Final RCM on Use of Nuclear Techniques to Develop Simple Tannin Assays for Predicting and Improving the Safety and Efficiency of Tanniniferous Forage (D3.10.22)

Report

This meeting was held from 7 to 11 June 2004 in Kars, Turkey.

Eleven Research Contract, Agreement and Technical Research holders participated. The objective of the meeting was to evaluate the work conducted in the second phase of the project in which the main aim was to develop strategies for detanninification of tree and shrub leaves and to use the promising ones to enhance the nutrient availability from these feed resources. The results from this programme will be published in a special issue of the Animal Feed Science and Technology journal, which is likely to be published in early 2005.

Main achievements from the second phase of the project are as follows:

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 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. Highest 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 soyabean meal increased daily gain of lambs fed on oaten hay-based diets by 55%, possibly as a result of protection of soyabean protein from degradation in the rumen by the leaf

tannins and increase in protein availability post-ruminally. To achieve such effects, soyabean meal should be distributed after entire consumption of acacia leaves.

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 ^{15}N label proved ineffective in the methodology due to poor precision associated with the high natural abundance of ^{15}N relative to the levels of enrichment achieved.

2. By contrast, in a parallel trial, the use of ^{125}I-labelled protein highlighted the value of the *in vivo* method. By using ^{125}I-labelled protein, *in vivo* rankings of tannins correlated with the ability of the same tannins to bind protein *in vitro*. Higher release rates of ^{125}I-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 ^{125}I-protein ranked tannins, on their ability to release protein, similarly to that observed from post-ruminal dosing of ^{125}I-protein via an abomasal cannula. Hence, tannins can be assessed on their ability to bind protein *in vivo* without the need to cannulate animals.

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 goat by 15%. Feeding cassava flour with the soaked leaves further improvement weight gain to 59%.

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

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 wet season was 2.65%.

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 were also similar.

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 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 ^{14}C-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 (23 and 11% of $^{14}CO_2$ mineralized, respectively, after 10 weeks incubation). Similarly, the rate of PEG mineralization was lower when it was added to the soil as complexed to tannins (0.16 %/d vs. 0.25%/d). For 50% of the initial amount of PEG to be mineralized to CO_2 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 the 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.

Mlambo and Makkar, Austria

1. The *in situ* tannin binding assay method based on ^{14}C-polyethylene glycol (^{14}C-PEG) binding has been simplified by reducing both the amounts of feed sample and ^{14}C-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.

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 it did not translate into body weight gain; b) did not change leptin levels, suggesting no effect on fat reserves from feeding oak leaves with or without PEG; and c) did not affect LH pulsatility and sign of oestrus.

Acamovic and co-workers, UK

1. Interaction between non-starch polysaccharide (pectin), rumen microbes and tannins *in vitro* was demonstrated using ^{15}N 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 (r^2=0.86) with the mobile bag technique for evaluation of postruminal digestibilty 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 given (e.g. Lotus spp., which was difficult to administer in adequate amounts due to high fibre content.

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

Hagerman and co-workers, USA

The ^{125}I 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, Phytochem. Anal. 15, 159-163).

Participants in the Second RCM

Name	Address	Tel, fax, e-mail
Dr. M. Raisul Alam	Dr. M. Raisul Alam Department of Animal Science Bangladesh Agricultural University Mymensingh 2202, Bangladesh	Tel: 008809153971 Fax: 008809155810 E:mail: mralam@royalten.net
Dr. Adibe Luiz Abdalla	Mr. Adibe Luiz Abdalla Centro de Energia Nuclear na Agricultura CENA/USP Av. Centenario 303 Caixa Postal 96 CEP 13400-970,Piracicaba, Sao Paulo, The Federative Republic of Brazil	Tel: +55 19 34294730 Fax: +5 19 34294610 E-mail: abdalla@cena.usp.br
Dr. Sedat Yildiz	Dr. Sedat Yildiz Kafkas Universitesi, Veteriner Fakultesi Fizyoloji Anabilim D. 36040 Kars, Turkey	Tel: ++(90) 474 2426800-1165/1062 Fax: ++(90) 474 2426846 E-mail: yildizsedat@hotmail.com
Dr. Budi Tangendjaja	Dr. Budi Tangendjaja Research Institute for Animal Production, Balai Penelitian Ternak P.O. Box 221, Bogor 16002 Indonesia	Tel: 62251240752 Fax: 62251240754 E-mail: budtang@bogor.wasantara.net.id
Dr. Hichem Ben Salem	Dr. Hichem Ben Salem INRAT, Laboratoire de Productions Animales et Fourragères Rue Hédi Karray, 2049 Ariana Tunisia	Tel: ++216 71230024 Fax: ++216 71752897 E-mail: bensalem.hichem@iresa.agrinet.tn
Dr. David McNeill	Dr. David McNeill Senior Lecturer in Ruminant Production Faculty of Veterinary Science The University of Sydney Camden, NSW 2570 Australia	Tel: 61246550633 Fax: 0061246552374 E-mail: d.mcneill@camden.usyd.edu.au
Dr. Tim Smith	Dr. Tim Smith School of Agriculture, Policy and Development, University of Reading, Early Gate, P.O. Box 236 Reading Berks, RG6 2AR Berkshire UK	Tel: 00441249714455 Fax: 0044118352421 E-mail: timsmith2@btopenworld.com timothy.smith@btinternet.com
Dr. A. Ray Till	Dr. A. Ray Till Department of Agronomy and Soil Science, University of New England Armidale, NSW 2351 Australia	Tel: (612 67) 732687 Fax: (612 67) 733465 E-mail: atill@pobox.une.edu.au
Dr. Thomas Acamovic	Dr. Thomas Acamovic Dept of Biochemistry and Nutrition SAC, Auchincruive, AYR KA6 5HW Scotland, UK	Tel: +44 (0)1292525103 Fax: + 44 (0)1292525098 E-mail: t.acamovic@au.sac.ac.uk
Dr. Harinder Makkar	Animal Production and Health Section International Atomic Energy Agency P.O. Box 100, Wagramerstr. 5 A-1400 Vienna, Austria	Tel: +43-1-2600.26057 Fax: +43-1-26007 E-mail: H.Makkar@iaea.org