

Final Technical Report

NRIL Contract Number: ZC0024

DFID Contract Number: R6421

Project Title:

Anti-nutritional components of tropical forage legumes: mechanisms by which secondary metabolites exert their effects and the means by which they can be ameliorated

RNRRS Programme: Livestock Production Programme

Production System: Semi-arid

Project Leader/Institution: Drs Mike Theodorou and Phil Morris/IGER

Counterpart Institution: Drs Emyr Owen and Carlos Lascano/Reading University/CIAT

Official Start Date: 1st Sept 1995

Official End Date: 31st Aug 1998

Actual Start Date: 31st March 1996

Actual End Date: 30th April 1999

Budget: £150,000

NB. There was a significant delay in the actual start date of this project while suitable candidates was recruited through CIAT. We were particularly keen to recruit from Latin America and ultimately we succeeded with the recruitment of Rolando Barahona and Solange Sanchez as documented in previous reports. However, financial resources were requested from NRI from the Official start date in order to permit travel, planning and recruitment procedures between the project initiators in Colombia and the UK.

Executive Summary

The lack of shrub legume species adapted to acid infertile soils in cattle producing regions of the semi-arid tropics has long been recognized as a major constraint against sustainable livestock production. Some legume species are recognized and do show promise but others that are high in protein also contain high levels of tannins, which might limit their value as a feed for livestock production. This is usually because of the negative effect of tannins on intake, digestibility, and nitrogen utilization in ruminants.

In order to select for tropical legumes low in tannins and to define more appropriate strategies for their utilization in farming systems, there is a need to understand how these and other anti-nutritional factors affect the nutrition of ruminants. Research thus far has provided much insight into these matters, but also directs us to the need for more detailed information. This research project investigates the key mechanistic issues related to tannin molecular structure and the role played by tannins in altering the rate and extent of digestion of plant biomass in the rumen.

Background

Seasonal scarcity of feed resources, particularly for ruminants, is a widespread constraint in tropical animal production systems. Scarcity is often exacerbated by the poor quality of the feeds that are available. The introduction and wider use of superior leguminous shrubs and trees into existing arable/grazing systems has the potential to improve feed supply and particularly quality in times of scarcity, e.g., during the dry season. There are therefore significant benefits that could arise if farmers were able to use shrub and tree legumes in a sustainable way as feed resources for livestock. An account of any significant research previously carried out and a description of the importance of the researchable constraint(s) that the project sought to address is given in the final report. This report is not available until the actual end-date of the project.

Project Purpose

The purpose of this project is to increase our understanding of the effects and importance of environment on anti-quality and anti-nutritive factors, especially condensed tannins, and their significance in livestock feeding systems. The information obtained is intended to contribute to our comprehension of how best to select leguminous shrubs and trees for better nutritive value. A full account of the project purpose and how it addresses identified development opportunities and identifies constraints to development is provided in the final report.

Research Activities

This research project has five main objectives, viz:

1. The influence of condensed tannins from a range of forage legumes on the fermentation kinetics of plant cell walls.
2. The influence of condensed tannins from a range of forage legumes on the kinetics of hydrolytic enzyme (plant cell wall degrading enzymes) from various axenic and mixed populations of rumen micro-organisms.
3. Effect of development and environment on the production of secondary metabolites.
4. The anti-nutritive components of *Cratylia argentea*.
5. Tanninases of plant and microbial origin and mechanisms of protection against tannins.

Rolando Barahona conducted the first three objectives at IGER and CIAT as part of his PhD programme. Alison Brooks, a senior research assistant at IGER, conducted objective four with materials provided by CIAT. Solange Sanchez, a Colombian employee of CIAT, conducted objective five at IGER and CIAT.

Outputs

All the anticipated outputs were achieved. These included research reports and publications as outlined in the original proposal and the transfer of gas production technologies to CIAT and IGFR (India). Barahona's thesis and a separate report covering Objectives 5 and 6 form the main report. A summary of recommendations regarding the value of feeding tanniniferous forages is presented below, followed by criteria for the selection of tanniniferous forages for breeding purposes.

Findings of particular relevance were:

1. The presence of condensed tannins has an overall negative relationship with in vitro degradability at high concentrations but does not necessarily result in a negative impact on animal productivity and can improve it at low concentrations.
2. The nutritional impact of condensed tannins should not be based solely on their concentration in plant tissue. Tannin structure (hydroxylation level and molecular weight) also needs to be considered, as these are equally important.
3. The effects of tannins on rumen enzyme activity is a function of the characteristics of both protein and condensed tannin amount and structure and may also be related to the living or dead state of the plant tissue on ingestion (i.e. grazed and fresh versus conserved and dead plant biomass).
4. The structure of condensed tannins varied when *Desmodium ovalifolium* was grown under different environments, even for the same cultivar/variety.
5. In vitro degradability is a function of the entire chemical composition of the plant and it is therefore important not to make assumptions where individual factors have been studied in isolation.
6. In vitro experiments should therefore be used as probes for detailed examination of specific aspects of a scientific problem and in making predictions to test in vivo.
7. Binding of tannins to cell wall carbohydrates may be as significant in relation to reducing cell wall digestibility as the effects of tannins are on reducing the rate of protein degradation.

Criteria for the selection of tanniniferous forages for breeding purposes.

One of the objectives of the Tropical Forage Project in CIAT and other similar programs is to develop multipurpose shrub legumes with broad adaptation to biotic and abiotic constraints in target agro-ecosystems of the tropics. Shrub legumes selected for adaptation to environmental constraints are then

screened for forage quality and subsequently evaluated with animals in researcher- led and farmer- led trials. A major contribution of this Project was to transfer new techniques to CIAT useful for assessing forage quality (i.e. gas production) and to provide new insights into the manner by which condensed tannins may affect forage quality of tropical legumes. The results demonstrated that both concentration and structure of condensed tannins varied with environment. This information will be key to facilitate the selection and targeting of shrub legume genotypes to different locations and production systems in the tropics. As a result new forage quality selection criteria can be or have been added to on going efforts to select shrub legumes for smallholder systems in the tropics.

Recommendations from the work for breeding tanniniferous forages were as follows:

1. Tannin amount in a species is not a predictor of performance of that species. Tannins from different species have very different effects at the same concentration due to differences in structure.
2. Low level of tannins of the correct structure can be beneficial in increasing animal performance. Optimum concentration should be determined for each species.
3. Environmental growth conditions (soil, growth temperature, drought) affect both tannin amount and tannin structure even in the same variety, resulting in unpredictable tannin levels and structures between harvests. This is particularly the case with perennial species.
4. Species with a high protein content reduce the negative impact of high tannin levels as it is the amount of tannin in excess of that capable of being bound to plant protein which is then available for binding to salivary proteins, reducing intake, or to rumen enzymes, reducing fermentation.
5. Breed plants with less variable responses to environmental regulation of tannin accumulation.
6. Breed plants with lower levels of tannins (<5%), and with low tannin hydroxylation levels (high cyanidin: pelargonidin ratio) and with high leaf protein.

The following agronomic and quality associated selection criteria are traits currently examined by CIAT and partner institutions in LAC for the selection of new shrub legume alternatives in multi-locational trials:

Agronomic Selection Criteria

1. Seasonal edible forage (Leaf and fine stems in the wet and dry season, following 6 to 8 weeks regrowth after cutting) in low fertility acid and moderately acid soils in humid and sub-humid environments.
2. Drought tolerance (leaf retention in the dry season)
3. Rhizobium requirements (response to N and to inoculation with selected rhizobium strains)
4. Seed yield
5. Seed quality

Quality Selection Criteria

1. In vitro (gas production) dry matter and cell wall (NDF) digestibility
2. Cell wall concentration (NDF content) and fiber composition (non- starch polysaccharide constituents- xylose, uronic acids)
3. Presence or absence of hydrolyzable (gallic acid)
4. Presence or absence of condensed (proanthocyanidin) tannins using Butanol- HCL
5. Monomer composition of condensed tannins as indicative of ability of tannins to bind protein and influence digestibility
6. Astringency of condensed tannins (radial diffusion assay using Rubisco as test protein)
7. Intake, in vivo digestibility and N utilization, (feeding forge of legumes grown in contrasting sites to small ruminants housed in metabolism crates)

Given that the work carried out in this project was all carried out *in vitro*, there is still a need to validate in vivo the significance of the major findings. For example, the impact on animal production of different monomer composition of condensed tannins needs to be determined. Similarly, the significance of protein- condensed tannin specificity and the significance of changes in tannin structure with changes in environmental conditions require elucidation. Currently we are examining with sheep the significance of differences in monomer composition between provenances of *Calliandra*, which have been associated in vitro with astringency (higher delphinidin: cyanidin ratio greater astringency).

Contribution of Outputs

We have generated a substantial amount of novel research information and this will have an impact on our comprehension of forage quality and the effects of tannin on livestock. The outcome of the project will be disseminated initially within the scientific and educational community via professional contact, reports,

abstracts and scientific publications and presentations at scientific meetings. There is a training element to this research from which the PhD student and recipient country will benefit.

Projected papers from the work are as follows: we will circulate these prior to their submission to our sponsors for approval.

1. **Barahona, R, Lascano, C.E., Theodorou, M.K., Morris, P. Owen, E. and Narvaez, N.** Concentration and distribution of condensed tannins, composition of non-starch polysaccharides and in vitro fermentation of mature and immature tropical forage legumes. Tropical Agriculture ?
2. **Barahona, R, Sanchez, M.S., Lascano, C.E., Theodorou, M.K., Morris, P. and Owen, E.** Effect of condensed tannins from six tropical legumes on the activity of fungal fibrolytic enzymes. (Tropical Forage Journal ??)
3. **Morris P, Barahona R. and Theodorou M.K.** Determination of proanthocyanidin ratios in condensed tannins by diode array HPLC of anthocyanins following acid hydrolysis. Phytochemical Analysis
4. **Barahona, R, Lascano, C.E., Theodorou, M.K , Morris, P. and Owen, E.** Comparison of the effects of condensed tannins from tropical forage legumes on the fermentation of *Festuca arundinacea* cell walls: relative nutritional implications of bound and soluble condensed tannins. JSFA?
5. **Barahona, R, Lascano, C.E., Theodorou, M.K., Morris, P** Monograph on tannins in tropical forage legumes. CAB Int.
6. **Barahona, R, Lascano, C.E., Theodorou, M.K., Morris, P.** Determination of the molecular weight and proanthocyanidin ratios in a range of condensed tannins from temperate and tropical forage species by GPC and diode array HPLC. (Phytochemical Analysis or JSFA).
7. **Barahona, R, Lascano, C.E., Theodorou, M.K., Morris, P. Owen, E. and Narvaez, N.** Environmental effects on condensed tannin accumulation by *Desmodium ovalifolium*. (Phytochem or Crop Science).

With respect to item 5 above, discussions with CABI have resulted in their wishing to commission the monograph, if there is a commitment to pre-purchase a number (50 – 100) of the books (the price is yet to be set).