The potential for tree forage supplements to manipulate rumen protozoa to enhance protein to energy ratios in ruminants fed on poor quality forages.

by R.A. Leng, S.H. Bird, A. Klieve, B.S. Choo, F.M. Ball, G. Asefa, P. Brumby, V.D. Mudgal, U.B. Chaudhry, S.U. Haryono and N. Hendratno

INTRODUCTION

It is probable that there are large numbers of forages with secondary plant compounds that may manipulate digestive function in the rumen to the benefit of the animal. There is a need to survey such forages as they could represent cost-effective supplements for ruminants on poor quality pastures/crop residues. The first priority is to balance the rumen to allow an effective use of the basal diet which requires fermentative digestion. The main point to be emphasised is the need to extract, by microbial digestion, the maximum nutrients from the basal diet. At the same time,the growth efficiency of the rumen microbes must be optimised to provide the animal with a high protein (amino acids) to energy ratio (P/E ratio) in the nutrients absorbed. This can be achieved by supplementation with a combination of non-protein nitrogen and minerals. Once this has been achieved then the animal appears to need a small amount of extra supplementary protein that escapes to the lower tract to optimise the P/E ratio in the nutrients absorbed and maximize the animal's efficient use of the diet. The major problem for most developing countries is the availability of the resources needed to provide these supplements. Tree forages, and in addition seeds and pods, are potential sources of supplements that could provide an array of minerals and

soluble nitrogen for the rumen microorganisms. The same materials can potentially also provide the bypass protein either naturally, when they contain tannins, or when induced by simple processing.

The P/E ratio in the nutrients absorbed by ruminants on forage-based diets depends largely on the microbial growth efficiency in the rumen and on the amount of bypass protein supplement included in the diet.

It has been demonstrated recently that the presence of rumen protozoa reduces the protein to energy ratio in the nutrients absorbed (Bird, 1991) through:

- their predation of bacteria in the rumen decreasing the flow of bacterial cells to the intestines (Coleman, 1975).
- their apparent retention in the rumen (only 30% of the protozoal cells actually move to the intestines) (Weller & Pilgrim, 1974; Leng, 1982).
- their ability to digest particulate protein in the rumen and thus convert protein and amino acids to VFA with a net loss of dietary protein (Ushida and Jouany, 1986). Bird (1991), in a recent review article, summarised the beneficial effects of removal of protozoa and maintenance of the unfaunated state on rumen production as follows:

"With respect to the protein economy of the animal the advantages of removing the protozoa from the rumen and maintaining the defaunated state are:

- increased utilisation of protein nitrogen in the rumen
- increased availability of dietary protein for intestinal digestion.
- an increased yield of microbial protein from rumen fermentation.
- an increased proportion of the pool of rumen microbes flowing to the intestines (a reduced loss of microbial protein through degradation in the rumen)."

It appears that it can now be stated definitely that there will be more microbial and dietary protein available to the ruminant when protozoa are absent from the rumen. It follows therefore that in the absence of protozoa in the rumen there is an increase in the total protein available relative to the VFA produced and absorbed from the rumen. The improved P/E ratio will benefit the animal where it is fed diets low in true protein. In such a feeding situation, the increased P/E ratio will

increase the efficiency of nutrient utilisation by the animal. Thus removal of protozoa or a decrease in protozoal density in the rumen can be expected to increase ruminant production under most feeding conditions pertaining to roughage fed ruminants (Leng, 1990).

Following considerable research that tested the above statements, research began about 1980 to seek natural defaunating agents.

THE SEARCH FOR ANTI-PROTOZOAL FORAGES

Research commenced with the establishment of a bioassay system to assess the effects of various additives on the viability of rumen protozoa in culture medium.

Culturing and maintaining protozoa *in vitro*

A stock culture protozoa isolated from the rumen was maintained *in vitro* in caudatum-type salt media using techniques of Coleman (1978) as follows:

Four Hungate roll tubes containing 3ml of fresh media were prepared. To each tube of fresh media a mixed protozoal population was added. The cultures were fed daily with 0.1mg dry ground lucerne and 0.05 ml of corn starch suspension, 1.5% (w/v), the tubes were gassed with CO2, sealed tightly with a rubber bung and incubated at 39°C. After three days, 3ml of fresh media was added to each tube, and culturing was continued for a further 4 days. After this time, cultures were examined for protozoal growth and were transferred to 50 ml conical flasks with 25ml fresh media. The daily amount of feed was increased to 1.5mg lucerne and 0.2ml of the starch suspension.

Four stock cultures were maintained in this way. These cultures were found to consist of mixed *Entodinia* spp., as other genera present in the original rumen fluid rapidly disappeared from the mixed culture.

From this point onward each stock culture was maintained by removing 15ml of the culture media every three days and replacing this with fresh media.

Anti-protozoal assay

60ml of protozoal culture was available twice a week for assaying material for potential anti-protozoal toxins. Protozoa were fed with the starch suspension and a mixture of the test forage and lucerne.

Assay of anti-protozoal effects of forages

A number of forages were obtained; these had been dried at 60°C and ground through a 1mm sieve. All samples were assayed, mixed with the lucerne and the nutrients added to the cultures daily. In most cases, the test forages were at levels to replace 1, 10 or 100% of the lucerne.

Fresh caudatum-type salt media was prepared and 8ml of media was added to each of a number of Hungate roll tubes which were incubated at 39°C for at least 1 hour prior to inoculation. To each tube, 2ml of well mixed stock culture was injected and fed with 0.1mg of the mixture of lucerne and test forage and 0.1ml starch (1.5 (w/w) suspension. The tubes were incubated at 39°C for three days. On the fourth day, a 1ml sample of well mixed culture was taken and added to 1ml of formal saline (10% (v/v) formaldehyde; 0.9% (w/v) NaCl), with a few drops of iodine solution. This solution (0.1ml) was placed on a microscopic slide and all the protozoa present were counted using a dissecting microscope at 40x magnification. The protozoa count multiplied by 20 gave the number of protozoa in 1ml of the original culture.

Results of the anti-protozoal assay

The number of protozoa cultured, when starch plus a fibre source of either 100% lucerne or 100% *Leucaena leucocephala* was included, were similar ($3.5x10^3$ respectively).

In preliminary studies, a sample of powdered leaves of *Lotus pedunculatus* and also *Acacia dealbata* showed anti-protozoal activity. The numbers of protozoa in culture were depressed by increasing the *Lotus* proportion of the forage source in the growth medium. *Lotus pedunculatus* forage at 100% of the forage apparently killed all protozoa in the medium.

Anti-protozoal properties of forages

The results of assays of forages which showed anti-protozoal activity are shown in the following tables. Forages that stimulated protozoal growth, had no effect on the number of protozoa in culture, or inhibited protozoal growth are shown in Tables 1, 2 and 3 respectively.

TABLE 1. **Forages that apparently promote protozoal growth**

Name of plant	Forage substitution rate for lucerne leaf powder in the substrate added to incubation medium	
	1%	10%
	protozoa numbers as % of control	
Acacia leveocloala	121	109
Brachychiton australis	111	113
Casuarina cunninghamia	114	130
Albizia lebbeck	112	102
Commersonia bastramia	124	106
Desmanthus unantiem	112	114
Gliricidia sepium	113	138
Samanea saman	110	114
Casuarina cunninghamiana	123	130
Cajanus cajan	126	104
Desmanthus virgatus	112	108
Soya bean (*Glycine max*)	118	122
Desmodium intortum (green leaf)	139	114
Centrosema viaginium	110	142
Macroptilium atropurpureum	112	121
Macroptilium lathyroides	111	114
Cassia brewsteria	112	115
Gejera parviflora	122	111

TABLE 2. **Forages with no effects on protozoal growth**

Name of plant	Forage substitution rate for lucerne leaf powder in the substrate added to incubation medium	
	1%	10%
	protozoa numbers as % of control	
Casuarina cristata	107	128
Alphitonia excelsa	108	123
Acacia excelsa	91	85
Acacia harpophylla	109	113
Brachychiton populeus	109	98
Acacia salicina	94	88
Acacia glavocarpa	109	143
Acacia bidwilli	107	108
Alphiltonia petriei	106	124
Albizia chimensis	108	90
Acacia harpophylla	102	110
Desmanthus virgatus	96	99
Sesbania sesban	107	130
Indigofera schemperi	98	97
Macroptilium lathyroides	95	73
Arachis hypogea	106	117

Identification of a potent anti-protozoal component in *Enterolobium cyclocarpum* leaf

Enterolobium cyclocarpum was identified as the most likely forage to be used as a rumen manipulator. It was quickly established that all ruminants would consume the leaf materials after an initial introductory period. Over the next 6 years, a series of trials were carried out to test the value of *Enterolobium* spp. as a potential rumen anti protozoal agent and the response of animals to consumption of small quantities.

PRODUCTION TRIALS USING ANTI-PROTOZOAL LEAF FORAGES

The following experiments illustrate the potential usefulness of this material in small quantities to stimulate ruminant productivity.

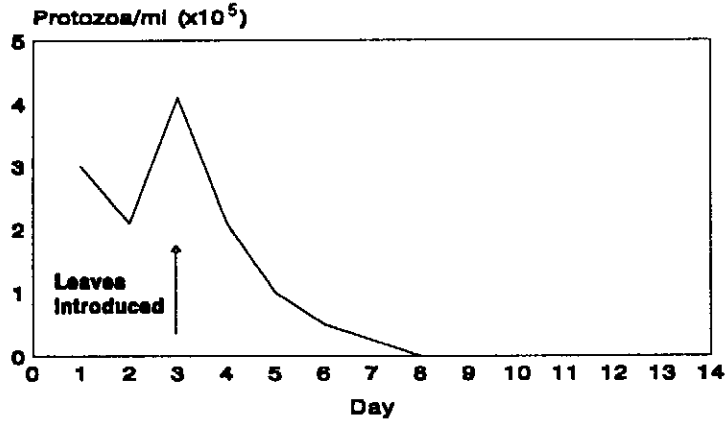
TABLE 3. **Forages with apparent anti-protozoal properties at 1% and 10% substitution rate for lucerne pasture of the in a culture medium**

Name of plant	Forage substitution rate for lucerne leaf powder in the substrate added to incubation medium	
	1%	10%
	protozoa numbers as % of control	
Acacia deanei	38	79
Acacia crassa	38	74
Acacia semilunata	72	77
Acacia spectabilis	56	73
Acacia chinchillensis	75	103
Desmanthus intortum	63	73
Centrosema pubescens	78	84
Casuarina ronophlora	79	111
Black wattle	51	8
Fern leaf	81	97
Indigofera schemperi	75	75
Macroptilium lathyroides	88	100
Leucaena leucocephala	69	99
Vigna parteri	55	86
Lotononis bainesii	75	78
Desmodium uncinatum	78	108
Aeschynomena falcata	82	99
Cassia rotundifolia	88	106
Enterolobium cyclocarpum	0	0
Enterolobium timbouva	0	0

Enterolobium cyclocarpum*: Anti-protozoal activity *in vivo

Four rumen fistulated buffaloes in Indonesia (Batan) (350kg LWt) were fed cut/carry grass with minerals. Samples of rumen fluid were removed daily through the rumen cannula for 3 days prior to the commencement of feeding the leaf forage. The buffaloes were then given 500 g of fresh leaves (30% DM) daily for seven days. The effects of feeding the leaf material is shown in Figure 1. Although rumen protozoa were markedly depressed over the 7 days when these leaves were provided, protozoa were not removed totally and rapidly returned to normal densities in rumen fluid following cessation of feeding of the leaf material.

FIGURE 1. **The effects of feeding 500g of fresh leaves from the tropical tree *Enterolobium cyclocarpum* to buffaloes given cut and carry grass in Indonesia**



In India (CIRB, Hisar), a trial was conducted feeding young buffaloes on chopped wheat straw based diets. Dried leaves (375g) of *Enterolobium timbova* fed for 3 days apparently totally removed protozoa from the rumen but defaunation was not complete (or security failed) as periodically protozoa appeared in the rumen within one week after the cessation of feeding the leaf materials. In Australia, fistulated sheep were fed small amounts of *Enterolobium cyclocarpum* leaves at various rates in a diet of oaten chaff. The results on protozoal densities in the rumen indicated that *Enterolobium* leaf materials at 75 g/d could reduce protozoal numbers in rumen fluid to negligible numbers but that it was most difficult to remove them entirely and they returned to normal population densities within 4 days of cessation of feeding the leaf-meal. It was concluded that it was unlikely that defaunation in ruminants would be effected by feeding the leaf meal from this tree.

Effects of leaf forage defaunation in buffaloes on productivity

No research has been reported of the effects of the presence of protozoa in the rumen of large ruminants fed poor quality forages. The potential for manipulating rumen protozoa and stimulating productivity is illust-

rated by research undertaken in the Central Institute for Research in Buffaloes, Hisar, India, which was carried prior to using the leaf meal as a potential rumen modifier.

Forty-eight Murrah buffalo heifers (average 105 kg live weight) were allocated to one of eight treatments (4 diets x 2 fauna states). Feed intake and live weight changes were monitored over a 106-day period. A basal diet of urea ensiled wheat straw, fresh green cut/carry grass (3 kg/day) and minerals was supplemented with groundnut cake (GNC) at four levels (g/day); 0, 250, 500 and 750. The buffalo were defaunated by dosing with a surface active agent.

The fauna-free condition which was created by drenching the animals with a surface active agent (Bird and Leng, 1978) increased live weight gain and improved feed conversion efficiency of heifers on all diets with the response being greatest in animals receiving the most GNC supplement (Table 4). Supplementing the basal diet with 750 g/day GNC increased the growth rate of the faunated animals from 220 g/day to 400 g/day (82%) and the growth rate of the defaunated animals from 263 g/day to 477 g/day (82%). A combination of defaunation and supplementation with 750 g/day GNC increased the growth rate of heifers from 220 g/day to 477 g/day (117%).

TABLE 4. **Growth rate (g/day) and feed conversion (FCE) of faunated (+F) and defaunated (-F) buffalo given straw-based diets.**

	Total DM intake (kg/day)		Growth rate (g/day)		FCE g DMI/g gain	
Diet	+F	-F	+F	-F	+F	-F
Basal[a]	3.13	2.57	220	263	18.2	11.1
Basal + GNC[b] (250g/d)	3.16	3.08	277	370	12.9	9.8
Basal + GNC (500g/day)	3.50	3.24	360	434	10.1	8.2
Basal + GNC (750g/day)	3.48	3.14	400	477	9.8	7.2

[a] Basal ration: urea-ensiled wheat straw, green fed 3kg, minerals.

[b] GNC = Groundnut cake.

Continual re-infection with protozoa during the study period meant it was necessary to drench each heifer (on average) four times during the 106-day period. During each drenching treatment (2-3 days), feed was not offered and animals took several days to regain full appetite following treatment. Regular drenching therefore greatly reduced feed intake of the heifers in the fauna-free group indicating that the differences reported may be minimal.

Results from these studies clearly show that defaunation can improve ruminant production from straw-based diets. Unfortunately there is no satisfactory method currently available for defaunating animals under field conditions. In these studies a detergent (sodium lauryl diethoxy sulphate) drenched directly into the rumen was used to defaunate animals. This method is not suitable for commercial use. The application of this technology is therefore dependent upon the development of a specific anti-protozoal agent. An alternative approach using leaf material is still being tested. However, the testing has been restricted to sheep because of the quantities of leaf material necessary to feed cattle.

Production of sheep given low quality forages supplemented with *Enterolobium* leaf material.

Cross-bred lambs, 10-11 months of age were dye-branded (technique for determining wool growth) while still in the paddock and then were individually penned in an animal house for 3 weeks prior to the experimental period. A pre-experimental rate of wool production was determined for all animals. Animals were divided into four dietary groups according to weight. The basal diet was oaten chaff (*ad lib.*), 1% urea and a mineral and vitamin mix.

The experimental diets were:

1. Basal diet (oaten chaff + 1% urea)
2. Basal + 125 g/d lupins (cracked)
3. Basal + 110 g/d lupins + (25 g/d) *Enterolobium cyclocarpum* (E.C.) leaves
4. Basal + (90 g/d) lupins + (75 g/d) *Enterolobium cyclocarpum* (E.C.) leaves (diets 2,3,4 were iso-nitrogenous)

Bodyweight change was monitored over an 8 week period and wool growth over the last 6 weeks of the experimental period. There were 9 animals in dietary groups 1 and 2 but only 4 animals in groups 3 and 4 (there was insufficient E.C. leaves to feed 9 animals/group). The results are shown in Table 5.

TABLE 5. **Effects of feeding *Enterolobium cyclocarpum* leaves (EC) on the rate of live weight gain and wool growth of lambs.**

	Dietary Group			
	Urea	Lupins	Lupins + 25g/d EC	Lupins + 75g/d EC
Initial weight	35	35	34	34
Final weight	39	40	41	41
Oaten chaff intake (g/d)	876	853	909	901
Total intake (g/d)	896	894	1029	1021
Growth rate (g/d)	63	93	103	115
FCR (g feed/g gain)	14.3	10.6	10.0	8.9
Wool growth* (clean wool) (g/d)	6	7	7.5	8.8
Rumen protozoa popn. x 10^5/ml	3.1	202	2.6	3.1

* Wool growth rates have been adjusted using pre-experimental wool growth as a covariate.

Discussion of results with sheep

Supplements of EC leaves were readily accepted by sheep. There was no apparent detrimental effects of EC leaves on rumen function even to the extent that protozoal numbers in the rumen did not change. Intake of oaten chaff increased with the addition of leaf.

Supplements of EC leaves significantly increased the rate of body weight gain (24%) and wool growth (27%). These responses appear to be due largely to an improved efficiency of feed utilisation and therefore probably represent a change in the protein to energy ratio in the nutrients absorbed. The supplements (25 and 75 g/d) of EC leaves did not apparently alter the numbers of protozoa in the rumen, so it appears that the leaf had either altered the behaviour of the protozoa (making them more efficient) or affected some other (unknown) aspect of rumen

function. This is an area that requires considerable further study to determine whether there are other effects of the leaf meal on the rumen protozoa.

DISCUSSION

Studies over a period of 15 years at the University of New England have shown that, on low true protein forage based diets in particular, removal of protozoa from the rumen of sheep and cattle and preservation of the fauna free state has improved animal productivity (see Bird *et al.*, 1990; Bird, 1991). The increased productivity appears to be associated with a greater supply of essential amino acids from microbial protein but, where the basal forage is high in protein, extra dietary protein escapes the rumen for digestion in the intestines in fauna free ruminants (Ushida and Jouany, 1986). The increased amino acid supply appears to stimulate productivity of traits that are limited by amino acid supply such as body weight gain, wool growth and milk production. The improved P/E ratio in the nutrients absorbed increases the efficiency of feed utilisation for production.

The identification of a naturally occurring anti-protozoal forage that is relatively easily obtained in tropical countries or can be grown as a crop would open up new potential strategies for increasing animal productivity from poor quality forages.

In the research presented here, only a small range of the forages that are potentially available in tropical countries have been examined and there is a real need for a survey of different groups of plants. At the present time, the active ingredient in the forage has been identified and is being prepared as a single compound for use in many intensive animal production systems.

One of the important aspects of study concerns protozoal ecology in the rumen. It is apparent that many factors in the feed affect the capacity of protozoa to lower the P/E ratio in the nutrients absorbed. Perhaps protozoal activity can be modified by inclusion of other compounds in feed that have been identified through research. Bentonite, a clay mineral, has an overall effect similar to defaunation when added to the

diet of sheep in small quantities (15-20g/d) (Fenn and Leng, 1990). In the studies now presented, sub-optimal levels of this anti-protozoal compound in the diet were almost as effective as the defaunated state in stimulating protein availability to sheep and increasing productivity.

SECONDARY PLANT COMPOUNDS IN TREE FORAGES

Secondary plant compounds are chemicals produced by plants that create an advantage for that plant in the ecosystem within which it has evolved. They are produced as protective agents in a number of plants to modify, limit or prevent damage to the plant against consumption or attack by insects, fungi bacteria, protozoa or grazing animals, and also may be produced to reduce competition by other plants.

In recent times it has been suggested that some plants respond to invasive actions in stimulating an increased synthesis of particular secondary plant compounds which then decrease consumption of the plant by graziers.

It would be surprising that the wide variety of secondary plant compounds produced by a diversity of tree forages were all detrimental to ruminants. It could also be expected that, where forages have been components of the diet of ruminants, microbial diversity and genetic selection would provide the rumen microorganisms with the ability to modify or degrade these secondary compounds.

In the same way, with plants seldom consumed by ruminants because of location of growth, growth habit or taste, it would be surprising if they did not affect selectively or totally the growth of microbes in the rumen and that, where intake of such plants/compounds is controlled, there is great potential to manipulate rumen function.

Bibliography

Bird, S.H. 1991. The role of protozoa in relation to the nutrition of the host animal. In: *Recent Advances in the Nutrition of Herbivores*. Y.W. Ho, H.K. Wong, N. Abdullah and A.Z. Tajuddin (eds.). Malaysian Society of Animal Production pp. 171-180.

Bird, S.H. and Leng, R.A. 1978. The effects of defaunation of the rumen on the growth of cattle on low-protein high-energy diets. *British Journal of Nutrition* 40: 163-167.

Bird, S.H., Nolan, J.V. and Leng, R.A. 1990. The nutritional significance of rumen protozoa. In: *The Rumen Ecosystem: The Microbial Metabolism and its Regulation*. S. Hoshino, R. Onodera, H. Minato and H. Itibashi (eds.). Japan Scientific Societies Press and Springer-Verlag, Tokyo. pp. 151-160.

Coleman, G.S. 1975. Interrelation ships between rumen ciliate protozoa and bacteria. In: *Digestion and metabolism in the ruminant*. McDonald, I.W. and Warner, A.C. (eds.), Univ. New England, Armidale, Australia. pp. 140-164.

Coleman, G.S. 1978. *Techniques in the study of mixed populations*. D.W. Lovelock and R. Davies (eds.). Society for Applied Bacteriology. Technical Series II.

Fenn, P.D. and Leng, R.A. 1990. The effect of bentonite supplementation on ruminal protozoa density and wool growth in sheep either fed roughage based diets or grazing. *Australian Journal of Agricultural Research* 41: 167-174.

Leng, R.A. 1982. Modification of rumen fermentation. In: *Nutritional Limits to Animal Production from Pastures*. J.B. Hacker (ed.). CAB, Farnham Royal, U.K. pp. 427-453.

Leng, R.A. 1990. Factors affecting the utilisation of 'poor quality' forages by ruminants particularly under tropical conditions. *Nutrition Research Reviews* 3: 277-303.

Ushida, K. and Jouany, J.P. 1986. Effects of protozoa on rumen protein degradation in sheep. *Reproduction, Nutrition and*

Development 25: 1075-1081.

Weller, R.A. and Pilgrim, A.F. 1974. Passage of protozoa and volatile fatty acids from the rumen of the sheep and from a continuous *in vitro* fermentation system. *British Journal of Nutrition* 32: 341-351.