the general applicability of protein precipitation assays and the Prussian blue total phenols assay, although neither of these can distinguish between condensed and hydrolysable tannins.

Tannins can be modified during sample preparation. Harvested oak leaves appeared to increase in total phenol and tannin content after 48 - 72 hours, then decline to near their original levels within 120 hours (Kleiner, 1991). Hagerman (1988) reported variable effects of drying at 40°C and freeze-drying on extractable tannin contents, although oak leaf tannins have been found to be resistant to change when leaves were dried (Makkar and Singh, 1991). Orians (1995) found that the condensed tannin content of willow leaves fell when samples were air-dried and recommended using a

freeze-drier but without pre-freezing the sample to minimise changes to tannins. When Bolivian tree leaves were oven dried at 50°C for 16 - 24 hours losses of extractable tannins ranged from 19 to 70% compared to fresh leaves, depending on species (unpublished NRI data). Thus the effect of tannins of sample preparation appears to depend on the precise nature of the tannins. While using a freeze-drier without pre-freezing may be the preferred method to dry samples, in many practical situations in less developed countries samples would have to be air-dried or oven-dried.

The author suggests that for nutritive value assessment there may be advantages in using *in vitro* measurements of feed degradation rather than trying to correlate tannin assays to degradation characteristics. These techniques appear to be less prone to modifications resulting from sample preparation than the tannin assays and the data obtained will be more readily applicable than tannin assay data. While tannin measurement will continue to be extremely useful for research purposes, it may be less suitable for routine nutritive value assessment.

3. Occurrence of tannins in ruminant diets

3.1 Types of feeds which contain tannins

Tannins are found widely in plant material, in both leguminous and non-leguminous species. Of most significance to ruminant feeds is that many, if not nearly all, species of forage and browse legumes contain tannins. Kumar and D'Mello (1995) concluded that hydrolysable tannins were abundant in leaves, fruits, pods and galls of dicotyledons such as oak, chestnut and other species; condensed tannins are even more widespread. Some species contain both types of tannin.

Makkar (1993) listed the polyphenolic contents of 62 species of trees and shrubs from India. The list included important fodder species such as various Acacias, Albizias, *Calliandra calothrysus*, *Ficus* sp. , *Gliricidia sepium*, *Leucaena leucocephala*, various species of Prosopis and Quercus. Ritter and Reed (1992) studied 72 West African fodder tree and shrub species (from Benin, Niger and Nigeria) and found polyphenols in all the species studied. Average polyphenolic contents did not appear to be different in the climatic zones studied. Wood *et al.* (1995) studied 20 tree leaf fodders from Bolivia, 26 from West Africa and 24 from Colombia. Only 9 of the 70 samples were found to have no extractable tannins (as gauged by protein precipitation activity; unpublished data). Tannins are not restricted to tropical feeds; lotus, sainfoin and other temperate species have been found to contain condensed tannins (Waghorn *et al.*, 1990). Thus, while not all tree and shrub fodders and browses contain tannins, most certainly do. The occurrence of tannins is not restricted to particular limited classes of plants or climatic zones. **Tannins are therefore highly likely to be consumed in all agricultural systems where trees and shrubs are used as livestock feed.**

Tannins are not confined to just trees and shrubs. Makkar (1993) lists 28 agro-industrial by-products which might be useful as feeds. 19 of these by-products, including various seeds and seed cakes, have been shown to contain polyphenols. Cassava leaves, a potentially useful forage, contain condensed tannins (Reed *et al.*, 1982). Sorghum grain and stover can also contain tannins (Mueller-Harvey and Reed,

1992). **Therefore several crop residues and by-products of considerable importance as livestock feeds in less developed countries contain tannins.**

3.2 Importance of tanniniferous feeds

Tanniniferous feeds are important to ruminants in many less developed countries. However, there are few published estimates on either the quantities or proportions of such feeds in the livestock diets. Devendra (1995) has noted some broad estimates of the importance of tree browses (shoots, twigs, leaves, fruits and pods of trees and shrubs), which are generally high in tannins and important in arid and semi-arid regions. In northern Africa browse forms 60 - 70% of rangeland production and 40% of the total available feed, other estimates put such feeds as making up 40 - 50% of the total available feed (FAO, 1992). Tree leaves and pods are widely consumed by ruminants in sub-Saharan Africa. Gutteridge and Shelton (1994) have reviewed the role of forage tree legumes, noting that at least 75% of the shrubs and trees of Africa serve as browse plants. In India 60 - 70% of the forage requirements for goats are tree browses, tree legumes being particularly important (Devendra, 1995). **Browses are particularly important in extensive livestock systems, and become more important when the supply of alternative feeds is restricted such as during dry seasons and periods of drought.** In Australia, mulga (*Acacia aneura*) is grown in stands for use as drought reserves for grazing sheep (Gutteridge and Shelton, 1994). The ability of animals such as goats and camels to survive on browse largely explains why these animals are preferred in drought prone areas.

Tree fodders are also important in forested highland regions. Panday (1982) estimated that about 40% of the fodder available annually in Nepal comes from forest trees, but this figure obscures regional and seasonal variations. Trees are particularly important during the dry season when alternative green fodders are often not available. This broad pattern of tree leaf usage is found in the mountainous forested lands of northern India; oak tree leaves are particularly important in the highland (approximately 1,500 to 4,000 m) areas during the February to April dry season. An ongoing NRI study in the middle hills (900 - 2000 m altitude) in eastern Nepal identified 14 major fodder tree species used principally in the winter and dry seasons (November - June) to supplement crop residues in ruminant feeds. In this region tree fodders contributed over 15% of the dry matter and over 20% of the crude protein to the diet, but actual usage on individual farms varied depending on the ethnic and social group of the farmers, possible as a result of different livestock holdings and opportunities for planting trees.

In the more humid tropical areas legumes are often planted specifically for forage in extensive grazing systems and in association with crops. Leguminous trees are planted by smallholder farmers along field borders or fence lines. Herbage is lopped in cut and carry systems and used as supplements to low-quality feeds such as crop residues. Tree fodders contribute an estimated 8.2 million tonnes/year of fresh feed in South-East Asia, about 4% of the available feedstuffs, excluding grasses (Devendra, 1993). Tree fodders are also widely used in South America, Australia and southern Africa in more extensive grazing areas. Reynolds *et al.* (1993) reported that 45% of smallholder farmers in the sub-humid coastal region of Kenya used browse from fodder trees. NRI field projects in some less arid regions report the following usages of tree fodders.

In Eastern Kenya dairy cows have been successfully fed on a diet containing 25 to 30% of daily intake as the fodder tree *Calliandra calothyrsus*, and growing heifers about 15% of intake. Tree browses form about 1 - 2% of the feed of cattle in The Gambia during the dry season, but for goats such feed may constitute 10% of the diet. In humid and sub-humid lowland regions of Bolivia beef steers at pasture with access to poor quality pasture and *Leucaena leucocephala* would eat about 30% of their diets as leucaena in the dry season. Wet season consumption was somewhat lower at about 20%, presumably due to the availability of more and better quality pasture. In Jamaica goats at pasture ate at least 30% of their diets as legumes. **Thus tanniniferous feeds can be an important part of the diets of livestock in higher potential areas, not just in areas where high usage often reflects a lack of alternative feeds.**

The importance of trees and legumes to agriculture and the environment in reducing soil erosion and fixing nitrogen, together with uses of trees for timber etc. must also be noted. Their introduction and greater use is widely seen as of major importance in maintaining and increasing the productivity of many tropical regions.

Sorghum and its by-products are widely produced, particularly in semi-arid regions. **Sorghum stovers are used to feed cattle in many African and Asian countries, particularly in the dry season.** Devendra (1991) estimated that sorghum stover constituted 4.1% of the cereal straws produced in Asia, an annual production of 36.5 million tonnes. It was particularly important in India, the 23 million tonne/year production constituting 11% of the total cereal straw production.

4. Anti-nutritive effects of tannins

The effects of tannins on ruminants have been reviewed by Makkar (1993), Hagerman and Butler (1991) and Kumar and D'Mello (1995); the following has been largely extracted from these reviews. It is a complex picture involving a wide variety of ill-defined chemical compounds and a wide range of effects on animals. It is also a picture with many gaps as comparatively little is known about the effects of tannins directly on animals, nor is much known of the degradation of tannins in animals and the fate of degradation products.

Tannins bind to proteins in the mouth reducing the palatability of the feed, hence potentially decreasing intake. However, while tannins may certainly affect selection, it is not clear that intake is lowered to a major extent in animals used to a tannin-rich diet when there is no alternative feed (Hagerman and Butler, 1991). Tannins can inhibit digestive enzymes, inhibit rumen microbes directly and bind to the feed hence inhibit its degradation in the rumen. The neutral pH of the rumen facilitates the formation of tannin-protein complexes. As well as binding to protein, tannins can also bind to carbohydrates. Due to a combination of these activities tannins are associated with decreased rumen degradabilities. These effects can be beneficial to ruminants as inhibition of protein degradation in the rumen reduces the occurrence of bloat and potentially increases the availability of protein for digestion in the lower gut.

Some low molecular weight polyphenols are readily degraded in the rumen, but the high molecular weight quebracho tannin (a condensed tannin) is relatively resistant to degradation (Parrinder *et al.*, 1993). Murray *et al.* (in press) concluded that low

molecular weight polyphenols of heather inhibit rumen microbes, but sugar groups which are often linked to naturally occurring phenols are readily fermentable and stimulate *in vitro* fermentation. Orcinol (a low molecular weight polyphenol), when administered intravenously, led to an increase in energy expenditure in sheep, but no such increase was observed when physiological levels of orcinol were administered into the rumen (Iason and Murray, in press). However, Mueller-Harvey and Reed (1992) found that the amount of the low molecular weight polyphenol butin in sorghum leaves was highly negatively correlated with *in vitro* digestibility. Whether this was due to any anti-nutritive properties of butin itself or whether it was due to the importance of butin as a precursor of condensed tannin (butin also correlated with condensed tannin content) was unclear. **Anti-nutritive effects on ruminants are therefore probably due to the tannins in the feed (and their breakdown products) rather than the lower molecular weight polyphenols in the feed. However certain lower molecular weight polyphenols in the feed could have direct toxic properties.**

Tannin-feed complexes pass into the abomasum, where some protein-tannin complexes can dissociate in the acidic environment and the protein degraded. However, the ingestion of tannins is associated with increased nitrogen excretion in the faeces, so overall there is an inhibition of protein degradation. This in turn may account for the inferior growth of animals on high tannin diets. However Hagerman and Butler (1991) have suggested that tannins or their breakdown products can be absorbed from the gut and inhibit metabolism directly and this could be the major effect of tannins. The fate of tannins in the feed appears to depend on the animal consuming them. Sheep excreted 40% of quebracho tannin added to alfalfa pellets, deer excreted all the tannin in their faeces. While some of the degraded tannin may be readily detoxified and excreted, other products may be toxic, although little is known about this.

Tannins can cause liver and kidney damage, hydrolysable tannins possibly being particularly damaging. Tannins have also been implicated in inducing cancer. Exceptionally high levels of tannin consumption can kill ruminants. For example, consumption of high tannin content young oak leaves in Northern India has been implicated in cattle deaths. Nevertheless some animals are thought to be able to adapt to high tannin diets by producing proline rich salival proteins to bind tannin, diet selection to avoid tannins and possibly other metabolic changes to limit their effects. Rumen microbes may also adapt to resist and degrade tannins, although there is little evidence that this does happen (for example Makkar *et al.*, 1995b, were unable to induce the degradation of quebracho tannin by rumen microbes in an *in vitro* system). An added complication is that tree fodders may also contain other anti-nutritive factors as well as tannins. Makkar *et al.* (1995a) showed that the effects of tannins and saponins were additive *in vitro*.

Lessons from studies on the effects of tannins on monogastrics may also be relevant. Jansman (1993), reviewing the subject, concluded the following:
a) It has not been conclusively demonstrated that tannins reduce feed intake.
b) Tannins reduce weight gains and impair feed conversion efficiency.
c) Tannins reduce apparent digestibility of protein and, to a lesser extent, energy.
Similar conclusions are valid for ruminants, although ruminants are more tolerant of tannins than monogastrics.

Thus, there are two different effects of tannins, firstly to decrease the availability of nutrients from feeds and secondly to have direct toxic effects on animals. The question of how important direct toxic effects are in ruminants compared to the effects of tannins in reducing nutrient availability is unclear. If toxicity is due to an appreciable extent to certain breakdown products from tannins, analysing the feeds may not detect the compounds of interest as they may not necessarily occur in the toxic form in the feeds themselves. However, if in practice the reduction in nutrient availability can account for the most commonly found effects of tannins, there may be a limited need to investigate the toxicity of the tannins. The more acute toxic effects could be as much a reflection of high quantities of tannins consumed as the occurrence of tannins which have particularly toxic breakdown-products.

5. Strategies for alleviating the anti-nutritive effects of tannins

5.1 Tannin binding agents

5.1.1 Effectiveness

Tannin binding agents, notable polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP), have been widely used as research tools to investigate the effects of tannins both *in vivo* and *in vitro*. Both compounds are commercially available in a range of molecular weights. Lower molecular weight polymers are highly water soluble and bind strongly to tannins. Jones and Mangan (1977) first used PEG (molecular weight, MW, 4000) to prevent the formation of sainfoin tannin and protein complexes and to release protein from the complexes. While PEG was able to prevent tannin-protein complex formation, its ability to release protein from such complexes related to the pH of the environment, amount of tannin in the complex and the age of the complex at the time of adding PEG. At pH 8.0 a maximum of only 30% of the protein was released from the complex by PEG. This may account for the variable responses to treating dried feed samples with tannin binding agents observed by Khazaal and Ørskov (1994) and Wood and Plumb (1995). While PEG MW 4000 has been the most widely used tannin-binding agent, Makkar *et al.* (1995c) showed that PEG MW 6000 was more effective in overcoming the effects of tannins at neutral pH *in vitro*. PEG was more effective at binding tannins than PVP.

Some *in vivo* trials using PEG are reviewed below. Barry and Duncan (1984) sprayed lotus herbage with a 0.3 g g^{-1} solution of PEG (MW 3350) at a rate of 2.4 g PEG per g condensed tannin to overcome the effects of the tannin. The treatment increased apparent digestibility of nitrogen by 0.26 and also led to modest increases in cellulose and hemicellulose digestibility. Oral administration of PEG (MW 3350) at the rates of 40 and 60 g per day to lambs grazing on sulla and pasture was used by Terrill *et al.* (1992b). PEG had no consistent effect on the sheep grazing tanniniferous sulla or (tannin-free) pasture. Silanikove *et al.* (1994) used PEG (MW 4000) at 12.5, 25, 32 and 50 g/day to supplement a sheep diet containing the tanniniferous carob leaves (containing about 20 g kg^{-1} total phenols). The PEG was administered each morning mixed with a small amount of concentrate prior to feeding. Supplementation with 25 g per day PEG increased digestible organic matter intake by two-fold due both to increased intake and digestibility. 32 g per day PEG led to further apparent increases

but these were not statistically significant; there were no further improvements with 50 g per day supplementation. Withdrawal of PEG resulted in a rapid return to the lower intakes and digestibilities of the unsupplemented controls. Prichard *et al.* (1992) used 12 and 24 g per day of PEG (MW 4000) to supplement mulga browse consumed by sheep and reported a 56 and 78% (respectively) increase in intake but with little effect of dry matter digestibility. Protein digestibility did, however, increase. Wool growth increased by 166 and 178% respectively as a result of PEG.

Thus treatment with tannin binding agents can be highly effective in overcoming the anti-nutritive effects of tannins leading to improved animal performance. However, their affects can be variable which may relate to the nature of the tannins, the nature of the tannin-feedstuff complexes which can form and the history of the feeds tested.

5.1.2 Commercial application

There is very limited published information of the possible commercial benefits of treatment with tannin-binding agents. Silanikove *et al.* (1994) concluded that PEG supplementation would cost about US$ 0.03 per day in Israel for a 25 g supplement (US$ 1.2 per kg), less than half the cost of supplementing with 300 g of digestible organic matter per day to achieve similar performance. Current UK price is about US$ 11 per kg PEG MW4000 (from Merck Ltd), almost ten times the price quoted by Silanikove *et al.* (1994). At about US$ 100 per kg PVP (MW 44000) is considerably more expensive than PEG. However, the price of such chemicals in less developed countries would depend very much on local circumstances and there is insufficient information to attempt an analysis of either the costs or the benefits. In Australia PEG treatment is said to be regarded as too expensive, Prichard *et al.* (1992) commenting that a cheaper tannin-binder with similar properties would be of considerable commercial interest to Australian sheep farmers who use mulga browse as feed.

As far as the author is aware it is only in Southern Africa that a commercial tannin binding product has been marketed. In Zimbabwe, Agricura market a product called "Browse Plus" which contains PEG (MW 4000), PVP, calcium hydroxide and an emulsifier. The product is added to drinking water for the consumption of cattle (mainly) at a dosage rate of 1 to 3 g of product per livestock unit per day. The product is said to have had beneficial effects on cattle particularly during a period of drought, when presumably high-tannin browses were a particularly important component of the diet. Cattle were said to consume browses which were not consumed without "Browse Plus" supplementation, and farmers reported increased feed intake and better animal condition. There is, however, a lack of data from properly designed scientific trials on the benefits of the product. It is said that low-cost grades of PEG can be used as feed supplements and that different costs of different grades may account for the diverse opinions as to the cost of treatment. It is also notable that the level of supplementation with "Browse Plus" is very much less than that used experimentally when the objective has been to overcome all the effects of the tannins. Less ambitious improvements with lower levels of supplementation may be more appropriate commercially. Also a cocktail of active ingredients, which is apparently the case in "Browse Plus", may be more effective than supplementation with PEG alone.

5.2 Feed mixtures

Free-ranging animals can select their diets so as to avoid the worst effects of tannins. It is said that incidence of fatalities in which tannins have been implicated occur when animals are very hungry and are unable to select alternative feeds. In the cut and carry feeding systems of Nepal farmers there are some indications that mixtures of different tree fodders are used on occasions, possibly in part as a strategy of avoiding possible toxicity of excessive amounts of some fodder species. Using tree fodders as supplements to roughages, a common feeding practice, may coincidentally help limit the intake of tannins. However, this is an area which has been little investigated.

5.3 Treating feeds to remove tannins

Makkar (1993) reviewed this topic. The tannin content of oak leaves could be reduced by up to 90% by treatment with alkali, oxidising agents and tannin-complexing agent. Wood ash, a source of alkali potentially available to farmers, was effective in reducing tannin contents. However, these treatments also remove nutrients so their overall effectiveness is unclear, their potential commercial applicability even less so. Makkar (1993) suggested that microbial or enzymic treatments to degrade tannins warranted investigation.

5.4 Plant breeding

Plant selection and breeding, possibly involving modern genetic engineering techniques, could be used to optimise the tannin content of feed materials, hence increase its nutritive value. This may include introducing tannins into feeds which can cause bloat and, more particularly in less developed countries, selecting for reduced tannin content. There may also be scope for selecting against particularly toxic tannins and breakdown products if it was established that there were specific tannins causing problems. Butler (1988) has described work on screening sorghum to determine which polyphenols are useful in conferring disease, pest and bird resistance and which ones can be usefully removed to increase nutritive value. It must be noted that tannins play a vital role in protecting the plant from herbivore and other attack, so it is an approach which must be treated with some caution. When introducing improved fodder tree varieties agronomic properties such as ease of establishment, disease and pest resistance and foliage production will probably be the most important selection parameters, and these may be impaired in low tannin varieties.

6. Conclusions on importance of tannins

6.1 Tannins are consumed in agricultural systems where tree, shrubs and forage legumes are used as livestock feed. Certain agricultural by-products such as some seed cakes and sorghum stovers also contain tannins. **Therefore tanniniferous feeds are widely consumed by ruminants in less developed countries. Their importance does, however, vary considerably regionally, seasonally and even between farms.** The major situations where tanniniferous feeds are important for ruminants are given below:

(a) Tanniniferous feeds are vitally important in arid and semi-arid areas in the dry season and during periods of drought. These types of feeds are often the major type of feed available under these circumstances and enable the animals to survive in periods of feed shortage.
(b) Tanniniferous tree leaves are a particularly important feed in upland forests, again particularly in dry seasons. Trees are used as sources of feed in forested regions in general.
(c) In regions of higher agricultural potential tanniniferous forage legumes can be an important part of the diet. Tanniniferous seed cakes may also be important in some regions. Such feeds are generally valuable sources of protein.

6.2 **Tannins are associated with poor animal performance apparently due to inhibition of feed degradation.** However, tannins and possibly their breakdown products can have direct toxic effects on animals although these are not well characterised and their practical importance not well understood.

6.3 There are several possible strategies for alleviating the negative effects of tannins as suggested below:
(a) Using feed mixtures to minimise the intake of particular tannins may be beneficial and there are some indications that such practices are used in some feeding systems. It is, however, a very poorly understood approach and its potential effectiveness is unclear.
(b) Tannin-binding agents can be effective. In droughts (in particular) they could boost performance sufficiently to improve survival rates of ruminants. However, their commercial application has only recently begun and their usefulness is not clear. There is a case for reviewing the costs and benefits of agents such as PEG to see if such supplements are likely to be of commercial interest.
(c) Microbial or enzymic treatments of feeds to degrade tannins may be developed in the longer term and warrant further investigation.
(d) In the longer term improved plant varieties with optimal types and levels of tannins could be produced, but the potentially vital positive effects tannins can have for plants must not be lost in reducing the negative effects on animals.

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