Use of nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on tanniniferous tree foliage (D3.10.22)

Overall objective
To improve the safety and efficiency of feeding ruminants on tanniniferous tree foliage.

Specific objectives
1. Refine and standardise nuclear, chemical and biological assays for measuring tannins in plant material and validate the usefulness of these techniques for predicting animal performance.
2. Develop strategies for enhancing utilization of tree leaves as livestock feed, using the validated tannin assays.

Main outputs

a) Main outputs from the first phase of the project:

All participating groups used the same protocols and standards for tannin assays, which enabled comparison of the results and provided meaningful information. A manual containing methodologies for the analysis of tannins using chemical-, protein precipitation/binding- and bio-assays recommended by the consultants has been published (Makkar, 2003) and was used in both the phases of the CRP.

1. A total of 48 species of tree leaves and browses were characterized for tannin levels and activity. Ten of them were also evaluated in in vivo studies. The apparent digestibility coefficients of N correlated best with the following tannin assays: total phenol, total tannins, condensed tannins, radiolabelled BSA method, and percentage increase in gas on inactivation of tannins using polyethylene glycol (PEG) in the in vitro method for measuring gas production. When these tannin values were adjusted according to the N level in the forages (tannin %/N %), correlations remained high but were not improved. None of these values was a good predictor of feed intake in the short term in vivo studies. Under the conditions of these short-term studies, using a seven-day adaptation period, the intake data should be interpreted with caution. The intake data gave variable relative values but there were indications that ruminants need a longer adaptation period to tanniniferous forages.

2. Total phenols and tannins correlated best with the percentage increase in gas production on inactivation of tannins by PEG in the in vitro gas production method, suggesting that these assays, which are the simplest amongst the group of assays used in the first phase, provide useful information on the biological activity of tannins in the rumen and also in the whole gastrointestinal tract. In one study using leaves from 37 tree and browse species, it was concluded that samples containing total phenols and tannin levels (measured according to the methods outlined in this manual) up to 4% and 2%, respectively, are not expected to precipitate protein nor cause increases in gas production on the addition of PEG in the in vitro gas production method, and, therefore, are not likely to adversely affect ruminant productivity (Getachew et al., 2002).

3. A spectrophotometric method based on rhodanine (Inoue and Hagerman, 1988) and an HPLC method (Makkar, 2003) for gallotannins has been compared for 38 tree and browse samples. The
specificity and sensitivity of the HPLC method were higher than those of the rhodanine method. However, similar results were obtained by the two methods for the samples containing considerable levels of gallotannins of physiological significance. Using the HPLC method, 24 samples had negligible gallotannin levels (as gallic acid equivalent) (<0.1%), 9 samples contained levels between 0.1 and 0.5%, Dichrostachys cinerea 0.73%, Acacia giraffae and Calliandra calothyrsus 2% and 1.6%, respectively, Eucalyptus macrophylla 3.6% and A. hockii 14%.

4. A new and simpler spectrophotometric method, based on methanolysis of hydrolysable tannins to methyl gallate and followed by its reaction with potassium iodate, has been developed under the project (Hartzfeld et al., 2002). A protein precipitation capacity method, based on the determination of protein by a modified dot blot method using amido black dye was also developed (Hoffman et al., 2002). These methods should be included in the battery of selected methods for use in the second phase.

5. The potential of tannins for use as anthelminths was recognised by the group, but it was not included as a mandatory component in the second phase due to the need to focus efforts and the time constraint.

6. It was agreed that the main thrust in the second phase (December 2001 to May 2004) should be on using the above mentioned tannin assays to develop simple and economically viable approaches to detannify tannin-rich tree leaves and browses, and to exploit the full benefits of tanniniferous plants as animal feed supplements and as strategic feed reserves in situations of fluctuating nutrient supply.

**b) Main achievements from the second phase (given group wise)**

**Ben Salem and co-workers, Tunisia**

1. Wood ash treatment decreased tannin levels substantially (up to 70%) in Acacia cyanophylla leaves. This treatment increased fibre and crude protein digestibility (14 and 8 percentage units, respectively), N-retention (from -0.4 g/day to +2.4 g/day) and microbial protein supply (15 percentage units) in sheep, but it did not increase the efficiency of microbial protein synthesis, probably due to a lack of energy. It is possible to use the same wood ash solution four or five times to deactivate tannins.

2. Early experience of consumption of tannin-containing diets by lambs (up to an age of four months) did not affect both the intake of A. cyanophylla leaves and growth of lambs later in life (up to eight months of age).

3. Chopping, storage under anaerobic conditions, and water treatment decreased tannin levels in A. cyanophylla leaves by 16, 28 and 27%, respectively. Further deactivation of tannins was observed on using these treatments in combination. The highest level of deactivation (75%) was obtained in chopped acacia leaves, which were sprinkled with water and anaerobically stored for at least seven days.

4. Feeding of 100 g of air-dried A. cyanophylla leaves with 200 g of soya bean meal increased daily gain of lambs offered oat hay-based diets by 55%, possibly as a result of protection of soya bean protein from degradation in the rumen by the leaf tannins and an increase in protein availability post-ruminal. To achieve such effects, soya bean meal should be distributed after consumption of the acacia leaves.
Alam and co-workers, Bangladesh

1. Albizia procera leaves contain deleterious levels of total tannins, particularly in the dry season (6.1%). Tannin level in the wet season was 2.7%.

2. Treatments with either calcium hydroxide or potassium carbonate were as effective as polyethylene glycol (PEG) treatment in reducing the content of tannin in A. procera leaves collected in the dry season. The treatments (alkali applied as a spray, 2% of leaf dry matter; PEG applied as a spray, 2 : 1 w/w, PEG : tannin) reduced the content of extractable total tannin by about 93%. This reduction was improved marginally to 97% when the leaves were subjected to drying in the sun for three days after the treatments.

3. Despite the dramatic reductions in extractable tannin content achieved following alkali treatment, in vivo assessment showed that tannins were still present and as active in A. procera leaves treated with calcium hydroxide as in untreated leaves. The growth rates and nitrogen utilisation of goats supplemented with A. procera leaves were similar whether they were fed untreated or calcium hydroxide treated leaves, and when the goats on these treatments were supplemented with PEG, improvement in performance was also similar.

Tangendjaja and Wina, Indonesia

1. Soaking of chopped Acacia villosa leaves in water overnight reduced tannins by 41-76%.

2. Feeding of the soaked A. villosa leaves improved body weight gain of goats by 15%. Feeding cassava flour with the soaked leaves further improved weight gain to 59%.

3. In vitro studies showed that tannins from fresh Calliandra calothyrsus leaves can be used to improve rumen undegradable protein from soya bean meal and tofu waste. The effect was higher with soya bean meal. The recommended proportion is 1 : 1 (w/w on a dry matter basis) for the leaves and the protein source.

Vitti and co-workers, Brazil

1. Shrub and tree forages harvested in the North-East Region of Brazil (e.g. Mimosa hostilis, Astronian urundeuva, Manihot pseudoglaziovii and Anadenanthera macrocarpa) are potential feed resource but are limited in use because of their high tannin content (5-19%). In the dry season, the content of tannins was higher than in the wet season (9-12% versus 2-5%).

2. For Jurema (M. hostilis, Benth), aroeira (A. urundeuva, Engl), maniçoba (M. pseudoglaziovii) and angico (A. macrocarpa) foliage, extractable tannins decreased by 45% following urea treatment, and the increase in gas production on addition of PEG was only 11% suggesting substantial inactivation of tannins by this treatment. This decrease in extractable tannins for oven, sun and shade dried samples was 80, 78 and 77%, respectively. The urea treatment was most effective.

3. The palatability of wood ash treated Leucaena leucocephala leaves when given wet was low.

4. Using 14C-PEG, evidence was presented that PEG is degraded in soil. The extent of PEG degradation was higher for free PEG than in a PEG-tannin complex (after 10 weeks incubation 23 and 11% of 14CO2 was mineralized, respectively). Similarly, the rate of PEG mineralization was lower when it was added to the soil as complexed to tannins rather than as free PEG (0.16 %/d vs. 0.25%/d,
respectively). For 50% of the initial amount of PEG to be mineralized to CO2 in the soil, the complexed PEG would take about 82 days and the free PEG 29 days.

**Smith and co-workers, UK**

1. Dichrostachys cinerea pods were the most widely available and most effective in increasing live-weight gain and reducing kid mortality.

2. Wide differences within and between tree species in pod yield from year to year were recorded.

3. Treatments of the pods with solutions of ash, PEG and sodium hydroxide in vitro were effective in reducing tannin activity as measured using the gas method coupled with PEG addition. In vivo, untreated D. cinerea pods resulted in higher N-retention than PEG or sodium hydroxide treated pods, possibly because the fibrous diet resulted in a shortage of energy for potential rumen microbial synthesis when the protein supply was enhanced.

4. The information on collection, storage and use of pods has been disseminated to farmers via participation in trials, meetings, the media and Farmer Field Schools.

**Yildiz and co-workers, Turkey**

1. Addition of PEG at 5 or 10% of oak (Quercus hartwisiana) leaves (w/w, dry matter basis) resulted in an increase in protein digestibility when added to 185 g of oak leaves in a medium quality basal diet. However, this effect was not observed when the consumption of oak leaves was increased to 375 g.

2. Inclusion of PEG in the diets containing oak leaves: a) increased microbial protein supply, but was not translated into body weight gain; b) did not change leptin levels, suggesting there was no effect on fat reserves from feeding oak leaves with or without PEG; and c) did not affect LH pulsatility and sign of oestrus.

**McNeill and co-workers, Australia**

1. A simple in vivo method, based on isotopically labelled protein, that ranks different tannins on their abilities to release protein for digestion was developed. Use of a 15N label proved ineffective in the methodology due to poor precision associated with the high natural abundance of 15N relative to the levels of enrichment achieved.

2. By contrast, in a parallel trial, the use of 125I-labelled protein highlighted the value of the in vivo method. By using 125I-labelled protein, in vivo rankings of tannins correlated with the ability of the same tannins to bind protein in vitro. Higher release rates of 125I-protein in vivo compared to in vitro indicated that the ability of tannins to release protein is greater than is suggested by in vitro studies.

3. Oral dosing of 125I-protein ranked tannins, on their ability to release protein, similarly to that observed from post-ruminal dosing of 125I-protein via an abomasal cannula. Hence, tannins can be assessed on their ability to bind protein in vivo without the need to cannulate animals.
Acamovic and co-workers, UK

1. Interaction between non-starch polysaccharide (pectin), rumen microbes and tannins in vitro was demonstrated using 15N labelled microbes. Pectin, tannins and PEG influenced the attachment of rumen microbes to cellulose, in vitro. These interactions influence degradation of cellulose. The interaction of tannins with non-starch polysaccharide may explain some of the variation in the effects of different tannins in the presence of different carbohydrates in monogastric and ruminant animals.

2. Tannins from forage legumes (Lotus spp.) protected protein in Lotus from degradation in the rumen, but reduced overall digestibility showing incomplete release of protein bound to tannins in the lower gastrointestinal tract. Similar effects were seen when quebracho, mimosa and myrabolam tannins, and tannic acid were added to diets containing lupin seed, peas, soya and chickpeas, where some protection was afforded in the rumen but digestibility of protein and amino acids was reduced in the lower gut. The effect is dependent on tannin type, concentration and seed species and is likely to be influenced by the type and content of non-starch polysaccharide.

3. A model for assessing the digestibility of nutrients in the lower gut of ruminants was developed. It was based on the precision feeding method for poultry and consisted of quantitatively feeding (by gavage) suspensions of tannins, proteins, ground feedstuffs or mixtures thereof, directly into the crop of chickens. The procedure gave a high correlation (r²=0.86) with the mobile bag technique for evaluation of post-ruminal digestibility of Lotus, pea, lupin, chickpea and soya protein, but there was considerable variability in the chick model probably due to the low amounts of some tanniniferous material that were given (e.g. Lotus spp., which was difficult to administer in adequate amounts due to its high fibre content.

4. Tannins when included in the diet influenced the microbial profile in the gut of chicks. This was also evident in vitro studies for poultry and ruminants.

Makkar and Mlambo, Austria

1. The in situ tannin binding assay method based on 14C-polyethylene glycol (14C-PEG) binding has been simplified by reducing both the amounts of feed sample and 14C-PEG by a factor of 10, enabling analysis of a greater number of samples at a lower cost. In addition, a new approach for estimating the level of PEG binding to tannin-containing foliage without the use of a tannin-free substrate to correct for non-specific binding has been proposed. Further studies are required to better understand the biological significance of the values obtained by this method.

Hagerman and co-workers, USA

The 125I based radiolabelled method has been simplified to eliminate the more difficult procedures of relatively high speed (13 000 g) centrifugation and manipulations of small volumes of radiochemical solutions by binding the radiolabelled protein to tannin immobilized on a paper disk. The amount of radioactivity on the paper disk is determined by gamma counting (Henson et al., 2004).


Impact of the CRP

A Laboratory Manual on Tannin Assays published through this project has become a reference manual for use in various laboratories around the world. It has also been used in following IAEA training workshops:

a) IAEA/RCA Regional Training Workshop on 'Nuclear and related methodologies for quantification of tannins in feedstuffs', under TC Project RAS/5/035, 1-12 December 2003, Faisalabad, Pakistan. Number of participants: 17 (from 12 Asian countries)

b) Training to over 30 scientists from developing countries at the University of Hohenheim, Stuttgart, Germany over the last 4 years.

As a result of this CRP, Member States proposed TC Projects in this area and five TC Projects have been initiated on the utilization of tree foliage as livestock feed.

In a new CRP (D3.10.24), three Research Contract Holders are attempting to use tannin-containing tree and browse leaves for reducing methane emission. These approaches are also being considered under a new Regional TC Project (RAS/5/044) for reducing nitrogen discharge to the environment by livestock.

Effectiveness of the CRP

The CRP has been successful in achieving the specific objectives. New tannin assays have been developed, and the new and old assays have been validated. Tannin assay(s) predicting the biological value of tannin-rich feeds when fed to livestock have been identified.

Approaches for detanninification of browses have been developed. Amongst the approaches used, treatments with alkali, urea, or polyethylene glycol were promising.

The CRP has also opened new exciting avenues for future research.
Relevance of the CRP

The utilization of unconventional feed resources holds great relevance to developing countries. The main constraint to livestock development in these countries is the scarcity and fluctuation of the quality and quantity of the year-around animal feed supply. There is a serious shortage in animal feeds of the conventional types such as soybean, cotton seed and groundnut meals, etc. In addition, the human population is increasing at a high rate and the arable land is decreasing due to soil degradation, urbanization and industrialization. The grains are mostly used for human consumption. Novel approaches developed for utilization of tree leaves as livestock feed, developed through this project, would bridge the wide gap between supply and demand of feeds in developing countries.

Resulting publications from the CRP


9. A special issue of the Animal Feed Science and Technology journal, Vol no. 122, issue no. 1-2, August 12, 2005 containing following articles:
Title of special issue: Predicting and Improving the Safety and Efficiency of Feeding Ruminants on Tanniniferous Tree Foliage

Preface

Use of nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on tanniniferous tree foliage: achievements, result implications, and future research, p. 3-12. H.P.S. Makkar

In vivo assessment of the ability of condensed tannins to interfere with the digestibility of plant protein in sheep, p. 13-27. S. M. Andrabi, M.M. Ritchie, C. Stimson, A. Horadagoda, M. Hyd and D.M. McNeill,

Calibration and validation of the 14C-labelled polyethylene glycol-binding assay for tannins in tropical browse, p. 29-40. V. Mlambo and H.P.S. Makkar

The influence of tannin, pectin and polyethylene glycol on attachment of 15N-labelled rumen microorganisms to cellulose, p. 41-57. M.H.L. Bento, T. Acamovic and H.P.S. Makkar

Effect of early experience and adaptation period on voluntary intake, digestion, and growth in Barbarine lambs given tannin-containing (Acacia cyanophylla Lindl. foliage) or tannin-free (oaten hay) diets, p.59-77. H. Ben Salem, A. Nefzaoui, H.P.S. Makkar, H. Hochlef, I. Ben Sale and L. Ben Salem

Effects of chopping, and soaking in water, hydrochloric acidic and calcium hydroxide solutions on the nutritional value of Acacia villosa for goats, p.79-92. E. Wina and B. Tangendjaja

Wood ash treatment, a cost-effective way to deactivate tannins in Acacia cyanophylla Lindl. foliage and to improve digestion by Barbarine sheep, p. 93-108. H. Ben Salem, Sourour Abidi, H.P.S. Makka and A. Nefzaoui

Attempts to deactivate tannins in fodder shrubs with physical and chemical treatments, p. 109-121. H. Ben Salem, L. Saghroun and A. Nefzaoui


Digestion and body weight change in Tuj lambs receiving oak leaves (Quercus hartwissiana) with and without PEG, p. 159-172. S. Yildiz, I. Kaya, Y. Unal, D. Aksu Elmali, S. Kaya, M. Cenesiz, M. Kaya and A. Oncuer

Benefit from the association of small amounts of tannin-rich shrub foliage (Acacia cyanophylla Lindl.) with soya bean meal given as supplements to Barbarine sheep fed on oaten hay, p. 173-186. H. Ben Salem, H.P.S. Makkar, A. Nefzaoui, L. Hassayou and S. Abidi

(In addition 10 conference papers/abstracts were published by the group)